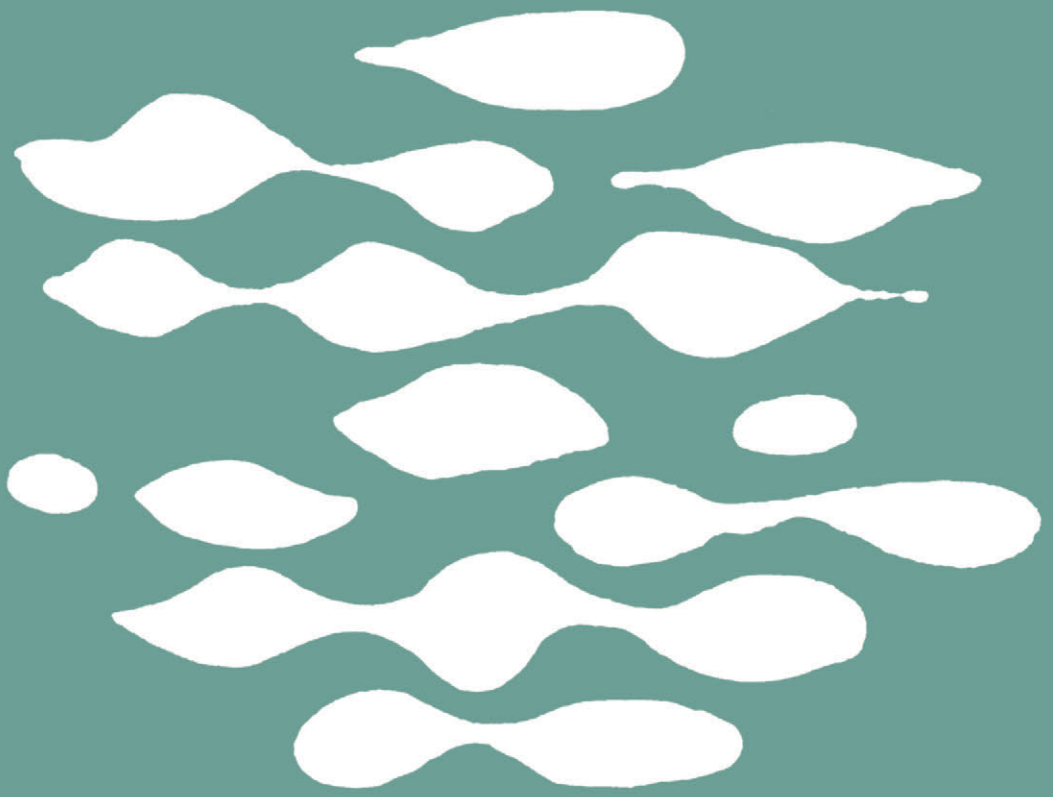
DEVELOPMENTS IN HYDROBIOLOGY

# Evolutionary Biology and Ecology of Ostracoda

edited by
David J. Horne and Koen Martens



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# Evolutionary Biology and Ecology of Ostracoda

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H. J. Dumont

Fifteen papers presented under Theme 3 of the 13th International Symposium on Ostracoda (ISO97), held at the University of Greenwich, Medway Campus, U.K., from 27 to 31 July, 1997. The conference organizers were David J. Horne and Ian Slipper (University of Greenwich), Alan Lord (University College London), Ian Boomer (University of East Anglia[1]) and Jonathan Holmes (Kingston University).

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# Evolutionary Biology and Ecology of Ostracoda

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# Preface: Ostracoda and the four pillars of evolutionary wisdom

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*Key words:* morphology, palaeontology, ecology, genetics, Ostracoda, evolution

**Abstract**

Morphology, palaeontology, genetics and ecology are the main scientific domains contributing theories, concepts and new data to evolutionary biology. Ostracods are potentially very good model organisms for evolutionary studies because they combine an excellent fossil record with a wide extant distribution and, therefore, allow studies on both patterns and processes leading to extant diversity. This preface provides an overview of the 15 contributions to the present volume and concludes that this set of papers supports the claim that ostracod studies are situated in all main evolutionary domains.

## Introduction

The so-called 'Modern Synthesis of Evolution', first coined by Huxley (1942) and later presented in a book edited by Mayr & Provine (1980), integrated Darwinian evolution and Mendelian genetics: evolution occurs by natural selection acting on genetic variability. Since then, several other disciplines have joined the evolutionary 'high table', as it was termed first by Maynard Smith (1984) and later by Eldredge (1995); molecular genetics was given a royal welcome as soon as the necessary techniques were available and palaeontology got a warm 'welcome back'. Ecology (evolutionary ecology in particular) tended to be a rather sleepy participant, but has been a major contributor to recent discussions, ever since handbooks like those of Cockburn (1991) and Pianka (1994) appeared. Other disciplines, such as developmental biology and biogeography, have had occasional invitations, but have yet to find their regular place; that will be merely a matter of time. At present, however, the four pillars supporting the Hall of Evolutionary Studies are morphology, genetics, ecology and palaeontology.

Ostracoda, small bivalved crustaceans, have much to contribute to all four scientific domains. Very few other animal groups can claim such a status. This is so because few other groups combine such an extensive fossil record with such a large extant diversity. Ostracods thus allow us to study pattern and process in space and time, investigating the origins and dynamics of biodiversity. Most of these exciting approaches are in general unavailable in other groups. But in ostracods, palaeoecological deductions (for the Quaternary) can be made with reasonable accuracy, using autecologies of extant taxa; molecular clocks can be calibrated, using phylogenetic branchings with absolute dating from fossils; and robust phylogenies can be established, integrating the results of no fewer than four different fields: morphology and ontogeny, genetics, palaeontology and (past and present) biogeography.

The present volume is one of three resulting from the Thirteenth International Symposium on Ostracoda (ISO97) which was held at the University of Greenwich (Medway Campus) in Kent, U.K., in July 1997. An international delegacy of over one hundred scientists attended ISO97: their diverse backgrounds, in the earth, environmental and life sciences reflect the current breadth of interest in Ostracoda. This special issue brings together 15 papers that were presented in Theme 3 of ISO97, entitled "*Evolutionary Biology and Ecology of Ostracoda*". Papers from themes 1 (*Non-Marine Ostracoda: Evolution and Environment*) and 2 (*Marine Ostracoda and Global Change*) have been published as special volumes of the journals *Palaeogeography, Palaeoecology, Palaeoclimatology*

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and *Marine Micropalaeontology*, respectively. Additional information about Ostracoda can be found in proceedings of the previous IRGO (International Research Group on Ostracoda) symposia (Puri, 1964; Neale, 1969; Oertli, 1971; Swain et al., 1975; Hartmann, 1976; Löffler & Danielopol, 1977; Krstic, 1979; Maddocks, 1983; Hanai et al., 1988; Whatley & Maybury, 1990; McKenzie & Jones, 1993; Ríha, 1995). Since the majority of these are not published in mainstream journals and may not be known to non-ostracod workers, we list the full details in our references.

We hope that this volume will help to place the Ostracoda firmly amongst those organisms, generally used to test evolutionary hypotheses, such as *Drosophila*, *Caenorhabditis, Arabidopsis* and others. In this, we follow a pioneer of evolutionary studies on ostracods, Dan Danielopol, who called one of his books: '*Cytherissa*, the *Drosophila* of Ostracoda' (Danielopol et al., 1990)!

## Genetic diversity

Ostracods were amongst the first aquatic invertebrate groups to be screened with allozyme electrophoresis techniques. We owe much of our knowledge on genetic diversity in Ostracoda to the pioneer work of the universities of Gdansk (Poland), Guelph (Canada) and Parma (Italy). The most interesting results from those studies, although this is a subjective choice, deal with the relationship between reproductive mode and genetic diversity: the discovery that sexual populations do not necessarily have a higher standing diversity than parthenogenetic ones, that different clones can have different autecologies and phenology, etc. More recently, DNA sequencing was successfully applied to two orders of the Ostracoda, Myodocopida and Podocopida, and, being a technique which provides higher resolution results on genetic change, this has again increased our understanding of ostracod evolution. Although there are no genuine genetic papers in the present volume, the keynote paper suggests several ways in which ostracod genetics can be used to study long standing questions in evolutionary ecology. We are happy to have this important contribution, written by two heavyweights in ostracod genetics: Paolo Menozzi is the head of the Parma school studying allozyme variability in non-marine ostracods, while the first podocopid DNA-sequences were obtained (by Isa Schön) in the laboratory of Roger Butlin in Leeds. Because of the advantages of the group, cited above (e.g. variety of reproductive modes, extensive fossil record), ostracod molecular genetics has a great future and will continue to help answer questions of broad biological relevance.

One aspect of ostracod genetics, however, is presently almost totally missing, namely karyology. Alicia Mouguilevsky has made important contributions on myodocopids during the past decade (e.g. Moguilevsky, 1995), but to find cytogenetic work on Podocopida, we have to go back to the papers by Tétart from the 1970s (e.g. Tétart, 1978) and before that to German papers from 1940 to 1950. Nevertheless, podocopid (and especially cypridinid) ostracods have remarkable karyotypes, with different kinds of sex determining systems and multiple sex chromosomes (Schön & Martens, 1998). Further understanding of the evolutionary ecology of reproductive modes in non-marine ostracods will depend largely on improved knowledge of podocopid chromosome patterns.

## Morphology and development

The papers in part I of this volume deal with three different disciplines: ontogeny, comparative morphology (of adults) and functional morphology.

Von Baer's law says that ancestral (general) characters originate earlier in the development than derived (special) characters. Haeckel translated this into the statement that ontogenetic development reflects phylogenetic relationships (Gould, 1977). Although this rule is not universally applicable, a certain congruence in patterns cannot be denied. Ostracods have determinate growth: there are no more moults after the animal reaches the adult stage and animals thus mature at or immediately after the final moult. Most podocopines have eight larval stages, the adult stage being the ninth. This rigid post-embryonic developmental pathway facilitates comparative analyses at high taxonomic levels. As for cytogenetic studies, ontogenetic research in ostracods was especially common in Germany in the first half of this century, but (with a few important exceptions) died out after that. Two complementary papers in the present volume revive this interesting branch of evolutionary research with reference to the non-marine ostracod *Eucypris virens*, by giving a description of a full ontogenetic series of limb development (Smith & Martens), and by following the development of the valve shape in different conditions (Baltanás et al.). Smith & Martens derive

some surprising results from their analysis, including seemingly conclusive evidence that the famous fifth limb in Cypridoidea is indeed thoracic in origin. This was recently foreshadowed by Meisch (1996), but thus far the available morphological evidence for the Cypridoidea indicated a cephalic origin.

One paper deals with comparative morphology. Podocopid ostracods have long been regarded as having lost any trace of abdominal segmentation, but Tsukagoshi & Parker, using detailed Scanning Electron Microscope investigations, show that this is not the case and discuss the phylogenetic implications of remnant segmentation in podocopids.

Research on functional morphology is often still a game of trying to decide between different possibilities, without any means to test hypotheses. Abe et al. have found that such apparent choices can be misleading and deduced that the upper lip in bioluminescent myodocopids has multiple functions.

In spite of the fact that there are at present no less than 25 different species concepts in use (Mayden, 1997), Patterson's recognition concept is the most popular for sexual organisms. It holds that conspecific mates recognise each other through a set of specific characters, called the 'Specific Mate Recognition System' (SMRS). Martens investigates the origin of different copulatory modules (=the morphological part of the SMRS) in related lineages. Also here, a pluralistic approach appeared necessary, as there are good indications that natural as well as sexual selection, stochastic effects and developmental constraint all contribute to some extent to determine which morphologies develop as part of the SMRS.

It is striking that most of the morphological papers in this book deal primarily with soft part morphology, since ostracodologists (those with palaeontological training, at least) have tended to concentrate on valve morphology, even to the extent that new (living) species were often described on these hard parts only. There are several reasons for this development, the main one being that more biologists have become involved in ostracod studies. This is a positive development, as long as it does not lead increasingly to valve morphologies being ignored. Both parts of ostracod morphology have their stories to tell. Some soft parts tend to be more conservative and can be used to derive higher order relationships, other limbs display specific differences and allow identification. Valves generally have more adaptive features and are a source of information on past and present water chemistry. In any case, if we want palaeontological and neontological classifications to remain integrated, then valves are the key as they are usually the only part of the animal that is available to palaeontologists.

## Palaeontology: the fossil evidence

Ostracods are unique in having such a long and detailed fossil record, without which they would be just one more extant crustacean group; fossil ostracods provide a powerful tool to investigate the origins and dynamics of extant diversities. We would thus do well to continue to pay particular attention to the history of ostracod evolution, as documented in a (post-Cambrian) fossil record spanning 400–450 myr! There are only three papers on fossil ostracods in this volume, but each touches upon a core problem of the history of life.

One of us has argued repeatedly that ancient lakes have particular advantages for the study of evolution of adaptive species flocks (Martens, 1997). Janz exemplifies this by summarising his earlier work on ostracod radiations in the fossil ancient lake Steinheim (Germany), with special reference to the beautiful leucocytherinid (Limnocytheridae) species flock. The paper by Khand refers to the origins of present-day (non-marine ostracod) faunas, which most likely are rooted in the Mesozoic (Whatley, 1992; Horne & Martens, 1998). One key question that still remains is what became of the large *Cypridea*-lineage? Why did they become so common and diverse during the early Cretaceous and, even more intriguingly, what caused their extinction (if at all) in the early Tertiary? Even during the most successful period of *Cypridea*, genuine cypridinid faunas were already present. This is best exemplified by the Early Cretaceous *Pattersoncypris* in which phosphatized appendages are beautifully preserved (Bate, 1972), recently redescribed by Smith (1998). The chaetotaxy of this animal is nearly identical to that of present-day Cyprididae, so that we have a documented case of almost 100 myr of morphological stasis. Present-day Cyprididae constitute nearly 80% of the total extant specific diversity of non-marine ostracods (Martens, 1998), so why did they fail to out-compete the *Cypridea* species in the Cretaceous? Molostovskaya's contribution, finally, deals with one of the most interesting aspects of ostracod biology, namely the presence of ancient asexuals. Fully asexual lineages are thought to be doomed to early extinction for several reasons (see Butlin et al., 1998), yet the Darwinulidae apparently managed to

persist for 100, maybe even 200 myr without any form of sexual reproduction. Only one other animal group claims a similar status, the bdelloid rotifers, but the absence of a useful fossil record makes it difficult to substantiate exactly how ancient this asexual lineage really is. The darwinulids, however, are the real thing! Molostovskaya summarises her extensive studies on the early (Palaeozoic) evolution of these darwinulids, in a time that the diversity of this group was much higher and sexuality still a common mode of reproduction. This paper is an important piece of the puzzle which was thus far missing.

## Ecology and palaeoecology

Once again it is the fossil record which makes this aspect of ostracod research especially relevant, as ecological patterns in the present can, to some extent, be extrapolated to the past. Relatively few organisms allow palaeoecological reconstructions, but ostracods offer no fewer than three commonly used techniques to unravel past environments, water chemistries and palaeoclimatic conditions: the use of indicator species, environmentally-influenced valve morphology and valve chemistry. The latter technique is illustrated in several papers in one of the other volumes derived from the 1997 Chatham meeting (Palaeogeography, Palaeoclimatology, Palaeoecology, 148 (1–3), April 1999). The second technique, mostly using valve ornamentation, appears at first glance most useful, but general application is still hampered by the lack of experimentally-derived causality between types of ornamentation and environmental factors. Perhaps the most intriguing problem in this context remains the exact meaning of nodosity, the occurrence of external nodes on the valves. Van Harten spent many years investigating this problem and now presents a novel solution, through the application of catastrophe theory, an approach increasingly used in evolutionary ecological models, for example to assess the impact of extant diversity on resilience of ecosystems (Schulze & Mooney, 1994). Majoran et al. investigated the effect of temperature and salinity on valve morphology in a well-known and extensively debated ostracod, *Krithe*. Such studies are most useful, as it is important to know how much of observed variation is due to genetic, environmental and interactive factors. Such studies are time-consuming, but we will need an extensive database in order to understand the contributions of these different factors to extant variability. A similar study, aimed at establishing the value of another marine ostracod as an experimental model organism, is that of Ikeya & Kato. Neil discusses critically the concept of 'environmentally-cued polymorphism', using examples from his work on Tertiary marine assemblages from Australia.

Reconstructing palaeoenvironments effectively demands from palaeontologists that they extract as much information as possible from their fossil assemblages. Three standard techniques have been cited above. Slack et al. summarise their work on the importance of the signal given by rare species, a signal which can only be obtained if the most common taxa are excluded from the analyses. Abe & Horiuchi, finally, discuss an example of coevolution, that of an isopod parasitic on a myodocopid ostracod – a rather apposite addition to our knowledge of ostracods, given that a whole family of ostracods, the Entocytheridae, is parasitic or commensal on isopods, amphipods and crayfish.

## Conclusions

It should be clear from the above discussion that ostracods have much to offer as a model group for evolutionary research. It should be equally clear that all aspects of ostracod research must be developed in order to fully exploit the potential of ostracods as an evolutionary model. The recent aversion towards micropalaeontology in academic curricula, for example, is in this context highly disturbing. Integrated studies of biological questions using ostracods and applying different techniques, moreover, will lift research to an even higher level. Such research networks should be promoted.

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and Paula Carey, for their assistance. The meeting was held under the aegis of the International Research Group on Ostracoda and the British Micropalaeontological Society. We wish to express our thanks to everyone involved in the peer-review of individual papers, to Hilary Foxwell (University of Greenwich) for redrafting several figures, and to the Editor-in-Chief of the journal for his support.

It is with sadness that we must note the tragic and untimely death of Katsumi Abe a year after attending ISO97. He was an innovative and internationally respected ostracod worker, and we are pleased to be able to include two of his papers in this volume.

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# Open questions in evolutionary ecology: do ostracods have the answers?

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*Key words:* sexual reproduction, parthenogenesis, sexual selection, speciation, metapopulation, diversity, species range

## Abstract

Ostracods have many advantages as study organisms in the field of evolutionary ecology, especially their excellent fossil record. In this review, we consider issues of current interest in several areas, show where ostracods are already providing valuable insights and suggest new ways in which they might be used. We concentrate on non-marine ostracods. The evolutionary maintenance of sexual reproduction is one of the biggest unsolved questions in evolutionary biology and, because they show a wide diversity of asexual forms, non-marine ostracods can help to provide the answer. Recent advances from studies based on fossil data, ecology and molecular genetic approaches are reviewed. Other areas in which ostracods might be used more than they have been to date and that are considered more briefly are: sexual selection on the large and complex genitalia and spermatozoa, the causes and rates of speciation, the consequences of metapopulation structure and the factors determining the boundaries to the geographic ranges of species and the local diversity of species.

## Introduction

A recent introductory textbook of evolutionary ecology describes it as the field for those who have "been fascinated and puzzled by the living world, and thought deeply enough . . . to ask the question 'why?'" (Cockburn, 1991: 2). It is the study of the interactions among living things and between living things and their abiotic environment (ecology), in the context of the historical processes that have resulted in the present patterns (evolution). The field is an exciting and active one, not least because, in Cockburn's words again: "the majority of problems in evolutionary ecology remain to be solved".

Cockburn's book contains only one reference to ostracods in its index. In a section on senescence (p. 174), there is a brief description of a comparison by Bell (1984) between the effects of age on survival rate in asexual organisms that reproduce by fission or by eggs, the latter group including an asexual population of the ostracod *Cypridopsis vidua*. A decline in survival rate with age was observed only in the egg-producing taxa. This is a tantalising glimpse into the potential of ostracods as model organisms for the study of general questions in evolutionary ecology. The field is otherwise dominated by studies of birds, mammals and a limited selection of insect taxa, especially *Drosophila* and the social insects.

Ostracods are unlikely to compete with established groups of study organisms on their 'home ground'. One would not wish to study the genetics of speciation in ostracods in preference to *Drosophila*, nor would one expect to compete with observations on the behavioural ecology of foraging using birds or fish. It will be some time before the geographical distributions of ostracods are known as well as those of flowering plants or butterflies. Nevertheless, ostracods have some important advantages as study organisms. They are very numerous, in a wide range of aquatic and semi-aquatic habitats. They are small, with determinate growth and, in at least some cases, can be cultured in the laboratory. They exhibit some fascinating patterns of variation within and between species which can be used to test ecological and evolutionary

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hypotheses, some of which we discuss below. Most important of all, these features are combined with a long and unusually complete fossil record characterised by *in situ* preservation of large numbers of individuals. The value of combining palaeontological and ecological approaches has been recognised previously (Evans & Griffiths, 1993).

Here we consider some of the current foci of attention in evolutionary ecology and either show how ostracods are being used to tackle 'big questions' or suggest how they could be used. We concentrate on non-marine ostracods since we know them better but we do not wish to imply that marine species have any less potential. We return repeatedly to the potential for combining ecological with broadly palaeontological information. Since the essence of *evolutionary* ecology is the addition of the historical perspective in answering questions about current patterns, the importance of the temporal dimension available for ostracods cannot be underestimated.

The areas we consider are:

1. sexual *versus* asexual reproduction,
2. sexual selection,
3. speciation,
4. metapopulation structure,
5. species boundaries, and
6. local diversity.

This is not intended to provide complete coverage of the status of the field. It is a selection of topics of interest to us which we hope will stimulate ostracodologists to place their observations in broader contexts and evolutionary ecologists to take note of the potential of ostracods as model organisms.

## Sexual and asexual reproduction

The great majority of eukaryotic species reproduce sexually: the life cycle includes meiosis, with the opportunity for re-assortment and recombination of genetic material, and syngamy, combining genetic material from two parental organisms. Asexual reproduction is relatively rare but is widely distributed across taxa: it has a 'twiggy' distribution on the phylogenetic tree. In apomixis, the organism dispenses with both meiosis and syngamy producing offspring that are genetically identical to their parent, except for mutation. Various intermediate modes of reproduction exist (Bell, 1982) but, for simplicity, we will concentrate on the contrast between sex, properly amphimixis, and apomixis.

Asexual reproduction is rare in marine ostracods (Horne et al., 1998) but common in freshwater species (Bell, 1982; Chaplin et al., 1994). Apomixis has been confirmed in a few cases and is assumed to be the mode of reproduction in all ostracods that lack males (Rossi et al., 1998). Chaplin et al. (1994) reported that seven out of 10 podocopid families, and 24 out of 29 North American genera, of freshwater ostracods include asexual lineages. An analysis of European data (NODE database – see Horne et al., 1998) using more up-to-date taxonomy indicates that no males have been recorded in 57% of 286 species of Cypridoidea, or in 28% of 50 species of Cytheroidea (the latter based on incomplete analysis at present). However, asexual lineages are known to occur in species that also have sexual populations, so these figures underestimate the occurrence of asexuality. Species which include both sexual and asexual lineages typically show a restricted geographical distribution for the sexual relative to the asexual form (termed 'geographic parthenogenesis', Vandel,1928). Well studied examples include *Heterocypris incongruens* (Turgeon & Hebert, 1994) and *Eucypris virens* (Horne et al., 1998; Schön & Butlin, 1998). The most likely explanation for these patterns is that asexual lineages are generated frequently from sexual species: indeed, freshwater ostracods may have the highest rate of origin of asexual lineages of any animal group (Chaplin et al., 1994). At the same time, the ostracods include a lineage of 'ancient asexuals', the Darwinulidae, with no sexual representatives for at least 25 M yr and probably longer (Butlin & Griffiths, 1993; Rossetti & Martens, 1996; Schön et al., 1996).

Finding the explanation for the near ubiquity of sexual reproduction, and the apparently restricted evolutionary potential of asexual lineages, is a major challenge in evolutionary ecology (Bell, 1982; Kondrashov,1993). In fact, there is no shortage of theories concerning the advantages and disadvantages of the different modes of reproduction, as evidenced by Kondrashov's (1993) compilation. The problem is to find ways of distinguishing the relative contributions of the various suggested sources of selection. Kondrashov argues that little further progress can be made by examining broad patterns of taxonomic or geographical distribution: instead, it is necessary to focus on the discrimination of specific predictions in suitable model organisms. We believe that ostracods may have some of the answers.

*Table 1.* Maynard Smith's two-way classification

| Origin of gene associations: | | Selection eliminates bad genes | Selection favours good gene combinations |
|---|---|---|---|
| | Chance | **Muller's Ratchet** | **Faster evolution** |
| | Selection | **Mutational load** | **Shifting optimum** |

This is not the place to review the costs and benefits of sex at length (see Kondrashov, 1993, or Butlin et al., 1998, for a simpler version). However, an outline may be helpful, using the two-way classification introduced by Maynard Smith (1989) for factors favouring sex. These factors have to work in opposition to the 'two-fold cost of sex': a gene in a sexual female gets into only 50% of her offspring, whereas a gene in an asexual female gets into all of them. At the organism level, this is equivalent to a cost of producing males (at least in anisogamous organisms without paternal care). Fundamentally, what sex does is to shuffle genes. It does not create variation – it is a common misconception that sexual populations are more variable than asexual ones (see below) – but rearranges variation, particularly by breaking up associations between variants at different genetic loci. These associations can be generated either by chance (that is, by mutation and/or genetic drift), or by natural selection which favours some combinations and removes others. The source of associations between genes is the first of Maynard Smith's axes. The second axis concerns the role of selection: does it favour the spread of new advantageous gene combinations, or remove disadvantageous genes? (Table 1).

All populations suffer from a continuous introduction of deleterious mutations. These tend to be removed by selection but, in an asexual population, selection can only act to favour the lineage with the least mutations. In a finite population, the best lineage may be lost by chance and then selection can only favour the second-best lineage: without sex and recombination, the number of deleterious mutations cannot be reduced. There is then some chance that the second-best lineage will also be lost, and so on. This is Muller's Ratchet. In the long term, it leads to progressive reduction in the fitness of asexual lineages.

In the shorter-term, and even in infinite populations, asexual lineages may suffer from the synergistic effects of deleterious mutations occurring in separate genes. These bad gene combinations are efficiently removed by selection in sexual populations, but cannot be broken up in asexual lineages and so represent a significant mutational load which reduces fitness.

The converse of this is that asexual populations are unable to bring together advantageous mutations that occur in separate lineages even if they have synergistic beneficial effects. In sexual populations, recombination can rapidly combine such advantageous genes and so the population can evolve more rapidly. This is the classic explanation for sex proposed by H. J. Muller in 1932, but it is a long-term explanation which only works in finite populations.

Finally, it is proposed that the optimum phenotype for a population is constantly changing, probably mainly because of the organism's interactions with competitors, predators and parasites. A sexual population is able to evolve rapidly in response to these changing selection pressures because of the continuous generation of new combinations of genes whereas, in an asexual population, the set of genotypes present can be modified only by mutation. This is akin to the 'Red Queen' hypothesis in ecology: constant evolution is necessary to stay fit in a changing environment (Van Valen, 1973).

Recently, an experimental investigation using yeast has demonstrated loss of fitness due to deleterious mutation in asexual populations but no improvement in the rate of adaptive evolution in sexual populations (Zeyl & Bell, 1997). However, given the short-term nature of the experiments and the inevitable simplicity of the selective environment, this result does not preclude a role for sex in promoting rapid evolution.

An important distinction between these ideas is the timescale over which they operate. The 'cost of males' is immediate and occurs in every generation. It can be counteracted in the short term by the 'shifting optimum' and 'mutational load' processes and in the long term by the 'Muller's Ratchet' and 'faster evolution' mechanisms. On balance, one can see that new asexual lineages might have an initial advantage, at least under some conditions, which enables them to

spread at the expense of their sexual progenitor but that this advantage may be lost over time. In outline, this explains the twiggy distribution of asexual taxa. However, it leaves many questions unanswered: What are the timescales involved? Why do some taxa or environments have more asexual 'twigs' than others? Which of the benefits is most critical in reality?

There are three areas in which there is much current interest and where ostracod studies are having an impact: the role of clonal diversity, the timescale from origin to extinction of asexual lineages and the paradox of ancient asexuals.

*Clonal diversity*

Clonal diversity varies widely both among asexual 'species' and between individual populations of the same species. The level of diversity within a species is a balance between the rate of origin of clones, either from sexual progenitors or by divergence from existing clones and the rate of extinction of clones. Within populations, the rate of immigration is also significant. It is a fairly simple task to measure clonal diversity using genetic markers such as allozymes or DNA sequence polymorphisms (Rossi & Menozzi, 1994), but possibly the most interesting aspect of clonal diversity studies is the question of ecological differentiation. This is important because of its potential influence on competition between asexual and sexual lineages.

The classic study in this area is the work of R. C. Vrijenhoek and his colleagues on *Poeciliopsis* fish in Mexican streams (see Vrijenhoek (1994) for a review). As in all asexual vertebrates, the clonal lineages in these fish arise by interspecific hybridisation. In this case, they can never replace the sexual forms since they remain dependent on sexual males for sperm to stimulate egg development, although the males contribute no genetic material to the offspring (this 'gynogenesis' is also known in some insects but has not yet been reported in ostracods). Vrijenhoek has shown that clones differ in their ecological requirements. Individual clones apparently can only exploit a limited range of available resources, but clones with different requirements can coexist and diverse clonal populations can exploit the environment more fully. The sexual populations are more flexible but appear to be unable to displace a clone from an environment to which it is well adapted. The result is that stretches of river containing many clones have only a low frequency of sexual individuals while localities with few clones have relatively large sexual populations. If it were not for the requirement for sperm, the sexual populations would surely be driven to extinction by clonally diverse asexual populations. This would cut off the source of new clones, in this case almost certainly from repeated hybridisations between the sexual parent species, and thus indirectly condemn the asexual lineage as a whole to extinction.

There is scope for comparable studies in ostracods. Clonal diversity in ostracods varies widely, reaching its highest levels in species with sexually reproducing populations (Rossi et al., 1998). For example, in *Eucypris virens* 211 clones have been identified electrophoretically within Europe, while in *Limnocythere inopinata* 125 clones have been detected in Italy, with the maximum diversity within a single site being 36 clones (Rossi et al., 1993; Rossi et al., 1998). Asexual lineages of this species are widespread across Europe, but only a few sexual populations are known all around the Mediterranean. In at least one of these localities, sexual and asexual lineages coexist (Rossi et al., 1998). It is also known that clones can differ in ecologically important parameters. Electrophoretically identified clones of *Heterocypris incongruens* differ in the response of life-history parameters to temperature and photoperiod, and these responses can be related to seasonal changes in abundance in the field (Rossi & Menozzi, 1990, 1993).

There remain great opportunities for studies in this area. The ecological interactions, in the laboratory and in the field, between sexual and asexual lineages should be the main focus of attention. However, there is also potential here to relate studies of the current interactions to the history of coexistence using information from the fossil record. Unfortunately, *Eucypris virens* is not a good candidate for this work because it is a pond species that does not preserve well. However, *Limnocythere inopinata* may be: it is a lacustrine species with a good fossil record and some ecologically distinct clonal groups may be identifiable from valve morphology (Geiger et al., 1998). Many questions could be addressed. Is clonal diversity related to the age of an asexual lineage? Are clonal lineages with close sexual relatives typically more persistent, or less? How well do clonal or sexual lineages colonise new habitats?

*Ages and origins of clones*

The costs and benefits of sexual reproduction operate over quite variable time scales. Although it is easy to see that an asexual lineage might have a short-term ad-

vantage but a long-term disadvantage with respect to a sexual lineage, it is much more difficult to say what actual times are involved. Until recently, this question could only be tackled through the fossil record and was, therefore, limited to taxa with good preservation and the potential to distinguish males and females on the basis of hard parts. Ostracods do fall into this category and have recently been used by Griffiths & Butlin (1995) to investigate the persistence of asexual and sexual taxa in 34 cores from the excellent Holocene sedimentary record in Europe. Sexual species are more persistent, more abundant and have less variable abundance through time than asexual species. This may be because of the ability of sexual populations to evolve in response to environmental change. However, this type of approach is necessarily limited by the fact that it can only follow 'species' which, for asexuals, are amalgams of unknown numbers of clones.

The new alternative approach to the age and origin of clones is the use of molecular data to reconstruct their phylogenetic and biogeographic history (Butlin & Griffiths, 1994). This approach has been used quite extensively to demonstrate the hybrid origin of asexual vertebrate lineages and estimate the ages of clones (Avise et al., 1992). Generally, these lineages appear to be young (<0.5 M yr), with some exceptions which may be explained by occasional sexual exchange of genetic material with their progenitor species. But asexual vertebrates may well be atypical and there are few studies of invertebrates at present.

In *Eucypris virens*, we have studied the molecular divergence among individuals from a wide range of European populations, including sexual populations in Spain and Sicily (Schön & Butlin, 1998). Two genes have been examined: a nuclear gene, the internal transcribed spacer of the ribosomal DNA and a mitochondrial gene, part of the cytochrome oxidase I locus. Based on the nuclear gene, the pattern of relationships indicates multiple origins of asexual lineages from sexual ancestors, apparently in some cases more recently than the isolation of the two sexual populations. This result was expected. Much more surprising is the extent of divergence observed, with up to 10% sequence divergence within Europe. It is difficult to convert this to an estimate of time since no calibration is available from other crustaceans, let alone ostracods. Using the rate of divergence observed for this gene in *Drosophila* gives a time of separation 4 M yr ago. At first sight, this suggests that asexual lineages of *Eucypris virens* have persisted for a remarkably long time, but this conclusion is uncertain because it is not known where in the ancestry of the individuals sampled the transition to asexual reproduction occurred. A more complete tree, that is one with more samples included, would help to overcome this problem although it can never be solved completely. Better calibration would also be desirable and is surely possible given the excellent ostracod fossil record, but may have to rely on species with a better record than *E. virens*.

The mitochondrial DNA data for *Eucypris virens* contain another surprise: the phylogenetic relationships implied are not consistent with the nuclear gene phylogeny (Schön & Butlin, 1998). Such inconsistency is to be expected in sexually reproducing species where mitochondrial gene exchange can occur independent of nuclear exchange. However, it should not occur in strictly apomictic lineages. In this case, the most likely explanation is that females in apomictic lineages can occasionally reproduce sexually with males of a sympatric sexual population. This has been suggested in other ostracods (Turgeon & Hebert, 1994, 1995). It is clearly important because it can provide an input of new genetic material to the asexual lineage, helping it to overcome Muller's Ratchet and generating new clones which increase its evolutionary potential. If the *Eucypris virens* lineages are really as much as 4 M yr old, this process may help to explain their persistence. It has been suggested, with reference to *Tonnacypris*, that this process might generate short-lived lineages with novel morphologies, possibly explaining some 'zone fossils' (Griffiths et al., 1998).

### *Ancient asexuals*

It is always worth examining apparent exceptions to general rules. In the debate about sexual reproduction, the 'ancient asexual scandals' (Judson & Normark, 1996), therefore, have a special place. A few taxa have apparently persisted without sex for very long periods of time and even managed to diversify. These 'ancient asexuals' are a scandal because all the theories suggest that asexual lineages should be doomed to extinction, either through mutation accumulation or failure to evolve in response to environmental change. Understanding how they have managed to keep up with environmental change and avoid accumulating deleterious mutations can potentially reveal a lot about these processes. The most celebrated ancient asexuals are the bdelloid rotifers – reputedly over 35 M yr old and with 363 apomictic species – but the ancient asexuals with the best fossil record are the darwinulid

ostracods. There are 25 extant darwinulid species and the fossil record of the family extends back to the Permian. Since females have a brood pouch, sex can be determined with some certainty from fossil valves.

The fossil record of darwinulids is a key advantage over other ancient asexuals, but the mechanisms that underlie their persistence have to be studied at the genetic and ecological levels. This work has recently begun. Clonal diversity in *Darwinula stevensoni*, as assessed by allozyme electrophoresis, is very low both in North America (Havel & Hebert, 1993) and in Europe (Rossi et al., 1998). DNA sequence data for the same two genes as we have used in *Eucypris* produce another surprise (Schön & Butlin, 1998). The rate of evolution of the mitochondrial sequence can be calibrated from the fossil record using two other darwinulid species as outgroups and suggests that clones within *D. stevensoni* have a maximum age of about 4.5 M yr (in our widest comparison between European and African samples). However, within these same samples, there is no variation in sequence for the nuclear region at all! One way in which ancient asexuals could escape Muller's Ratchet would be to have a low nuclear mutation rate (Schön & Martens, 1998; Schön et al., 1998), so maybe there is evidence that *D. stevensoni* has achieved this. Before this idea is confirmed, it is necessary to demonstrate that other nuclear genes also show low evolutionary rates and that the lineage is genuinely apomictic. Apomictic reproduction in the present populations can be tested by observing allozyme or DNA marker genotypes of families, while long-term apomixis can be tested using the 'Meselson effect', the progressive increase in heterozygosity and divergence between alleles that is expected in the absence of sex and is now potentially detectable by molecular methods (Judson & Normark, 1996).

These examples show that ostracods can play a major part in the study of the evolutionary ecology of sexual reproduction – one of the biggest outstanding questions in the field. We have dealt with this topic at some length, partly because of its importance and partly because of our own involvement with the topic. Below, we consider five other areas of current interest in less detail.

## Sexual selection

One consequence of sexual reproduction and the evolution of anisogamy is a conflict of interests between males and females. In most species, males compete for access to females: because sperm are cheap their reproductive success is limited mainly by the number of matings they can obtain. In contrast, females often need to mate only once to obtain enough sperm for their full complement of eggs: their reproductive success is influenced more by the 'quality' of the male they mate with than by the number of matings. This conflict results in strong selection pressures, especially on males, which can act in opposition to other components of natural selection. For example, sexual selection favours large size and big antlers in red deer males, but both of these have costs in terms of juvenile mortality and adult longevity respectively.

Sexual selection has been one of the most productive areas of evolutionary ecology in the last 20 years (Andersson, 1994). Some aspects of sexual selection are well understood but there is still much debate surrounding the so-called 'paradox of the lek'. The problem here is to explain why females exhibit strong preferences among males and males have very elaborate and expensive ornaments as a result of these preferences, when males contribute nothing but sperm to the offspring. It is called the paradox of the lek because it is most apparent in species of birds in which males form aggregations, 'leks', solely for the purpose of mating. Black grouse are a classic example (Alatalo et al., 1991). It is difficult to prove that females gain no direct benefits or, equivalently, no reduction in mating costs from choosing males. However, if males provide no direct input that might increase the number or quality of offspring, female preferences must be driven by some advantage in terms of the genetic fitness of their offspring. Two types of model compete to account for this advantage: either the male traits chosen by females act as 'honest signals' of the condition of the male or the male traits are arbitrary. The first idea requires that the traits are costly, otherwise they can be faked and are no longer reliable signals and that the condition of the male is genetically correlated with the fitness of offspring he sires. The second idea, the 'Fisher runaway process', works primarily because choosy females have sons who are themselves chosen and thus have high fitness through their high mating success. Thus, there is no need for the male signal trait to be associated with other components of fitness. There is no clear discrimination between these mechanisms yet.

A recent extension of this thinking has been to consider the possibility for female choice during or after copulation, and conversely the opportunity for

males to influence the fate of their ejaculates within females. Eberhard (1985, 1996) argued that much of the extraordinary variability in morphology of animal genitalia could be a result of 'cryptic female choice'. In effect, he suggested that males continue to signal to females during copulation through the interaction between their external genitalia and the female genital opening. For an interesting recent study see Arnqvist et al. (1997). Females may react to such signals by varying the duration of copulation and thus the amount of sperm transferred, or by altering the subsequent utilisation of sperm. There is now evidence that females do influence usage of sperm from different ejaculates both within species (in sand lizards: Olsson et al., 1996) and where a female has been mated by males of different species (in grasshoppers and crickets: Howard & Gregory, 1993).

Ejaculates typically contain much more than just sperm. The accessory gland secretions of males may provide nutrients to females but they may also influence her behaviour by, for example, promoting oviposition. If, as may often happen, the interests of the males and females do not coincide, these interactions lead to 'antagonistic selection' – an 'arms race' between males and females. In *Drosophila*, components of the accessory fluid decrease the longevity of females (Chapman et al., 1995) and sexual conflict results in coevolution of males and females, as predicted (Rice, 1996; see also Stockley, 1997).

In general, ostracods do not have striking external signal traits comparable to peacock's trains, although the bioluminescence of some marine ostracods might well qualify (Cohen & Morin, 1993). Two features of ostracods suggest that they have great potential for research in this area: large and complex genitalia, and large and complex sperm. The intromittent apparatus of male ostracods is, indeed, impressive. In some species, such as *Candona suburbana*, the complex paired hemipenes may reach 35% of the body length and the reproductive system as a whole, one third of body volume (Kesling, 1965; Cohen & Morin, 1990). The suggestion that "the complexity and size of the reproductive system result in part from the small size of the animal and difficulty of copulating while enclosed in a bivalve carapace" (Kaesler, 1987: p. 248; quoted in Cohen & Morin, 1990) really cannot be a sufficient explanation. Another traditional alternative is that the complexity is due to selection for reproductive isolation between species. This is considered and rejected by Eberhard (1985): species may be a consequence of divergence in genital morphology (see below), but for selection for reproductive isolation to drive divergence depends on the widespread operation of the process of reinforcement (selection for assortative mating caused by the production of unfit hybrid offspring; Butlin, 1989; Butlin & Tregenza, 1998). A more plausible explanation is that complexity and size of genitalia are a result of sexual selection by female choice. There is an excellent opportunity to test this hypothesis in species that show variation in hemipenis morphology, such as *Cythere omotenipponica* (Tsukagoshi, 1988). Martens (2000) considers sexual selection in Limnocytherinae operating on the whole copulatory module.

Until recently, ostracods held the record for the longest sperm, relative to body size, in the animal kingdom: *Propontocypris monstrosa* has sperm 5–7 mm long but a body length of only 0.6 mm (Wingstrand, 1988). Unfortunately, this position has recently been usurped by *Drosophila bifurca* whose sperm are 58 mm long: about 20 times the male body length (Pitnick et al., 1995). There are three types of explanation for these giant sperm: they may be a form of paternal investment in the zygote, they may ensure that paternal mitochondria are represented in the zygote or they may have an advantage in competition between ejaculates in multiply mated females. The first two explanations are made less likely, at least as general theories, by the observation that the proportion of the total length of sperm that enters the egg varies widely among *Drosophila* species: in *D. bifurca* only 1.6 mm of the 58 mm sperm enters on average (Karr & Pitnick, 1996). Sperm competition, a type of post-mating sexual selection, is the most likely explanation for the origin of giant sperm although they may obtain other functions subsequently. The extraordinary variation among ostracods in sperm size and sperm morphology that has been so beautifully documented by Wingstrand (1988), cries out for experimental investigation along these lines. The enigmatic Zenker's organ, apparently a sperm pump (Kesling, 1957; Matzke-Karasz, 1997), also deserves further functional study.

## Speciation

Under Mayr's Biological Species Concept, the origin of species is equivalent to the evolution of new barriers to gene exchange. These barriers are usually divided into those that occur before fertilisation, 'prezygotic' and those that occur later through the inviability or infertility of hybrids, 'postzygotic' barriers. Until re-

cently, research into speciation concentrated on the geographic context in which reproductive isolation evolves: is speciation allopatric, parapatric or sympatric? This approach tended to neglect the nature of the reproductive barriers, phenotypically and genetically, and the selection pressures that caused their evolution. However, significant progress has been made in the last 10 years, perhaps dating from the publication in 1989 of 'Speciation and its Consequences' (Otte & Endler, 1989) and a paper by Coyne & Orr (1989; and see Coyne & Orr, 1997).

Coyne & Orr (1989) reviewed studies of pre- and post-zygotic isolation between more than 100 pairs of *Drosophila* species and related the strength of isolation to the time of divergence of the species pair, as assessed by allozyme genetic distance. Species pairs were also categorised on the basis of overlaps in their ranges as either sympatric or allopatric. The results were striking: postzygotic isolation accumulates steadily with time and does not differ between allopatric and sympatric pairs but prezygotic isolation is much stronger in recently diverged sympatric species than in similar aged allopatric species. Prezygotic isolation is the primary cause of isolation among recently diverged sympatric species but both forms of isolation contribute to speciation in allopatry.

At the same time, genetic studies of postzygotic isolation, especially hybrid sterility, were also gathering pace. A fairly consistent pattern has now emerged (Wu & Palopoli, 1994). Postzygotic isolation is typically influenced by many genes and sterility or inviability of hybrids is a result of interactions among these genes rather than the effects of single loci. This was predicted by Th. Dobzhansky and H. J. Muller about 60 years ago! It is consistent with the gradual accumulation of small genetic changes in diverging populations which *incidentally* cause reductions in fitness when brought together in hybrids. Gradual accumulation explains the strong correlation with time of divergence in Coyne & Orr's (1989) study.

Progress in explaining prezygotic isolation has been less impressive, despite the fact that it seems to be the key to speciation in many organisms (Butlin & Ritchie, 1994). There are three major classes of explanation: it may be a side-effect of rapid divergence by sexual selection or sexually antagonistic selection, it may be a side-effect of natural selection, or it may be a direct consequence of selection to avoid production of unfit hybrids (reinforcement). Each of these possibilities is supported by recent research. Sexual selection may underlie the rapid diversification of cichlid fishes in the African rift valley lakes where closely related species pairs differ mainly in male sexual coloration (Turner & Burrows, 1995). Sexually antagonistic selection may play a part in *Drosophila* speciation (Rice, 1996) and could be responsible for 'assortative fertilisation' in crickets (Howard & Gregory, 1993) where sperm from heterospecific matings are only successful in fertilisation in the absence of homospecific sperm. Natural selection for divergent 'benthic' and 'limnetic' specialist morphologies in three-spine sticklebacks in Canadian lakes may incidentally alter the pattern of mate choice, thus reducing gene exchange and promoting further divergence, ultimately leading to sympatric speciation (Schluter, 1996). Finally, the controversial idea of reinforcement has received recent support from a study of flycatchers in Europe whose plumage divergence in an area of range overlap may have been driven by selection against hybrids, and thus against birds that mate heterospecifically (Sætre et al., 1997; and see Butlin & Tregenza, 1998).

Speciation is a process which happens slowly, at least in the majority of cases. A temporal dimension is helpful to its study and in most taxa can only be provided by the 'molecular clock', as in Coyne & Orr's (1989) review which concludes that speciation typically takes 1.5–3.5 M yr in *Drosophila*. In many ostracod taxa, a temporal view of divergence in valve morphology can be added to this, with a resulting increase in potential insights. Molecular data have little temporal resolution and limited geographic resolution. We believe there is enormous potential for studies of ostracods that combine surveys of variation in morphology across time and space, with data on genetic differentiation of extant populations and studies of the actual barriers to gene exchange between species, or between divergent populations. There is ample material for such studies: ostracods have apparently speciated extensively in many lakes such as *Limnocythere* in the Galla and Awassa basins of Ethiopia (Martens, 1990), *Cytherissa*, *Candona* and others in Lake Baikal (Mazepova, 1994) or *Candona* in Lake Ohrid (Mikulic, 1964). Marine systems offer alternative opportunities such as the different forms of geographic separation identified by Cronin (1988) on isolated oceanic islands or across the Isthmus of Panama (where other important speciation studies have been carried out, e.g. Lessios & Cunningham, 1990). Martens (1994) argues that speciation is a slow process in ostracods in ancient lakes, but also that gradualistic evolution has not been observed, leaving a paradox. By contrast, Cronin (1985, 1988) and What-

ley (1988) consider that speciation in marine ostracods can be punctuational or gradual and can be completed in less than 0.5 M yr. These conclusions are typically based on morphological comparison: it is very important that species status is also assessed genetically wherever possible and this may well hold some surprises.

We advocate a shift in emphasis from documentation of interspecific differences to experimental study of intraspecific variation. Once speciation is complete, it is extremely difficult to distinguish alternative hypotheses concerning the mode of evolution of reproductive isolation, or even to identify those changes that caused speciation among those that have accumulated since speciation. Systems such as that described by Tsukagoshi (1988) would be an excellent starting point: interspecific comparisons suggest that divergence in genital morphology is associated with sympatry between closely related species of *Cythere*. Within *Cythere omotenipponica*, there is geographic variation in genital morphology. Does this variation influence gene exchange as judged by genetic markers? If this species can be observed in captivity, does genital morphology influence mating success in interpopulation crosses? Is there heritable variation within populations? Are features of the genitalia under sexual selection (see Martens, 2000)? If these questions, and others like them, cannot be tackled in *Cythere*, there must be many other ostracod taxa in which they can be approached and where the answers will be of general importance.

## Metapopulations

Evolutionary change, including speciation, is strongly influenced by population structure, by which we mean the spatial distribution of individuals and the genetic variation they contain (Hewitt & Butlin, 1997). Currently, much attention is focused on a specific class of population structures known as 'metapopulations'. Put simply, a metapopulation is a population of populations. The importance of metapopulations lies in the persistence of the population as a whole despite the inevitable extinction of each of its component parts. Consider a weevil that feeds on a single species of plant. The food plant exists in discrete patches each of which can support a small population of beetles. Now, the population of beetles in any one patch will go extinct with some probability in each generation, either because of stochastic variation in numbers or because of changes in the ecology of the patch. In the absence of migration between patches, the whole population will eventually go extinct when all of its component patches have died out. But, with migration, empty (or newly formed) patches can be colonised. If colonisation is sufficiently frequent, a balance between extinction and colonisation is possible in which some patches will be occupied by beetles and some will be empty at any one time. This provides a different way of thinking about the way a species persists in a complex ecological landscape which is particularly valuable in the context of conservation: human activity may divide a once continuous habitat into a series of patches or remove patches from a pre-existing network, thus prejudicing the long-term persistence of organisms that rely on it.

An excellent concrete example of metapopulation thinking is provided by the study of the silver-spotted skipper butterfly *Hesperia comma* (Thomas & Hanski, 1997). This species is endangered in southern England, mainly because of loss of habitat, but has been recovering recently and occupying new patches of suitable habitat. Suitable habitat patches can be identified readily: the butterfly only occurs on south-facing calcareous grassland where its food plant, *Festuca ovina*, grows in a defined microhabitat. Metapopulation theory predicts that larger patches (where larger populations go extinct less frequently) and patches close to their nearest neighbours (which are colonised more readily) are more likely to be occupied than smaller or more isolated patches. This is true for *H. comma* on the chalk downs of southern England. Theory also predicts that small patches might be unable to sustain populations despite the availability of sufficient resources, because they suffer greater depletion of numbers from emigration. Again, this explains the observed pattern of population sizes in patches of different area in *H. comma* better than a model that does not allow for emigration.

Metapopulation structure also has genetic consequences. If new populations are founded by individuals drawn from many patches, metapopulations may maintain as much or more variation than equivalent undivided populations. However, it is more likely that new populations are founded from adjacent patches in which case metapopulations lose variation by extinction of local populations and the colonisation process tends to reduce differences among subpopulations. An example of the genetic structure of metapopulations is also provided by a study of endangered butterflies (Brookes et al., 1997).

The evolutionary significance of metapopulation structure is two-fold: it influences the amount of genetic variation maintained in the population as a whole and the way it is distributed among subpopulations and it gives many opportunities for partially isolated populations to diverge and become ecologically specialised. A metapopulation is essentially the structure envisaged in Wright's 'Shifting Balance' view of evolution. It potentially allows genetic changes fixed by chance in small local populations to open up new adaptive opportunities. An evolutionary innovation that arises in one local population can then spread to other populations. Metapopulation structure can increase the ability of asexual species to persist in the face of changing environments (Ladle et al., 1993).

Ostracods that live in small, discrete water bodies, such as rain pools or small ponds, almost inevitably have a metapopulation structure. Species that live in lakes or in the sea may have a less obvious substructure, but in many cases it may still be there. These environments are by no means uniform and samples from different parts of a lake are notoriously variable in species composition. The spatial scale at which ostracod metapopulations are structured will be dependent on dispersal and may be greater for species with desiccation-resistant eggs (Proctor, 1964; Baltanàs, 1998). In fact, genetic analysis shows little geographic structure for such species (Sywula, 1990; Rossi et al., 1998; Schön et al, in prep.). Once again, the fossil record of ostracods has the potential to add a temporal dimension to any study of the spatial patchiness of distributions which is not available for most organisms. Metapopulation thinking has yet to make much impact among ostracodologists, but interest is beginning to grow (Martens, 1997; Baltanàs, 1998).

## Geographic boundaries of species

The prospect of global climatic change, and the retrospective realisation of natural climate change in the past, is focusing much attention on the way in which animal and plant distribution limits will be, or have been, influenced (e.g. Harrington & Stork, 1995). One cannot predict changes in species ranges unless one understands the parameters that determine the current range boundaries. This is a problem that can be viewed at two levels. It can be seen as a purely ecological problem: what are the key factors that result in positive intrinsic rates of increase in one environment and negative rates in another. This is itself a difficult question to answer because of the very large number of potential influences. However, it does seem that climatic variables play an important part: phytophagous insects, for example, typically have ranges that are markedly smaller than those of their host plants and are influenced by climate (Quinn et al., 1997). Metapopulation thinking can help to explain species boundaries: it may be that the boundary represents the point at which patches of suitable habitat become so sparse or small that extinction outweighs colonisation (Lennon et al., 1997).

The alternative view poses the question in a more evolutionary way: why is the species unable to evolve the ability to exploit the environment beyond its current limits? Explicit modelling of species boundaries from this perspective has recently presented new openings for empirical study. In some cases, populations at the edge of a species distribution may be poorly adapted to their local habitat and only be maintained by immigration from populations in habitat that suits the species better. Populations that are maintained by immigration are called 'sink' populations. Holt & Gomulkiewicz (1997) investigate the influence of gene flow from 'source' populations into 'sink' populations at the species boundary. This gene flow can prevent adaptation of the sink population to its environment by swamping new adaptive mutations that arise. But immigration is essential to maintain the sink population, so the species cannot extend its range by adapting to new environments encountered at its margin. Holt & Gomulkiewicz (1997) question this picture: their model suggests that the availability of genetic variants with large effects on fitness in the new environment is the most important limiting factor. Increased immigration can actually favour adaptation by supplying more genetic variation from the source population and making available a larger population size in which mutation can occur.

Empirical studies of populations in marginal habitats with varying degrees of connection to populations in favourable habitats are needed, especially where environmental or gene flow parameters can be modified experimentally. If these could be combined with historical information on range spread, which may be possible in ostracods, they would be all the more powerful. There is now an excellent database available for the distributions of European non-marine ostracods, both extant and Quaternary (the NODE database constructed by D. J. Horne and other members of the European Union network programme 'Evolutionary Ecology of Reproductive Modes in Non-Marine

Ostracods') (Horne et al., 1998; Griffiths & Horne, 1998). One might expect that many species would have clear range boundaries, which they had reached within the last 10 000 years as a result of postglacial expansion. The arrival of species in their current localities may be documented in the Holocene sedimentary record. New precisely dated habitats are frequently created by the construction of ponds and reservoirs and could be constructed experimentally. Expansion and the nature of current range boundaries can be compared between sexual and asexual taxa. There is enormous potential here, but unfortunately, despite the size of the NODE database, most species have not been reported sufficiently frequently for the limits to their distributions to be interpreted with confidence. Common species are reported from throughout the range covered by the database, but a species like *Cypris bispinosa*, apparently restricted to the Mediterranean region, has only 39 records and its range margins must be in doubt (Baltanàs, pers. comm.). Every effort must be made to improve the quality of data available on such a fundamental question as this.

## Species diversity

Finally, an old problem continues to attract attention: how do many species coexist within a habitat? This is part of a larger question concerning the variation in species diversity among habitats, both on small and on global scales (Gaston, 1996). In a way, it is the same as the question of species boundaries: diversity at any one location is determined by the number of species whose ranges (geographical and ecological) encompass that locality. From the palaeontological perspective, it is also a question of species boundaries but, for fossil species, limits in time are important, as well as spatial boundaries.

There are two broad perspectives on species diversity: either it reflects the diversity of ecological opportunity and is limited by interspecific competition or it reflects a balance between colonisation and extinction by species from a regional 'pool' as in the MacArthur & Wilson (1967) model of 'island biogeography'. The latter perspective has gained support recently, partly because of the realisation that species do not always have to occupy distinct niches to coexist. For example, Shorrocks (1996) has shown how independent patterns of aggregation of two potentially competing species can allow them to coexist on a patchy resource, even though one of them is always the superior competitor when they occur on the same patch. It is also more consistent with the idiosyncratic changes in ranges of individual beetle species, and thus the variable composition of communities, during range expansion from glacial refugia (Coope & Wilkins, 1994). Indeed, the growing awareness of the influence of Pleistocene glacial cycles on current ecological communities has a profound effect on the way species diversity is viewed. It is probably no longer tenable to view communities as equilibrium assemblages, or indeed to view populations as optimally adapted to their present habitats (Hewitt & Butlin, 1997).

Naturally, it is possible to consider combinations of chance and competition. Studies of island faunas, such as the anole lizards of the Caribbean (Losos, 1996), tend to show that island size and isolation are important, as expected from models of extinction and colonisation, but that the diversity of habitats within islands also influence diversity. An excellent study of this type on non-marine ostracods of the Canary Islands has recently been published (Malmqvist et al., 1997).

Like most of the questions that we have discussed, species diversity is a question which can only be answered by a combination of studies on present patterns, and mechanisms at the level of species interactions, with analysis of the historical sequence of events that has led to the present state. We consider freshwater ostracods living in lakes almost unique in the combination of features that suits them for such work: they have an excellent Holocene record and they occur in discrete patches of habitat. Thus, they combine the advantages of island populations, such as those of the Caribbean island anole lizards, with the historical record available for tree pollen or beetles. To make best use of ostracod data, it will be necessary to combine historical data from many localities and analyse them quantitatively. The potential for such analyses has been demonstrated by our work on sexual *versus* asexual lineages (Griffiths & Butlin, 1995) and we are currently applying statistical methods developed for pollen data (Gradstein et al., 1985) to ostracod assemblages using the same data set. It would be valuable, and is certainly feasible, to combine such investigations with work on current communities of ostracods. There are many questions outstanding. For example, do the species that arrive first influence the ability of later arriving species to colonise a water body? Do the same, or similar, species assemblages recur independently in different lakes, or at differ-

ent times, indicating ecologically compatible groups? Are there pairs or larger groups of species that show correlated patterns of presence or abundance across time or across lakes? Even simple questions remain largely unanswered: does species diversity increase, or decrease, with the age of a water body or with its size? (See Martens (1997) for a consideration of some similar questions in relation to ancient lakes.)

## Concluding remarks

We hope that this brief survey of topics will whet the appetite of ostracod specialists for general issues in evolutionary ecology, or persuade ecologists seeking model systems to investigate the potential of ostracods. Our survey has been biased toward topics that we find interesting, and toward examples from European freshwater ostracods of which we have some knowledge. However, we feel that the advantages of ostracods are generic: good fossil record, abundance, culturability, mix of reproductive modes, life-histories and life-styles, discrete habitats and so on. The major proviso is the limitation of most palaeontological analysis to valve morphology. It is very important to establish for extant species the extent to which valve morphology reflects genetic and ecological differentiation. The future will surely bring many fascinating studies of ostracod ecology that will provide insights for evolutionary ecologists everywhere.

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# Trunk segmentation of some podocopine lineages in Ostracoda

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*Key words:* Crustacea, Maxillopoda, Ostracoda, Podocopina, trunk segments, segmentation

## Abstract

The trunk segments of podocopine ostracods, which have previously been regarded as having a non-segmented body, are comprehensively illustrated in some lineages for the first time. Descriptions are given of the trunk segmentation of representatives from eight podocopine families: Bairdiidae, Eucytheridae, Leptocytheridae, Cytheridae, Hemicytheridae, Cytheruridae, Loxoconchidae and Xestoleberididae. As observed in the family Leptocytheridae, the maximum number of segments in the Podocopina examined is 11. The boundary between the thorax and abdomen of Podocopina is not distinct. It was found that in Podocopina, the paired copulatory organs are derived from between the fifth and seventh most terminal segments in females and the second most terminal segment in males. It is suggested that the female body plan of Podocopina is plesiomorphic because it is common to platycopid structure and approximately satisfies the supposed ground plan of the Maxillopoda.

## Introduction

Extant ostracods are divided into four orders (Maddocks, 1982): Myodocopida, Palaeocopida, Platycopida and Podocopida, with Podocopida containing the most species (recently extant punciids (Kirkbyocopina) tend to be understood as a primitive Podocopida rather than Palaeocopida: McKenzie et al., 1984; Hanai & Tabuki, 1995). According to Maddocks (1982), the order Podocopida, with a high diversity and abundant fossil record, is classified into two suborders: Metacopina and Podocopina. Metacopina flourished in the Palaeozoic, but has since become less diverse. In contrast, Podocopina rapidly increased in species diversity after the Palaeozoic, and 44 out of the 54 podocopine families are extant (Schram, 1986). At present, podocopine ostracods are highly abundant and inhabit almost all types of aquatic environment, worldwide.

The cephalic region of the crustacean body bears a number of complex and obviously specialized structures such as eyes, and consequently carcinologists tend to pay most attention to this region. Though the post-cephalic or trunk region is important for its reproductive features and often its locomotory appendages, phylogenetical or evolutionary understanding of this region is relatively limited.

There are few papers dealing with trunk segmentation in ostracods. In Podocopina, this is in part because the external demarcation of body segments is problematic. It is difficult to locate segments using an optical microscope because the trunk part of the body is extremely reduced and often many (if not all) of the segments are fused (Figure 1). This simplification of the body plan of Podocopina seems to be a result of paedomorphic evolution. In comparison, *Saipanetta* (Sigillioidea: primitive Podocopida) and Platycopina (Platycopida) have obvious external signs of trunk segments, as demonstrated by McKenzie (1967), Maddocks (1972, 1973), Schulz (1976), Maddocks & Iliffe (1986) and Swanson (1991, 1993). Additionally, Schulz (1976) presented fine illustrations of chitinous parts of podocopid ostracods and indicated that Podocopina (*Saipanetta brooksi* Maddocks, 1973) and Platycopina (*Cytherella pori* Lerner-Seggev, 1964) have 7 and 11 trunk segments (including the structure he called the 'telson'), respectively. This work by Schulz (1976) provided a foundation for the understanding of the phylogeny and body plan of Ostracoda.

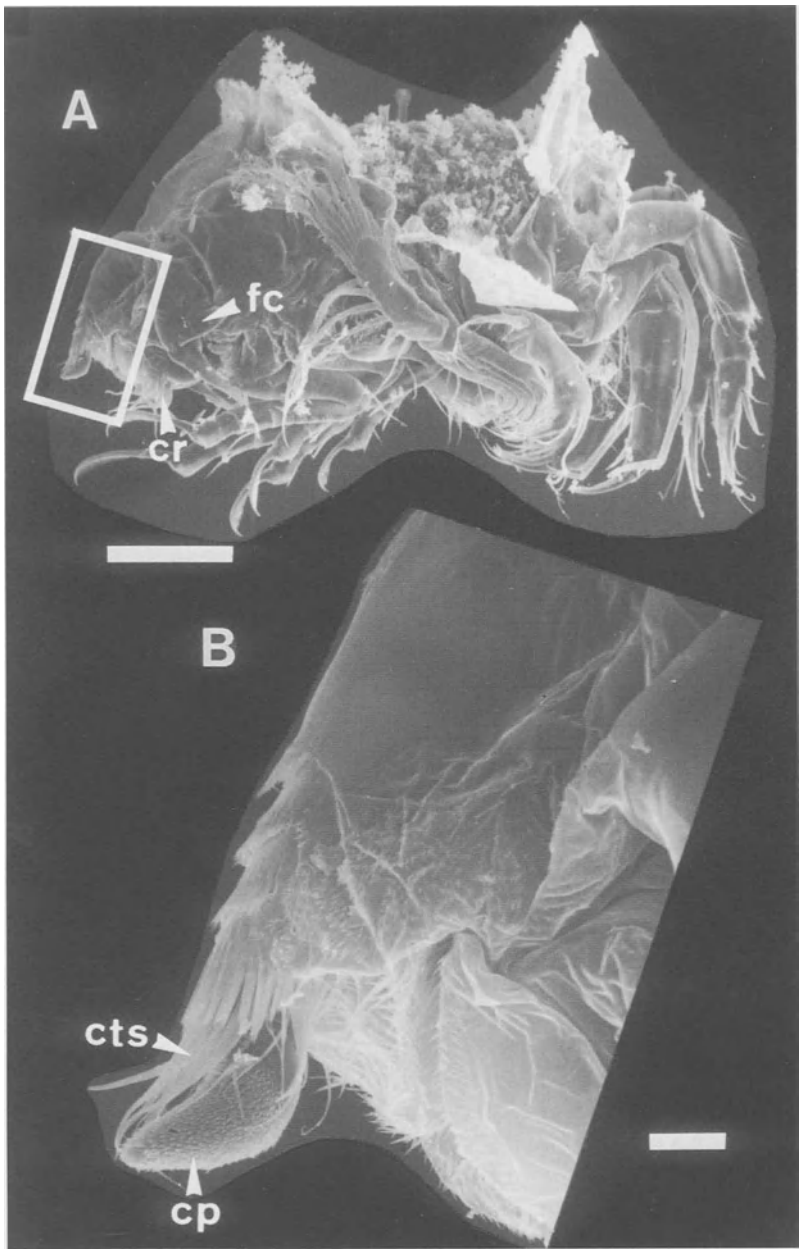


*Figure 1.* The chitinous part of the podocopine ostracod *Cythere sanrikuensis* Tsukagoshi & Ikeya, 1987, from Loc. 1 (UMUT RA 27564); carapace removed. (A) Lateral view of the complete chitinous body. (B) Detail of trunk region, as marked by the oblong in A. Abbreviations: cp – caudal process; cr – caudal ramus; cts – comb row of terminal segment; fc – female copulatory organ. Scale: 100 $\mu$m for A and 10 $\mu$m for B.

A few trunk segments of modern Podocopina (*Cythere* species) are obvious in the figures of Schornikov (1974), where one or two comb rows of spines on the caudal part of the exoskeleton were illustrated. Tsukagoshi & Ikeya (1991) redescribed a species of *Cythere* and revealed several obvious segments on the abdomen using a scanning electron microscope (S.E.M.), although this particular work did not enable a comprehensive examination of all trunk segments. Therefore, the aim of the present study is to provide a detailed examination of podocopine segmentation, and to possibly determine the segment(s) from where the reproductive organs are derived.

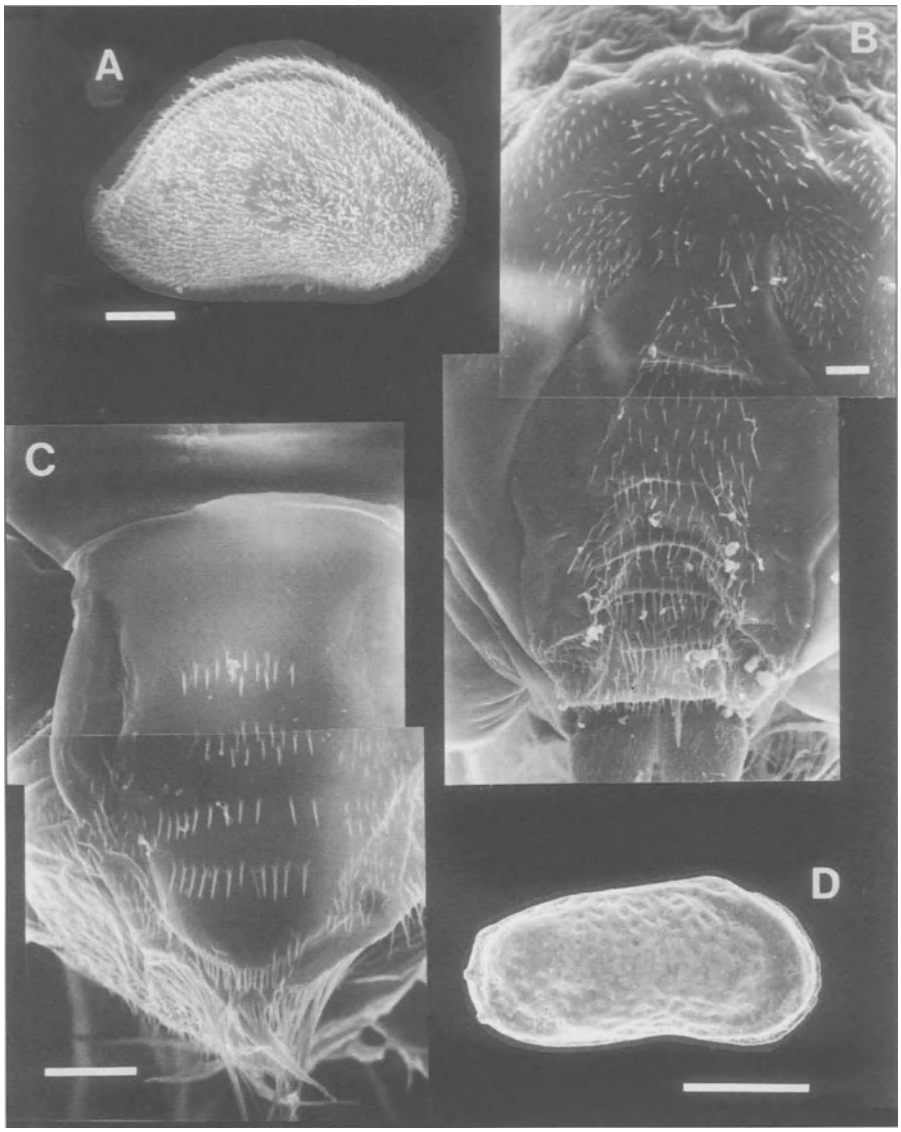


*Figure 2.* Complete animals and trunk regions of females of Bairdiidae (A and B) and Eucytheridae (C and D). (A) and (B) *Neonesidea oligodentata* (Kajiyama, 1913) from Loc. 2 (A) right lateral view of carapace, UMUT RA 27565; (B) dorsal view of trunk region, UMUT RA27566). (C) and (D) *Munseyella hatatatensis* Ishizaki, 1966 from Loc. 3 (C) dorsal view of trunk region, UMUT RA 27567; (D) right lateral view of carapace, UMUT RA 27568). Scale: 200 $\mu$m for A and D, 10 $\mu$m for B and C.

## Materials and methods

The extant species in Table 1, representing eight families of Podocopina with a long fossil record, were studied (a brief account of their geological history is also given to aid evolutionary comparisons). General geological distribution of each taxon was mainly derived from Moore (1961), Morkhoven (1963) and Schram (1986). The order of taxa is based on Hanai et al. (1977).

All examined specimens were obtained from algae or bottom sediments in intertidal zones. The algae were cut near their rhizoids and the bottom sediments were collected using a small bottom sampler with 50 $\mu$m mesh. The substrate samples taken and their localities are in Table 2.

The specimens were studied in a HITACHI S-430 S.E.M. with HITACHI EP-1050 image processor. It is necessary to clearly observe all the external boundaries of trunk segments in order to understand the

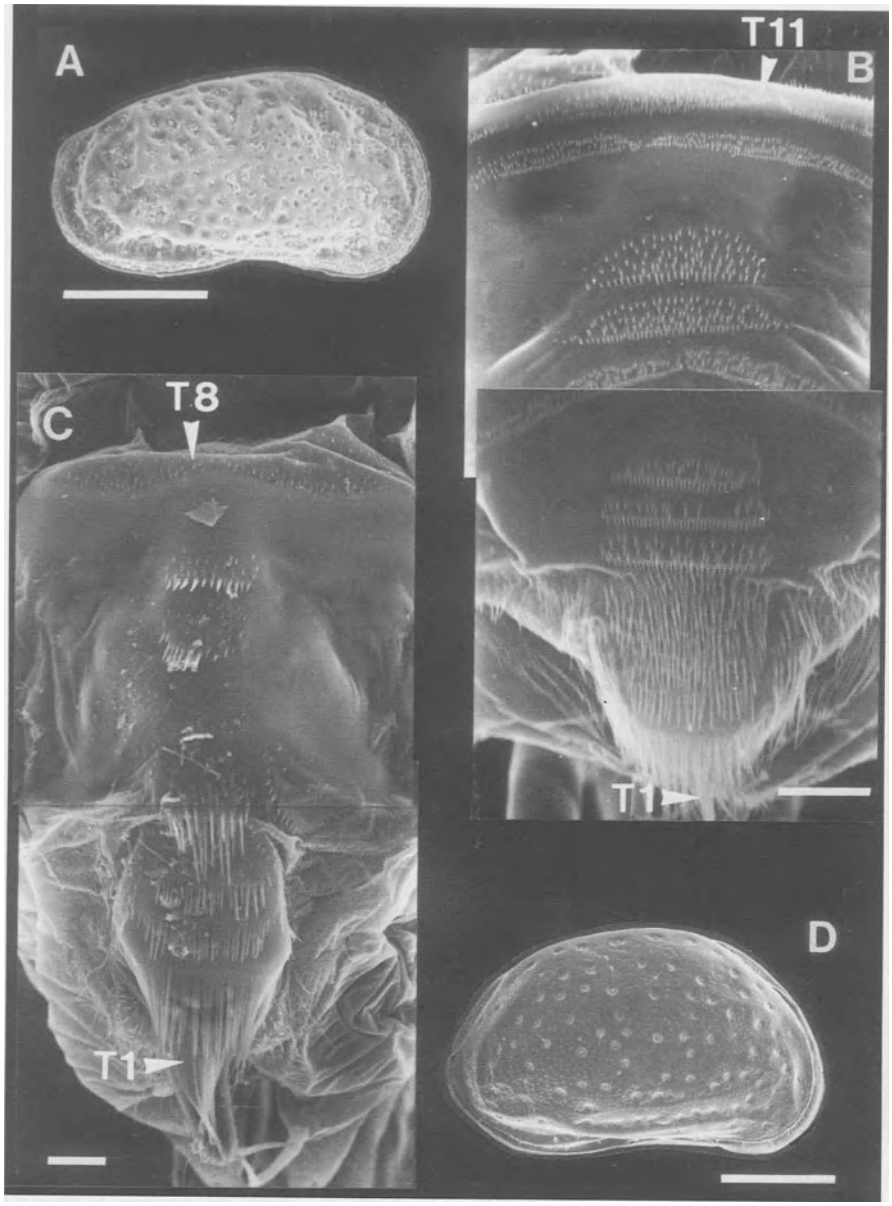


*Figure 3.* Complete animals and trunk regions of females of Leptocytheridae (A and B) and Cytheridae (C and D). (A) and (B) *Callistocythere setouchiensis* Okubo, 1979, from Loc. 4 (A) right lateral view of carapace, UMUT RA 27569; (B) dorsal view of trunk region, UMUT RA 27570). The eleventh, most anterior segment (T11) can be observed in B. (C) and (D) *Cythere sanrikuensis* Tsukagoshi & Ikeya, 1987 from Loc. 1 (C) dorsal view of trunk part, UMUT RA 27571; (D) right lateral view of carapace, UMUT RA 27572). Scale: 200 $\mu$m for A and D, 10 $\mu$m for B and C.

segmentation of podocopine ostracods. Therefore, to prevent shrinkage and consequent wrinkling of the exoskeleton (which may obscure the segment boundaries), each specimen was treated in a freeze dryer (HITACHI ES-2030) or critical point dryer (HITACHI HCP-2). The chitinous part of each specimen was completely separated from the carapace before coating with platinum.

For determination of the number and morphology of trunk segments, female specimens of the eight species were used because their copulatory organs are relatively small and do not provide an obstacle for observation of the arrangement of segments. Male specimens of two taxa were also studied to determine the segment from which their copulatory organs are derived.

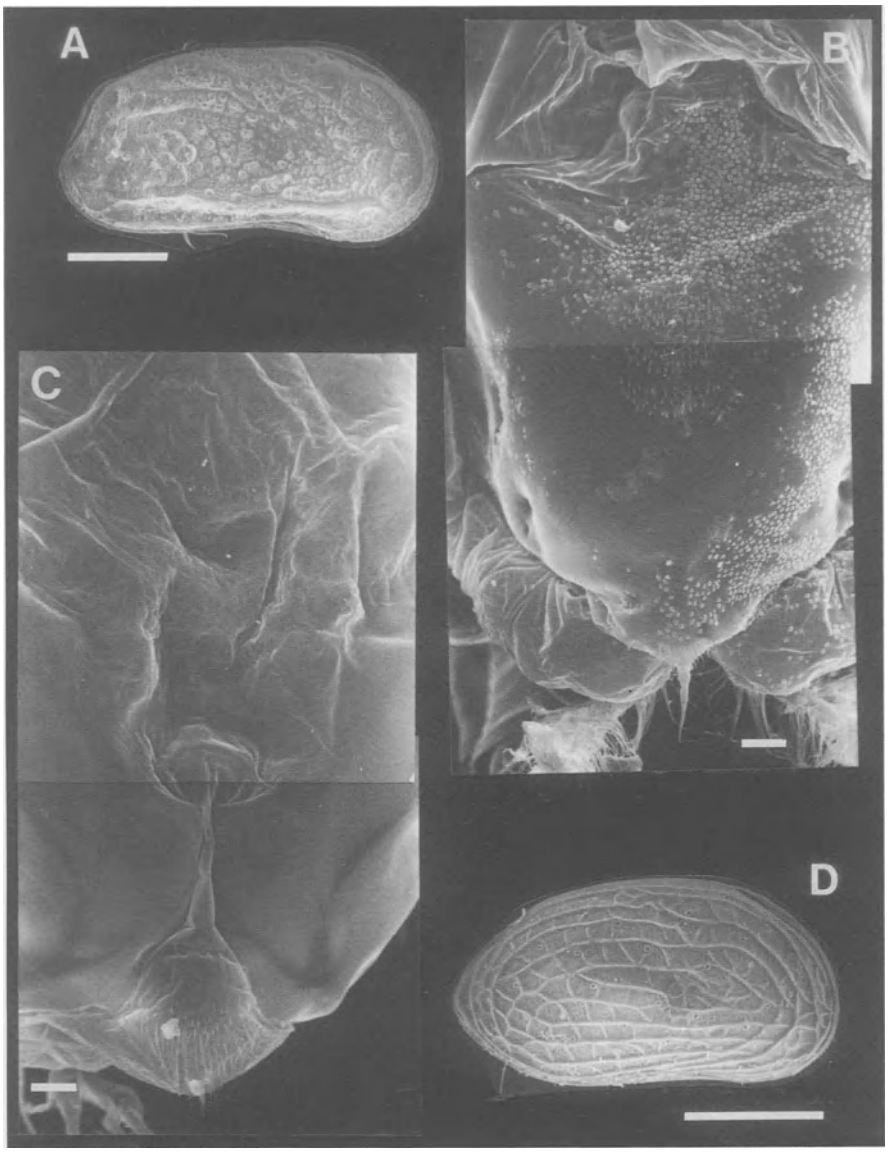


*Figure 4.* Complete animals and trunk regions of females of Hemicytheridae (A and B) and Cytheruridae (C and D). (A) and (B) *Hemicythere* sp. from Loc. 5 (A) right lateral view of carapace, UMUT RA 27573; (B) dorsal view of trunk region, UMUT RA 27574). (C) and (D) *Angulicytherura miii* (Ishizaki, 1968) from Loc. 6 (C) dorsal view of trunk region, lacking any sign of segmentation, UMUT RA 27575; (D) right lateral view of carapace, UMUT RA 27576). Scale: 200 $\mu$m for A and D, 10 $\mu$m for B and C.

The illustrated specimens have been deposited in the collections of the University Museum, the University of Tokyo (UMUT-numbers).

## Terminology

The trunk region of the crustacean body is generally less understood than the cephalic region. This is certainly true of podocopine ostracods, where terminology of the trunk body parts is confused. Schram (1986) summarized the terminology for crustacean morphology, and in particular revised definitions for the telson, caudal rami, uropods and furcae. His definitions were based on the relative positions of these structures to the anal segment: the telson is the last body unit in which the anus is not terminal; the anal segment is the last body unit in which the anus is terminal (by this definition the anterior region of the telson is homologous to the anal segment); the rami (=

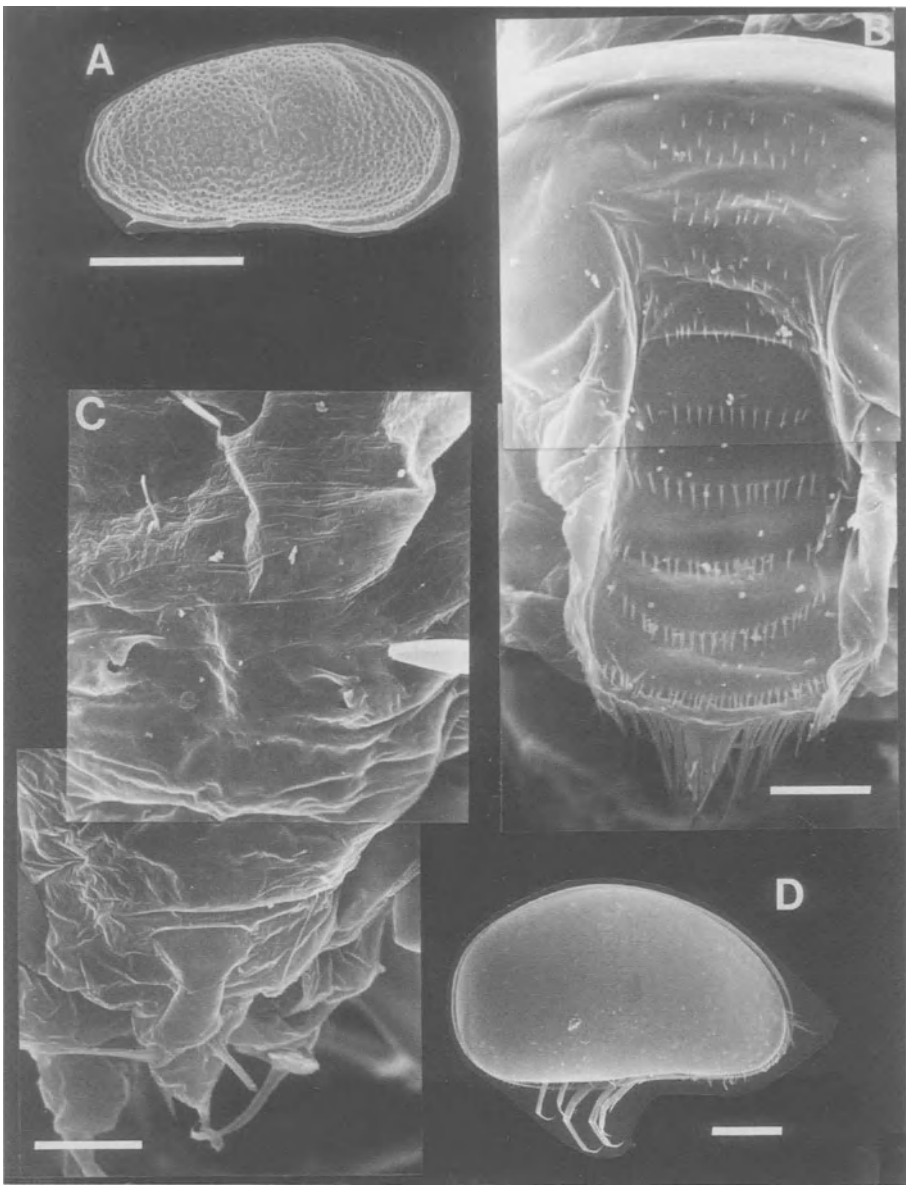


*Figure 5.* Complete animals and trunk regions of females of Loxoconchidae (A and B) and Xestoleberididae (C and D). (A) and (B) *Cytheromorpha* sp. from Loc. 3 (A) right lateral view of carapace, UMUT RA 27577; (B) dorsal view of trunk region, UMUT RA 27578). (C) and (D) *Xestoleberis hanaii* Ishizaki, 1968 from Loc. 2 (C) dorsal view of trunk region, UMUT RA 27579; (D) right lateral view of carapace, UMUT RA 27580). Scale: 200 $\mu$m for A and D, 10 $\mu$m for B and C.

caudal rami) are appendage-like structures that arise from the anal segment or near the base of the telson and serve to assist locomotion; the rami are analogues of uropods, which are specialized appendages of the segment just anterior to the anal segment or telson; the furcae are small lobes or spines that are located near the terminus of the telson (Schram, 1986) (Figure 6). By this definition, podocopine ostracods possess a pair of caudal rami, which have been frequently regarded as furcae, and a tiny protrusion (which is termed the caudal process herein) on the posterior margin of the anal segment.

Traditionally, crustacean body segments are numbered from the anterior, i.e. the anteriormost segment is called the first. In modern Podocopina, the anterior part of the trunk region is generally reduced and the arrangement of, and boundaries between, anterior segments of the trunk are particularly unclear;

*Table 1.* Explanation of the examined taxa

| Species name | Family | Earliest records of genus | Comments |
|---|---|---|---|
| *Neonesidea oligodentata* (Kajiyama, 1913) (Figure 2A) | Bairdiidae | Miocene? | Considered to be primitive within Podocopina because some closely related genera (based on shell characteristics) are known from Ordovician and, therefore, represent some of the earliest podocopines. |
| *Munseyella hatatatensis* Ishizaki, 1966 (Figure 2D) | Eucytheridae | Palaeocene | Fossil eucytherids appear in the late Cretaceous. |
| *Callistocythere setouchiensis* Okubo, 1979 (Figure 3A) | Leptocytheridae | Late Cretaceous | |
| *Cythere sanrikuensis* Tsukagoshi & Ikeya, 1987 (Figure 3D) | Cytheridae | Early Miocene | A closely related genus (*Schizocythere*; see Tsukagoshi & Kamiya, 1996: p. 350-352) is known from the Late Cretaceous. |
| *Hemicythere* sp. (Figure 4A) | Hemicytheridae | Late Pliocene | Fossil hemicytherids are known from the Late Cretaceous, but a species group closely related to *Hemicythere villosa* (Sars, 1866) (type-species) first appeared in the Late Pliocene. |
| *Angulicytherura miii* (Ishizaki, 1968) (Figure 4D) | Cytheruridae | Late Pliocene | |
| *Cytheromorpha* sp. (Figure 5A) | Loxoconchidae | Late Cretaceous | |
| *Xestoleberis hanaii* Ishizaki, 1968 (Figure 5D) | Xestoleberididae | Late Cretaceous | In terms of species diversity and numbers of individuals, it is one of the most successful of Recent podocopine genera, and is ovoviviparous. |

in contrast, the segmentation of the posterior part of the trunk region is often well defined. Therefore, it is simpler to standardize the terminology of homologous segments within Podocopina if the terminal (posteriormost) segment is regarded as a basal point for numbering of the trunk segments. Namely, in this paper, the posteriormost segment is denoted T1 and the anteriorly next adjacent segment T2, and so forth (Figure 6).

## Results: trunk segmentation

In most species examined, the trunk region of the body exhibits cuticular folds which are interpreted as the external boundaries of segments. An external character of each segment, as deduced from species with particularly clear cuticular folds, is the possession of a comb row, or well-defined assemblage, of spines at the posterior end (Figures 2–5). The schematic illustrations of the trunk parts are shown in Figure 7. The number of segments recognised in each species are as follows:

*Callistocythere setouchiensis* Okubo, 1979 (Figures 3A, B, 7A, 8A and 10A): Leptocytheridae.

This species has the highest number of trunk segments of the eight species examined. Eleven segments were identified by the presence of dense stands of spines orientated perpendicular to the longitudinal axis of the body. Segment T1 is extremely reduced and has long setae. Segment T3 bears particularly numerous spines. Segments T6 and T9 are relatively long. Seg-

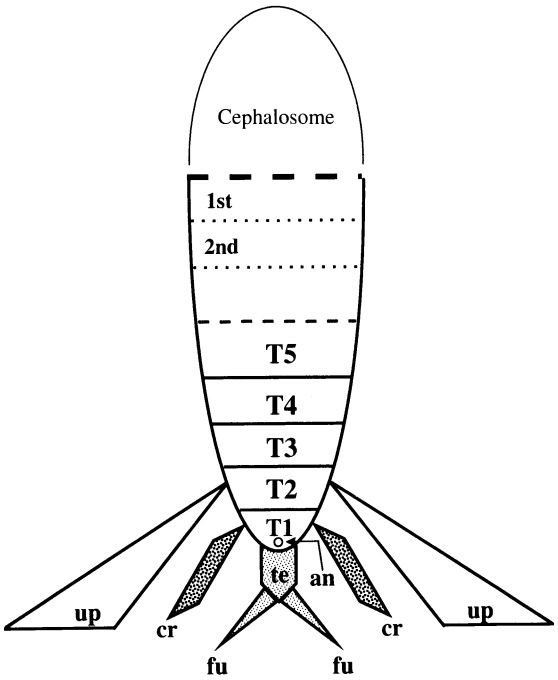


*Figure 6.* Diagrammatic trunk body plan of Crustacea, dorsal view (modified after Schram, 1986). The terminal segment is denoted as T1 and the next anterior segment as T2 and so forth. an – anus; cr – caudal ramus; fu – furca; te – telson; up – uropod.

ments T7, T10 and T11 extend to both lateral sides of the trunk. Segments T10 and T11 are extremely longitudinally compressed.

*Cytheromorpha* sp. (Figures 5A, B and 7B): Loxoconchidae

A total of 10 segments were recognized. Apart from T1, segments and their vestiges are arranged at approximately regular intervals. Segment T1 is reduced and has relatively long setae. Segments T2–T6 possess comb rows of spines at their posterior ends. Segment T7 has spines on its posterior margin. Indistinct scattered assemblages of spines form the vestiges of anterior than segment T7.

*Cythere sanrikuensis* Tsukagoshi & Ikeya, 1987 (Figures 1A, B, 3C, D, 7C, 8B, 9 and 10B): Cytheridae

Nine segments were clearly evident. Segment T1 is reduced and has very long setae and a thumb-like stout caudal process. Segments T2–T5 also have relatively long setae or spines on their posterior margins. The posterior margin of segment T5 forms a relatively acute fold. Segments T5 and T7 are extended longitudinally and extend to both lateral sides of the trunk. Segments T8 and T9 are extremely compressed.

*Neonesidea oligodentata* (Kajiyama, 1913) (Figures 2A, B and 7D): Bairdiidae

Five clear cuticular folds and some assemblages of spines were recognised. Segment T5 is relatively long. Segment T7 is long and wide. The numerous rows of spines on each segment made the comb rows on the posterior ends of the segments difficult to interpret. There are no cuticular folds or distinct comb rows of spines anterior to segment T7. A short and narrow caudal process occurs on the posterior margin of segment T1.

*Munseyella hatatatensis* Ishizaki, 1966 (Figures 2C, D and 7E): Eucytheridae

Six segments were identified by the presence of six comb rows of spines. Cuticular folds were not evident. Segment T1 is relatively short. The comb rows of spines on segments T5 and T6 are relatively scattered. Segments anterior to T6 are not apparent.

*Hemicythere* sp. (Figures 4A, B and 7F): Hemicytheridae

One and three assemblages of small spines are present on the posterior and middle regions of the massive urosome, respectively. These assemblages can be regarded as vestiges of segments, but the segments themselves have become completely fused, making it difficult to determine which assemblage corresponds to which segment. The posterior end of the body bears a narrow caudal process.

*Angulicytherura miii* (Ishizaki, 1968) (Figures 4C, D and 7G): Cytheruridae

Except for an assemblage of setae on the posterior end, the urosome is homogeneous and has no vestige of segmentation. Fusion and simplification have become complete.

*Xestoleberis hanaii* Ishizaki, 1968 (Figures 5C, D and 7H): Xestoleberididae

Segments are completely fused and there are neither comb rows nor assemblages of spines. A small caudal process is present.

## Results: derivation of the copulatory organs

In the above species, segmentation of the trunk is most easily seen in *Callistocythere setouchiensis* and *Cythere sanrikuensis*. Consequently, the segmentation of both sexes of these two species was examined

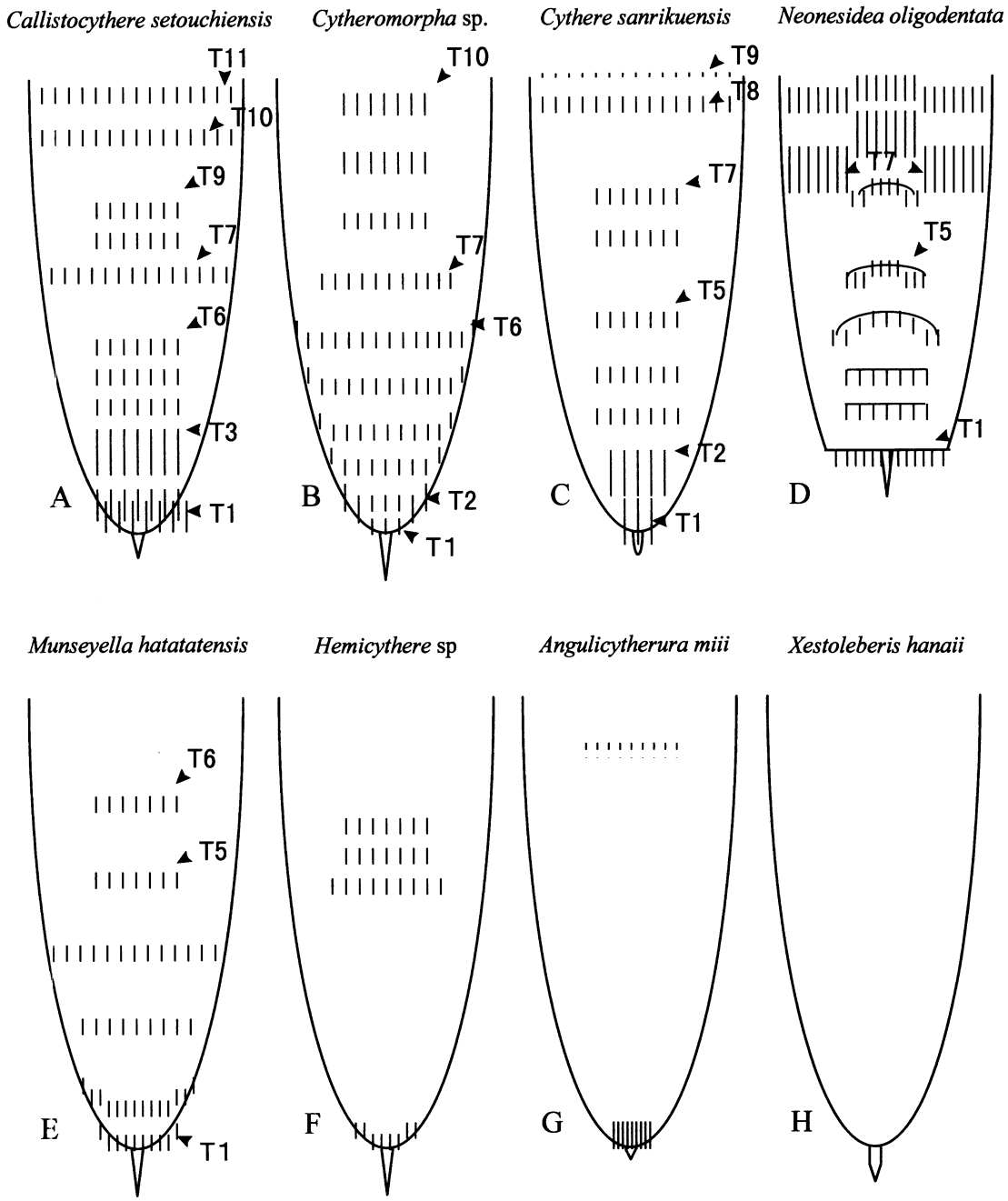


*Figure 7.* Schematic diagram of trunk body aspects of eight species examined. (A) *Callistocythere setouchiensis*. (B) *Cytheromorpha* sp. (C) *Cythere sanrikuensis*. (D) *Neonesidea oligodentata*. (E) *Munseyella hatatatensis*. (F) *Hemicythere* sp. (G) *Angulicytherura miii*. (H) *Xestoleberis hanaii*. Refer to S.E.M. photos of Figures 2–5.

in further detail, and the segments from which their copulatory organs are derived were identified.

In females of *Callistocythere setouchiensis*, segments T6 and T9 are relatively long, and segments T7, T10 and T11 extend to both lateral sides of the trunk (Figures 7A and 8A); in females of *Cythere sanrikuensis* it is segments T5 and T7 which are particularly long (Figures 7C and 8B). A long female segment T7 was also observed in *Neonesidea oligodentata* (Figure 2B). A pair of female copulatory organs appear to be located at the ventral side of T5 or T7 in *C. setouchiensis* and *C. sanrikuensis* (Figures 8A, B and 9).

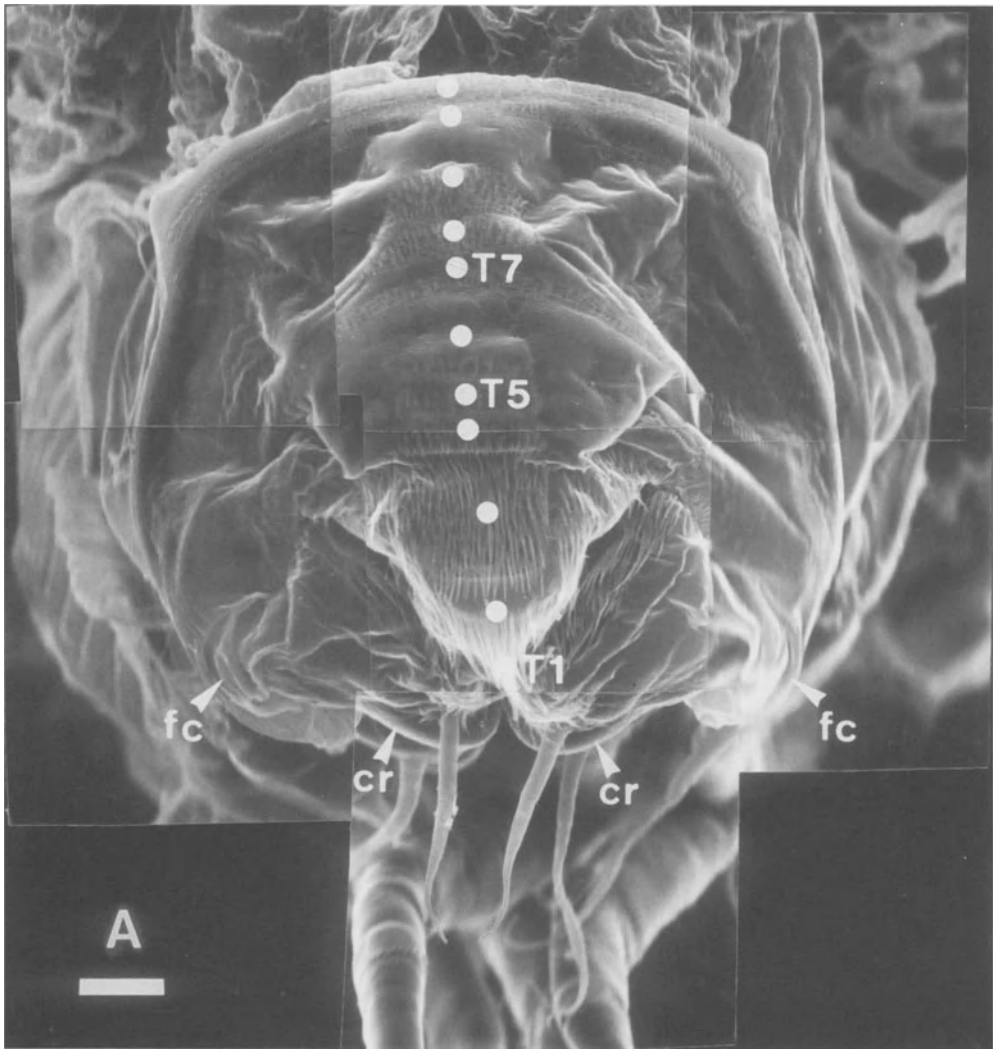


*Figure 8.* Comparison of female trunk segmentation of *Callistocythere* and *Cythere*; dorsal views of trunk regions are shown. (A) *Callistocythere setouchiensis* Okubo, 1979, from Loc. 4 (UMUT RA 27581). (B) *Cythere sanrikuensis* Tsukagoshi & Ikeya, 1987, from Loc. 1 (UMUT RA 27582). cp – caudal process; cr – caudal ramus; fc – female copulatory organ. Each white dot denotes a segment. Scale: 10 $\mu$m.

In *Callistocythere setouchiensis*, the upper part of the zygum (a petiole-like part) of the male copulatory organs lies across the anterior part of segment T2 (Figure 10A). At first sight, the paired male copulatory organs of *Cythere sanrikuensis* appear to derive from segment T4. However, the posterior end of segment T5 attaches to the narrow anterior end of segment T4. Segments T3 and T4 are compressed and lifted up, probably by the extremely well developed copulatory organs (Figure 10B). The fact that the upper part of the zygum can not be found and is probably covered by the cuticle of the segments T3 and T4 supports this inference. Therefore, in *Cythere sanrikuensis* and *Callistocythere setouchiensis*, the male copulatory organs are probably derived from segment T2.

## Discussion

Based on our analysis of eight species, our plan of the pattern of trunk segmentation of the examined podocopine ostracods is shown in Figure 11; there is a maximum of 11 segments in the trunk region. The number of trunk segments in some non-podocopine ostracod taxa has previously been determined. Eleven segments occur in Platycopida (Schulz, 1976; includ-

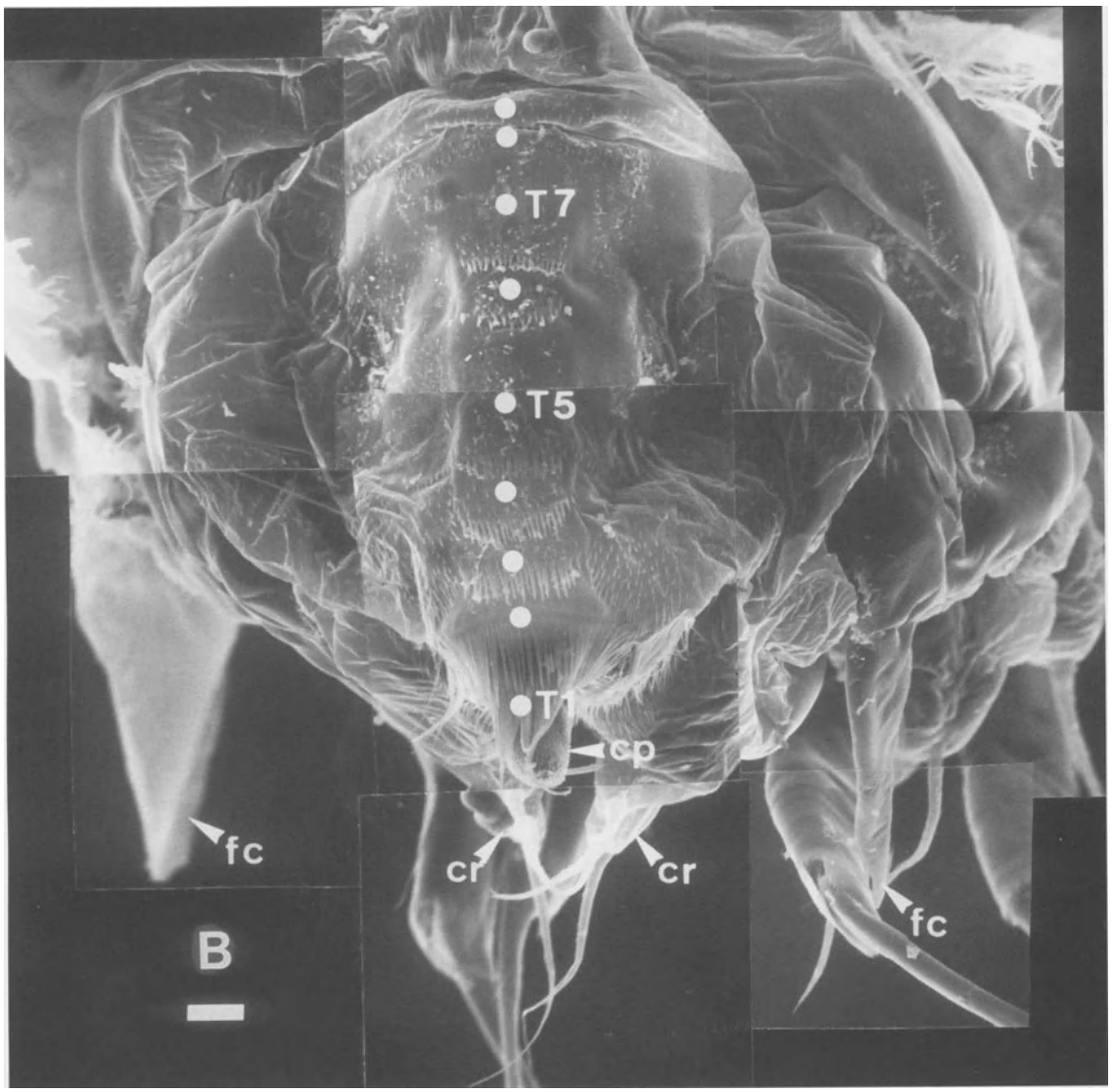


*Figure 8* (*continued*).

ing the telson as the last segment as interpreted by Schram, 1983) and Kirkbyocopina (Swanson, 1989, 1990). Therefore, 11 segments can be regarded as plesiomorphic within Ostracoda. Consequently, the common ancestor of ostracods must have possessed 11 trunk segments. Podocopine ostracods include many highly derived taxa and the majority abandon segmentation (McKenzie, 1972, 1983) as a result of progenesis. The reduction in the clarity of segmentation within the anterior part of the trunk is particularly notable; the fusion of segments within Hemicytheridae, Cytheruridae and Xestoleberididae is a typical example. Our results support the trend that the more derived taxa, as determined from the fossil record, have abandoned segmentation. Hemicytheridae and Cytheridae are relatively younger taxa, as determined from the fossil record (Table 1). They may have lost their segmentation as a result of paedomorphic evolution to simpler ground plan taxa. In contrast, xestoleberidid taxa have a relatively long fossil record, starting from the Late Cretaceous in spite of the disappearance of segments. Possibly, their urosome may be specialized in connection with their ovoviviparous (brood care) mode of reproduction.

We suggest that the segment from which the pair of female copulatory organs originates in Podocop-

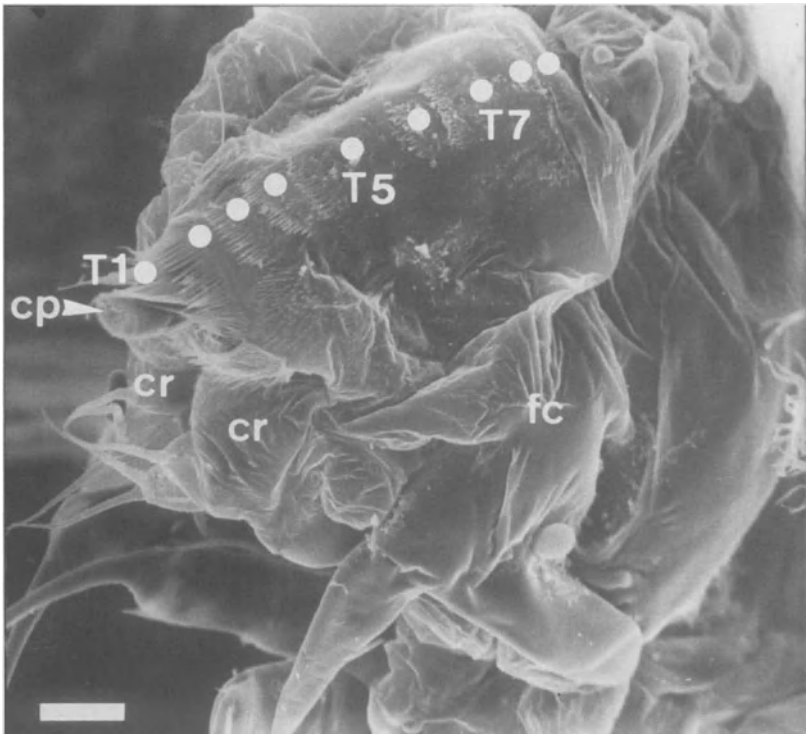


*Figure 9.* Dorso-lateral view of a female *Cythere sanrikuensis* Tsukagoshi & Ikeya, 1987 from Loc. 1 (UMUT RA 27582) showing the segment bearing the copulatory organ; female copulatory organs are located below the swollen segment T5. Each white dot denotes a segment. cp – caudal process; cr – caudal ramus; fc – female copulatory organ. Scale: 20 $\mu$m.

ina is conservative because other ostracod taxa also have their male and female copulatory organs at approximately the same position. For example, the male copulatory organ is near the fifth (T7) or the sixth (T6) trunk segment in Platycopida (Schultz, 1976), and between the fifth (T7) and seventh (T5) segments in Kirkbyocopina (Swanson, 1989). It is, therefore, probable that the pair of female copulatory organs in Podocopida is homologous to the copulatory organs of both sexes of Platycopina and Kirkbyocopina. Since in both primitive taxa the fifth (T7) segment is suspected to derive male and female copulatory organs, segment T7 seems to be the most probable one from which the female copulatory organs originate. This is somewhat different from Walossek & Müller's (1998) interpretation, who suggested that most maxillopodans have the gonopores on the seventh thoracomere (segment T5 in this paper). Further comparative anatomical information on primitive ostracods is needed. In contrast, the position of the male copulatory organs in the examined podocopine taxa (i.e. segment T2) is probably a derived character.

The 5-6-5 segmentation (5 cephalic, 6 thoracic and 5 abdominal segments) is regarded as the typical body plan of Maxillopoda by Newman (1983). Recently, the 5-7-4 segmentation was proposed as a maxillopodan body plan by Walossek & Müller (1998). By these definitions, as ostracods are believed to be maxillopodans, the podocopine segments T1–T5 or T1–T4 comprise the abdomen, and the segments T6 to T11 or T5–T11 make up the thorax (Figure 11). This pattern of segmentation can be compared with other crustaceans, although the external boundary between thorax and abdomen is not distinguishable in the examined podocopine ostracods. Assuming that ostracods conform to the 5-6-5 or the 5-7-4 body plan, segment T7, which probably bears the female copulatory organs, belongs to the thorax. This is consistent for Maxillopoda. The copulatory organs of male Podocopina, derived from segment T2, are comparable to the uropods of eumalacostracans or the sixth abdominal appendages of phylocaridans. Whatever the case, the origin of the extremely well-developed male copulatory organs of Bairdioidea and Cytheroidea is different from that of other ostracods.

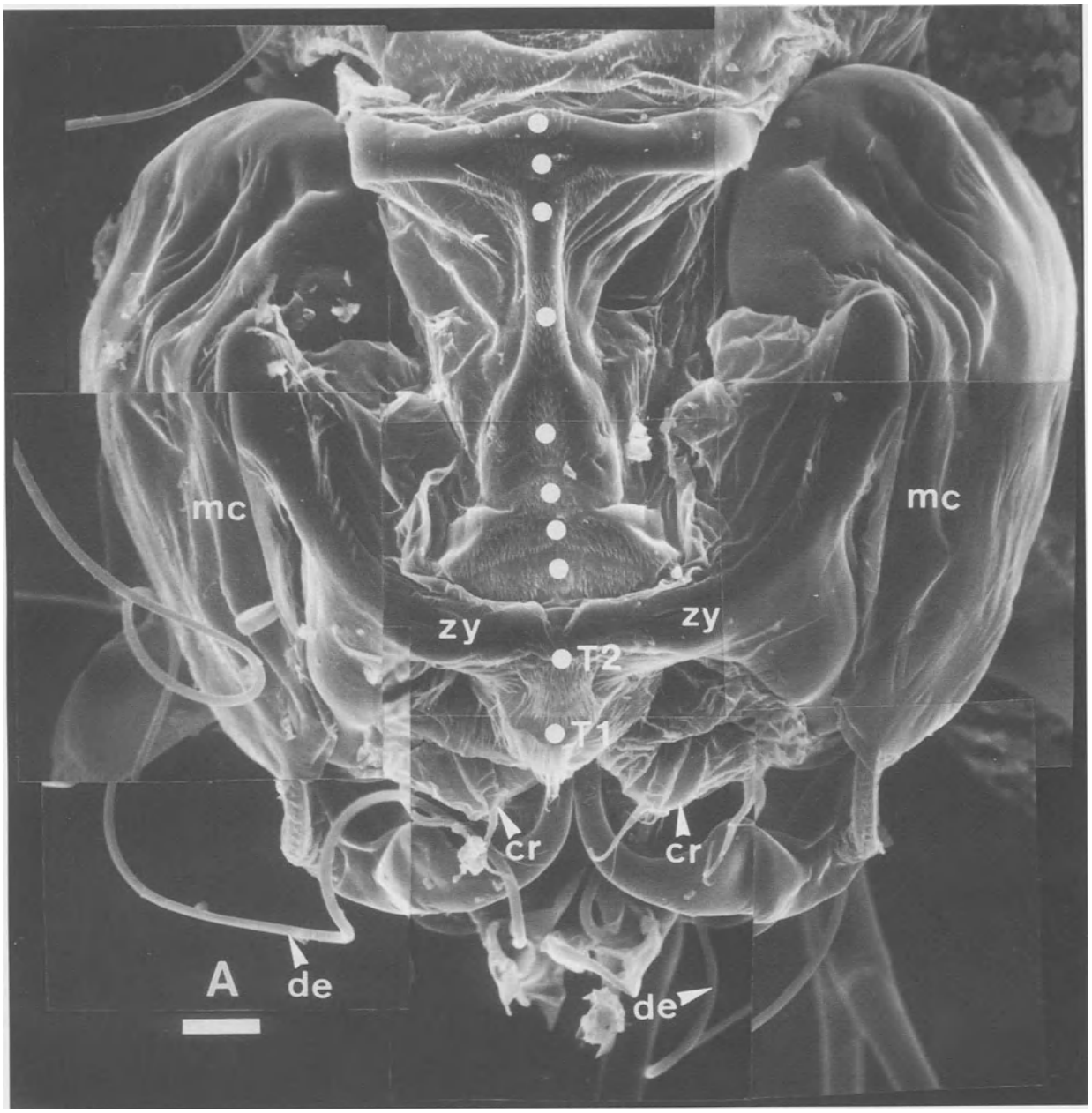


*Figure 10.* Comparison of male trunk segmentation of *Callistocythere* and *Cythere*; dorsal views of trunk regions are shown. (A) *Callistocythere setouchiensis* Okubo, 1979, from Loc. 4 (UMUT RA 27583). (B) *Cythere sanrikuensis* Tsukagoshi & Ikeya, 1987, from Loc. 1 (UMUT RA 27584). ca – clasping apparatus; cp – caudal process; cr – caudal ramus; de – ductus ejaculatorius; mc – male copulatory organ; zy – zygum. Each white dot denotes a segment. Scale: 10 $\mu$m.

A further reason for the lack of study of segmentation in Podocopina may be that the well-developed pair of male copulatory organs obscures the boundaries of segments. However, since female copulatory organs of Podocopina are relatively small, females would appear highly suitable for the general analysis of segmentation. It is necessary to examine the segmentation of other ostracod taxa in detail, especially the position of male and female copulatory organs, in order to make a resolved comparison of ostracods with other crustaceans, and in particular the maxillopodan group (there is some debate as to which taxa com-

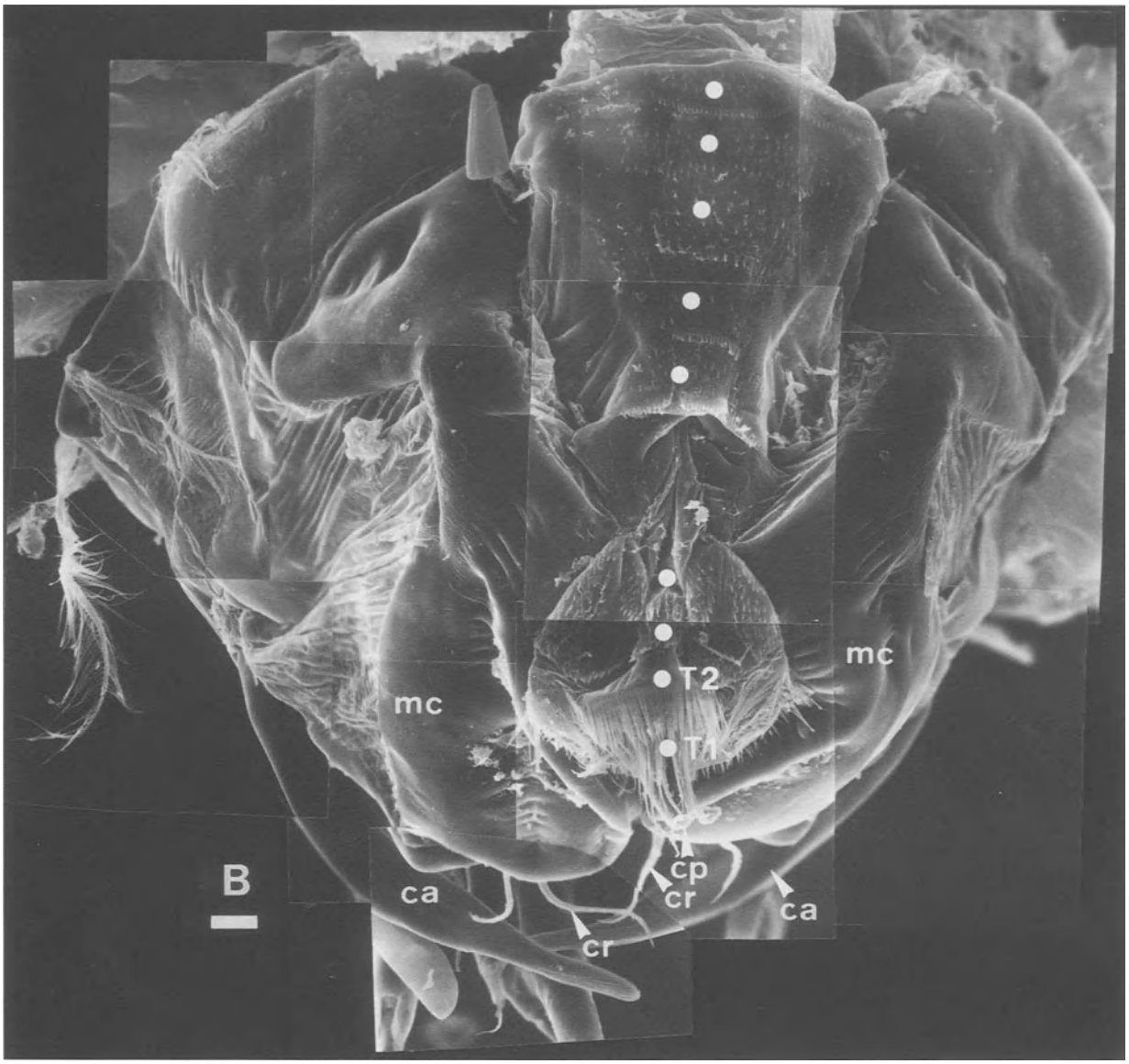


*Figure 10* (*continued*).

prise the Maxillopoda, but the taxa possessing the 11 post-cephalic segments are limited). Determination of non-ostracod crustacean appendages which are homologous to those of ostracod appendages is problematic but essential, as indicated by Maddocks (1982). Of course, a comprehensive analysis which may involve embryology, histology, functional morphology, paleontology and/or molecular biology is required to unequivocally determine the phylogenetic position of Ostracoda.

## Conclusions

The Podocopina possess 11 trunk segments, thus conforming to the pattern in Platycopida and Kirkbyocopina.

We propose that the male and female copulatory organs of Podocopina are derived from the second and seventh most terminal segments, respectively.

The position of the female copulatory organs in the Podocopina is the plesiomorphic state.

*Table 2* List of the sample information

| Loc. no. | Type of sample | Water depth | Collecting date | Loc. name (in Japan) | Lat. (N) | Long. (E) |
|---|---|---|---|---|---|---|
| 1 | Algae in rocky shore | 0.5 m | 21 June 1986 | Tokkarisho Point, Muroran City, Hokkaido | 42° 18.4′ | 141° 00.8′ |
| 2 | Algae in rocky shore | 0.3 m | 3 April 1991 | Jonouchi, Misaki-cho, Kanagawa Pref. | 35° 09.5′ | 139° 00.8′ |
| 3 | Bottom sediments | 10 m | 30 June 1992 | Nakanose, center of Akkeshi Bay, Hokkaido | 43° 00.0′ | 144° 48.0′ |
| 4 | Algae in rocky shore | 0.3 m | 3 April 1991 | Aburatsubo Inlet, Misaki-cho, Kanagawa Pref. | 35° 09.3′ | 139° 36.9′ |
| 5 | Algae in rocky shore | 0.2 m | 21 June 1986 | Yaoi, Suttsu Bay, Hokkaido | 42° 48.2′ | 140° 13.2′ |
| 6 | Lagoonal sediments | 0.5 m | 3 April 1993 | The mouth of Obitsu River, Chiba Pref. | 35° 24.5′ | 139° 54.4′ |

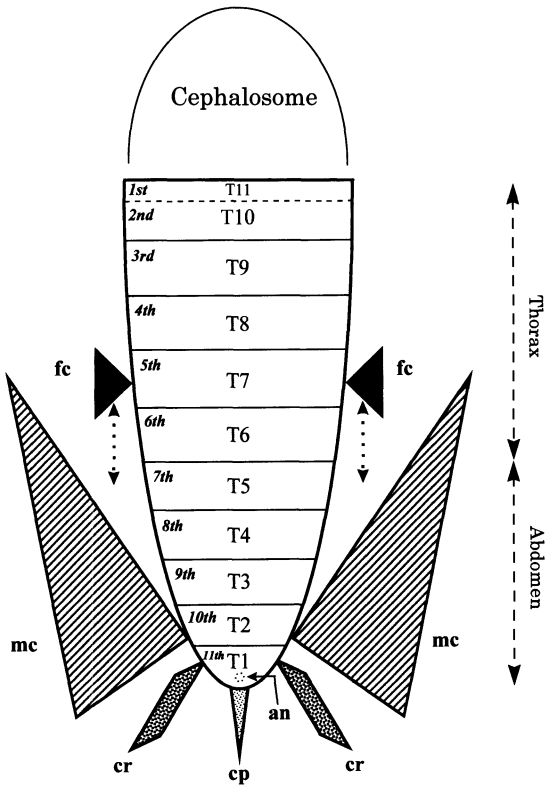


*Figure 11.* Diagrammatic trunk body plan of podocopine Ostracoda, dorsal view, as determined from this study. The male and female copulatory organs are derived from segments T2 and T5, T6 or T7 respectively (arrow indicates range of possible location of the male copulatory organs). an – anus; cr – caudal ramus; cp – caudal process (maybe homologous to telson); fc – female copulatory organ; mc – male copulatory organ.

## Acknowledgements

We would like to thank Dr Jim Lowry for his advice regarding Crustacea and for giving us the opportunity to study in the Australian Museum. Gratitude is also expressed to the late Dr Tatsunori Ito for his suggestions about the importance of segmentation in Crustacea, and Prof. David Siveter and Drs Koen Martens and David J. Horne for their critical reviewing of the manuscript. Additional thanks go to Drs Kazuyoshi Hashizume, Takahiro Kamiya, Kyosuke Ikuta and Prof. Noriyuki Ikeya for their valuable advice and encouragement. The staff members of the Paleobiological Laboratory of the University of Tokyo and the Marine Invertebrate Department of the Australian Museum provided helpful advice and various facilities to the authors. This study was partly funded by the Grant-in-Aid for Scientific Research (No. 06740643 and No. 07740653) of the Ministry of Education, Science and Culture, Government of Japan.

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# The ontogeny of the cypridid ostracod *Eucypris virens* (Jurine, 1820) (Crustacea, Ostracoda)

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*Key words:* morphology, ontogeny, Ostracoda, evolution, fifth limb, crustacean phylogeny

## Abstract

The chaetotaxy (shape, structure and distribution of setae) of appendages and valve allometry during the post embryonic ontogeny of the cyprididine ostracod *Eucypris virens* are described. It is shown that the basic ontogenetic development of *E. virens* is very similar to that of other species of the family Cyprididae. During ontogeny, the chaetotaxy shows continual development on all podomeres of the limbs with the exception of the last podomere on the antennulae. The long setae on the exopodite and protopodite of the antennae have a natatory function until the actual natatory setae develop in later instars. Aesthetascs (presumed chemoreceptors) *ya* and *y3* are the first to develop and may have an important function in the first instars. Cyprididae require a pediform limb in the posterior of the body presumably to help them to attach to substrates and this is reflected by the pediform nature of one limb at all times throughout all instars. This study has also shown that the fifth limb is most probably of thoracic origin and hence ostracods have only one pair of maxillae.

## Introduction

As arthropods, ostracod growth is characterised by a number of moults. Ostracods exhibit determinate growth, which means that there is a terminal ecdysis. The number of juvenile stages in each species is fixed. Most podocopine ostracods have nine post embryonic life stages: eight juvenile and one adult stage (Figures 1 and 2). Roessler (1982a, b) identified the occurrence of a pre-nauplius stage within the egg in *Chlamydotheca* and *Heterocypris*, two genera of the family Cyprididae. These short-lived (circa 1 h) stages could occur in all podocopine ostracods and might be of importance for phylogenetic discussions on (supra-) ordinal level (Roessler, 1998). The pre-nauplius stage is not figured here as this paper concentrates on stages after hatching.

The morphology of juvenile stages in Ostracoda remains poorly investigated. Nevertheless, identifying juveniles can be necessary, for example in studies on population dynamics and life cycles of certain communities and species. The history of ostracod research has also demonstrated that in various cases, juveniles of a certain species or genus can be described as a new taxon. A striking example is given by Fox (1964: 174–175), who re-investigated the validity of the genus *Siphlocandona*, and determined that these specimens were juveniles of a species of the genus *Herpetocypris*.

Early studies involving limb morphology of juvenile instars of ostracods of the suborder Podocopina include those of Claus (1868) on *Dolerocypris fasciata* (Müller, 1776), *Cyclocypris ovum* (Jurine, 1820) and *Cypridopsis vidua* (Müller, 1776); Müller (1894), who studied an unidentified species of the Cyprididae; and Schreiber (1922) on *Heterocypris incongruens* (Ramdohr, 1808). Cannon (1925) studied the glands of the juveniles and adults of the ostracods *Cypridopsis vidua, Bradleystrandesia fuscata* (Jurine, 1820) (as *Cypris fuscata*) and *Heterocypris incongruens* (as *Cyprinotus incongruens*). The soft part morphology of the instars of *Limnocythere inopinata* (Baird, 1843) and *Darwinula stevensoni* (Brady & Robertson, 1870), (see Scheerer-Ostermeyer, 1940), *Cypridopsis vidua*,

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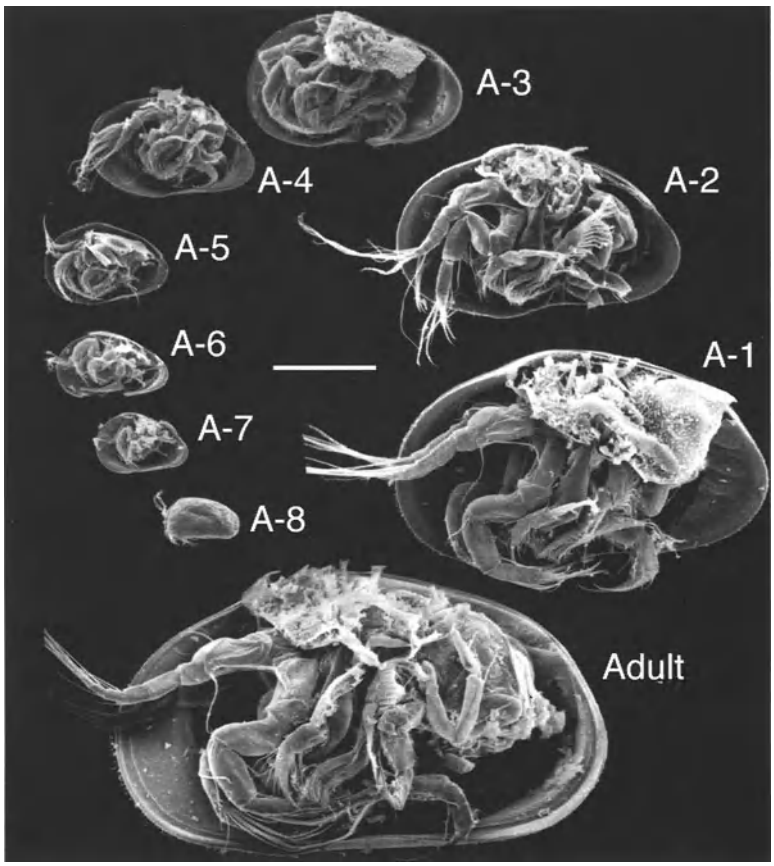


*Figure 1.* Instars of *Eucypris virens*, specimens critical point dried with left valve removed. Scale bar=330 $\mu$m.

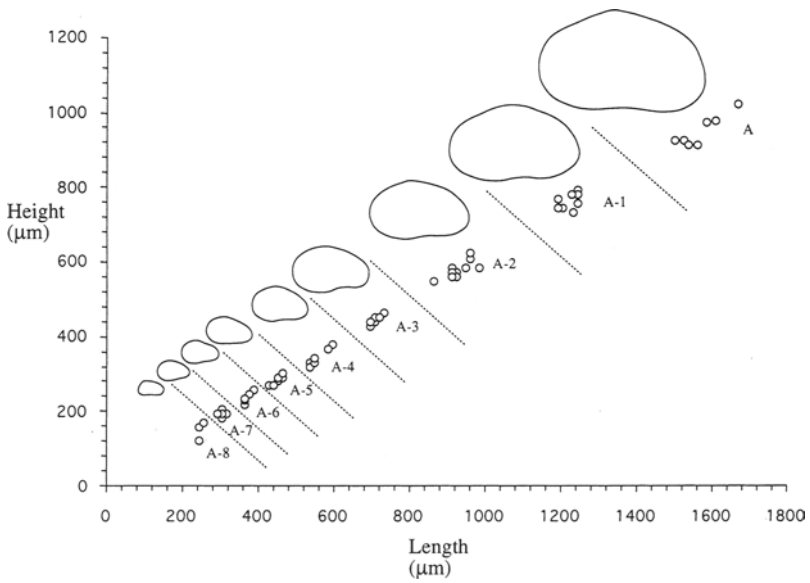


*Figure 2.* Carapace sizes of the instars of *Eucypris virens*, anterior to left.

(see Kesling, 1951), *Cyprideis torosa* (Jones, 1850) (as *C. littoralis* – see Weygoldt, 1960), *Herpetocypris chevreuxi* (Sars, 1896) (as *H. agilis*), *Heterocypris incongruens* and *Cypridopsis vidua* (see Fox, 1964) have also been documented. McKenzie (1968) reviewed and summarised many of the previous ontogenetic studies of soft parts and Ghetti (1970) subsequently contributed studies on *Stenocypris* Sars, 1889, species. Broodbakker & Danielopol (1982) documented the pincer development of the cleaning limb of *Herpetocypris chevreuxi* in detail and Roessler (1983) studied the ontogeny of *Heterocypris bogotensis* Roessler, 1982.

Previous work on ostracod ontogeny most often dealt with species of the family Cyprididae, but generally provided only fragmentary data. The present paper aims to complement this by providing an ontogenetic study of the carapace and of detailed chaetotaxy of the limbs of a complete series of juvenile stages (from stage one onwards) of the cypridid ostracod, *Eucypris virens*. Furthermore, this paper also aims to determine the homology and origin of the fifth appendage of (Podocopina) ostracods. Some authors believe this limb is of cephalic origin (Hartmann, 1977, Maddocks, 1982), while others argue that it has a thoracic origin (Kesling, 1951; Fox, 1964; Athersuch et al., 1989; Meisch, 1996). This issue has important phylogenetic implications; if the fifth limb is a thoracic appendage, then ostracods have only four pairs of cephalic appendages. Although within other groups of crustaceans such as the Notostraca, Anostraca, Conchostraca and Cladocera, some species have only four cephalic appendages, there are, however, some species in all these groups with at least a reduced or vestigial fifth cephalic appendage (McLaughlin, 1982). Thus ostracods would be the only crustacean group in which *all* species have only four cephalic appendages.

## Material and methods

The species *Eucypris virens* was selected for this study because it was one of the key-organisms of which morphological and genetic variability, life cycle, distribution, behaviour, etc. were studied in the framework of a European research network on reproductive ecology in non-marine ostracods (Horne & Martens, 1994). Furthermore, the animal is relatively large, which means that even the earlier juvenile instars could still easily be handled, investigated and dissected. Finally, the parthenogenetic populations of this species are easy to culture in the lab, as they are very sturdy and resistant, and produce both resting and subitaneous eggs, which means continuous reproduction is possible and a nearly constant supply of various juvenile instars is available for study.

A number of specimens of *Eucypris virens* were collected from a temporary pool in a quarry at Ketton, Lincolnshire, U.K. on 3 March 1995 by D. J. Horne (University of Greenwich). The specimens were transferred to a tank and a continuously reproducing population was established. A total of 93 specimens were used in the present study. Soft-parts (limbs) were mounted in glycerine on glass slides, sealed using clear nail varnish and drawn using a camera lucida. The carapaces were mounted on stubs and sputter-coated in gold. Carapaces with soft parts intact were critical point dried prior to coating. Specimens were photographed using a 'Hitachi 520' Scanning Electron Microscope.

All figured and referred material has been deposited in the collection of the Department of Palaeontology, Natural History Museum, London, U.K. (OS 15122–OS 15162) and in the Ostracod Collection of the Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium (OC 2002 and OC 2003).

## Terminology

Chaetotaxy of the limbs follows the model proposed by Broodbakker & Danielopol (1982), revised for the antennae by Martens (1987). Abbreviations used herein are as follows: An1 – Antennula; An2 – Antenna; Md – mandible; RLO – rake-like organs; Mx – Maxilla; L5 – 5th limb; L6 – walking leg; L7 – cleaning limb; CR – caudal ramus (furca); LV – left valve; RV – right valve.

## Results: description of post embryonic ontogeny in *Eucypris virens*

The present section does not offer an exhaustive description of the chaetotaxy of each instar (this can be deduced from the illustrations), but indicates the most important changes in existing limbs or newly formed structures. Furthermore, newly formed segments, setae and claws are indicated with arrows in Figures 3–19. An extensive description of valves and

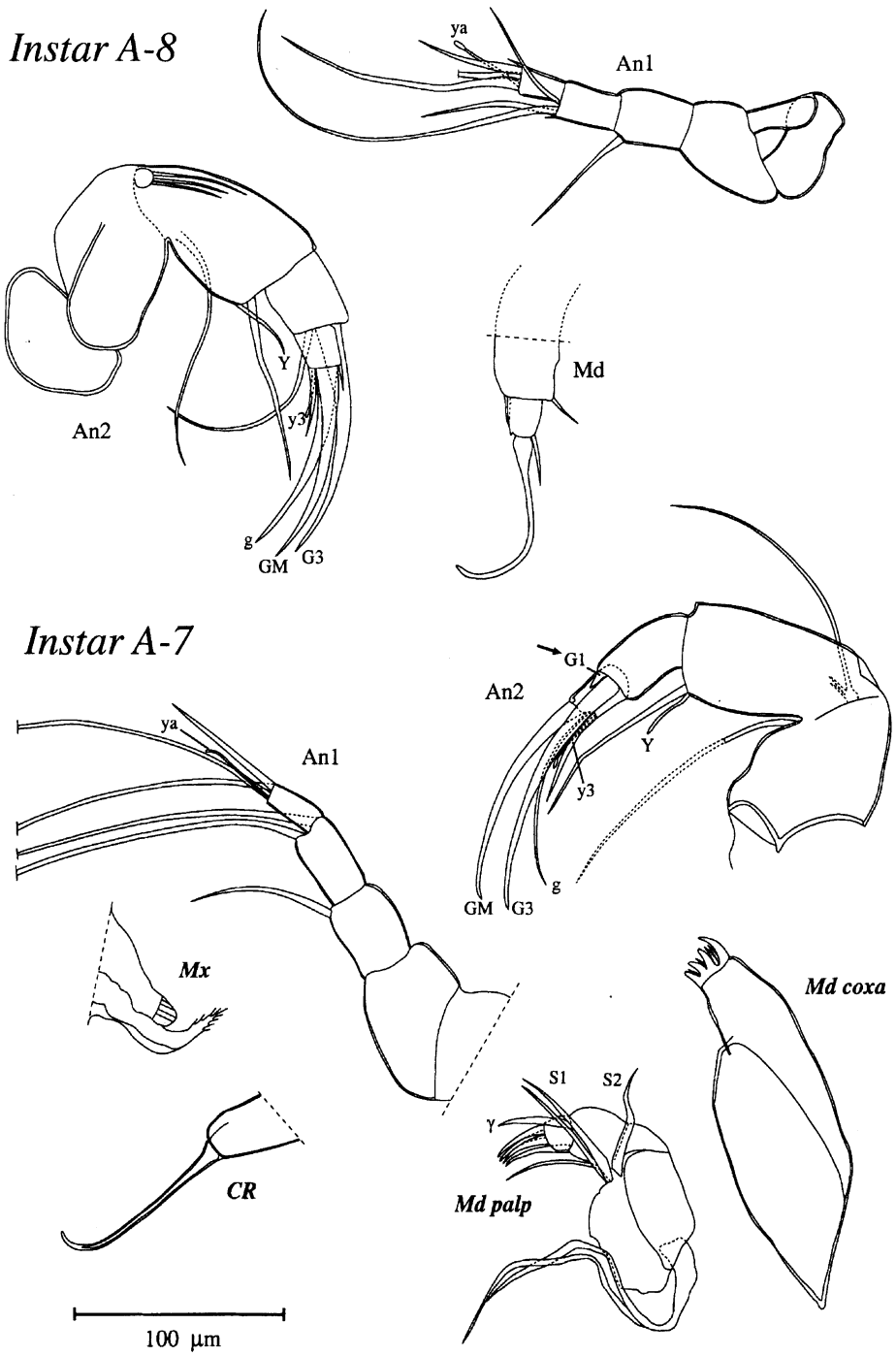


*Figure 3. Eucypris virens* Instar A-8. An1 (OS 15132); An2, outer face (OS 15132); Md (OS 15133). *Eucypris virens* Instar A-7. An1 (OS 15134); An2, inner face (OS 15135); Md, palp inner face (OS 15136), coxa (OS 15137); Mx, outer face (OS 15137); CR (OS 15137). Arrows indicate the first appearence of a feature. Bold italics indicate new limbs.

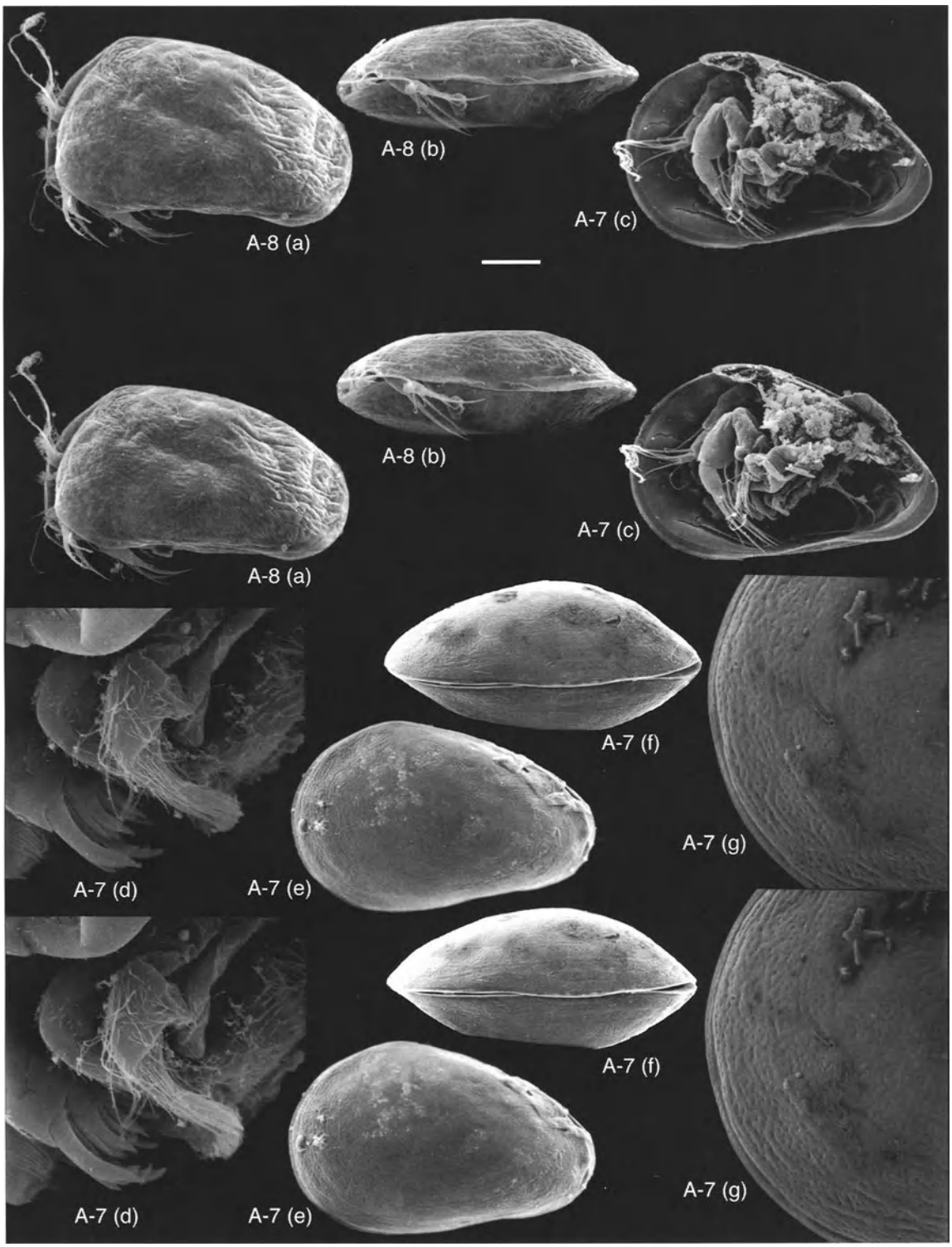


*Figure 4. Eucypris virens*. A-8 (a), critical point dried specimen, right valve (OS 15122) Scale bar=50 $\mu$m; A-8 (b), critical point dried specimen, ventral view (OS 15122) Scale bar=50 $\mu$m; A-7 (c), critical point dried specimen, left valve removed (OS 15123) Scale bar=60 $\mu$m; A-7 (d), critical point dried specimen, detail of maxilla *Anlage*, (OS 15123) Scale bar=7.5 $\mu$m; A-7 (e), right valve, (OS 15155) Scale bar=60 $\mu$m; A-7 (f), ventral view, (OS 15155) Scale bar=60 $\mu$m; A-7 (g), detail of anterior region, (OS 15156) Scale bar=24 $\mu$m. Stereo pairs.

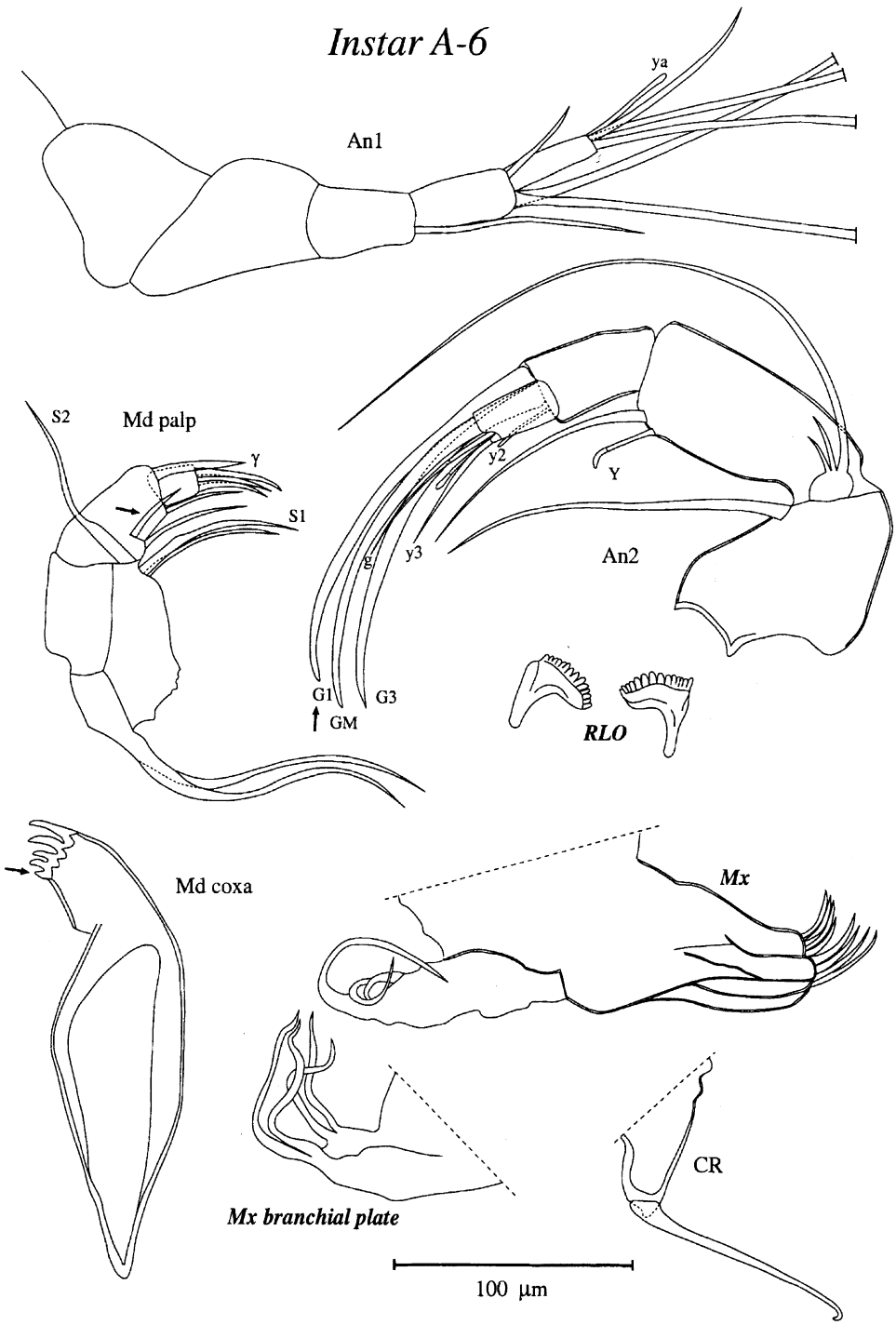


*Figure 5. Eucypris virens* Instar A-6. An1 (OS 15138); An2, outer face (OS 15138); Md, palp inner face (OS 15138), coxa (OS 15138); RLO (OS 15138); Mx, outer face (OS 15138), branchial plate (OS 15138); CR (OS 15138). Arrows indicate the first appearence of a feature. Bold italics indicate new limbs.

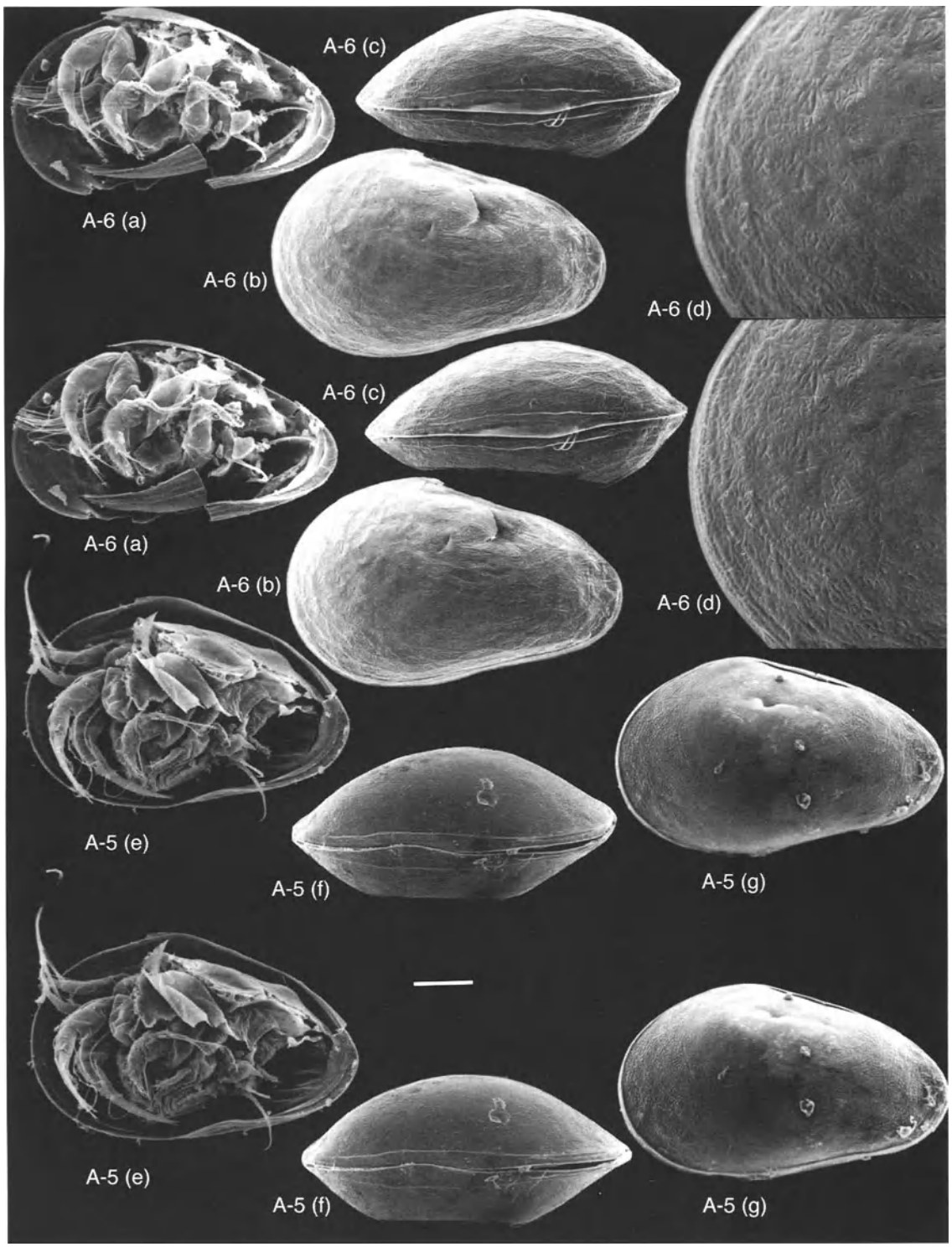


*Figure 6. Eucypris virens*. A-6 (a), critical point dried specimen with left valve removed. (OS 15124) Scale bar=70 $\mu$m; A-6 (b), right valve, (OS 15157) Scale bar=65 $\mu$m; A-6 (c), ventral view, (OS 15157) Scale bar=65 $\mu$m; A-6 (d), detail of anterior region (OS 15157) Scale bar=30 $\mu$m; A-5 (e), critical point dried specimen with left valve removed. (OS 15125) Scale bar=75 $\mu$m; A-5 (f), ventral view, (OS 15158) Scale bar=80 $\mu$m; A-5 (g) right valve, (OS 15158) Scale bar=80 $\mu$m. Stereo pairs.

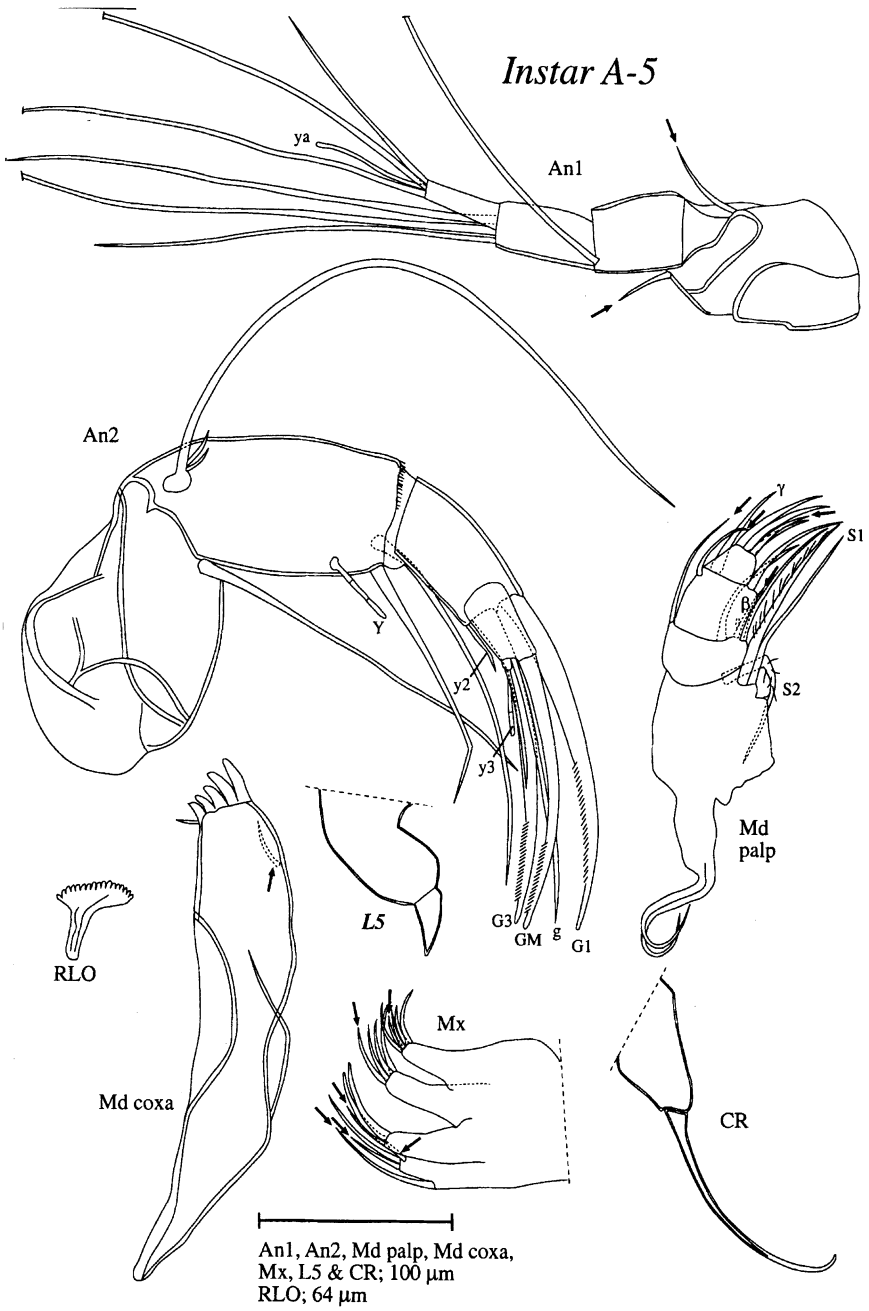


*Figure 7. Eucypris virens* Instar A-5. An1, inner face (OS 15139); An2, outer face (OS 15139); Md, palp outer face (OS 15139), coxa (OS 15139); RLO (OS 15140); Mx, outer face (OS 15139); L5, (OS 15141); CR (OS 15139). Arrows indicate first appearence of a feature. Bold italics indicate new limbs.

limbs of the adult stage has already been given by Smith & Martens (1996).

*First instar (A-8)* (Figures 2, 3 and 4a, b)

Carapace subrectangular in lateral view. Length 225–240 $\mu$m; maximum height 160 $\mu$m in the anterior third of the carapace. Valves uncalcified, flexible, with adductor muscles attached at the mediodorsal region. LV overlapping RV. Surface of valves covered with small pits and partly wrinkled, especially posteriorly and with a few normal pores with setae.

Eye already formed as a darkened bulbous spot towards the anterodorsal region of the body. Uniramous An1 protruding from a projection of the body, just in

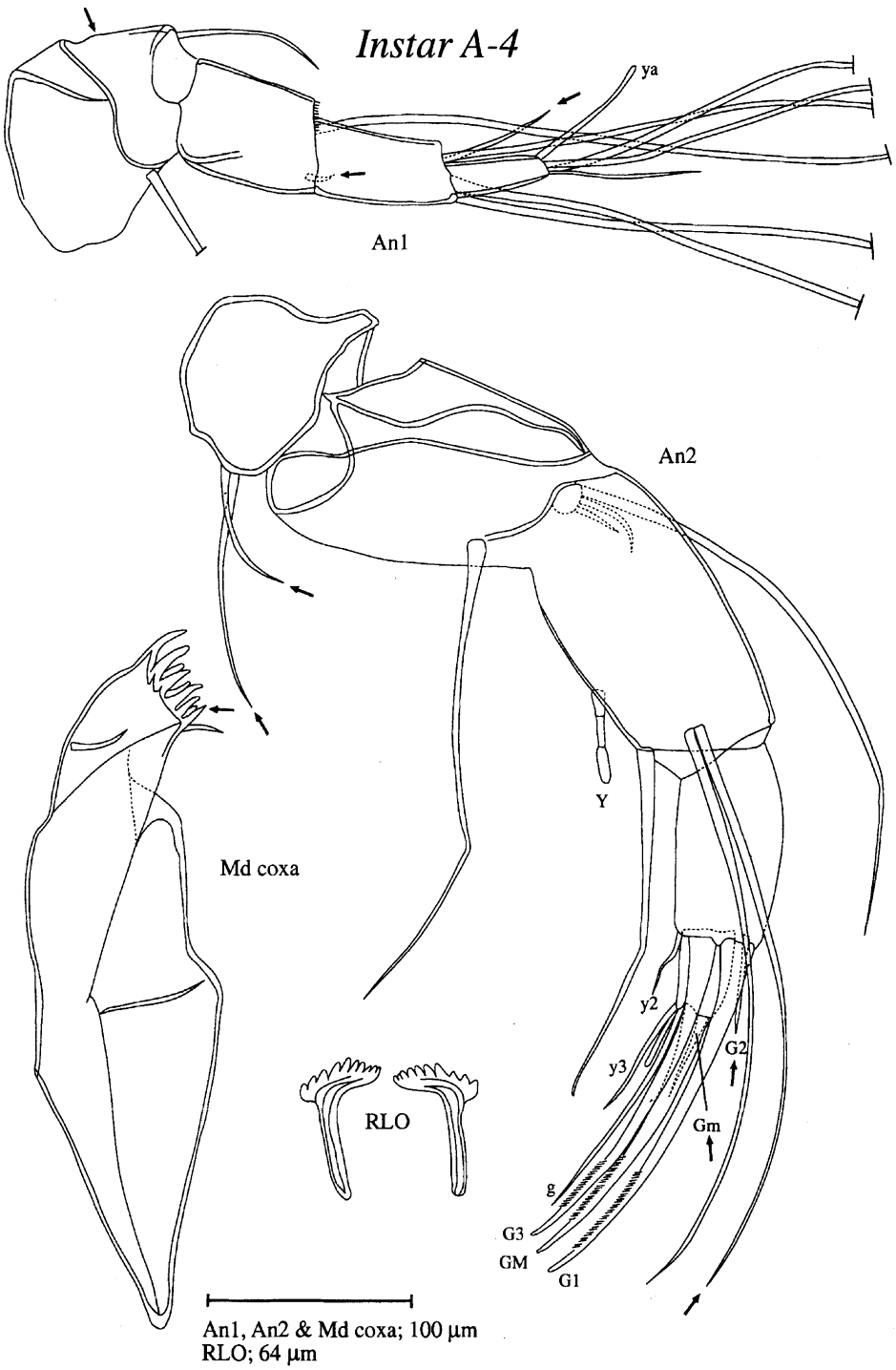


*Figure 8. Eucypris virens* Instar A-4. An1, inner face (OS 15142); An2, inner face (OS 15142); Md, coxa (OS 15142); RLO (OS 15142). Arrows indicate the first appearence of a feature.

front of the eye. An1 consisting of four podomeres, the first broad and subtriangular, the other three rectangular. Single subapical seta on the ventral side of the second podomere. Third podomere with three long apical setae and one very short spine on the ventral side. Fourth (apical) podomere with two long setae,

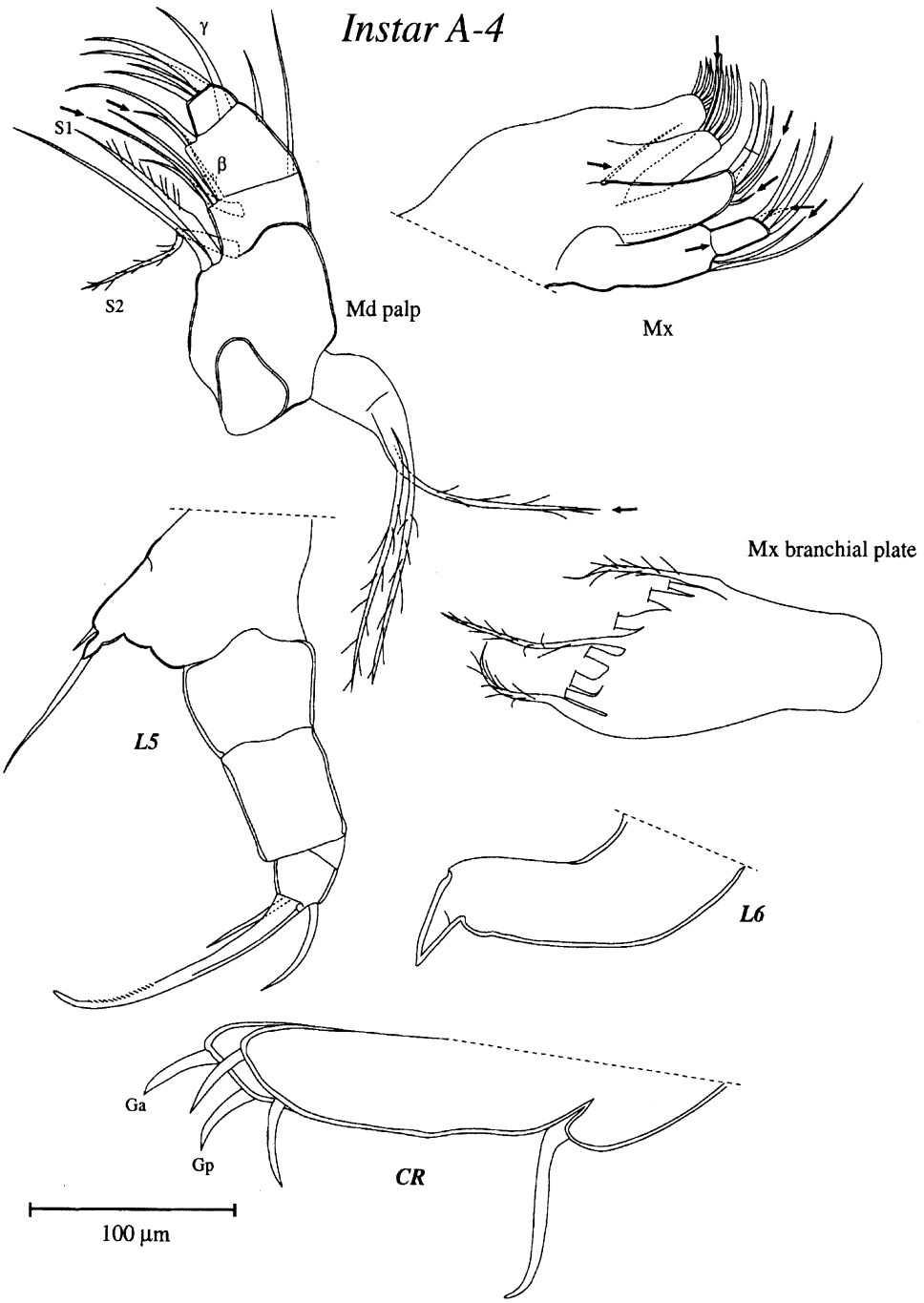


*Figure 9. Eucypris virens* Instar A-4. Md, palp outer face (OS 15142); Mx, outer face (OS 15142), branchial plate (OS 15143); L5 (OS 15144); L6 (OS 15143); CR (OS 15145). Arrows indicate the first appearence of a feature. Bold italics indicates new limbs.

one medium length seta and an aesthetasc (*ya*). Upper lip present in a basic form in the anteroventral region of the body and just in front of the An2.

An2 is biramous, consisting of a protopodite, endopodite and a small exopodite. Segmentation between protopodite and endopodite is not distinct. Protopodite consisting of two podomeres, with a long seta protruding from the ventral apical corner of the distal-most one. Exopodite is on the apical outer face of the protopodite and consists of a small base with

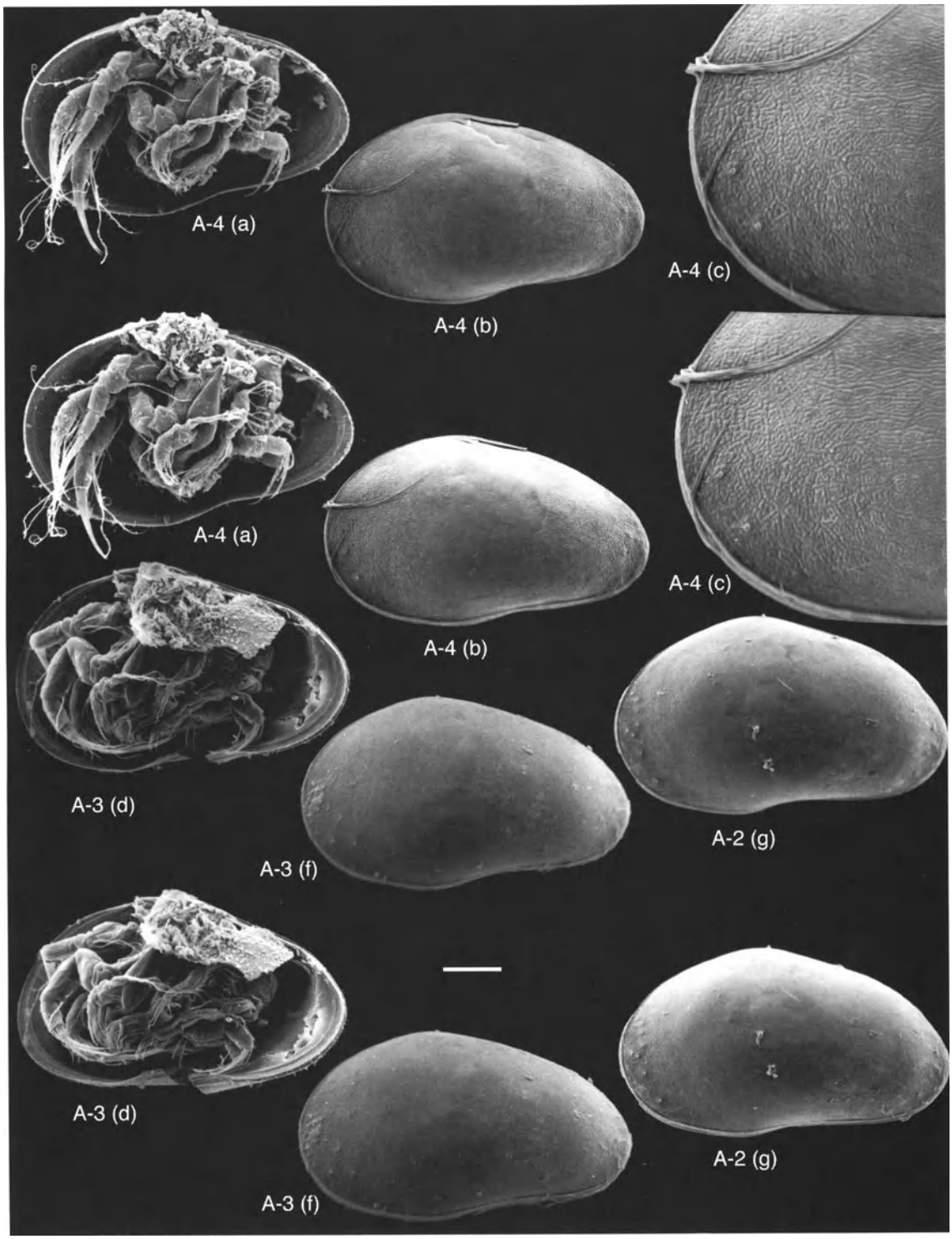


*Figure 10. Eucypris virens*. A-4 (a), critical point dried specimen with left valve removed, (OS 15126) Scale bar=100 $\mu$m; A-4 (b), right valve, (OS 15159) Scale bar=105 $\mu$m; A-4 (c), detail of anterior region, (OS 15159) Scale bar=48 $\mu$m; A-3 (d), critical point dried specimen with left valve removed, (OS 15127) Scale bar-130 $\mu$m; A-3 (e), right valve, (OS 15160) Scale bar=136 $\mu$m; A-2 (f), right valve, (OS 15161) Scale bar=172 $\mu$m. Stereo pairs.

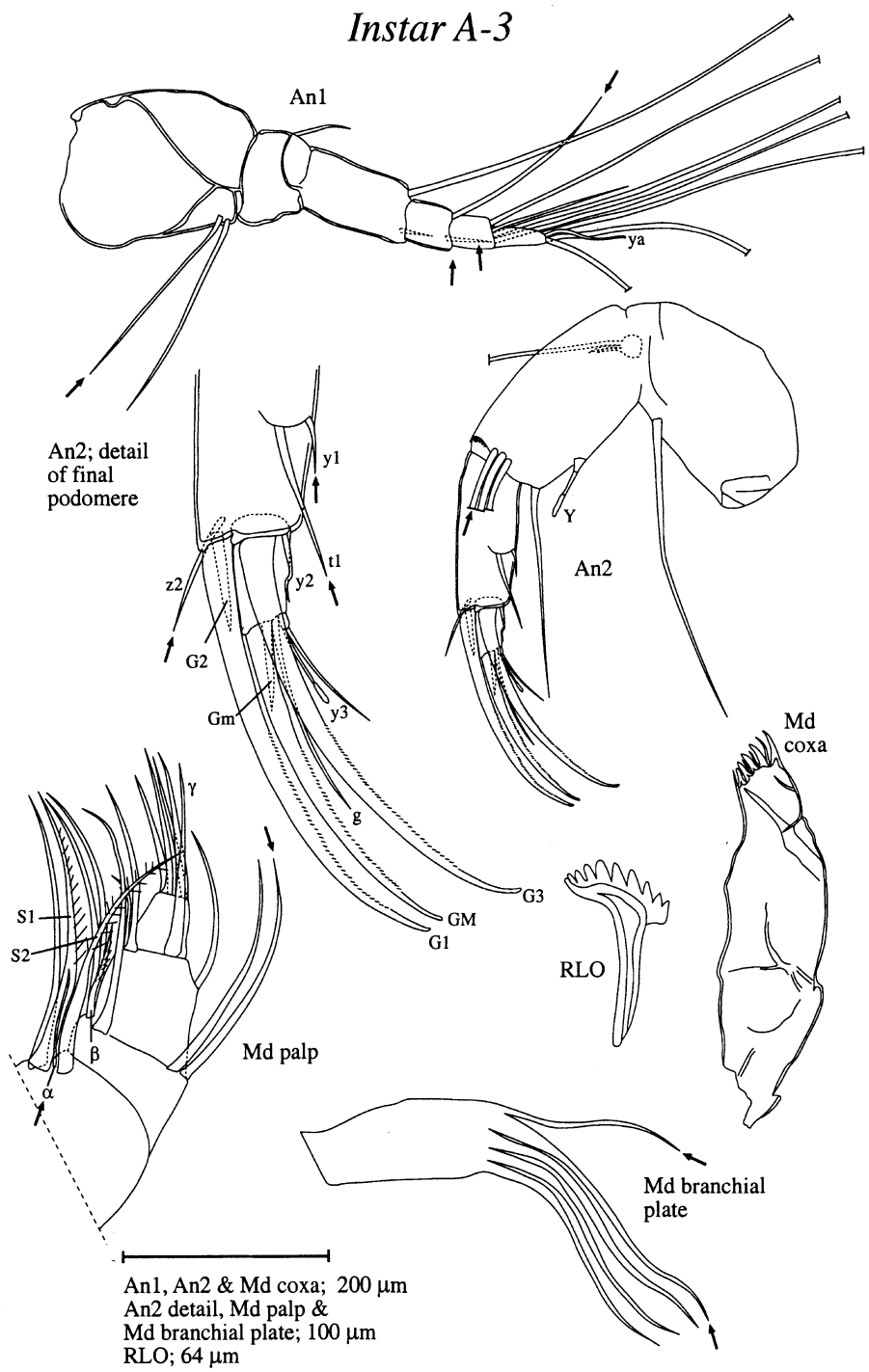


*Figure 11. Eucypris virens* Instar A-3. An1, inner face (OS 15146); An2, inner face (OS 15146); Md, palp inner face (OS 15146), branchial plate (OS 15147), coxa (OS 15146); RLO (OS 15146). Arrows indicate the first appearence of a feature.

three sub-equal length setae. Endopodite consisting of three podomeres; the first broad and subrectangular and with one long seta and one short seta (*Anlage* of aesthetasc *Y*) protruding from its apical ventral edge. Second podomere with one large, dorso-apical claw (*G3*) and one long, ventral seta. Terminal podomere

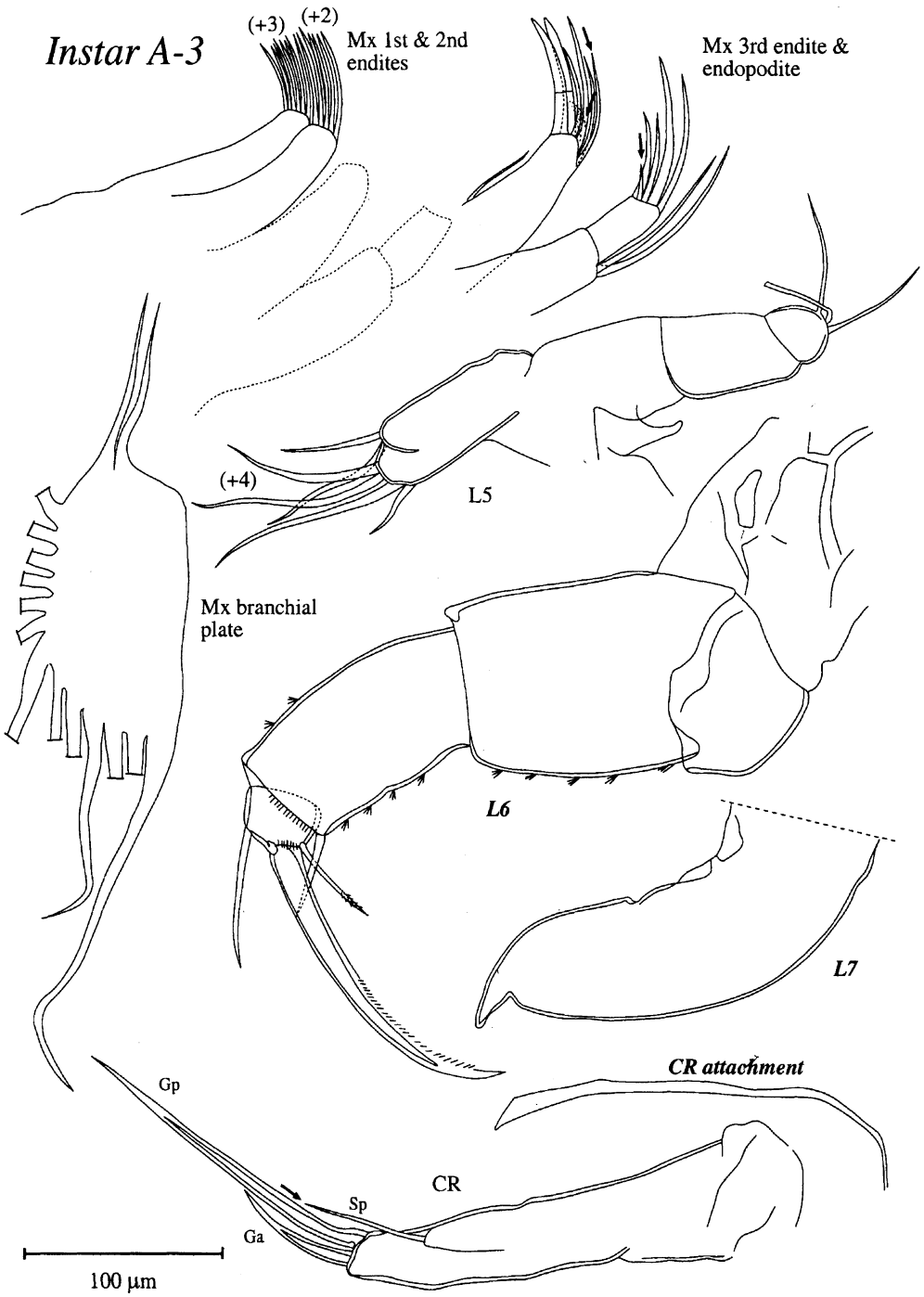


*Figure 12. Eucypris virens* Instar A-3. Mx, 1st, 2nd and 3rd endites outer face, endopodite outer face and branchial plate (OS 15146); L5 (OS 15146); L6 (OS 15146); L7 (OS 15146); CR and attachment (OS 15146). Arrows indicate the first appearence of a feature. Where it is not possible to determine which setae are new, a bracketed number indicates how many more setae are present e.g. (+1). Bold italics indicate new limbs.

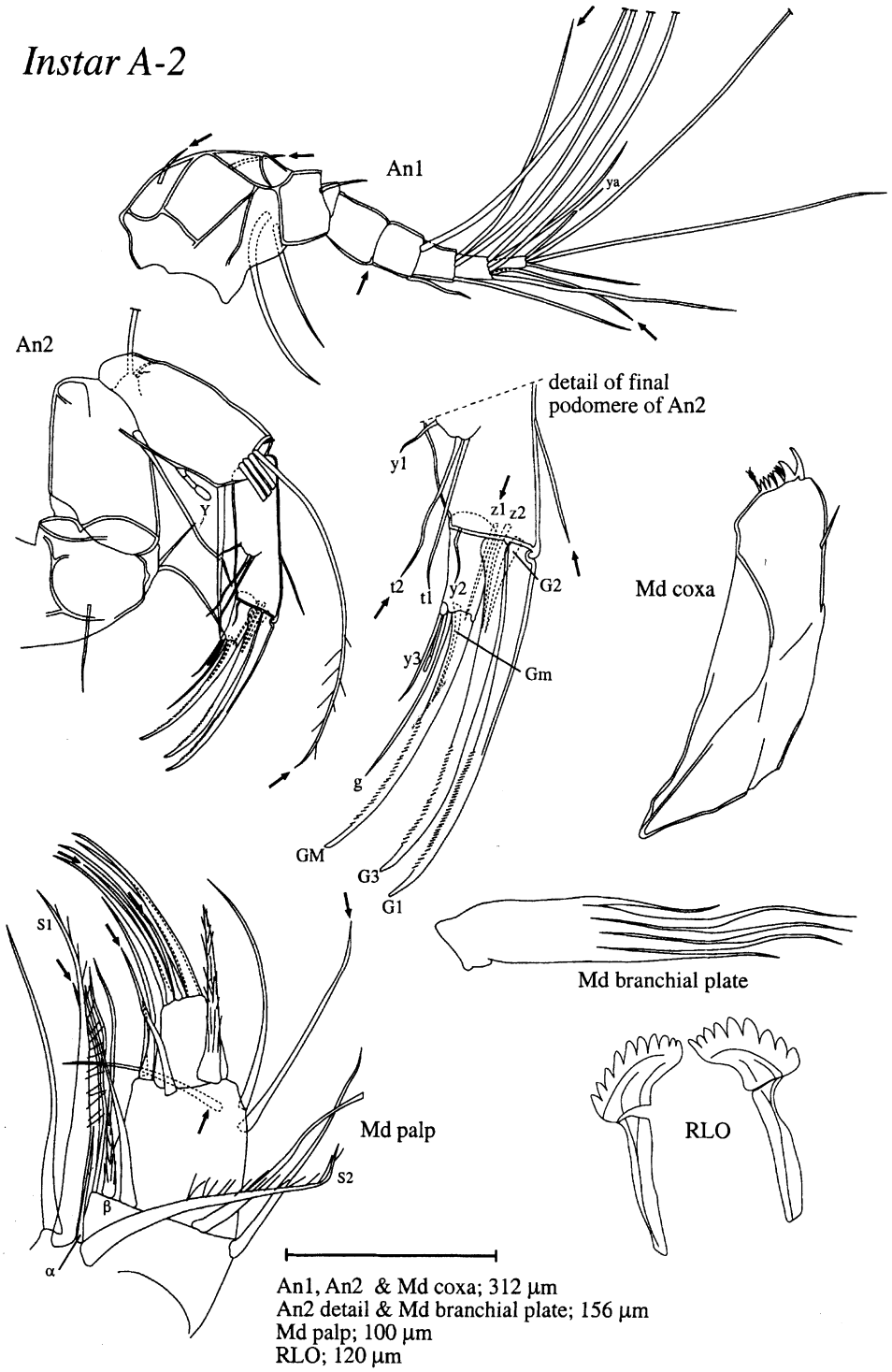


*Figure 13. Eucypris virens* Instar A-2, An1, outer face (OS 15148); An2, inner face (OS 15148); Md, palp inner face (OS 15148) branchial plate (OS 15149) coxa (OS 15148); RLO (OS 15148). Arrows indicate the first appearence of a feature.

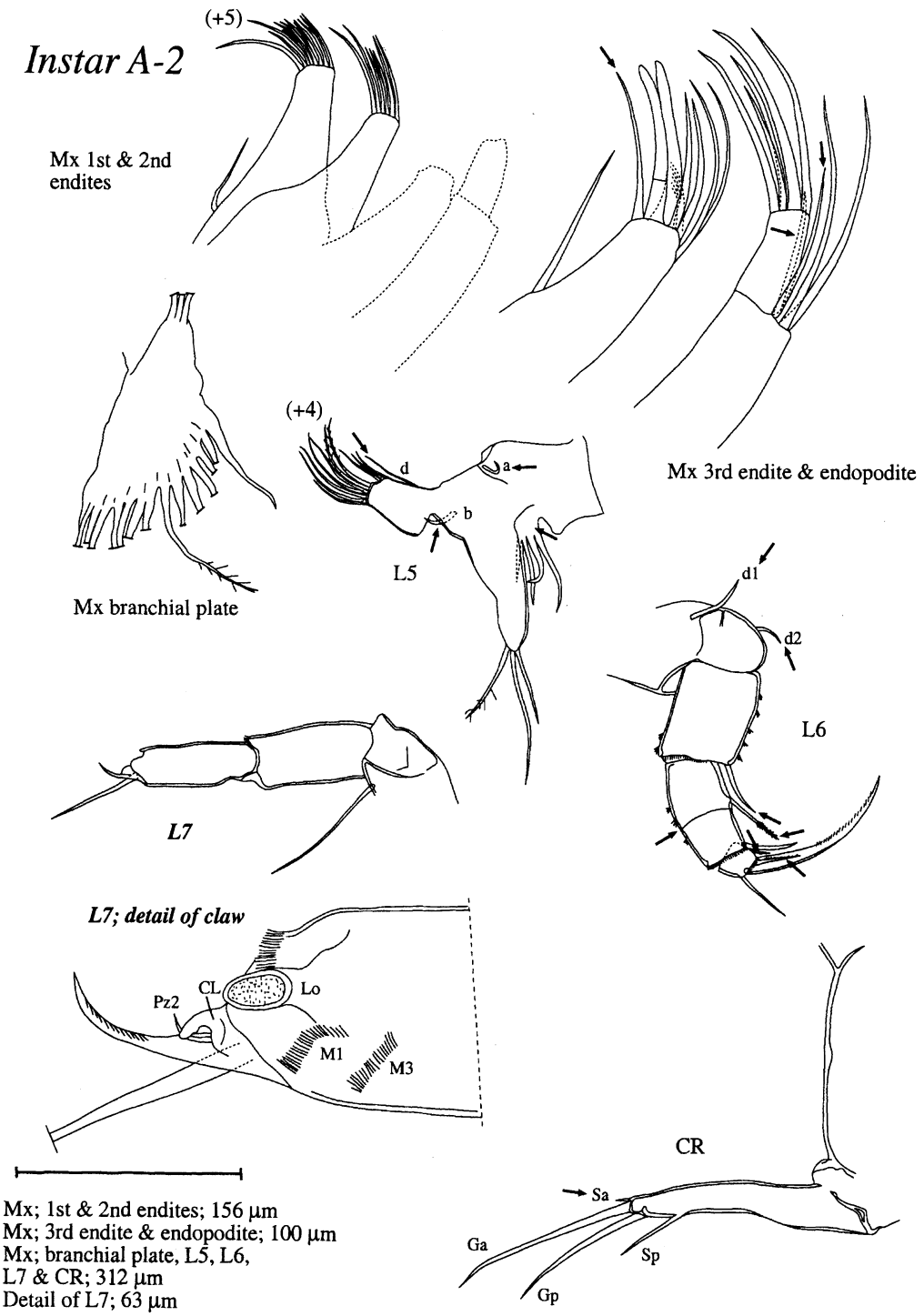


*Figure 14. Eucypris virens* Instar A-2. Mx, 1st, 2nd and 3rd endites, endopodite outer face and branchial plate (OS 15148); L5 (OS 15148); L6 (OS 15148); L7 (OS 15148); CR and attachment (OS 15148). Arrows indicate the first appearence of a feature. Where it is not possible to determine which setae are new, a bracketed number indicates how many more setae are present e.g. (+1). Bold italics indicate new limbs.

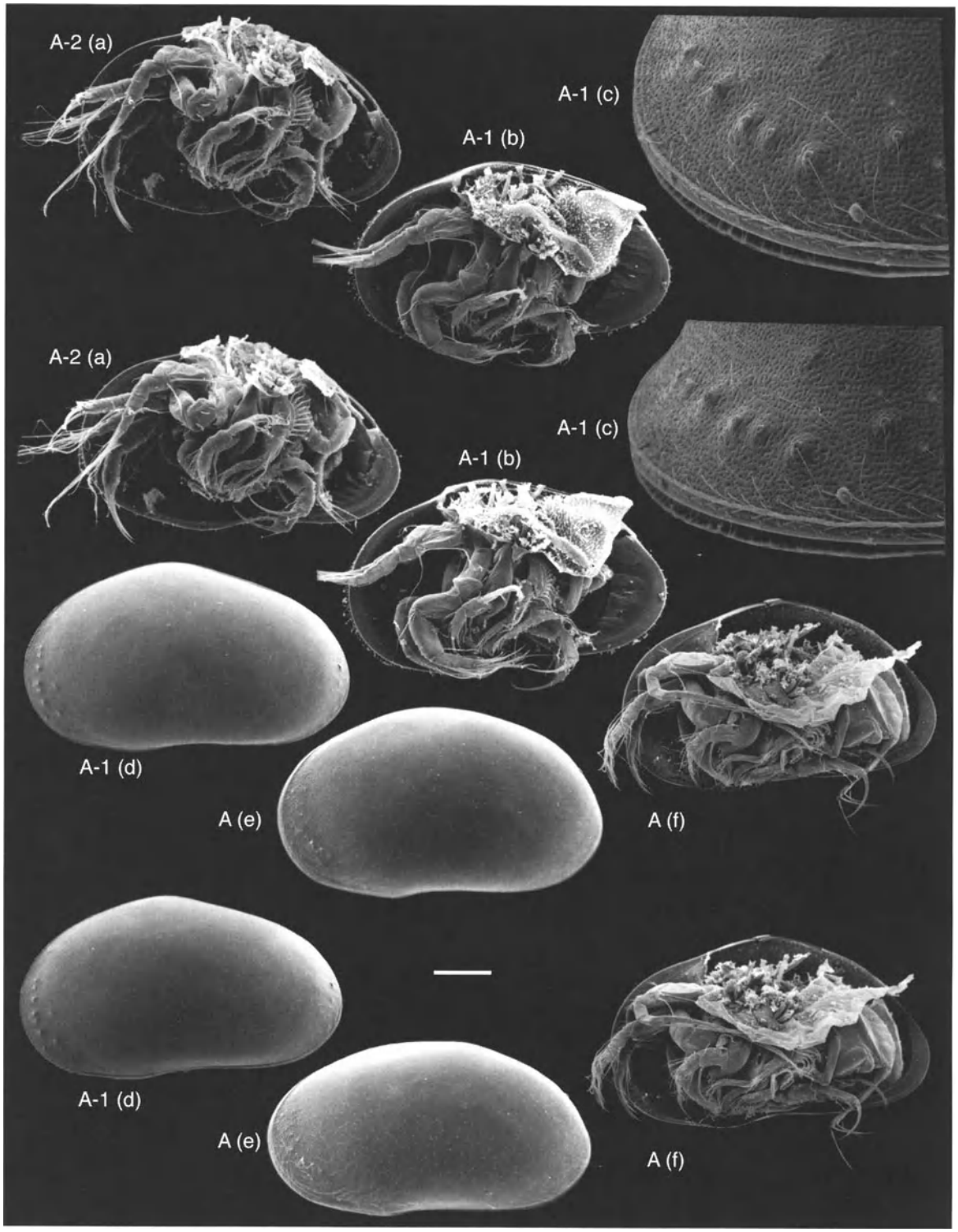


*Figure 15. Eucypris virens*. A-2 (a), critical point dried specimen with left valve removed. (OS 15128) Scale bar-152 $\mu$m; A-1 (b), critical point dried specimen with left valve removed. (OS 15129) Scale bar-227 $\mu$m; A-1 (c), detail of anterior region. (OS 15162) Scale bar=48 $\mu$m; A-1 (d), right valve, (OS 15162) Scale bar=238 $\mu$m; A (e) right valve, (OC 2002) Scale bar-285 $\mu$m; A (f), critical point dried specimen with left valve removed. (OS 15130) Scale bar=326 $\mu$m. Stereo pairs.

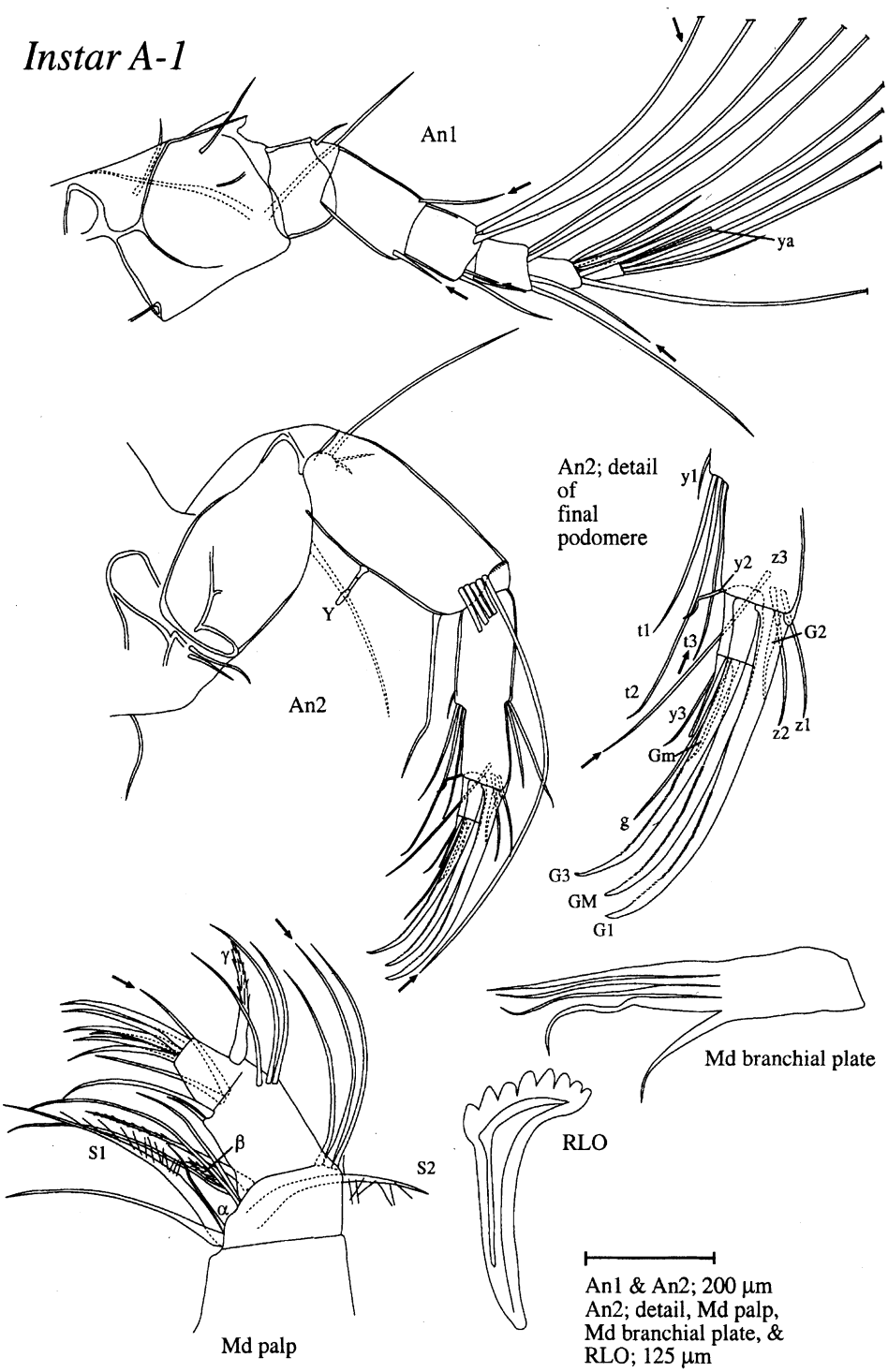


*Figure 16. Eucypris virens* Instar A-1. An1, outer face (OS 15150); An2, inner face (OS 15150); Md, palp outer face (OS 15150), branchial plate (OS 15151); RLO (OS 15151). Arrows indicate the first appearence of a feature

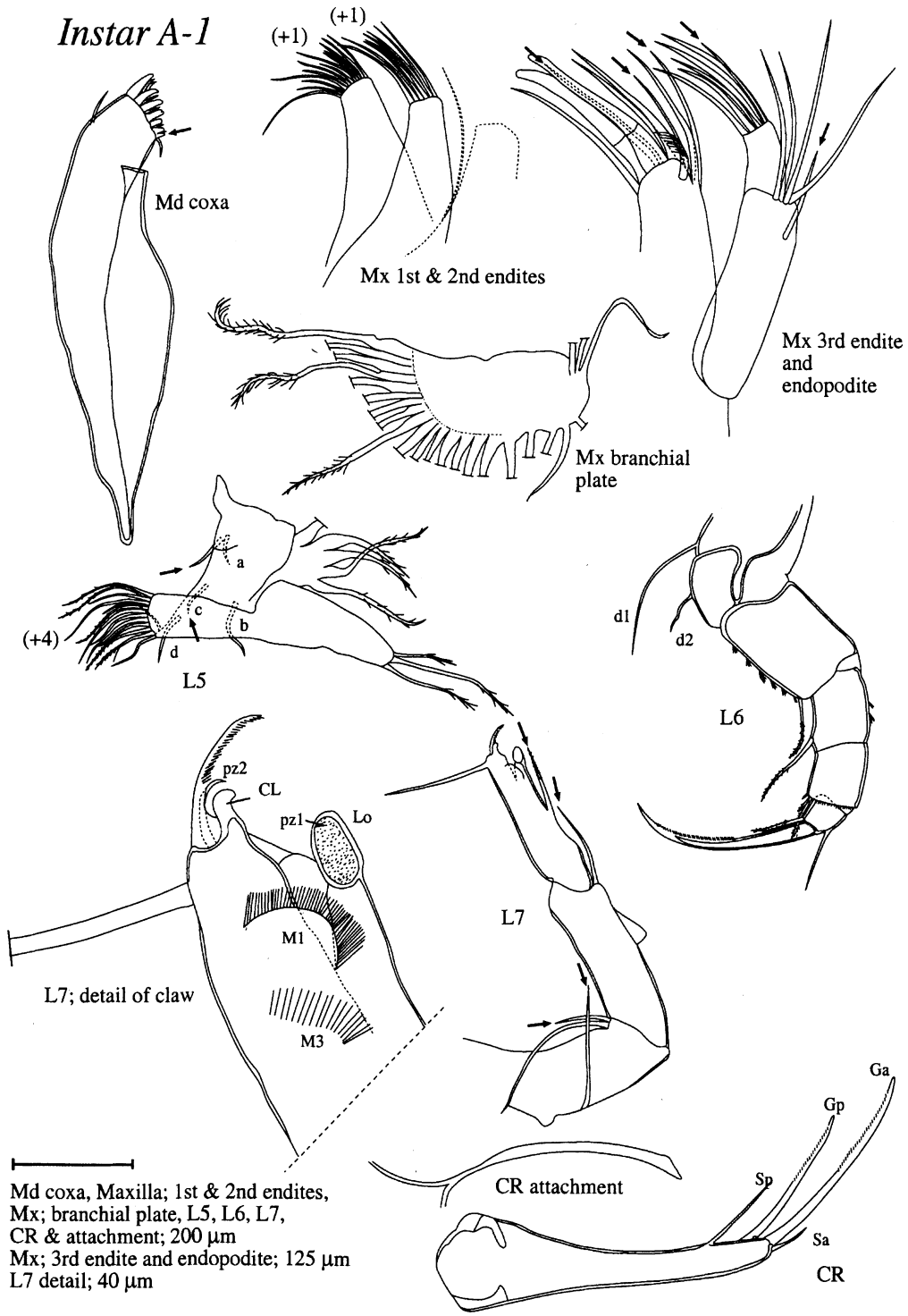


*Figure 17. Eucypris virens* Instar A-1. Md, coxa (OS 15151); Mx, 1st and 2nd endites outer face (OS 15150) 3rd endite, endopodite outer face & branchial plate (OS 15151); L5 (OS 15150); L6 (OS 15151); L7 (OS 15151); CR and attachment (OS 15150). Arrows indicate the first appearence of a feature. Where it is not possible to determine which setae are new, a bracketed number indicates how many more setae are present e.g. (+1).

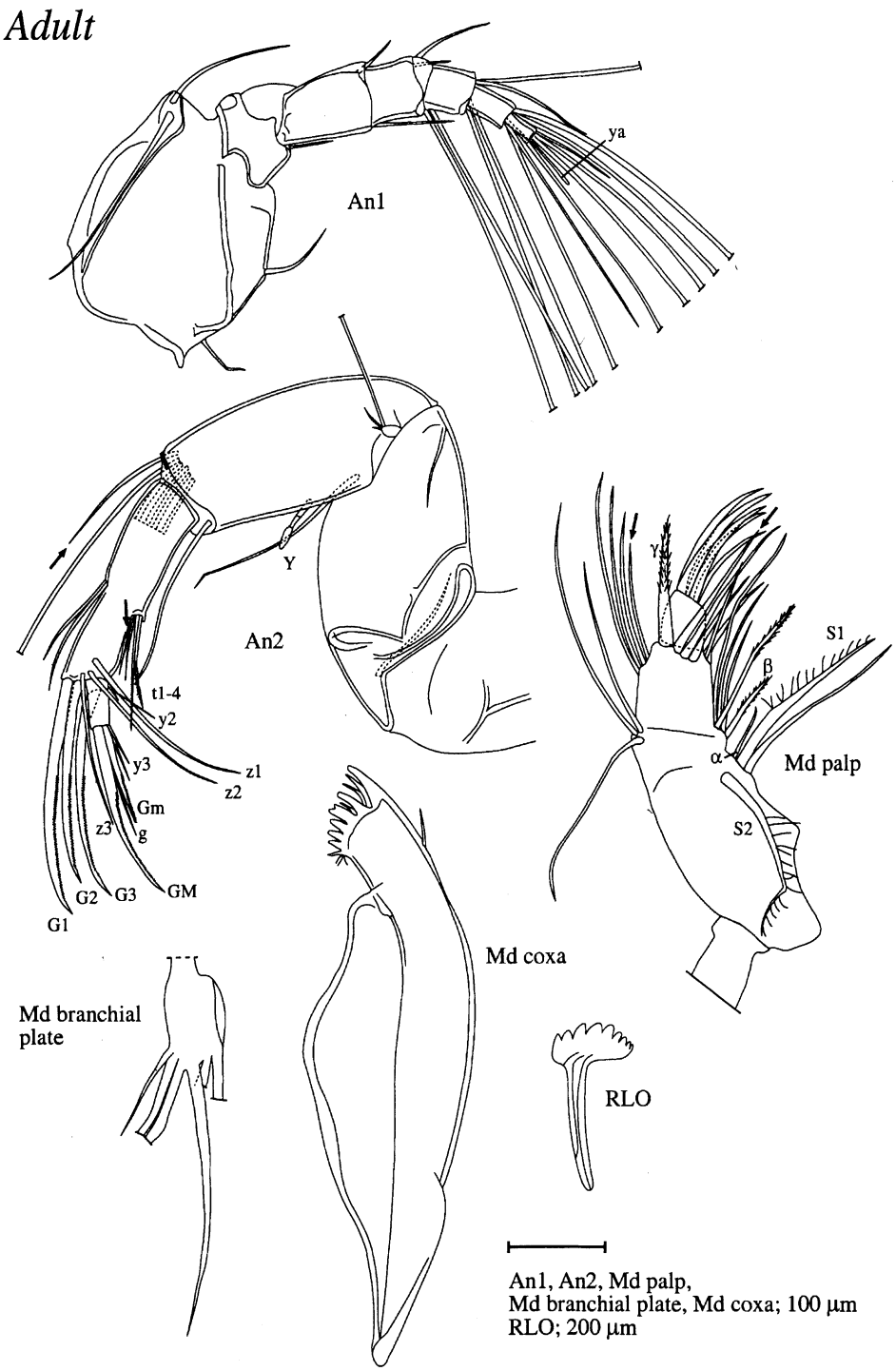


*Figure 18. Eucypris virens* Adult instar. An1, inner face (OC 2002); An2, outer face (OC 2002); Md, inner face (OC 2002), branchial plate (OS 15152), coxa (OC 2002); RLO (OC 2002). Arrows indicate the first appearence of a feature.

apically with a short spine on the dorsal edge and two dorsally positioned large claws, (*GM* and *g*) and a bifurcating aesthetasc (*y3*).

Md points backwards and is attached to the body just above and to the side of the mouth region. Md consists of a long, strongly curved claw protruding

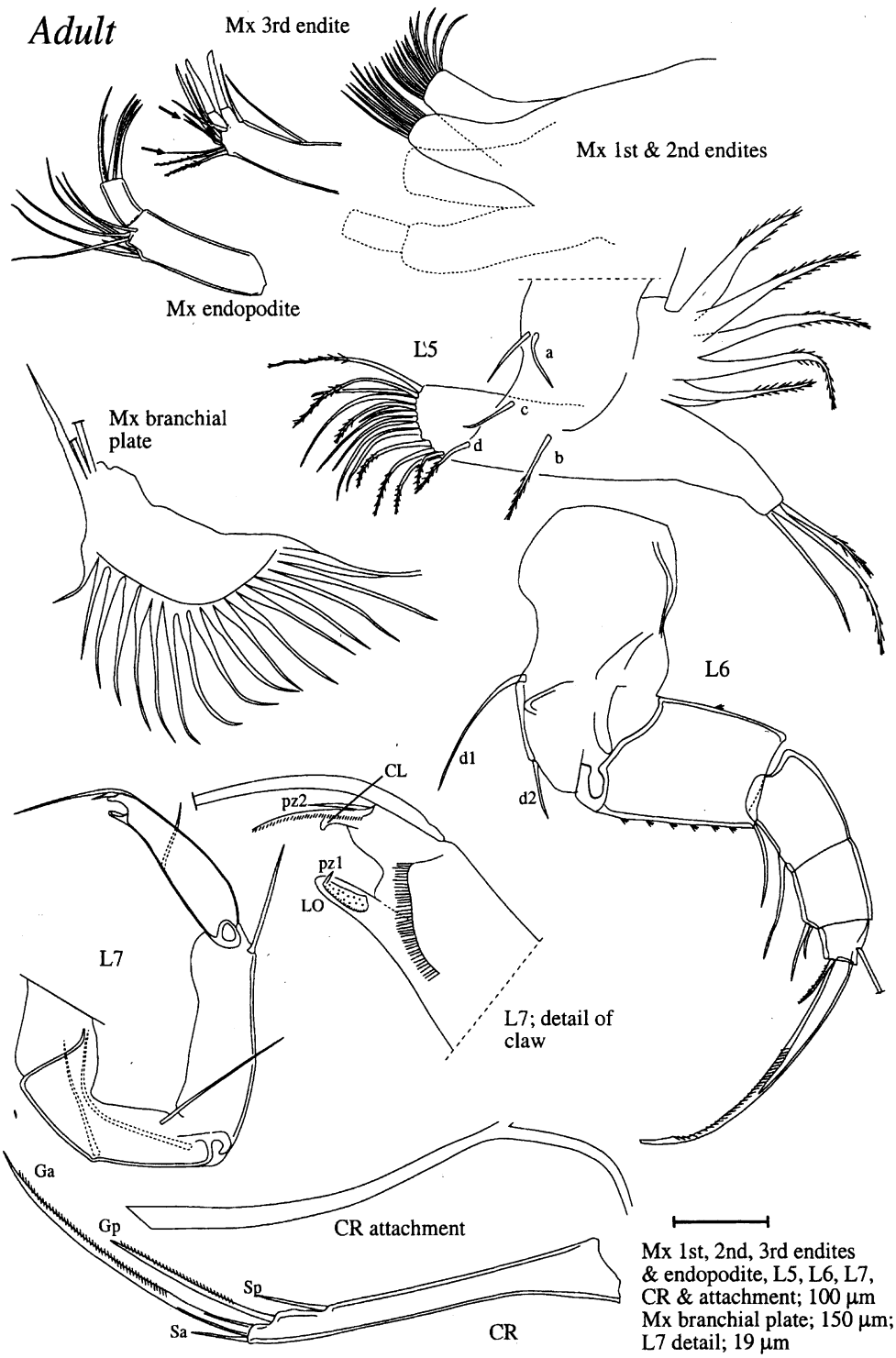


*Figure 19. Eucypris virens* Adult Instar. Mx, third endite, endopodite outer face (OC 2002) and branchial plate (OS 15154), first and second endite outer face (OS 15153); L5 (OC 2003); L6 (OC 2002); L7 (OC 2002); CR and attachment (OC 2002). Arrows indicate the first appearence of a feature.

from a jointed base, the latter with three subquadrate segments, distally decreasing in size; first podomere with one apical seta, second podomere with two apical setae. Body behind the Md is rather large and bulbous in lateral view.

*Second instar (A-7)* (Figures 2, 3 & 4c–g)

Length of carapace 300–320 $\mu$m; maximum height 180–200 $\mu$m, in the anterior third of the carapace which slopes posteriorly from this point. LV overlapping RV. Posterior part of the carapace forming a small slit when the valves are closed. Surface ornamentation simple, consisting of numerous small pits and granules, especially towards the anterior and posterior regions. A limited number of normal pores with setae also present in the central anterior region. Contact margins of the valves simple with an incipient marginal rim.

An1 similar to those in instar A-8, the only change occurring at the end of the third podomere with two long setae replacing one long and two shorter setae and the spine.

An2 exopodite now consisting of one long and two much shorter setae protruding from a rounded base. First endopodal podomere similar to instar A-8. Second podomere of the endopodite with claw *G3* as in the previous instar, but the seta accompanying it in instar A-8 is now missing. A small node, possibly the *Anlage* of claw *G1*, present on the dorsal edge. Claw *g* present on the final podomere of the An2 in the previous instar, now transformed into a seta, thus with only two claws on this appendage.

Md developed into a feeding appendage, consisting of an expanded coxa and a palp. Coxal endite bearing four teeth with intervening setae, protruding on either side towards the mouth, just behind the more developed upper lip; coxa pointing dorsally and slightly raked backwards. First palp segment is large, with setae *S1* and *S2* present apically on the ventral edge, together with an accompanying smooth seta. Exopodite (respiratory plate) consisting of two long setae, protruding from the dorsal edge of the protopodite. Endopodite two-segmented; first segment with one long and one shorter seta near the dorsal proximal edge and apically with a broad, stout $\gamma$ seta on the ventral side and a stout seta on the opposite (dorsal) side; final segment with three short, stout, apical claws.

*Anlagen* of the Mx consisting of an elongated, curved, stout palp, with a bared surface towards the tip. Posteriorly, lying along side the palp is a shorter process with a rounded end. Mx *Anlagen* lie in folds on each side of the hypostome and terminate near the mouth.

Pediform *Anlage* of the CR points downwards and backwards from the posterior region of the animal and consists of an elongate, basal segment with a long, terminally curved claw.

*Third instar (A-6)* (Figures 2, 5 and 6a–d)

Length of carapace 366–390 $\mu$m; height 219–256 $\mu$m. Outline similar to instar A-7, but anterior end slightly more inflated. Ventral outer list developed. Surface ornamentation more pronounced than in previous instars, with numerous pits and grooves again especially towards the anterior and posterior regions.

An1 very similar to those of instar A-7, but with an additional seta on the apical end of the third podomere.

An2 larger than in A-7 and with inflated base to exopodite which has its long seta proportionally longer. Aesthetasc *Y* segmented at the tip and slightly shifted in position towards the protopodite. *G1* developed into a large claw and accompanied by claw *G3* and also for the first time with aesthetasc *y2*. Chaetotaxy of terminal podomere unchanged.

Md slightly bigger than in instar A-7. Endopodite with an additional medio-dorsal seta on the first endopodal podomere (=second palp segment). Coxa now with five teeth, but overall shape remaining very similar.

Rake-like organs appearing for the first time, present in the anterior region of the hypostome. Rake like organs are small, with a short root and relatively wide top edge with 14 small, rounded teeth.

Mx with endopodite and three endites, all elongate. Endopodite and third endite each with a single claw-like seta. Second endite with two terminal setae. First endite with four terminal setae. Branchial plates elongate, with four long setae at the posterior end.

CR is unchanged from instar A-7 and still an *Anlage*.

*Fourth instar (A-5)* (Figures 2, 6e–g and 7)

Carapace length 427–463 $\mu$m; height 268–304 $\mu$m. Maximum height marked by a dorsal inflation and situated approximately at one-third of the length from the anterior margin, carapace sloping posteriorly from this point. Shell ornamentation most strongly developed towards the anterior and posterior margins and consisting of a gentle reticulate pattern and numerous pits. Normal pores with setae more numerous on the

anterior portion of the carapace. Muscle scars not distinct and situated in the postero-central region of the carapace.

Protopodite of the An1 large and subrectangular, bearing two small setae, one on the ventral and one on the dorsal surface, towards the apical edge. Second podomere more quadrate than in instar A-6 and with apical seta much longer. Third podomere similar to second podomere, but with the three apical setae all long. Final podomere unchanged.

Exopodite of An2 with a proportionally larger base than in instar A-6. Aesthetascs *Y* now three-segmented as in the adult and shifted further towards the protopodite. First natatory seta appearing on the inner edge of the first podomere of the endopodite. Chaetotaxy of the final two podomeres of the endopodite remaining unchanged from instar A-6.

Endopodite of Md with three recognizable segments, resulting from the splitting of the first endopodal podomere in instar A-6 (second palp segment). First endopodal podomere (second palp segment) with additional $\beta$ seta on the proximal dorsal edge (forming a group of three) and additional long seta on proximal ventral edge. Second endopodal podomere (third palp segment) with an additional seta on the ventral edge in a subapical position. Third endopodal podomere (fourth palp segment) with an additional seta on the apex. Coxa similar to instar A-6, but with an additional subapical seta on the outer edge.

Rake-like organs with a wider upper edge than in instar A-6, bearing up to 17 small, rounded teeth and with a relatively short root.

Elongated endopodite of Mx still unsegmented, terminating with one long dorsal, subapical seta and three apical setae, one of these longer than the other two. Third endite with two smooth and curved *Zahnborsten*, accompanied by a medium-length seta. Second endite with four sub-equal length setae. First endite with two medium-sized and three shorter setae. Branchial plates relatively large and consisting of four long, posterior setae, lying alongside the body, reaching back as far as the CR.

*Anlagen* of the L5 protrude from behind the base of the Mx and point posteriorly, terminating near the CR base. L5 consist of an elongate, curved podomere, capped by a smaller triangular podomere.

CR is situated just behind L5s, still as an *Anlage* and unchanged from instar A-6.

*Fifth instar (A-4)* (Figures 2, 8, 9 and 10a–c)

Carapace length 536–597 $\mu$m; height 317–378 $\mu$m. Shape similar to instar A-5. Outer lists more developed and continuing around the valves to the anterior and posterior regions, terminating at the hinge. Surface ornamentation similar to that in instar A-5, but with the addition of pustules in the anterior region. Muscle scars still in a postero-central position and not distinct, although slightly more developed.

An1 with five podomeres, with the protopodite now divided into two podomeres; one larger subquadrate basal podomere and a smaller, distal subtriangular podomere; two setae on the protopodite longer than in instar A-5. Next podomere with an additional very small seta, opposite the long apical seta. Third podomere with an additional medium-length seta accompanying the three longer setae. Final podomere remaining unchanged.

An2 protopodite with two ventrally positioned setae, of medium length, one longer than the other, on the first podomere. First endopodal podomere with aesthetascs *Y* shifted to a position about 2/3 along the ventral margin and no longer proximal to the sub-apical, ventral seta; two natatory setae in a mid-apical position present on the inner face. Seta *G2* present on the apical side of the second endopodal podomere, accompanying aesthetascs *y2*, and the two large claws *G3* and *G1*. Final endopodal podomere with the addition of a stout seta *Gm*.

Md endopodite with additional seta with $\beta$ group at apex of first podomere (second palp segment). Another shorter seta present on the dorso-apical corner of the second podomere (third palp segment), forming a group of two. Respiratory plates of Md with an additional long seta, forming a group of three. Md coxa, although larger than with instar A-5, still similar in shape, but with the addition of an extra tooth on the endite (total of 6).

Rake-like organs well-developed, with a long root and with an upper edge supporting 10 small, triangular teeth which lean towards the inner edge.

Endopodite (palp) of Mx two-segmented; first podomere large, elongate and subrectangular, with one long and one medium-length seta on its outer, apical edge; second podomere smaller, sub-rectangular, bearing three, stout, medium-length and one short seta. Third endite with two well-developed *Zahnborsten*, smooth and curved, with a collar 1/3 the way up, and one very short and two long setae. First and second endites with approximately four and five apical setae, respectively. Branchial plates broader and more elongate than in instar A-5 and fringed on the posterior edge by nine hirsute (covered with setules) setae.

L5s developed into walking legs, consisting of four podomeres. First podomere rounded and with a small projecting base (endite), bearing one long and one short setae. Second podomere subquadrate. Third podomere rectangular. Final podomere trapezium-shaped and bearing a long serrated claw and two short setae.

*Anlagen* of L6 appearing in this instar, morphologically very similar to *Anlagen* of L5 in instar A-5, consisting of a basal podomere with a terminal, triangular-shaped cap and protruding from just behind the base of the L5.

CR altered from its *Anlage* state to a form similar to that in adults. CR lying along the body and terminating between the bases of the L5s. CR consists of elongate, broad rami with a rounded end, bearing two small claws (*Ga* and *Gp*) which are equal in length and curved towards the posterior. Note: the CR looks relatively lightly sclerotized and hence, may not be functioning in this instar, a fact further corroborated by the absence of any supporting attachments of the endoskeleton.

*Sixth instar (A-3)* (Figures 2, 10d, e, 11 and 12)

Carapace length 695–731 $\mu$m; height 427–463 $\mu$m. Posterior region more inflated and anterior region slightly more compressed in dorsal view than in instar A-4. Valve margins more developed and set with marginal pore canals and setae. Surface ornamentation less reticulate than in previous instars, but with more normal pore canals with setae and pustules on the anterior region. Muscle scar position moved slightly towards the anterior and now distinct; pattern similar to that seen in adults, consisting of seven elongate scars.

An1 with six podomeres, resulting from the fourth podomere splitting into two smaller quadrate podomeres. First podomere large, broad and subrectangular in shape and supporting two long, subapical setae on its ventral edge. Second podomere wider than long and bearing a single, short seta on the dorsal margin. Third podomere an elongate rectangle and approximately twice as long as it is wide, apically supporting a long seta on the dorsal corner and a small seta towards the ventral edge. Fourth podomere subquadrate, bearing two apical setae, one long and one short. Fifth podomere with two long and one medium-length setae. Sixth (final) podomere, as in previous instars.

Protopodite and exopodite of An2 similar to those in previous instars. First endopodal segment with aesthetascs *Y* closer to the protopodite than with instar A-4; additional natatory seta present on the inner surface (total of 3). Second endopodal segment with seta (*t1*) on the ventral side, accompanied by a much smaller seta (*y1*); this segment apically with the large claw *G1*, seta *G2*, and the other large claw *G3*, a relatively long aesthetascs *y2* and the first appearance of a short seta *z2*. Final podomere, as in instar A-4, with claw *GM*, setae *g* and *Gm* and aesthetascs *y3*.

First palp-segment of the Md with an additional small seta $\alpha$ accompanying *S1* and *S2*. First endopodal segment (second palp-segment) with $\beta$ seta more hirsute and with additional seta on the apical ventral edge (forming a group of two) second endopodal segment as in instar A-4. Final podomere with an additional claw on the apex, now totalling three claws and two setae. Respiratory plate consisting of 4 long setae and one shorter seta. Coxa unchanged.

Rake-like organs with a long root and outer edge set with 10 small, rounded teeth, the larger of which are towards the centre.

Endopodite (palp) of Mx with an additional seta on the apex of the final segment. Third endites with two additional setae (now total of five) accompanying the *Zahnborsten*, smallest seta with unidirectional setules pointing towards the inner surface. First and second endites of Mx each with approximately seven terminal setae. Branchial plates wider than in instar A-4 and with 13 long setae along the posterior and ventral edges and with two reflexed setae on the anterior edge.

L5 changed in form and now recognizable as a maxilliped, consisting of an endopodite pointing posteriorly and a feeding process (endite) pointing anteriorly. Elongated endopodite three-segmented, terminating with three long setae. Endite bearing six setae; three long, two of medium length and one short. Protopodite with the *Anlage* of the respiratory plate, consisting of a simple, elongate process.

L6 transformed from *Anlagen* into walking legs. First podomere subtriangular and fitting into the top of the second podomere to form a knee-like joint; second podomere subrectangular. Third podomere, although narrower, more elongate than the second podomere and with a short, stout seta on its ventro-apical corner. Fourth podomere small and subquadrate, supporting a large, curved, terminally serrated claw, a ventro-apical seta and a subapical seta.

L7 appearing in *Anlagen* state, similar in shape to that of the L6 in instar A-4. L7 protrudes from just behind the base of the L6 and consists of an elongate stump, capped with a triangular podomere.

CR more heavily sclerotized than in instar A-4. CR long, distally bearing two claws and one seta

(*Sa*); distal claw (*Ga*) short, broad and curved towards the posterior; adjacent claw (*Gp*) approximately four times longer. Seta (*Sp*) on the dorsal edge of medium length. CR now with a long and non-bifurcating attachment.

*Seventh instar (A-2)* (Figures 2, 10f, 13, 14 and 15a)

Carapace length 866–987 $\mu$m; height 549–622 $\mu$m. Outline similar to that of instar A-3. Surface ornamentation no longer reticulate, but a large number of small pits still present, especially anteriorly and posteriorly. Pustules slightly more numerous in the anterior region. Muscle scars now in a central position.

An1 with seven podomeres, as a result of the third podomere (as seen in instar A-3) dividing to form two quadrate podomeres. First podomere with two additional setae on the dorsal side. Fifth and sixth podomeres (fourth and fifth in instar A-3) each with an additional long seta at the apex. Final podomere unchanged.

An2 with one additional seta on the ventral margin of the protopodite. First podomere of endopodite with the aesthetascs *Y* situated further towards the protopodite than before and with four long natatory setae protruding from the inner-dorsal margin (three in instar A-3). Additional seta two-thirds down dorsal edge of the second podomere and opposite edge now with two *t*-setae; apical chaetotaxy of this second podomere as in instar A-3, except for the addition of a *z1* seta on the outer face, of approximately the same length as the seta *z2*. Chaetotaxy of third podomere unchanged.

First Md endopodal podomere (second palp segment) with an additional seta with the $\beta$-group (total of five). Second endopodal podomere (third palp podomere) with two additional setae near ventro-apical corner (total of three), one additional seta on inner face, near dorso-apical corner. Terminal podomere with six claw-like setae as opposed to four in instar A-3, due to the addition of two setae in a median position. Mandibular branchial plates and mandibular coxae similar to those in instar A-3.

Rake-like organs similar to those of instar A-3, but larger.

Mx similar to those of instar A-3, but endopodite (palp) with two additional setae on top of the first podomere; second podomere apically with the same number of setae, but now with three long and two shorter setae. Third endite with an additional seta on the inner edge, close to the two, stout *Zahnborsten*. First endite now with approximately 12 terminal setae. Second endite unchanged. Branchial plates larger than before, with 15 setae protruding from the posterior edge and with one additional reflexed seta on the anterior edge.

L5 further developed in this instar, with endopodite now unsegmented. Branchial plate on protopodite with three long and one shorter setae. Protopodite with two small setae, *a* and *b*; endite with four additional long setae, forming a group of seven, two inner-most of which are distally hirsute. Proximal to this group (on the inner side) group of three, medium-length setae and one isolated, curved seta (*d*).

L6 now with five podomeres (third podomere in instar A-3 divided into two quadrate segments). Additional seta (*d1*) on the protopodite and another additional shorter seta (*d2*) on the first podomere of the endopodite. Second podomere with an additional seta on the ventral apical corner. Third podomere with an additional hirsute seta on the ventro-apical corner and a smaller seta in a medio-apical position. Fourth podomere unchanged.

L7 developed into a cleaning limb, consisting of three, elongate podomeres. First podomere with a seta protruding from the apical region. Final podomere capped in a fully developed cleaning pincer as seen in the adults.

CR more elongate than in instar A-3. Distal claw (*Ga*) longer than the proximal claw (*Gp*) in this instar (reversed situation in instar A-3). CR attachment bifurcates distally.

*Eighth instar (A-1)* (Figures 2, 15b–d, 16 and 17)

Carapace length 1195–1244 $\mu$m; height 731–793 $\mu$m. Compared with instar A-2, the posterior part of carapace is more rounded, marginal setae more numerous, pustules more pronounced and muscle scars more distinct.

An1 with two additional setae on the ventro-apical and ventro-dorsal corners of the third podomere, which is now more rectangular. One additional long seta and one much shorter seta also appearing on the apex of the fourth podomere. One long seta appearing on the apex of the fifth podomere. Four long setae on the apex of the sixth podomere joined by an additional medium-length seta. Terminal podomere unchanged.

An2 larger than in instar A-2. Aesthetasc *Y* again shifted position closer to the protopodite. Additional natatory seta on the inner face of the first podomere of the endopodite. Second podomere with setae *t3* and *z3* appearing, as well as another seta (total of two) opposite the *t*-setae. Terminal podomere unchanged.

Md with an additional long seta (now total of three) on the outer edge of the second palp-segment and one seta in $\beta$ group now hirsute. Third palp-segment with an additional apical seta, forming a group of two setae on the inner side. Final podomere with the short seta (added in instar A-2) now proportionally longer. Branchial plates unchanged. Coxal endites with one small additional tooth on the inner side.

Rake-like organs larger than in instar A-2, but otherwise unchanged.

Endopodite (palp) of Mx with an extra seta on the outer edge of the first podomere. Second podomere of endopodite with one extra seta on the outer edge. Apical setae on third endite increased to 10, with three additional setae (two smooth, one hirsute) positioned anteriorly of the *Zahnborsten*. First and second endites with additional terminal setae, approximately 13 and 8, respectively. Branchial plates more developed, with a more semicircular shape, bearing 21 setae progressively getting shorter towards the anterior.

L5 protopodite now with two *a*-setae and with addition of *c* seta. Endite apically with one large group of setae as opposed to the two smaller groups of setae seen in instar A-2, comprising 14 setae in this instar (10 in instar A-2), five of which are hirsute. Branchial plates more developed, with five hirsute setae, longer than before.

L6 larger than in instar A-2, but mostly unchanged, apart from seta *d1* being proportionally longer, now approximately three times the length of seta *d2*.

First podomere of L7 with two extra setae (total of three). Second podomere with an extra apical seta. Third podomere with an additional medio-ventral seta, but apical chaetotaxy (pincer) as in instar A-2.

CR larger than in instar A-2. Terminal seta *Sa* proportionally longer. CR attachment also very similar to that in instar A-2.

*Ninth instar; the Adult (A)* (Figures 2, 15e, f, 18 and 19 – also see Smith & Martens, 1996)

Carapace length 1500–1671 $\mu$m; height 914–1024 $\mu$m. Carapace elliptical, with well developed pustules on the anterior region. Ventral margin sinuously curved in the anterior third. Carapace in dorsal view anteriorly more pointed than posteriorly.

An1 similar to that in instar A-1, but with last five podomeres slightly more elongate and the shorter apical seta on the sixth podomere is proportionally longer.

An2 with aesthetascs *Y* nearer protopodite than in instar A-1. Final (short) natatory setae appearing dorsally on the inner face of first podomere. Second podomere with additional seta *t4*; apically with three longer *z* setae and another large claw developed from the stout seta *G2* (seen in instar A-1), joining the two other claws on this podomere. Third podomere also with another claw developed from a stout seta *Gm* (in instar A-1), but this claw only half the length of its accompanying claw.

Md palp third segment with one additional seta joining the $\gamma$ group and one additional seta appearing in the subapical group of setae on the ventral edge (total of four). Three apical claws on terminal podomere more strongly developed than in instar A-1. Branchial plates and coxae larger, but otherwise unchanged.

Rake-like organs larger than in instar A-1, but otherwise unchanged.

Mx endopodite with an extra apical seta on the first podomere, forming a group of five. Third endite with two additional short setae anterior to the *Zahnborsten*. Branchial plates large and now with 19 setae extending from the most posterior point, along the ventral edge to the anterior.

L5 unchanged in size and chaetotaxy, except two setae on the endite of the protopodite now being hirsute (smooth in instar A-1).

L6 larger than in instar A-1, otherwise unchanged.

L7 larger than with instar A-1 and with proportionally smaller *pz2* seta at the tip of the third podomere.

CR more elongate than in instar A-1. CR attachment unchanged.

## Remark

Through the ontogeny of the last two instars, the posterior part of the animal becomes proportionally longer and wider, as the sexual organs develop. As a result of this, there is a relatively larger gap between the L7s and the base of the CR.

## Discussion

*Ontogeny of non-marine ostracods*

For the Cyprididae, the following ontogenetic sequence has previously been recognized (Kesling, 1951; Ghetti, 1970; Roessler, 1983) (Table 1). The first instar, sometimes termed the nauplius stage (A-8), is already entirely enclosed within a bivalved carapace. The valves are not calcified and are flexible. The

ostracod at this stage has relatively well-developed An1s and An2s, as well as the *Anlagen* of the Mds. The eye is also developed and the upper lip is recognizable. The second instar (A-7) has a lightly calcified carapace. In addition to the well formed An1s and An2s, the Mds are fully developed with an endopodite and basal coxa and the *Anlagen* of the Mxs and the CR also appear. Instar A-6 is marked by the appearance of recognizable Mxs. The fourth instar (A-5) is characterized by the appearance of the *Anlagen* of the L5s. In addition to the well developed An1s and An2s, the Mds and Mxs are almost fully formed. In the fifth instar (A-4), the L5s take on the appearance of walking legs and the *Anlagen* of the L6s develop. In the sixth instar (A-3), the L5s have changed from walking legs to maxillipeds. The L6s have become fully developed walking legs. The *Anlagen* of the L7s have also appeared. The seventh and eighth instars (A-2 and A-1) have all appendages present in a recognizable form, apart from the sexual organs. Additional setae and size are the only features that change within the morphology of the limbs. Other changes are related to the size and shape of the carapace.

The basic ontogeny of *E. virens* is similar to that of other cypridiidine ostracods (Claus, 1868; Müller, 1894; Schreiber, 1922; Kesling, 1951; Fox, 1964; Ghetti, 1970; Roessler, 1983). Some of the more notable ontogenetic differences between *Eucypris virens* and *Heterocypris bogotensis* (see Roessler, 1983) and *Cypridopsis vidua* (see Kesling, 1951) are as follows:

1. Roessler (1983) identified a more complex Md *Anlage* for the first instar (A-8) of *Heterocypris bogotensis* than this study revealed for *Eucypris virens*, although the pediform nature of this limb in both species is very similar.

2. Roessler interpreted very small, rounded projections of the body in instars A-8, A-6 and A-5 as the first occurrences of the *Anlagen* of the Mx, L5 and L6, respectively. We have been unable to locate such *Anlagen* in these instars, in spite of thorough investigations using both critical point dried specimens with S.E.M. and undissected specimens with light microscopy.

3. Kesling (1951) stated that in the third instar (A-6) the natatory setae of the An2 appear in abbreviated length and in the fourth instar there are three natatory setae. It is not clear which setae on the An2 he means and he could be referring to the long seta of the exopodite, as well as the long setae on the protopodite and one on the endopodite.

4. Kesling (1951) stated that, in instar A-6, there are six setae on the exopodite plates of the Mx in *C. vidua*. *E. virens* has only four setae on this plate at this stage. Unfortunately, Kesling (1951) does not mention the number of setae on this plate for the other juveniles, but the adult form has 20 setae along the ventral edge with five reflexed setae at the anterior edge. *Eucypris virens* has 19 setae on the ventral edge and just three reflexed setae. Note, however, that these setae (rays) are weakly sclerified and that it is easy to miscount them. Furthermore, the number of respiratory rays on this plate is also known to vary intra-specifically.

5. In instars A-5 and A-4, Kesling noted that *C. vidua* has An1s consisting of five podomeres. *Eucypris virens* has only four podomeres in the An1s in instar A-5, but five podomeres in instar A-4. However, in instar A-3, both *C. vidua* and *E. virens* have six podomeres. *Cypridopsis vidua* is then noted by Kesling (1951) to have six podomeres in instar A-2, whereas *E. virens* has seven, the same number as seen in the adult. Presumably *C. vidua* has seven podomeres in its An1s at instar A-1, as Kesling (1951) stated that all limbs are in their definitive (adult) form at this stage.

*The development of the An1*

Although the chaetotaxy of *E. virens* shows continued development through ontogeny in most limbs (i.e. acquiring more setae and/or claws at each moult), this is not the case for the final podomere of the An1s, which has a fully developed chaetotaxy in the first instar (Figure 20). The chaetotaxy of the final podomere consists of two long, and one shorter setae and the aesthetascs *ya* from the first instar onwards and this pattern remains unchanged through the entire ontogeny. This is the only exception to the rule of continued development throughout ontogeny.

The podomeres of the An1s do not originate in one place, but proliferation of the number of segments occurs through splitting of extant segments (Figure 20). Hence, what was previously called 'fused' segment is actually most likely an incompletely differentiated podomere.

Additionally, podomeres do not necessarily originate along a distal-proximal gradient (or vice versa), for example podomere 3 is formed before 6 and 7 are split, the latter differentiation occurs before 4 and 5 split etc. (Figure 20). Finally, the pattern may become even more obscured by secondary fusion of previously differentiated podomeres, e.g. during the moult from A-6 to A-5, podomere 2 secondarily fuses with podomere 1. Such a mosaic of apparently inconsequential differentiation and fusion events could indicate that the

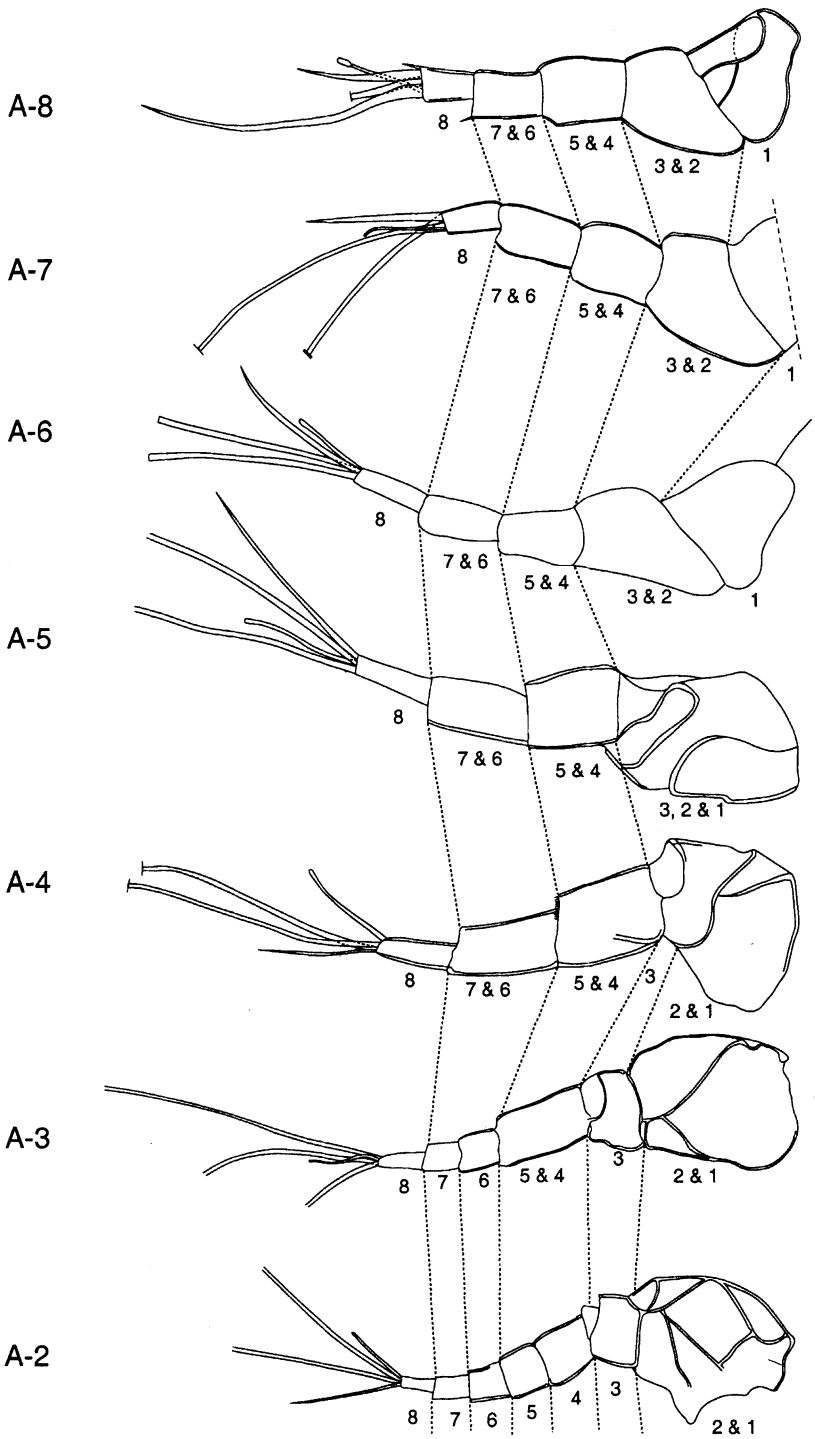


*Figure 20.* The development of the An1 of *Eucypris virens* during ontogeny. (Not drawn to scale).

present post embryonic ontogeny of the Cypridoidea, which conservatively consist of 9 instars (including the adult stage) is actually a compressed version of a previously more extensive ontogeny involving more instars. For example, the first instar (nauplius) has well developed An1s and An2s and *Anlagen* of the Mds. As in the remainder of the ontogeny, generally one pair of limbs appears as *Anlage* per instar, this could

mean that this stage is a compressed version of at least three instars, of which the prenauplius stage (Roessler, 1982a, b) could be a remnant. In this particular case, such a compression could be due to the fact that the first juvenile stage needs at least those three pairs of limbs to be competitively viable as a free-swimming organism. No new appendages are formed in the last 3 instars, during which the final differentiation of podomeres takes place and the reproductive organs are formed. However, it is not impossible that previously more instars occurred, but that these disappeared and that ontogeny was further compacted.

*An2 chaetotaxy*

During the moult from A-8 to A-7, the claw *g* on the terminal podomere of the An2 is transformed into a seta, and this seta remains present all through the life of the ostracod, while other claws develop later on. The loss of a claw on the An2 from instar A-8 to A-7 may indicate that the ancestors of this group had an additional claw in the adult form, when compared to extant adult forms. This discovery is of further importance when sexual dimorphism in distal An2 chaetotaxy is taken into account. Martens (1987) showed that the difference in chaetotaxy in the male, when compared to females, occurs during the final moult and involves only claws and setae on the subapical segment, i.e. *z*-setae, etc. These alterations are, therefore, newly formed differences (apomorphic) and not recurrences of plesiomorphic conditions, as seta *g* on the terminal segment, for example, is not re-instated as a claw.

The long setae on the An2 (one on the exopodite and one on the protopodite) act as natatory setae in the early juveniles before the main natatory setae on the endopodite develop. In early juveniles, these setae are proportionately much longer than seen in later instars. As the natatory setae on the endopodite do not start to develop until instar A-5, these setae have a significant function in swimming. As the main natatory setae develop, they take over this function and the setae on the endopodite become proportionately shorter.

Additionally, the An2 natatory setae can be used to discriminate late instars. It is relatively easy to determine the developmental stage up to instar A-3, by looking at presence or absence of appendages and their *Anlagen*. However, in the last three instars, all limbs are present and more or less fully formed. Fox (1964) noticed that the An2 natatory setae can be used to determine these late instars, and this is corroborated by the ontogeny of *E. virens*. Adult specimens of *E. virens* have five long and one short natatory setae, instar A-1 has five long setae; instar A-2 has four long setae; instar A-3 has three long setae; instar A-4 has two long setae and instar A-5 has one long seta. They are relatively large, easy to find and have been noted to change in a homologous way in other cypridid species (Fox, 1964; Ghetti 1970), including *C. vidua* (R. Smith, pers. obs.). This ontogenetic model is also valid for cypridid species in which all natatory setae are short (e.g. *Psychrodromus*, *Herpetocypris reptans*, etc.).

*Aesthetascs*

The only aesthetascs present in the first instar are *ya* on the An1s and *y3* on the An2s. The main aesthetascs *Y* on the first endopodal segment of the An2s first appears in the first instar as a seta on the ventro-apical corner. Later on in the ontogeny, it develops first into a two-segmented aesthetasc (instar A-6), then into a three-segmented aesthetasc (instar A-5). As this development occurs, the aesthetasc shifts its position from the apical corner to a site about 2/3 along the ventral edge of the segment in the adults. This shows that the single aesthetasc *Y* in the Cyprididae, and maybe in the whole of the Cypridoidea, originates from an apical seta on the segment, and is not homologous with any of the aesthetascs from the proximal cluster (clump *Ac*), reported from other Podocopine groups, such as the Macrocypridoidea, *Saipanetta* (Sigilloidea), *Darwinula* (Darwinulocopina), etc. (see Rossetti & Martens, 1996, for a brief review). Note that during the ontogeny of *Darwinula stevensoni* clump *Ac* originates from the base of the podomere (Scheerer-Ostermeyer, 1940) and not from apical setae. It should now be checked if the single aesthetasc *Y* in the Pontocypridoidea is homologous with the aesthetasc *Y* in Cypridoidea (i.e. derived from an apical seta) or with the clump *Ac* in Macrocypridoidea (as was previously assumed for the Cypridoidea). As both types of structures are not homologous and if the clump *Ac* in Macrocypridoidea is homologous with that in Darwinulocopina and Sigilloidea, then the position of the former group in the Cypridocopina (together with Pontocypridoidea and Cypridoidea) becomes less certain.

Aesthetascs *y2* and *y1* on the An2s first occur in instars A-5 and A-3, respectively. Therefore, the presence of the aesthetascs *ya* (on An1s) and *y3* (on An2s) in instar A-8 probably highlights the functional importance of these two aesthetascs, which are fully formed in the earliest stage. The first two instars of *Cypridopsis vidua* have been noted to spend more time

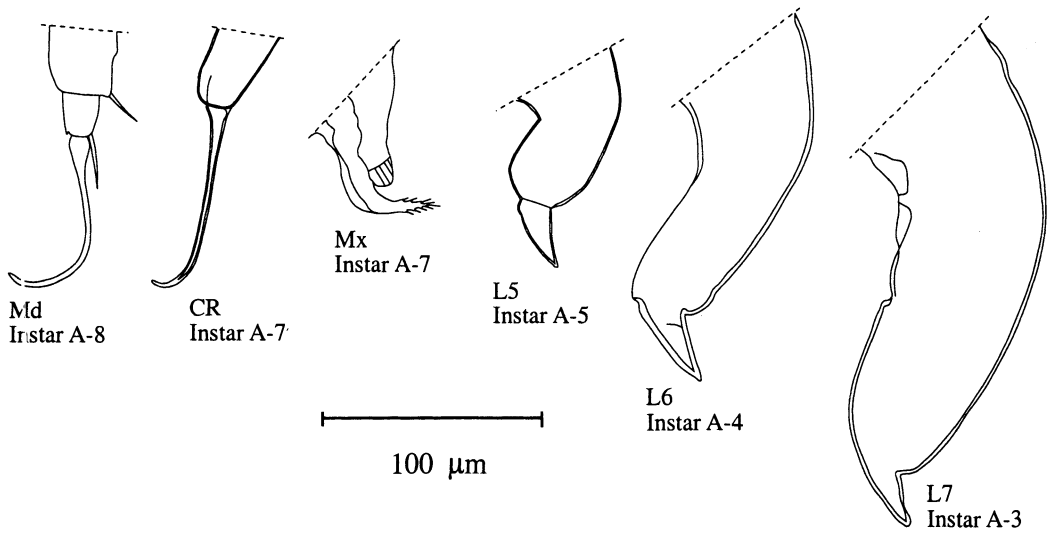


*Figure 21.* Anlagen of the limbs of *Eucypris virens*. Md (OS 15133); CR (OS 15137); Mx (OS 15137); L5 (OS 15141); L6 (OS 15143); L7 (OS 15146).

swimming than later instars (Kesling, 1951), and this is also true for *Eucypris virens* (R. Smith, pers. obs.). Furthermore, the first instars of cypridid ostracods still carry substantial amounts of yolk and supposedly do not feed (Schreiber, 1922; Martens et al., 1985). As the rake-like organs occur in the third instar (A-6), this is most likely when the animal starts feeding. The development of aesthetascs *ya* and *y3* in the earliest instars is thus probably related to a free-swimming life style. The other aesthetascs develop as the ostracod changes to a more benthic life style and starts feeding.

*A posterior pediform limb is present in all instars*

The pediform nature of the *Anlage* of the Md in the first instar indicates that it is not used for feeding, but rather as a limb for walking on (or attachment to) a substrate. Schreiber (1922) cultured the first instars of *Heterocypris incongruens* without any food and they moulted into the next instar, but without putting on additional weight. This indeed suggests that the first instars feed on stored yolk, leaving the *Anlagen* of the Mds available for other functions. In the second instar, the *Anlage* of the CR is similar in appearance to that of the pediform Md in the first instar (Figure 21) and this points towards a similar function. As the Md in the second instar is a fully developed feeding appendage, the *Anlagen* of the CR may assume the former function i.e. the attachment to substrates. The CR remains in this *Anlage* state up to the fifth instar, where it is transformed to a state resembling that of the adult furca. This happens as the L5s develop into walking legs and can presumably take over the function of the *Anlage* furca. The L5s do not transform into feeding appendages until the L6s have developed into walking legs and hence, takes over the L5s original function. This may indicate the importance of having an appendage in the posterior region of the animal, with a long claw, that can be used, possibly in conjunction with the An2s, for walking and attachment to substrates. Kesling (1951) reached a similar conclusion for *Cypridopsis vidua*, a species with a reduced CR in the adults. The development of the CR in this species is very similar to that in *Eucypris virens* up to the fifth instar i.e. an *Anlage* with a strongly curved terminal claw. After this, the CR, instead of developing further into an elongate structure, reduces to its flagelliform adult shape. This could again be related to the appearance of the L5s (pediform in the fifth instar), which then assumes the CRs previous function.

*The L5 of the Podocopine ostracods is thoracic in origin*

There has been much debate as to whether the L5s are either of thoracic (Kesling, 1951; Fox, 1964; Athersuch et al., 1989) or of cephalic origin (Hartmann, 1977; Maddocks, 1982). The situation is highly confused, not in the least because morphological terminology, with attendant implications for the origins of the limbs, has been indiscriminately applied to the L5s. Most confusion stems from the fact that the limb has a very different morphology (and function) in different podocopine groups. In Bairdiocopina and

Cytherocopina, the L5s, L6s and L7s are walking limbs in both males and females, and researchers of marine ostracods (where these groups are most common) mostly consider these appendages as three pairs of thoracic appendages. Martens (1990) furthermore showed for the genus *Limnocythere* (belonging to the most important non-marine cytherid radiation), that an elongated skeletal structure separates the Mxs and the L5s. It consists of a ventral, rectangular plate on which two lateral arms, running in dorsal direction, are inserted. Tendons connecting with the Mds and the Mxs are inserted on the arms. Kaufmann (1896) named this structure 'sternum' in *Leucocythere* and Schultz (1976) illustrated it for a large number of different ostracod taxa. None of these authors further elaborated on the significance of this structure. However, if this separation agrees, for example, with the division between head and thorax, then the L5s in this group are indeed the first thoracic limbs. Furthermore, the last three limbs of ostracods are all connected by the chitinous endoskeleton within the thorax (Athersuch et al., 1989).

In most Cypridoidea, comprising the majority of non-marine ostracods, however, the L5s are modified into feeding appendages, which in the males, are also used as clasping organs during copulation. Most researchers working on non-marine ostracods thus consider the L5s as maxillae, referring to the first pair of maxillae as maxillulae. The latter opinion seemed to be corroborated by the position of cephalic glands. Some groups of crustaceans are known to have two segmental, excretatory glands, associated with the An2 (antennal gland) and with the second Mx (maxillary gland). Certain ostracods have similar glands associated with the An2 and the L5 (Cannon, 1925; Hartmann, 1977). It is, therefore, argued that if these glands are homologous with other crustacean groups, the L5 must be cephalic and represents the second maxilla. Bergold (1910) was the first to name the latter gland in ostracods the maxillary gland. However, as Kesling (1951) first pointed out, the sole reason for doing so is that the orifice of the gland is located immediately behind the Mx. Cannon (1925) also called this gland the maxillary gland, but considered it to be associated with the L5 (second maxilla of Cannon), not the Mx. This confusion is due to ostracods having no segmental boundaries, thus to which limb the maxillary gland is associated with is open to interpretation.

The only remaining confusion thus originates from the shape and function of the L5s in Cypridoidea. During ontogeny of *E. virens*, the L5s change from walking legs in A-4, to feeding appendages in A-3. In the walking leg state, they have a morphology similar to that of the L6s walking legs that appears in A-3: it has the same number of segments, which furthermore have a similar shape and a very similar terminal claw (Figure 22). Fox (1964) documented a similar development for the L5 for *Herpetocypris chevreuxi*.

The *Anlagen* of the L5s, when appearing in the fourth instar (A-5), consist of a posteriorly directed, elongate base or sheath, terminated by a small triangular segment. This is very similar to the morphology of the *Anlagen* of L6s and L7s as they appear in instars five (A-4) and six (A-3), respectively (Figure 21). The *Anlagen* of the Mxs, however, as seen in the second instar (A-7), have a very different morphology. Ontogeny thus shows, together with a reassessment of arguments based on adult anatomy, that the L5 of the Cyprididae has a thoracic and not a cephalic origin.

Additionally, Meisch (1996) demonstrated that the L5 of the Macrocypridoidea also is thoracic in origin. This highlights that two superfamilies within the infraorder Cypridocopina (Macrocypridoidea and Cypridoidea) as well as the infraorder Cytherocopina have only four cephalic appendages and strongly supports the hypothesis that all podocopes have a thoracic origin for the L5.

This immediately implies that Ostracoda only have one pair of Mx, which has important phylogenetic consequences, as generally, the Crustacea have five pairs of cephalic appendages. This creates a number of problems. Firstly, it is not known if ostracods had two pairs of Mx during their early history and lost one pair of Mx later on. A comparison to the ontogeny in other ostracod lineages, i.e. the Myodocopida, could contribute to resolving this problem. Secondly, it is not immediately clear which pair of Mx is now absent: the first or the second. If we accept that the appearance of limbs during post embryonic ontogeny occurs uniformly and sequentially in a caudal direction, then the fact that no *Anlage* of limbs appear in instar A-6 (while the extant Mx appear in A-7, the L5s in A-5), most probably indicates that it is the second pair of Mx which is missing. Note that in view of the previously hypothesized reduction of the number of instars, the persistence of instar A-6, in which no limb is added, is puzzling. This could indicate that the loss of the Mx is indeed a relatively recent event in Podocopine history.

Finally, does the presence of only four pairs of cephalic appendages mean that (podocopine) Ostracoda do not belong in the Crustacea s.str.? Although a partial re-definition of this group might be necessary, allowing for more morphological flexib-

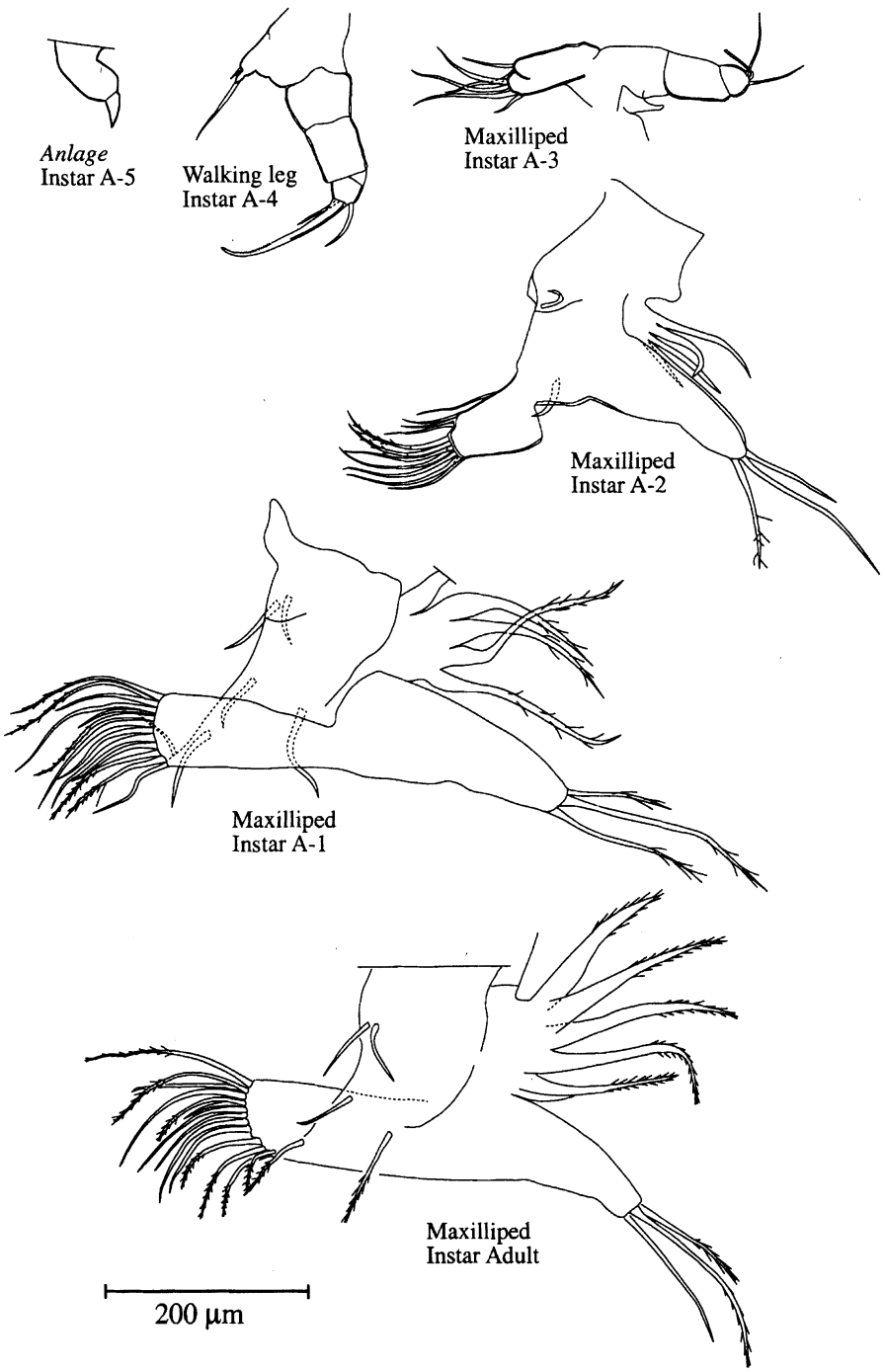


*Figure 22.* Development of L5 in *Eucypris virens* Anlage A-5 (OS 15141); Walking leg A-4 (OS 15144); Maxilliped A-3 (OS 15146); Maxilliped A-2 (OS 15148); Maxilliped A-1 (OS 15150); Maxilliped Adult (OC 2003).

ility, the loss of one pair of cephalic appendages during history should not be regarded as a fundamentally different body plan, only as a modification of an existing one. Other crustacean groups (e.g. Notostraca, Anostraca, Conchostraca and Cladocera) contain some species which have vestigal, reduced or absent Mx (McLauglin, 1982). Note, moreover, that several thoracic appendages and almost all abdominal appendages have disappeared in the Ostracoda, most likely to make the body fit within the enclosure of two interlocking valves. From a functional point of view, the development of a calcified carapace indeed imposes severe spatial constraints. It would thus have been more surprising if none of the five cephalic appendages had disappeared. Gould (1989), in his reinterpretation of the fauna of the Burgess Shale, did consider this type of modification as a fundamentally different body plan, thus arriving at the concept of the Cambrian explosion of variety in life-forms. Later work (for example see Briggs et al., 1993), however, has shown that most of Gould's so-called new (and extinct) body plans are little more than small modifications of existing ones. The present argument, that extant podocopine ostracods have only four pair of cephalic appendages, corroborates the latter view.

## Conclusions

The following conclusions can be formulated. 1. The basic ontogenetic development of the limbs of *Eucypris virens* is similar to that of other Cyprididae species. 2. Chaetotaxy shows continuous development on all podomeres during ontogeny with the exception of the last podomere on the An1. 3. The long setae on the exopodite and protopodite of the An2 function as natatory setae in early instars. This function is taken over by the main natatory setae on the endopodite in later instars. Other aspects of interest in chaetotaxy of the An2 deal with the presence of an additional claw *g* in early instars, present as a short seta in later instars and the adults. 4. Aesthetascs *ya* and *y3* are the first to develop (in early, non-feeding, free-swimming) instars, while aesthetascs *Y*, *y1* and *y2* form in later instars, which feed and have a primarily benthic mode of life. An2 aesthetasc *Y* originates as a distal seta in early instars, and later on in ontogeny assumes a different morphology and position. This aesthetasc is, therefore, probably not homologous to the clump of aesthetascs (*Ac*) in some other podocopine groups. 5. The L5 in Ostracoda is thoracic in origin; this group does not have a second Mx and thus has only four pairs of cephalic appendages.

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# Ontogenetic changes in the carapace shape of the non-marine ostracod *Eucypris virens* (Jurine)

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*Key words:* shape, size, ontogeny, outline analysis, non-marine Ostracoda

## Abstract

Developmental changes in carapace form (size+shape) during ontogeny have been explored in *Eucypris virens* (Crustacea, Ostracoda) using elliptic Fourier analysis. Clones from different geographic localities raised under controlled constant conditions (temperature and photoperiod) were used to characterize developmental pathways in the species. A larger data set including field populations and laboratory populations cultured under a range of environmental conditions were used to infer influence of environmental factors on carapace shape changes during ontogeny. Size changes between consecutive juvenile stages support empirical laws describing the doubling of ostracod volume at each moult. Ontogenetic changes point out the remarkable influence of environmental conditions on carapace shape.

## Introduction

The vast array of organisms in nature display a wide diversity of life-history tactics which evolved from the tension between adaptation and constraint (Stearns, 1992). Evolution of life-histories must be studied using the comparative approach (Harvey & Pagel, 1991). However, if life-history traits and ontogeny are tied to some extent, as some theories predict (Gould, 1977), we could also follow evolution of life-histories by tracking changes during development (Schweitzer et al., 1986). Because morphological changes during ontogeny can be preserved in the fossil record, to use the ontogenetic approach has some advantages, namely it allows the direct exploration of life-history evolution on a geological scale.

Obviously not all groups of organisms are suitable for such an approach but ostracods (Crustacea, Ostracoda) certainly are. First, Ostracoda have an extensive fossil record from the Ordovician to the Recent. Second, they inhabit a wide range of habitats (from saline to freshwater habitats and from high mountain lakes to the deep-sea), covering a large spectrum of environmental conditions and, hence, are subjected to a wide range of selective pressures. Third, like other crustaceans, ostracods grow by ecdysis, but their growth is determinate (i.e. there is no growth after maturation) and the number of moults is fixed so each carapace can be assigned to a certain ontogenetic stage. That means that the ontogeny of different individuals can be compared on the basis of homologous stages. However, apart from frequent studies using carapace length–height measurements (e.g. Kesling, 1951; Anderson, 1964; Sandberg, 1964), there are few morphometric studies dealing with the ontogenetic changes in the carapace shape of ostracod species, most of them referring to marine species (e.g. Schweitzer et al., 1986; Maness & Kaesler, 1987; Schweitzer & Lohmann, 1990). Most remarkable is the work of Hounsome (1975) who explored in a quantitative way ontogenetic changes in the carapace shape of the non-marine ostracod *Eucypris virens*. Here we also search for growth patterns in *E. virens*, but whereas Hounsome's approach was in the realm of traditional morphometrics (Hounsome, 1975), we will be within the framework of the so-called 'new morphometrics' (Rohlf & Marcus, 1993).

*Eucypris virens* (Jurine, 1820) is a nonmarine species with a wide distribution which has received significant attention concerning its taxonomy (Smith &

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Martens, 1996), karyotype – the chromosome complement of an individual – (Tétart, 1978), morphology (Tétart, 1982) and life-history (Otero et al., 1998), making it the standard for studies among the Cypridoidea.

Our aim, therefore, is twofold:

1. to describe developmental pathways in terms of changes in size and shape, and
2. to address ontogenetic variability both at the individual and the population level in this species.

## Material and methods

*Eucypris virens* is a species which mainly lives in lentic waters (pools, ponds, marshes, swamps, etc.) although it is also known to occur in running waters (Bronstein, 1947), marsh springs (Wendling & Scharf, 1992), hyporheic habitats (Gagneur & Chaoui-Boudghane, 1991) and even in hot-water springs (Gülen, 1985). It prefers temporary or semipermanent waters with salinity ranges from 0 to 5 g $l^{-1}$ (De Deckker, 1983; Carbonel et al., 1988). Nüchterlein (1969) defined it as a thermoeuryplastic species. Lifespan is about 8–10 weeks (Griffiths & Evans, 1991). Populations of *E. virens* develop on muddy or sandy substrates but seem to prefer vegetated areas when available: *Eleocharis* (Benzie, 1989), *Phragmites* beds (Martens & Dumont, 1984; Griffiths & Evans, 1991) and charophytes (our observations). Population density is low (13 ind $m^{-2}$, in Benzie, 1989; 200 ind $m^{-2}$, in Baltanás, 1994) when compared to other ostracod species (e.g. *Cyprideis torosa* in Heip, 1976; *Heterocypris carolinensis* in McLay, 1978).

### *Material*

The material used in the present study was obtained from laboratory cultures (Sets A & B) and field populations (Set C) (see Table 1 for locations):

*Set A*

Four different all-female European populations of *Eucypris virens* were sampled and laboratory clones from individual females were obtained. Genotypes for the different clones were assessed using starch gel electrophoresis. One clone from each population was selected and reared at constant photoperiod (6:18 LD) and temperature (16 °C). From each clone, 12 neonates were isolated and reared until death. Animals in the laboratory were reared isolated in separate wells of tissue culture plates with fragments of the mat-forming alga *Tolypothrix tenuis* supplied as food. Carapace moults of isolated individuals were kept and preserved in 70% alcohol for subsequent recording of outlines.

*Set B*

*Eucypris virens* at different development stages, collected from five different field sites were kept isolated in the lab and followed until death. Culture protocol was similar to 'Set A' but with a wider range of environmental conditions (culture plates were kept at a constant temperature of 4 °C, 12 °C or 18 °C). Moults were kept and preserved for outline analysis.

*Set C*

Three temporary ponds in an area near Madrid (Spain) (distance between ponds less than 1 km) were sampled for *E. virens* using a Mondsee-corer (28.5 sq cm) at several dates (November 96 through March 97). One of these ponds provide specimens for experiment set A (see Table 1). After collection animals were preserved in ethanol (70%).

Given the bilateral asymmetry in valve size and shape that is characteristic of most ostracods, only right valves were used for further analysis. Valve outlines were recorded with a standard video based system (CCD video camera 600 l.h.r.; PCVision-Plus frame grabber and *Morphosys* software) as chain codes of 800–2000 points. Chain codes were decoded to *x,y*-coordinates and the system origin was placed at the centroid (centre of gravity) of each valve. The area enclosed by the outline was calculated.

### *Size*

Ostracod size is commonly measured as carapace length. This is done by orientating the valves with their ventral margins resting on a base line and length is measured as the longest horizontal axis cutting the valve outline at two points (Farkas, 1974). However, there is not an objectively-defined 'unique' base line, especially when small juvenile stages are involved, so different observers are likely to vary in their measurements. An alternative, which is neither dependent on reference points of the valve nor on the subjectivity of researchers, is outline area. Valve area can be easily recorded using image analysis techniques which are widely available nowadays (Rohlf, 1990); or simply by drawing the carapace outline (using a *camera lucida*) onto millimeter paper and estimating the corresponding surface.

*Table 1.* Location of sampling sites of *E. virens*. Last column includes both number of specimens used for outline analysis and to which experimental set they belong

| Site | Locality | Country | Latitude | Longitude | N (Set) |
|---|---|---|---|---|---|
| Berzosa-1 | Hoyo de Manzanares (Madrid) | Spain | 40° 37′ N | 3° 56′ W | 11 (C) |
| Berzosa-N | Hoyo de Manzanares (Madrid) | Spain | 40° 37′ N | 3° 55′ W | 65 (A)/43 (C) |
| Berzosa-GL | Hoyo de Manzanares (Madrid) | Spain | 40° 37′ N | 3° 56′ W | 53 (C) |
| Morcuera | Miraflores de la Sierra (Madrid) | Spain | 40° 48′ N | 3° 45′ W | 108 (B) |
| Murone | Vercelli (Piamonte) | Italy | 45° 16′ N | 8° 11′ E | 82 (A) |
| Ventina | Terni (Umbria) | Italy | 42° 30′ N | 12° 40′ E | 80 (A) |
| Colorno | Parma (Emilia Romagna) | Italy | 44° 56′ N | 10° 22′ E | 24 (B) |
| Bigliana | Reggio Emilia (Emilia Romagna) | Italy | 44° 50′ N | 10° 31′ E | 31 (B) |
| Capraia | Grosseto (Toscana) | Italy | 43° 02′ N | 9° 50′ E | 3 (B) |
| Bramhope | Leeds (W. Yorkshire) | U.K. | 53° 50′ N | 1° 35′ W | 86 (A)/79 (B) |

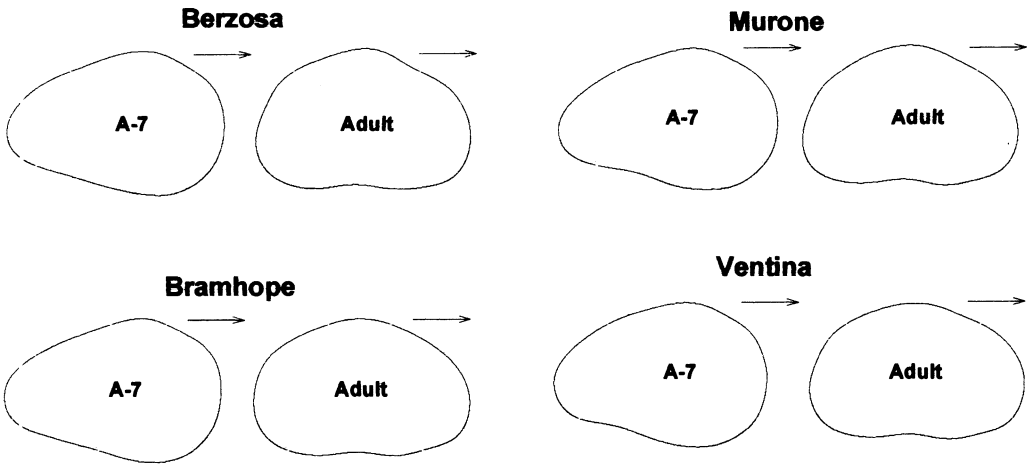


*Figure 1.* Outlines (standardized for size) for the second juvenile instar (A-7) and the adult of *E. virens* in four clonal cultures from different European localities.

Most size measurements in the ostracod literature are linear dimensions (length, height and width), so we scaled size to a linear dimension, the square root of outline area ($L'$=Area$^{1/2}$). Growth during ontogeny was evaluated by fitting such a linear dimension ($L'$) to a juvenile stage ($S$) using linear regression analysis (least squares). Although there exists a huge number of growth equations to describe change in size (Gompertz, Von Bertalanffy, logistic, Weibull, etc.), here we used the exponential model:

$$L' = ae^{bS},$$

where $a$ and $b$ are parameters of the model, because it provides a coefficient of proportionality between consecutive juvenile stages ($e^b$). Additionally, the exponential model has been also previously used to describe growth in *E. virens* (Hounsome, 1975) and several other ostracod species (Kesling, 1951; Szczechura, 1971; Farkas, 1974; Marín, 1984) making comparisons possible.

*Shape*

A numerical representation of the shape of outlines was achieved by performing an elliptic Fourier analysis (Kuhl & Giardina, 1982; Rohlf & Archie, 1984) on recorded $x,y$-coordinates. Ten harmonics – each of the orthogonal Fourier components into which a wave form is decomposed – were obtained for each outline because, in another extensive study on shape variability in adult *E. virens* carapaces (Baltanás et al., in prep.), we found that this number of harmonics described valve outlines very well. The elliptic Fourier coefficients were mathematically normalised to be in-

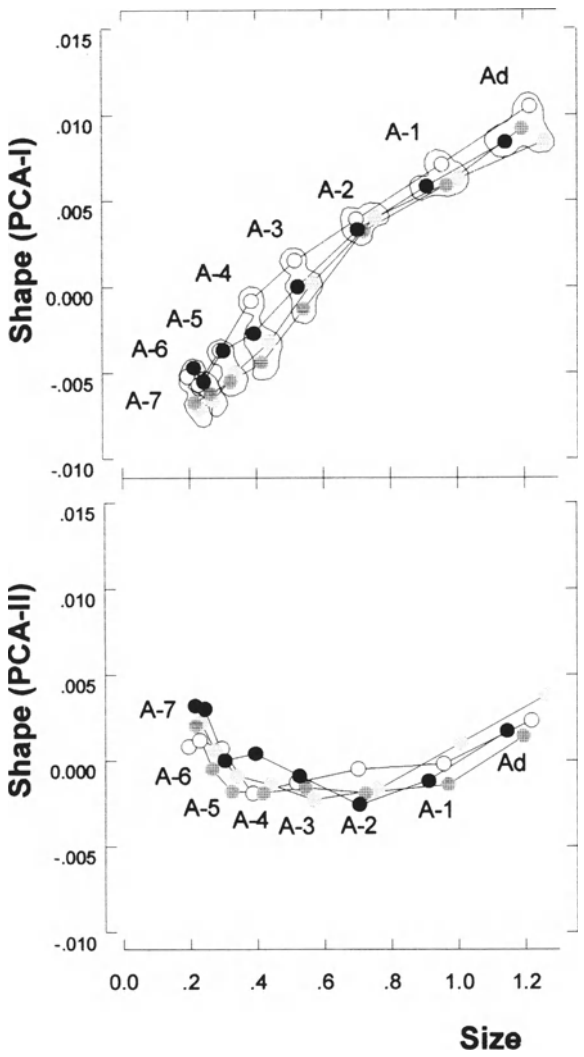


*Figure 2.* PCA scores for average shapes of each ontogenetic stage and clone of *E. virens*. Colour codes: white=Berzosa; light grey=Bramhope; dark grey=Murone; and black=Ventina.

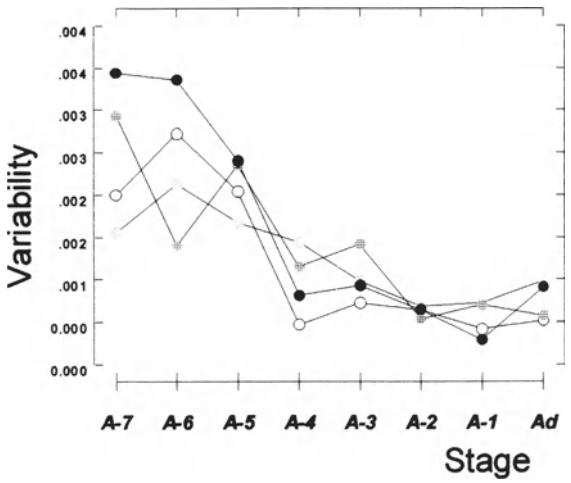


*Figure 3.* Shape variability for each ontogenetic stage and clone of *E. virens* estimated as the sum of variances for all harmonic coefficients (colour codes as in Figure 2).

variant to size, rotation and starting position of the outline trace (Ferson et al., 1985). This resulted in a matrix with 37 nontrivial normalised coefficients. Coefficients $A_1$, $B_1$ and $C_1$ (for the first harmonic) are invariant and can be ignored. $A_0$ and $C_0$ (the terms for the zeroth harmonics) determine the starting position for the digitisation trace along the outline and do not provide information on shape itself.

Principal component analysis was performed on harmonic coefficients in order to show in a simple way patterns in shape variability among individuals. There are two main factors influencing shape variability within a given taxa: genotype and environment (and their interaction). In order to disentangle their effects, ontogeny was described using monoclonal cultures under constant environmental conditions ('Set A'). Despite that, some phenotypic variability is still expected due to stochasticity of biochemical and developmental pathways (Gabriel & Lynch, 1992). Therefore, average shapes were used as representative of each juvenile stage. These were computed by averaging harmonic coefficients elementwise (Ferson et al., 1985).

Morphological variability within each instar was measured as the total variance (sum of univariate variances for all dimensions in morphospace) which is proportional to mean square Euclidean distance (Van Valen, 1974; Foote, 1993).

In order to reflect population variability, outlines from both 'Set B' and 'Set C' were projected onto the morphospace defined by average instar shapes.

## Results

### *Size change during ontogeny*

Regression analysis (exponential model) resulted in the following parameter estimations:

$$L = 0.118e^{0.257S},$$

where $n$ (sample size)=313; $r^2$ (coefficient of determination)=0.992 and $p$ (significance level) <0.0001.

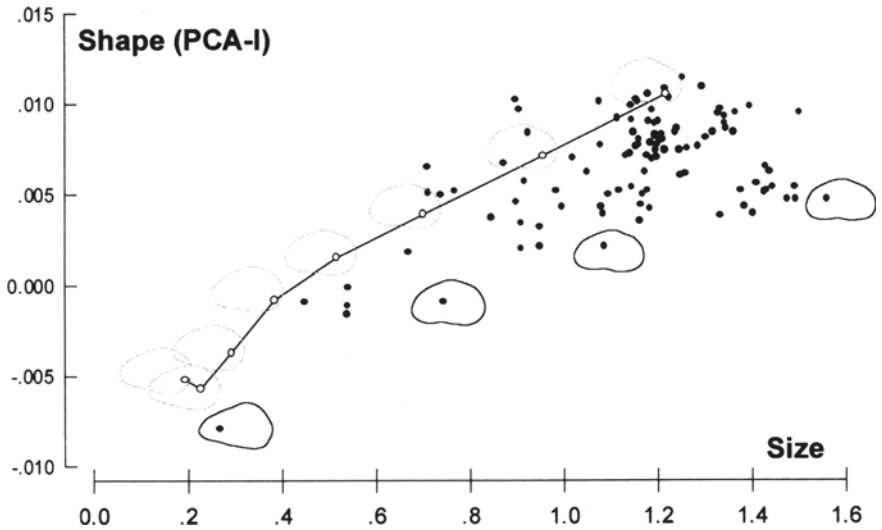


*Figure 4.* Projections of *E. virens* outlines onto morphospace (size & shape). A set of geographically close field populations (black dots) are displayed together with the development pathway inferred for a lab clone from the same area (Berzosa, Spain). Some outlines are included to allow for comparisons (grey outlines=average shapes of the cultured clone; black outlines=actual shapes from field populations).

Growth coefficient between consecutive juvenile stages is 1.293, meaning that ostracod linear dimensions increase by a factor close to 1.3 or, in terms of volume, that it grows 2.16 times each moult. These figures are in agreement with empirical laws which propose that arthropods' linear dimensions increase at each moult by a fixed proportion (Brooks' law) or that moulting results in a doubling of the animal's volume (Przibram's law) (Kesling & Takagi, 1961; Kurata, 1962; Hounsome, 1975).

*Shape change during ontogeny*

Shape change of the carapace during ontogeny in all clones can be described simply as a process of differential growth (positive allometry) of the rear parts of the carapace. As generally shown in most podocopid ostracods, the anterior part of the valves is rounded from the very beginning of development, whereas the posterior part starts off more pointed (Figure 1). Through ontogeny, the posterior part, more precisely the postero-dorsal quadrant, enlarges its size in parallel with the addition of limbs and the development of reproductive structures.

Principal component analysis performed on harmonic coefficients representing mean shapes for each developmental stage ('Set A') resulted in the ordination displayed in Figure 2. There is a high degree of congruence or similitude in the developmental pathway exhibited by all four clones. The first axis explains 78% of the variance in the raw data and it is mainly associated with ontogenetic changes. The second axis, which explains a minor proportion of the total variance (7.8%), seems to correspond to the 'arch effect' – a quadratic distortion of the first PCA axis caused by the inability of PCA to handle nonlinear responses – characteristic of some factor plots (Reyment & Jöreskog, 1993).

Shape variability for each juvenile stage and each clone is represented in Figure 3. Because of the genetic homogeneity of each clone and the constancy of culture environmental conditions, this measure represents random individual variation in shape. The first juvenile instars are much more variable in shape, irrespective of their genotype. This is probably due to poorly calcified valves, typical for the juveniles, but it should be checked that it is not an instrumental artifact caused by the difficulties of accurately recording shape in small organisms. From A-4 onwards, shape variability seems to decrease to a constant level.

Variability due to genetic diversity and environmental fluctuations was explored using 'Set B' and 'Set C' (Figures 4 and 6). Concerning carapace size and shape, both data sets reflect higher variability than 'Set A'. In 'Set C' (Figure 4), it could be expected that *E. virens* from the field would show size-shape features randomly distributed around development pathways established for laboratory populations, where genotypic and environmental fluctuations are minimal. However, if laboratory cultures are used as reference,

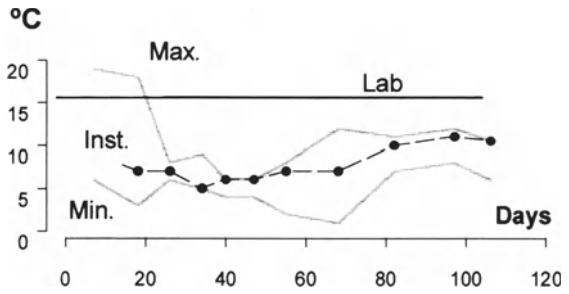


*Figure 5.* Temperature ranges measured in the field for populations of *E. virens* displayed in Figure 4. Lab temperature was kept at 16 °C and is displayed for reference.

those specimens coming from the field tend to have larger sizes and more 'juvenile' shapes. It seems that, under the lower and more variable temperatures experienced by the field populations (Figure 5) compared to those in the laboratory (constant temperature 16 °C), the rate of development of shape decreases while rate of size growth increases.

## Discussion

### *Size change during ontogeny*

A 'quasi' constant growth factor for length has been assessed for several ostracod species (Kesling, 1952; Szczechura, 1971; Hounsome, 1975; Heitkamp, 1979; Herman & Heip, 1982; van Harten, 1983; Marín, 1984; Maness & Kaesler, 1987). It has been suggested that such a developmental constant between age classes has the same ecological basis as Hutchinson's constant for competing species (Maiorana, 1978; Van Harten, 1983), i.e. represents the minimum size difference which allows coexistence by reducing competition between conspecific juvenile instars and congeneric species, respectively. Unfortunately, similar size distribution patterns can be also produced by non-biological processes (e.g. children's bicycles, sets of kitchen skillets and sets of plates) (Horn & May, 1977; Maiorana, 1978). Benthic ostracods mainly feed on organic detritus, which seems difficult to be partitioned according particle size. On the other hand, information available on interspecific ostracod competition suggests that size differences of an order of magnitude similar to those observed between consecutive juvenile instars are not sufficient to allow for coexistence and that other mechanisms (e.g. segregation in microhabitat or in timing for population growth, etc.) must also contribute to the final output (McLay, 1978). Our data cannot solve the question of whether growth constant is selected to reduce competition between development stages, but certainly adds new evidence on the generality of Brook's Law.

*Eucypris virens* from field populations attained larger sizes than those reared in the laboratory. This is supposedly a consequence of growth at lower temperatures, as it has been shown that low temperatures result in a higher rate of size increase (Hounsome, 1975; Martens, 1985). Nevertheless, the effect of temperature on ostracod development is still not well known and sometimes lower temperatures produce smaller, not larger size (Martens, 1985). The importance of environmental factors in ostracod growth has long been stressed (Skogsberg, 1920) and should be carefully considered not only when dealing with Recent populations but especially with fossil assemblages. In words of Hounsome (1975), ". . . size differences are not always reliable taxonomic indicators".

### *Shape change during ontogeny*

Shape is a unique feature for many species (Van Morkhoven, 1962), and we rely on its constancy at the specific level for taxonomic work. During ontogeny, all four clones of *E. virens* followed a similar, well-defined pathway through the morphospace (Figure 2). There is, however, some source of variability which apparently affects the smaller juvenile instars much more than the last ones. It could be that methods for shape features extraction are less accurate when dealing with very small objects and that the observed variability is, at least partly, due to instrumental errors. This possibility should be tested with further studies.

When overall shape variation is broken up into its main components (PCA) most of it reflects ontogenetic changes. Schweitzer & Lohmann (1990) found a very similar pattern of shape variability in field populations of *Cyprideis margarita*. These authors identified the variance not explained by ontogeny as a result of sexual dimorphism and seasonal variation. Our data, however, were obtained from four clonal lineages (all-female) raised under homogeneous controlled conditions, so no environmental variability or sexual features are involved.

Ostracod carapace shape, like any other phenotypic trait, must be influenced by both genotype and the environment. Although there is no information on the genetic identity of all the clones included in data 'Set B' and 'Set C', it is likely that those from the same geographic area ('Set C') share a higher pro-

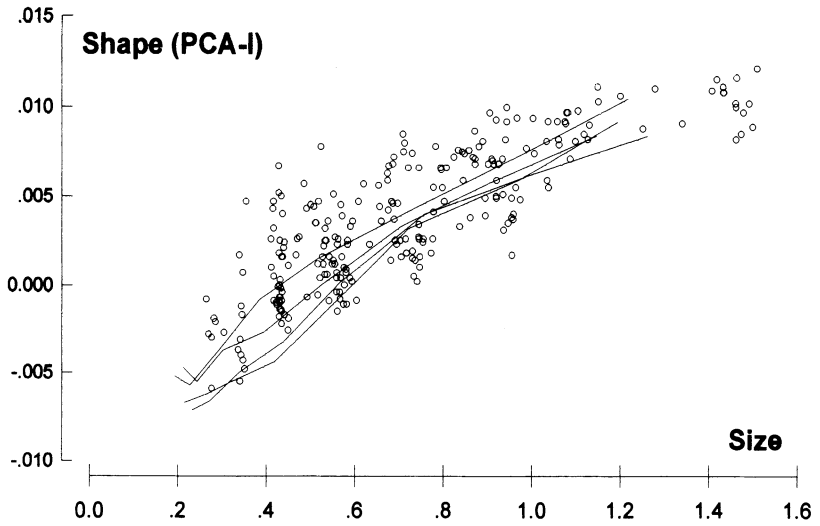


*Figure 6.* Projections of *E. virens* outlines onto morphospace (size & shape). Lines represent development pathways obtained from clonal cultures; open circles correspond to individuals from field populations ('Set B').

portion of alleles and, therefore, are genotypically more similar than those coming from distant localities ('Set B'). If genotype tightly controls carapace shape through ontogeny and environmental conditions have a minor influence on the process, we should expect to find less variability among individuals in 'Set C' (genotypically similar but raised under fluctuating environmental conditions) than among individuals in 'Set B' (genotypically less similar but raised under controlled constant environmental conditions). However, the opposite seems to occur suggesting that environmental factors (temperature, conductivity, food supply, etc.) play a major role in shape determination in *Eucypris virens*.

## Conclusions

During ontogeny, *E. virens* doubles its carapace size with each moult adding new empirical evidence to the wide applicability of Brooks' law among crustaceans. Unfortunately, ultimate mechanisms responsible for such pattern remain unknown.

Concerning carapace shape, development pathways seem to be fairly similar for clones from a wide geographical range. However, shape variability increases significantly when organisms are raised under fluctuating environmental conditions. We still know little about quantitative contribution of external factors (e.g. temperature, salinity, photoperiod, . . .) on ostracod carapace shape but these observations stress the importance of carapace shape analysis for a number of applications, especially for palaeoenvironmental reconstructions.

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# Multifunctions of the upper lip and a ventral reflecting organ in a bioluminescent ostracod *Vargula hilgendorfii* (Müller, 1890)

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*Key words:* reflecting organ, upper lip, Myodocopa, chemical cues, evolution

## Abstract

Multifunctions of the upper lip in a bioluminescent myodocopid *Vargula hilgendorfii* were studied by video observation and histological method. The localization of luciferin and luciferase gland cells within the upper lip was partly successful. Two long protrusions of the upper lip, both of *V. hilgendorfii* and a non-luminescent species of the same family, immediately anterior to the mouth, were found to show very flexible movement especially while eating, as if smearing on the food surface a secretion from the protrusions (glands), which may support the hypothesized secretion of digestive enzymes from the upper lip. This hypothesis is further supported by the new finding of a pair of ducts which connect the basal part of the upper lip with the posterior digestive duct (stomach). Comparative studies of *V. hilgendorfii* with several sympatric non-luminescent species of the same family have also revealed that it has a characteristic reflecting organ immediately posterior to the anus. It is a conical small protrusion, as if dangling from the ventral edge of the abdomen at the apex of the cone. It is observable only in live specimens, when the furca, which is located outwardly to the organ, is sufficiently transparent. When illuminated, the reflecting organ reflects the distinct light. The diameter of the mirror (chemical composition provisionally analyzed) is about 6–8% of the carapace length. The organ develops from the very first stage of its ontogeny without reference to sex, which suggests that the function may be related to intraspecific signaling or predatory deterrence.

## Introduction

Easy use and reasonable price of the video camera has brought about a new approach to studies of microscopic marine invertebrates such as Ostracoda. It was on the basis of video observations that the evident courtship behaviour (Morin, 1986; Morin & Cohen, 1991) and copulation (Parker, 1995) were recognized in some myodocopids, and interpreted with respect to asymmetrical appendages of a podocopid, *Bicornucythere bisanensis* (Abe & Vannier, 1991). Video observations enabled functional morphology of appendages to be discussed in detail in some myodocopid species (Vannier & Abe, 1992, 1993; Parker, 1997) and the anastomosed pattern on the myodocopid carapace was proved to be a vascular network (Abe & Vannier, 1995), leading to discussion of the early evolution of the Palaeozoic ostracods (Vannier & Abe, 1995).

Such video observation has the potential to reveal what has long remained uncertain concerning the function of some conspicuous organs, such as the upper lip of myodocopid ostracods. The upper lip is well known (Kornicker, 1975 and a series of his work on myodocopid Ostracoda; Cohen & Morin, 1993 for Caribbean bioluminescent species), a well developed soft part located anterior to the mouth, but its exact function has remained unknown. One of the known facts is that the light-producing materials, luciferin (substrate) and luciferase (enzyme) are synthesized, stored in and emitted from the upper lip of bioluminescent myodocopes, although a definite localization of luciferin and luciferase has not yet been determined (Yatsu, 1917; Okada, 1927; Takagi, 1936; Saito

† Deceased

et al., 1986a, b) except for the case of two species immunocytochemically studied by Huvard (1993).

This fact does not explain, however, why non-luminescent myodocopids also have a well developed upper lip. A comparative study of luminescent and non-luminescent species, therefore, would be a good approach to understanding the principal function of the upper lip. This is one of the aims of our study.

During the course of the study, we found a 'ventral reflecting organ' in a bioluminescent species, *Vargula hilgendorfii* (Müller, 1890). Its structure is briefly described for the first time with the results of a provisional analysis of the chemical composition and the assumed ecological functions. The evolutionary origin of the acquisition of the reflecting organ and the luminous ability are discussed in relation to chemical cues.

## Materials and methods

*Vargula hilgendorfii* and several non-luminescent undescribed species (Cypridinidae) were collected from Tateyama Bay (139° 51′ E, 34° 59′ N) in the Boso Peninsula, Sotoura Bay (138° 59′ E, 34° 41′ N) and Nabeta Bay (138° 56′ E, 34° 39′ N) in the Izu Peninsula, central Japan. They were collected by a baited trap (Vannier & Abe, 1993; Abe et al., 1995), and transferred to laboratory in aerated sea water, examined under a microscope with a fiber illuminator and video recorded (for detailed procedure, see Vannier & Abe, 1993). Fixed specimens were processed by critical point drying methods (Vannier & Abe, 1993) or with hexamethyldisilazane (HMDS) (Nation, 1983) for the observation by SEM (scanning electron microscope, JEOL JSM-35CFIIA). A dry fracturing method (Toda et al., 1989) was used for the internal obvservation of the reflecting organ. For light microscopy, the specimens were fixed with Bouin's solution and embedded in soft paraffin. Serial sections about 6 $\mu$m thick each were stained with Mayer's hematoxylin and eosin (H-E). Computer-aided 3-D image was reconstructed by Luzex F (NIRECO) and computer software 'Voxel view' (IMAGE & MEASUREMENT). The chemical composition of the reflecting organ was provisionally analyzed by HPLC (high performance liquid chromatography) (JASCO 860, L-column ODS, 4.6×150 mm), following the method of Chae et al. (1996).

Among more than 10 non-luminescent cypridinid species, one undescribed species was selected for detailed comparative study with *V. hilgendorfii*. The species was the third most abundant myodocopid collectable by our sampling method, following *V. hilgendorfii* and another undescribed species. It closely resembles *V. hilgendorfii* in general appearance as observed laterally by a binocular microscope, except for the color in the integument of the upper lip. The lip is bright yellow in *V. hilgendorfii*, while dark brown in the non-luminescent species. Other taxonomically important characters are to be described elsewhere (Hiruta, in prep).

In the following main text, two species names are abbreviated as HIL (*Vargula hilgendorfii*) and NLC (non-luminous cypridinid).

## Results

### *Upper lip*

Although observations of live undissected specimens under a binocular microscope did not detect any difference between HIL and NLC in the outer appearance (lateral view) of the upper lip except for its color (Figures 1a–d), SEM photos showed a considerable discrepancy especially in the number and the disposition pattern of the openings (nozzles) of gland cells (Figures 1e,f and 3) and the morphological chracteristics of the tubercle(s) on which the openings were located. The ventral side of the lip has anteriorly an unpaired and posteriorly a paired field in HIL, while only one large unpaired field in NLC. In both species, a pair of tusk-like protrusions (longer in NLC than in HIL) were located at the posteriormost region. The nozzles sometimes showed variation in number, as indicated in lower case in Table 1.

The histological observation revealed that one nozzle accomodated one to a few gland cells, of the same type (most cases) or different types (in the case of type A). Gland cells were classified into six types in HIL (A1, A2, B, C, D, E) and four in NLC (K, L, M, N), both principally based on how the tissues were stained with hematoxylin and eosin, and granule size and its density (see the caption of Table 1 for the criteria) (Figure 2). The main characters in each type of gland cell were summarized as in Table 1 (see also the explanation of Table). As for type A1 and A2 in HIL, these two types of cells were always coupled (thus named under the same letter), meeting together in six nozzles in the unpaired filed and two nozzles in the paired field. The only exceptional case was found in the paired field, where two cells of type A2 were

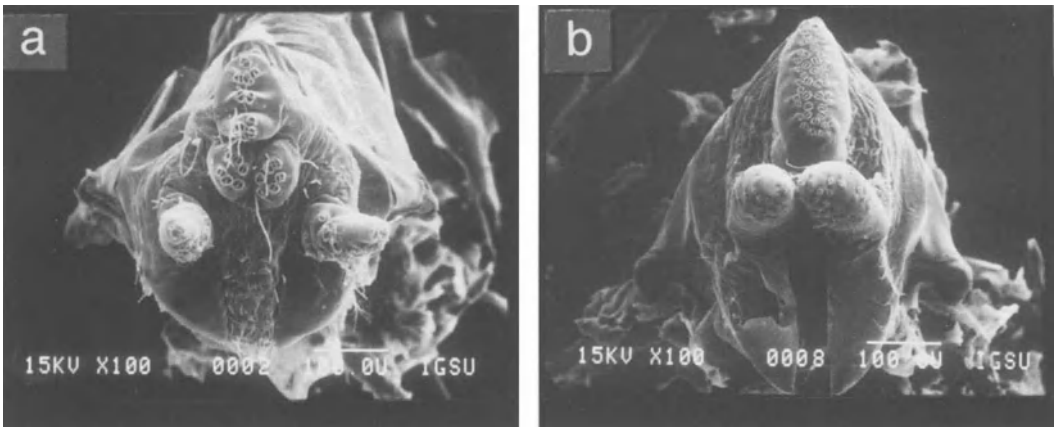



*Figure 1.* Comparison of HIL (*Vargula hilgendorfii*) and NLC (non-luminous cypridinid species). (a) (c) (e) HIL; (b) (d) (f) NLC. (a) (b) left side view of live specimens. (c) (d) close view of the upper lip, left valve removed. (e) (f) SEM photos of glandular cells openings (nozzles) of the upper lip.

coupled into one nozzle within each counterpart (Figure 3). In the case of other types of gland cell, a few cells reached a single nozzle, but the exact number was not determined.

Light microscopy of live specimens of HIL revealed that A2 type cells contained a lot of large (approximately 10 $\mu$m in diameter) yellow granules (Figure 2d).

A definite correspondence was not found in those glandular cell types between the two species in terms of either the disposition pattern or how they were stained. Some of the cell types were confined within the tusk (E of HIL and M of NLC), while others were found in every region (C of HIL, L and N of NLC). The two nozzles of type C found at the tusk were restricted its position to its tip, while nine nozzles of type E were sporadically scattered on the whole surface of the tusk. Another possibly important result was that type B seemed to be always associated with a coupled nozzle of Type A1 and A2 (see 'Discussion').

Two long tusks which are developed among many cypridinid myodocopes at the posteriormost part of the upper lip were video-observed to show extremely flexible movement in both HIL and NLC, being more evident in the latter. They smeared onto food what is probably a secretion from the tusk glandular cells.

*A pair of ducts*

The 3-D image constructed, based on a series of paraffin sections (> 90), revealed the presence of a pair of thin ducts which emerge at the basal part of the upper lip and run posteriorly until near the surface of a stomach (Figure 4). The duct is very thin and seems circular in a section. It was not clear whether or not the ends of the ducts connect with the stomach.

*Ventral reflecting organ*

It was found for the first time that HIL has a characteristic organ that reflects light at a point immediately posterior (ventral) to the anus. It is a small conical protrusion, as if dangling from the ventral edge of the abdomen at the apex of the cone (Figure 5). It was observable only in live specimens, when the furca, which is located outward of the organ, was transparent enough. When illuminated, the reflecting organ was video-observed to reflect the distinct light at the circular bottom surface of a conical structure. The surface appearance of a metallic lustre resembled that of a naupliar eye of the species.

The diameter of the mirror was about 6–8% of the carapace length. The outer surface of the conical organ, except for the circular bottom, was coated with a thin layer of dark brown pigmental cells, in which small granules of equal size (about 1 $\mu$m in diameter) were contained. Internally the organ seemed to be composed of three major parts from the apex: 1. the basal layer of irregularly shaped crystalline-like platelets (about 0.2 $\mu$m in thickness), 2. well-stained flattened cells layer, and 3. a combination of larger spherical cells and smaller well-stained cells (Abe & Yamamura, in prep). The last part showed somewhat honeycomb arrangement and attained more than a half of the total organ in volume. To the surface of this

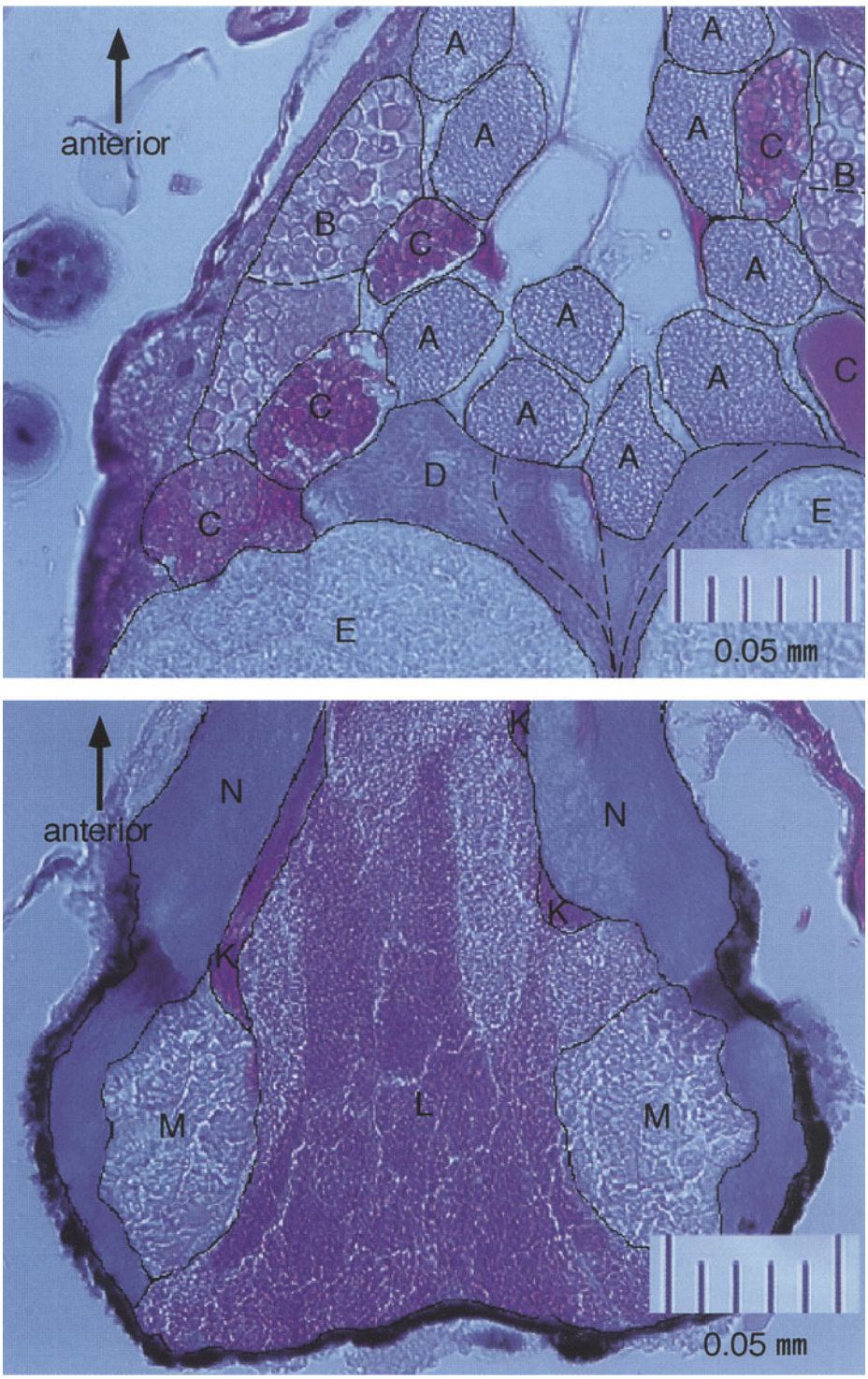



*Figure 2.* Histological observation (paraffin sections) and close light microscopy of the upper lip: (a) (b) (d) HIL; (c) NLC. (a) (b) (c) horizontal sections; (d) A2 cells with large granules. A–E and K–N represent a group of gland cells classified based on the granule size and how the tissues were stained with hematoxylin and eosin. B and D may be subdivided. At the basal part and the intermediate part of the lip A1 and A2 are found separately, but they meet together one by one at six nozzles in the unpaired field and two nozzles in the paired field. In the photo a, most of the contents of A2 are lost during the preparation, but they are seen in photo b.

*Table 1.* Type of gland cells (A–E, K–L) and number of nozzles with criteria on which grouping was made. 'A' indicates the coupling of type A1 and A2 cells. These two different type cells meet together at a single nozzle (see text). In the column of tubercular region of *V. hilgendorfii*, the number of nozzles are given in three subdivided regions, i.e. anterior unpaired, posterior right and left (in parenthesis). As for a non-luminous species, the exact number of the nozzles of each cell type was not known based on the paraffin section method because of too large a number but the total number could be counted. Variation in the total number of nozzles is indicated in lower case. Type E has nine nozzles on each tusk in most cases, but 10 in some specimens

| *Vargula hilgendorfii* | | | | | Non-luminous cypridinid species | | | |
|---|---|---|---|---|---|---|---|---|
| | | tuberuclar region | columnar region | | | | tubercular region | columnar region |
| A | small granules weakly stained with eosin | 10 (6,2,2) | 0 | ⟷ | K | stained pink with eosin | present | 0 |
| B | large granules weakly stained with eosin | 4 (2,1,1) | 0 | ⟷ | L | large granules stained pink with eosin | present | 0 |
| C | large granules stained pink with eosin | 4 (2,1,1) | 4 | ⟷ | M | large granules weakly stained with eosin | 0 | present |
| D | stained purple with hematoxylin | 4 (0,2,2) | 0 | ⟷ | N | stained purple with hematoxylin | present | present |
| E | stained pale purple with hematoxylin | 0 | 18+2 | | | | | |
| | total number of nozzles | 21-22-23 | 22-24 | | | total number of nozzles | 46-48 | 80 |

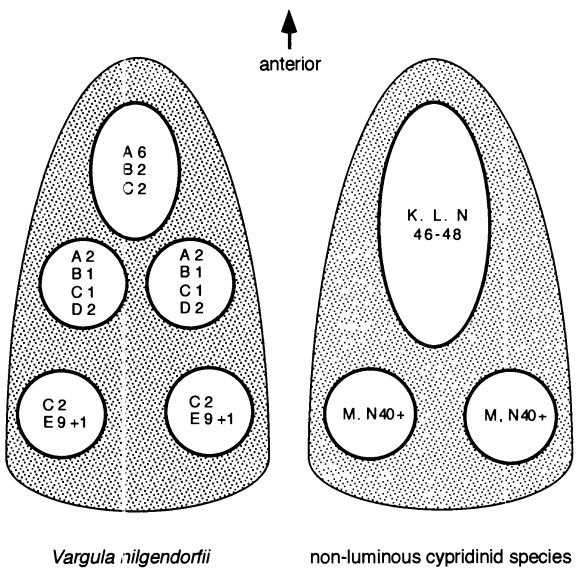



*Figure 3.* Schematic diagrams of the upper lip of *Vargula hilgendorfii* and a non-luminous cypridinid species, with the number of nozzles. In *V. hilgendorfii*, the upper three ellipses indicate the frontal unpaired field and the paired field (left and right) and the lower two tusks. In a non-luminescent species, there is one unpaired field and two tusks. Arrow indicates anterior.

spherical cell membrane, a lot of granules of variable size (up to 3.5 $\mu$m in diameter) were found attached.

The spherical cells may be the origin from which a transparent fluid came out during the dissection of this part. A provisional HPLC analysis of the organ, based on the comparison of the result when the whole part of the stomach was processed and the result when the reflecting organ and its surrounding alone was processed, revealed that the organ contained hypoxanthine (possibly the platelets and solution described above).

Although the organ attaches to the ventral part of the stomach at the apex with supporting structure (Figure 5), its reflecting surface was movable, changing the direction of the reflection according to the movement of the furcae which covers the organ. In this sense, the 'working angle' of the mirror ranges about 90 degrees (Figure 6).

The organ was found to develop from the very first stage of ontogeny without reference to sex. No sexual dimorphism was observed in any aspects. Similar organs were found in another luminescent undescribed species, but never found in any nonluminous species collected together with HIL and NLC.

## Discussion

### *Multifunction of the upper lip*

As shown by Cohen & Morin (1993) and a series of the intensive work on myodocopids by Kornicker (e.g. 1975), the general morphology of the upper lip varies greatly. Therefore, it may be difficult to find homologous relationships in glandular cell types between

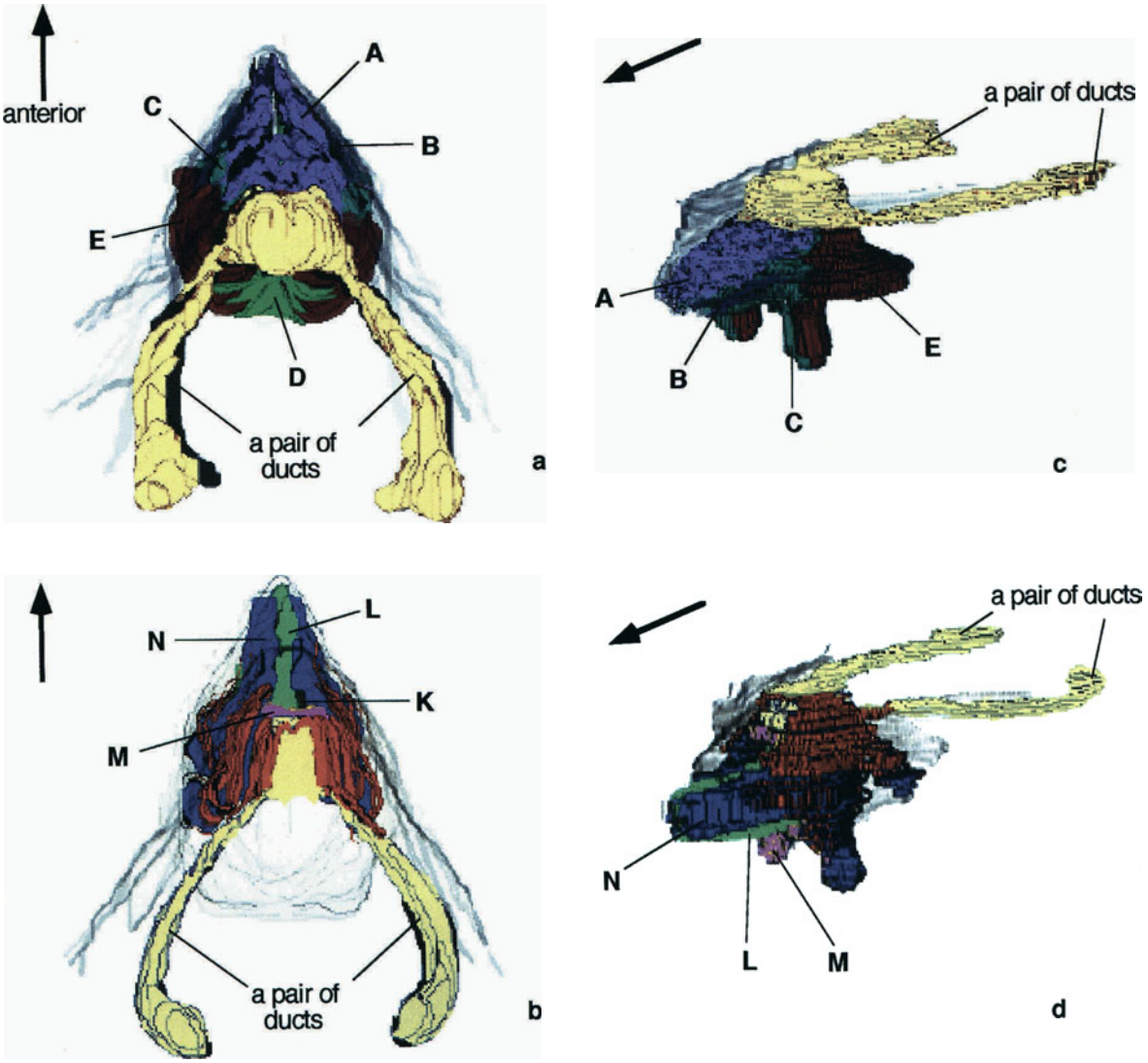
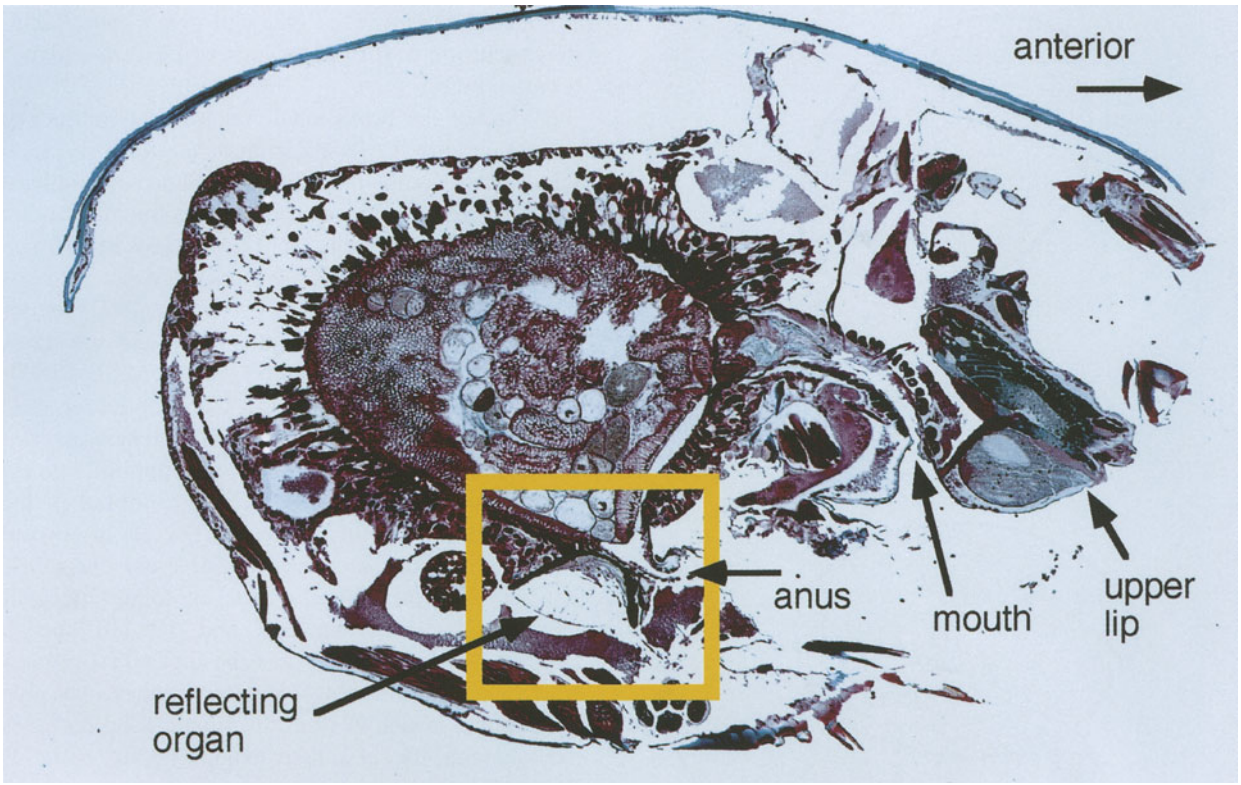



*Figure 4.* 3D diagrams of a pair of ducts at the basal part of upper lip in *V. hilgendorfii* (a, b) and a non-luminous cypridinid (c, d). (a) (c) oblique view; (b) (d) dorsal view. Selection of colour is arbitrary. Arrow indicates anterior.

the two species examined in this study. The internal structure (e.g. paraffin section or TEM) of the upper lip of non-luminescent species have attracted few workers so far and thus only limited information is available.

It has long been attempted to localize the cells secreting luciferin and/or luciferase within the upper lip of HIL (Yatsu, 1917; Okada, 1927; Takagi, 1936; Saito et al., 1986a, b) and other bioluminescent myodocopids (e.g. Huvard, 1993). The exact knowledge, however, has not been obtained yet. Some of them assumed that the yellow colored cells which contain large granules should be luciferin (Yatsu, 1907; Okada, 1927), which is supported by the fact that the color of the lip in the animal that emitted light becomes pale yellow because of the consumption of luminous substances. If this assumption (luciferin is yellow) is true, A2 among six types of cell is exclusively responsible for the secretion of luciferin (Figure 1d).

Since the process of bioluminescence is a chemical reaction (oxidization) of the substrate (luciferin) with the aid of the specific enzyme (luciferase), the effective reaction is likely to require the nearby occurrence of the two chemicals. In this sense, type C and E cells are not very likely to be responsible for luciferase secretion, because they are found in the



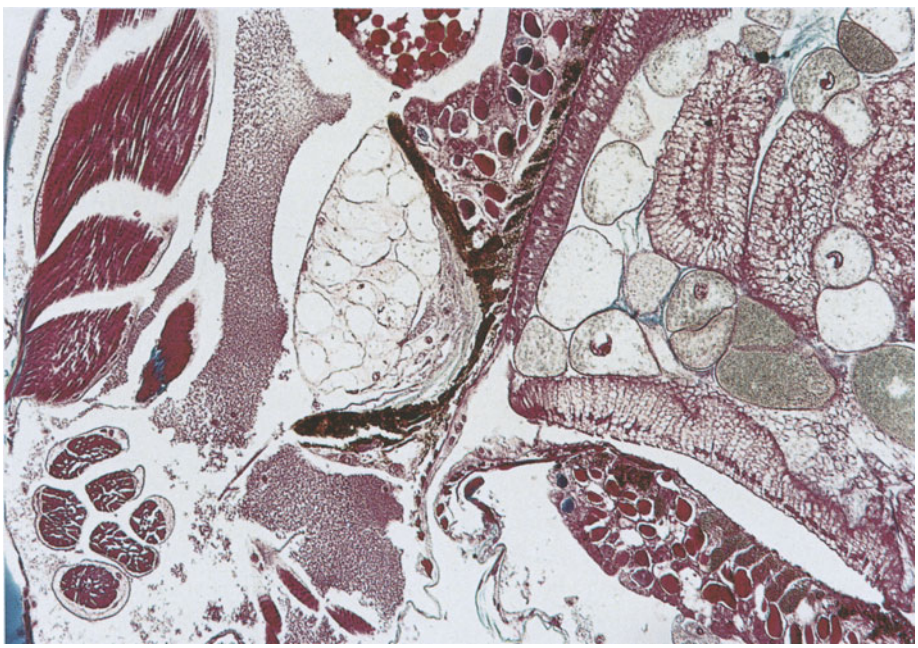



*Figure 5.* Paraffin sections of the whole body of *Vargula hilgendorfii* (sagittal section) (a), and a close view of the ventral reflecting organ (b), bold square area in (a).

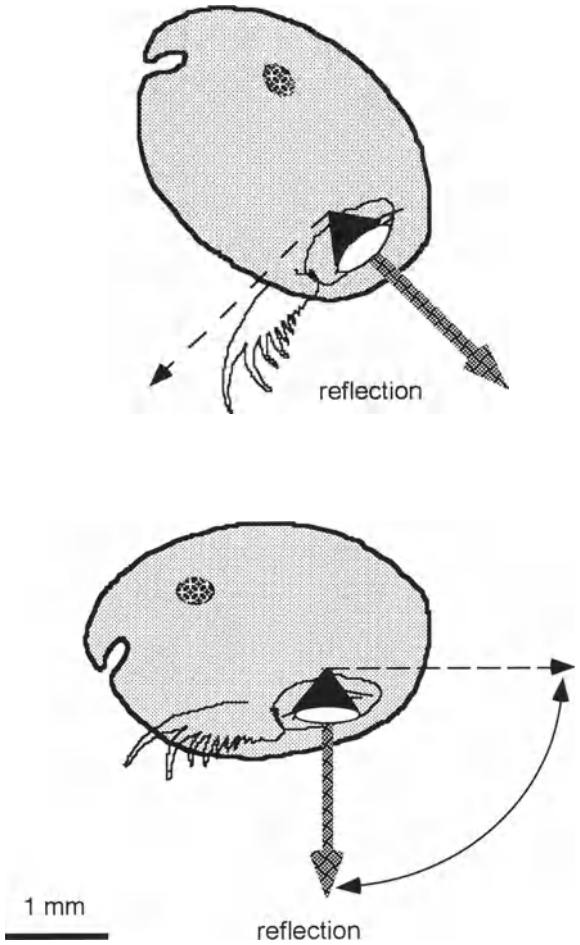



*Figure 6.* Working angle of the ventral mirror to reflect the light. Direction of the mirror surface changes according to the movement of furcae. The furcae are rotated by about 90 degrees, for instance, when the animal takes off from the substrate into the water column.

tusk where A2 cells are entirely absent. However, it is interesting to note that Huvard (1993), using immunocytochemical techniques, found luciferase in cells (her B cells) which are also found in the tusks of *Vargula graminicola* and *V. tsujii*.

On the contrary, the fact that A1 and A2 are almost always found coupled with each other is very suggestive. It is possible that A1 is responsible for luciferase, and the two substances react upon exposure to oxygen in the sea water. Nonetheless, one exception (a nozzle of A1 cells alone) in the paired field remains unexplained. One more possible candidate of luciferase cells is type B, because type B cells are also associated with A2. However its number of nozzles seems too small (Table 1 and Figure 3 ) for the chemical reaction required. An immunocytochemical method as conducted by Huvard (1993) will give a more definite conclusion as for the location of luciferase glands (Ono, in prep).

Whether our provisional conclusion is correct or not concerning the localization of luciferin and luciferase, what are the other types of gland cells (at least C, D, E) for? We must explain some functions other than the bioluminescence for those glands in HIL and all of four types in NLC. We consider that some of them may secrete mucus (see Huvard, 1993) and/or function as digestive enzymes to different substrates from the food (Claus's opinion cited in Muller, 1894; Okada, 1927; Abe et al., 1995). The peculiar repeated movement of two tusks located immediately anterior to the mouth may support this assumption. Indeed, because some of the gland cells are limited in the tusks (E and M in this study and D cells of Huvard (1993)), they may secrete a digestive enzyme to assist the ingestion of food. However, some types still remain unexplained, that is, C and D in HIL and K, L, N in NLC. They may also be digestive enzymes which work on different substrates, or they may produce mucus which is useful to obtain food particles (see Cannon, 1931) and to make a 'bolus of light' (Huvard, 1993) at least in luminous species.

Regarding the common presence of possible digestive enzymes in both luminous and nonluminous myodocopids as one piece of evidences, it has been hypothesized based on the provisional biochemical analysis that a luminous enzyme, luciferase might have evolved from a digestive enzyme (Abe & Vannier, 1993; Abe, 1994; Abe et al., 1995, 1996), resulting in luminous ability which should be useful for the nocturnal life of HIL. This hypothesis may be supported by the fact that the cells responsible for luciferase in *V. graminicola* and *V. tsujii* is (her B cells) also found in the tusks (Huvard, 1993).

### *The pair of ducts*

The presence of the ducts has been noticed by other workers, too (e.g. by A. Cohen, pers. comm.). It may be a key discovery to understand the interrelationship of the upper lip and stomach, thus the hypothesis about the origin of luminescent enzyme mentioned above. Although it was not clear in our study whether the ducts reached and opened at the stomach, we hypothesize that they are actually connected to the digestive duct and that some substance such as digestive enzyme may be introduced between the upper lip and the stomach. The reason that the non-luminescent species has a

similar morphology of the upper lip is well understood in such a context. It is not yet concluded, however, whether the luminous ability had been acquired among non-luminous species or the latter had lost the ability in the evolutionary processes.

*Structure, function and origin of the ventral mirror*

The similarity of metal-like appearance of the mirror surface with the nauplius eye may suggest the similarity of chemical components of them as well (not analyzed yet).

Another luminescent species (undescribed) collected during this study has also a similar mirror organ, but non-luminescent myodocopid species seem never to have one (among more than ten species collected together during this study).

It is interesting to consider why such a well-developed ventral mirror organ in HIL has not been noticed by earlier researchers. One reason is that the organ is located inward of the furcae, which is transparent in vivo but easily turns cloudy after death and preservation in alcohol. Another reason may lie in the ordinary observing angle under a binocular microscope, in which researchers usually lay the specimens down and look at them in the lateral position. The reflection by the mirror cannot be observed in this direction.

The presence of working angle may have its meaning, if any, when the reflecting direction changes in a daily life. The time of such changing of direction is known to be restricted to when the animal kicks off and departs from the substrate or the bottom sediment surface to swim upward, or when it moves around the food to search for a good point to eat or cut pieces off it. They do not rotate the furcae while swimming. Though the reflected light itself is strong enough to be detected by the surroundings, the flashing effect may be more effective which is made by the quick back-and-forth movement of the furcae.

The ecological significance of the mirror organ should be interpreted in terms of functions not related to the reproductive behaviour (courtship) since it shows no apparent relationship to age and sex. Since HIL is a nocturnal species (resting within the bottom sand layer in the daytime), the reflection of the light can function only at night or at dim light. One plausible effect of the reflection is signaling to conspecific members, e.g. an alarm. Recent video observations made it clear that HIL emitted a light at the same time as being spat from the mouth of a gobioid fish, as described by Morin (1983, 1986) in the Caribbean sea ('burglar alarm effect'). It is highly probable that the gobioid fish was stimulated with some chemicals emitted by the prey and spat it out, resulting in the ostracod escaping from being completely swallowed (NLC was also observed to be spat out by the fish, though). Those chemicals may be either luciferin or luciferase or both, and the light bomb was made as a result. However, those escaping animals swim with their furcae folded and thus without a flashing effect of the reflected light. Moreover, the reflection by the mirror seems too small compared with the light source (light bomb) to enhance the alarm effect to the conspecific animals.

One possible situation where the blinking (with furcal movement) can function as signalling to the surrounding conspecifics is the simultaneous commencement of foraging behaviour at dim light (30 min after the sunset). When they come out of the bottom sand layer with their furcae intensely moving back-and-forth (Vannier & Abe, 1993), the dim light may be reflected by the mirror organ giving a blinking effect. Such a blinking light could be responsible for the simultaneous commencement of foraging activity, which must enhance the efficiency of the 'hunting' of large wounded animals (annelids, fish, molluscs etc.).

Hypoxanthine is commonly produced in the course of degradation of purines to uric acid. These substances are regarded as waste products in the animal body, suggesting a relationship between the digestive duct and the reflecting organ. The close position of the reflecting organ to the anus may suggest that the origin of hypoxanthine should be sought in waste material within the digestive organ. Uric acids derived from hypoxanthine may form crystals, comprising the thin layers of irregular platelets at the basal part of the mirror. Such platelets can produce a peculiar reflection by multiple thin-layer interference. Solution of hypoxanthine within spherical cells may contribute to the renewal of the crystals. It has been reported that some male copepods have a regular hexagonal structure of guanine (Chae et al., 1996) in the dorsal integument which contributes to the display of iridescent colors by ta similar physical mechanism (Chae & Nishida, 1994 and refs. therein).

Our SEM observations found a similar structure of platelets plus surrounding pigment cells also in the tapetal cell of nauplius eye (cf. Huvard, 1990) in HIL which has a metal-like lustre like a ventral mirror. Therefore, we examined the possibility that the ventral reflecting organ might serve as a visual sensory organ.

But neither nervous cells nor any related structures were observed in the paraffin section.

The above two examples (luciferase from digestive enzyme and mirror from waste product) discussed in terms of chemical triggers of the origin of new characters (luminous ability and reflector) may indicate hopeful approaches to the study of evolution by rather simple devices such as a binocular, video observations and field survey.

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# Factors affecting the divergence of mate recognition systems in the Limnocytherinae (Crustacea, Ostracoda)

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*Key words:* stochasticity, natural selection, sexual selection, developmental constraints, phylogenetic constraints, speciation

**Abstract**

Specific Mate Recognition Systems (SMRS) consist of a set of morphological, behavioural and physiological traits which allow mate recognition. The Limnocytherinae, a lineage of non-marine podocopid Ostracoda, have a relatively wide diversity of copulatory modules, a concept largely congruent with the morphological part of the SMRS. The present paper describes the various copulatory modules in some detail and discusses potential mechanisms responsible for the divergence of these modules. Although none of the processes was thus far demonstrated directly, resulting patterns provide indirect evidence that four different mechanisms contribute. Stochastic processes (chance) as well as developmental and other phylogenetic constraints are involved in the initial selection (choice) of modified structures. Subsequent (positive) directional sexual selection on traits of the recognition systems causes radiative speciation within lineages. At all times, natural selection acts on the development of these structures, either stabilising or negative directional. A number of potential tests for these hypotheses are suggested.

## Introduction

When Darwin (1859) developed his theory on speciation by natural selection, he was left with the problem as to why males and females of the same species can be so different in appearance. Even more puzzling was how extravagant male traits can evolve, for example, the bright colours, large feathers and fins or conspicuous vocal signalling, none of which improve survival, rather the opposite. Therefore, he introduced the concept of *sexual selection* (1871). Whereas natural selection implies competition over natural resources with the aim of survival, sexual selection refers to competition over mates, aiming towards reproduction. If an individual is unfit under natural selection, it will die. An individual with a low sexual fitness will fail to reproduce.

Sexual selection can act at several moments during the reproductive process and in various ways. Most commonly, it occurs before the actual mating, during courtship, either by *contests* (males fight over possession of females, e.g. in deer) or by *female choice* (the male attempts to stimulate the female into accepting him for copulation) (Eberhard, 1985). It has been argued that 'choice' is a very human-contrived term, and that 'preference' or 'selection' would be more appropriate in this context (Paterson, 1993b). A combination of both processes is found in *leks*, where several males attempt to demonstrate their fitness in a so-called arena, while females observe and eventually select a mate (Höglund & Alatalo, 1995). Other (prezygote) mechanisms of competition over mates more recently recognised are *scrambles* (early search and swift location of mates), *endurance rivalry* (ability to remain reproductively active during a large part of the season) and *sperm competition* (includes mate guarding, frequent copulation, mating plugs etc., but also the ability to remove rival sperm) (Andersson, 1994).

Sexual selection is still largely defined in terms of competition over mates, but the definition should really be refined to competition over gametes (Eberhard, 1996), as the process by no means ends with successful mating. Females of most species will have much greater opportunities to exert influence over events in the post intromission phase. Especially in

*Table 1.* Types of SMRS signals and mechanisms of sexual selection in Recent Ostracoda. Data for Myodocopa based on Cohen & Morin, 1990a,b and Morin & Cohen 1991; for Podocopa based on McGregor & Kesling 1969, Cohen & Morin 1990a, Danielopol 1980 and Horne et al., 1998

| | Myodocopa | Podocopa |
|---|---|---|
| *Signal* | | |
| auditive | No | No |
| visual light patterns | Yes | No |
| tactile morphology | Yes | Yes |
| behaviour | Yes | Yes |
| physiological pheromones | ? | ? |
| *Mechanisms of sexual selection* | | |
| scrambles | Yes | Yes |
| endurance rivalry | Yes | Yes |
| contests leks | Yes | No |
| mate choice | Yes | Yes |
| sperm competition | Yes | Yes |

prezygotic (but post-intromission) conditions, such females can largely control whether successful copulation will eventually also result in offspring, but they even have some degree of postzygotic control, i.e. by induced abortion. These aspects of *cryptic female choice* (i.e. which cannot be measured from successful mating), potentially of large evolutionary importance, are extensively reviewed by Eberhard (1996), but are only marginally dealt with here. Most of the present essay concentrates on pre-intromission recognition and selection.

Note, also, that the discussion on whether or not sexual selection is a process independent from natural selection has become largely semantic, as even Andersson, in his extensive review, refers to 'sexual selection and other natural selection'. At the same time he thus recognises the special character of sexual selection and stresses the intimate relationship between the two types of processes (1994: 8).

Sexual selection in ostracods has thus far been demonstrated in Myodocopida (exclusively marine ostracods) only. The effects of sexual selection on the development of bioluminescent courtship display, and the resulting rapid specific radiation in the myodocopid Cypridinidae (not to be confused with the podocopid family Cyprididae), was extensively discussed by Kornicker (1969, 1975, 1981, 1985), Cohen & Morin (1990b, 1993, 1997), Morin & Cohen (1991) and Parker (1995, 1997). Males in this group have specific bioluminescent displays during set periods of the night and this courtship display can be considered as lekking, although it was not recognised as such by Höglund & Alatalo (1995). Females remain mostly in the benthos and only seek out the preferred male for a short time. Initial species recognition and pre-intromission mate selection thus primarily occur through this visual display. However, Morin & Cohen (loc. cit.) also report the presence of several conspecific, non-signalling males around other signalling males, which means that at least a second stage in the process of mate selection will occur, either through scramble, contest or female choice, or a combination of these mechanisms. Although the actual copulation has not been observed *in situ*, these assumptions seem logical as the available evidence adds up to a classical situation of sexual selection, where many males are competing for few females, using several types of signals: certainly, initially, visual (bioluminescence) and possibly, subsequently, also tactile (Table 1). More speculative (direct evidence is lacking) is the hypothesis that the hardened spermatophore in these and many other myodocopids serves as a *mating plug*, which might have evolved in response to sperm competition in the past. Morin & Cohen (1991) further argue that the mating system in this lineage has evolved through sexual selection and that the extensive and rapid radiation of this group in the Caribbean is at least partly due to the effect of sexual selection on recognition systems, albeit combined with low dispersal ability.

The effect of sexual selection on the evolution of reproductive isolation, and hence on speciation, is controversial (summary in Andersson, 1994) and in any case will depend on which species concept is applied. At least 22 species concepts are in use to date (Mayden, 1997). They differ considerably with regard to theoretical significance, generality, operationality and applicability and, hence, in their respective resulting accounts of past and present diversity. This diversity of species concepts, of course, reflects the range of diversity of the taxa to which they relate, as well as that of methods used and underlying theory applied. The most commonly used concepts are those dealing with multicellular biparental organisms, the so-called *biological species concepts* (termed *sexual species concepts* by Bell, 1997), including the isolation and the recognition species concepts. The *isolation concept* holds that two individuals of opposite gender belong to different species, if they are repro-

ductively isolated from each other, i.e. will not or cannot mate. If insemination occurs, it will produce invidious or sterile hybrids (Mayr, 1969), or hybrid breakdown (reduced fertility in later generations of hybrids) will occur (Dobzhansky, 1970). According to the *recognition concept*, two individuals of opposite gender belong to the same species if they recognise each other as potential mates through a set of morphological, behavioural and physiological characteristics (Paterson, 1993a). Such sets of characteristics are called the *SMRS* (=*Specific Mate Recognition Systems*) in the recognition species concept. 'Recognition' and 'isolation' concepts are thus two sides of the same coin (but see Masters et al., 1990).

Danielopol et al. (1990) coined the term *copulatory module*, a concept largely congruent with the morphological part of the SMRS. The copulatory module includes all aspects of morphology which are involved in the mating process and, for example in arthropods, can comprise a wide variety of aspects of body and appendage morphology, other than the copulatory appendage alone, such as limbs, valve morphology, etc. (McGregor & Kesling, 1969; Kornicker, 1985; Cohen & Morin, 1990a). It could be argued that the entire phenotype, including its extensions as defined by Dawkins (1982), takes part in this process. Yet, certain traits are more intensively involved in the process than others and can generally be recognised as such. Traits which are part of the copulatory module have a species-specific morphology, are usually sexually dimorphic and are used by the male to stimulate the female into accepting him for copulation, and hence for reproduction. It is useful to apply this concept of Specific Sexual Dimorphism (SSD) to recognise traits of the copulatory module (and of the SMRS), because the majority of invertebrate species is still known from their morphology only. If nothing is known about their behaviour or on any other part of the SMRS, morphology is the only aspect that can be assessed.

Much debate has focused on the evolution of recognition systems: are these the result of chance, natural selection or sexual selection or of a combination of these (and other) processes? Extensive reviews of the history of these discussions are given, for example, by Bell (1997) on a micro-evolutionary scale and by Andersson (1994) on a macro-evolutionary scale. Especially the possibility that sexual selection can cause divergence of recognition systems is controversial (Paterson, 1993b). Most work on sexual selection has been done on vertebrates and insects, little on other invertebrates. Nevertheless, there are good indications that also in those groups sexual selection can be an important mechanism driving evolution and, ultimately, speciation. Sexually selected traits generally contribute directly to better chances for fitter offspring (e.g. stronger males have larger territories, fitter males build better nests for brooding, etc.), but even when males contribute nothing but sperm towards reproduction, as is the case in many invertebrates, there is still considerable sexual selection on various traits, because such characters are supposed to signal indirectly for higher fitness (Andersson, 1994).

The present paper discusses the divergence and diversity of the copulatory modules in a representative podocopid non-marine ostracod lineage, the Limnocytherinae and attempts to distinguish the effects of different processes on the evolution of copulatory modules: chance, preadaptation, developmental constraints and natural and sexual selection.

## Material and methods

### *Podocopida and Limnocytherinae*

Five infraorders are presently recognised in the Podocopida (Martens, 1992b; Martens et al., 1998), three of these have representatives in non-marine habitats. At least 75% of the non-marine species belong to the Cypridocopina, about 20–25% belong to the Cytherocopina, another 1–3% are Darwinulocopina (Martens, 1998a). Most non-marine Cytherocopina are in the family Limnocytheridae Klie, 1938; the taxonomy of this group has remained confused for a long time. Colin & Danielopol (1978, 1980) first introduced order by establishing the presence of two subfamilies: the Limnocytherinae Klie, 1938 and the Timiriaseviinae Mandelstam, 1960.

Danielopol et al. (1990) divided the Limnocytherinae into four tribes: Limnocytherini, Leucocytherini, Cytheridellini and Dinarocytherini (change of rank). Since the Cytheridellini have been transferred to the Timiriaseviinae (Martens, 1995), the nominate subfamily is left with three tribes, which are mainly distinguished on the basis of the hinge. An updated, but still preliminary, reassessment of the genera in the Limnocytherinae was presented in Martens (1996) and is here summarised in Table 2. The present discussion will focus on the two extant tribes of the Limnocytherinae.

Podocopid ostracods have a relatively uniform morphology. The entire body is enveloped by two cal-

*Table 2.* Preliminary generic reassessment of post-Cretaceous Limnocytherinae Klie, 1938 (modified after Martens, 1996)

1. Tribe **Limnocytherini** Klie 1938

Abbreviated Diagnosis: hinge antimerodont, hinge bar smooth, act present or absent, pct nearly always present. Hemipenis with copulatory process never spiralled.

1.1. *Limnocythere*-lineage

Characteristics: caudal ramus in hemipenis small, marginal pore canals straight, clasping organ generally well developed and consisting of several processes.

Genera: *Limnocythere* Brady, 1868 (=*Limnicythere* Brady, 1868; =*Limnicytheridea* G.W. Müller, 1912; =*Acanthopus* Vernet, 1928); *Galolimnocythere* Schornikov, 1973; *Limocytherina* Negadaev-Nikonov, 1967.

1.2. *Paralimnocythere*-lineage

Characteristics: marginal pore canals branched; ventral setae on basal sement of at least P2–3 strongly reduced or absent; setae of caudal ramus in hemipenis relatively large.

Genera: *Paralimnocythere* Carbonnel, 1969 (=*Relictocytherina* Negadaev-Nikonov, 1968 (fossil); non *Paralimnocythere* Wang, 1989); *Kiwicythere* Martens, 1992.

1.3. *Neolimnocythere*-lineage

Characteristics: hemipenis with caudal ramus large to very large, marginal pore canals straight. Genera: *Neolimnocythere* Delachaux 1928, *Paracythereis* Delachaux, 1928 (=*Pampacythere* Whatley & Cholich, 1974 (fossil)).

2. Tribe **Leucocytherini** Danielopol & Martens, 1990

Abbreviated Diagnosis: hinge bar crenulate, act present or absent, pct with 1–3 lobes. A1 and P3 often (not always) with sexual dimorphism; hemipenis with setae of caudal ramus large, UR and LR simple.

Genera: *Leucocythere* Kaufmann, 1892; *Ovambocythere* Martens, 1989; *Potamocythere* Schornikov, 1986; *Athalocythere* Schornikov, 1986, *Prolimnocythere* Karmischina, 1970 (fossil); *Elkocythereis* Dickinson & Swain, 1967 (fossil).

3. Tribe unknown:

Characteristics: hinge adont. A2 with sexual dimorphism in endclaws and exopodite (no sexual dimorphism in A1 or T3); hemipenis with simple structure, with clasping organ completely reduced.

Genera: *Korannacythere* Martens, 1996.

4. Tribe: **Dinarocytherini** Krstic, 1987 (exclusively fossil)

Abbreviated Diagnosis: hinge amphidont, hinge bar crenulated, act and pct present; LV with anterior (+sometimes posterior) cardinal tooth.

Genera: *Dinarocythere* Krstic, 1987; (=*Scordiscia* Krstic & Schronikov, 1993); *Cladarocythere* Keen, 1972.

cified valves which hinge along the dorsal margin. Most of the body consists of the head, which has four pairs of appendages: Antennula (A1), Antenna (A2), Mandibula (Md) and Maxillula (Mx1). The exact homology of the fifth limb is subject to discussion (reviewed in Cohen et al., 1998), as it is a maxilliped in the Cypridoidea (and was thus considered a head appendage), but takes the form of a walking limb in Cytheroidea, in which case it would be a first thoracopod. Smith & Martens (this volume) show in *Eucypris virens*, a member of the Cypridocopina, that the fifth limb is ontogenetically a thoracopod. In this group, this is the only limb which can be sexually dimorphic and be part of the copulatory module. In other groups, such as Cytherocopina, several other limbs can have a function in mate recognition.

Podocopid ostracods have paired male reproductive systems, including two large hemipenes (Cohen & Morin, 1990a). Long spermatozoa occur in certain Cypridocopina: an animal of 1 mm long can have spermatozoa of up to ten times its own size (Wingstrand, 1988; Schön & Martens, 1998, see further discussion in Butlin & Menozzi, 2000). Within the five lineages of the Podocopina (see Martens, 1992b), two have developed paired sperm-pumps, called Zenker's organs, which are separate from the actual hemipenes (Cypridocopina and Sigilloidea); two other lineages have incorporated the sperm-pumps into the male copulatory appendages (Cytherocopina and Bairdiocopina). The fifth lineage, Darwinulocopina, has abandoned sex at least since the end of the Mesozoic and is not discussed here. These different solutions have several consequences. In the groups where the copulatory

appendages have the sperm-pumps incorporated, all parts of the actual copulatory complex (see below) are well sclerified and external. In the Cypridocopina and Sigilloidea, on the other hand, the hemipenes are independent of the sperm-pumps and have all parts of the copulatory complex enveloped by the sheets of the peniferum; the actual copulatory process only leaves the envelope when the hemipenes are in erection. While it is not entirely clear how this difference affects the reproductive biology of the animal itself, although a rapid qualitative assessment indicates that the groups with external copulatory complex show significantly more morphological variability in these structures, it certainly facilitates the investigation of the morphology of this external copulatory complex.

*Morphology and terminology of the hemipenis in Limnocytherinae*

The functional morphology of the hemipenis in the Limnocytherinae was studied and a terminology was developed by Danielopol et al. (1990) and Martens (1990a, 1998b). As an understanding of the morphology of the limnocytherinid copulatory appendage is essential for the present discussion, this model and its terminology are here summarised.

The hemipenes occupy up to one third of the male body cavity and consist of an outer sheet, the *peniferum*, in which different *rami* are incorporated, a solid mass of different *muscles*, an internal *labyrinth* and the externally situated *copulatory complex*.

The copulatory complex is situated dorsally in the relaxed position of the hemipenis (Figure 1, top). It is the most important structure for the present discussion and consists of upper and lower ramus of the clasping organ, copulatory process and three setae of caudal ramus. The *upper ramus* is present in certain taxa as a long tentacle-like process (e.g. in *Limnocythere inopinata*); more often, however, it is either reduced to a small, transparent lobe, or completely absent. The *lower ramus*, however, is more often well developed, in *Limnocythere* s.s. it consists of two parts: a large *hook-like process* and a 3-lobed *lateral process*, both of variable morphology. The *copulatory process*, the actual penis, is an elongated, sclerified trabecule in which a *ductus ejaculatorius* is clearly visible. The *glans*, the distal part and the actual intromitting organ, can be of highly variable morphology. The *caudal ramus*, with three *setae*, is present in all groups and can be of variable size and shape.

*Abbreviations used in text and figures*

HP=Hemipenis: dl=distal lobe; pl=proximal lobe; cp=copulatory process; CR=caudal ramus; f(1–3)=setae 1–3 of the caudal ramus ('furca'); hp=hook-like process; lp=lateral process; LR=lower ramus of clasping organ (=hp+lp); mt=movable trabecule; UR=upper ramus of clasping organ. Other soft parts: A1=Antennula; A2=Antenna; Md=Mandibula; Mx1=Maxillula; T1-3=thoracopods. Exo=exopodite. Valves: act=anterior cardinal tooth (of hinge); H=height of valves; L=length of valves; pct=posterior cardinal tooth (of hinge); RV=Right valve; LV=Left valve.

## Results

This section presents descriptions of different copulatory modules in Limnocytherinae; these can be more extensively viewed in the original descriptions of the various taxa (see references in Table 2). An overview of the different limbs involved in the copulatory modules of the different lineages is given in Table 3. Examples of hemipenis morphology in the different lineages, showing gradual increase of shape and size of the caudal ramus, are given in Figure 1.

Not all specific and sexually dimorphic traits are necessarily directly involved in mating and thus belong to the copulatory module. For example, certain traits can be ecological adaptations (West-Eberhard, 1983). However, all specific sexually dimorphic traits at least have the potential to be used for recognition during mating and I here consider them part of the copulatory module and further consider this set of traits as the morphological part of the mate recognition systems. Such an approach could over-estimate the diversity of recognition systems to some extent, but as this paper compares recognition systems within related lineages, all with comparable ecologies, the potential bias will be limited.

*Limnocytherini* – Limnocythere-*group* (Figure 1)

Within this lineage, the morphological radiation occurs in the copulatory complex, i.e. the copulatory process and the clasping organ of the hemipenis. The upper ramus can either be present as a tentacle-like structure (e.g. *Limnocythere* s.s. and *Limnocytherina*) or be absent (e.g. in *Galolimnocythere*, see Martens & Mazepova, 1992). The lower ramus can be differentiated into hook-like and lateral processes, as in *Limnocythere* s.s., or into one large and bizarrely shaped

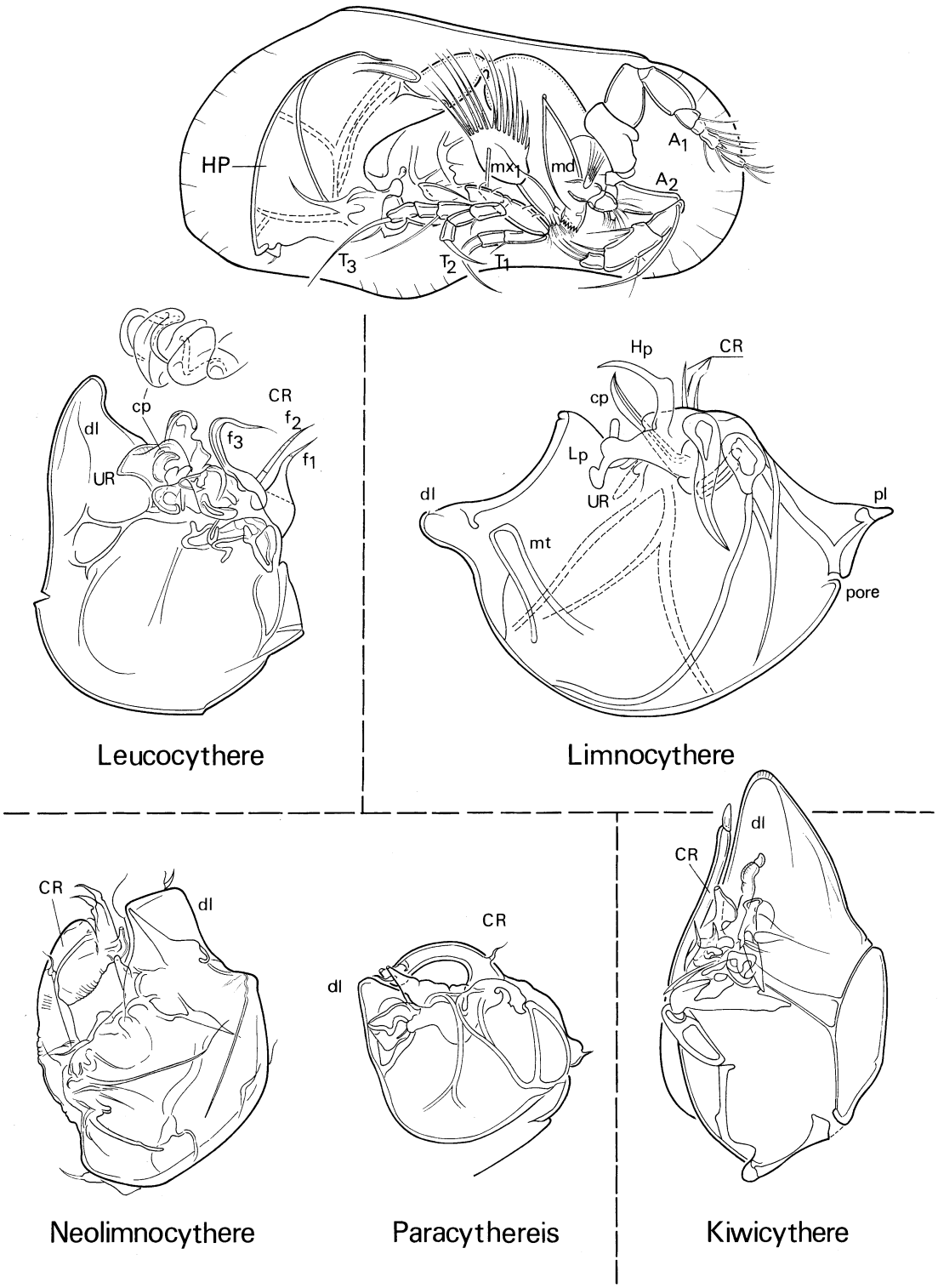


*Figure 1.* Top: Schematic representation of body plan in Limnocytherinae (example of male Limnocythere, right valve removed, one limb of each pair drawn) (after Martens, 1990a). Middle and bottom: hemipenis in the different lineages of Limnocytherinae, showing shape of caudal ramus, clasping organ and copulatory process (middle row: *Limnocythere* and *Leucocythere*) and increase in size of caudal ramus (bottom row: *Kiwicythere, Paracythereis, Neolimnocythere*). Redrawn after several authors, not to scale.

*Table 3.* Specific and sexually dimorphic morphologies in copulatory modules of Recent Limnocytherinae (Ostracoda)

| | Valves | A1 | A2 | T3 | Hemipenis | | |
|---|---|---|---|---|---|---|---|
| | | | | | Cp | CO | Fu |
| LIMNOCYTHERINI | | | | | | | |
| *Limnocythere* | V | – | – | (T3) | Cp | LR | – |
| *Limnocytherina* | | | | | | | |
| *Galolimnocythere* | | | | | | | |
| *Paralimnocythere* | V | – | – | – | Cp | – | Fu |
| *Kiwicythere* | | | | | | | |
| *Neolimnocythere* | V | – | – | – | – | – | Fu |
| *Paracythereis* | | | | | | | |
| LEUCOCYTHERINI | | | | | | | |
| *Leucocythere* | V | A1 | – | T3 | Cp | UR | Fu |
| *Ovambocythere* | | | | | | | |
| *?Potamocythere* | | | | | | | |
| UNKNOWN TRIBE | | | | | | | |
| *Koranacythere* | – | (A1) | A2 | – | – | – | – |

process, as in *Limnocytherina* (see Delorme, 1971; Danielopol et al. 1990). The sometimes large expansions of the hemipenis are correlated with significant sexual dimorphism in valve shape. Male valves are often more elongate and have ventro-caudal expansions to accommodate the elaborate hemipenis structures. The copulatory module appears to consist entirely of the copulatory complex and the carapace; the morphology of the caudal ramus in the hemipenis, of the Antennae and of the T1–T2 shows little or no specific sexual dimorphism; some SSD is seen in the T3 of some species groups.

*Limnocytherini* –
Paralimnocythere/Neolimnocythere-*group* (Figure 1)

The second group in the Limnocytherini, taxonomically characterised by branched pore canals, shows a gradual increase in size of the caudal ramus in the hemipenis. The Palaearctic *Paralimnocythere*, especially speciose in the Balkan Lakes, has a relatively small caudal ramus in the hemipenis (Martens, 1992a), but the Pacific *Kiwicythere* has a hyper-developed caudal ramus. Inter-specific differences are mainly in the shape of the actual copulatory process; the clasping organ shows no significant radiation. Although the presence of the branched radial pore canals clearly differentiates the *Paralimnocythere*-lineage from the *Neolimnocythere*-branch, the tendency towards hyper-development of both basis and setae of the caudal ramus in the hemipenis is furthered in the latter lineage, which consists of the South American *Neolimnocythere* and *Paracythereis* (see Delachaux, 1928). The radiation of Neotropical limnocytherids is poorly understood and will prove highly informative for the evolution of the Limnocytherinae as a whole (Martens et al., 1998). From the available information, it is clear that the radiation in this lineage is primarily exemplified by the hyperdevelopment of the male (hemipenal) caudal ramus. Most of these taxa also have well-developed surface ornamentation (spines, etc.), which is at least partly related to environmental factors, but which could also play a role in the (tactile) mate recognition.

*Leucocytherini* – Leucocythere *group* (Figure 2)

In the second tribe, the Leucocytherini, the diversification of the copulatory complex of the hemipenis is restricted to the copulatory process and, to a lesser extent, to the upper ramus of the clasping organ and the caudal ramus. However, in this lineage, the copulatory module also comprises the sexually dimorphic A1 and T3. The southern African *Ovambocythere* has the most plesiomorphic character states, with a nearly straight copulatory process, and little sexual dimorphism in either A1 or T3 (Martens, 1989, 1991). Unfortunately, the hemipenis morphology of the Asian genera

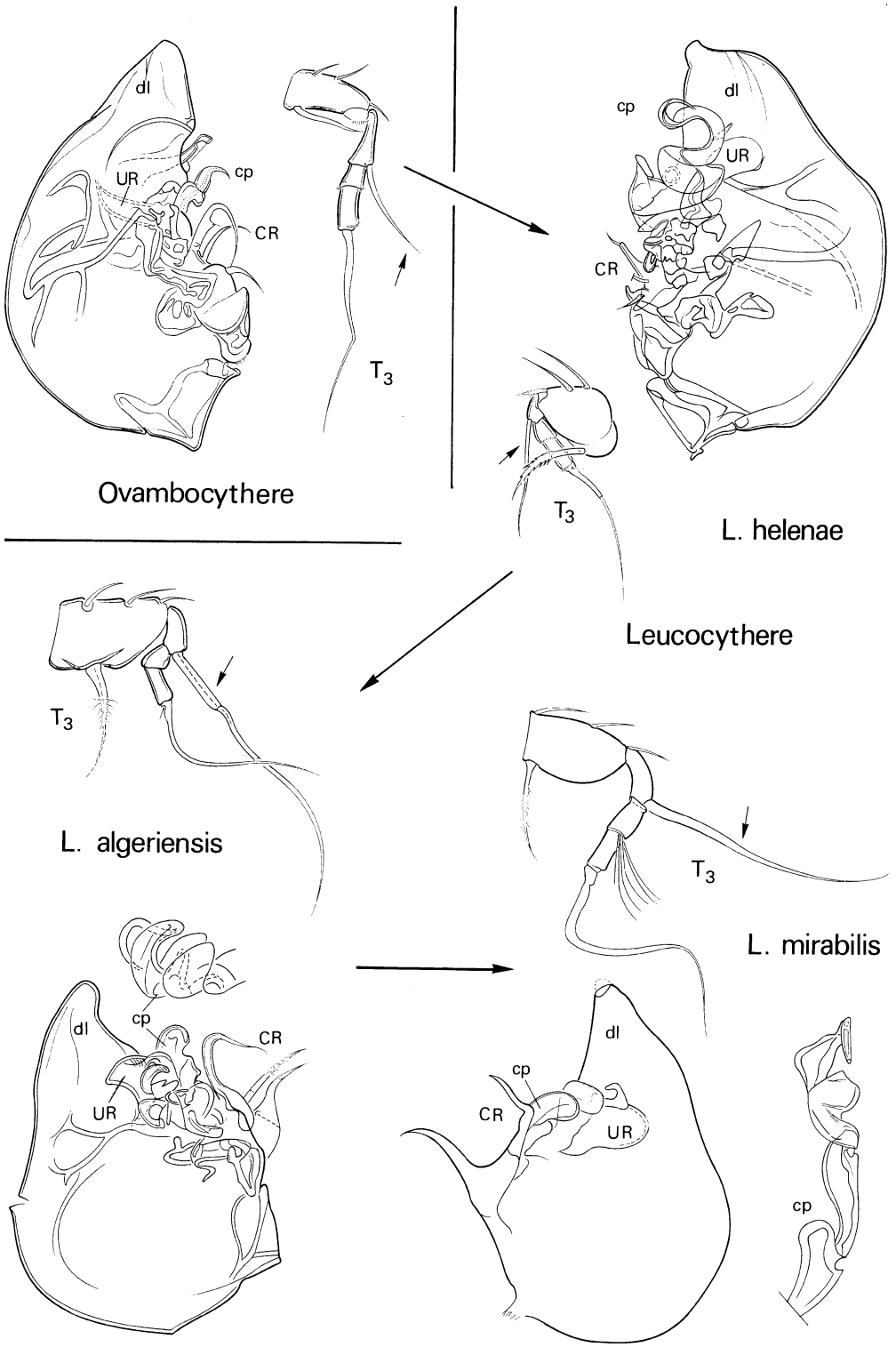


*Figure 2.* Specific sexually dimorphic features in *Ovambocythere* and *Leucocythere*: Hemipenis, copulatory process and T3 (redrawn after Danielopol et al., 1990; Martens, 1989, 1991).

*Potamocythere* and *Athalocythere* is insufficiently (or not at all) known, so these important taxa cannot be included in comparative analyses.

*Unknown Tribe* – Korannacythere (Figures 3 and 4)

This genus occurs in temporary pools of the Drakensberg region (South Africa). Three species are thus far known, each with a restricted distribution; all have a very special copulatory module (Martens, 1996). There is almost no sexual dimorphism in the valve shape and the hemipenis structure is simple and largely non-specific. The entire clasping organ (both lower and upper ramus) has disappeared, the caudal ramus consists of a simple base with three setae and the copulatory process is a sickle-shaped organ with an undiversified glans. The absence of prominent species-specific (dimorphic) characters in valves and hemipenis, however, is amply compensated by interspecific differences in the morphology of the A2; especially in the length of the exopodite, length and shape of apical claws and the shape and setulation of the three major ventral setae of the endopodal segments. In all species, the exopodite in the female is longer than in the male. The sexual dimorphism is strongest in *K. devriesi* (restricted to the north-western part of the Drakensberg area), less developed in *K. ugiensis* (occurring in the southern berg) and almost absent in *K. hamerae* (eastern part of the area). As these exopodites are long in both sexes of all other genera of the Limnocytherinae, and short exopodites can thus be considered apomorphic, a tendency in decreasing exopodite size from East to West becomes apparent (Figure 4). This tendency is paralleled by a dimorphic change in the length of the end claws, which become shorter and more stout, and a progressive reduction of the smallest accompanying seta of the lateral aesthetasc on second endopodal A2 segment.

The taxonomic position of *Korannacythere* is at present dubious. Most soft part features (size of lobe dl in hemipenis, absence of clasping organ, . . .) would suggest a proximity to Leucocytherini, but the adont hinge prevents its inclusion in this tribe. It is here kept as 'Uncertain Tribe' within the Limnocytherinae.

## Discussion

*The podocopid model: reproductive behaviour*

The reproductive behaviour of podocopids was recently reviewed by Horne et al. (1998) and no visual displays (Cohen & Morin, 1990a) or pheromones have thus far been reported in these ostracods, although the large glands associated with the A2 in Cytherocopina could produce such specific chemicals. Males seem to find females by simply crawling or swimming over the sediment or plant surface. They will generally grab anything that resembles a potential mate: females of other species, other males, even little sand grains etc., but occasionally also a genuine conspecific female. The male then proceeds to manipulate the female, stimulating her into accepting him as a potential mate. During this manipulation, the male also attempts to position her in the correct mating position. This position depends on the lineage studied, and can be: dorsal/ventral, posterodorsal/ventral, ventral/ventral, reverse ventral/ventral, etc. (McGregor & Kesling, 1969; Danielopol, 1980; Cohen & Morin, 1990a). Several appendages can be used by the male to keep the female in the correct position, but only the female decides whether or not to open the carapace to allow insertion of the hemipenes between the valves and, eventually, intromission. Males will only attempt insertion in the (specific) correct mating position. In most cases, initial insertion occurs very carefully, as rapid closing of the valves by the female when hemipenes are inserted could cause permanent damage (Horne, unpubl. data).

In the few cases where mating of Limnocytherinae was observed (Geiger et al., unpubl. data on *L. inopinata*), the posterodorsal/ventral position was always assumed, but only after a considerable period of manipulation, which included also ventral/ventral positions. This manipulation will allow females to have tactile contact with various parts of the male morphology, also with anterior appendages, which cannot be checked in the actual mating position. Part of recognition and selection may thus occur during this initial manipulation, but this is further discussed below. A more extensive description of the different stages of the mating processes in podocopid ostracods is presented in Horne et al. (1998).

*The podocopid model: variability in copulatory modules of the Limnocytherinae*

Table 3 summarises the morphological features (limbs, valves and parts of hemipenis) here postulated to be involved in the copulatory module of the different lineages in the Limnocytherinae. The group shows a mosaic of recognition traits, scattered over

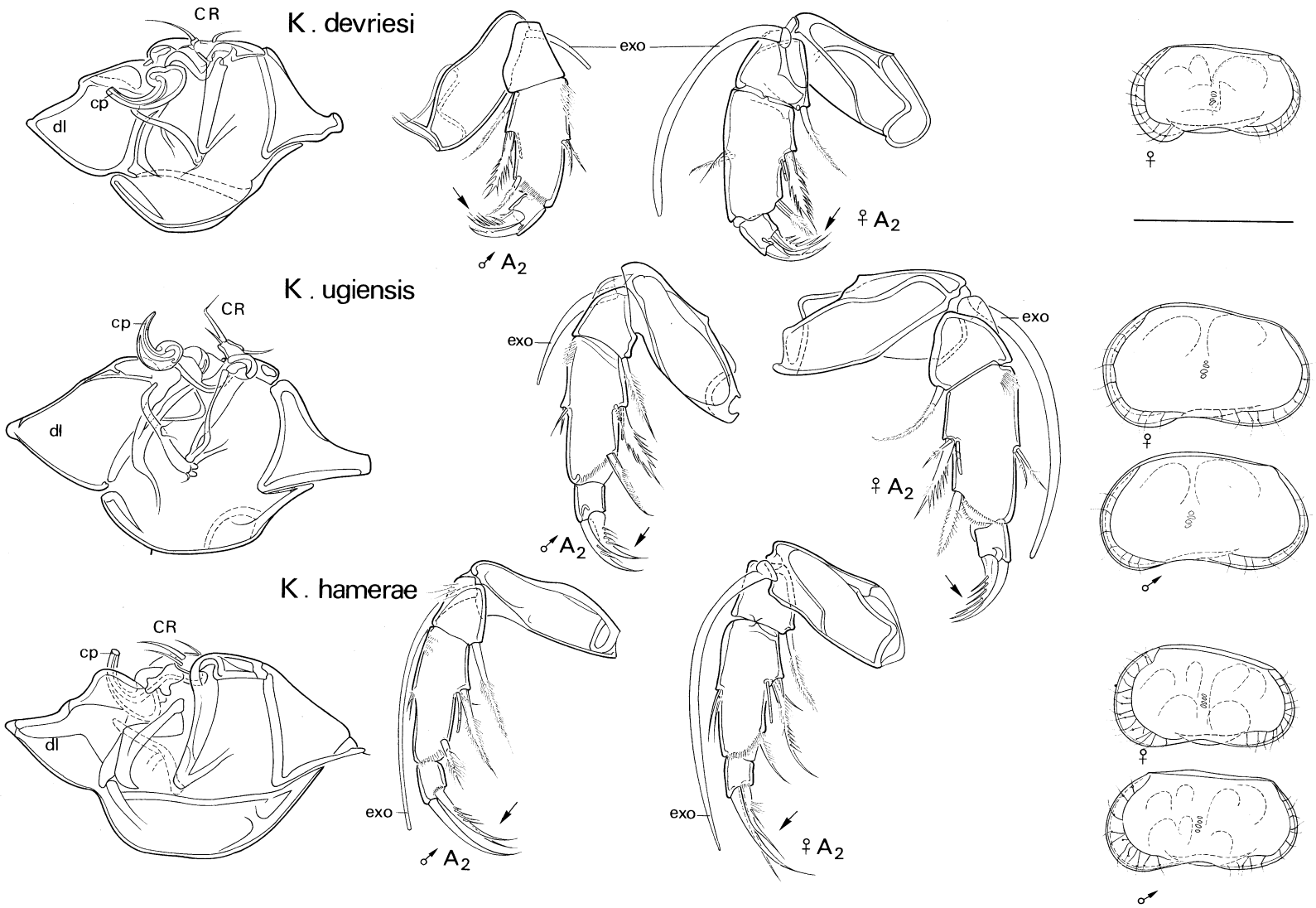


*Figure 3.* Copulatory modules in three species of *Korannacythere*: hemipenis (right), male and female A2 (middle) and right valve, internal view (right). Scale=33 $\mu$m for hemipenes and A2, 315 $\mu$m for valves. Modified after Martens (1996).

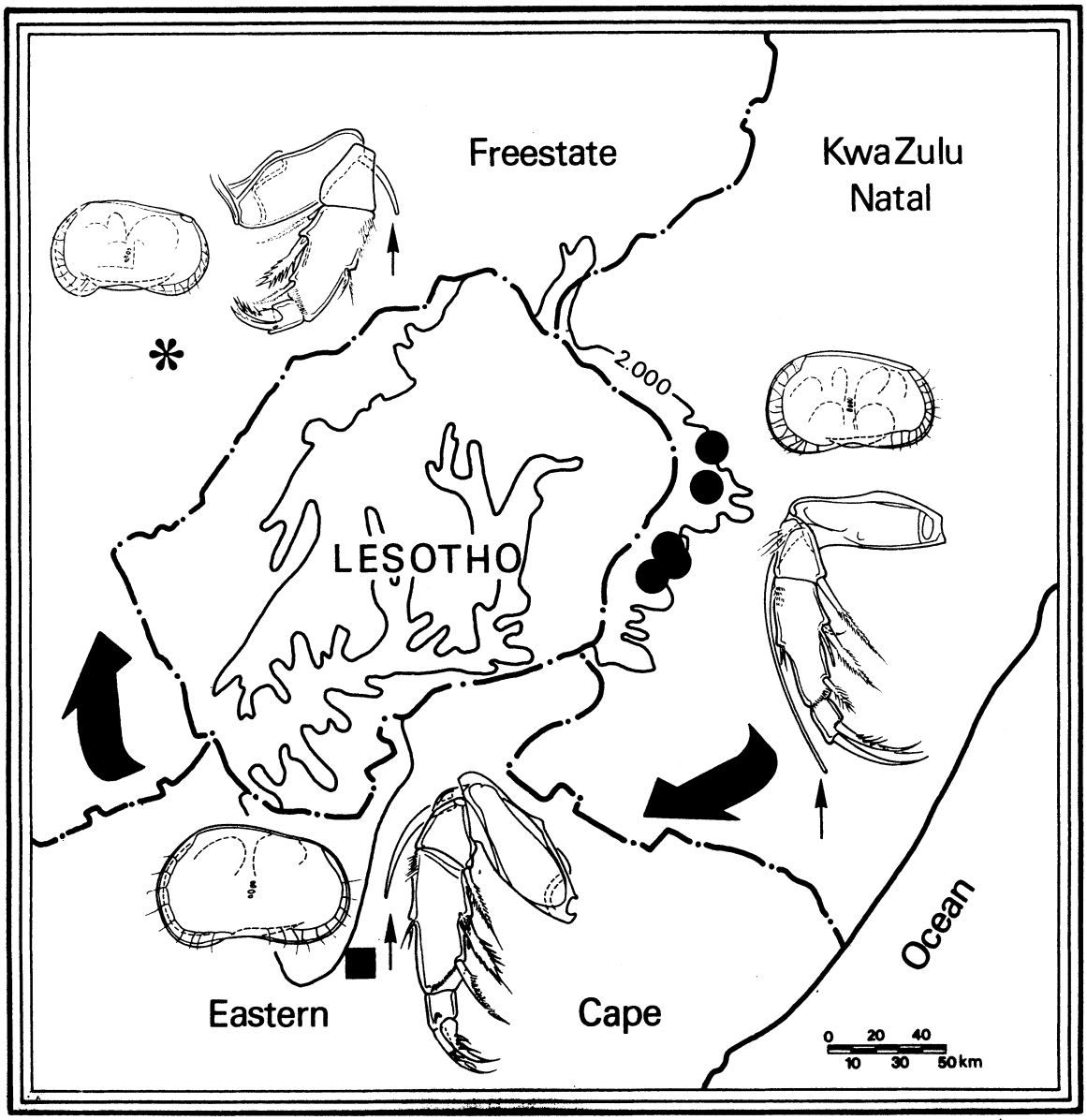


*Figure 4.* Geographic distribution of species of *Korannacythere* around the Drakensberg (South Africa), showing female right valve and male A2. Asterisk: *K. devriesi*, squares: *K. ugiensis*, dots: *K. hamerae*. Modified after Martens (1996).

the different evolutionary lineages. All, apart from *Korannacythere*, have clearly dimorphic valve and have at least some part of the copulatory complex of the hemipenis which shows clear specific differences. Some groups (*Limnocythere, Leucocythere* s.l.), but strangely enough not all, have species-specific copulatory processes. I hypothesise that, apart from mate recognition, the extended and heavily spiralled (corkscrew-like) copulatory process in the genus *Leucocythere* might have a function in removal of sperm of previous males. This form of sperm competition has not yet been directly observed or shown in podocopids, but the inferred functional morphology of at least the leucocytherid copulatory process strongly hints at this.

In the *Paralimnocythere* and the *Neolimnocythere* lineages, the hemipenal caudal ramus shows directional enlargement. The clasping organ shows diversification in two lineages: in the lower ramus in *Limnocythere* s.l. (upper ramus absent or uniform) and

in the upper ramus in *Leucocythere* s.l. (lower ramus absent or uniform). Significant modifications in other limbs occur in A1 and T3 in the *Leucocythere* group, in the A2 in *Korannacythere*.

In spite of the fact that Limnocytheridae is an old group (c 300–350 Ma – Whatley & Mouguilevsky, 1998), such a wide range of elements in the copulatory module is exceptional in the Podocopina, which normally only have hemipenal features (moreover internally situated in the Cypridocopina), combined with specific sexual dimorphisms in valves and one pair of limbs (in Cypridocopina the fifth limb, modified to asymmetrical clasping organs). Even in the sister group to the Limnocytherinae, the Timiriaseviinae, morphological sexual dimorphism is mostly limited to valves, hemipenes and, in some cases, one pair of limbs (mostly T3, Martens, 1995). This pattern of recognition systems is the result of a combination of stochasticity, selection processes and constraints.

*Stochasticity, preadaptation and developmental constraints determine which structures are involved in the mate recognition systems*

The initial choice of the structure which will be incorporated into the copulatory complex, and which will undergo morphological diversification, happens either 1. through the existence of some preadaptation in that structure, or 2. by chance, while 3. certain structures can be excluded through developmental or other phylogenetically linked constraints.

Certain somatic limbs in the copulatory complex might have had sexually dimorphic *preadaptations* for other functions, prior to their inclusion in the copulatory module. One could speculate that, for example, the T3 could have developed a cleaning function (for the hemipenis) in the males of certain groups. However, no evidence for any of the limbs presently known to be involved in mate recognition is available for the Podocopina. The potential relevance of preadaptations during further stages of the divergence of SMRS are discussed below.

Preadaptation is most likely not an important factor in the choice amongst the different hemipenis structures, as this organ does not have a function other than during courtship and actual copulation. At least in the hemipenis, the initial choice of structure occurs largely by *chance*. In *Limnocythere*, the lower ramus of the clasping organ has known a hyper-development and has even diversified into two different processes, while the upper ramus is completely absent or remains undiversified. The complete opposite has occurred in *Leucocythere*. Similarly, specific morphologies in the caudal ramus of the hemipenis are unknown in the entire *Limnocythere*-group, while the same appendage has a (gradual) hyper-development in other lineages in the Limnocytherini. Note that also somatic traits (limbs) can become incorporated in the copulatory module by chance, for example by a mutation in a gene complex responsible for the development of such limbs.

The interpretation of the stochastic origin of specific hemipenal morphologies is challenged by the 'mate check' hypothesis (Jocqué, 1998), which holds that parts of the SMRS not only signal suitability as a potential partner for reproduction, but actually convey information of hidden, but crucial (behavioural), traits. Following this hypothesis, female choice is but a secondary effect of this 'mate check'. Initial choice of secondary sexual characters that eventually show morphological radiation in the lineage would then not be random, but linked to somatic traits of which information needs to be conveyed. Again, there are at present no data indicating the validity of this hypothesis for the podocopid Ostracoda.

A final type of effects on the divergence of recognition systems are *developmental and phylogenetic constraints*. I treat these together, as Williams (1992) argued that the former is a special case of the latter. Developmental constraints could, for example, explain the aberrant morphology of *Korannacythere*. The sexual and specific morphological differences in the A2 of *Korannacythere* replace the normal differences in hemipenis morphologies, which is highly simplified, through complete reduction of the entire clasping organ. This simplification of the hemipenis is related to a reduction in carapace size and a simplification of the (male) valve shape. The ability to produce sexually dimorphic valves in the male could have been lost in the final ontogenetic stages of an ancestor of this group. This would at the same time have been a pre-adaptation to life in ion-poor environments, as smaller carapace size would be advantageous. Weakly calcified small valves are more easily strengthened by shape and sculpture than large ones (Danielopol, 1976). Species of *Korannacythere* indeed live in temporary rock pools on the Drakensberg sandstone of South Africa, which have a very low ionic content of the water (EC=c 10 $\mu$S/cm, Martens, 1996). But such neotenic valve shapes would in their turn impose secondary constraints on hemipenis development. This is

an alternative to the adaptationist scenario developed below.

As such a copulatory complex thus far developed in *Korannacythere* only, this can also be considered an example of a phylogenetic constraint. However, better examples are necessary, as Williams (1992) has calculated that, in order to obtain a level of significance of $P < 0.05$, at least six lineages with either trait must be available. The assessment of the present group does not fulfil these conditions entirely, but Table 3 indicates that the absence of somatic limbs in the SMRS in the Limnocytherini, as opposed to the inclusion of A1, A2 or T3 in Leucocytherini, is most likely phylogenetically constrained.

Wills (1989), finally, coined the term *evolutionary facilitation* for processes that enhance evolutionary potential for adaptation to rapidly changing environments. These processes involve restructuring the genome in ways that allow integrated parts of the genome to change fast and cause rapid adaptations in *certain* aspects of an organism's morphology and biology. The existence of such evolutionary toolboxes, as Wills (loc. cit.) called them, would explain enigmatic phenomena such as mimicry, repeated parallel evolution and even punctuated equilibrium, but can also be applied to the concept of phylogenetic constraints. Furthermore, while these concepts were thus far thought to be adaptive and moulded by natural selection, similar processes can be invoked in the evolution of mate recognition systems. The presence of different genetic toolboxes could thus explain why different parts of the body experience morphological differentiation in related lineages. This remains entirely speculative. Wills' concept has thus far been ignored in evolutionary biology, this in spite of the fact that it has potentially great explanatory power for various paradoxical processes.

### *Sexual selection causes divergence of recognition systems and, ultimately, radiative speciation*

While preadaptation, stochasticity and developmental (phylogenetic) constraints initially determine which structures in the recognition system will be subject to modification in a given lineage, further morphological diversification within the group will then occur primarily through sexual selection. This is a controversial issue. Paterson (1993b) argued that sexual selection, as defined by Darwin, is an intraspecific process, while recognition clearly acts interspecifically, and acolytes of the recognition concept school thus refute the potential effect of sexual selection on recognition systems. Following this distinction, acceptance of a male by a female as a mate (*female choice*), thus involves two cognitive actions (decisions) of the female: the potential mate must first be recognised as conspecific (*recognition*), then must possess a sufficiently high fitness within the pool of potential conspecific males (*selection*). Ryan & Rand (1993) argue against this distinction, and instead suggest using the terms *recognition* when one individual considers another an appropriate mate, even if mistaken, and *preference* when one individual tends to mate with one individual rather than another, thus implying a comparison. Recognition can, therefore, be used for species recognition (interspecific), but can also be applied to recognition of sex, kin etc., i.e. intraspecific. Preference is then directly related to sexual selection and to speciation. The possibility that sexual selection can actually lead to diverging recognition systems through signal-receptor coevolution, was extensively discussed by Andersson (1994). Several observations (discussed below) support the, at least partial, relevance of such mechanisms in the development of the different recognition systems in the Podocopina in general and in the Limnocytherinae in particular.

Sexual selection, and the subsequent divergence of recognition systems, requires an initial degree of intraspecific variability within the SMRS. This variation can either be geographically or ecologically linked, but must necessarily have a genetic component; otherwise neither sexual, nor other natural selection can act upon it. The existence of such variability within SMRS seems paradoxical, and was indeed refuted by several authors, e.g. by Paterson (1993b): if conspecific mates recognise each other through the SMRS, than its efficiency would be higher with larger uniformity, as the risk of errors in mate recognition would be reduced. There are indeed examples to support this view. Gray & Cade (1999) found that females of certain cricket species prefer males with average number of pulses in their song. This is a case of female preference imposing stabilising sexual selection, leading to decreasing variability in the male trait and not causing divergence of mate recognition systems.

Nevertheless, variability within the SMRS does exist in other groups and has meanwhile been described from several studies (for example Ryan & Rand, 1993). For sexual selection to increase variability of mate recognition traits, it will have to be positive directional (e.g. for size of body or of ornaments) (Weatherhead et al., 1987). In podocopid ostracods, the

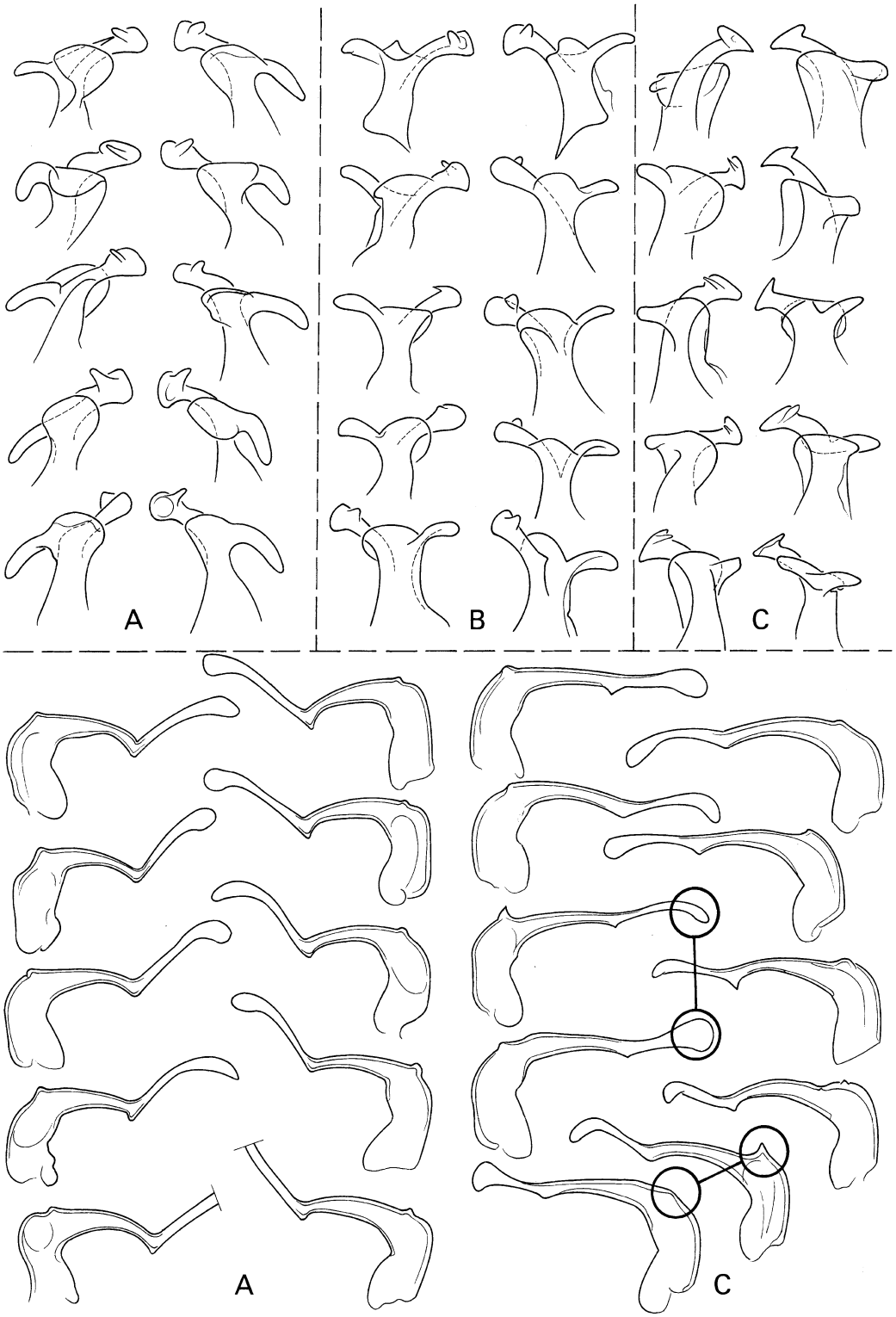


*Figure 5.* Variability in lateral process (top) and hook-like process (bottom) of lower ramus in hemipenis of subspecies of *Limnocythere borisi* from Ethiopian lakes. (A) *L. borisi borisi* (Lake Abijata), (B) *L. borisi awassaensis* (Lake Awassa), (C) *L. borisi shalaensis* (Lake Shala). Left and right structure of each pair belong to the same individual (left and right hemipenis). Examples of important variation amongst and within individuals indicated with circles. Modified after Martens (1990a).

presence of geographical variability in hemipenis morphology has been demonstrated in *Cythere omotenipponica* by Tsukagoshi (1988). Both geographical and intra-populational variability in the copulatory complex of the *Limnocythere borisi-thomasi* complex in a geographically limited area (the Zwai-Shala basin) was illustrated by Martens (1990a,b) (Figure 5).

In the presence of such variability, slightly diverging preferences amongst receptors (females), can cause initial deviations from normal distributions in variations in male signalling traits within and amongst populations, as signals and receptors coevolve in normal conditions. Several mechanisms can then further this evolution into speciation, or will at least accelerate this process, mostly in sympatric or at least parapatric conditions. *Reinforcement* occurs in conditions with gene flow between populations (i.e. different species have not yet been formed), which will diverge owing to selection against hybrid offspring (e.g. Butlin, 1989). This selection can be the result of decreased fertility of hybrids (in the first or second generation), but also because hybrids may be less successful in competition over mates. Subsequent sympatry of both lineages can then further enhance the divergence through the process of *(reproductive) character displacement*, a mechanism which increases the magnitude of specific signals between isolated species (i.e. without gene flow) in sympatry. Both processes, but especially the latter, will reduce wasted reproductive efforts which would lead to (infertile) hybrids.

Tsukagoshi (1988) reported character displacement in Japanese species of the podocopid genus *Cythere*. Although his data do not fully support a classical case of character displacement, they still indicate that the morphology of the copulatory complexes of hemipenes in this cytherid genus are subject to selection processes, which are not related to ecology of the abiotic environment, and are, therefore, most likely of sexual nature. The (mono- or at least paraphyletic) *Limnocythere* radiation in the Zwai-Shala basin of East Africa (Martens, 1990a,b) constitutes another example of the diversification of copulatory modules, most likely through sexual selection. As it was demonstrated that lake level fluctuations have united and separated at least 4 of the 5 lakes at various stages during the Pleistocene (summary in Martens, 1990b), there has been ample occasion for both the sympatric and the allopatric processes to have been active.

Both examples provide circumstantial evidence only and actual sexual selection in Podocopida remains to be demonstrated directly.

*Natural selection constrains divergence of recognition systems*

It has been argued (West-Eberhard, 1983) that certain dimorphic morphologies might be due to different biological functions of the sexes, for example: different feeding strategies, brooding, superior ability of males to locate females (i.e. different locomotory modules, rather than copulatory modules – Danielopol et al., 1990). Although the potential of such preadaptations cannot be ruled out (see above), there are several arguments against applying this idea to the specific and dimorphic morphologies discussed here.

Morphologies of the male A1, A2 and T3 as observed in the Limnocytherinae will cause a reduced mobility, rather than increased agility. For example, the smaller male exopodite in A2 in *Korannacythere* species will not increase mobility and hence will not make that males are better equipped to locate females. The same is true for the aberrant morphologies of male A1 and T3 in *Leucocythere* s.s.: although some setae have become larger, these freak appendages will rather obstruct than facilitate locomotion. The sexual dimorphism in the chaetotaxy of the A2 in many Cypridocopina (Broodbakker & Danielopol, 1982; Martens, 1987), on the other hand, is most likely simply a functional feature during mating which has been subject to natural selection, not to sexual selection, as these morphologies are sexually dimorphic, but never have species-specific shapes and hence cannot serve in mate recognition.

There are, furthermore, no indications that males and females have different feeding strategies. Both sexes occur on the same sediments and no habitat segregation is apparent (Martens, 1990a for *Limnocythere*). Limnocytherinae are not brooders, but in the extant brooding Cytherocopina (including Timiriaseviinae), it is always the female which has the brooding cavity, so that (apomorphic) male adaptations are not related to brooding.

Natural selection in mate recognition traits mostly works as a stabilizing (Moore, 1990) or negative directional force (Weatherhead et al., 1987) and always puts constraints on the maximum development of the trait. In the case of podocopid ostracods, for example, the ornaments on the hemipenis can never become so large that they no longer fit in the closed carapace. Together with Vermeij (1987) and Williams (1992), I see merit in pondering about why certain body plans do not exist. In this particular case, one could ask why certain limbs (for example Mx1, Md, T1 or T2) are not

involved in the SMRS. It could be that the functional constraints imposed by the fact that these limbs are heavily involved in other modules, respectively feeding and locomotory, are too important to allow them to play a role in the copulatory module. Modification to such limbs would decrease fitness and natural selection would weed out such traits. In Limnocytherinae, T3 can be modified, but then both T1 and T2 are needed for crawling. In most male Cyprididae, the T1 is modified to a clasping organ, while T3 is a cleaning limb, but these groups primarily use swimming locomotion, while heavily developed caudal rami are most important in crawling. Alternatively, however, the fact that such species are thus far unknown could entirely be a matter of chance: either such SMRS have not yet been developed, or have not yet been discovered. The aberrant body plan of *Korannacythere* was only described in 1996 and preliminary results indicate that various other copulatory modules might exist in the Limnocytherinae of the South American Altiplano.

*An adaptationist's view on the evolution of* Korannacythere

The overall size reduction and the loss of the sexually dimorphic shape of the valves in *Korannacythere* could be a pre-adaptation, as discussed above. However, I argue that this is an adaptation, through natural selection, to the low ionic content of these pools. The latter indeed affects the species of this genus, as they often live with weakly calcified to nearly uncalcified valves. It probably also explains the nearly adont hinge structure in this lineage. Smaller carapaces are more easily strengthened. For example, the unisexual valve shape might be closer to the ideal (most strengthened) rounded valve shape. The small valves without dimorphic shape then constrain the development of the copulatory appendages. There are no reasons why then the A2 started to differentiate amongst populations, as this is an absolutely novel feature within the entire subfamily. But likewise, there is no evidence to date that the morphological differentiation of this limb can be linked to any ecological or biological feature, other than to recognition and/or sexual selection through female choice. Speciation most likely occurred in a geographical cline, from east to west along the Drakensberg (Figure 4), so that these processes must have occurred parapatrically, i.e. with a certain degree of gene flow. Following this working hypothesis, *Korannacythere* thus shows all three elements acting on the development of recognition systems: adaptation to ion-poor habitats through natural selection prevented normal (usual for the lineage) development of specific recognition traits in the hemipenis; stochasticity caused the A2 out of all other limbs to be subjected to sexual selection (a novel situation in the lineage), and the latter processes caused it to be part of the copulatory module.

*Testing these hypotheses*

There are various ways in which parts of the above hypotheses can be tested. Most obvious is the need to directly observe the use of the different parts of the copulatory module during mating: do males of *Leucocythere* effectively use their A1 and males of *Korannacythere* their A2 to stimulate females? Are the copulatory modules effective as reproductive barriers, i.e. will females of one species of *Korannacythere* effectively refuse copulation with allospecific males? Some of these experiments are presently being carried out. Other breeding experiments could analyse the degree of sexual selection in species with geographical variation in the copulatory modules, like in the case of *Cythere omotenipponica* (Tsukagoshi,1988). Molecular phylogenies will allow mapping of the different morphologies on phylogenetic trees and correlation of morphological similarity of the recognition system to phylogenetic relatedness. This will allow to test for the importance of either stochasticity or phylogenetic constraints. However, this requires more complete phylogenies than are available at present.

Most tests, however, will of necessity be non-experimental and will rely on new discoveries, for example of species of *Korannacythere* in pools with higher ionic content. The case-study of the *Limnocythere*-cluster in the East African Zwai-Shala lakes can be followed up by analysing the Quaternary fossil record. Timing of appearance of the different extant species in the history of these lakes will provide information on the tempo of speciation and can be linked to hydrological situations: did the species appear during high lake stands (sympatric conditions) or during periods of low lake levels (allopatric in separate lake basins).

These examples are not exhaustive, but only indicate that there are indeed alternative methods to test such hypotheses. The fact that only a limited number of experimental approaches are possible (mostly breeding experiments) is not necessarily a problem, as discoveries of new species, new localities or of new fossil evidence are as much falsifications of hy-

potheses in inductive sciences (like phylogeny and biogeography) as are experiments in deductive sciences (Martens & Danielopol, in press).

## Conclusions

The subfamily Limnocytherinae shows a wide range of morphological traits, which form part of the copulatory module, (i.e. all aspects of morphology involved in mating, and which is thus largely congruent with the morphological part of the Specific Mate Recognition System). Although there is as yet no direct evidence for any of the processes, various elements support the importance of stochastic processes (chance), preadaptations and developmental constraints in the initial choice of structures and of subsequent impact of sexual selection on recognition systems, causing radiative speciation within lineages. Natural selection at all times puts constraints on the development of structures and systems and thus directs, or at least influences, speciation.

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# An example of intralacustrine evolution at an early stage: the freshwater ostracods of the Miocene crater lake of Steinheim (Germany)

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*Key words:* Steinheim basin, freshwater ostracods, ancient lake, palaeo-ecology, speciation, intralacustrine evolution

**Abstract**

I present the findings of two detailed studies on Steinheim lake ostracods carried out over the last eight years. The 178 samples studied cover a combined section about 30 m thick, comprising the whole sequence of seven planorbid beds. Of the 53 species found, 44 occur in the basic sediment layers, the *kleini* beds. In contrast, all of the subsequent beds contain 16 species, and only seven species withstood the extinction at the *kleini–steinheimensis* boundary. This may be mainly due to the loss of littoral habitats brought about by a constantly increasing lake level, as the majority of the species in the *kleini* beds were shallow water dwellers. Long-term lake level fluctuations are considered the most important external factor of the post-*kleini* period. Density fluctuations of the ostracods *Pseudocandona steinheimensis* and *Potamocypris gracilis* broadly reflect these water level fluctuations. At the beginning of the *sulcatus* period, when the lake level was very high, some species show morphological changes in their carapaces. During this time *Leucocythere immigrata* split into the daughter species *L. sieberi* and *L. esphigmena*. In the following *trochiformis* period, when the water level was lowest, the most curious carapace sculptures appear. The morphological changes detected, however, cannot simply be interpreted as ecophenotypic. The speciation event in *Leucocythere*, the non-recolonization with ubiquitous species during the low water level stage (*trochiformis* period), as well as the types of morphological changes indicate mainly evolutionary processes, as also the planorbid molluscs prove. Moreover, ostracods and planorbids show convergent evolutionary patterns. Compared with data on ostracod speciation in ancient lakes, the ostracod assemblage of Lake Steinheim seems to be a good palaeontological example of intralacustrine evolution at an early stage.

## Introduction

Ostracod and gastropod shells are the most abundant fossils among the Miocene freshwater deposits of the Steinheim basin. Since Hilgendorf (1863, 1867) postulated intralacustrine evolution of the Steinheim planorbids, scientific interest has mainly focused on the planorbid gastropods. Hilgendorf's (1867) phylogenetic tree, in which he explained his hypothesis, is one of the first palaeontological documentations of gradual speciation. His hypothesis, heavily disputed at that time, was largely confirmed over the last two decades. Mensink (1984) proved the gradual transition of Hilgendorf's main branch planorbids by means of biometrical investigations. This data set was reinvestigated by means of multivariate methods (Povel, 1993). By S.E.M. analysis of the protoconch structures Gorthner (1992) and Nützel & Bandel (1993) were able to show that both Hilgendorf's main branch and side branch planorbids are valid species. Because of the similarity of the heavily sculptured species of the Steinheim lake with endemic species of ancient lakes, Gorthner & Meier-Brook (1985) postulated that the Steinheim lake was a 'long-lived' lake.

Although they are well preserved, the Steinheim ostracods had been studied only from a small section of the lake deposits in the past, and the two earlier studies (Lutz, 1965; Sieber, 1905) did not refer to Hilgendorf's hypothesis. In order to improve our knowledge of Steinheim ostracods, the author conducted a first detailed bed-by-bed investigation from 1989 to 1990. Ostracod samples of all planorbid beds, except the basal *kleini* beds, were studied (Janz, 1992a). Over the last 2 years a second study, dealing with

ostracods of the *kleini* beds, was carried out, which completed the profile (Janz, 1997).

The present paper summarizes the findings of both studies. As the Steinheim ostracod assemblage shows both a relation to abiotic factors and a similar pattern of shell alterations as in the planorbids, discussion focuses on the convergent evolutionary patterns of ostracods and gastropods, and the possible causes and mechanisms which might have provoked the phenomena detected. According to the review of ostracod speciation in ancient lakes by Martens (1994) the ostracod assemblage of Lake Steinheim is interpreted as an example of intralacustrine evolution at an early stage.

*The Steinheim basin*

The Steinheim basin is situated on the Swabian Alb in southern Germany (latitude 48° 41′ N, longitude 10° 04′ E) (see Figure 1). It was formed by a meteorite impact during the Middle Miocene, presumably simultaneously with the bigger Ries crater, 40 km away (Groschopf & Reiff, 1966). Thus, the radiometric age of the Ries (15.1 ± 0.1 Ma) (Staudacher et al., 1982) is considered to be also valid for the Steinheim basin. The Steinheim basin is a complex impact crater structure with an almost circular outline, a diameter of about 3.5 km and a central uplift of about 50 m visible height. Originally, the immediate crater impact was about 220 m deep. The impact debris (thickness 40–50 m) covered the bottom during the first few seconds. Then the crater filled with water and became a lake. As there is no evidence for creek or river influx, it is supposed that the water supply came from the subterranean karst system and from precipitation.

The preserved lake sediments reach a thickness of 30–40 m. When the lake period had ended, it is probable that the basin was completely filled with lake sediments. That the basin can be recognised again today, is due to partial erosion during the Quaternary, caused by a river entering the basin from the NW. It bifurcated at the central hill, one branch running through the western, the other through the eastern part of the basin. Thus, the latest sediments (*supremus* beds) are preserved only near the SE margin of the basin (Knill). In contrast, in the western basin, where erosion was most marked, only the oldest sediments (*kleini* beds) are still preserved (Bahrig et al., 1986; Reiff, 1976, 1988, 1992).

The lake sediments consist of calcareous siltstones, arenites and limestones. Lithostratigraphically, the deposits cannot be subdivided, but using the planorbids as markers, a biostratigraphic subdivision is possible. Hilgendorf (1867) defined 10 planorbid zones by using in each case the first occurrence of a new planorbid morph of his main branch as a marker. According to Mensink (1984) the subdivision into seven planorbid beds is applicable (from the lowermost to the uppermost beds): *kleini, steinheimensis, sulcatus, trochiformis, oxystoma*, *revertens* and *supremus*.

It is not exactly known for how long the lake actually existed. Gorthner (1992) suggested between some hundreds of thousands to more than one million years. If it is true that the Steinheim deposits included two mammal units (MN 6 and MN 7), the lake must have existed even longer, about two million years (Heizmann & Hesse, 1995; Reiff, 1988). While the well studied *trochiformis* and *oxystoma* beds serve as a reference locality for MN 7, however, no striking evidence for MN 6 has been found in the older beds so far.

The lake's history is mainly characterized by long-term lake level fluctuations, presumably due to tectonic activities (Reiff, 1988), and to a minor extent, to evaporation as well (Bajor, 1965). These factors will be discussed in more detail below.

## Material and methods

The material studied originates from: seven pits on the western margin of the basin (Ga–Gg), one drilling core some hundreds of meters NE of these pits (B), three sections around and from the former Pharion's sand pit at the slope of the central hill (SF, S, Ph), and one pit at the SE margin of the basin (K) (see Figure 2).

Sections B, SF and S could be directly correlated using characteristic layers with fish and leaf residues, while the remaining sections were ranged in by the planorbid markers. The resulting combined section comprises about 30 m thickness, and covers the whole sequence of all seven planorbid beds. Altogether 178 samples have been studied. For further details concerning location and description of the sections, as well as the methods of sample treatment and analysis, see Janz (1992a, 1997).

## Results

*Species spectrum and stratigraphic range*

Table 1 lists the 53 ostracod species found in system-

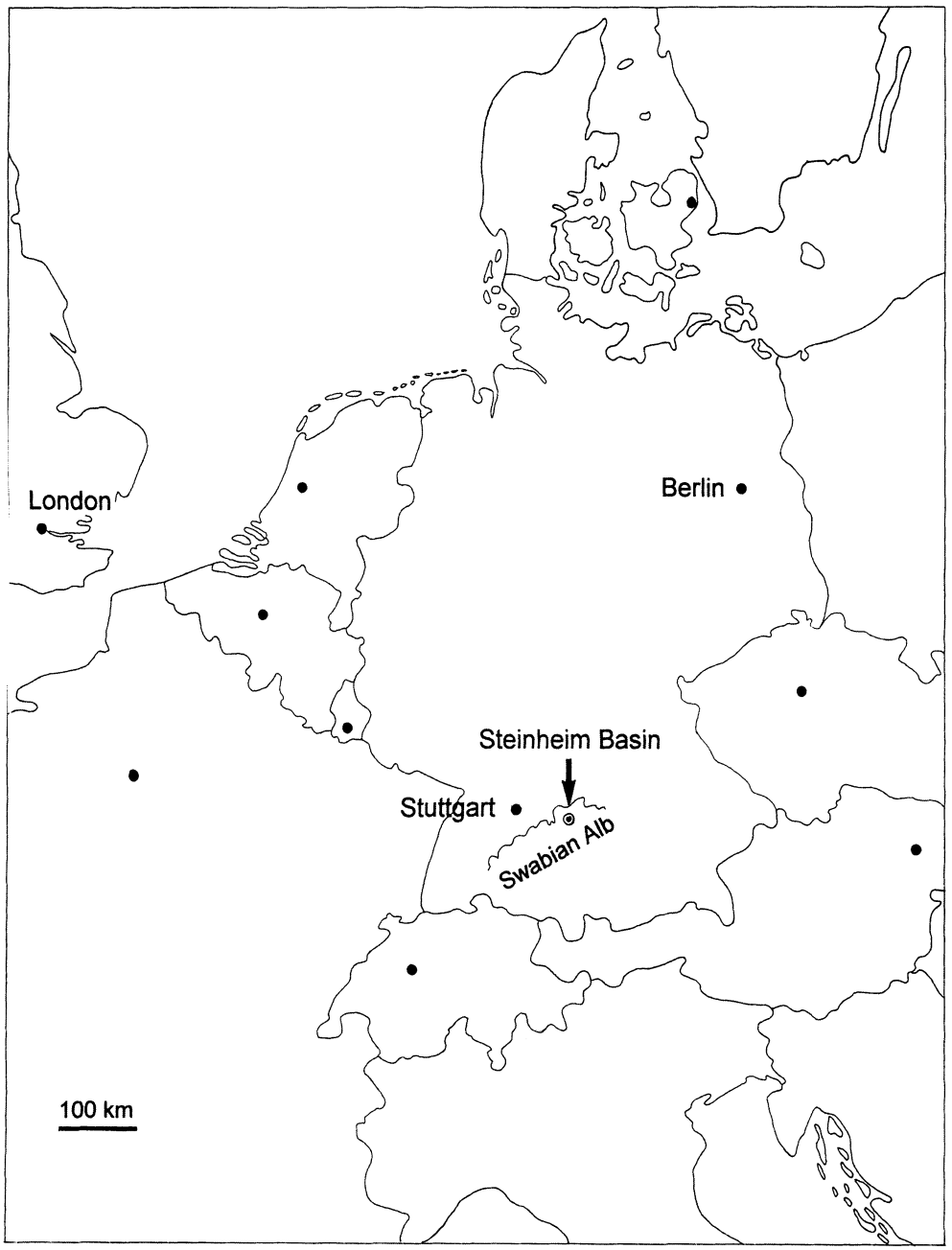


*Figure 1.* Location of the Steinheim basin.

atic order. Species of all three Ostracoda superfamilies with freshwater species, Cytheroidea (four spp.), Darwinuloidea (two spp.) and Cypridoidea (47 spp.), were found. Most of the species belong to the families Candonidae (20 spp.) and Cyprididae (24 spp.). Owing to the still insufficient knowledge of Miocene freshwater ostracods, 21 species had to be retained in open nomenclature. Two species, *Leucocythere immigrata* Janz 1992 and *Cypris falki* Janz 1997, have been described as new. All species are characterised and documented, and if possible, larval stages were delimited by means of height-length diagrams, by Janz (1992a, 1997).

The diagram in Figure 3 shows the stratigraphical range of the 53 species within the planorbid beds. From the *kleini* beds onwards, a drastic reduction in

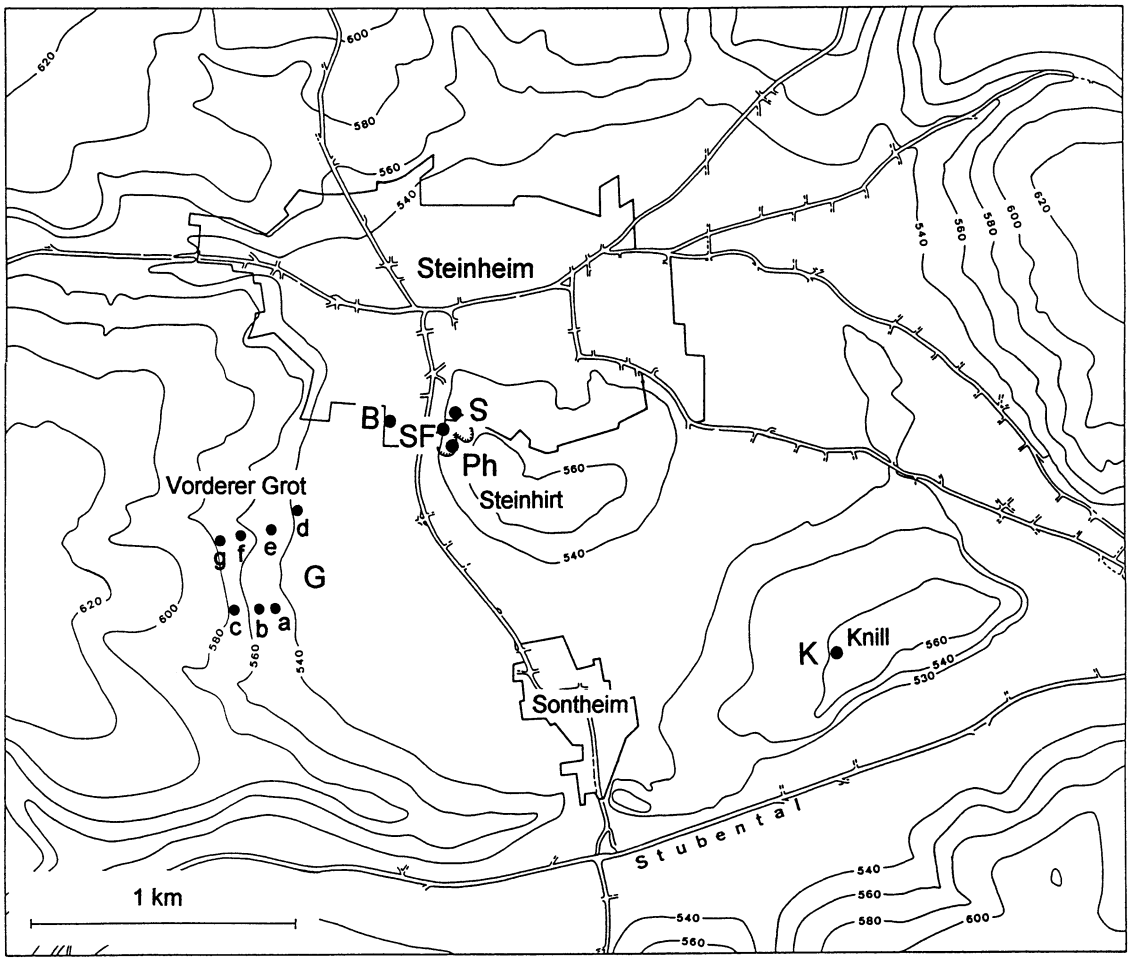


*Figure 2.* Topographical map of the Steinheim basin and situation of the sampled sections.

number of species at the end of the *kleini* period and the beginning of the *steinheimensis* period occurred. Of the 44 species of the *kleini* beds, 37 species (70% of all species discovered) occur exclusively in these layers, and only seven species withstood the extinction at the *kleini-steinheimensis* boundary. In the post-*kleini* period, nine further species occur, four of which are only recorded by a single valve. It is worth mentioning that of the non-persisting species of the *kleini* beds, only a single species, *Cyprinotus inaequalis*, resettled at a later period (no. 38 in the *oxystoma* beds).

## *Ecology*

Despite the diminution in the number of species at the *kleini–steinheimensis* boundary, the rich fossil record and good state of preservation generally suggest suitable living conditions in the crater lake throughout its history. This seems obviously due to the calcium-rich water (Bajor, 1965). Long-term lake level fluctuations are considered the most important factor influencing the biotope both directly and indirectly.

### *The* kleini *period*

The section of the *kleini* beds studied did not include the earliest sediments deposited in a low level stage, which was described as 'early lake stage' by Bahrig et al. (1986). Although most of the species found in the *kleini* beds can be classified as shallow water dwellers, their distribution pattern within the seven pits proves that the crater lake had already formed a distinct littoral and profundal zone at that time. The high species diversity as well as the abundant occurrence of typical littoral species prove that the littoral zone is represented by Gc, Ge, Gf and Gg, while the profundal zone, Ga, Gb and Gd, shows a low diversity and a lack of littoral species. Additionally, the latter zone is also characterized by the occurrence of *Nitellop-*

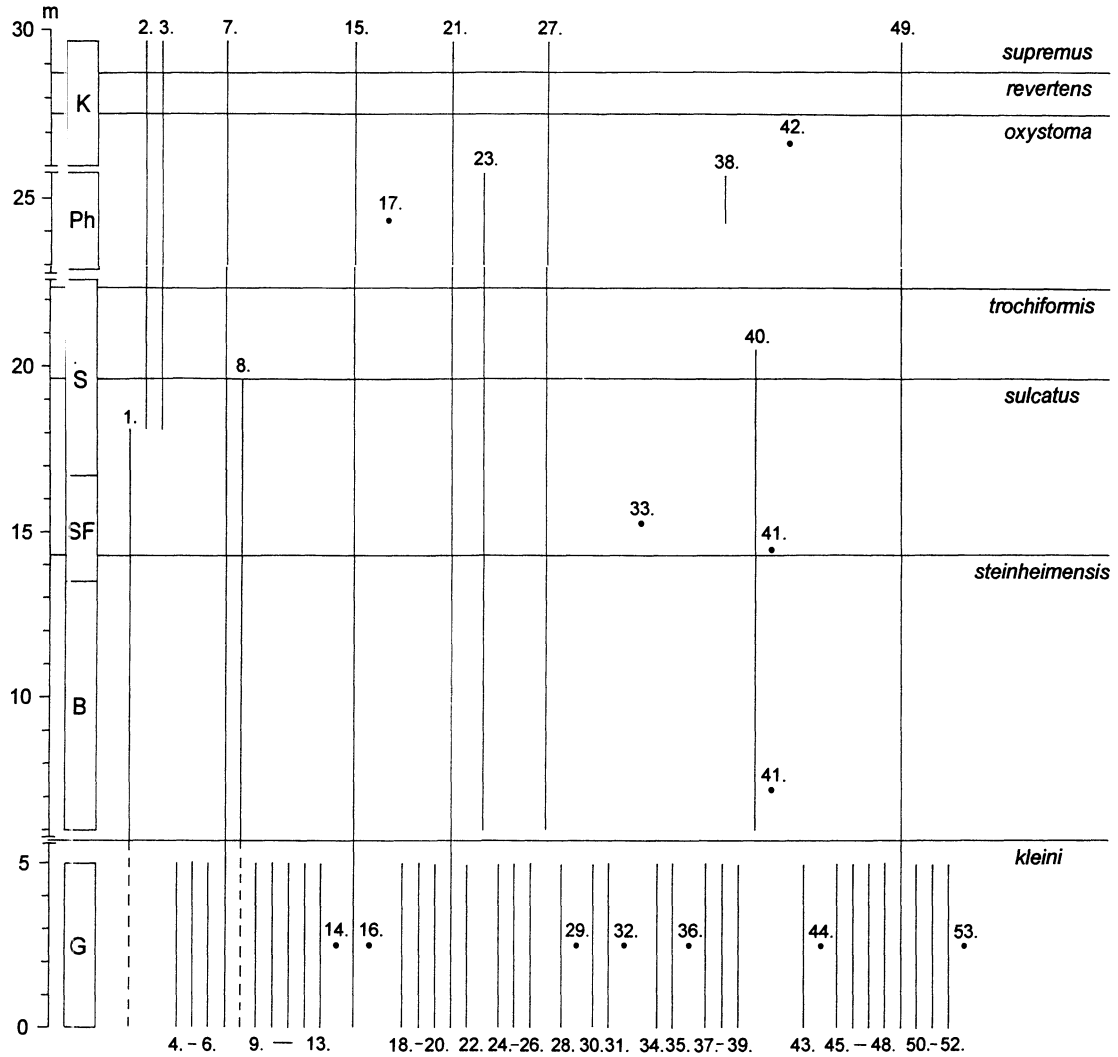


*Figure 3.* Correlation of the sections and stratigraphic range of the ostracod species within the planorbid beds. The numbers refer to Table 1.

*sis* charophytes which are indicative of deeper water conditions (see Schudack & Janz, 1997). This means that the section studied represents the middle to late *kleini* period, when the second lake stage according to Bahrig et al. (loc. cit.), the 'transgression stage', had already started.

The occurrence of *Cavernocypris subterranea* in Gb and Gc supports the hypothesis of water supply through a connection of the lake's basin to the subterranean karst system and/or an inflow of cold springs.

*The* kleini–steinheimensis *boundary*

What could have happened at the *kleini–steinheimensis* boundary? Are there drastically changing variables which could be responsible for the decrease in the number of species?

Although sediment chemistry of the *kleini* beds is still insufficiently understood, the sediments of both *kleini* and *steinheimensis* beds are of the same kind, and provide no evidence of a radical change in water chemistry. Nevertheless, two factors might have contributed to the disappearance of three-quarters of the species at that time.

(1) A loss of littoral habitats. Because of the steep crater rim the gradually rising water level could have led to a loss of littoral habitats causing the disappearance of shallow water dwellers.

(2) A temperature drop. The large number of species with a preference for warm conditions among

*Table 1.* The ostracod species of the Miocene lake deposits of the Steinheim basin listed in systematic order

Superfamily **CYTHEROIDEA**

Family **Limnocytheridae**
Subfamily Limnocytherinae
1. *Leucocythere immigrata* Janz 1992
2. *Leucocythere esphigmena* (Sieber 1905)
3. *Leucocythere sieberi* (Lutz 1965)

Subfamily Timiriaseviinae
4. *Metacypris cordatoides* Carbonnel 1969

Superfamily **DARWINULOIDEA**

Family **Darwinulidae**
5. *Darwinula stevensoni* (Brady & Robertson 1870)
6. *Darwinula cylindrica* Straub 1952

Superfamily **CYPRIDOIDEA**

Family **Ilyocyprididae**
7. *Ilyocypris binocularis* Sieber 1905
8. *Ilyocypris* sp.

Family **Candonidae**
Subfamily Candoninae
9. *Candona* (?) sp. 1
10. *Candona* (?) sp. 2
11. *Candona* (?) sp. 3
12. *Fabaeformiscandona fabaeformis* (Fischer 1851)
13. *Fabaeformiscandona* cf. *balatonica* (Daday 1894)
14. *Fabaeformiscandona* (?) sp.
15. *Pseudocandona steinheimensis* (Sieber 1905)
16. *Pseudocandona* cf. *marchica* (Hartwig 1899)
17. *Pseudocandona* cf. *ratisbonensis* (Lutz 1965)
18. *Pseudocandona* sp. 1
19. *Pseudocandona* sp. 2
20. *Candonopsis* cf. *kingsleii* (Brady & Robert. 1870)
21. *Candonopsis arida* Sieber 1905
22. *Paracandona euplectella* (Brady & Norman 1889)

Subfamily Cyclocypridinae
23. *Cyclocypris nitida* Sieber 1905
24. *Cyclocypris ovum* (Jurine 1820)
25. *Cyclocypris* cf. *labialis* Sywula 1981
26. *Cypria dorsalta* Malz & Moayedpour 1973
27. *Physocypria suborbicularis* (Sieber 1905)
28. *Physocypria* sp.

Family **Notodromadidae**
29. *Notodromas monacha* (O.F. Müller 1776)

Family **Cyprididae**
Subfamily Cypridinae
30. *Cypris falki* Janz 1997

Subfamily Eucypridinae
31. *Eucypris dulcifons* Diebel & Pietrzeniuk 1969
32. *Eucypris* sp.
33. *Moenocypris* (?) sp.

Subfamily Dolerocypridinae
34. *Dolerocypris* sp.

Subfamily Cypricercinae
35. *Strandesia spinosa* Stchepinsky 1960
36. *Strandesia* sp.
37. *Strandesia* (?) sp. juv.

Subfamily Cyprinotinae
38. *Cyprinotus inaequalis* (Sieber 1905)
39. *Cyprinotus* cf. *vialovi* Schneider 1961
40. *Heterocypris steinheimensis* (Lutz 1965)
41. *Heterocypris* sp. 1
42. *Heterocypris* (?) sp. 2
43. *Heterocypris* sp. 3
44. *Heterocypris* sp. 4

Subfamily Cypridopsinae
45. *Cypridopsis biplanata* Straub 1952
46. *Cypridopsis cucuroni* Carbonnel 1969
47. *Cypridopsis* sp. 1
48. *Cavernocypris subterranea* (Wolf 1920)
49. *Potamocypris gracilis* (Sieber 1905)
50. *Potamocypris* cf. *arcuata* (Sars 1903)
51. *Potamocypris* sp. 1
52. *Potamocypris* sp. 2
53. *Pseudocypretta* sp.

those which disappeared indicate a warmer climate during the *kleini* period than during the following *steinheimensis* period.

### *The post-*kleini *period*

According to Bahrig et al. (loc. cit.) the transgression stage continued during the *steinheimensis* period, and the highest lake level was reached during the following *sulcatus* period. In the subsequent *trochiformis* period a regression took place causing shallow water conditions to return, presumably throughout the basin. After this 'regression stage' the lake level rose again to a mid-level which was maintained during the *oxystoma* and *revertens* period. This stage is called the 'stagnation stage', and probably included even the *supremus* period, according to Bahrig et al. (loc. cit).

These lake stages, postulated by means of sediment investigations, are largely supported by the ostracod findings. Figure 4 shows the density fluctu-

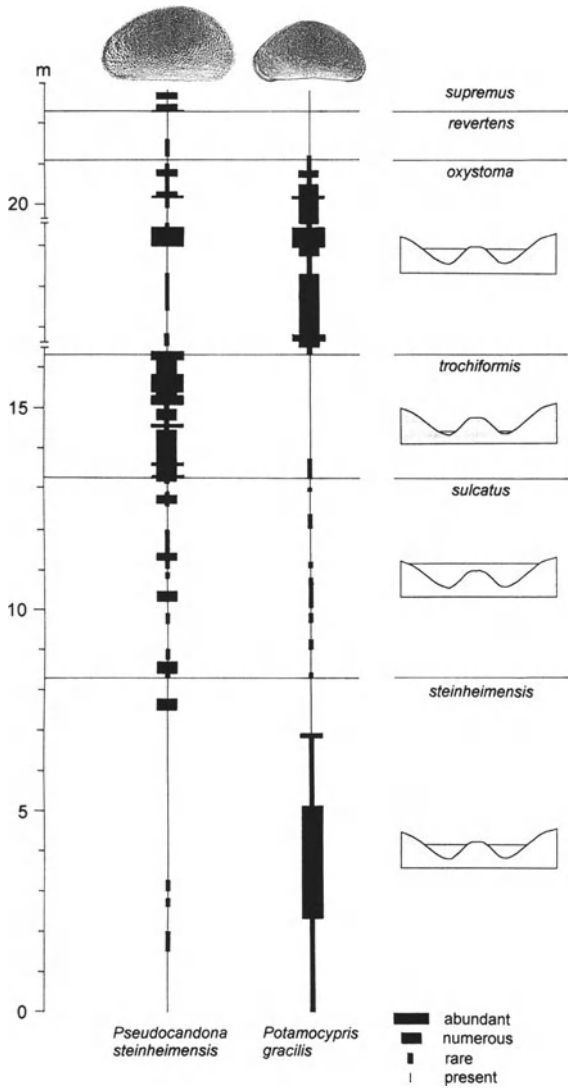


*Figure 4.* Density fluctuations of *P. steinheimensis* and *P. gracilis* during the post-*kleini* period with the lake level stages, symbolised according to Bahrig et al. (1986).

ations of *Pseudocandona steinheimensis* and *Potamocypris gracilis* through the post-*kleini* sequence with the lake stages symbolised beside it. Using the actualistic principle it is suggested that *P. steinheimensis* was a littoral species like its most similar modern-day species *Pseudocandona compressa*. *Potamocypris gracilis* could not be evaluated in this way, but because its density pattern is largely contrary to that of *P. steinheimensis* it is suggested that it was a deep water dweller. Both interpretations are supported by the new data of the *kleini* beds where *P. steinheimensis* mainly occurred in the littoral and *P. gracilis* in the profundal.

Both indicators roughly reflect the long-term lake level fluctuations of the post-*kleini* period. While *Pseudocandona steinheimensis* is most abundant in the *trochiformis* beds (regression stage), *Potamocypris gracilis* is found in the *steinheimensis* and *oxystoma* beds which were deposited in mid-level stages. Although used as indicator of deep water conditions, *P. gracilis* was not very abundant in the *sulcatus* period, when the lake was deepest. There is evidence that at that time an oxygen deficiency existed in the profundal which can provide an explanation for the low density of *P. gracilis*. The oxygen deficiency is supported by the occurrence of one leaf layer and two fish layers occurring in the *sulcatus* beds, indicating a deficiency in the decomposition process. Moreover, meromictic circulation conditions of the lake can be postulated by means of limnological considerations. Taking into account that the surface/depth relation of the lake (surface, 3500 m diameter; depth, min. 120 m to max. 160 m at that stage) was very low (22–29), holomictic circulation conditions are unlikely during that period.

The most recent lake deposits, the *supremus* beds, are incomplete and therefore hardly known. Contrary to Bahrig et al. (loc. cit.), who interpreted them as still belonging to the stagnation stage, the ostracods would rather indicate the beginning of a renewed drop in lake level.

### *Morphological changes of the carapace*

During the post-*kleini* period morphological changes of the carapace in some species were detected in the sequence, and for *Leucocythere immigrata* a splitting event into the daughter species *L. sieberi* and *L. esphigmena* was postulated (Janz, 1992a). As with the planorbids, morphological changes became obvious at the beginning of the *sulcatus* period, and the most aberrant forms occur during the *trochiformis* period.

### Ilyocypris *sp. and* Heterocypris steinheimensis

*Ilyocypris* sp. and *Heterocypris steinheimensis* show similar stratigraphical distribution patterns in the post-*kleini* period. Neither survived the subsequent *trochiformis* period. While the carapaces of *Ilyocypris* sp. are non-tuberculate during the *steinheimensis* period, during the *sulcatus* period specimens with three tubercles occur besides the normal forms. At the same time the density of this species decreased.

Although of similar stratigraphic distribution pattern, the morphological changes in *Heterocypris steinheimensis* are different. From the beginning of the

*sulcatus* period to the early part of the *trochiformis* period a continuous widening of both the anterior and posterior inner lamella took place, resulting in more pointed or even beak-like ends of the carapace in dorsal view. As a result the crenulation of the margins of the right valve, which is a generic character of *Heterocypris*, became more and more indistinct. Moreover, coinciding with these morphological changes a change in the mode of reproduction from bisexual to parthenogenetic, as well as a density increase, was observed.

### Candonopsis arida *and* Ilyocypris binocularis

In contrast to the previous example, *Candonopsis arida* and *Ilyocypris binocularis* persist throughout the profile. In *C. arida* higher-shaped specimens with a strongly convexed dorsal margin occurred exclusively during the *trochiformis* period. The most extreme of these forms reach a height/length ratio (*h/l*) of 0.54, whereas the normal forms range between *h/l* 0.42 to 0.49. The presence of intermediate forms in the *trochiformis* beds indicate that the higher forms also belong to *C. arida*.

In *Ilyocypris binocularis*, the most abundant ostracod species of the lake deposits, morphological changes were difficult to determine at first. Most samples contained a variety of differently sculptured carapaces, and if the material was not so rich, one would have been inclined to describe them as different species. But such a concept would not have worked. In a later study, using the characteristic 'marginal ripplets' on the inner lamella, the hypothesis that all these morphs belong to one species was proven (see Janz, 1994).

In order to assess whether there is a special distribution pattern of the different morphs through the profile, four sculpture types have been defined: (1) Without tubercles; (2) With weakly expressed tubercles; (3) With distinct but rounded tubercles; and (4) With distinct tubercles, and a spine-like pointed posterodorsal tubercle.

From 53 samples, the percentages of these four types were calculated by means of evaluation of 30–40 left valves in each case.

As a rule, the heavily sculptured carapaces (types 3 and 4) represent well over 50% in the *steinheimensis, sulcatus, trochiformis* and lower *oxystoma* beds. Within the three samples of the *steinheimensis* beds which could be evaluated in this way, 90% are heavily sculptured, and within one of those samples type 4 reaches 60%. While in the *sulcatus* beds type 3 is more abundant than type 4, this is reversed in the *trochiformis* beds. During the *oxystoma* period the percentage of weakly sculptured carapaces (types 1 and 2) increases steadily, and in the late *oxystoma* and the *revertens* beds type 1 was found only. With the beginning of the *supremus* period all other types reappeared, and again the heavily sculptured carapaces represent over 50% (see Figure 5).

It can be concluded that at least from the *trochiformis* period onwards the distribution pattern of the different morphs of *I. binocularis* shows a dominance sequence from heavily sculptured over weakly and again to heavily sculptured carapaces. This sequence corresponds fairly well with the planorbid sequence from *Gyraulus trochiformis* (heavily sculptured) over *G. oxystoma* and *G. revertens* (weakly sculptured) to *G. supremus* (again more sculptured).

### *The* Leucocythere immigrata *lineage*

As with *Ilyocypris* sp. and *Heterocypris steinheimensis*, *Leucocythere immigrata* also exhibits morphological changes of the carapace at the beginning of the *sulcatus* period, evidenced by an increase in size, a relative increase of the height of the posterior part of the valve, and a strengthening of the dorsal margin and hinge structures. During the *sulcatus* period this species split into the daughter species *L. sieberi* and *L. esphigmena*, both of which persisted for the remaining life of the lake. All three *Leucocythere* species reproduced bisexually.

As the exact time of the split, which is the beginning of reproductive isolation of the daughter species, is not determinable, morphological distinctiveness of the daughter species was used instead as a criterion for the dating of the new species. When considering the character *h/l* ratio of *Leucocythere* shells through the profile the splitting hypothesis becomes graphical. Figure 6 shows this character from 256 female right valves. The *h/l* ratio of *L. immigrata* (0.57–0.63; mean value, 0.6) takes an intermediate position between *L. sieberi* (0.54–0.58; mean value, 0.56) and *L. esphigmena* (0.58–0.66; mean value, 0.62). Although the first clear delimitation is recognizable in the upper *sulcatus* beds (at about 12–13 m in the profile), the speciation will naturally have taken place earlier, probably in the middle *sulcatus* beds (at about 10–11 m). This is the time when an oxygen deficiency existed in the profundal, proved by a lot of fish and leaf residues.

The daughter species *L. sieberi* appears more similar to the stem species *L. immigrata* than *L. esphigmena*, and does not show remarkable morpho-

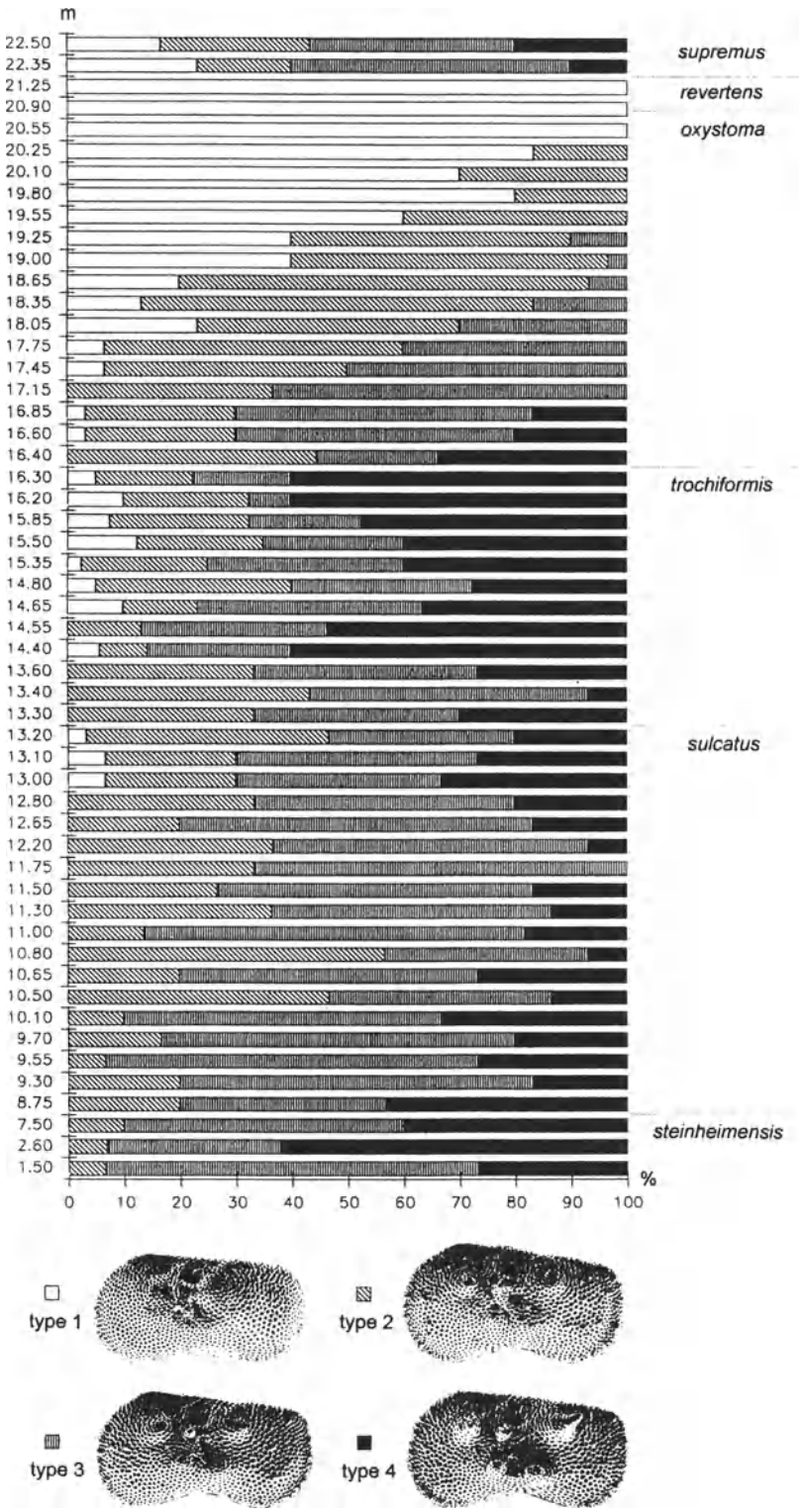


*Figure 5.* Distribution pattern of the percentages of the four sculpture types of *I. binocularis*, calculated by means of evaluation of 30–40 left valves in each case. The metres plotted at the ordinate refer to the combined profile of the post-*kleini* period (see Figure 4).

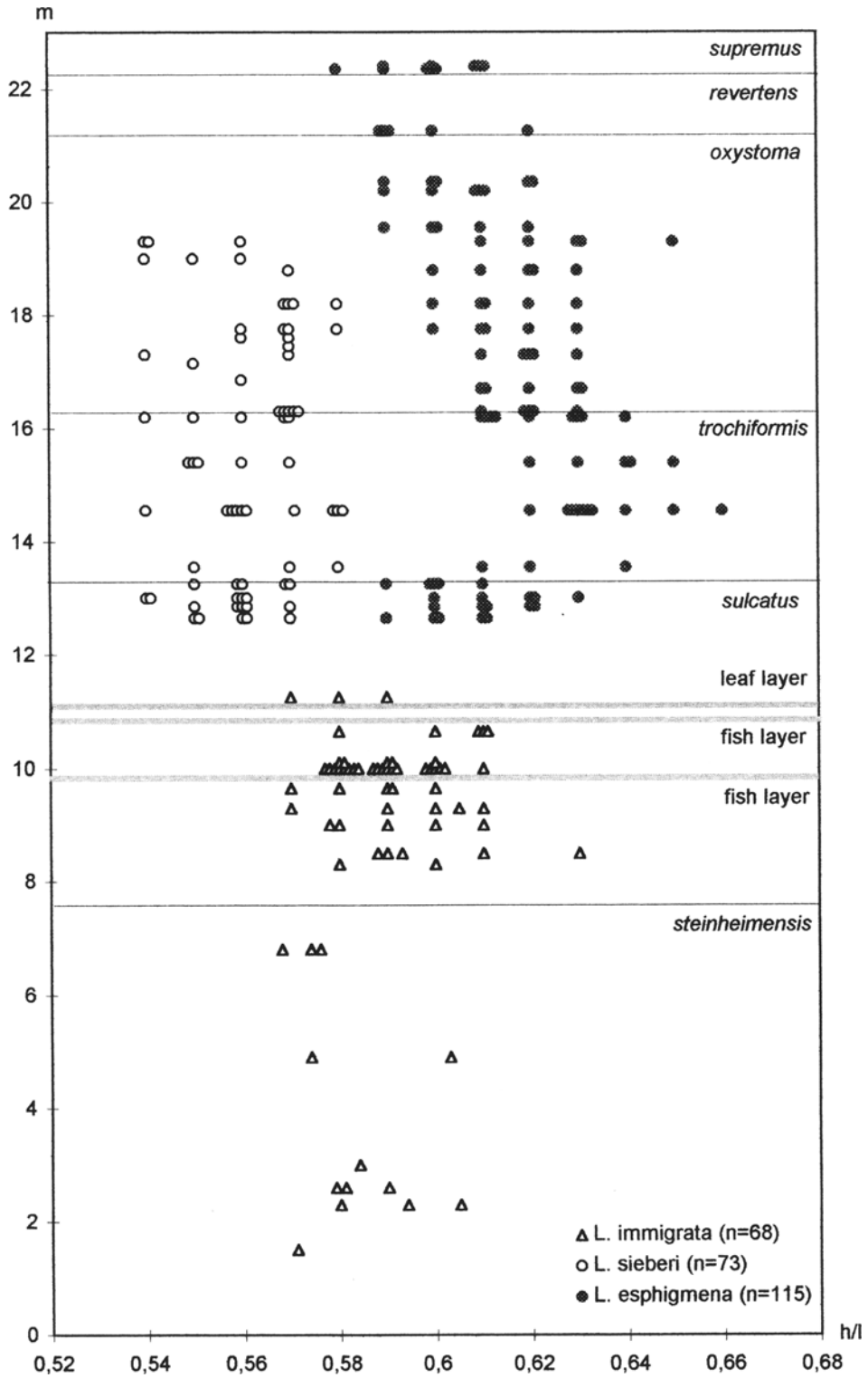


*Figure 6.* Height/length ratios of female right valves of *Leucocythere* during the post-*kleini* period.

logical changes in the further sequence. In contrast, *L. esphigmena* differs widely from *L. immigrata*, and changes appreciably through the subsequent beds. Some characteristic features of *L. esphigmena* are: a pronounced dorsal concavity, a very strong dorsal margin and strong hinge structures, a keel-like ventrolateral expansion, a strong surface reticulation, and a relatively high anterior part of the valve.

These features are most distinct in the *trochiformis* beds, and the morphs of these layers fulfill best the original description of *L. esphigmena* given by Sieber (1905). During the following *oxystoma* beds the typical features became continuously weaker and a reduction in size occurred. At the end of this stage the carapaces of *L. esphigmena* look completely different. The dorsal margin is straight and the lateral expansion weak and unkeeled. Subsequently, the samples of the *supremus* beds again contained valves with a dorsal concavity and keel-like expansions ventrolaterally. Thus, *L. esphigmena* shows a similar sequence of morphological changes as found in *I. binocularis* and known for the planorbids as well.

The morphological changes briefly described for the right female valves can also be observed in the left valves and male valves. Because of a marked sexual dimorphism in all three species of *Leucocythere*, females and males are easily distinguished.

To sum up, it may be said that the splitting hypothesis is supported by the occurrence of gradual transitions between the *L. immigrata* forms of the *steinheimensis* beds and the lower *sulcatus* beds, and the morphologically intermediate position of *L. immigrata* between *L. sieberi* and *L. esphigmena* (Figure 7). Moreover, a detailed study on the microfeatures of these *Leucocythere* species by Viehofen (1997) revealed two types of *L. immigrata*, a 'robust' and a 'filigrane' type, from which both daughter species are derivable. Owing to the also gradual morphological changes in *L. esphigmena* in the further sequence, it is very unlikely that the detected changes in *Leucocythere* species could be the result of immigration and extinction events.

## Discussion

The ostracod findings show both evolutionary patterns convergent to the gastropod assemblage, as well as a clear relation to the lake's ecology. In contrast to former discussions on intralacustrine evolution in Lake Steinheim dealing with planorbids only, a new approach, taking into account both groups, is now possible.

### *Convergent evolutionary patterns of ostracods and gastropods*

Both groups exhibit the following characteristics:

(1) A drastic reduction in species number at the *kleini–steinheimensis* boundary. While in the *kleini* beds the aquatic gastropods comprise 15 species from 13 genera, the *steinheimensis* beds contain six species from three genera (see Finger, 1998; Mensink, 1984; Nützel & Bandel, 1993). This is a reduction to 40% of the species and 23% of the genera. In ostracods a reduction to 23% of the species and 35% of the genera occurred.

(2) Morphological changes of the shell during the post-*kleini* period. Morphological changes in both groups become obvious at the beginning of the *sulcatus* period, and most aberrant forms occur during the *trochiformis* time; subsequently, a reduction of sculpture occurs during the *oxystoma* period leading to normally shaped and non-sculptured forms at the *revertens* period, and finally, at the *supremus* period, again sculptured forms occur.

(3) Speciation. While the genus *Gyraulus* of the planorbids displays a radiation affecting 18–20 endemic species (see Hilgendorf, 1867, Nützel & Bandel, 1993), in ostracods only one single speciation event (two endemic species) occurred.

Despite the striking similarities, however, gastropods and ostracods show a different spectrum of responses to the challenges of the post-*kleini* period which might reflect differences in genetic plasticity as well as ecological valency of the species. From the taxa considered *Gyraulus* has to be regarded among the genetically most plastic. In contrast, the persistence of the two gastropod species *Limnaea dilatata* and *Pseudamnicola pseudoglobulus* (see Mensink, 1984) seems to be due to their large ecological valency, because significant morphological changes are lacking. In ostracods four different kinds of responses can be distinguished:

(1) Persistence without morphological changes (*Pseudocandona steinheimensis, Potamocypris gracilis*): although similar to *Limnaea dilatata* and *Pseudamnicola pseudoglobulus*, both ostracod species, in contrast, exhibit density fluctuations coinciding with the changes in level of the lake.

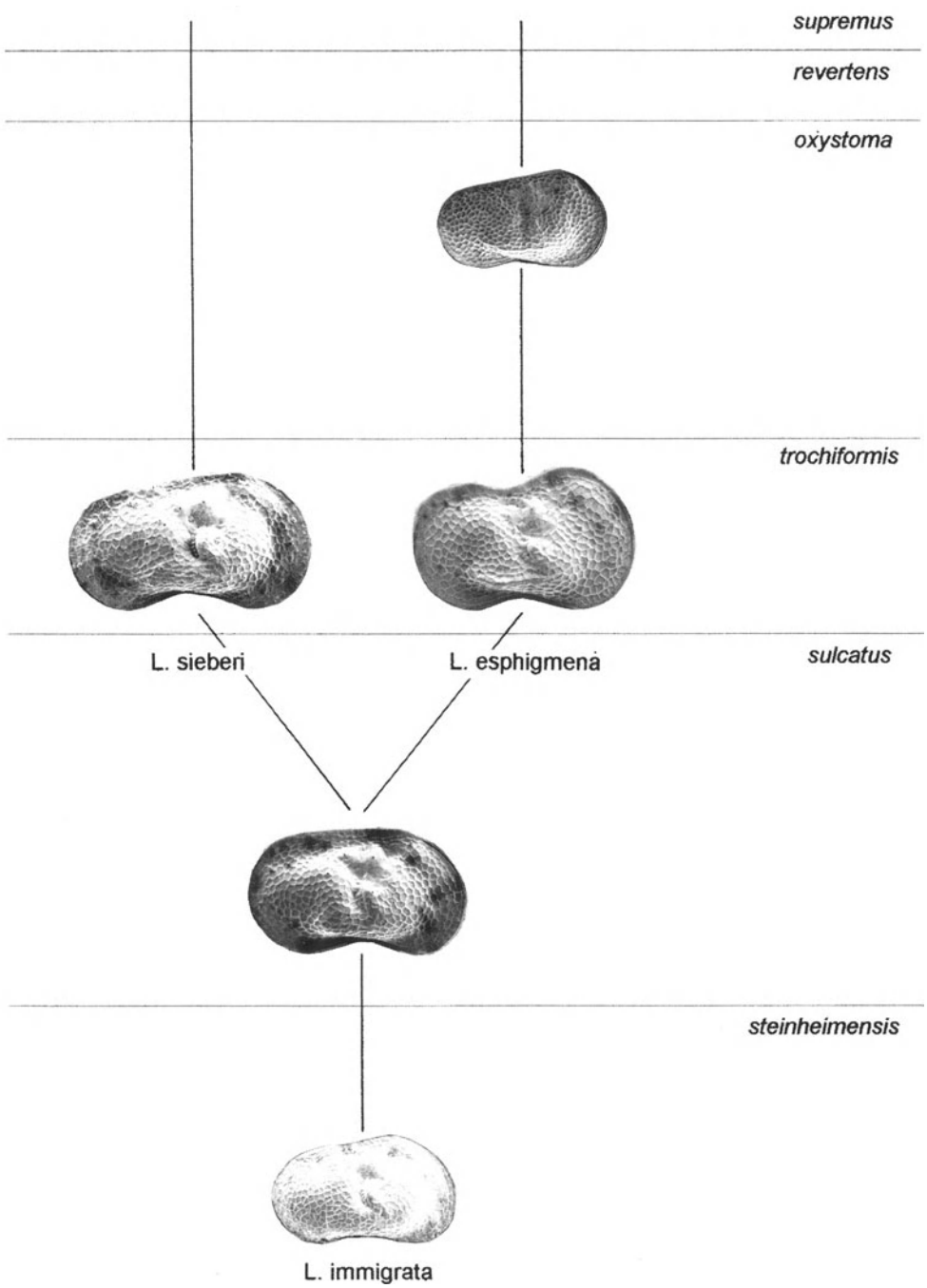


*Figure 7.* The splitting of *L. immigrata* into *L. sieberi* and *L. esphigmena* (female right valves).

(2) Persistence with morphological changes (*Candonopsis arida, Ilyocypris binocularis*): this case is not reported for the snails. As in *C. arida* the occurrence of high-shaped valves is restricted to the *trochiformis* beds, these aberrant forms might be mainly caused by the low Ca/Mg ratio (see below). The distribution pattern of the different morphs of *I. binocularis*, on the other hand, indicates genetic polymorphism, which would also make plausible

the great success of this species throughout the lake's history.

(3) Persistence till the beginning of the early *trochiformis* period, and morphological changes at the beginning of the *sulcatus* period (*Ilyocypris* sp., *Heterocypris steinheimensis*); this is also not reported for the snails. The morphological changes show that both species are genetically plastic to a certain degree, but obviously not sufficiently plastic to overcome the challenges of the *trochiformis* period.

(4) Morphological changes and speciation (*Leucocythere*); the responses of *Leucocythere* are similar to those of *Gyraulus*, although *Leucocythere* is not as speciation prone as *Gyraulus*.

*Impact of external factors*

Bajor (1965) has investigated several chemical parameters of the Steinheim sediments and planorbid shells. He did not find a consistent correlation between any chemical parameter and shell morphology. Best correlation is with the Ca/Mg ratio at the *trochiformis* period when Ca/Mg ratio was lowest, and the morphologically most aberrant forms of both planorbids and ostracods occurred. Nevertheless, it cannot be deduced from this that the morphological changes on the whole are ecophenotypical, because there is evidence for intralacustrine evolution in either group. In planorbids the crucial arguments are the modes of morphological changes as documented by biometrical data (Mensink, 1984), as well as the different protoconch structures shown by Gorthner (1992) and Nützel & Bandel (1993); in ostracods the main fact is the splitting of *Leucocythere immigrata* (Janz, 1992a; Viehofen, 1997).

As outlined above, long-term lake level fluctuations caused several biotope changes. This factor should be accepted as one of the most important external factors. I conclude direct influence of lake level increase by reduction of littoral habitats to be mainly responsible for the species reduction at the *kleini–steinheimensis* boundary. What additional role a temperature drop might have played should be checked by a new study of oxygen isotopes.

In the post-*kleini* period parameters influenced by the lake level became limiting factors. At the *sulcatus* period oxygen deficiency in the profundal may have represented the second big challenge for the Steinheim lake dwellers. Besides the density decrease in *P. gracilis*, it is possible that the splitting of *L. immigrata* could have been triggered by this change.

The crucial abiotic factor of the following period, the regression stage, could have been the reduced concentration of calcium. Danielopol et al. (1990) consider deficiency of calcium to be the responsible factor having directly provoked the curious *L. esphigmena* shells. They consider *L. esphigmena* a paradigmatic example for their holistic carapace model. Thereafter, changes in one of the structural submodules of the carapace edifice may have forced changes in other submodules, in order to maintain the functional role of the carapace. In *L. esphigmena* insufficient calcification combined with the heavy load from the soft body led to a carapace deformation with a pronounced dorsal concavity. According to this model, the typical features of *L. esphigmena* valves from the *trochiformis* beds, namely the strong dorsal margin and hinge structures, the keel-like expansion ventrolaterally and the strong reticulation of the surface, have to be interpreted as compensatory elements to maintain carapace stability. This is indeed a very compelling explanation, and I agree that at the regression stage direct influence of chemical factors may have played a more important role than at other stages.

However, the daughter species of *L. esphigmena*, *L. sieberi*, as well as some other species (*P. steinheimensis, C. nitida, P. suborbicularis* and *P. gracilis*) apparently did not suffer calcification problems. Furthermore, *P. steinheimensis* and *C. nitida* exhibit marked density increases (Janz, 1992a: Figure 20) which illustrate fairly benign conditions for some littoral dwellers at that time. This fact, on the other hand, raises the question, why then did no invasion or recolonization of ubiquitous species take place, if there were again littoral habitats available? As it seems unlikely that abiotic factors like the Ca/Mg ratio were the final hindrance, I suppose that biotic factors played a key role.

Although, contrary to the planorbids, most of the Steinheim ostracods were non-endemic, presumably they were well adapted and had occupied all niches. Possibly, some of the ostracod species had specialized on snail faeces or dead snails for food, or interspecific relationships between ostracods and other species had established. There are some examples of recent ostracod–snail relationships (Deschiens, 1954; Janz, 1992a; Sohn & Kornicker, 1975). As palaeontological studies are not appropriate to clarify food preferences and interspecific relationships, in future, studies on

modern assemblages from ancient lakes should pay more attention to these questions.

While in gastropods no immigration or recolonization is reported for the whole post-*kleini* period, in ostracods *Cyprinotus inaequalis*, which was present in the *kleini* beds, reappeared for a limited period in the middle *oxystoma* period. In the *kleini* period, this species occurred in both the littoral and profundal, and it was classified as a species with a preference for warmer conditions. Therefore, the reappearance of *C. inaequalis* may confirm similar conditions for the middle *kleini* and middle *oxystoma* period. The niche of this species, which is a good swimmer, was obviously not occupied.

From a holistic point of view Gorthner (1992) suggested that in the planorbids, morphological shell changes were non-adaptive and non-functional. The main requirement for such changes is a long-lasting stable environment. In the post-*kleini* period, after an initial period of intralacustrine speciation, endemic species had occupied all niches, and thus, potential immigrants had no chance to settle. As a consequence and in the course of time, selective pressure was reduced in the endemic fauna, and thus neutral mutations could be tolerated. Although Lake Steinheim was a 'long-lived' lake, existing for some hundreds of thousands or even two million years (Gorthner & Meier-Brook, 1985; Janz, 1997; Schudack & Janz, 1997), it was not a stable long-lasting biotope throughout the post-*kleini* period, and the ostracod findings do not support Gorthner's (1992) hypothesis.

On the contrary, the above discussion shows that no general solution can be provided to explain all phenomena. There are a lot of factors and mechanisms involved, and there should be an attempt to assess the relative importance of each of them. Nevertheless, as the planorbids are more prone to speciation than the ostracods, the possibility cannot be excluded that even the span of time covered by a single lake stage was sufficient for an evolution pattern in *Gyraulus* as postulated by Gorthner (loc. cit.). Gorthner's concept, however, has provoked a new view, and has drawn attention to a comparison of the Miocene fauna of Lake Steinheim with extant faunas of ancient lakes.

### *Comparison with other ancient lake ostracod assemblages*

According to Martens (1994) most of the endemic ancient lake ostracods belong to three groups: the Candonidae, the Limnocytheridae and the Cytherideinae. They have in common that they are not able to swim, they have, as a rule, bisexual reproduction, and they are not able to produce desiccation-resistant eggs (not yet conclusively proved for Candonidae). Therefore, in contrast to the bulk of non-marine ostracods, they lack the ability of rapid dispersal and colonization of new habitats, and rapid multiplication. On the other hand, as they follow the concept of K-selection, they are better able to adapt and more prone to speciation in a long-lasting stable environment. Martens (loc. cit.) postulated a succession of candonid/limnocytherid fauna at an initial stage during the history of ancient lakes, which was replaced by candonid/cytherideinid fauna at a second stage. An example of radiation of the former assemblage is the extant ancient Lake Ohrid (2–3 My), while extensive radiations of Candonidae and Cytherideinae were found in the oldest ancient lakes Baikal (25–30 My) and Tanganyika (9–12 My).

In the former crater lake of Steinheim, Cytherideinae are totally absent and, of the Candonidae and Limnocytheridae, only the latter group exhibits a limited radiation. Whether there is a trace of initial radiation in Steinheim candonids cannot be assessed, and, even if there were, it would be difficult to recognise from valves only. Compared with Martens' concept, the ostracod assemblage of Lake Steinheim seems to be an example of intralacustrine evolution at an early stage.

## Conclusions

Studies of ancient lakes have revealed that endemic species can exhibit curious morphologies. In order to reconstruct the development of these phenomena, it would also be necessary to study the fossil record of ancient lakes. The basic requirement for this, a complete sedimentary record and well-preserved fossils, is not common. The fossil Lake Steinheim, however, provides a good example of a 'fossil long-lived' lake, and its fossil record of well-preserved ostracod and gastropod shells enables a detailed documentation along the time axis. From this follows not only a better understanding of the former lake itself, but also of intralacustrine evolution pattern in general. On the other hand, the assessment of causes and mechanisms can only be achieved through studies on Recent (living) organisms from ancient lakes. Therefore, both approaches, neontological and palaeontological, complement one another, and should be more frequently

combined in future. Ostracods form excellent subjects for this purpose.

## Acknowledgements

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# The origins of modern nonmarine ostracod faunas: evidence from the Late Cretaceous and Early Palaeogene of Mongolia

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*Key words:* Cretaceous, Tertiary, Cyprideidae, Cyprididae, Talicyprideinae, Mongolia

## Abstract

The Cretaceous and Tertiary development of Mongolian non-marine ostracod faunas is reviewed. During the Late Cretaceous and Early Palaeogene, representatives of the Cypridoidea were widespread and common, Cytheroidea less so and the Darwinuloidea comparatively rare. The evolutionary history of the subfamily Talicyprideinae is considered, with reference to the genera *Talicypridea*, *Altanicypris*, *Khandia* and *Bogdocypris*. It is suggested that the extinct Talicyprideinae were related to the mid-Cretaceous to Recent subfamily Cypridinae (e.g. the genus *Cypris*), both belonging to the family Cyprididae. It is shown that early representatives of the Cyprididae, one of the most diverse non-marine cypridoidean families today, were present from Early Cretaceous onwards (e.g. *Lycopterocypris*, *Mongolocypris*), alongside the dominant Cretaceous cypridoideans, the Cyprideidae (e.g. *Cypridea*), which became extinct in the Palaeogene.

## Introduction

Stratigraphical studies of the Cretaceous and Palaeogene nonmarine ostracods of Mongolia have revealed significant temporal changes and geographical heterogeneity. Two phases of ostracod evolution can be discerned: a Late Cretaceous phase (represented by the deposition of the Barun Bayan, Bayan Shire, Bayan Zag, Barun Goyot and Nemegtu formations) and a late Palaeocene phase (represented by the Naran Bulak Formation) (Badamgarav et al.,1995). The two phases are recognized on the basis of substantial differences in the taxonomic character of their respective ostracod assemblages.

### *The Late Cretaceous Phase*

At the transition between the Early and Late Cretaceous a gradual change of ostracod assemblages is observed. By the end of the Early Cretaceous, widespread genera such as *Limnocypridea*, *Theriosynoecum* and *Ilyocyprimorpha* had disappeared. There was also a marked drop in species diversity of the genera *Cypridea* (*sensu lato*), *Mongolianella*, *Timiriasevia* and others, but some species belonging to these genera survived into the Late Cretaceous (e.g. *Cypridea fracta, C. prognata*, *C. occollata*, *Timiriasevia polymorpha*). During the Late Cretaceous, extinctions and new appearances of ostracod genera and species continued, although generally the Late Cretaceous phase is characterized by relatively gradual development of the ostracod faunas, reflecting more or less stable conditions in the lake basins of the Gobi during that interval. Apparently the best environmental conditions for the ostracods existed during the formation of the Nemegtu deposits, which yield the most abundant and diverse ostracod assemblages.

In strata of early Late Cretaceous age (Barun Bayan, Bayan Shire formations), the ostracod faunas still have much in common with Early Cretaceous ones, both on the generic and, to some extent, the species level. However, beginning in the Bayan Shire Formation, new genera appear, some characteristic of Late Cretaceous faunas (e.g. *Mongolocypris*, *Talicypridea*, *Gobiocypris*, *Altanicypris*), others representing taxa which have survived into modern faunas: *Eucypris*, *Candona*, *Cypria*, *Cyclocypris*, *Cyprinotus*, *Limnocythere* (all *sensu lato*). In the Bayan Shire Formation *Mongolocypris*, *Talicypridea*, *Candona*, *Mediocypris*, *Cypria* and *Eucypris* appear, followed

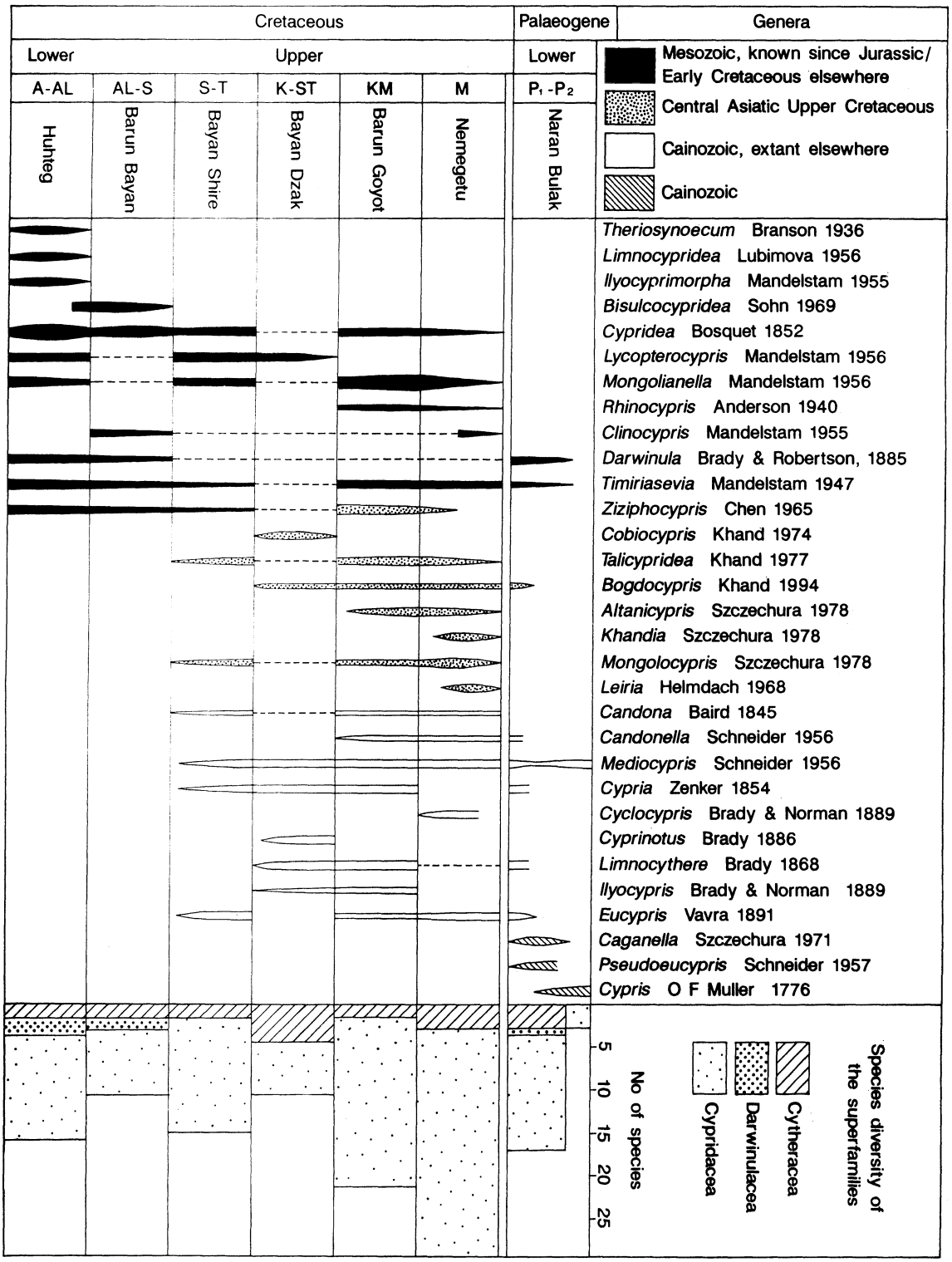


*Figure 1.* Stratigraphical distribution of non-marine ostracod genera in the Late Cretaceous and Early Palaeogene of Mongolia.

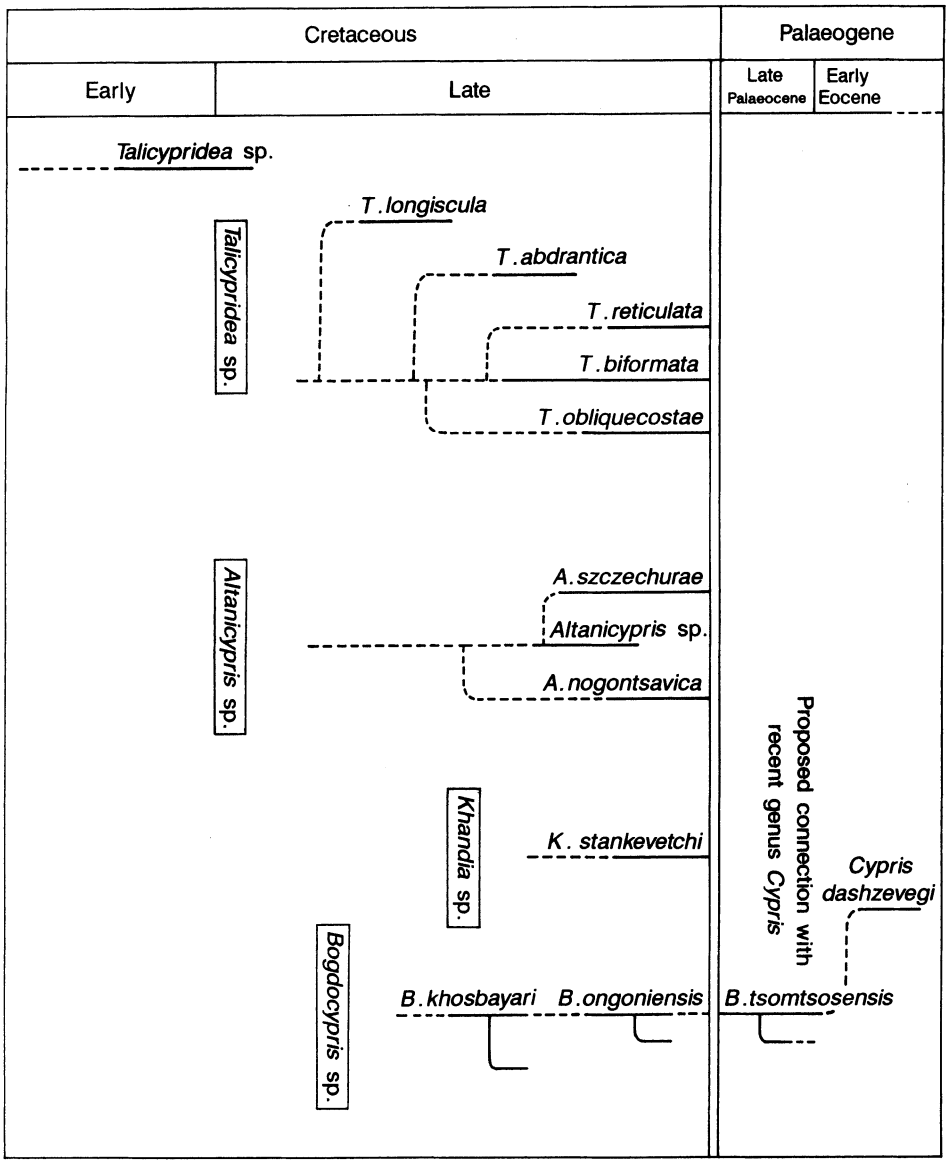


*Figure 2.* Postulated evolutionary relationships within the Talicyprideinae in the Late Cretaceous and Early Palaeogene of Mongolia.

by *Gobiocypris*, *Limnocythere*, *Ilyocypris* and *Cyprinotus* in the Bayan Zag Formation (Figure 1). In the succeeding Barun Goyot and Nemegtu formations, the first appearances of *Altanicypris*, *Candoniella* and *Cyclocypris* are recorded (Figure 1). The genus *Eucypris* becomes widespread in the Nemegtu Formation, four species being found abundantly at almost all of the localities studied.

*The Early Palaeogene Phase*

The Early Palaeogene phase of ostracod evolution occurred during the deposition of the Naran Bulak Formation and is marked by substantial reduction of taxonomic diversity, compared to the Late Cretaceous phase. The big difference between the Late Cretaceous and Early Palaeogene ostracod assemblages is partly accentuated by the presence of a gap in sedimentation at the junction of these intervals. The Palaeogene (Upper Palaeocene-Lower Eocene) deposits are distributed only in the south-western part of Mongolia, so the Early Palaeogene ostracod assemblages cannot give a complete picture of ostracod evolution during that time. Representatives of *Limnocythere*, *Timiriasevia*, *Caganella* and *Eucypris* are widely developed, being

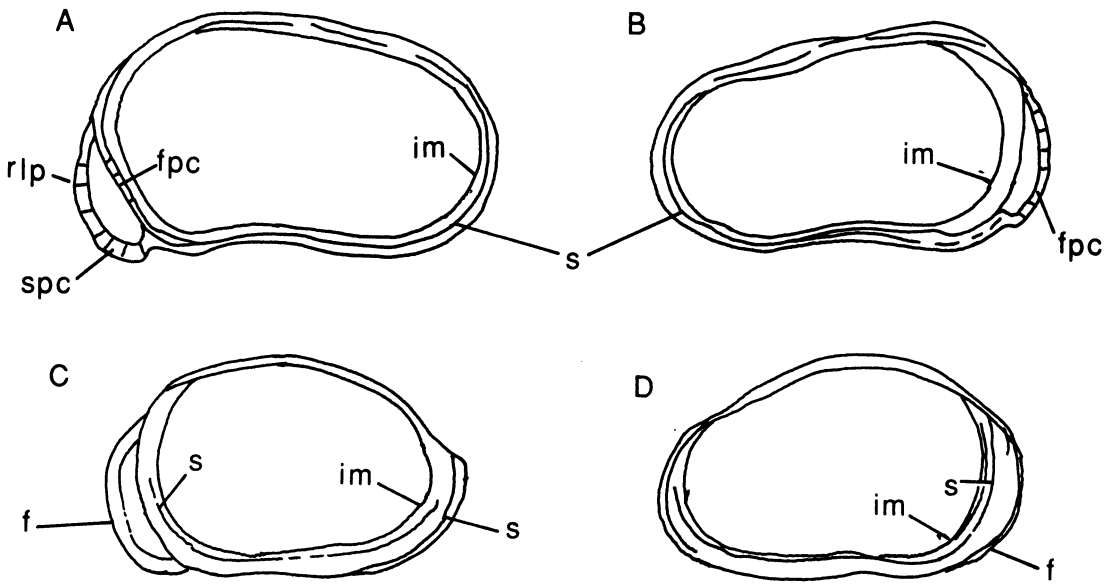


*Figure 3.* A comparison of the internal valve structure of the genera *Talicypridea* and *Cypris*. (A), (B), *Talicypridea biformata* (Szczechura & Blasyk, 1970); (A), right valve internal lateral view; (B), left valve internal lateral view. (C), (D), *Cypris subglobosa* Sowerby, 1840 (after Okubo, 1972); (C), right valve external lateral view; (D), left valve external lateral view. Key to abbreviations: fpc = first pore canal zone; spc = second pore canal zone; s = selvage; rlp = rostrum-like process; f = flange; im = inner margin; l = list.

found in abundance and with quite high specific diversity. *Ilyocypris*, *Candona*, *Cypris* and *Mediocypris* are also represented.

The established phases of evolution of the non-marine ostracods facilitate the recognition of local stratigraphical divisions in Mongolia and also characterize the change of ostracod faunas at the Mesozoic-Cenozoic boundary.

## The evolution of the Talicyprideinae

Especially interesting are the morphology, biostratigraphy and evolution of the subfamily Talicyprideinae (Hou, 1982), widely distributed in the Late Cretaceous of Mongolia and adjacent regions (China, Trans-Baikalia), and possibly representing a transition between Mesozoic and Recent Cyprididae. Their evolution is analyzed in detail below (Figure 2). The subfamily Talicyprideinae includes the genera *Talicypridea*, *Altanicypris*, *Bogdocypris*, and *Khandia* which share similar general shell morphology; their right valve is equipped with a 'rostrum-like' process, which previously was considered as a lip-like extension (Szczechura,1978). The rostrum-like process superficially resembles the rostrum of Mesozoic Cyprideidae (e.g. the genus *Cypridea*), but its internal structure reveals similarities with that of the anterodorsal part of the anterior end of the valves of Recent Cyprididae. The form, size and arrangement of the rostrum-like process, and also the characteristic shape and ornament are different for different genera and show certain trends of change in time, allowing the evolution of lineages within this subfamily to be recognized (Figure 2).

One lineage (*Talicypridea biformata*, *T. oblique-costata*, *T. reticulata*, *T. longiscula*, *T. abdarantica*) is characterized by rounded-oval shell shape and circular-cellular external ornament; the posterior third of the carapace shows the maximum inflation and the rostrum-like process is crescent-shaped. A second lineage (*Altanicypris szczechurae*, *A. multispina*, *A. nogontsavica*, *A. ostrea*) is characterized by an evenly inflated carapace, smooth or spinose ornament and wedge-shaped rostrum-like process. A third lineage, represented by *Khandia stankevitchae* has a large, deep carapace with spinose ornament and a rostrum-like process in the middle of the anterior end of the right valve. The fourth lineage (*Bogdocypris khosbayari*, *B. ongoniensis*, *B. tsomtsosensis*) consists of species with smooth or coarsely reticulate valves, elongate-oval shape and moderate, even inflation. The rostrum-like process is perpendicular to the long axis

*Table 1.* Systematic listing of non-marine ostracod genera from the Cretaceous and Palaeogene of Mongolia

- Order Podocopida G.W.Müller, 1894
  - Suborder Cytherocopina Gründel, 1967
    - Superfamily Cytheroidea Baird, 1850
      - Family Limnocytheridae Klie, 1938
        - Subfamily Limnocytherinae Klie, 1938
          - Genus *Limnocythere* Brady, 1868
        - Subfamily Timiriaseviinae Mandelstam, 1947
          - Genus Theriosynoecum Branson, 1936
          - Genus *Timiriasevia* Mandelstam, 1947
      - Family Cytherideidae Sars, 1925
        - Subfamily Cytherideinae Sars, 1925
          - Genus *Gobiocypris* Khand, 1974
  - Suborder Darwinulocopina Sohn, 1988
    - Superfamily Darwinuloidea Brady & Norman, 1889
      - Family Darwinulidae Brady & Norman, 1889
        - Genus *Darwinula* Brady & Robertson, 1885
  - Suborder Cypridocopina Jones, 1901
    - Superfamily Cypridoidea Baird, 1845
      - Family Ilyocyprididae Kaufmann, 1900
        - Genus *Ilyocypris* Brady & Norman, 1889
        - Genus *Rhinocypris*Anderson, 1940
      - Family Cyprideidae Martin, 1940
        - Subfamily Cyprideinae Martin, 1940
          - Genus *Cypridea* Bosquet, 1852
          - Genus *Bisulcocypridea* Sohn, 1969
      - Family Candonidae Kaufmann, 1900
        - Subfamily Candoninae Kaufmann, 1900
          - Genus *Candona* Baird, 1845
          - Genus *Candoniella* Schneider, 1956
        - Subfamily Cyclocypridinae Kaufmann, 1900
          - Genus *Cyclocypris* Brady & Norman, 1889
          - Genus *Cypria* Zenker, 1854
      - Family Cyprididae Baird, 1845
        - Subfamily Cyprinotinae Bronstein, 1947
          - Genus *Cyprinotus* Brady, 1886
          - Genus *Leiria* Helmdach, 1971
        - Subfamily Eucypridinae Bronstein, 1947
          - Genus *Eucypris* Vavra, 1891
          - Genus *Lycopterocypris* Mandelstam, 1956
          - Genus *Pseudoeucypris* Schneider, 1957
          - Genus *Clinocypris* Mandelstam, 1955
        - Subfamily Cypridinae Baird, 1845
          - Genus *Cypris* O. F. Muller, 1776
          - Genus *Mongolocypris* Szczechura, 1978
        - Subfamily Talicyprideinae Hou, 1982
          - Genus *Talicypridea* Khand, 977
          - Genus *Altanicypris* Szczechura, 1978
          - Genus *Khandia* Szczechura 1978
          - Genus *Bogdocypris* Khand, 1994
      - Family Cypridopsidae Kaufmann, 1960
        - Subfamily Cypridopsinae Bronstein, 1947
          - Genus *Cypridopsis* Brady, 1868
        - Subfamily Trapezoidellinae Sohn, 1979
          - Genus *Ilyocyprimorpha* Mandelstam, 1955
          - Genus *Limnocypridea* Lubimova, 1956
        - Subfamily Mongolianellinae Neustrueva, 1989
          - Genus *Mongolianella* Mandelstam, 1955
      - Family uncertain
        - Genus *Caganella* Szczechura, 1971
    - Superfamily uncertain
      - Genus *Ziziphocypris* Chen, 1965

of the valve. The Palaeogene *B. tsomtsosensis* is more elongate and has a reduced rostrum-like process compared to the two Late Cretaceous species. *Bogdocypris khosbajari* has the rostrum-like process situated lower, though still perpendicular to the long axis of the valve. The genus *Bogdocypris* may occur in the Late Cretaceous of Argentina, recorded as '*Altanicypris* sp.1' and 'gen. et sp. indet.' by Musacchio & Simeoni (1989).

In the *Talicypridea* and *Altanicypris* lineages, which are restricted to the Late Cretaceous, there is a marked tendency towards increasing prominence of the rostrum-like process and towards decreasing carapace size. In contrast, the *Bogdocypris* lineage, which persisted into the Palaeogene, shows elongation of the carapace and a reduction of the rostrum-like process. The internal valve structure of the Talicyprideinae carapace, with its rostrum-like process and double marginal pore canal zone, resembles that of Recent *Cypris* (Figure 3). It can, therefore, be suggested that modern Cypridinae inherited the morphology of the Mesozoic Talicyprideinae. If this relationship is justified, it must be concluded that the modern Cypridinae have more ancient roots than was considered until now. More detailed study of these relationships is required. In particular the current assignment of the Cretaceous *Mongolocypris* to the Cypridinae (Table 1) must be questioned, since it appears in Mongolia at the same time (Cenomanian) as *Talicypridea* (Figure 1). The position of the Early Cretaceous (Aptian) cypridid *Pattersoncypris* also needs to be considered (Bate, 1972).

## Conclusions

The Late Cretaceous is thus seen as an important phase in the evolution of non-marine ostracods in Mongolia. It begins in the early part (Cenomanian), when extant taxa (e.g. *Candona*, *Eucypris*, *Cypria*) first begin to appear, alongside more familiar 'Wealden'-type faunas (e.g. *Cypridea*, *Timiriasevia*) surviving from the Early Cretaceous. Also, during the Late Cretaceous in Mongolia a radiation of the Talicyprideinae is seen. The phase ends at the Cretaceous-Tertiary boundary, when many representatives of the Cretaceous faunas became extinct. Many of these extinctions are probably only of local significance, however; *Cypridea* s.l., for example, is known to have survived into the Palaeogene in China. Central Asia may, therefore, be considered as a possible centre of origin for many modern non-marine ostracods. A similar mid-Cretaceous faunal turnover has been documented in other parts of the world, such as France (Babinot et al., 1996), however, and Cretaceous ancestry for some lineages has been postulated in other areas such as Alaska (Brouwers & De Deckker, 1996) and Europe (Horne & Martens, 1998). For future study, taxonomic and stratigraphical comparisons on a global scale are likely to be rewarding.

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# The evolutionary history of Late Permian Darwinulocopina Sohn, 1988 (Ostracoda) from the Russian Plate

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*Key words:* Permian, Darwinulocopine ostracods, Russian Plate, lakes

## Abstract

Evolutionary trends in Late Permian Darwinulocopina are summarised with reference to extensive collections from eastern European Russia, from the White Sea in the North to the Cis-Caspian in the South. They inhabited large, shallow lakes in which the variety of habitats was favourable for high ostracod diversity. Three superfamilies are represented: the Darwinuloidea preferred lakes with terrigenous sedimentation and insignificant bicarbonate, the Suchonelloidea inhabited lakes with increased bicarbonate and could also live in low-sulphate waters, and the Darwinuloidoidea inhabited high-bicarbonate water bodies and could also survive in low-magnesium waters. Different evolutionary trends account for the different ages of the crucial stages of development of each superfamily: the beginning of the mid-Tatarian for the Darwinuloidea, the late Tatarian for the Suchonelloidea and Darwinuloidoidea.

## Introduction

The Darwinulocopina were among the earliest non-marine ostracods, first appearing in the Carboniferous (possibly the Devonian) and still existing today, represented by, e.g., the genus *Darwinula* Brady & Robertson, 1870. More than 400 fossil and living species of *Darwinula* have been described (Kashevarova & Neustruyeva, 1982). Much of this diversity is restricted to the Carboniferous and Permian; post-Palaeozoic Darwinulocopina appear to have maintained a persistent but low-diversity presence in comparison with other non-marine ostracods (Podocopina: Cypridoidea and Cytheroidea), with only about 28 species known to be extant (Rossetti & Martens, 1998). The higher diversity of the Permian apparently coincided with the presence of sexuality in at least some groups in this lineage (Abushik, 1990), although it is often difficult to identify sexual dimorphism in poorly preserved material. The genus *Darwinula* could be as much as 350 million years old, although Molostovskaya (in Abushik, 1990) placed most Palaeozoic *Darwinula sensu lato* in the genus *Palaeodarwinula*. The longevity of the genus *Darwinula* may be related to its mode of reproduction: all modern Darwinulocopina are exclusively parthenogenetic, and have apparently been so since late in the Mesozoic. On the other hand, Permian Darwinulocopina have been reported to be diverse at species level and commonly to exhibit sexual dimorphism (Sohn, 1988). In view of the apparent longevity of parthenogenesis in this group, a phenomenon difficult to reconcile with current evolutionary theory (Butlin & Menozzi, 2000), the evolutionary history of Late Permian Darwinulocopina from the Russian Plate is of special interest.

## Material and methods

The material used in this study was collected from 170 exposed sections and wells (boreholes) in eastern European Russia, from the White Sea in the North to the Cis-Caspian in the South. It comprises 12 000 samples, each yielding 100–400 ostracod shells. The Darwinulocopina are represented mainly by complete carapaces and more rarely by disarticulated valves, moderately well to perfectly preserved, with internal and external structural details observable.

The research was carried out in three stages: (1) observations in field exposures, (2) study of ostracod specimens on rock samples with a binocular micro-

scope, magnification 25–50×, and (3) microscopic studies of washed specimens mounted on micropalaeontological slides (in incident and transmitted light) and in orientated microsections (in transmitted light).

In the field, special attention was paid to the precise horizon of each ostracod sample, the extent and thickness of productive layers and the mode of occurrence of the microfaunal remains (uniform distribution, nests, lenses, interlayers, shells strewn on bedding planes or accumulations in suncracks, etc.). In addition to bulk collections, oriented (way-up) samples were taken.

Under the binocular microscope such details were noted as lithological peculiarities, assemblage densities, valve orientations, patchiness of distribution on bedding planes, shell colour, alteration and mode of preservation. Whenever possible, qualitative or quantitative assessments of the relative abundance of genera were made; this is important since destruction of fragile shells during processing often gives a misleading impression of assemblage composition. Binocular microscope studies of rock specimens were often complemented by observations of thin sections made at various orientations relative to the bedding planes. Additional information was gathered on associated fossil remains; such observations are particularly useful when dealing with core material in which microfaunal remains are rare.

Washed microfaunal preparations were picked and mounted on standard micropalaeontological slides, where special attention was paid to shell colour and mineralogy (preservation). Taphonomic and palaeoecological details were recorded for each locality.

For the study of the systematics, evolution and ecology of the Darwinulocopina, attention was concentrated primarily on ostracod assemblages which had been buried in situ or in which post-mortem transport was minimal, so that they could be assumed to represent a single biotope. Morphological features of ostracod shells were examined and recorded, including general outline, details of valve margins, positions and shapes of the greatest convexities, angles formed at the anterior and posterior terminations of the carapace, shape of carapace in longitudinal cross-section, valve asymmetry and overlap, hinge, muscle scars, ornament and ontogenetic stage.

Considering all of these features, four morphological groupings were established, corresponding to superfamilies, families, genera and species (Molostovskaya, 1982, 1990) and reflecting evolutionary relationships. In reconstructing the evolutionary history of Late Permian Darwinulocopina, a combination of endogenic (genotypic) and exogenic (ecological) factors was considered.

*Table 1.* Permian families and genera of the three superfamilies of the Dawinulocopina

| |
|---|
| Superfamily Darwinuloidea Brady & Norman, 1889 |
| Family Paleodarwinulidae Molostovskaya, 1990 |
| Genus *Paleodarwinula* Molostovskaya, 1990 |
| Genus *Garjainovula* Molostovskaya, 1990 |
| Family Suchonellinidae Kuchtinov, 1985 |
| Genus *Suchonellina* Spizharskyi, 1937 |
| Genus *Wjatkellina* Molostovskaya, 1990 |
| Superfamily Suchonelloidea Mishina, 1972 |
| Family Suchonellidae Mishina, 1972 |
| Genus *Suchonella* Spizharskyi, 1937 |
| Genus *Dvinella* Molostovskaya, 1990 |
| Genus *Tatariella* Mishina, 1967 |
| Family Praesuchonellidae Molostovskaya, 1990 |
| Genus *Praesuchonella* Molostovskaya, 1980 |
| Superfamily Darwinuloidoidea Molostovskaya, 1979 |
| Family Darwinuloididae Molostovskaya, 1979 |
| Genus *Darwinuloides* Mandelstam, 1956 |

## Results and discussion

Permian Darwinulocopina are represented by three superfamilies: the Darwinuloidea Brady & Norman, 1889, the Suchonelloidea Mishina, 1972 and the Darwinuloidoidea Molostovskaya, 1979. Families and genera belonging to these three superfamilies are listed in Table 1.

### Darwinuloidea

The Permian Darwinuloidea developed through aromorphosis and increasing ecological valency. During some time intervals, ecological factors had only limited influence, exerting no significant pressures on the main evolutionary trends of the group. The rates of evolution of the Darwinuloidea were not steady, so that two phases may be recognised: a slow evolutionary phase in the Paleodarwinulidae during the Ufimian, Kazanian and early Tatarian stages, and a rapid phase in the Suchonellidae during the later

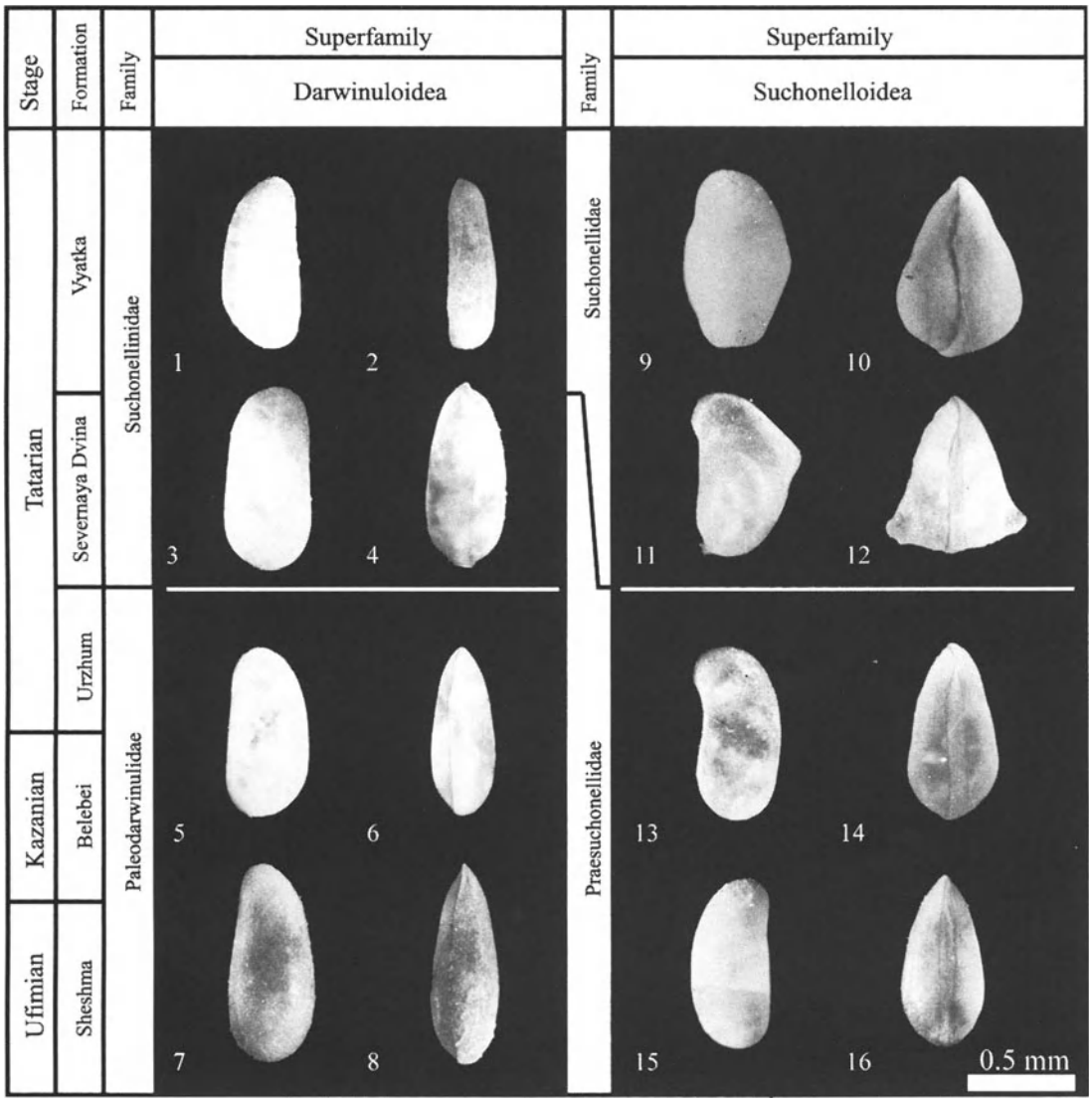


*Figure 1.* Evolutionary trends in Darwinulocopina during the Late Permian: representative species shown in reflected light. Each pair of illustrations shows a lateral and a ventral view of a valve or carapace. (1, 2) *Wjatkellina vladimerina* (Belousova), The Sukhona, Fedosovo, Vyatka Formation; (3, 4) *Suchonellina parallela* Spizharskyi, Orenburg region, Sorochinsk, Severnaya Dvina Formation; (5, 6) *Paleodarwinula fragiliformis* (Kashevarova), Orenburg region, Sorochinsk, Urzhum Formation; (7, 8) *Paleodarwinula lubimovae* (Kashevarova), Orenburg region, Marjevka, Sheshma Formation; (9, 10) *Suchonella typica* Spizharskyi, North Dvina, Zabelino, Vyatka Formation; (11, 12) *Dvinella cyrta* (Zekina), The Sukna, Fedosovo, Vyatka Formation; (13, 14) *Praesuchonella nasalis* (Sarapova), Orenburg region, Sorochinsk, Urzhum Formation; (15, 16) *Praesuchonella tichwinskaja* (Belousova), Oreburg region, Schestemir, Sheshma Formation.

Tatarian (Severnaya Dvina and Vyatka times) (Figures 1 and 2).

The carapaces of the Paleodarwinulidae are lenticular in dorsal view, widest in their posterior third. Their valves are subsymmetrical with narrow, barely visible, anterior marginal pore-canal zones, bipartite hinges with fluted ridges, and eight or nine wedge-shaped adductor muscle scars. The Suchonelliniidae are characterized by carapaces which are almost cylindrical in dorsal view and widest in the posterior quarter, asymmetrical valves, broad anterior marginal pore-canal zones, 10–12 wedge-shaped adductor muscle scars and a tripartite, fluted-ridge, hinge. Their valves are thickened at the anterior and dorsal margins. Thus the Darwinuloidea can be seen to exhibit evolutionary trends of increasing carapace size, hinge complexity and valve asymmetry, as well as dorsal flattening, a shift of greatest convexity/width from the

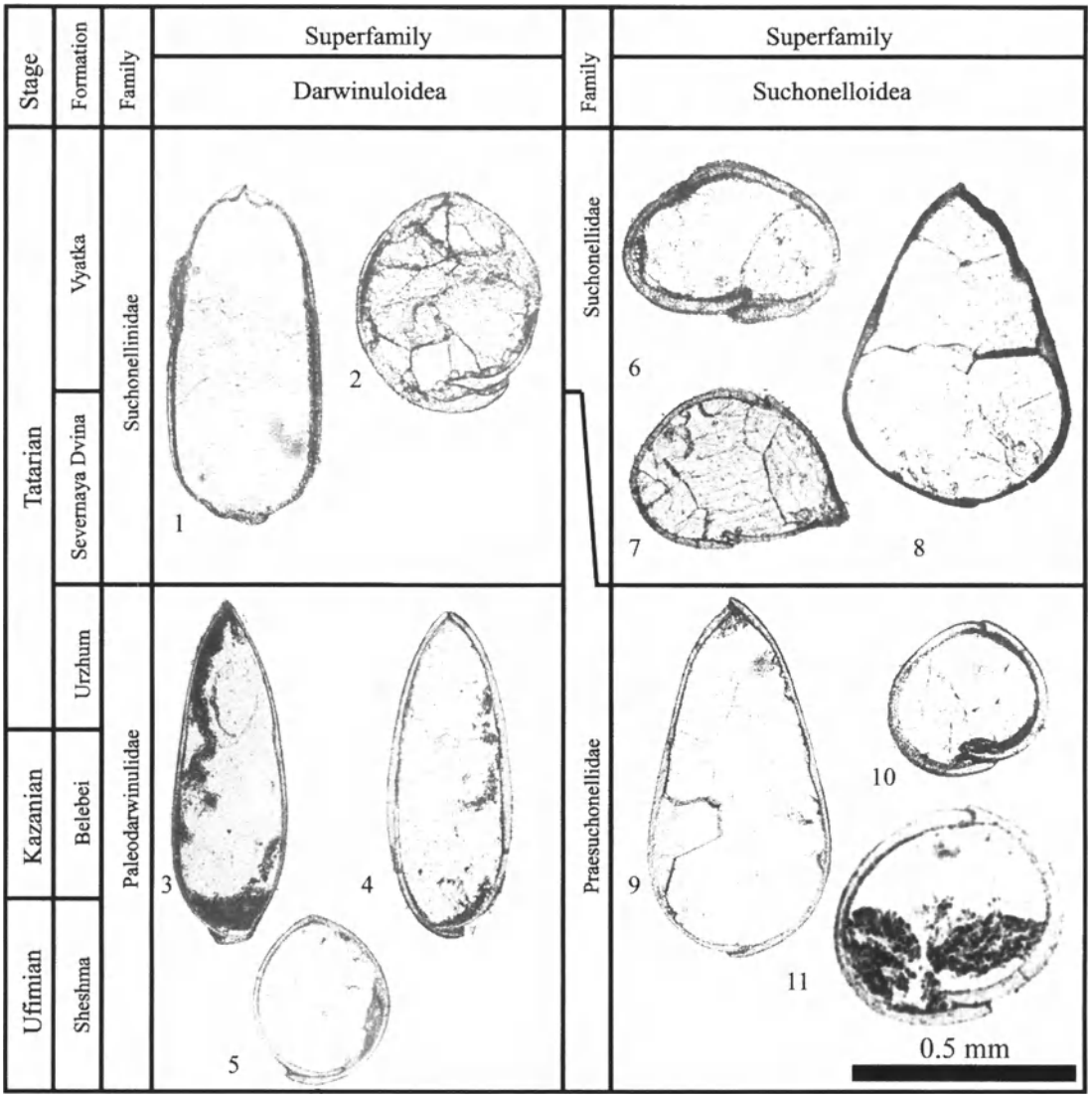


*Figure 2.* Evolutionary trends in Darwinulocopina during the Late Permian: carapaces of representative genera shown in longitudinal and transverse section, in transmitted light. (1, 2) *Suchonellina* Spizharskyi; (3–5) *Paleodarwinula* Molostovskaya, 1990; (6, 8) *Dvinella* Molostovskaya; (7) *Suchonella* Spizharskyi; (9–11) *Praesuchonella* Molostovskaya.

posterior third towards the posterior end and greater development of marginal pore-canal zones. This morphological progression may be related to adaptations to a broadening environmental and distributional range.

## Suchonelloidea

The Suchonelloidea also show two phases of evolution in the Permian: the slow development of the Praesuchonellidae during the Ufimian, Kazanian, and early to mid-Tatarian (Urzhum and Severnaya Dvina times), and the more rapid evolution of the Suchonellidae in the late Tatarian (Vyatka times) (Figures 1 and 2). Praesuchonellidae carapaces are teardrop-shaped in dorsal view, with the greatest convexity in the posterior third; valves are weakly asymmetrical with bipartite, fluted-ridge, hinges. Carapaces of the Suchonellidae are wedge-shaped in dorsal view, widest (greatest convexity) posteroventrally and flattened ventrally; their valves are strongly asymmetrical and overlapping, their bipartite hinges are more complex with fluted ridges supplemented by shelves. The main evolutionary trends of the Suchonelloidea

are thus an increase in convexity or inflation of the carapace, a posteroventral shift of the maximum inflation, increasing valve asymmetry and hinge development.

Unlike the Darwinuloidea, the Suchonelloidea do not show a broadening environmental valency, but rather a progressive morphological divergence and speciation through adaptation to more precisely defined ecological niches. The evolutionary development of *Suchonella blomi* Molostovskaya may be considered as an example. This species existed at the beginning of Vyatka time and is characterized by moderately thick, elongate valves with broadly rounded anterior and posterior extremities of almost equal height. It was rather euryfacial, occupying a variety of ecological niches. Detailed observations of morphology and palaeoecology at different stratigraphical horizons reveal two gradually divergent trends. In one group the posterodorsal cardinal angle became less distinct, the dorsal margin straightened, the height of the anterior extremity increased and the anterodorsal cardinal angle became more distinct; the valves became thinner and lighter. This trend resulted in the species *Suchonella auriculata* (Sharapova) which inhabited soft, silty substrates in relatively deep and calm hydrodynamic regimes. A second group shows increasing shell strength and thickness, especially posteriorly, the anterodorsal cardinal angle becomes less distinct, the dorsal margin is straightened, the posterodorsal cardinal angle becomes more sharply marked and is opposed by a tubercle on the larger valve. This trend culminates in a new genus and species, *Dvinella cyrta* (Zekina), which inhabited firm carbonate substrates in well-oxygenated, shallow waters with abundant influx of detritus.

Further evolution of *Suchonella auriculata*, following the same trend, resulted in *Suchonella typica* Spizharskyi, a species with a slender carapace, high, compressed anterior end and hollow spines in the posteroventral region. This species preferred soft clayey silts in calm parts of a lake. No further development of *Dvinella cyrta* is known and it is likely that a lack of suitable biotopes in late Vyatka lakes resulted in its extinction.

### Darwinuloidoidea

The evolution of the Darwinuloidoidea consisted of progressive adaptation without pronounced divergence in any one adaptive feature. The Darwinuloididae, representing this superfamily in the Late Permian, have carapaces which are oval in lateral view, with the greatest inflation in the posterior half, thickened valve terminations and 10–12 adductor muscle scars (Molostovskaya, 1990). Their carapaces became larger and inflation increased and shifted towards mid-length. This group was especially widespread during the late Tatarian (Vyatka time), their optimum conditions being shallow-water areas of lakes with high bicarbonate levels.

### *Ecological development of the* Darwinulocopina

The ecological history of the Darwinulocopina is associated with the development of numerous Late Permian lakes of different sizes on the Russian Plate. This process was influenced by a marine regression, with the sea retreating northwards, and by the influx of fresh waters and terrigenous sediments from the Ural mountains. The lakes left behind by the withdrawing sea, initially saline, developed bicarbonate and bicarbonate–sulphate freshwater chemistries. The geographical distribution of the different types of lake formed a mosaic; thus, in the southwestern part of the Russian Plate, fresh, low-carbonate lakes characterized by terrigenous sedimentation dominated, but occasional high-sulphate waterbodies occurred in which gypsum was precipitated (e.g., the Buzuluk Depression).

The diversity of lakes and ecological niches was favourable for ostracod diversity. The Darwinuloidea preferred lakes with terrigenous sedimentation and insignificant bicarbonate. They are found mostly associated with clays and siltstones, more rarely with marls and limestones. The Suchonelloidea inhabited lakes with increased bicarbonate and could also live in low-sulphate waters; they are encountered mainly in limestones and marls. The Darwinuloidoidea inhabited high-bicarbonate waterbodies and could also survive in low-magnesium waters. They occur predominantly in limestones, more rarely in marls, and occasionally in dolomitic limestones.

Most of the Late Permian lakes were probably large in areal extent, but shallow. Besides ostracods, they were inhabited by bivalves and gastropods. Charophyte algae occurred occasionally. The lakes sometimes dried up, leaving ripple marks, raindrop marks and desiccation-cracks in their sedimentary records. Many of the lakes were interconnected repeatedly during periods of high lake levels, according to the evidence of correlation of some sedimentary sequences. In spite of the diversity of lakes and the mosaic pattern of their distribution, however, there is a clear general

trend of freshening through time, as well as geographically from South to North, following the regressing sea. These trends are reflected in the ecological development and distribution of the Darwinulocopina.

## Conclusions

Late Permian Darwinulocopina are represented by three superfamilies: Darwinuloidea, Suchonelloidea and Darwinuloidoidea, each with its own distinctive evolutionary history. The Darwinuloidea developed through aromorphosis and increasing ecological valency. The Suchonelloidea developed through adaptive radiation and consolidation of ecological niches. In the Darwinuloidoidea, development was by progressive adaptive adjustment. These different evolutionary trends account for the different ages of the crucial stages of development of each superfamily. For the Darwinuloidea, this was the beginning of the mid-Tatarian (Severnaya Dvina Formation), marked on the Russian Plate by major geological reorganization (Molostovsky, 1983; Molostovskaya, 1997) and by significant changes in the evolutionary development of terrestrial vertebrates (Ochev, 1976) and ichthyofauna. This boundary was unimportant for the Suchonelloidea, however; the turning-point in their development occurred much later, at the end of Severnaya Dvina time. The Darwinuloidoidea developed more steadily, although the most important changes are also associated with the end of Severnaya Dvina time.

## Acknowledgements

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# Variable noding in *Cyprideis torosa* (Ostracoda, Crustacea): an overview, experimental results and a model from Catastrophe Theory

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*Key words:* noding, salinity, physiological response, *Cyprideis torosa*, cusp model

## Abstract

Various anomalohaline ostracod species will, under certain conditions, develop hollow, outward flexions of the lateral surface of their carapace that are called nodes. While the potential positions on the shell surface of such nodes are normally fixed, their number and relative strength are variable. This phenomenon, which is called variable noding, is best known in *Cyprideis torosa* (Jones, 1850) but it actually features in several more species of *Cyprideis* and other cytherideinid genera, both Recent and fossil. Alternately explaining it as ecophenotypic or genotypic, nearly all primary sources in the literature associate variable noding with low environmental salinity. Culturing results confirm that the phenomenon reflects a direct physiological response rather than a genotypic adaptation. From the cultures, it also appears, however, that there is yet another factor active, in addition to and interfering with environmental salinity. This factor is provisionally called 'factor X'. The experiments suggest that factor X may represent either the pH or the $CO_2$ content of the ambient water, and hence be directly related to dissolved $CaCO_3$. With two interfering factors, variable noding can possibly be described by the cusp model of Catastrophe Theory with salinity and factor X as the controls and noding capability as the resultant reaction surface. Lacking sufficient calibration, the model must remain qualitative for the time being. Further experimentation and careful observation in the field should allow its quantification, thus clearing the path for palaeoecological application.

## Introduction

Benthonic Ostracoda are minute crustaceans possessing bivalved, calcareous carapaces, which are capable of excellent fossilization. The euryhaline species *Cyprideis torosa* (Jones 1850), now known from Europe, Asia and Africa, first appeared in the late Miocene. It inhabits anomalohaline waters (i.e., saline waters of divergent salinities and possibly aberrant solute composition; van Harten, 1990). Under certain conditions, individuals of this species will develop nodes: localised, raised areas on the lateral surfaces of their valves (Figure 1). These nodes are essentially hollow, outward flexions of the carapace with shell thickness remaining approximately the same across them (for cross sections through noded shells, see Kilenyi, 1972: pl. 1, figures 4–6). They are variable in number and size but their potential positions on the shell are fixed (Kilenyi, 1972; Sandberg, 1964; Vesper, 1972, 1975). Contrary to Kilenyi's (1972) assertion, nodes seem to be more frequent on right than on left valves (Sandberg, 1964; Vesper 1972, 1975; this paper). The phenomenon is known as variable noding.

The occurrence of noded and unnoded (i.e., smooth) variants within the species has caused considerable nomenclatural confusion. In the context of the species as a whole, both noded specimens and noded populations seem to be rarer than are smooth ones. Soon after Jones had first described the species as *torosa* (meaning *swollen, with swellings*), Brady (1868) proposed the name *littoralis* for the more common smooth forms. Despite the fact that the latter author (in Brady & Robertson, 1870) withdrew his misnomer almost immediately, the ensuing taxonomic tangle lasted for nearly a century, particularly in zoological literature. It was not until Sandberg (1964) published his classic monograph on the genus *Cyprideis* in the Americas that the two variants were

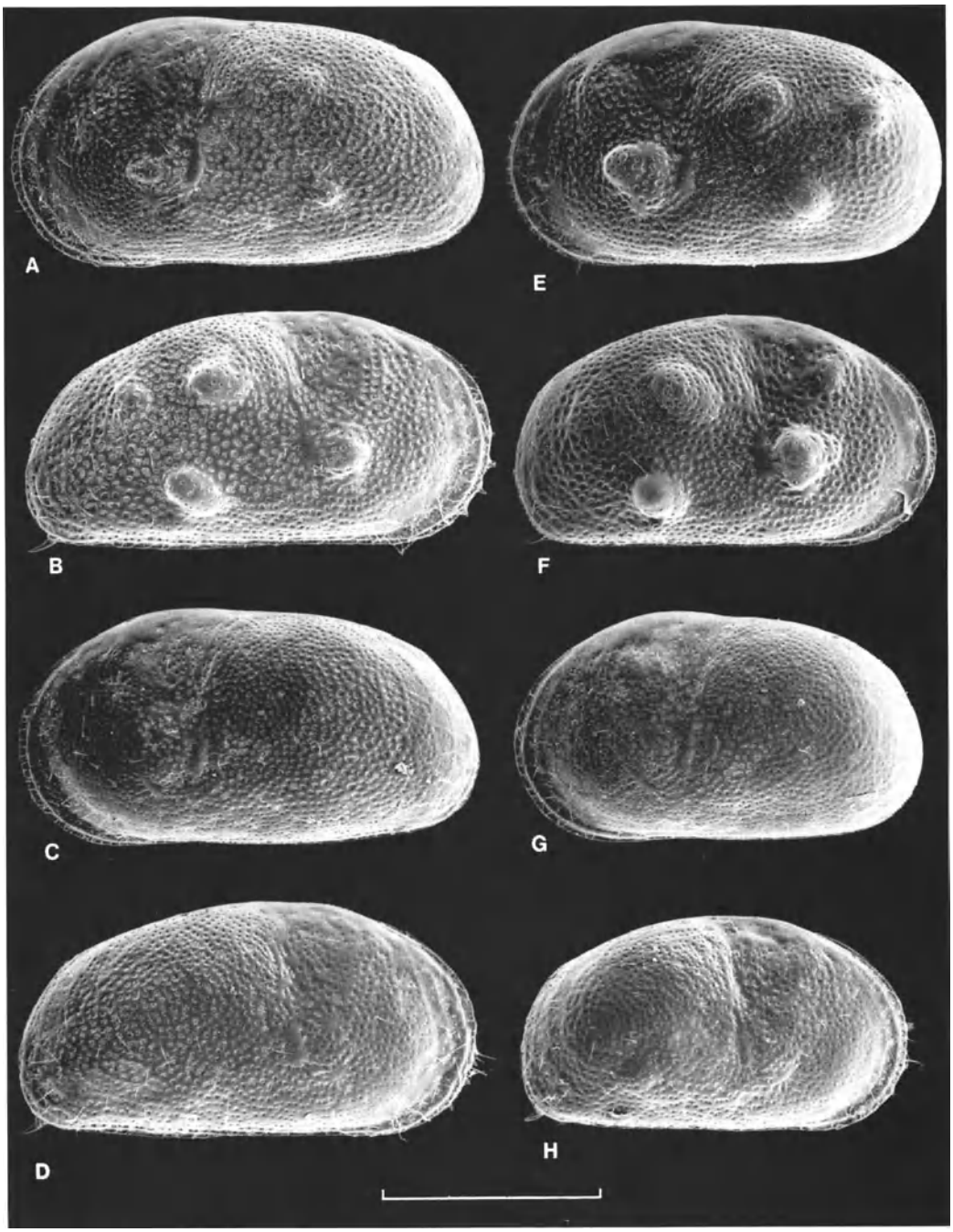


*Figure 1. Cyprideis torosa*. External views of noded and smooth adult valves retrieved from the approximately 13 g $l^{-1}$ culture. (A–D) Males; (E–H) Females; (A, C, E, G) left valves; (B, D, F, H) right valves. Scale bar = 0.5 mm.

widely recognised as conspecific. For synonymy and an enlightening review of the species' nomenclatural history, see Sandberg (op. cit.).

Noded and smooth individuals are sympatric and often occur together in the same or neighbouring local populations. Whereas 100% smooth populations are common, 100% noded ones have never been found. The highest figure quoted in literature is approximately 98% noded (Vesper, 1972, 1975).

Variable noding seems fairly wide spread among anomalohaline ostracods in general. It features in several *Cyprideis* species, both fossil and Recent. Nodes similar to those in *Cyprideis*, and probably homologous, also occur in other cytherideinid genera such as *Hemicyprideis* and *Cytherissa*. Hollow nodes in the limnocytherids *Limnocythere* and *Theriosynoecum* may or may not be homologous (see van Morkhoven, 1963). The wart-like outgrowths on the shells of the Oligocene cytherideinid *Neocyprideis williamsoniana* (Bosquet) that have often been mentioned in this context are massive rather than hollow, however, and consequently fall into a different class (see Keij, 1957: pl. 18, figure 20).

Nowadays, most authors agree that variable noding is somehow associated with low ambient salinity. This relationship is of obvious palaeoecological significance since it suggests the possibility of estimating palaeosalinity from fossils. Unfortunately, there are several major obstacles. The process that causes noding is still poorly understood, and there is little agreement about the salinity level inductive to node formation. Furthermore, conspecificity of smooth and noded dimorphs is often even harder to ascertain in fossil than in Recent forms.

In this paper I critically review some controversial opinions in the light of results from culturing experiments and propose a new model to explain variable noding. For more exhaustive historical reviews, the reader is referred to Kilenyi (1972) and Vesper (1972, 1975).

## Genotype or ecophenotype

The apparent association with low salinity suggests that node development is somehow triggered by the environment. The question remains whether a genetic adaptation is involved or a mere physiological response to low external salinity.

Foremost among proponents of genetic control are Hartmann (1964) and Kilenyi (1972). Their hypotheses are based on the notion, originally emanating from Triebel (1941), that the genus *Cyprideis* is thalassogenic (i.e., of marine descent) and that noded populations are somehow en route from marine to freshwater habitats. Noding would be an adaptive feature connected with life in less saline environments and be controlled by natural selection. Kilenyi (op. cit.) compared part of his model with industrial melanism in the lepidopteran *Biston betularia*, which is, of course, the classic example of dynamic genetic dimorphism. In the case of variable noding, however, the crucial problem is that the function, hence the possible selective advantage, of the nodes is absolutely unknown and probably even non-existent (van Harten, 1996). The only physical function that was ever proposed, viz. a mechanism to adjust the weight of the ostracod to a lower specific gravity of the medium (Triebel, 1941), was convincingly disproved by Sandberg (1964) and Kilenyi (1972).

The nodes are probably a direct, physiological response to the environment. Sandberg (1964: p. 42) suggested that noding represented "a physiologically controlled, abnormal but not pathological reaction to a deficiency of some environmental constituent or constituents". Van Harten (1996: p. 193), on the basis of culturing experiments, called the nodes a "cast of the past", a frozen memento of an accident of sorts that happened during the previous moulting.

This does not detract from the fact that the *positions* at which the nodes may occur on the shell must be presumed to be fixed genetically or anatomically. Sandberg (op. cit.) established a combined total of seven different potential node positions that form a relatively constant pattern in the various species of the genus *Cyprideis*. Different species show different combinations of implemented positions, and no species are known to fill all seven positions simultaneously. *Cyprideis exuberans* van Harten from the Spanish Neogene seems an exception: it exhibits seven nodes but their arrangement on the shell deviates somewhat from Sandberg's scheme (van Harten, 1980).

Before examining the evidence that noding is a physiological response to specific external salinity levels, it is useful first to discuss the salinity range involved.

## The critical salinity level

The literature is next to unanimous in recognising a

negative correlation between variable noding and ambient salinity, i.e., noding becomes more pronounced as salinity decreases. One exception is Kruit (1955) who found noded *C. torosa* in both weakly saline and polyhaline to strongly saline environments in the Rhône delta. That author reported salinity levels as high as 46 g $l^{-1}$ but the correlation with noding is questionable since he did not discriminate between dead and living specimens. The actual salinity at which his noded specimens were living remains in doubt.

The link between noding and low salinity poses the obvious question of whether or not there is a critical salinity level below which nodes are able to develop, the level, therefore, that would divide smooth from partly noded populations. There is little unanimity here. Opinions vary from a mere 1 g $l^{-1}$ (Wagner, 1957) through 5 g $l^{-1}$ (Schäfer, 1953), 6–9 g $l^{-1}$ (Vesper, 1972, 1975) and 8 g $l^{-1}$ (van Harten, 1996), to a denial of the existence of any critical level (Kilenyi, 1972). It is this controversy in particular that stands in the way of routine application of variable noding as a palaeosalinometer.

Part of the problem is that there is an unfortunate tendency among workers to regard measured salinity as gospel. In the anomalohaline waters that constitute the typical habitat of *Cyprideis*, salinity is often significantly variable due to tidal influences and especially the opposing effects of precipitation and evaporation. Anomalohaline salinity levels tend to be lowest, therefore, in winter and highest in summer (see, for instance, van Harten, 1975).

In an ostracod's lifetime, the only occasion when nodes can be formed must be in the brief intervals of ontogenetic moulting, when the old shells are shed and new ones formed (see also Sandberg, 1964: p. 41, for a statement to the same effect; and Kilenyi, 1972: p. 58, for an expression of doubt). If environmental salinity is at all relevant to noding, then it must be salinity during moulting, or perhaps immediately before, that affects the presence of nodes in the next instar. Subsequent salinities may or may not be different but are quite irrelevant to node formation. For this reason, readings taken in waters that are prone to salinity variation should be interpreted with caution.

Kilenyi (1972) collected noded specimens at salinities ranging from 7 to 34 g $l^{-1}$ in the Thames estuary. Although he reported that salinity increased eastward in the estuary, he did not pay special attention to possible in situ changes over time. Yet such changes must have been rather universal in the estuary, due to interaction of such agents as tides, wind, and river runoff. His material, or part of it, may even have been transported by currents from areas with a different salinity than that at the sampling locality. Given the general seaward increase, and assuming that such transport would be predominantly in a seaward direction, then chances are that salinities at which the nodes formed were lower, on average, than those at which the noded specimens were eventually collected. It follows that Kilenyi's higher salinity quotes, on which his negation of the critical level was based, might somewhat overestimate the values at which node formation actually took place.

Vesper (1972, 1975) studied the phenomenon of noding in *C. torosa* in several inland brackish waters in North Germany. Foremost among his localities was the River Schlei, the salinity of which he reported as increasing from about 2 to 14 g $l^{-1}$ in a seaward direction, over a distance of around 40 km. While he too did not allow for fluctuations, there is no reason to assume that his data are significantly compromised by transport.

Setting aside a few local variations, Vesper found a sharp correlation between salinity and noding. Noded and smooth forms both occurred over the whole of the salinity range of the River Schlei but "above the limit of 5 g $l^{-1}$ salinity the unnoded form predominates, below the 5 g $l^{-1}$ limit almost exclusively the noded form of *Cyprideis torosa* is present" (1975: p. 212). Similarly, the maximum number and strength of the nodes tended to be greatest at lower salinity.

The correlation of salinity and noding reported by Vesper is non-linear: the ratio of smooth relative to noded specimens changes very abruptly in the approximately 5–9 g $l^{-1}$ salinity range (Figure 2). For this reason, Vesper considered the 5–9 g $l^{-1}$ range as most critical for noding, although he did mention some localities with 4 g $l^{-1}$ salinity at which he found only smooth specimens.

## Culturing experiments

Van Harten (1996) first cultured *C. torosa* at different salinities in the laboratory and obtained noded juveniles in the offspring of 100% smooth parents that came from a locality where no noded specimens had ever been found living. Salinity in the source locality ranged from around 12 g $l^{-1}$ in winter to around 22 g $l^{-1}$ in summer. In cultures kept at 6.5 and 3.25 g $l^{-1}$ salinity, about 10% of the first generation A-1

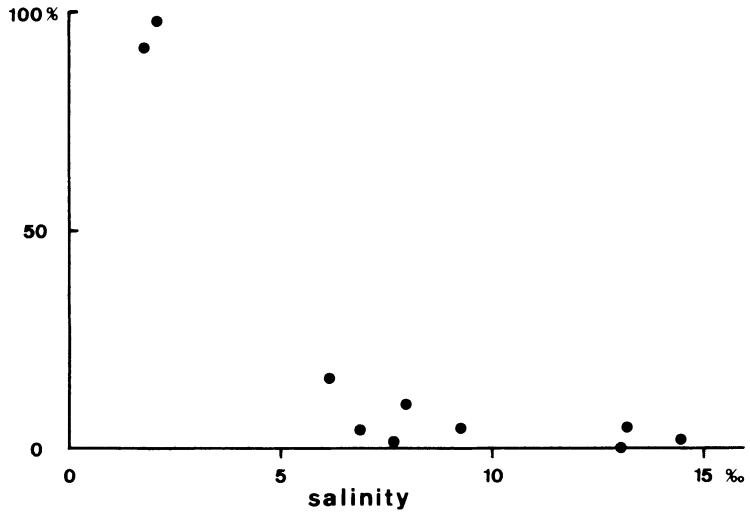


*Figure 2. Cyprideis torosa.* Relationship between noding (in terms of percentage of noded specimens per sample) and salinity in the River Schlei. After data in Vesper (1972, 1975).

juveniles and an indeterminate portion of earlier instars developed nodes. This experiment demonstrated that noding may develop instantly in smooth-shelled populations if they are subjected suddenly to lower salinity, and suggested that the phenomenon reflects a direct physiological response, unconnected with any particular genetic background. Because noded specimens did not arise in a culture kept at 13 g $l^{-1}$ (i.e., the salinity in the source locality on the date of collecting) it was supposed at the time that the critical salinity level below which nodes could develop must lie somewhere between 6.5 and 13 g $l^{-1}$. Relating noding to osmosis, van Harten (op. cit.) proposed 8 g $l^{-1}$, a value that equals the internal osmoregulation level that brackish-water ostracods are able to maintain according to Aladin (1993).

The results of a more recent experiment, however, make it necessary to reconsider the issue. As opposed to the former experiment, the new cultures were started from all-noded materials, with the aim to make the nodes disappear and to ascertain at what salinity level this would happen. A series of four cultures was set up at around 13, 10, 7, and 3 g $l^{-1}$ salinity, respectively, the last mentioned value being close to the salinity of the natural water from which the starting material was collected (around 4 g $l^{-1}$, November 1995). Procedures were essentially the same as those used in the first experiment described in van Harten (1996). For a protocol of the present experiment, the reader is referred to the Appendix.

*Table 1. Cyprideis torosa.* Offspring (A-1 instar) of four batches of 15 all-noded adult females each that were cultured at approximate salinities indicated (see Appendix); $N_{LV}$ and $N_{RV}$ are total numbers of left and right valves retrieved from the cultures after termination; % noded refers to valves showing nodes, irrespective of number and intensity. Salinity correlates positively with yield (in terms of retrieved number of valves) and negatively with relative frequency of noded specimens. Note that noding is more frequent on right than on left valves

| Salinity (g $l^{-1}$) | $N_{LV}$ | $N_{RV}$ | % noded LV | % noded RV |
|---|---|---|---|---|
| 3 | 60 | 54 | 78 | 98 |
| 7 | 184 | 172 | 66 | 88 |
| 10 | 185 | 183 | 39 | 74 |
| 13 | 310 | 294 | 30 | 64 |

Noded specimens arose in the first-generation offspring in all cultures, but their relative frequencies appeared to increase with reduced salinity (Table 1). This instant reaction to salinity change is in line with the results of the older experiment in confirming that noding is a direct physiological response. However, the complete disappearance of nodes above 8 g $l^{-1}$ salinity, which is predicted by the osmosis theory, did not materialise. Nodes were also found in the cultures kept at around 10 and 13 g $l^{-1}$ salinity. This discrepancy can only be explained if an extra factor is involved that modifies the physiological effect of salinity. This cannot be food, as van Harten (1996)

tentatively proposed, because imperfect nutrition can hardly be expected to result in the appearance of nodes where none should be. Node building takes energy, and energy ultimately comes from food. Although it is possible to make an informed guess about its true identity (see below), the extra factor will henceforth be referred to as 'factor X'.

The yield, and hence the population density, of the cultures was proportional to salinity (Table 1). This somewhat surprising correlation may be artifactual, however, rather than truly salinity-related. Water for the 13 g $l^{-1}$ salinity culture was taken from a pond that is known to support a huge and flourishing population of *C. torosa*. For the other cultures, media were made up by progressively diluting this 13 g $l^{-1}$ water with low-saline water taken from a nearby ditch. Not a single specimen of *C. torosa* was found in this second source at the time of collecting, retrospectively suggesting the presence of a substance detrimental to occupation (e.g., agricultural fertiliser or pesticide). Because of the negative correlation between amount of admixed water and population density in the cultures, the density differences may reflect progressive worsening with dilution. This issue obviously needs further study and experimentation.

The tanks used for culturing were quite small (around 100 ml of medium) and population density is likely to have had differential effects on living conditions. Partial $O_2$ and $CO_2$ pressures are most likely to have been affected. It seems probable that $O_2$ was lower and $CO_2$ higher in the more populous cultures than in the more sparsely populated ones due to the animals' respiration. The amount of $CO_2$ influences the pH of the water and, thereby, the solubility of $CaCO_3$. It should be noted in this connection that small decreases in environmental oxygen do not necessarily exert a negative effect on *C. torosa*, due to the species' high capacity for long-term anaerobiosis (Jahn et al., 1996).

Remarkably, $CaCO_3$ has been associated with noding before. Vesper (1975: p. 215), in one apparently far-seeing phrase, opined that "a physiological control of noding, i.e., an influence of environmental factors, *either* salinity *or* the amount of $CaCO_3$, within sensitive periods of the animals could be imagined" (emphasis added by the present author). He may have been wrong only in co-ordinating the environmental factors, not accumulating them. The present culturing results are consistent with the assumption that factor X in reality represents pH or $CO_2$ content and, therefore, is directly related to dissolved $CaCO_3$.

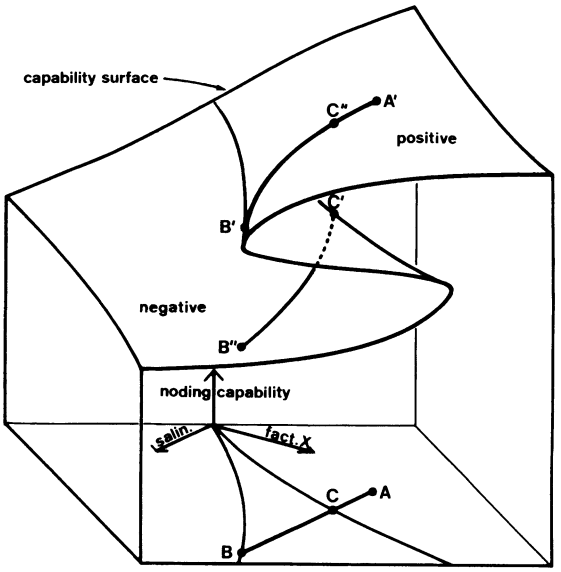


*Figure 3.* Variable noding in *Cyprideis torosa* as descibed by the cusp model of Catastrophe Theory. See text for explanation.

Whatever the factor's nature, its mere existence is the main point of the present discussion. The combination of two driving factors that modify each other's effect makes the noding phenomenon a potential candidate for description by the cusp model of Catastrophe Theory. This candidature is seconded by a set of other qualities: the presence of bimodality (the shells are either noded or smooth), the suddenness of the transition and the apparent occurrence of hysteresis (the transition from smooth to noded seems to take place under different conditions than does the reverse). For an account of the significance of these characteristics, see Zeeman (1976).

## Noding as a cusp catastrophe

"Things that change suddenly, by fits and starts" (Zeeman, 1976: p. 65) often can be explained through Catastrophe Theory. It seems possible indeed to describe variable noding by the so-called cusp model of this theory with salinity and factor X as the controls and noding capability as the reaction surface (Figure 3).

Salinity and factor X are plotted at right angles on the horizontal plane, perpendicular to which stands a third axis that represents the resulting reaction. The latter is expressed as noding capability (capacity or incapacity to form nodes). The pleated surface on top is called the capability surface since it is the locus of the

noding capability that results from any combination of the controls on the base plane. Extremely low salinity levels combined with any value of factor X result in positive capacity for noding, whereas extremely low values for factor X lead to incapacity, irrespective of salinity. In such extreme cases there is no interference by the other variable. This is true of all control intersections that are overlain by only one sheet of the capability surface. Things are different, however, in the middle of the drawing, under the pleat. Here, all possible control intersections are overlain by three sheets of the capability surface, the upper and lower ones of which represent capacity and incapacity respectively. The third or middle sheet has no practical significance (it exists only to provide a theoretical, continuous transition between the other two parts of the surface).

Under the pleat, both capability modes are possible, and noding may or may not occur (this does not necessarily refer to partly noded and partly smooth populations; the bimodal option basically applies to any individual specimen). In the model, it depends on how the particular control combination has come into being, which of the two modes will actually materialise. Consider point A on the control surface. It is lying outside the projection of the pleat, and a specimen sitting there has positive noding capability, hence may be noded. Now let salinity increase, or the specimen migrate to a higher salinity area, keeping factor X constant. The matching point of intersection of the noding capability axis with the capability surface (A′) will move to the front left in the drawing until it reaches the edge of the pleat (B′). Here the top sheet vanishes, and the path of the point drops abruptly to the bottom sheet (B″). In terms of noding this translates into saying that the capability for noding is retained until the salinity level that corresponds with the edge of the pleat is reached, whereupon it suddenly disappears, and noding is no longer possible (B). In real life, the specimen will, of course, keep its nodes, if it has any, but it (i.e., its later ontogenetic instars, if any) will be unable to develop new ones in future. The same is true of its descendants.

The phenomenon of hysteresis becomes visible by going over the trajectory the other way. The point of intersection (B″) now travels on the bottom sheet until it reaches the right edge of the pleat (along the whole of this part of the trajectory there is no capability for noding, and the ostracod stays smooth). At the edge of the pleat (C′), the bottom sheet vanishes, and noding capability must jump to the top sheet (C″): the capability to form nodes suddenly appears. This happens at a lower salinity level than the one where the nodes disappeared (which is precisely what occurred in the culturing experiments). The space between the points of appearance and disappearance represents the hysteresis interval.

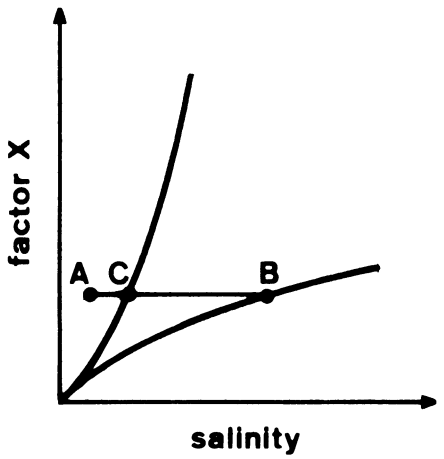


*Figure 4.* Base plane of cusp model of Figure 3 showing salinity and factor X as the controls and the bifurcation set bounding the hysteresis area. See text for explanation.

The projection of the pleat edges onto the control plane is the cusp-shaped curve to which the model owes its name. It is easier to work from there than from the three-dimensional diagram (Figure 4). Inside the bifurcation set of the cusp is the hysteresis area, the width of which depends on the value of the controls. The foremost property of the bifurcation set is that nothing happens upon entering: the capability mode remains the same. It is only upon leaving the bifurcation set, when the control trajectory intersects with the second curve of the set, that the catastrophic change occurs, and the mode suddenly turns into its alternate state.

The bifurcation set might seem to divide a 'smooth' from a 'noded' area on the control plane. It is important to note that these areas in reality represent mere noding capability, not absence or presence of nodes. In the 'smooth' area this capability is absent, so under these conditions all specimens are of necessity smooth. But, despite the positive capability for noding in the 'noded' area, this faculty need not necessarily be implemented, and smooth specimens may still occur. The constraint probably is that it takes energy to form nodes. According to the osmosis hypothesis (van Harten, 1996) a specimen must first attain osmoregulation (through the uptake of salt in its food) in order to be able to actually construct nodes. This explains

why 100% noded natural populations have never been found in *C. torosa*, although 100% smooth populations are common.

The present model is strictly qualitative and it may be oversimplistic in that it uses only two controls and a symmetrical bifurcation cusp. The latter might be skewed, and more than two control parameters might be involved. Only quantification can show whether the model runs true to form as is or needs to be adjusted. Catastrophic events driven by more than two control parameters can be accommodated in higher-order models that use more than three dimensions (for further elucidation on this point, see Zeeman, 1976). Calibration of the model should come from further experimentation and careful observation in the field.

## Conclusions

Continued experiments confirm the earlier conclusion that noding in *C. torosa* is a physiological response. Nodes can be produced or made to disappear instantly by changing the environmental salinity. There is no evidence of genetic control. On the other hand, the present results strongly indicate that, besides salinity, an additional factor, provisionally termed 'X', is involved. The cusp model of Catastrophe Theory seems able to explain what happens.

Its present lack of calibration notwithstanding, the cusp model goes a long way to explain the bewildering diversity of reports on the critical salinity level associated with noding. The model predicts that there really is no such critical level. Whether any given salinity level may or may not lead to node formation depends on the interaction of salinity with factor X, assuming that osmoregulation has been attained. The model also satisfactorily explains the hysteresis between appearance and disappearance of nodes found in the culturing experiments and sheds a new light on some of the more extreme salinity quotes in existing literature.

The absence of a truly critical salinity level for the occurrence of nodes does not necessarily preclude variable noding as an index in palaeoecology although the wish-dream of an easy-to-use palaeosalinometer had best be forgotten. There may, of course, be situations in which one really is faced with the impossible task of having to solve the proverbial equation with two unknowns. If the present model turns out well, however, and once it is calibrated, then one might be in a position to judge one's 'palaeoprobabilities' with far greater reliability than ever before.

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## Appendix: protocol of culturing

From November 17, 1995 to July 30, 1996, *C. torosa* was cultured from live material taken from a drainage canal near the Dutch North Sea coast (Hondsbosscheveaart near Camperduin; salinity on collecting 4–4.4 g $l^{-1}$). Culturing media consisted of mixtures of natural brackish waters, initial salinity levels being set at 3.4, 6.7, 10.0 and 13.2 g $l^{-1}$, respectively. These levels were roughly maintained in the experiment by adding demineralised water to compensate for evaporation. Terminal values were 2.6, 5.9, 9.0 and 12.6 g $l^{-1}$, respectively. Ambient temperatures were mostly in the 20–30 °C range.

Culturing occurred in glass jars containing about 100 ml of medium and crushed Palaeozoic shale as a substrate. Some fragmented egg-shell was added to the substrate to provide an excess of $CaCO_3$. The food was garden compost (the actual nutritious substance probably being bacterial biomass). Each culture started from 15 females and four males, all adult and showing one or more nodes.

The two higher salinity cultures tended to be the most active ones during the whole of the experiment. They were also the first to show reproduction. Freely moving juveniles were first seen by the second half of February 1996 in the approximately 13 g $l^{-1}$ culture. For numbers of adult specimens retrieved after termination, see Table 2.

*Table 2. Cyprideis torosa.* Numbers of adult specimens, in terms of valves, retrieved from cultures after termination

| Salinity ($g^{-1}$) | $N_{males}$ | $N_{females}$ |
|---|---|---|
| 3.4–2.6 | 10 | 29 |
| 6.7–5.9 | 38 | 84 |
| 10.0–9.0 | 32 | 109 |
| 13.2–12.6 | 138 | 287 |

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# The effect of temperature on shell size and growth rate in *Krithe praetexta praetexta* (Sars)

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*Key words:* Crustacea, Ostracoda, *Krithe praetexta praetexta*, life cycle, ontogeny, temperature

**Abstract**

The effect of temperature on growth rate, shell size and shell shape in *Krithe praetexta praetexta* (Sars) was studied in four thermocultures. From July 1995 to June 1996, the cultures were kept in a continuously flowing open system pumping water from the intermediate watermass of the Gullmarn fjord, west coast of Sweden. Three cultures were kept at constant temperatures of 5, 10 and 14 °C, respectively. The fourth (reference) culture largely followed the natural variation in temperature. At the termination of the experiment, all living ostracods from a 125 $\mu$m sieve were sampled from the cultures. Population age structures were analysed for the various thermocultures of *K. praetexta praetexta*. These were more shifted towards later ontogenetic stages with higher temperature, i.e. the ontogenetic development was more rapid in the warmer cultures. An alternative explanation is due to diapause causing cohorts to accumulate in some ontogenetic stages only when the temperature is constant. The differences in shell size of *K. praetexta praetexta* among the thermoconstant cultures were not statistically significant.

## Introduction

The genus *Krithe* Brady, Crosskey & Robertson, is most common in deep-sea habitats where it is one of the most abundant and diverse ostracod genera. *Krithe* is known to show substantial variation in the size of the carapace and the internal anterior vestibule; the significance of the latter variation is a matter of controversy (see Peypouquet, 1977; McKenzie et al., 1989; Whatley & Zhao, 1993; Van Harten, 1995, 1996). Several authors have noted a positive correlation between size of species of the genus *Krithe* and depth (see Van Morkhoven, 1972; Kaesler & Lohmann, 1976; Peypouquet, 1977). Whatley & Zhao (1993) also noted that some species of *Krithe* show an increase in size with depth and explained it as due to a depth correlated decrease in temperature.

We have followed the taxonomy of McKenzie et al. (1989) in identifying *Krithe praetexta praetexta* (Sars) as a discrete subspecies of *Krithe praetexta* (Sars). This is a rather unusual representative of the genus; it occurs at relatively shallow depths of, for example, > 15 m in Scandinavian fjords (see Elofson, 1941), while in the open ocean it occurs at depths from 50 to 500 m (Elofson, 1941; Athersuch et al., 1989). It is a taxon typical of the boreal eastern Atlantic and known from off NE England, Scotland, Denmark, Sweden and Norway (Athersuch et al., 1989) in a total range of water temperatures from +1 to 18 °C (Elofson, 1941). Preliminary observations from aquarium cultures on the ecology and size variation of this subspecies are given by Majoran & Agrenius (1995). The objective of this study was to describe a methodology of culturing and to show the first experimental results of the isolated effect of temperature on the ontogenetic development, shell size and shell shape of *K. praetexta praetexta*.

Little is known about the durations of life cycles and reproductive strategies of representatives of the genus *Krithe*. Uffenorde (1972) found that *K. similis* (G.W. Müller) from the Limski Kanal, Northern Adriatic Sea, has continuous reproduction throughout the year. The life cycle of *K. praetexta praetexta* is yet to be determined in detail. Elofson (1941: 384–385) found the adults, A-1 and A-2 stages in the Gullmarn fjord during the whole year, which may indicate

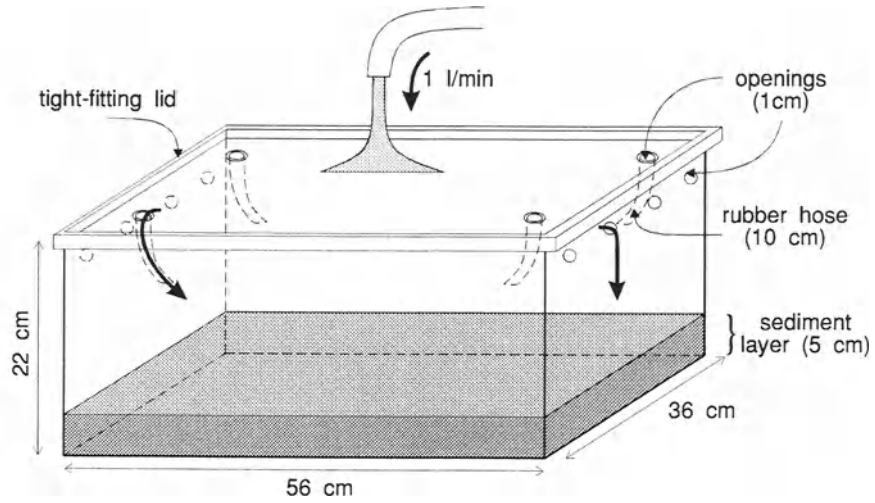


*Figure 1.* Schematic diagram showing the basic setup of the cultured aquaria. Note that both the aquaria and the lids were composed of non-transparent plastic material.

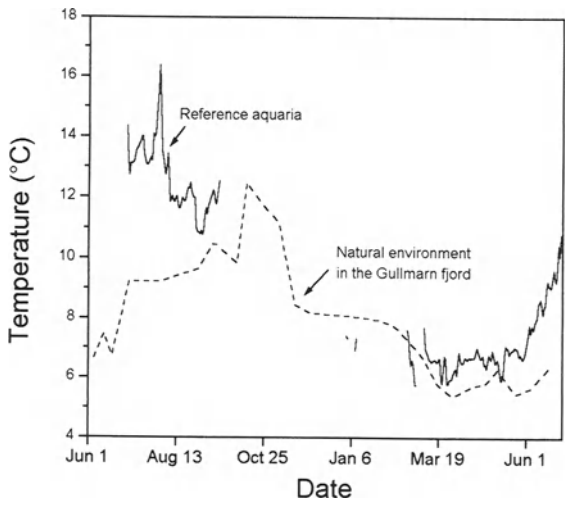


*Figure 2.* Seasonal variation in temperature from July 1995 to July 1996 at a depth of 40 m in the Gullmarn fjord and in the reference aquarium (the temperature monitoring system was out of order most of the time in the reference aquarium from 19 September, 1995 to 22 February, 1996).

that this subspecies also has continuous reproduction throughout the year.

## Material and methods

This study is based on observations of four thermocultures of *K. praetexta praetexta* grown in aquaria at the Kristineberg Marine Research Station, on the Gullmarn fjord, Sweden. The cultures were prepared as follows:

1. Non-transparent plastic boxes (7 in total, each 56×36×22 cm) were used as aquaria in this study (Figure 1). They were provided with lids to reduce the penetration of light to prevent algal growth, in order to mimic the natural light conditions at a depth of 40 m in the Gullmarn fjord. There is no algal growth at a depth of 40 m in the Gullmarn fjord since the mean Secchi depth is only 7.5 m (Lindahl, 1995).

2. The aquaria were provided with surface sediment (0–5 cm) obtained from a depth of 40 m in the Gullmarn fjord (58 °15.85′ N; 11 ° 28.55′ E) on 12 June 1995. The sediment consisted of 11% sand (> 63 $\mu$m), 37% silt (63 > 3.9 $\mu$m) and 52% clay (< 3.9 $\mu$m). The sediment was sieved through a net with a mesh size of 1 cm to remove small pebbles, large shells and macrobiota, then frozen for storage and thawed several weeks later to finally constitute a 5 cm bottom layer in the aquaria (Figure 1).

3. A mixed meiobiota, sieved through a mesh size of 1 mm, containing ostracods was randomly added to the aquaria. By this we assume that all aquaria were characterised by a similar population structure of *K. praetexta praetexta* at the beginning of the experiment. The cultured ostracods derive from the same location in the Gullmarn fjord as the bottom sediment of the aquaria. They were sampled by an epibenthic sledge on 7 July 1995 with a mesh size of 0.5 mm; this mesh inevitably became clogged by sediment during sampling.

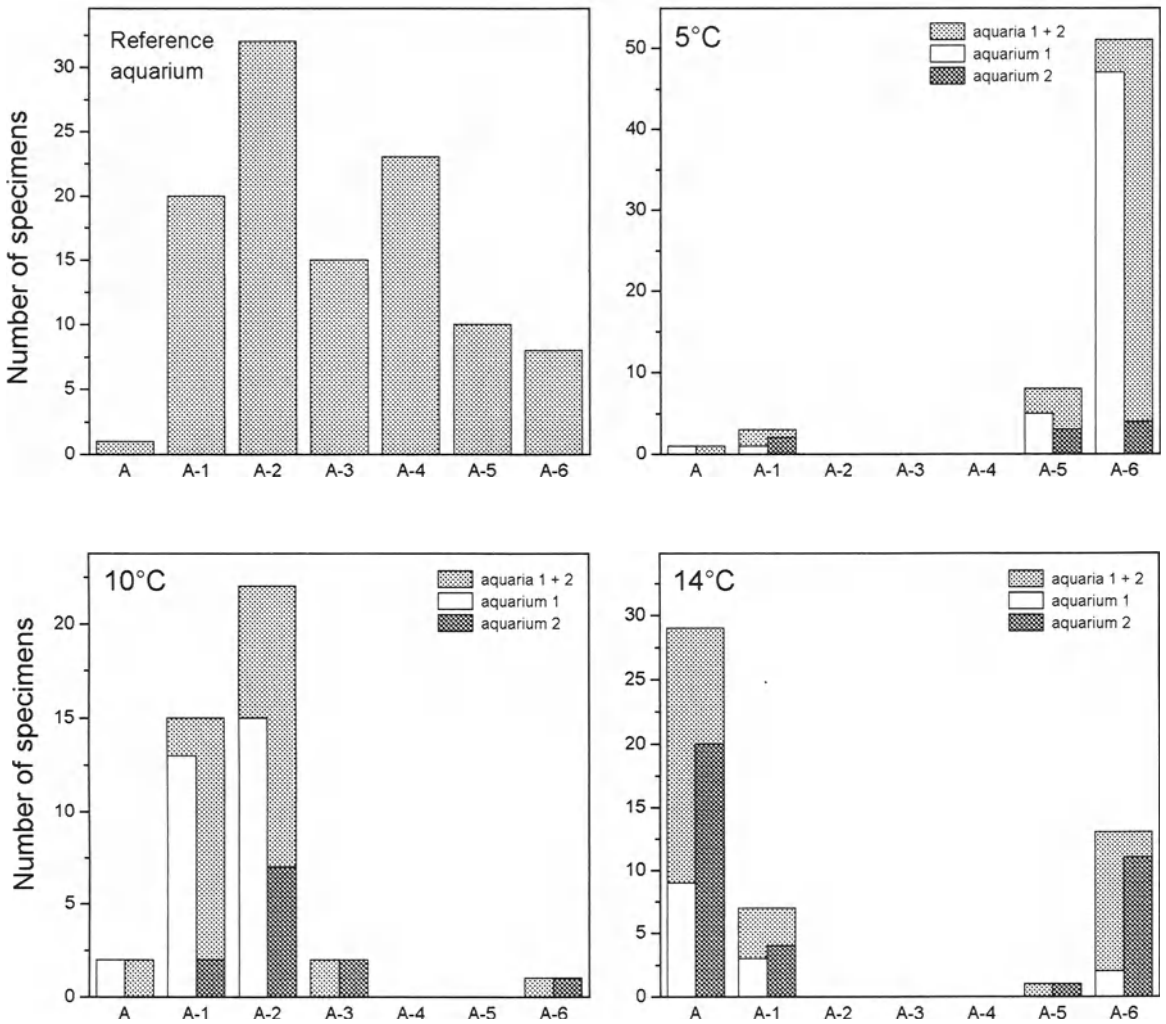


*Figure 3.* Population age structures of living *Krithe praetexta praetexta* in the various thermocultures at the end of the experiment in June 1996.

4. Between July 1995 and June 1996, the aquaria were maintained in a continuously flowing, open system pumping water from the intermediate watermass of the fjord (from which the ostracods derive). This watermass is normally confined to depths between 15 and 50 m (Svansson, 1984). From July 1995 to July 1996, the salinity of the intermediate watermass varied between 32.7 and 34.8 g $l^{-1}$, the oxygen content between 3.6 and 6.4 ml $l^{-1}$, and the temperature between 5.4 and 12.5 °C at a depth of 40 m in the Gullmarn fjord (58 °15.5′ N; 11° 26.0′ E) (Data from the Pelagic Monitoring Kristineberg). The water in the aquaria was renewed by approximately 1 l/min. To maintain well-oxygenated conditions, the water was flowing in a thin layer on the entire lid before entering the aquaria. The water entered the aquaria through four holes (each with a diameter of 1 cm) equipped with 10 cm long rubber hoses, one hole at each of the four corners of the lid (Figure 1). The water left the aquaria through a series of holes (1 cm in diameter) located directly below the lid overlap on both of the shorter sides of the aquaria (Figure 1).

5. Four different thermocultures were maintained. Three cultures (two aquaria for each culture) were kept at constant temperatures of 5, 10 and 14 °C, respectively, by thermoregulation of the inflowing water and by keeping constant temperatures in the storage rooms. Temperature was automatically monitored every hour in these three cultures from which the following means (m) and standard deviations (s) were

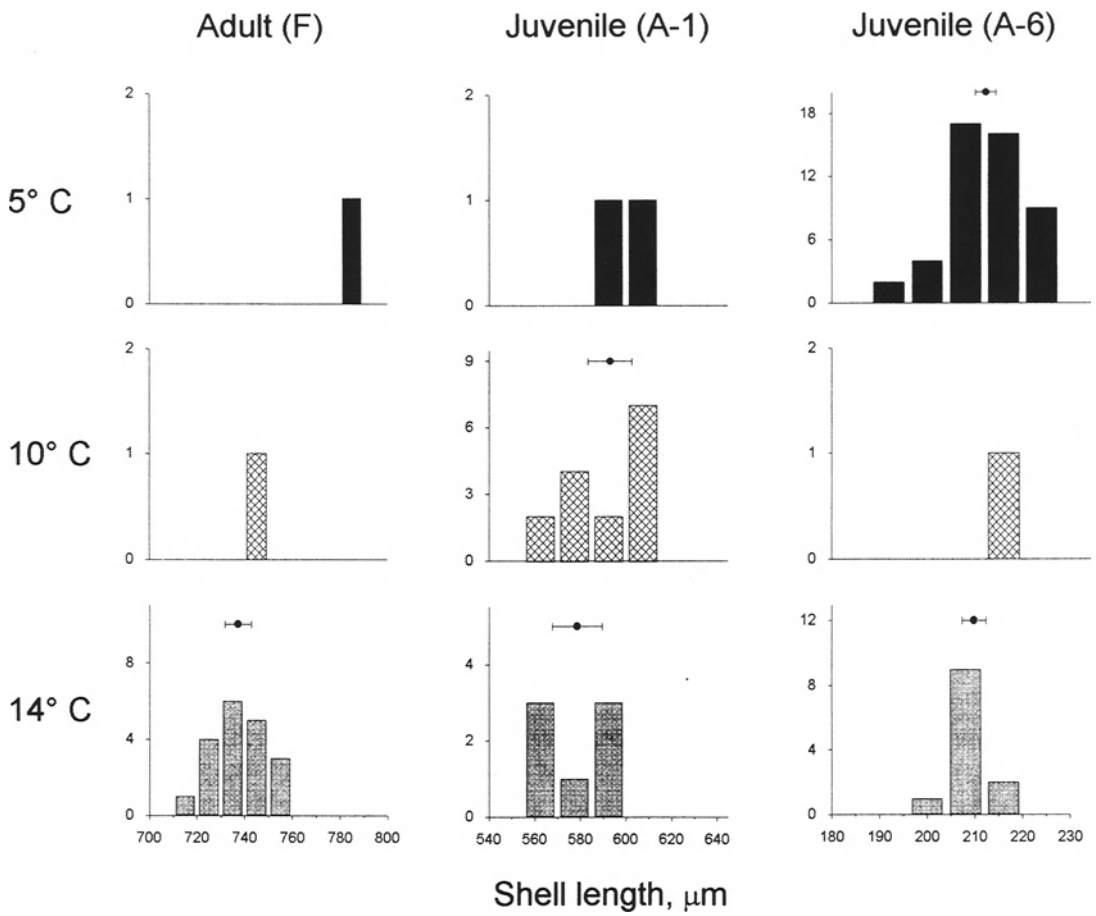


*Figure 4.* Histograms showing variation in the length of adult females and of ontogenetic stages A-1 and A-6 of *Krithe praetexta praetexta* among the three thermoconstant cultures of 5, 10 and 14 °C, respectively. Means and 95% confidence limits are shown in histograms containing more than two specimens.

calculated: m = 5.01 °C with s = 0.24 (for the 5 °C culture) , m = 10.16 °C with s = 0.98 (for the 10 °C culture) and m = 14.10 °C with s = 0.48 (for the 14 °C culture). The fourth (reference) culture (one aquarium) approximately followed the natural variation in temperature (Figure 2). The temperature in this culture ranged between 6 and 9 °C during the last two months of the experiment. The temperature monitoring system for this culture was out of order most of the time between 19 September, 1995 and 22 February, 1996. Summer temperatures were somewhat higher in the reference aquarium compared to the natural environment because of a temperature increase during the transport and storage of inflowing water (Figure 2).

6. The experiment was terminated in June 1996. No sampling was performed until the experiment was terminated. Then, the top 3 cm of the aquarium sediment were removed using a siphon passed carefully over the bottom surface. *Krithe praetexta praetexta* is infaunal but occurs not deeper than approximately 2 cm in the sediment (see also Majoran & Agrenius, 1995). All living ostracods, including stages A-6 to adults of *K. praetexta praetexta,* retained from a 125 $\mu$m sieve were picked and stored. We have no information concerning ontogenetic stages A-7 and A-8.

Population age structures were determined for *K. praetexta praetexta* in the various thermocultures. A mean ontogenetic stage index (MOS-index) was calculated for each culture to describe differences in the advancement of the ontogenetic development:

$$\mathrm{MOS} = (1 \bullet N_{\mathrm{A}} + 2 \bullet N_{\mathrm{A\text{-}1}} + \cdots + 7 \bullet N_{\mathrm{A\text{-}6}})/N_{\mathrm{tot}}$$

where $N_{\mathrm{tot}} = N_{\mathrm{A}} + N_{\mathrm{A\text{-}1}} + \cdots + N_{\mathrm{A\text{-}6}}$.

$N$ denotes the number of individuals in each ontogenetic stage. A MOS-index of, for example, 2.5

indicates that the mean is exactly between ontogenetic stages A-1 and A-2.

Eventual differences in the shell size and shape of *K. praetexta praetexta* among the various thermocultures were documented as follows: Light microscope images of all specimens were captured by a TV camera connected to a frame grabber. The images were imported and stored in a PC computer. We used length as a measure of size and compared ontogenetic stages A-6, A-1 and adults (females). Outlines of the left valves of adult females and the A-1 stage were digitized from a homologous starting point (at the maximum curvature of the posteroventral angularity) and converted to sequences of 100 equally spaced points. To study shape variation among the outlines, the method of eigenshape analysis (Lohmann, 1983) based on the covariance matrix was used.

## Results

The species composition in the various thermocultures at the end of the experiment is shown in Table 1. The population structures of living *K. praetexta praetexta* at the end of the experiment are shown in Figure 3.

A total of 109 living *K. praetexta praetexta* were found in the reference culture. All stages from A-6 to adults are represented, but with a predominance of stages A-4–A-1.

A total of 63 living specimens were found in the 5 °C culture of which 51 belong to the A-6 stage. Other ontogenetic stages were rare or absent.

A total of 42 living specimens were found in the 10 °C culture, mainly from stages A-1 and A-2. Earlier ontogenetic stages and adults were rare or absent.

A total of 50 living specimens were found in the 14 °C culture, predominantly adults but with a notable contribution from stages A-1 and A-6.

The population structures are consequently more shifted towards the later ontogenetic stages with higher temperature and vice versa (Figure 3). Figure 4 shows that within the ontogenetic stages A-6, A-1 and adults of *K. praetexta praetexta*, the average size is greatest in the 5 °C culture and smallest in the 14 °C culture. The differences in size among the various thermocultures are, however, not statistically significant in any of these stages (except for the one large adult female of the 5 °C culture), as seen from the overlapping 95% confidence limits (Figure 4). Figure 5 shows the effect of temperature on the MOS-index, which follows a cyclic pattern in a comparison among the three thermoconstant cultures. Figure 5 also shows the relationship between length and temperature for the A-1 stage.

The eigenshape analysis did not reveal any segregation among the three thermoconstant cultures with respect to the shape of the shell for neither the A-1 stage nor the adult females (Figure 6).

## Discussion

The moult cycle of all crustaceans that have been examined appears to be under hormonal control (Fingerman, 1987). The timing of the moult cycle is regulated by a moult-inhibiting hormone (MIH) which suppresses the biosynthesis of ecdysteroids (i.e. moulting hormones). The release of MIH seems to be primarily nervously regulated and secondarily dependent on ecdysteroid feedback and on environmental stimuli (Aiken & Waddy, 1992). Of environmental stimuli, temperature is among the most important (e.g. Skinner, 1985; Aiken & Waddy, 1992).

The most significant results of the present investigation are revealed by Figure 3. Our interpretation of Figure 3 is that the growth rate in *K. praetexta praetexta* is positively correlated with temperature. In the thermoconstant cultures, relatively discrete cohorts move through ontogeny with different speed at different temperatures. However, such discrete cohorts are not obvious from the reference culture. Diapause or hibernation may cause cohorts to accumulate at some ontogenetic stages. Thus, diapause could explain that the population structure observed in the three thermoconstant cultures are restricted to just a few ontogenetic stages (Figure 3). Constancy in temperature may interfere with the normal breakdown of diapause. In the reference culture, on the other hand, temperature fluctuates enough to breakdown diapause, leading to a more continuous population structure. An alternative explanation may be that the population structure of the reference culture is intermediate between the structure in the 5 and 10 °C cultures. This is in agreement with the circumstance that the temperature in the reference culture during the last months (spring of 1996) of the experiment varied between 5 and 8 °C.

It is known that some ostracod species that hibernate as eggs require certain minimum temperatures for the eggs to develop. Laboratory cultures of *Heterocypris salina* (Brady) require temperatures > 5 °C for the eggs to hatch (see Ganning, 1971). The euryhaline *Cyprideis torosa* (Jones) and the two

*Table 1.* Species composition of ostracod faunas in the four thermocultures sampled during June 1996

| Species/culture | Reference | 5 °C (I) | 5 °C (II) | 10 °C (I) | 10 °C (II) | 14 °C (I) | 14 °C (II) |
|---|---|---|---|---|---|---|---|
| *Acanthocythereis dunelmensis* | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Argilloecia conoidea* | 2 | 1 | 1 | 0 | 1 | 3 | 8 |
| *Bonnyannella robertsoni* | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Cluthia cluthae* | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| *Cytheromorpha fuscata* | 0 | 3 | 2 | 1 | 0 | 0 | 1 |
| *Cytheropteron latissimum* | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Elofsonella concinna* | 7 | 20 | 18 | 12 | 2 | 11 | 10 |
| *Jonesia acuminata* | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Krithe praetexta praetexta* | 110 | 54 | 9 | 29 | 13 | 14 | 36 |
| *Palmoconcha laevata* | 0 | 2 | 2 | 1 | 1 | 3 | 1 |
| *Paracytherois arcuata* | 1 | 0 | 0 | 10 | 11 | 0 | 10 |
| *Paracytherois flexuosa* | 0 | 2 | 0 | 1 | 2 | 2 | 2 |
| *Robertsonites tuberculatus* | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Sarsicytheridea bradii* | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| *Semicytherura nigrescens* | 0 | 4 | 4 | 0 | 0 | 8 | 8 |
| Total number of specimens | 120 | 90 | 37 | 57 | 30 | 44 | 77 |
| Number of specimens/$m^2$ | 606 | 454 | 189 | 288 | 152 | 222 | 389 |
| Number of *Krithe*/$m^2$ | 550 | 270 | 45 | 145 | 65 | 70 | 180 |

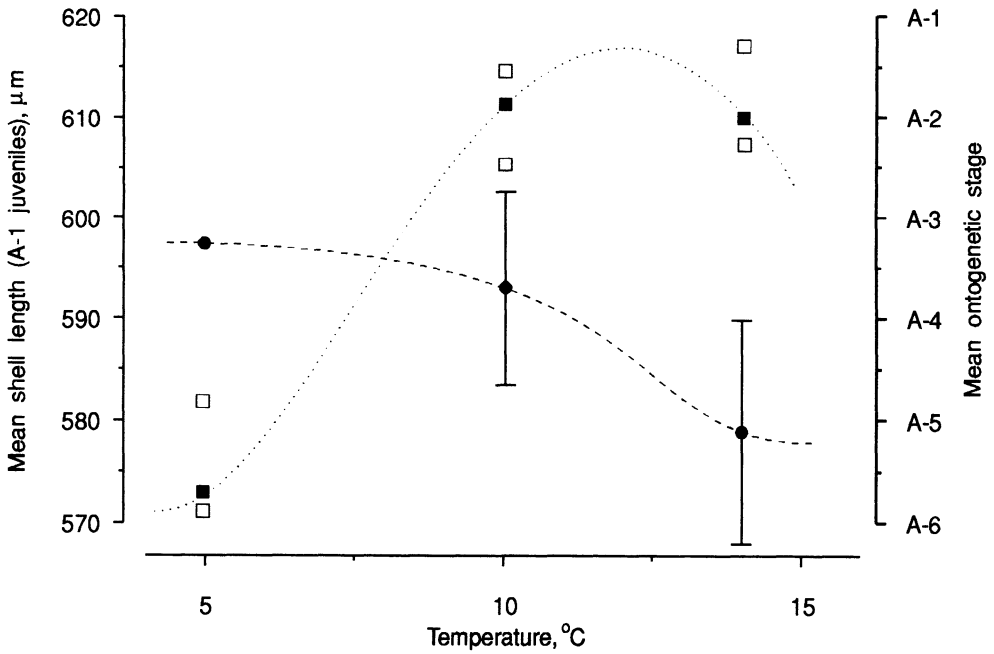


*Figure 5.* The variation in MOS-index among the three thermoconstant cultures (a filled square represents the MOS of aquarium 1 + 2 in a culture; the empty squares above and below the filled square represent the MOS of aquarium 1 and 2, respectively, in the corresponding culture). The variation in length of the A-1 juveniles (filled circles provided with 95% confidence intervals) is also shown.

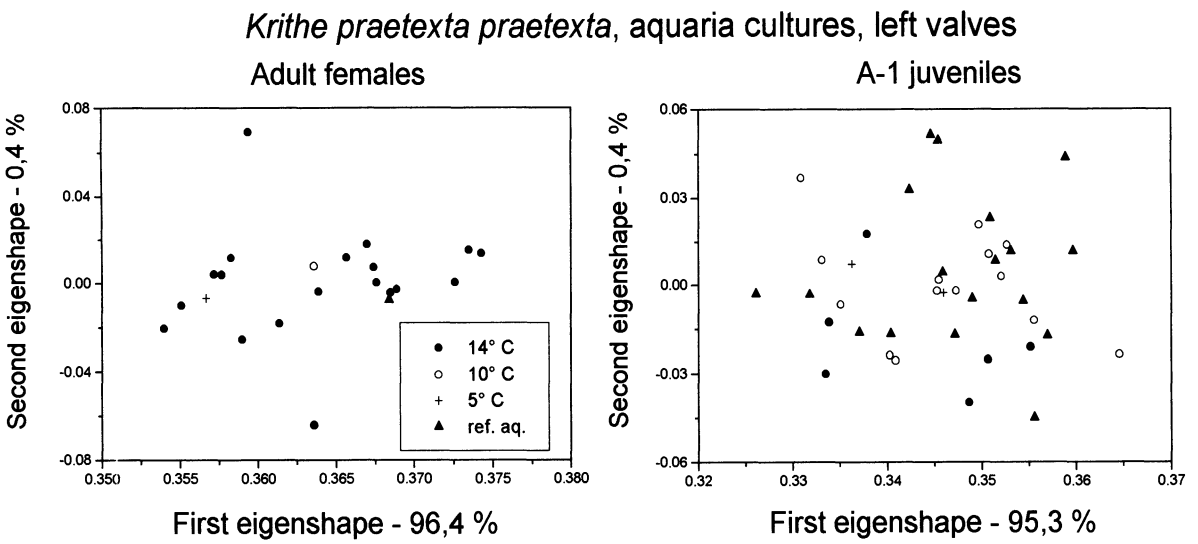


*Figure 6.* Distribution of adult females and A-1 juveniles in the plane of the first and the second eigenshape, respectively. In both cases, the first eigenshape accounts for more than 95% of the total variability and the second eigenshape for less than 0.5%.

brackish-water (euryhaline) species *Loxoconcha elliptica* (Brady) and *Elofsonia baltica* (Hirschmann) require minimum temperatures of 12, 8 and 4–5 °C, respectively, for eggs to develop (Theisen, 1966). It was not the object of this paper to determine the minimum temperature for egg development in *K. praetexta praetexta*. However, a temperature of 5 °C is near the lowermost annual temperature in the environment from where the fauna originates in the Gullmarn fjord (Figure 2). The presence of abundant *K. praetexta praetexta* juveniles at the A-6 stage in the 5 °C culture strongly indicates that eggs and instars of this species can develop at this temperature. It indicates that temperature does not prohibit ontogenetic development to occur throughout the whole year in the Gullmarn fjord which is consistent with Elofson (1941: 384–385).

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# The life history and culturing of *Xestoleberis hanaii* (Crustacea, Ostracoda)

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*Key words:* marine Ostracoda, life history, laboratory culture, embryogenesis, oogenesis, growth rate and time

## Abstract

*Xestoleberis hanaii* Ishizaki, 1968 is one of the most abundant species on the Japanese coast and can be collected in all seasons from intertidal calcareous algae on rocky shores. Several characteristics make this species a suitable 'experimental animal' in the laboratory: (1) adaptability to artificial environments (room temperature, petri dish, artificial seawater, single cultured food-type), (2) high fertility (active copulatory behaviour, egg brooding within the carapace, high egg productivity) and (3) rapid growth rate. Females mate after the final moult (when the reach sexual maturity); oviposition of fertilized eggs takes place over a period of four days after the final moult. Eggs (about 40 in total) are laid a few at a time in the postero-dorsal brood space of the carapace; they hatch in about 9 d as A-7 instars which are then discharged from the brood space within a day or two. Seven moults take place within the next approx. 33 (female) or 39 (male) days to reach adulthood. Adult females live for about 18 weeks and may repeat the reproductive cycle three times; adult males live for about 14 weeks.

## Introduction

The use of *Drosophila* in cultivated experiments has played an important role in the progress of evolutionary biology. In order to elucidate the evolutionary processes of Ostracoda, it has become desirable to develop a similar capability for the culturing of experimental animals. The initial aim of this research was to select a suitable ostracod taxon for such use.

It is relatively easy to cultivate fresh- and brackish-water ostracods, less so for marine species. The marine ostracod *Xestoleberis hanaii* Ishizaki, 1968 lives among intertidal calcareous algae on rocky shores of Japan; we have successfully cultivated it under controlled laboratory conditions for five generations, allowing us to determine details of its life history including oogenesis, ovulation, oviposition, embryogenesis, mating behaviour and ontogeny.

## Material and methods

In its natural habitat, the reproductive rate of *Xestoleberis hanaii* varies seasonally, but a complete range of instars can generally be found throughout the year. Its rapid growth rate makes it especially suitable for culturing. Our culture was started from specimens collected in May 1994 from the Omaezaki Promontory, Shizuoka Prefecture. For the histological study, some specimens collected from Shimoda (Izu Peninsula) were used. In both instances, intertidal algae growing on rocky shores (particularly the short, tufted calcareous algae) were torn off at the holdfast and washed in a bucket of seawater; the resulting material was sieved (16 and 250 mesh) and the residue containing the ostracods was stored in seawater for return to the laboratory. Living ostracods were then extracted by pipette under a binocular microscope.

Laboratory cultures were established as follows:

1. All were maintained at a constant 25 °C, avoiding direct sunlight but receiving natural daylight from the north.

2. Cultures were kept in flat-bottomed plastic petri dishes with lids; single specimens were grown in 24-well tissue culture plates (17 mm diameter, 20 mm deep), group cultures in 6-well plates (37 mm diameter, 20 mm deep).

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3. Artificial seawater was prepared to approximately 34 g $l^{-1}$ to match that of the locality from which the specimens were obtained. The water in each petri dish was refreshed every other day; removal of residual food and waste products was accomplished by transferring the ostracods by pipette to new dishes once a month.
4. Cultures were fed with cultured *Tetraselmis* sp. (Prasinophyceae), prepared as follows: ammonium sulphate (50 mg), calcium superphosphate (15 mg), urea (5 mg) and *crewat* 32 (5 mg) were dissolved in 1 l of distilled water; 10 ml of this solution were then mixed with 1 l of artificial seawater (34 g $l^{-1}$) and *Tetraselmis* sp. was added and allowed to multiply for at least 2 weeks. A drop of seawater containing $6.8 \times 10^6$ cells per ml was added to the culture dish by pipette once a week; more frequent aplications were unnecessary since *Tetraselmis* continued to multiply in the ostracod culture dishes.

Individual A-3 or A-2 instars were isolated and grown to adulthood. A single virgin male and female were transferred to a petri dish, where mating commenced. Newly hatched juveniles resulting from this union were isolated from their parents and cultured as a group. Repetition of this procedure allows populations of known pedigree to be maintained.

The contents of the adult brood space were viewed under the microscope, both *in situ* (through the adult valves) and by removing eggs, embryos and instars.

In order to examine the relationship between copulation and the oviposition cycle, the following experiments were carried out:

1. A virgin female and male were placed together. After the first oviposition was observed in the female carapace, the male was removed.
2. A virgin female and male were kept together and the oviposition cycle of the female was observed.
3. A virgin female and several males were kept together and the oviposition cycle of the female was observed.

In each case the dates of copulation and first oviposition were recorded.

To investigate the relationship between oogenesis in the adult ovary and the cycle of oviposition, adult females (both from cultures and from field collections) were fixed with Bouin's solution: just after the final moult, more than five days after the final moult and at five stages during the oviposition cycle (see Figure 9):

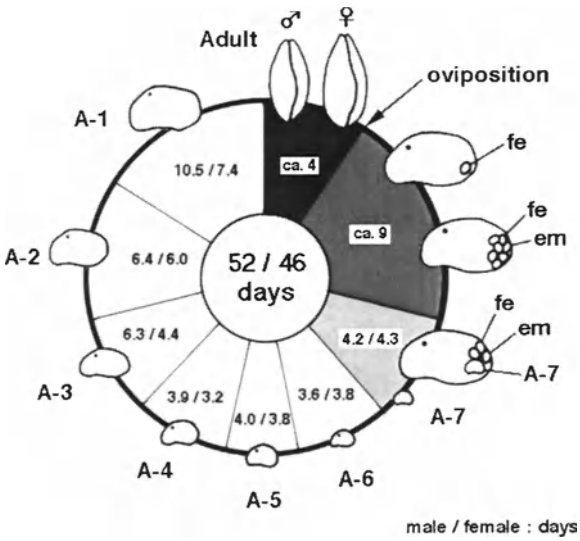


*Figure 1.* Summary of life history of *Xestoleberis hanaii* in culture. A: adult; A-1 to A-7: juvenile instars (fe: fertilized egg, em: embryo; numbers (male / female) inside the disc show duration of each stage in days).

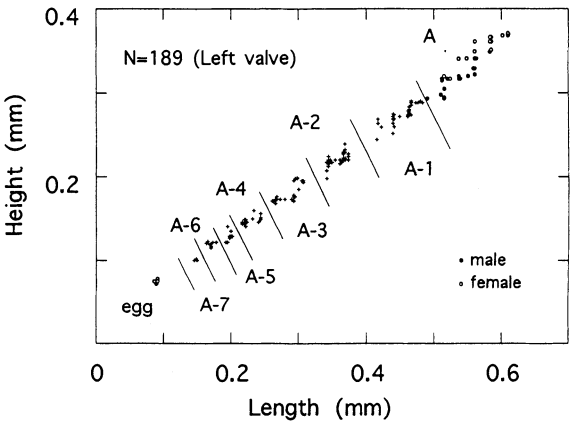


*Figure 2.* Size distribution of eggs and instars of *Xestoleberis hanaii* in culture (all measurements, except eggs, are of left valves).

1. Just after a few eggs first apeared in the brood space.
2. After the first few embryos with eyespots were observed in the brood space.
3. After the first A-7 instar was discharged from the brood space.
4. When all eggs in the brood space had developed into embryos or A-7 instars.
5. After all A-7 instars had been discharged from the brood space.

The fixed specimens were rinsed in 90% ethanol, dehydrated in graded ethanol-n-butanol series and em-

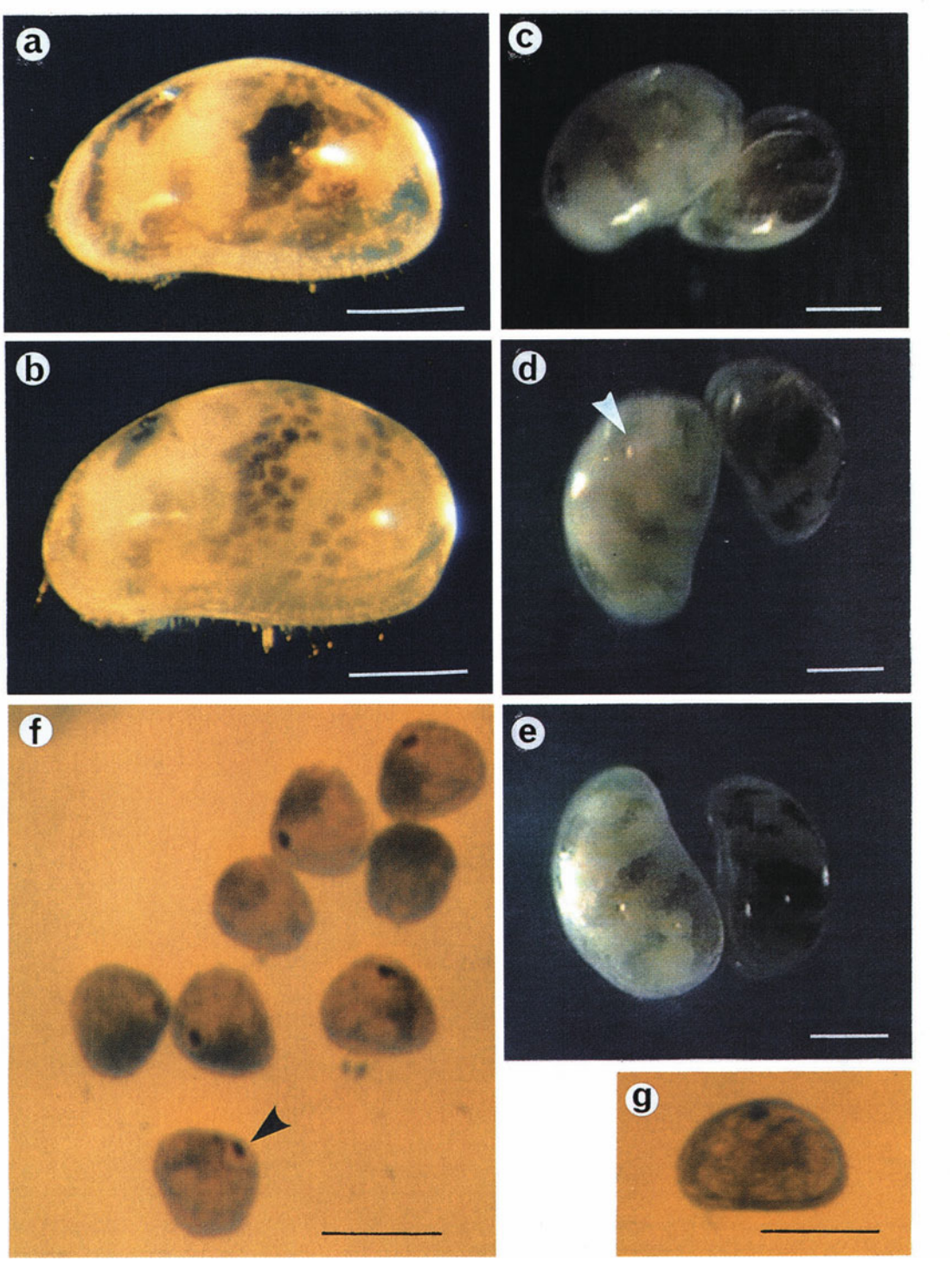


*Figure 3. Xestoleberis hanaii*: (a) male left valve, lateral; (b) female left valve, lateral; (c)–(e) stages in mating behaviour (eggs in female carapace arrowed); (f) eggs and embryos from inside a female carapace (eye spot arrowed); (g) A-7 instar. Scale bars: (a)–(e) 200 $\mu$m, (f) (g) 100 $\mu$m.

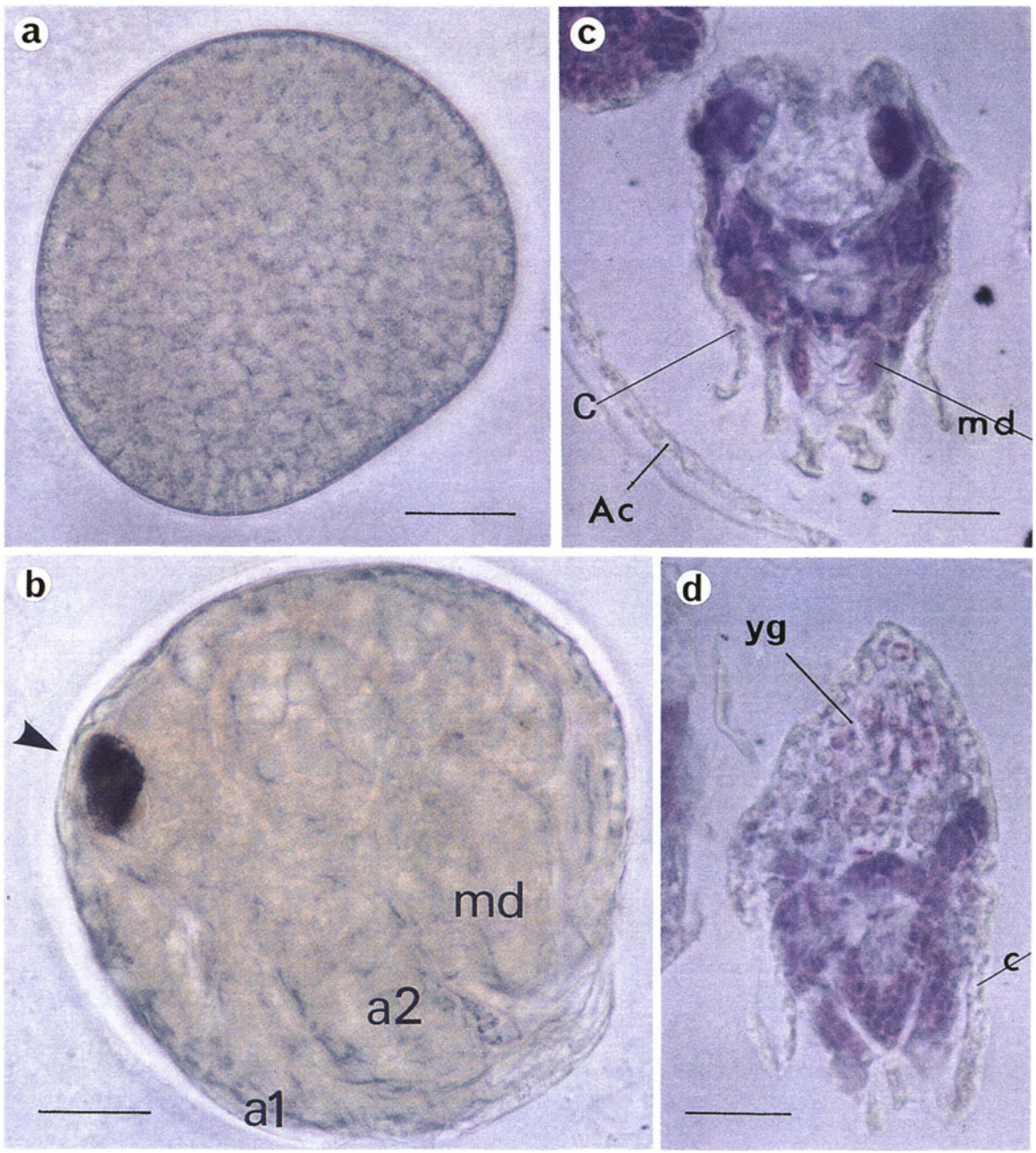


*Figure 4. Xestoleberis hanaii*: (a) egg filled with yolk; (b) embryo (eye spot arrowed; a1: antennule, a2: antenna, md: mandible); (c) embryo in the brood space (paraffin section; md: mandible, c: forming carapace, Ac: valve of adult female); (d) A-7 instar in the brood space (paraffin section; yg: yolk granules, c: carapace). Scale bars: all 20 $\mu$m.

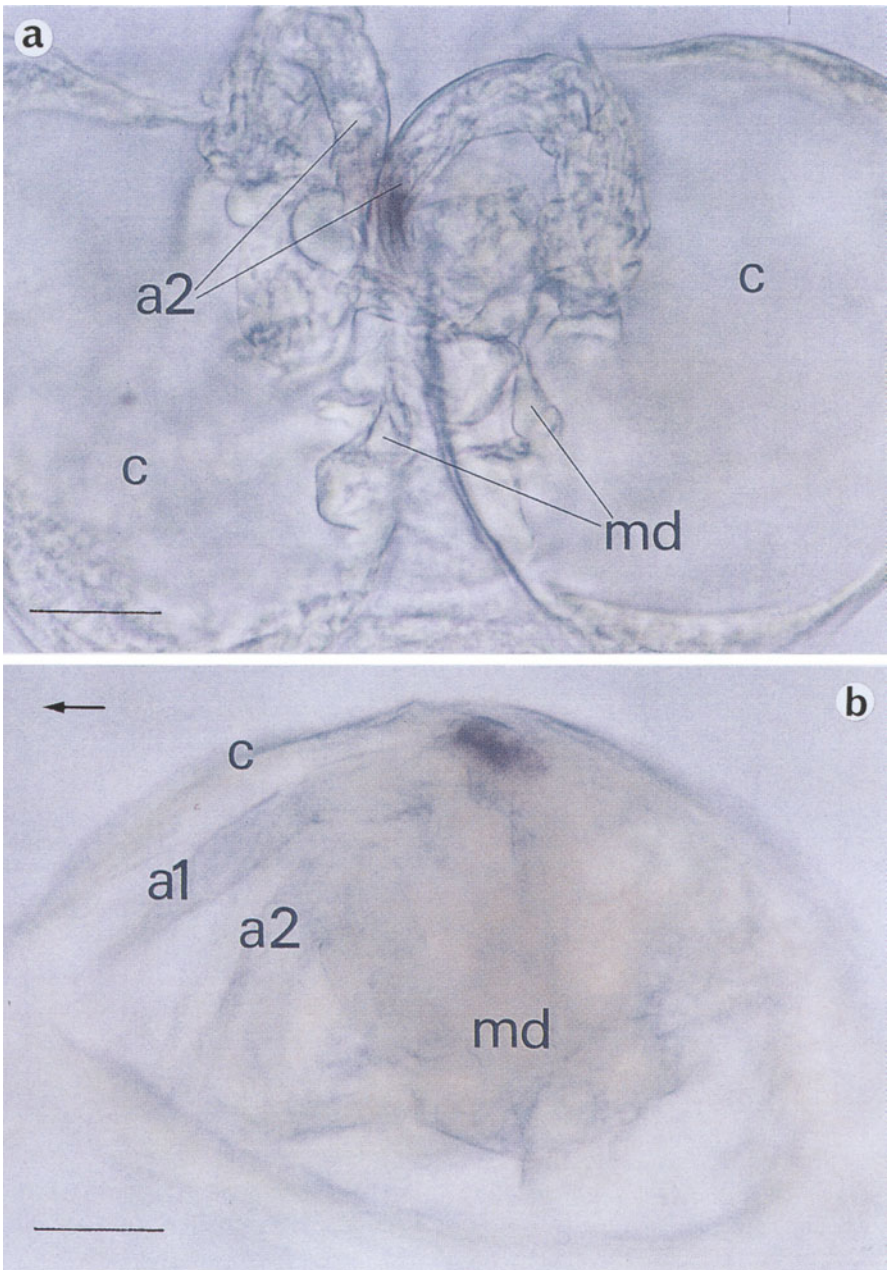


*Figure 5. Xestoleberis hanaii*: (a) embryo opened along the ventral margin (c: forming valves, a2: antenna, md: mandible; antennulae present but out of focus); (b) A-7 instar, left lateral view (arrow indicates anterior; c: carapace, a1: antennule, a2: antenna, md: mandible). Scale bars: both 20 $\mu$m.

*Table 1.* Duration (days) of juvenile instars (calculation based on 26 specimens cultured from A-7 to adult)

| Male specimen | Duration of each stage (days) | | | | | | | Total days |
|---|---|---|---|---|---|---|---|---|
| | A-7 | A-6 | A-5 | A-4 | A-3 | A-2 | A-1 | |
| M13 | 2.0 | 4.0 | 4.0 | 2.0 | 8.0 | 4.5 | 7.0 | 31.5 |
| M12 | 2.0 | 4.0 | 5.5 | 2.0 | 7.0 | 7.5 | 6.0 | 34.0 |
| M11 | 2.5 | 2.0 | 4.0 | 5.0 | 3.0 | 8.0 | 7.5 | 32.0 |
| M10 | 2.0 | 4.0 | 3.0 | 3.0 | 7.5 | 4.0 | 11.0 | 34.5 |
| M9 | 2.0 | 4.0 | 3.0 | 3.5 | 7.0 | 7.0 | 8.0 | 34.5 |
| M8 | 6.5 | 4.0 | 4.0 | 2.5 | 3.5 | 5.0 | 9.5 | 35.0 |
| M7 | 2.5 | 3.5 | 6.0 | 3.0 | 6.0 | 5.5 | 11.0 | 37.5 |
| M6 | 6.0 | 3.0 | 2.0 | 4.0 | 5.0 | 5.0 | 13.0 | 38.0 |
| M5 | 3.0 | 3.0 | 6.0 | 5.0 | 6.5 | 6.5 | 9.0 | 39.0 |
| M4 | 10.0 | 3.0 | 4.5 | 4.0 | 4.5 | 5.0 | 8.0 | 39.0 |
| M3 | 5.0 | 2.5 | 4.0 | 7.0 | 7.0 | 6.0 | 11.5 | 43.0 |
| M2 | 5.0 | 5.5 | 4.0 | 5.0 | 5.5 | 7.0 | 16.5 | 48.5 |
| M1 | 6.5 | 4.0 | 2.5 | 4.5 | 11.0 | 12.0 | 18.0 | 58.5 |
| Mean | 4.2 | 3.6 | 4.0 | 3.9 | 6.3 | 6.4 | 10.5 | 38.8 |

| Female specimen | Duration of each stage (days) | | | | | | | Total days |
|---|---|---|---|---|---|---|---|---|
| | A-7 | A-6 | A-5 | A-4 | A-3 | A-2 | A-1 | |
| F13 | 6.0 | 3.5 | 4.0 | 4.0 | 2.0 | 4.5 | 5.0 | 29.0 |
| F12 | 2.0 | 2.5 | 2.5 | 5.0 | 2.5 | 6.0 | 5.5 | 26.0 |
| F11 | 3.0 | 3.0 | 3.5 | 3.0 | 4.0 | 4.0 | 7.5 | 28.0 |
| F10 | 5.5 | 4.0 | 4.0 | 1.0 | 3.0 | 4.5 | 7.0 | 29.0 |
| F9 | 5.0 | 4.0 | 4.0 | 2.0 | 2.0 | 8.0 | 5.0 | 30.0 |
| F8 | 5.5 | 5.0 | 4.0 | 2.0 | 4.0 | 4.0 | 7.0 | 31.5 |
| F7 | 2.0 | 4.0 | 4.0 | 3.0 | 4.0 | 6.0 | 8.5 | 31.5 |
| F6 | 5.0 | 4.5 | 3.0 | 3.0 | 5.0 | 6.0 | 6.0 | 32.5 |
| F5 | 5.0 | 3.5 | 4.5 | 5.0 | 4.5 | 5.0 | 7.0 | 34.5 |
| F4 | 3.0 | 3.5 | 5.0 | 3.0 | 4.0 | 5.0 | 11.5 | 35.0 |
| F3 | 5.5 | 4.0 | 4.0 | 4.5 | 5.5 | 7.0 | 7.5 | 38.0 |
| F2 | 5.0 | 4.0 | 1.0 | 3.5 | 11.0 | 8.0 | 8.0 | 40.5 |
| F1 | 3.5 | 4.0 | 5.5 | 3.0 | 6.0 | 10.0 | 11.0 | 43.0 |
| Mean | 4.3 | 3.8 | 3.8 | 3.2 | 4.4 | 6.0 | 7.4 | 33.0 |

bedded in paraffin; they were then serially sectioned at 3–5 $\mu$m thickness, stained with Mayer's hemotoxylin eosin and analyzed under the microscope to determine the numbers of previtellogenic and vitellogenic oocytes and of mature eggs.

## Results

In our cultures (Figure 1), female *Xestoleberis hanaii* laid fertilized eggs over a period of 1 d after mating, 4 d after the final moult. Up to 40 eggs were laid a few at a time in the postero-dorsal brood space of the carapace. The eggs hatched after about 9 d as A-7 instars which were then discharged from the brood space within 1 or 2 d. Subsequently the juveniles moulted seven times within approximately 33 (female) or 39 (male) d to become adults, which lived for about 18 weeks (females) or 14 weeks (males) after the final moult.

Each instar is identifiable by the size and shape of its valves (Figures 1 and 2), changing from cir-

*Table 2.* Differentiation of growth stages of oocytes: numbers of oocytes and mature eggs in adult females at five stages (see Fig. 9). O.S.: observed specimens; e.v.o.: early vitellogenic oocytes; l.v.o.: late vitellogenic oocytes; M.E.: mature eggs; v.f.s.: virgin female specimen; m.f.s.: mature female specimen more than 5 days after final moult

| O.S. | e.v.o. | l.v.o. | M.E. |
|---|---|---|---|
| v.f.s. | 0 | 0 | 0 |
| m.f.s. | 7 | 1 | 1 |
| m.f.s. | 10 | 7 | 1 |
| m.f.s. | 11 | 4 | 5 |
| Stage 1 | 5 | 3 | 1 |
| Stage 1 | 6 | 4 | 4 |
| Stage 1 | 7 | 4 | 4 |
| Stage 2 | 2 | 0 | 1 |
| Stage 2 | 1 | 2 | 0 |
| Stage 2 | 6 | 3 | 0 |
| Stage 3 | 6 | 2 | 0 |
| Stage 3 | 3 | 3 | 0 |
| Stage 4 | 4 | 0 | 0 |
| Stage 4 | 7 | 2 | 0 |
| Stage 4 | 0 | 1 | 0 |
| Stage 5 | 2 | 3 | 4 |
| Stage 5 | 7 | 3 | 2 |
| Stage 5 | 5 | 1 | 1 |

cular in early instars to oval in later ones. Sexual dimorphism is not apparent until the adult instar (Figures 3a, b), when females are longer and higher than males, as well as being more rounded posteriorly with an inflated postero-dorsal brood space. Experimental results are given below.

*Embryogenesis*

Newly laid eggs were oval (90×75 $\mu$m) and whitish yellow, filled with yolk and stained with eosin when serially sectioned (Figure 4a), but became tinged with yellow within a few days and then became semitransparent. Embryos were ovoid (98×81 $\mu$m) with a slight angle corresponding to the mid-dorsal margin. Inside the egg membrane, the early development of the antennulae, antennae and mandibles could be seen, together with a brownish-red eye-spot near the mid-

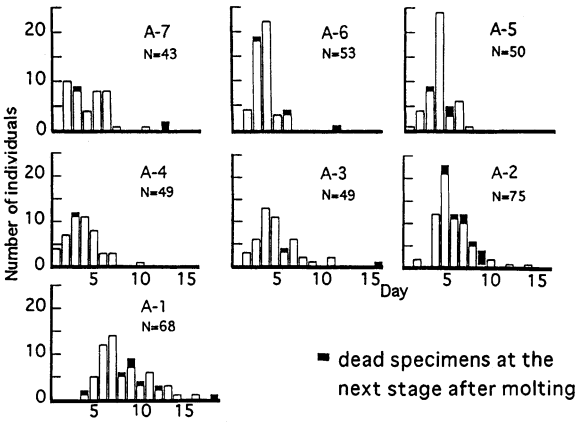


*Figure 6.* Development times of juvenile instars in culture.

dorsal margin (Figure 4b). A ventral marginal crack was observed just prior to hatching, corresponding to free margin of the valves in subsequent instars, along which the carapace could be opened (Figure 5a). In sections, the egg membrane of the embryo could not be confirmed but some yolk granules were present (Figure 4c). The A-7 instar was ovoid and slender, with well-developed antennulae, antennae and mandibles visible inside the carapace (Figure 5b); in section, yolk granules were not observed and the majority of tissue was stained with hematoxylin (Figure 4d).Three different stages (eggs, embryos and instars) were observed in the brood space at the same time. Newly hatched A-7 instars separated from the brood space were able to walk around in a petri dish.

*Growth rate and development time*

Observations on 97 cultivated individuals were made to determine the development time for each instar; 51 of these came from a single adult pair and were cultivated in individual dishes from the A-7 stage onwards, while additional specimens were selected from a mass culture (13 × A-6, 2 × A-4, 1 × A-3, 28 × A-2 and 2 × A-1) for further separate cultivation. Cultures were checked twice a day to determine the time of moulting. Growth rate is demonstrated by the measurements of length and height of the moulted carapaces (Figure 2). Development time for each instar was variable (from one up to a maximum of 18 d) but for the majority of individuals, the earlier moults took place more rapidly than the later ones (Figure 6). Individuals that took a long time to moult often died soon after moulting. Out of the 51 specimens cultivated from the same parents, 43 survived to the A-6 stage and 26 of these survived to adulthood, resulting in an approximately even sex

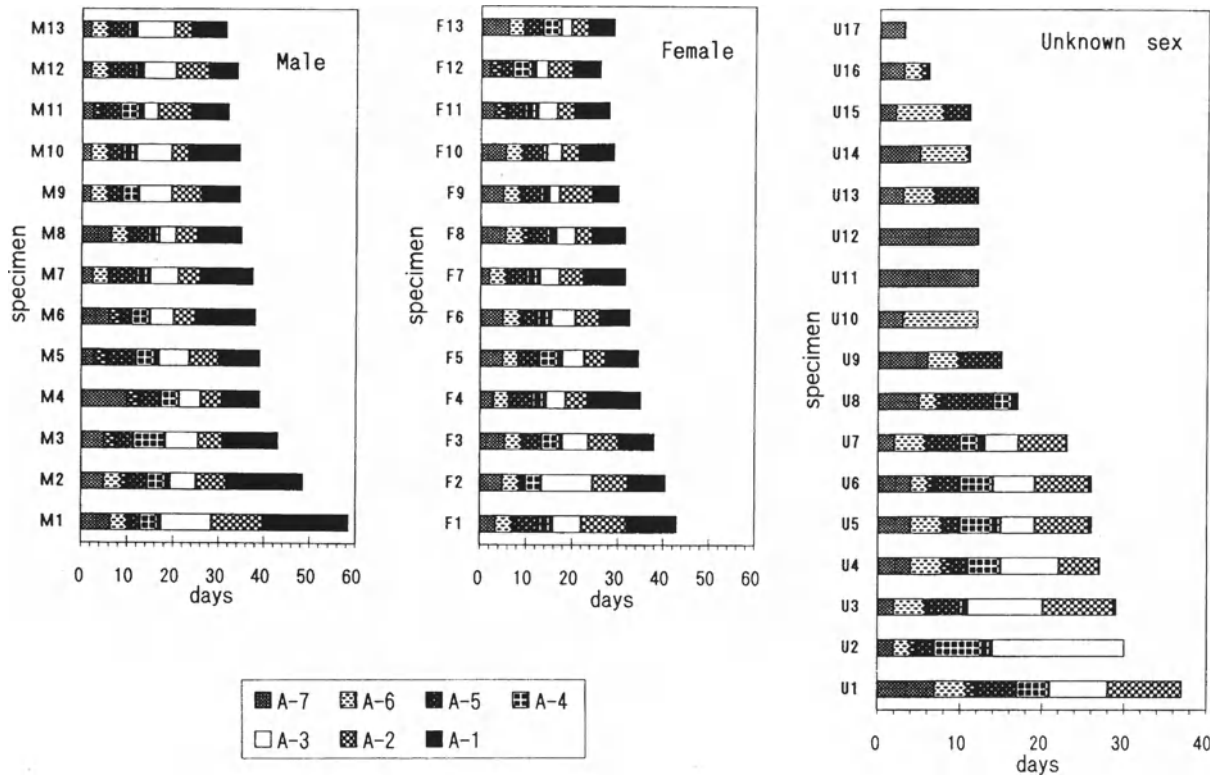


*Figure 7.* Sexual differentiation of development time of juvenile instars (see Table 1) (unknown sex: juveniles that died before reaching adulthood, so sex could not be determined).

ratio, the entire development from A-7 to adult taking 26–58.5 d. Males moulted more rapidly than females in the A-7 to A-5 stages, but this situation was reversed after the A-5 stage (Figure 7, Table 1). Mortality rates appear to be higher for the earlier instars. No post-adult moulting was observed.

*Copulation*

Males were observed to mate within a day of the final moult. Two particular courtship behavioural patterns were observed. In the first, the male approached a stationary female and slowly rotated the closed female carapace until a suitable mating position was reached. In the second, the male chased a walking female, mounting from behind into a lateral position and then rotating the carapace into the mating position. Once in position, the male rubs the postero-ventral part of the female carapace with his first pair of legs and then inserts the copulatory organs. The postero-ventral areas of the male and female carapaces are brought close together, the male copulatory organs protrude from a broadly gaping carapace and are introduced between the slightly gaping valves of the female. Copulation generally takes 19–20 min, but a duration of more than 1 h was recorded. Copulation took place lying on the bottom or clinging to the side (with threads) of the petri dish. Both males and females (including those with eggs in the brood space) mated repeatedly, with more than one individual.

*Relationship between copulation and oviposition*

After females had hatched their eggs and discharged the juveniles from the brood space, a resting period of about 10 d was observed, following which oviposition began again. In the case of newly-moulted females introduced on the same day to males, mating occurred within 1 or 2 d and oviposition began 4 or 5 d later. Females kept isolated for 5 d after moulting were observed to mate within a day of being paired with males and oviposition then occurred within another day or two. After about 40 eggs have been laid by one female, oviposition stops for a time. Eggs hatch in the order in which they were laid; the resulting A-7 instars are released from the adult carapace at a rate of

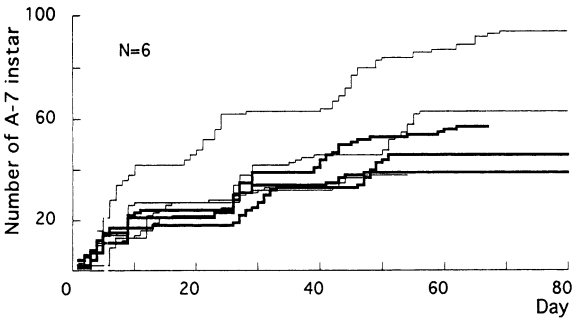


*Figure 8.* T ming of discharge of A-7 instars from the brood space (thick lines ndicate females that mated only once, thin lines those that mated twice or more).

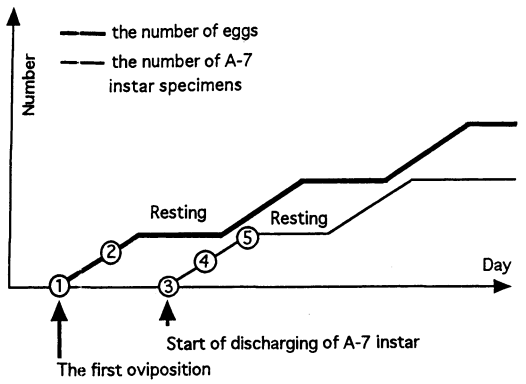


*Figure 9.* Five stages based on the growth condition of the eggs, embryos and A-7 instars (see text for explanation) (thick line: eggs; thin line: A-7 instars).

two or three per day, and are replaced in the brood space by new eggs. Discharging all A-7 instars (18–43 per adult) took 10–15 days, following which a 10-day 'rest period' ensued before further discharge of instars began. Eggs were occasionally observed to have fallen out of the female carapace; in most cases these failed to hatch. Adult females repeated this reproductive cycle three times, so that each produced 39–94 juveniles in total (Figure 8). Three oviposition periods sometimes occurred after only one copulation, showing that females can store spermatozoa; in one case, however, a female only commenced a third period of oviposition after a second mating had occurred.

*Oogenesis*

The 1 or 2 d period after the final moult before the female becomes reproductively active, and the 10 d 'rest period' after each reproductive phase, may be due to oogenesis in the adult ovaries, which are situated on both sides of the thorax above the alimentary canal and in which growing oocytes of various sizes can be observed (Figures 10a, b). Early vitellogenic oocytes are ellipsoidal, 27–35 $\mu$m in diameter, and contain germinal vesicles (11–16 $\mu$m) and nucleoli (5 $\mu$m) in the central region; the cytoplasm stains with hematoxylin , the growing yolk granules around the germinal vesicles stains well with eosin (Figure 10a). Late vitellogenic oocytes are spheroidal and larger (40–58 $\mu$m) although the size of the germinal vesicles and nucleoli remains much the same, and the cytoplasm is filled with fine yolk granules (Figure 10c). Mature eggs are 47–67 $\mu$m in diameter and the cytoplasm is filled with large yolk granules, while the germinal vesicle has dwindled (Figure 10d). Immediately after the final moult, only pre-vitellogenic oocytes could be seen in the inner ovary of the female, but through the five stages at which specimens were fixed (Table 2), various combinations of early and late vitellogenic oocytes, and mature eggs, were seen. Broadly similar results were obtained from specimens collected in the field, although no mature eggs were observed in any of the five stages.

## Discussion

It has been claimed that *Xestoleberis* differs from other podocopid ostracods in having only seven (compared to the normal eight) juvenile instars (Kesling, 1961; McKenzie et al., 1983; Hiruta, 1984). Elofson (1941) found only seven juvenile stages and noted that the A-7 instar already has well-developed mandibles, on the basis of his study of three species which he identified as *X. aurantia* (Baird, 1843), *X. pusilla* sp.nov. and *X. depressa* Sars, 1866 (his *X. aurantia* = *X. nitida* (Liljeborg, 1853) while his *X. pusilla* = the true *X. aurantia*; see Whittaker, 1978 and Athersuch et al., 1989). Okubo (1984), however, who studied the life cycle of *X. hanaii*, described it as having eight juvenile instars; according to him the A-8 instar had a reddish eye spot and underdeveloped antennulae and antennae, but lacked mandibles or a carapace. We have observed eggs, embryos and A-7 instars together in the brood space of female *X. hanaii*; since the embryo, just before hatching, has antennulae, antennae and mandibles as well as a carapace, it seems clear to us that Okubo's observation was in error. We concluded that *X. hanaii* does not have an A-8 instar. According to Fox (1964) who discussed the moulting stages of crustaceans, including ostracods, podocopid ostracods develop the 'nauplius stage' (antennulae + antennae + mandibles)

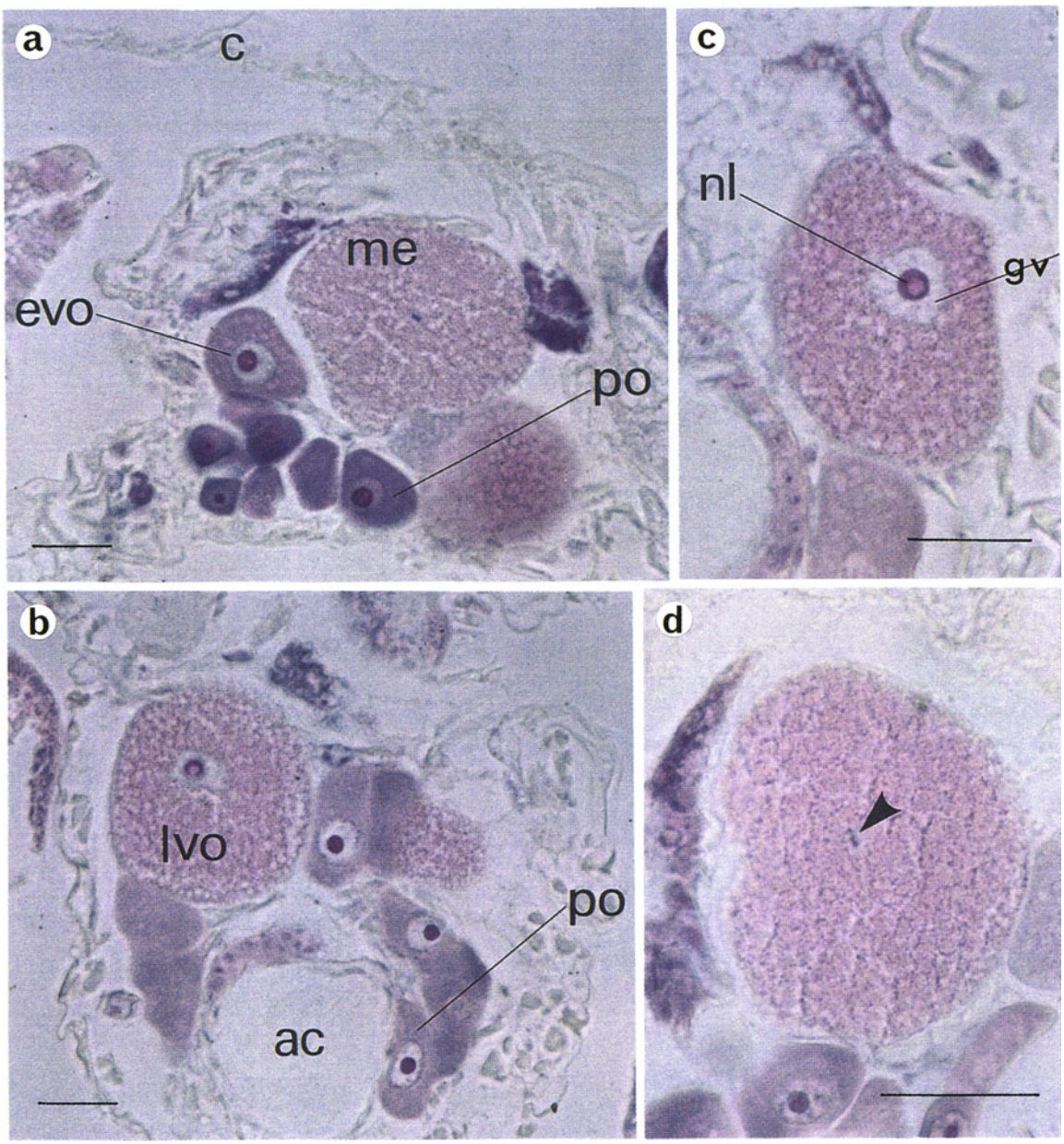


*Figure 10.* Sections of ovaries showing growth stages of oocytes: (a) longitudinal; (b) transverse; (c) late vitellogenic oocyte; (d) mature egg. Mitotic spindle arrowed; ac: alimentary canal, c: adult carapace, evo: early vitellogenic oocyte, lvo: late vitellogenic oocyte, me: mature egg, po: pre-vitellogenic oocyte, gv: germinal vesicle, nl: nucleoli. Scale bars: 20 $\mu$m.

at the A-8 instar; ostracods hatching at the A-7 stage must incorporate the 'nauplius stage' into the embryo.

However, it has been pointed out to us (D. J. Horne & K. Martens, pers. comm.) that the size gap between the egg and the A-7 instar seems very large (see Figure 2), that the A-8 instar might have been missed because it is usually very short-lived (< 1 h), and that the embryo with a 'ventral crack' (Figures 4b and 5a) might in fact be the A-8 instar. We measured weakly calcified A-7 instar carapaces which had been released from the brood space (Figure 5h), but not instars which had just hatched within the brood space; we certainly found some size variation among the embryos we measured (Figure 3f). Although developed embryos, just prior

to hatching, have a distinct egg membrane, we cannot confirm the presence of a membrane in the embryo sectioned within the brood space (Figure 4c), so that particular one could be considered to be a hatched A-8 instar. Further investigation of the early developmental stages is clearly required to resolve this problem.

In *X. hanaii*, the manipulation of the female by the male during courtship appears to be by pushing with the first pair of legs (fifth limbs); this is in contrast to otherwise similar behaviour in *Bicornucythere bisanensis*, in which the male rolls the female around by pulling with the first legs (Abe & Vannier, 1993).

## Conclusions

*Xestoleberis hanaii* is a suitable animal for laboratory culture experiments, although our studies have raised a number of questions requiring further study, particularly that of the apparent absence of an A-8 instar. Future laboratory studies of the life histories of ostracod species, particularly their ontogeny and growth rates, will yield information of value in the interpretation of evolutionary lineages and speciation rates.

## Acknowledgements

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# Factors influencing intraspecific variation and polymorphism in marine podocopid Ostracoda, with particular reference to Tertiary species from southeastern Australia

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*Key words:* Ostracoda, intraspecific, Tertiary, ornament

**Abstract**

Work on intraspecific variation in ornate ostracods over the last 30 years is reviewed. Polymorphism (discontinuous variation) due to environmental and genetic factors (and combinations thereof) is discussed. Specimens of the ornate marine genera *Cletocythereis*, *Hermanites*, *Notocarinovalva*, *Neobuntonia*, *Chapmanella*, *Actinocythereis*, *Spinobradleya* and *Loxoconcha*, from Tertiary deposits of southeastern Australia, are examined for examples of significant intraspecific morphological variation. Variations in the degree of spinosity, the nature of spines, the degree and type of aggradation (celation), the occurrence of microreticulation, the shape of fossae, the form of eye tubercles and subcentral tubercles, combinations of reticulation and nodules, and the relationships between punctation, reticulation and spinosity, are described and illustrated. These variations are considered in relation to location, time, facies, inferred palaeoenvironment and assemblage. It is shown that the complex of factors connecting these variations with their expression by the phenotype does not allow their unqualified use in establishing palaeoecological parameters, especially where strict polymorphism is not established, or where the timescale of the variations may be several orders of magnitude out of step with the palaeoecological changes inferred from them. The necessity of high magnification illustrations is stressed. The entire range of research into intraspecific variation must be accessed if robust palaeoecological inferences are to be drawn.

## Introduction

This paper discusses the different hypotheses which have been put forward to explain intraspecific variation, not only in ostracods, but also in other invertebrate taxa. Whilst it is generally conceded that all such variation must ultimately have a genetic basis within the organism itself, differing views are held concerning the triggers which cause the variation to occur. If some specimens in a population have thicker shells, for instance, one hypothesis attributes this to the effects of the environment, whilst another takes the view that the variation is a polymorphism triggered by evolutionary factors. The discussion here focuses on morphological variation of the shell in marine podocopid ostracods, but it should be noted that some authors have investigated such variation in non-marine ostracods, too (e.g. Carbonel & Peypouquet, 1983). Variation in 'soft part' anatomy is a significant part of the total picture, but it is not considered here.

Any consideration of intraspecific variation in ostracods must establish whether or not the variation is polymorphic. Polymorphism is defined as discontinuous variation within a population, with the rarest morph existing at a frequency greater than that which can be maintained by recurrent mutation alone (Clark, 1976). If the variation in a population or assemblage is more or less continuous between two extremes (smooth to reticulated, for example), then it is not polymorphism. Even discontinuous variation is not necessarily polymorphic, since fossil and living assemblages are time-averaged to a greater or lesser extent; apparent discontinuity of variation may be because specimens that would illustrate the continuum of change have not been preserved or collected. This difficulty can sometimes be obviated by collecting large

numbers of specimens. Alternatively, one might refer to 'morphotypes', which may or may not be polymorphic, depending on whether or not they occur in the same population. Clark's (1976) definition implies a biological, rather than geological, time scale; furthermore, it refers to genetically determined changes and excludes inter-population variations such as geographical races where environmental factors might be determinants, although even these variations have a genetic component. What I regard as intraspecific variation may or may not be polymorphism, even though it is discontinuous, since time-averaged fossil assemblages may or may not represent populations. All discontinuous variation represents either genotypic change due to mutation (usually regarded as giving rise to new species rather than just new morphotypes) or the result of the interaction of environmental stimuli with the genotype.

This paper focuses on morphological variations and the various explanations which have been given for them in the past, as well as providing hypotheses for such variations in the context of this study. Since only single or very few specimens are examined, the variations they exemplify are used to illustrate rather than provide statistical confirmation of the ideas discussed. The examples, concerned mainly with the expression of a given reticulation pattern or the development of a given style of ornamentation, form a basis for discussion of previous explanations of variation and of attempts to utilise such explanations for palaeoecological analyses.

*Review of previous work*

Over the last 35 years, the subject of intraspecific variation in ostracods has received continued attention, both for the palaeoecological data it provides and for its taxonomic significance. Pokorny (1964) foreshadowed later work in suggesting that the reticulation pattern in ornate ostracods is constant within a species and is the ancestral form from which later variations are derived. He referred briefly to the gradual loss, or intensification, of the basic reticulation pattern in several lineages of the Trachyleberididae from the Upper Cretaceous of Bohemia. Hartmann & Köhl (1978) and Hartmann (1982) stressed the importance of considering variation in the surface ornament of individuals in populations before drawing phylogenetic inferences about such variations. Their examples were Recent *Mutilus pumilus*, *Xestoleberis chilensis austrocontinentalis* and *Hiltermannicythere bassiounii* from coastal locations in west, south and east Australia. Hartmann concluded that "it is impossible to correlate ornamental and size variation with any single environmental factor" (1982: 369); he found no connection between valve surface relief and the ecological conditions in which the ostracods were found.

Keen (1982) considered intraspecific variation and polymorphism in a wide range of Tertiary species and reviewed previous work. He established the significance of time-averaging in fossil assemblages and its effect in blurring the relationships between such assemblages and living populations. Contrary to Hartmann, Keen said that "size can be strongly influenced by the environment and is probably usually a phenotypic character", although he also stated that "size is presumably the result of both genotypic and phenotypic variability" (1982: 384). He found that brackish-water ostracods exhibit the highest level of intraspecific variability and the closest relationship between morphology and environment. He has also claimed that punctate and smooth forms of the same brackish-water species were environmentally determined, the smooth forms correlating with lime-rich waters (Keen, 1977). However, Garbett & Maddocks (1979) refer to the opposite relationship for *Limnocythere floridensis* Keyser from Texas bays, where ornamentation appears to decrease with decreasing salinity, although sampling was not extensive enough to establish this as a statistically significant fact. Carbonel (1975) correlated such loss of ornamentation with rising water temperatures. Keen (1982) was unable to correlate variations in ornamentation (such as loss of reticulation in certain areas of the carapace, development of spines, strengthening or weakening of ridges) with substrate, sediment type or time. Another complicating factor in Keen's discussion is whether variation is polymorphic or continuous. Ducasse & Rouselle (1978) saw a correlation between populations exhibiting well-developed polymorphism and marine incursions, whilst monomorphism was associated with regresssions. More recently, however, they have been much less inclined to attribute variation solely to environmental factors, giving increasing weight to varying genotypic responses (including heterochrony) by individuals; they state: "it is difficult to find an adaptive pattern common to the different species studied" (Rouselle & Ducasse, 1993: 225).

The work of Japanese ostracodologists has focused on interspecific, rather than intraspecific, variation. For instance, Tsukagoshi & Kamiya (1996) dealt with heterochrony of ostracod hinges; Tsukagoshi &

Ikeya (1987), Tsukagoshi (1990) and Kamiya & Hazel (1992) investigated pore systems; Irizuki (1993, 1994) and Irizuki & Sasaki (1993) studied cell-reflecting sculptures and marginal pores. Okada (1981, 1982a) established the direct correspondence between cell arrangement and reticulation patterns. This body of research provides substantial evidence linking cell structure and pore systems with reticulation patterns, and highlights the persistence of such patterns at species level, something which must be taken into account in discussing intraspecific variation.

Benson (1974, 1975, 1981, 1983) has investigated the causes of change in the form, function, architecture and ornamentation of the ostracod shell. He focused attention on the mechanical and structural analysis of the shell as a biological and evolutionary response to the various stresses which must be overcome by the ostracod, whose carapace must adequately protect it and enable it to function efficiently. This approach provides explanations for relatively sudden changes in fossil sequences and underlines causal factors, which the ecophenotypic scenario does not usually take into account, such as mechanical accommodation under stress.

A hypothesis linking the vestibule size and shape of the genera *Krithe* and *Parakrithe* to the dissolved oxygen content of seawater was proposed by Peypouquet (1975). Studies by Peypouquet (1979, 1983), Riha (1989) and McKenzie et al. (1989) applied this hypothesis to estimating the palaeoenvironments of a variety of fossil ostracod assemblages. However, Whatley & Zhao (1993) and Van Harten (1995) have questioned the validity of Peypouquet's hypothesis and the conclusions drawn from its application.

Work by several French authors reflects a strong focus on relationships between forms of, and variations in, ornamentation and environmental factors. Carbonel (1975) studied the circumstances associated with the disappearance of ornamentation. The relationship between morphology and environmental parameters was studied by Babinot & Colin (1983), that between ornamentation and water chemistry by Carbonel & Peypouquet (1983). The aggradation/degradation of shell ornament was investigated by Peypouquet et al. (1988). Ecophenotypic variations in reticulation, tuberculation and spinosity were considered by Braccini & Peypouquet (1991, 1996). The term 'environmentally-cued polymorphism' (Peypouquet et al., 1988) has been used as an umbrella term for these studies, though not all of the variations can be regarded as polymorphic (*sensu stricto*). More recent studies (Peypouquet et al., 1986, 1988; Ducasse, 1995; Braccini & Peypouquet, 1991, 1995, 1996) draw detailed palaeoecological inferences from limited morphological data, with little statistical environmental analysis and restricted geographical coverage.

*Defining the problem*

The following factors must be taken into account if conclusions with wide applicability are to be reached.

1. Taxonomy. There must be agreement on the diagnostic characters of the species and on the acceptable degree of intraspecific variability.
2. Uniformitarianism. Palaeoecological inferences are generally, though not invariably, based on uniformitarian principles. If special circumstances apply to a fossil occurrence or assemblage, they should be distinguished from those which apply to living forms.
3. Interrelationship of factors. It should be recognized that the treatment, in isolation, of any given factor influencing variability, is a matter of methodological convenience. In most cases, the environmental factors involved are multiple and complex.
4. Co-occurrence. Intraspecific variability in one species may suggest conclusions which are either contradicted or re-inforced by variability in another species in the same assemblage. The methodology employed should indicate whether or not such co-occurrences have been taken into account.
5. Other factors.
   (a) Fossil assemblages are time-averaged when considered on the time-scales of many of the environmental changes their variability (or that of their component species) is supposed to reflect. High resolution sedimentary sequences can provide seasonal, annual or decadal sampling, so as to minimise time-averaging effects; such opportunities should be utilised.
   (b) The numbers of specimens examined, and the size of the samples from which they are drawn, are often significant in reaching conclusions, even where mathematical procedures are not involved.
   (c) The continuity or discontinuity of variability within populations helps to establish polymorphisms (see above).
   (d) Circular arguments are prone to occur when the methodology includes both empirically determined environmental factors (e.g. oxygen isotope ratios) from which environmentally-cued variation

is inferred, and morphological variations empirically linked to given environmental conditions and from which palaeoenvironmental conditions are inferred.

## Material and methods

More than 2700 specimens from 16 Tertiary localities (details are given in plate explanations) in southeastern Australia, and 30 specimens of one species from a Recent beach sand deposit at Darwin, Northern Territory, form the assemblage from which 79 specimens were chosen for detailed SEM study. Details of the assemblages and localities are given by Neil (1995). Morphological variability was studied in eight species representing eight genera:

1. *Chapmanella flexicostata* (Chapman, 1914). Eight specimens from seven locations; Late Oligocene to Late Middle Miocene.
2. *Cletocythereis caudispinosa* (Chapman & Crespin, 1928). Seven specimens from six locations; Early Miocene to Late Middle Miocene.
3. *Hermanites glyphica* Neil, 1994. Eleven specimens from eight localities; Oligocene to Late Middle Miocene.
4. *Spinobradleya nodosa* Neil, 1994. Sixteen specimens from three localities; Early Middle Miocene; and a Recent species for comparison, *Actinoleberis arafurae* Howe & McKenzie, 1989.
5. *Neobuntonia batesfordiense* (Chapman, 1914). Five specimens from four localities; Early to Middle Miocene.
6. *Notocarinovalva yulecartensis* Neil, 1994. Nine specimens from nine localities; Oligocene to Middle Miocene.
7. *Actinocythereis* sp. Nine specimens from nine localities; Oligocene to Late Middle Miocene.
8. *Loxoconcha* sp. Thirteen specimens from 11 localities; Oligocene to Late Middle Miocene.

The assemblages from which these species were obtained were mostly deposited in marine, shallow water, high energy environments (based on studies by other workers: Quilty, 1971; McKenzie & Peypouquet, 1984; Abele, 1988; Warne, 1993), but one (Fossil Beach, Mornington) represents a lower energy, deeper water environment (Whatley & Downing, 1983; McKenzie & Peypouquet, 1984). The specimens were recovered from unconsolidated marls or calcareous sandstones. Some evidence of recrystallization was evident in the Middle Miocene assemblage from the Pata Limestone at Kingston-on-Murray in South Australia, and some specimens from the Early Miocene (Longfordian) at Cape Grim, Tasmania, show post-depositional deformation. The species chosen were widely distributed amongst the assemblages from the different localities, particularly *C. flexicostata*, *Actinocythereis* sp. and *H. glyphica*. The Middle Miocene assemblages from Muddy Creek and Grange Burn are represented by much larger collections than those from the other localities, but as no statistical analysis of assemblages/populations is made here, this bias in numbers does not affect the conclusions drawn.

The specimens were examined by SEM in order to describe and illustrate the variation apparent in shell ornamentation, specifically reticulation, spinosity, tuberculation and punctation. The work of Benson (1977, 1983) and Okada (1981, 1982a,b) has established the invariant character of the reticulation pattern (as opposed to its strength of expression) in a given species and its relationship to the underlying cell structure of the ostracod shell. No attempt has been made, therefore, to document changes in reticulation pattern; intraspecific variations studied include those affecting the thickness and height of the muri (the walls forming the reticulation), the development of second-order or micro-reticulation and aggradation or celation which produces projections, flanges, strap-like extensions and other modifications of the muri. Sylvester-Bradley & Benson (1971) defined 'tubercle' as a rounded, well-defined projection or protuberance on the external surface of the valve which is usually matched by a corresponding depression on the interior valve surface. Eye tubercles and subcentral tubercles are considered here. Puncta are defined as "small circular pits, semi-circular in cross-section" (Sylvester-Bradley & Benson, 1971) as seen on the external surface of the carapace.

## Results

### *Reticulation*

#### *Hermanites glyphica*

The primary pattern of muri (first order reticulation) is present, but not strongly expressed, in later instars, only reaching its full development in adults; in some cases (which may be gerontic forms) the muri are

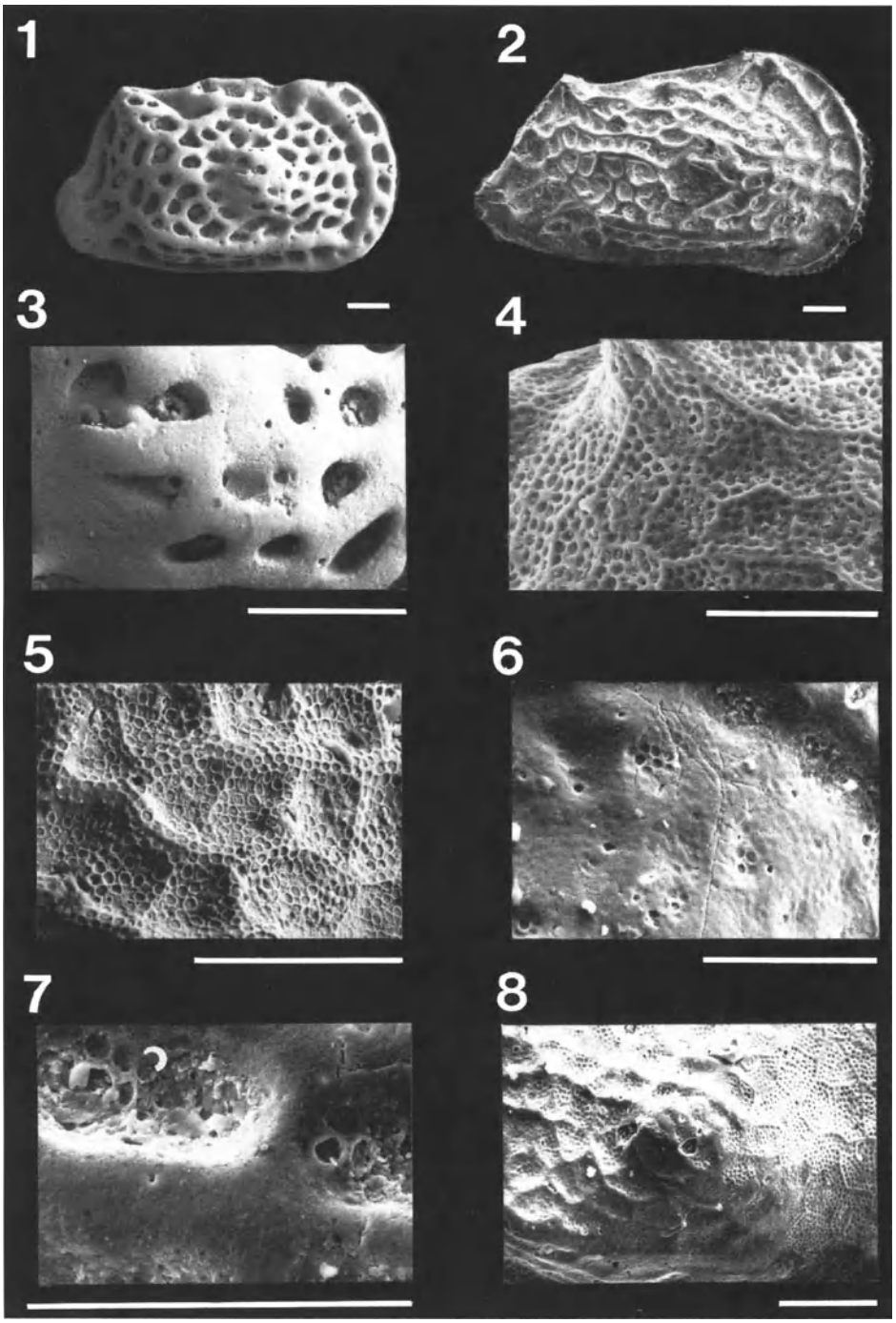


*Plate 1. Hermanites glyphica.* All Middle Miocene unless otherwise stated. *Figures 1 and 3.* Adult (gerontic specimen?), Muddy Creek, Victoria; (1) right valve; (3) smooth muri in area of subcentral tubercle. *Figure 2.* Late juvenile instar, right valve, Mitchell River, Victoria. *Figure 4.* Primary and secondary reticulation on late juvenile instar, Muddy Creek, Victoria. *Figure 5.* Microreticulation partially obscured by aggradation, adult, Late Oligocene, Fossil Bluff, Tasmania. *Figures 6 and 7.* Microreticulation obscured by aggradation, Early Miocene, Fishing Point, Victoria. *Figure 8.* Differential development of primary and secondary reticulation, late juvenile instar, Warrambine Creek, Victoria. All scale bars=100 $\mu$m.

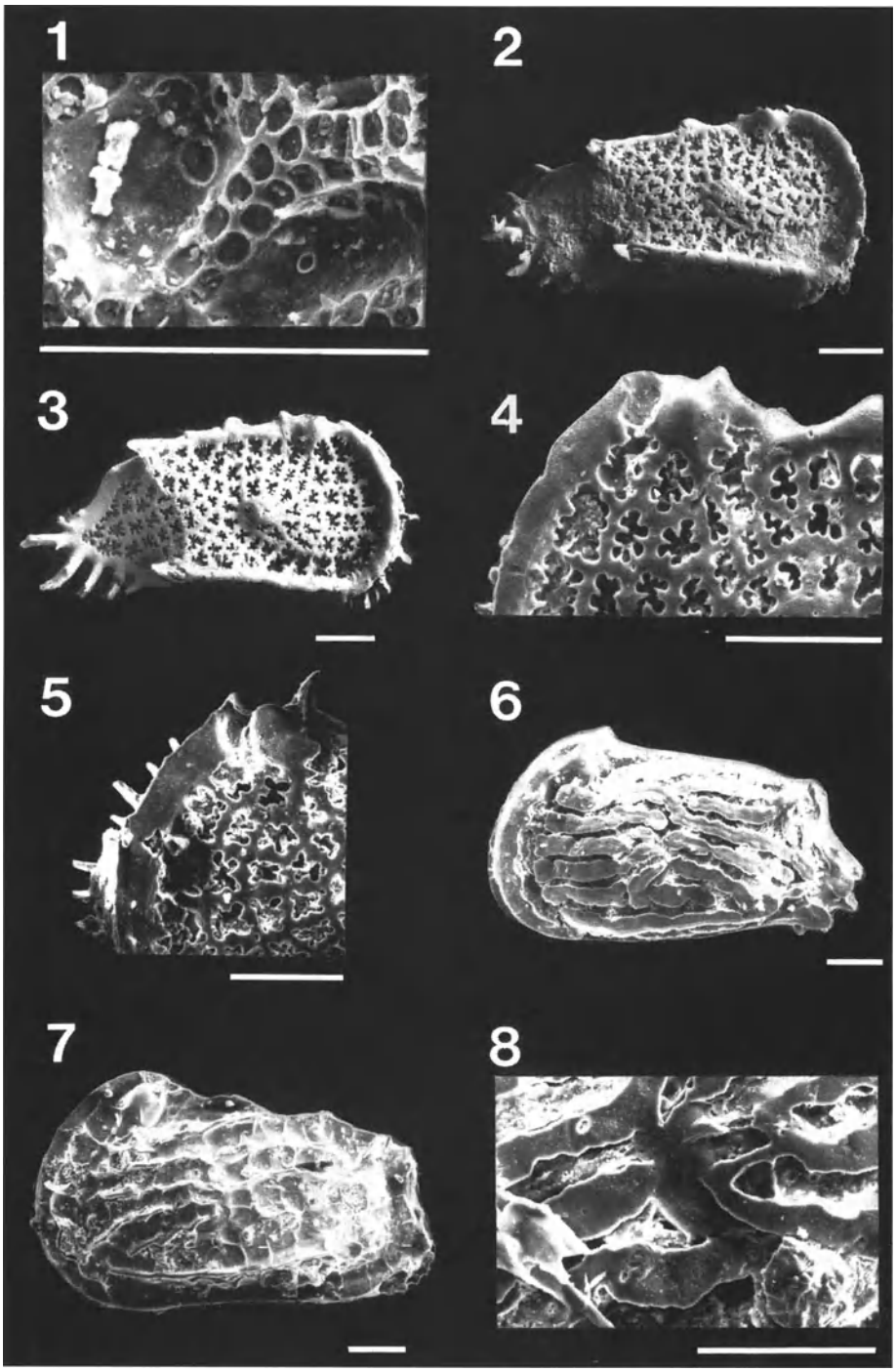


*Plate 2.* All Middle Miocene unless otherwise stated. *Figure 1. Hermanites glyphica*, detail of microreticulated muri and smooth fossae, Native Hut Creek, Victoria. *Figures 2–5. Cletocythereis caudispinosa*; (2) juvenile right valve, Warrambine Creek, Victoria; (3) adult right valve, Muddy Creek, Victoria; (4) left valve, rounded anterior ridge and low profile eyespot, Native Hut Creek, Victoria; (5) left valve, arcuate anterior ridge, Early Miocene, Fishing Point, Victoria. *Figures 6–8. Chapmanella flexicostata*; (6) adult left valve with broadly celated ribs, Muddy Creek, Victoria; (7) adult left valve with narrow ribs, Lower Oligocene, Fossil Bluff, Tasmania; (8) celated ribs on late juvenile instar, Warrambine Creek, Victoria. All scale bars=100 $\mu$m.

massive and rounded (Pl. 1, Figures 1–3). Second order reticulation is also present in later instars (Pl. 1, Figure 4) and is more strongly expressed than primary reticulation in the earlier stages of ontogeny. Where the second order reticulation is lost (partially or fully) in some adult specimens, this appears to be due to aggradation of the surface, in other words, additional shell material obscures the underlying pattern of microreticulation (Pl. 1, Figures 5–7) One specimen (Pl. 1, Figure 8) shows the second order pattern predominant in the anterior of the shell, with the first order pattern dominant in the posterior. In some cases, the microreticulation extends over the fossae as well as the muri, whereas in others (Pl. 2, Figure 1) the first order muri are completely microreticulated but are distinct from the floors of the fossae which undercut the large muri and are free of any microreticulation.

*Cletocythereis caudispinosa*

This species is characterized by a pattern of celation or aggradation marked by angular and pointed outgrowths from the muri of the reticulation, parallel to the lateral surface of the shell (Malz, 1980). These outgrowths appear on both adult and juvenile specimens from all of the assemblages in which it occurred (nine out of the 16 studied) (Pl. 2, Figures 2 and 3). The anterior marginal ridge also exhibits two forms in adult specimens: either rounded or arcuate in cross-section (Pl. 2, Figures 4 and 5). The rounded form is more common and there are no transitional forms.

*Chapmanella flexicostata*

This hemicytherid, widely distributed in the Tertiary sediments of southeastern Australia, has a reticulation generally characterized by celation in the form of distinctive strap-like developments of the muri (Pl. 2, Figures 6 and 7). These features are not identified in the original description and figure by Chapman (1914), where the ribs (muri) of the reticulation are referred to as 'costae' and show no evidence of such celation. The presence of strap-like growths is a variable characteristic in the assemblages studied here and the width of the straps varies between specimens. It is not possible to quantify the variation, but the illustrations (Pl. 2, Figure 8; Pl. 3, Figures 1 and 2) suggest that it may be continuous and, therefore, not polymorphic. Those specimens without celation clearly reveal that the muri develop around the margins of the underlying cells, so that dividing line is visible along each murus (Pl. 3, Figures 3 and 4). Even where the celation has produced the broadest 'straps', this dividing line is still visible, suggesting a different orientation of the calcite crystals corresponding to the underlying cell boundaries (Pl. 3, Figure 5). The celation is not present on juvenile instars (Pl. 3, Figure 6). It represents an adult response, possibly an environmentally-cued one, though not a polymorphism in the strict sense of the term. Few of the adult specimens in this study reveal the original mural structure, but none of the juvenile instars show significant celation.

*Spinobradleya nodosa*

The variability in this species relates to the development of spines from papillae, rather than to any intraspecific variation in the reticulation, which becomes increasingly strongly expressed throughout ontogeny and reaches its maximum in the adult (Pl. 3, Figure 7).

*Spinosity*

*Spinobradleya nodosa*

The fundamental pattern is a reticulation with pierced conjunctive and disjunctive spines (Pl.3, Figure 8). Adults and juveniles show both reticulation and spines. The sequence of development of the spines is from papillae through sharp, pointed spines, to large, rounded, pierced tubercles (Pl. 4, Figures 1–3). The sequence for the reticulation is from low, distinctly expressed muri, through rounded muri bordering sharply defined fossae, to very rounded ridges with indistinctly defined fossae (Pl. 4, Figure 5). The two sequences are concurrent, the stages coinciding with successive moults. There are no specimens displaying more than one stage in either sequence. At high magnifications juvenile specimens show concentric lines of deposited material on spines, muri and fossae, the significance of which is unknown (Pl.4, Figures 6 and 7); this is not evident on adults. The morphologically similar Recent species *A. arafurae* is shown for comparison (Pl. 5, Figures 1 and 2). Patterns of both reticulation and papillae/spines/tubercles are consistent amongst all the specimens; the only variations are in terms of the stages referred to above, which relate directly to ontogeny. Thus, these specimens do not show intraspecific variation, even though at low magnifications such variation seems to be present.

*Actinocythereis sp.*

Since the genus *Actinocythereis* is by definition spinose, the fundamental pattern of lateral spines remains

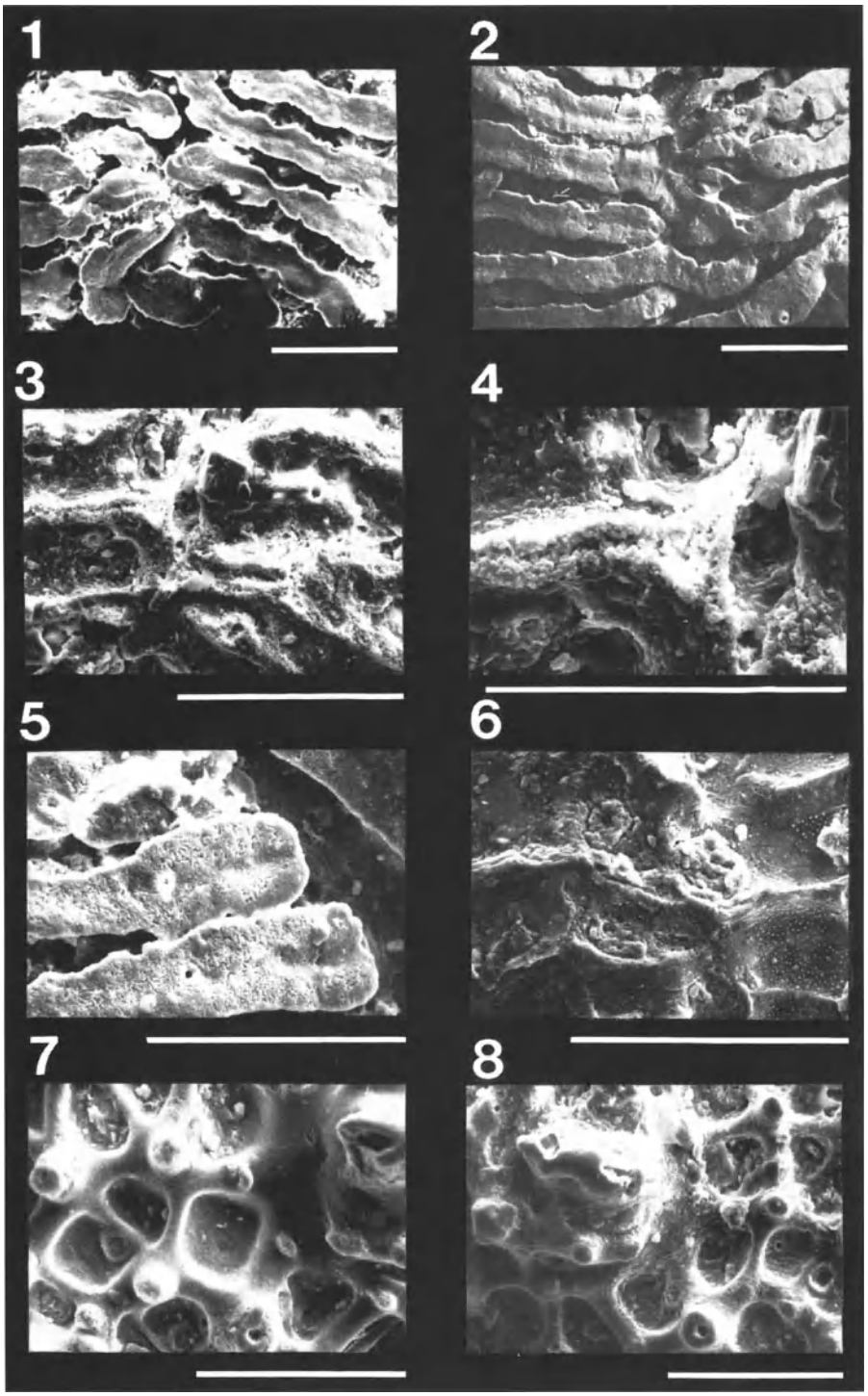


*Plate 3.* All Middle Miocene unless otherwise stated. *Figures 1–6. Chapmanella flexicostata*; (1) celated ribs, adult, Muddy Creek, Victoria; (2) celated ribs on small adult, Mitchell River, Victoria; (3) (4) uncelated ribs, showing mid-rib junction, Kingston, South Australia; (5) spatulate celated ribs, Muddy Creek, Victoria; (6) uncelated ribs on juvenile, Lower Oligocene, Fossil Bluff, Tasmania. *Figures 7 and 8. Spinobradleya nodosa*; conjunctive and disjunctive spines on full reticulation, adults, Muddy Creek, Victoria. All scale bars=100 $\mu$m, except Figure 4=50 $\mu$m.

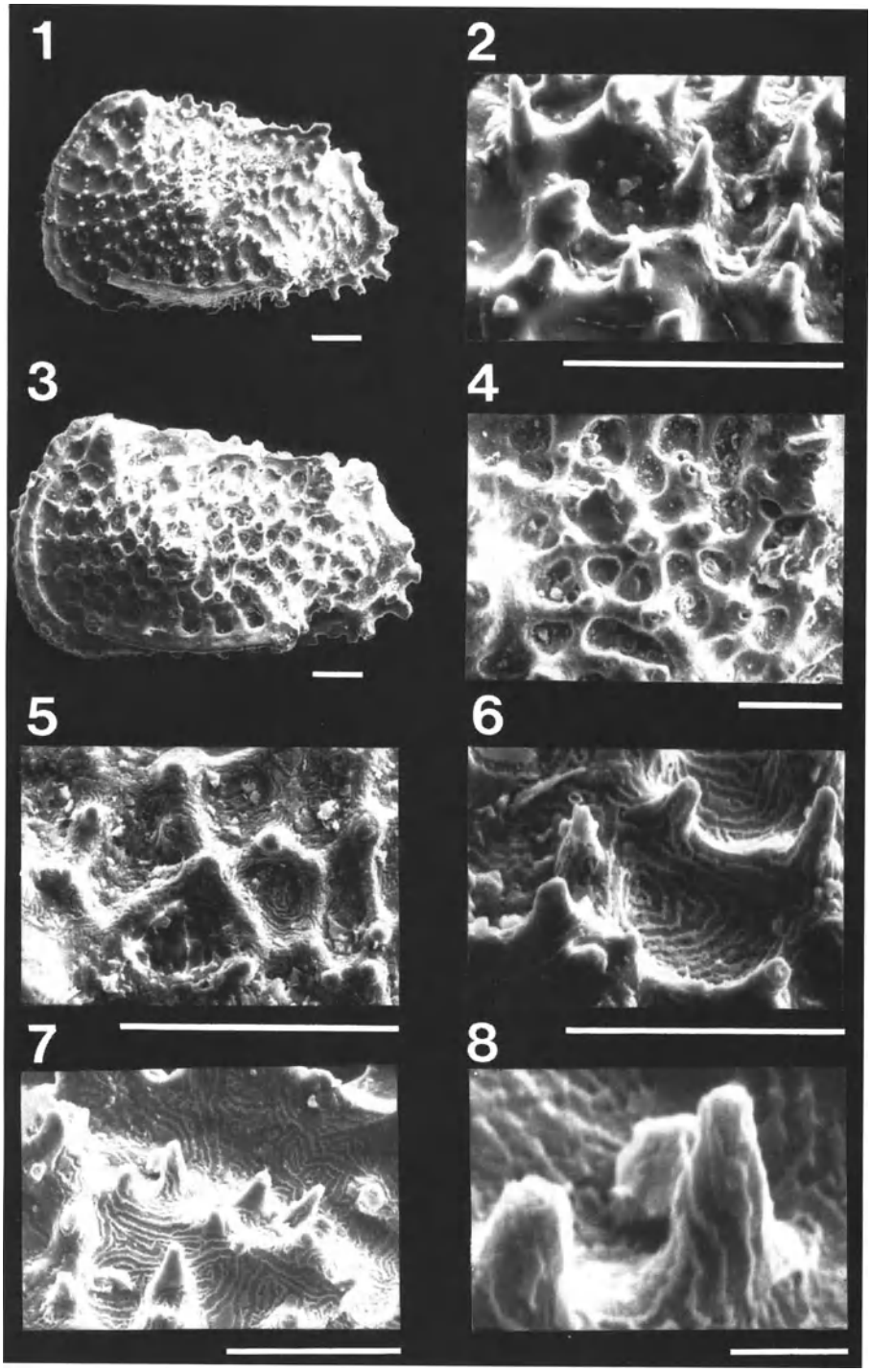


*Plate 4. Spinobradleya nodosa*, all Middle Miocene, Muddy Creek, Victoria. *Figure 1*. Late juvenile instar, left valve, with papillae. *Figure 2*. Papillae on early juvenile instar. *Figures 3 and 4*. Adult; (3) left valve; (4) detail of tubercles and reticulation. *Figure 5*. Rounded muri and indistinct fossae, adult. *Figures 6–8*. Details of late juvenile instar showing concentric lines, possibly of deposition. Scale bars: 1–5=100 $\mu$m; 6, 7=50 $\mu$m; 8=10 $\mu$m.

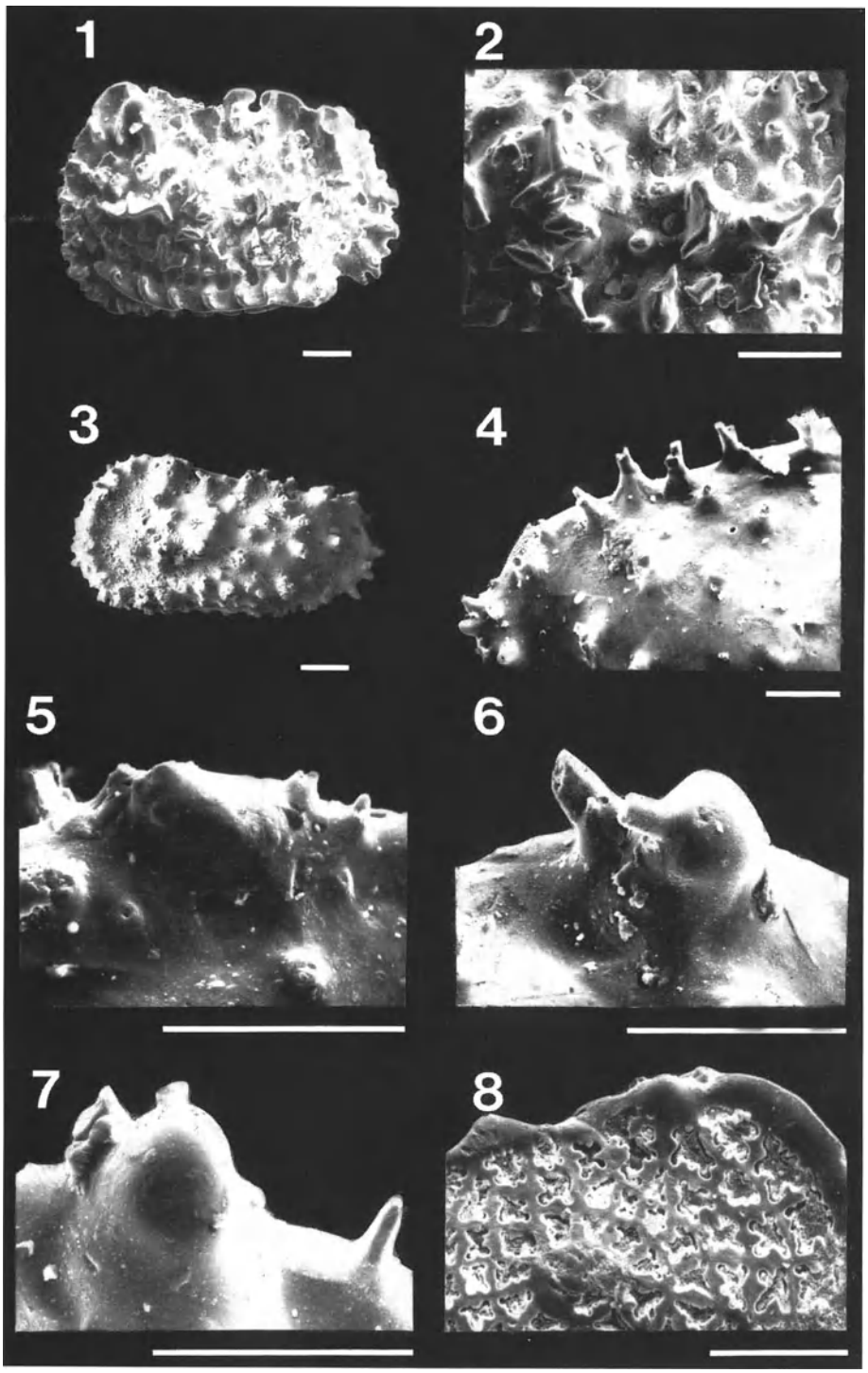


*Plate 5.* All specimens Middle Miocene unless otherwise stated. *Figures 1 and 2. Actinoleberis arafurae*, adult, Recent, Dripstone Beach, Darwin, Northern Territory; (1) left valve; (2) detail of reticulation and tubercles. *Figures 3–7. Actinocythereis* sp.; (3) adult left valve, Oligocene, Port Willunga, South Australia; (4) polyfurcate spines, Native Hut Creek, Victoria; (5) blunted tubercles, Lower Oligocene, Bells Headland, Victoria; (6) spine on eye tubercle, Muddy Creek, Victoria; (7) eye tubercle, Muddy Creek, Victoria. *Figure 8. Cletocythereis caudispinosa*, right valve showing low profile eyespot, Warrambine Creek, Victoria. All scale bars=100 $\mu$m.

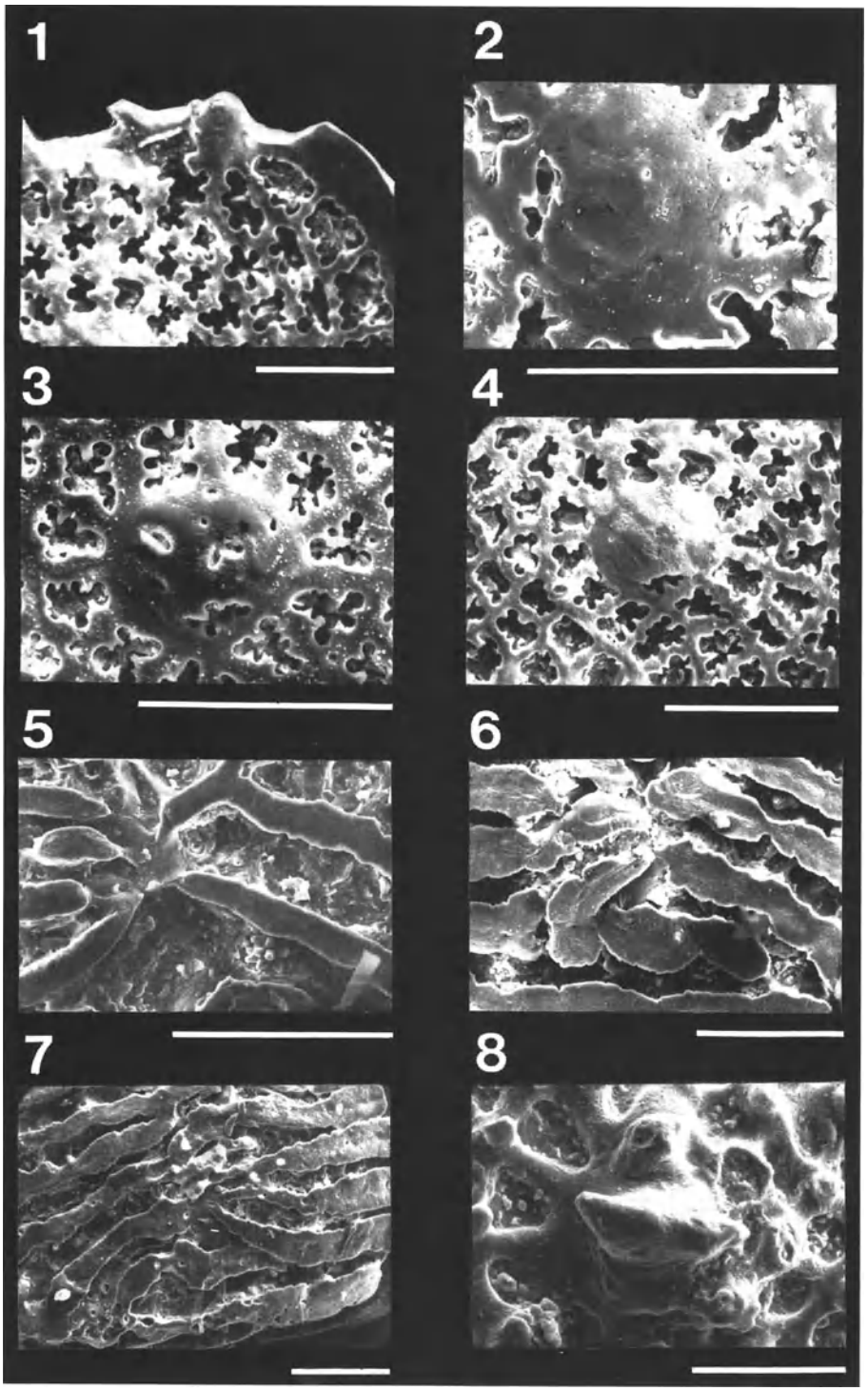


*Plate 6.* All Middle Miocene unless otherwise stated. *Figures 1–4. Cletocythereis caudispinosa*; (1) high profile eyespot, Fossil Beach, Victoria; (2) subcentral tubercle, Early Miocene, Fishing Point, Victoria; (3) subcentral tubercle, Muddy Creek, Victoria; (4) subcentral tubercle, Fossil Beach, Victoria. *Figures 5–7. Chapmanella flexicostata*; (5) celated ribs broken at subcentral tubercle, Fossil Beach, Victoria; (6) (7) variations at the subcentral point, Muddy Creek, Victoria. *Figure 8. Spinobradleya nodosa*, subcentral tubercle, Muddy Creek, Victoria. All scale bars=100 $\mu$m.

unchanged (Pl. 5, Figure 3). The spines themselves do vary, however, from specimen to specimen. Some are polyfurcate at the ends (Pl. 5, Figure 4), some are blunted and rounded in adult specimens (Pl. 5, Figure 5), and in one case an angular nodule has developed on the eye tubercle (Pl. 5, Figure 6). However, many of the specimens in these assemblages show evidence of post-mortem transport, abrasion and damage. This makes it difficult to gauge actual variation in growth form, since well-preserved specimens are rare.

*Tubercles*

The eight species studied all have eye tubercles, ranging from inconspicuous to prominent. Intraspecific variation is minimal, as one would expect with a feature which has such a clearly defined and vital function as vision. It is not feasible to draw any palaeoecological inferences from the slight intraspecific variations in eye tubercles described here. *Actinocythereis* sp. has a prominent eye tubercle which exhibits a range of sizes (Pl. 5, Figures 5 and 7). The eye tubercle of *C. caudispinosa* has a high, sub-hemispherical dome associated with the arcuate form of the anterior ridge, but a much lower dome in specimens with the rounded anterior ridge (Pl. 2, Figure 4; Pl. 5, Figure 8; Pl. 6, Figure 1). In the other species studied, the eye tubercle is an inconspicuous feature showing little variation.

Subcentral tubercles are usually an external reflection of the point of attachment of the adductor muscles to the inner surface of the shell. There are considerable differences between species in the expression of the subcentral tubercle, as well as some intraspecific variation. Three of the species studied (*N. batesfordiense*, *N. yulecartensis* and *Loxoconcha* sp.) have no subcentral tubercle.

*Cletocythereis caudispinosa*

The subcentral tubercle is characteristically smooth and lacks reticulation. The degree of protrusion is generally slight. The main variations are the presence of some puncta or micropuncta (Pl. 6, Figure 2) and the encroachment of the reticulation on some margins (Pl. 6, Figures 3 and 4). These differences can be attributed to the celation of the original reticulation pattern. As suggested above, celation is present in all specimens, and only the size and shape of the outgrowths indicate any difference in the amount of aggradation occurring. Consequently, variations in the subcentral tubercle are a reflection of the aggradation of the whole shell; no separate inferences can be drawn.

*Chapmanella flexicostata*

No subcentral tubercle, as such, occurs in the reticulation pattern of this species, but the muri converge on the site that would normally be occupied by such a tubercle. The convergent area exhibits variations which are probably caused by different degrees of celation or aggradation of the muri. Some specimens (Pl. 2, Figure 8) show strap-like growths continuous across the point of convergence. Others (Pl. 6, Figure 5) are discontinuous, leaving an area without celation at the convergence. The uncelated specimens (Pl. 3, Figure 3) show the transverse muri to be continuous across the convergent area. The junctions of the muri also exhibit variation (Pl. 6, Figures 6 and 7). None of these intraspecific differences is clearly discontinuous within the assemblages studied and, therefore, none can be described as polymorphic. They, like the variations in the strap-like outgrowths on the muri themselves, reflect different levels of aggradation, which may be environmentally-cued.

*Spinobradleya nodosa*

The subcentral tubercle of this species is characterized by a short, massive, narrow, smooth ridge, with or without sculpture (Pl. 6, Figure 8). It is quite unlike those of *C. caudispinosa* and *C. flexicostata* and does not seem to be directly related to the adductor muscle attachment point on the inside of the valve. It does not exhibit any marked intraspecific variation.

*Punctation*

Puncta are a significant feature of the external surface of *Loxoconcha* sp., *N. batesfordiense* and *N. yulecartensis*, and some intraspecific variation occurs in each of these species.

*Loxoconcha sp.*

The puncta of the central portion of the valve become progressively larger and the area between them more like a reticulation towards the anterior and posterior, whilst there is a tendency for them to become smaller or more widely spaced towards the dorsal margin. Valve size and shape are diagnostic features of this species, as is the punctate surface which exhibits intraspecific variation. The most uniformly punctate form is the norm, as has been established with a large 'population' of nearly 800 specimens from the two Middle Miocene localities at Muddy Creek (Pl. 7, Figures 1 and 2). Variations in this material include

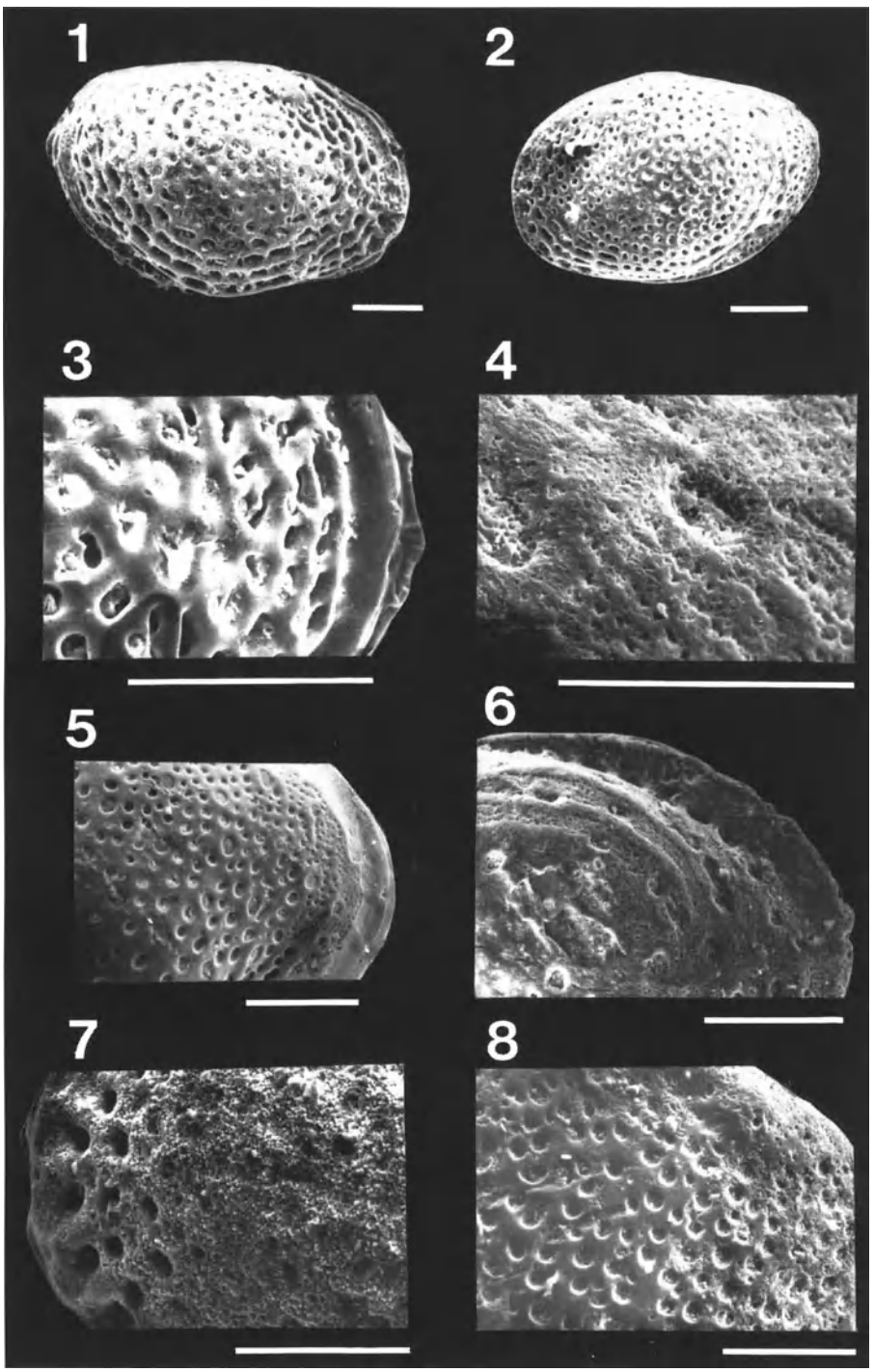


*Plate 7.* All Middle Miocene unless otherwise stated. *Figures 1–7. Loxoconcha* sp.; (1) adult right valve showing punctate/reticulate pattern, Oligocene, Port Willunga, South Australia; (2) adult left valve showing punctate/reticulate pattern, Late Oligocene, Point Addis, Victoria; (3) deep fossae with rounded muri, Native Hut Creek, Victoria; (4) small, indistinct puncta with micropunctation, Mitchell River, Victoria; (5) small puncta over whole surface, Early Miocene, Fossil Bluff, Tasmania; (6) aggraded specimen, posterodorsal margin, Mitchell River, Victoria; (7) diagenetically altered specimen, Kingston, South Australia. *Figure 8. Notocarinovalva yulecartensis*, small puncta with flat interareas, Late Oligocene, Point Addis, Victoria. All scale bars=100 $\mu$m, except 4=50 $\mu$m.

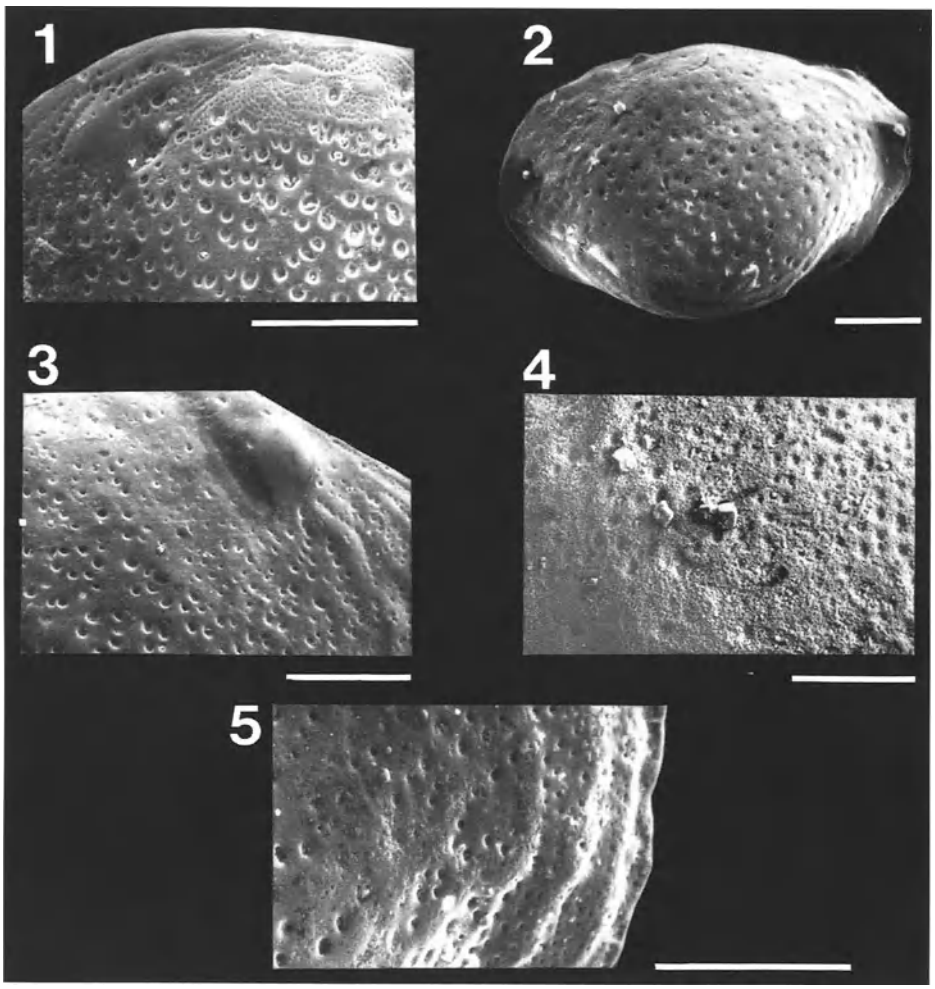


*Plate 8.* All Middle Miocene unlesss otherwise stated. *Figures 1 and 4. Notocarinovalva yulecartensis*; (1) dense uniform puncta with some micropunctation, Early Miocene, Fossil Bluff, Tasmania; (4) small puncta, widely scattered, Oligocene, Port Willunga, South Australia. *Figures 2, 3 and 5. Neobuntonia batesfordiense*; (2) left valve, showing small, regularly distributed puncta, Muddy Creek, Victoria; (3) small puncta, irregularly distributed, Early Miocene, Fossil Buff, Tasmania; (5) small puncta with weak ribbing, Muddy Creek, Victoria. All scale bars=100 $\mu$m.

a trend towards an overall reticulation with deep fossae bounded by rounded muri (Pl. 7, Figure 3); small puncta with some microreticulation on the interareas (Pl. 7, Figure 4); a small central punctate area surrounded by a broad reticulate margin (Pl. 7, Figure 1); small puncta over the whole lateral surface of the valve (Pl. 7, Figure 5); and what appears to be an aggraded form in which the underlying pattern of punctation/reticulation is largely obscured by material showing micropunctation (Pl. 7, Figure 6). Some specimens show diagenetic alteration of the surface (Pl. 7, Figure 7). None of these variations is demonstrably discontinuous within the assemblages studied. If a uniformly punctate surface is the norm, then these examples represent the ends of a continuum between reticulate and smooth surfaces.

*Notocarinovalva yulecartensis*

The lateral surfaces of the Middle Miocene forms display small puncta with generally flat interareas (Pl. 7, Figure 8), but the earlier Oligocene - Early Miocene form is more densely and uniformly punctate, with some micropunctation (Pl. 8, Figure 1). The two forms do not occur together in any one assemblage and the variation may represent evolutionary change, since the environment of deposition for both forms was similar.

*Neobuntonia batesfordiense*

The variations in punctation reflect a similar pattern to those displayed by *Loxoconcha* sp. The norm is for small puncta, regularly distributed (Pl. 8, Figure 2). Variations include small puncta of differing sizes, irregularly distributed (Pl. 8, Figure 3); very small puncta widely scattered (Pl. 8, Figure 4); and small puncta in groups, with some weakly expressed ribbing on the anterior (Pl. 8, Figure 5). Unlike the pattern of variation in *Loxoconcha* sp., there is no age correlation in *N. batesfordiense*. Since the degree of punctation is fairly constant and the variations are confined to the grouping or distribution of puncta, and some minor variation in the size of puncta, it is unlikely that aggradation/degradation is involved. It is also difficult to attribute the variation to environmental changes.

## Discussion

The intraspecific variations detailed here reflect the many factors involved in any analysis of the causes of variation. Consideration of all of these factors (and probably others not identified here) is needed if robust inferences about palaeoecological, morphological and ontogenetic relationships are to be drawn.

*The morphology of the variations*

This can only be assessed adequately on the basis of SEM images, at appropriate levels of magnification. The level of spinosity in *S. nodosa*, for instance, is shown to be an ontogenetic development and not an intraspecific variation. On the other hand, the microreticulation of *H. glyphica* is shown at high magnification to be present throughout ontogeny and hence, not an aggradational response. Sometimes the evidence is not conclusive. For example, *C. caudispinosa* shows angular outgrowths on the reticulation, apparently indicating celation or aggradation of the original pattern, but the presence of these outgrowths on specimens from all the assemblages raises the question of whether they might be genetically controlled, irrespective of the environment. Description of morphs as 'reticulate', 'spinose' and 'smooth' without high magnification images (Braccini & Peypouquet, 1996) provides an inadequate basis for other workers to utilise them in making palaeoenvironmental inferences.

*The relationship of variations to ontogeny*

If a variation is present only in adult forms, as with the aggradation of first-order reticulation in *H. glyphica*, then it is likely to be an environmentally-cued response, provided that the time scale for the development of juvenile instars is short in comparison with the life span of adults and the environmental change is long-lasting. The specimens of *H. glyphica* show that intraspecific variation in the reticulation is not demonstrably discontinuous among adult specimens and is unlikely, therefore, to reflect polymorphism. The presence of different levels of aggradation and microreticulation on the same shell also suggest that it can occur between moults, reinforcing the interpretation that the aggradation is an environmentally-cued response. The presence of microreticulation, on the other hand, must be genetically-cued, being present across moult boundaries and persistent in adults. This view differs from that expressed by Braccini & Peypouquet (1996) who proposed that microreticulation is an aggradational phenomenon developing only in a carbonate-saturated environment. The sequence of variations in relation to the ontogeny of *H. glyphica* is an essential factor in determining whether aggradation or degradation is taking place.

Conversely, if a feature ('variation') is present in juveniles (generally only later ones) as well as adult forms, as with aggradational outgrowths on the reticulation of *C. caudispinosa*, then it is unlikely to be an environmentally-cued response, especially if assemblages from different localities covering a geological (rather than ecological) time range all display the same feature. Since celation (outgrowths) were found on adult and juvenile specimens of *C. caudispinosa* from all of the assemblages studied, one might assume that these features are not indicative of any intraspecific variation, but reflect the expression of the genotype for this species. The original description and figure do not identify or show these outgrowths (Chapman & Crespin, 1928). However, outgrowths of this kind characterize a number of species from different genera, e.g. *'Hermanites' lungulata* (McKenzie, Reyment & Reyment, 1991) and *Costa* sp. (illustrated by Sylvester-Bradley & Benson, 1971), in which some specimens are free from the outgrowths (Neil, 1994). This could be interpreted as signalling an environmentally-cued response. In the present study, however, *C. caudispinosa* occurred in nine assemblages representing varying depositional conditions in terms of depth and facies, so the idea of an environmentally-cued response is not suppor-

ted. But another species of *Cletocythereis* (*C. rastromarginata*) does not display celation in any of the assemblages studied here, although it does show outgrowths in recent forms (Malz, 1980; Yassini & Jones, 1995). The evidence is thus inconclusive.

In *C. flexicostata*, the apparent restriction of celation to adults may represent an environmentally-cued response or a normal stage of the ontogeny, although the presence of a few adults without celation suggests the former is more likely.

Such inferences require a study of juvenile as well as adult instars and need support from data on species generation times. Some morphological changes are directly related to ontogeny, such as the development of tubercles from papillae in *S. nodosa*. These changes may be mistakenly referred to as intraspecific variations if juveniles and adults are not correctly identified.

*Time scales of intraspecific variations and palaeoenvironmental changes*

The period over which a cytheracean ostracod species develops to the adult stage (generally through eight juvenile instars and one adult instar) may be anything between 40 days and 3 years (Kesling, 1961). For palaeoenvironmental inferences to be drawn from intraspecific variations, the length of the life cycle and the evidence of juvenile, as well as adult valves, may need to be considered, since the time-scale of the environmental changes may be of a different order of magnitude.

*The co-occurrence of intraspecific variations in assemblages*

If several species of ostracod, showing variations, occur in an assemblage, then the inferences based on these variations should not be made in isolation from one another. Aggradational and spinose variations are inferred to have mutually exclusive environmental causes by Braccini & Peypouquet (1996; 68), yet in Figure 1 of the same paper (1996: 67) they are shown as having the same cause. Co-occurrence in a given assemblage may rule out mutually exclusive environmental triggers, if the time scale of the changes is of a similar order to the inevitable time-averaging of the composition of the assemblage. If, however, the time-scales differ by one or more orders of magnitude, then co-occurrence would not rule out mutually exclusive triggers.

*Time-averaged assemblages*

All fossil assemblages are time-averaged to some extent, so that the occurrence of different kinds of intraspecific variation reflecting different environmental cues is more likely than not, especially if the environmental changes occur over a short ecological time range compared with the geological range of most assemblages. Braccini & Peypouquet make use of this characteristic in their 'Morph Method' tables in Figures 7A and 7B (1996: 72). However, their assertion that "the reaction of ostracodes to environmental changes is drastic and quick" (1996: 69) needs further supporting evidence. One must assume their use of the term 'reaction' is intended to cover the physiological responses of the animals to changes in the environment. Changes in the order of days or months (Peypouquet et al., 1986) are claimed to give rise to 'ornamental overloading', but other workers are sceptical of variations occurring over such short time intervals (Whatley & Zhao, 1993: 285).

*Polymorphism and continuous variation*

As pointed out above, it is difficult to establish whether intraspecific variations in a time-averaged assemblage are strictly polymorphic, even when the variation is discontinuous. Hence the use of the term 'environmentally-cued variation' is to be preferred to 'environmentally-cued polymorphism'. Since polymorphism is understood to refer to the production of different morphs at the same ontogenetic stage (Clark, 1976), variations occurring between moults are not polymorphic. Although most workers agree, according to Clark (1976), that polymorphism is not concerned with continuous variations, which is generally under polygenic control, it is evident from some of the specimens studied here (e.g. Pl. 1, Figure 8) that aggradational features are not necessarily fully expressed at a given ontogenetic stage, and that an aggradational process might show evidence of continuous variation. A polymorphic response implies a threshold figure for the environmental trigger, whereas continuous variation does not. If aggradation or degradation occurs as a polymorphic response, then that one response at a constant level identifies the trigger. On the other hand, the presence of differing levels of aggradation or degradation within the one assemblage would indicate that the environmentally-cued response could occur at different levels. This does not alter the fundamental relationship which is inferred between the ostracod's response and the particular environmental

factor involved. Variations in the subcentral tubercle of *C. caudispinosa* (P. 6, Figures 2–4) are a case in point.

*Hypotheses for causes of variation*

Over the last 25 years, two broadly-based theories have received consistent support from workers dealing with intraspecific variation in ostracods. The first seeks to link variations with changes in the environment, especially in relation to the extent to which calcium and carbonate ions are available, but also in relation to water temperature, salinity and depth. Recently the umbrella term 'environmentally-cued polymorphism' (Peypouquet et al., 1988) has been used for this relationship. Generally, the intraspecific variations identified are of the 'aggradation/degradation' type (Peypouquet et al., 1980) in which shell material is either added to the existing structural patterns of reticulation and ribbing, or removed from them. The second theory, not mutually exclusive of the first, has been most consistently advocated by Benson (1974, 1975, 1981, 1983) and proposes that structural variations of reticulation or ribbing are due to the shell responding to mechanical stress, caused either through ontogenetic development, or through physical changes in the environment such as increasing water depth. Such responses tend to occur rapidly, so that assemblages will show discontinuous variation.

Environmental cues such as excess or deficiency of carbonate may trigger aggradation or degradation responses and these may or may not be related to the ostracod's reaction to increased or decreased mechanical stress on the shell. An adaptationist viewpoint would focus on aggradation or celation as related to this change in mechanical stress, whereas an emphasis on palaeoecological interpretation would focus on the morphological changes simply as feedback from environmental changes. The inter-relationship between these approaches is complex and inferences about the palaeoenvironment which do not take the structure and style of ornamentation of the ostracod shell into account may oversimplify the palaeoecology inferred (McKenzie & Peypouquet, 1984; Reyment et al., 1988).

It has been proposed that spines are developed as a defence against predators or to extend sensory setae (Benson, 1981). Alternatively, Braccini & Peypouquet (1996) proposed that spines develop to screen the animal from an increased flow of particulate matter in the surrounding water. These functions are not mutually exclusive, though their probability is linked to the combination of factors that apply in a particular environment, and to the palaeoenvironmental inferences drawn from the assemblage of which they are part. For example, the occurrence of spinose, reticulate and smooth species in one assemblage may represent environmentally-cued responses at different stages during the time over which the assemblage accumulated (the time-averaging effect). This is even more probable if the spinose, reticulate and smooth forms are morphs of a single species. On the other hand, if differently-ornamented specimens are from different species and the environment can be demonstrated to have been stable over time, then an explanation based on a genetic response has a higher probability of being correct.

Variations in the number and nature of anterior and posterior spines do occur. Reyment et al. (1977) established for the genus *Cytheridea* that there is no correlation between any such variations in anterior and posterior spines, nor with lateral spines, and this is likely to be the case with *Actinocythereis* sp., and ontogenetically within the genus *Actinocythereis*, although no statistical analysis has been carried out. Irrespective of whether or not the variations observed in the spines of *Actinocythereis* sp. represent polymorphism, they do represent potential palaeoenvironmental indicators. It is difficult to reconcile the suggestion that spines function as a means of screening particulate matter (Braccini & Peypouquet, 1996) with the view that they are a defence against predators (Benson, 1981). The former is a very specific environmental factor, the latter a very general one. The former idea refers to the appearance of spines on previously non-spinose forms, the latter to the body plan of the animal, genetically determined throughout ontogeny. There is no clear pattern or sequence in the variations seen in *Actinocythereis* sp. It is, therefore, unwise to draw conclusions about environmentally-cued responses, although the co-occurrence of species showing such responses may support the hypothesis that the changes in the spines are aggradational features.

Another fundamental question is raised by the work of Simkiss (197, 1986) and others on the relationship between biomineralization and detoxification. Simkiss (1977: 199) writes: ". . . calcium is a toxic ion that must be removed from most cells and the occurrence of calcium deposits may therefore represent a form of detoxification. Biomineralization may be a cellular detoxification mechanism for calcium". This detoxification mechanism could then

be involved in the variety of purposes normally associated with biomineralization. This would render much more complex the relationship between aggradation, environmentally-cued responses and reaction to changes in mechanical stress. The 'carbonate supersaturated' environment would represent a threat rather than an opportunity. A 'carbonate undersaturated' environment, on the other hand, would simply indicate that the ostracod would have an inadequate supply of calcium carbonate to construct the full genotypic expression of the shell, since there would be no 'pumping out' of toxic ions. The connection between biomineralization and detoxification is beyond the scope of the present paper, but it is likely that the strength and validity of palaeoecocological inferences from ostracod variations would be enhanced by exploring this connection further. Similarly, as referred to in the introduction, a study of variation in the 'soft part' anatomy of ostracods would provide a broader and more valid basis for hypotheses about intraspecific variation/polymorphism. However, the limitation of work to essentially fossil 'populations' and assemblages means that these aspects have been excluded from the present discussion.

## Conclusions

It will be important for workers in the area of intraspecific variations and their evolutionary and palaeoecological significance to take account of a wide range of existing research. The interconnections between the various branches of this research are usually significant and if ignored may lead to contradictory results. High magnification illustrations are essential for adequate interpretation, and large sample sizes from widely scattered locations give a sounder base for inferences, especially if statistical work on populations is to be carried out. These points, although quite obvious, are sometimes ignored.

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# Trend, signal and noise in the ecology of Ostracoda: information from rare species in low-diversity assemblages

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*Key words:* Ostracoda, sample size, species diversity, Nile Delta, Egypt, rare species

## Abstract

Samples of Ostracoda from nearshore marine environments, where the water is more likely to be brackish or hypersaline, typically have low species diversity and are dominated by such species as *Cyprideis torosa*. In high-diversity samples from normal-marine environments, rare species are likely to contribute to environmental noise. Evaluation of low-diversity samples from Lake Manzala, Egypt, however, shows that the environmental signal provided by rare species can be masked by the overwhelmingly dominant species, *C. torosa*. In such instances, the dominant species should be removed from the data set and studies based on a large sample of the rare species.

## Introduction

The data from any suite of samples used to interpret the environment, whether modern or fossil, can be partitioned into three components: trend, signal and noise. The task of the ecologist or paleoecologist is to resolve the data from the samples into these three components. Thus, much of applied aquatic ecology, environmental science and paleoecology can be regarded as an attempt to discriminate the environmental signal from the noise in the hope of identifying long-term trends that are useful either for predicting the environments of the future or for retrodicting those of the past.

We are interested in rare species such as one encounters in studies of ostracods from brackish-water or hypersaline, marginal-marine environments. Are rare species part of the environmental noise? If so, what should be done with them? If they are not mere noise, can a signal be extracted from quantitative information on the abundance and distribution of rare species? In studies where a goal is to assess quantitatively the similarities and differences among high-diversity samples from normal-marine environments, rare species have been widely regarded as a source of noise. Because their occurrence in a sample is dependent in large part on the vagaries of sampling, the data on the occurrence of rare species are seen as hampering the quantitative assessment of community structure. This is especially true if similarities among samples are to be compared through the use of presence and absence data. To remove operationally the rare species from a data set, a number of schemes, some of them quite arbitrary, have been devised. Thus, Haack & Kaesler (1979) deleted from their study any species with a mean relative abundance over all the samples of less than 0.01, except when the standard deviation of relative abundances of the species was greater than 0.005. Using these criteria, they eliminated uniformly rare species from their study but retained species that were rare overall but relatively abundant in a few samples.

Here we investigate the occurrence of rare species using the assemblage of Holocene ostracods of Lake Manzala, Egypt, as test data (Kontrovitz et al., 1992; Slack et al., 1995). We show that in some instances the rare Ostracoda provide a different signal from that of the total Ostracoda fauna, which in these samples is dominated by a single, abundant species, *Cyprideis torosa*. Under the appropriate circumstances, abundant species, which can mask the environmental signal from the rare ostracods, are best regarded as noise.

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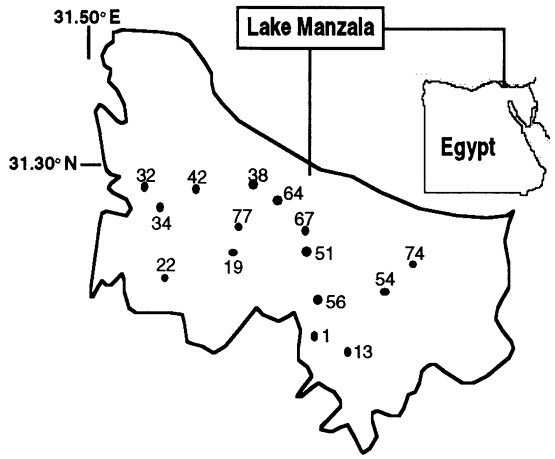



*Figure 1.* Map of Manzala Lake, Egypt, showing locations of grab samples and the top of one core (formerly sample IV, here renumbered 77). Not shown are numerous, small, low islands that prevent the free flow of water from one part of the lake to another.

Thus, to eliminate rare species from a data set is a valid procedure only when one is dealing with samples that have a high species diversity. In such samples as these, the rare species, in fact, do contribute to the noise. Samples that have very low species diversity, however, are typically marked by few species, one or two of which are abundant; and others that are quite rare. The rare species may convey appreciable environmental information that is masked by the overwhelming abundance of the common species. We demonstrate that rare species can be the key to unlocking a different environmental signal. This is not meant to suggest, however, that abundant species provide no useful information.

## Material and methods

### *Description of sites studied*

Kontrovitz et al. (1992) and Slack et al. (1995) described the geological setting and the Quaternary geological history of Lake Manzala (Figure 1) and illustrated the Ostracoda that occur there (Table 1). Shaheen & Yosef (1978), Kerambrun (1986) and Stanley & Warne (1993) have dealt with the environmental implications of the area's geological history and subsequent changes induced by human activities. For that reason, here we need only review the salient aspects of the environment. The salinity of Lake Manzala ranges from 1.4 to 21.0 g $l^{-1}$, depending on the distance from sites of influx of fresh water. The lake averages 1.3 m in depth and is alkaline with a pH that ranges from 7.8 to nearly 9.0. The water is highly oxygenated and the temperature follows that of the air rather closely. Numerous small islands disrupt the free flow of water from one part of the lake to another. The area is one of long-standing environmental change. Coutellier & Stanley (1987), for example, pointed out that the coastal margin of the Nile Delta has moved northward as much as 50 km during the past 5000 years, an average of some 10 m per year (see also Slack et al., 1995). Because of the long history of agriculture in the area, how much of this change was induced by human activity is difficult to assess. Nevertheless, such recent events as the completion and closure of the Aswan High Dam in 1964, clearly an activity that is under human control, have intensified changes in the environment and threatened fisheries in the lake.

### *Methods*

Whether to regard rare species as signal or noise depends very much on the number of specimens available in the sample. Because rare species are rare, one may have to process an inordinately large amount of sediment to find enough specimens to provide a useful sample of the Ostracoda. Another way of expressing the goal of our study is to determine which species ought to be used for analysis from among those occurring in a sample or suite of samples.

Slack et al. (1995) have reviewed much of the literature that supports micropaleontologists' long-standing inclination to pick from their samples 300 specimens for study. Dennison & Hay (1967) dealt with sampling theory in terms of the binomial equation. Consider this question: how many Ostracoda must one pick from a sample to be 95% certain of detecting species (i.e. find at least one specimen of each) that comprise one percent of the sample? Equation (1) describes the binomial relationship as:

$$Y = (1 - p)^n, \tag{1}$$

where $Y$ is the probability of failure (in this instance 1.00–0.95=0.05), $p$ is the proportion the target species comprise of the sample (in this instance, 1% converted to the proportion 0.01) and $n$ is the needed sample size. Solving this equation for $n$ gives Equation (2):

$$n = \log Y / \log(1 - p). \tag{2}$$

The result is that $n$=log(0.05)/log(0.99)=298.07≈300, which is the justification for picking 300 Ostracoda.

*Table 1.* Number of ostracods found in 16 samples, total ostracods, and mean per sample

| Sample number | Total Ostracoda | *Cyprideis torosa* | *Sylvestra* | *Loxo-concha* | *Ilyocypris* | *Propontocypris* | *Darwinula* | Hemicytherid | *Cypridopsis* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 817 | 770 | 45 | 2 | 0 | 0 | 0 | 0 | 0 |
| 7 | 1692 | 1657 | 29 | 0 | 6 | 0 | 0 | 0 | 0 |
| 13 | 2267 | 2210 | 57 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 1386 | 1300 | 74 | 0 | 2 | 10 | 0 | 0 | 0 |
| 22 | 2546 | 2188 | 306 | 4 | 4 | 1 | 43 | 0 | 0 |
| 32 | 781 | 630 | 52 | 7 | 66 | 5 | 21 | 0 | 0 |
| 34 | 800 | 473 | 100 | 5 | 213 | 5 | 4 | 0 | 0 |
| 38 | 786 | 654 | 78 | 44 | 6 | 4 | 0 | 0 | 0 |
| 42 | 609 | 453 | 96 | 21 | 33 | 6 | 0 | 0 | 0 |
| 51 | 810 | 772 | 35 | 3 | 0 | 0 | 0 | 0 | 0 |
| 54 | 1260 | 1256 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 1579 | 1534 | 43 | 1 | 0 | 1 | 0 | 0 | 0 |
| 64 | 5808 | 5666 | 122 | 17 | 0 | 3 | 0 | 0 | 0 |
| 67 | 2264 | 2062 | 154 | 43 | 4 | 1 | 0 | 0 | 0 |
| 74 | 1646 | 1597 | 33 | 5 | 0 | 7 | 3 | 1 | 0 |
| 77 | 2563 | 1821 | 399 | 316 | 1 | 25 | 0 | 0 | 1 |
| Total | 27514 | 25043 | 1627 | 468 | 335 | 68 | 71 | 1 | 1 |
| Mean | 1726 | 1565 | 102 | 29 | 21 | 4 | 4 | 0 | 0 |

Thus, by picking 300 specimens from a sample, one can be 95% certain of detecting species that comprise as little as 1% of the sample.

Following Pielou (1966a, b, c, 1967, 1969, 1975), we have used Brillouin's (1962) index of species diversity from information theory. This index has been employed successfully in previous assessment of the aquatic environment (e.g. Kaesler & Herricks, 1976, 1979) and has been applied to the study of Ostracoda as well, both from the fossil record (e.g. Haack & Kaesler, 1979) and from modern environments (e.g. Kaesler & Mulvany, 1977). Using the computer programs written by Kaesler & Mulvany (1976a, b), we determined the mean value of 50 species diversities that were computed from randomly selected subsamples of size 35 and 300 for the total Ostracoda fauna and of size 35 for the rare species. Thus, we were able to adjust samples to a uniform size, a necessary step because Brillouin's index is influenced by sample size. The subsample size of 35 was selected arbitrarily to allow us to include most of the samples in analysis of the rare species.

In addition, we computed product-moment correlation coefficients between a number of characteristics of our samples, especially valves per gram and $\log_{10}$ of valves per gram (Tables 2–4). Use of logarithms smoothed the data and allowed us to use the same scale for valves/g as for species diversity (Figures 5 and 6). These coefficients allowed us to identify instances in which the information content of data on rare species departed from that of the total sample dominated by the abundant species, *Cyprideis torosa*.

## Results and discussion

To assess the extent to which data on the rare Ostracoda comprise signal or noise, we have compared results of analyses done with the total Ostracoda fauna and with only the rare species. Table 1 shows that of the eight species found in Lake Manzala, only *Cyprideis torosa* can be regarded as abundant. *Cyprideis torosa* comprises more than 90% of the ostracod fauna, whereas the other seven species average only 1.3% of the fauna.

The ostracods collected in Lake Manzala do not provide a strong environmental signal. This may result in large part from the presence of numerous, small islands scattered across the lake, preventing the free flow of water and hampering the free taphonomic transport of ostracod valves and carapaces from one

*Table 2.* Data on each of the 16 samples in the study. $H_{35}$ is the mean of 50 Brillouin's species-diversity values computed from randomly selected subsamples of size 35

| Sample number | Sediment, wet (g) | Valves/g | Total rare species | % rare species | $H_{35}$, all species | $H_{35}$, rare species |
|---|---|---|---|---|---|---|
| 1 | 0.20 | 4051 | 47 | 5.75 | 0.17 | 0.16 |
| 7 | 1.07 | 1576 | 35 | 2.07 | 0.06 | 0.60 |
| 13 | 0.35 | 6450 | 57 | 2.51 | 0.09 | 0.00 |
| 19 | 0.19 | 7365 | 86 | 6.20 | 0.18 | 0.46 |
| 22 | 0.22 | 11604 | 358 | 14.06 | 0.36 | 0.53 |
| 32 | 0.94 | 834 | 151 | 19.33 | 0.56 | 2.63 |
| 34 | 0.68 | 1185 | 327 | 40.88 | 0.82 | 1.74 |
| 38 | 0.38 | 2064 | 132 | 16.79 | 0.49 | 2.43 |
| 42 | 0.21 | 2942 | 156 | 25.62 | 0.70 | 2.30 |
| 51 | 0.15 | 5263 | 38 | 4.69 | 0.16 | 0.27 |
| 54 | 1.29 | 976 | 4 | 0.32 | | |
| 56 | 1.23 | 1288 | 45 | 2.85 | 0.18 | 0.16 |
| 64 | 1.06 | 5461 | 142 | 2.44 | 0.05 | 0.51 |
| 67 | 0.54 | 4208 | 202 | 8.92 | 0.30 | 0.99 |
| 74 | 1.21 | 1361 | 49 | 2.98 | 0.17 | 1.63 |
| 77 | 0.93 | 2767 | 742 | 28.95 | 1.42 | 2.53 |
| Sum | | | 2571 | | 5.70 | 17.04 |
| Mean | 0.67 | 3712 | 161 | 11.52 | 0.38 | 1.14 |

*Table 3.* Valves/g, all species and rare species; $r$=0.65. Column 2, grams of wet sediment, has been rounded from 4 to 2 decimal places. Values of *valves/g (total)* and *valves/g (rare)* were computed from unrounded values and are, thus, correct

| Sample number | Sediment, wet (g) | Total Ostracoda | Total rare species | Valves/g (total) | Valves/g (rare) |
|---|---|---|---|---|---|
| 1 | 0.20 | 817 | 47 | 0451 | 233 |
| 7 | 1.07 | 1692 | 35 | 1576 | 33 |
| 13 | 0.35 | 2267 | 57 | 6450 | 162 |
| 19 | 0.19 | 1386 | 86 | 7365 | 457 |
| 22 | 0.22 | 2546 | 358 | 11604 | 1632 |
| 32 | 0.94 | 781 | 151 | 834 | 161 |
| 34 | 0.68 | 800 | 327 | 1185 | 484 |
| 38 | 0.38 | 786 | 132 | 2064 | 347 |
| 42 | 0.21 | 609 | 156 | 2942 | 754 |
| 51 | 0.15 | 810 | 38 | 5263 | 247 |
| 54 | 1.29 | 1260 | 4 | 976 | 3 |
| 56 | 1.23 | 1579 | 45 | 1288 | 37 |
| 64 | 1.06 | 5808 | 142 | 5461 | 134 |
| 67 | 0.54 | 2264 | 202 | 4208 | 375 |
| 74 | 1.21 | 1646 | 49 | 1361 | 41 |
| 77 | 0.93 | 2563 | 742 | 2767 | 801 |
| Sum | | 27614 | 2571 | | |
| Mean | 0.67 | 1726 | 161 | 2595 | 242 |

*Table 4.* Values of logarithms (base 10) of valves/g and 1000 times species diversity used to prepare Figures 3–6

| Sample number | $\log_{10}$ (v/g) all spp. | $\log_{10}$ (v/g) rare spp. | $\log_{10}$ (1000 $H_{35}$) all species | $\log_{10}$ (1000 $H_{35}$) rare species |
|---|---|---|---|---|
| 1 | 3.6 | 2.4 | 2.228 | 2.193 |
| 7 | 3.2 | 1.5 | 1.799 | 2.776 |
| 13 | 3.8 | 2.2 | 1.934 | – |
| 19 | 3.9 | 2.7 | 2.258 | 2.658 |
| 22 | 4.1 | 3.2 | 2.556 | 2.723 |
| 32 | 2.9 | 2.2 | 2.748 | 3.420 |
| 34 | 3.1 | 2.7 | 2.913 | 3.240 |
| 38 | 3.3 | 2.5 | 2.686 | 3.404 |
| 42 | 3.5 | 2.9 | 2.842 | 3.362 |
| 51 | 3.7 | 2.4 | 2.199 | 2.438 |
| 54 | 3.0 | 0.5 | – | – |
| 56 | 3.1 | 1.6 | 2.260 | 2.204 |
| 64 | 3.7 | 2.1 | 1.672 | 2.708 |
| 67 | 3.6 | 2.6 | 2.481 | 2.995 |
| 74 | 3.1 | 1.6 | 2.233 | 3.211 |
| 77 | 3.4 | 2.9 | 3.153 | 3.403 |

sampling site to another, thus reducing the mappability of the ecological patterns. In this kind of distributional ecology based on subfossil organisms, one looks for mappable patterns. The sedimentological situation formed by the many islands in Lake Manzala, however, may have produced unmappable patterns at the scale at which the lake was sampled. The use of both abundance of valves (as measured by valves per gram of sediment) and species diversity demonstrates that the rare ostracods and the total ostracod fauna send subtly different signals about the environment.

Slack et al. (1995: Figure 2, p. 399) investigated the abundance of Ostracoda in samples of Lake Manzala as valves per gram (valves/g) of the total Ostracoda. They found the greatest abundance in samples 13, 19 and 22, as well as a trend toward greater abundance in the middle part of the lake than toward the eastern and western margins. Here we compare valves/g of the total Ostracoda fauna with the rare Ostracoda, with *Cyprideis torosa* deleted from the data set (Figure 2). The product-moment correlation coefficient ($r$) of valves/g of all species vs. valves/g of rare species is 0.65. The coefficient of determination, $r^2$, is 0.33. This is the proportion of the variation in one of these variables that is explained by the other. This low value suggests that the rare Ostracoda convey a different environmental signal from that of the total fauna.

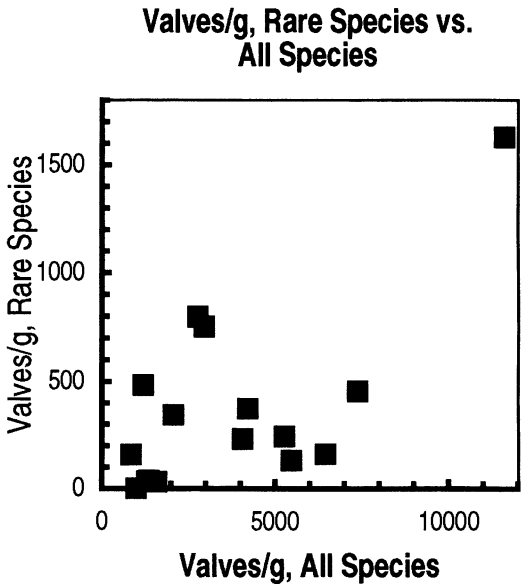
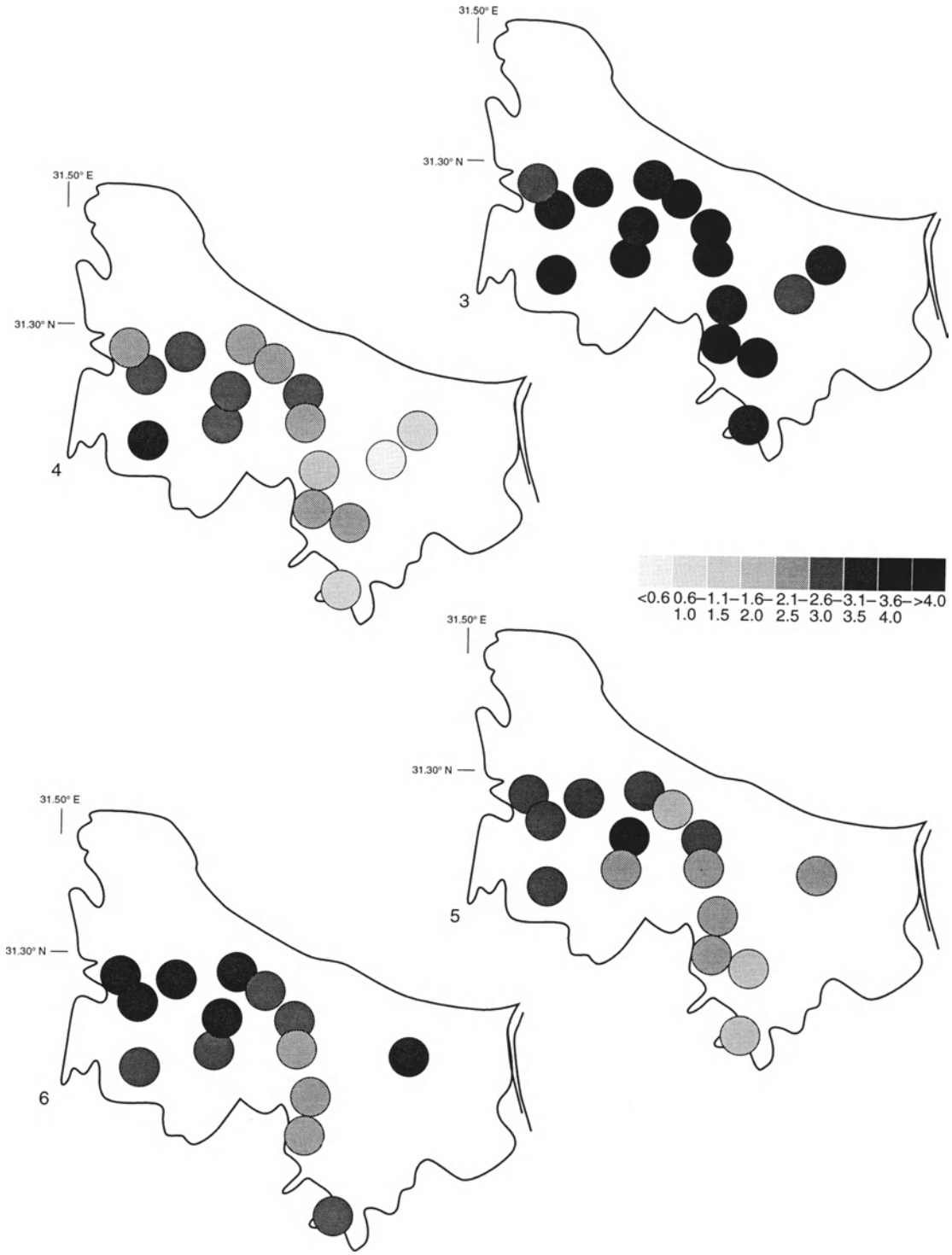



*Figure 2.* Scatter diagram of valves per gram of rare species (excluding *Cyprideis torosa*) vs. valves per gram of all species. The correlation coefficient, $r$, is 0.65; $r^2$, the coefficient of determination, is 0.33.

Figure 3 shows, plotted on the map of Lake Manzala, the logarithm (to the base 10) of valves per gram of all Ostracoda, including *Cyprideis torosa*. Figure 4 shows the same kind of plot but includes only the valves of rare species (see also Table 4). We have




*Figures 3–6.* (3) $\text{Log}_{10}$ (valves/g) for all species including *Cyprideis torosa*. (4) $\text{Log}_{10}$ (valves/g) for rare species with *Cyprideis torosa* deleted from the data set. (5) $\text{Log}_{10}$ (1000 $H_{35}$), computed for all species including *Cyprideis torosa*; $H_{35}$ is the mean of 50 randomly replicated Brillouin's indices of species diversity computed for 35 individuals. (6) Same as Figure 5 but with *Cyprideis torosa* deleted from the data set. Data for all figures in Table 4.

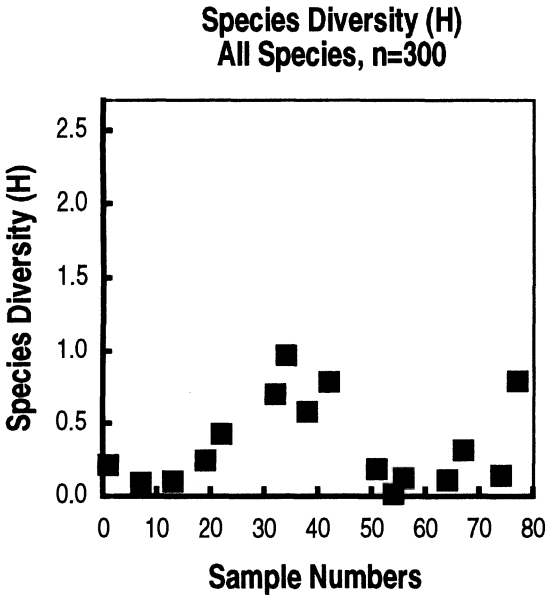



*Figure 7.* Brillouin's indices of species diversity ($H$) expressed as the mean of 50 replications using randomly selected subsamples of size $n$=300; all species used, including *Cyprideis torosa*.

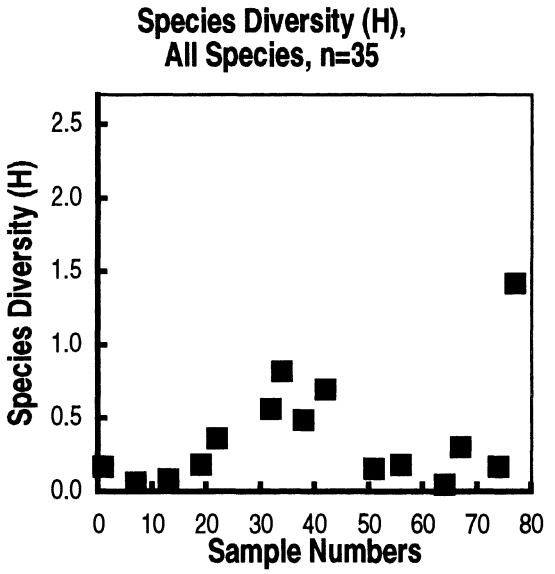



*Figure 8.* Brillouin's indices of species diversity ($H$) expressed as the mean of 50 replications using randomly selected subsamples of size $n$=35; all species used, including *Cyprideis torosa*.

shown the data for each sample as isolated points rather than as continuous patterns to avoid implying continuity from one sample site to the other. As was found by Slack et al. (1995), we have observed that ostracods are most abundant near the southern shore of Lake Manzala and the abundance decreases markedly both to the east and to the west of the lake's center. The pattern of abundance of the rare species is quite different. Abundance is lowest in the eastern part of the lake, and it increases rather steadily to the west and south. Only in the single, western-most sample does the abundance again decrease.

The dependency on sample size of an index of species diversity necessitates the use of samples of uniform size. Figures 7–9 show the mean species diversity of 50, randomly selected subsamples of each sample (Kaesler & Mulvany, 1976b). Figure 7 is based on random subsamples of size 300 from the total ostracod fauna. Figure 8 is based on subsamples of size 35. The product-moment correlation coefficient ($r$) between the two estimates of diversity is 0.87, which gives a coefficient of determination ($r^2$) of 0.75. Thus, one set of species diversities explains 75% of the variation in the other set: the two measures of diversity, both based on the total Ostracoda, are clearly sending nearly the same environmental signal.

Figure 9 shows similar indices of diversity based on subsamples of 35 specimens of rare species. The di-

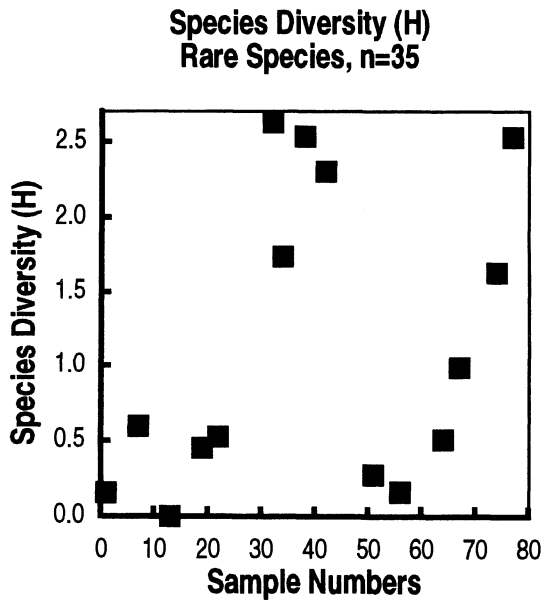



*Figure 9.* Brillouin's indices of species diversity ($H$) expressed as the mean of 50 replications using randomly selected subsamples of size $n$=35; only rare species included; *Cyprideis torosa* deleted from the data set.

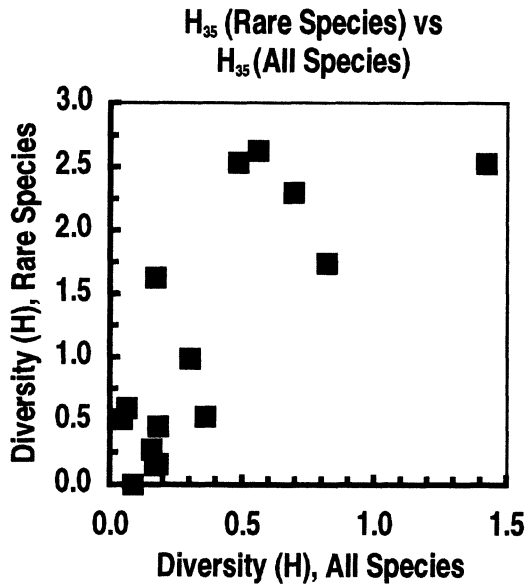



*Figure 10.* Scatter diagram of species diversities of rare species v. all species. Brillouin's indices of species diversity ($H$) expressed as the mean of 50 replications using randomly selected subsamples of size $n$=35.

versities of the rare species are much higher and much more widely scattered because the strongly dominant species, *Cyprideis torosa*, has been deleted from the data set. The correlation coefficients with the other sets of species diversities in Figures 7 and 8 are, respectively, 0.80 and 0.75, giving $r^2$ values of 0.65 and 0.57. The species diversity of the total Ostracoda fauna explains only a little more than half of the variation among the rare species, again implying that the total and rare Ostracoda faunas are sending different environmental signals. Figure 10 shows a scatter diagram that compares the species diversities in Figures 8 and 9 (see also Table 2). The scatter in this figure again confirms the observation that the species diversities of the rare species is conveying a different signal from that of the total ostracod fauna.

Figures 5 and 6 show the geographical distribution of a measure of the species diversities in Lake Manzala (see also Table 4). To make the values more nearly comparable with the information in Figures 3 and 4, we have plotted the logarithm of 1000 times the species diversity: $\log_{10}(1000\ H)$. Figure 5 shows a general increase in species diversity of the total Ostracoda from the southeastern to the western part of Lake Manzala. The figure also shows samples of both low diversity and high diversity interspersed with other samples and superimposed on the overall trend. These samples make the actual pattern of species diversity largely unmappable, presumably in part because of the wide range of environmental tolerance of *Cyprideis torosa*. This species is known for its ecophenotypic plasticity. A future step in our research will be to consider ecomorphs of this dominant species as distinct entities in the hope of finding a means of interpreting the species diversity of the total ostracod fauna. Besides having generally higher values (Figure 6), the species diversities of the rare species are more readily mappable. Lowest species diversities occur in the south-central part of the lake. These low values are more or less ringed by concentric patterns of increasingly higher species diversity.

## Conclusion

In September, 1861, in a letter to Henry Fawcett, Charles Darwin wrote:

> "About 30 years ago there was much talk that geologists ought only to observe and not to theorise; and I well remember some one saying that at this rate a man might as well go into a gravel-pit and count the pebbles and describe the colours. How odd it is that anyone should not see that all observation must be for or against some view if it is to be of any service!"

It is more than mere tautology to say that what one does depends on what one is doing and that what one is doing determines what one should do. Darwin's lines to Fawcett are important to consider when one is faced with the study of low-diversity samples. When picking Ostracoda from a sample, we unabashedly discard quartz grains, foraminifera and minute shell fragments because the Ostracoda – 300 of them – are the target of the study. Where the goal of a study is to assess quantitatively the similarities and differences among samples of quite low species diversity, one ought to consider assessing whether the dominant species is masking the environmental signal of the rare species. If so, one should discard the dominant species and evaluate rare species, which in the past have been widely regarded as noise in the study of high-diversity samples. It is clear that more work remains to be done to determine under what other circumstances discarding dominant species from analysis of low-diversity assemblages might be prudent.

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# Reproductive strategy of an isopod *Onisocryptus ovalis*, parasitizing a bioluminescent myodocope ostracod *Vargula hilgendorfii*

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*Key words:* parasite, isopod, metamorphosis, functional morphology, Myodocopa

## Abstract

The functional morphology and the reproductive strategy of a parasitic isopod *Onisocryptus ovalis* in a bioluminescent ostracod *Vargula hilgendorfii* as its final host were studied based on video and SEM observations. During its lifetime, *Onisocryptus ovalis* dramatically metamorphoses several times, changing sex from male to female in the final host's carapace. At nearly the last ontogenetical stage, the parasite anchors its body with a pair of thoracopods to the posterodorsal region of the host ostracod's trunk and loses all the other appendages and thus its mobility as well. Thereafter, the parasite reverses bodily orientation during the final moulting so as to locate its mouth in the midst of the host eggs, and finally consumes them, leaving only the egg membrane. Such a mode of feeding of the parasite following the fixation of the body is interpreted in terms of the adaptation to escape elimination from the ostracod carapace by the host's cleaning appendages (the seventh limbs) and to obtain as much space as possible for the parasite's own eggs/embryos at the sacrifice of the mother's mobility. The synchronization between the timing of metamorphosis of the parasite and the reproductive cycle of the host animal can be expected to guarantee the parasite the opportunity to exploit sufficient nutrition from the eggs of the host.

## Introduction

In a series of studies on *Vargula hilgendorfii* (G. W. Müller, 1890), one of the authors (KA) has attempted to understand the species from various viewpoints, including morphometry (Reyment & Abe, 1995), functional morphology (Vannier & Abe, 1993), ecology (Abe et al , 1995), biochemistry of secretions (Abe et al., 1996), circulatory system (Abe & Vannier, 1995) and early evolution of Ostracoda (Vannier & Abe, 1992, 1995).

*Onisocryptus ovalis* was first described by Shiino (1942), then assigned to the present genus by Strömberg (1983). It has been paid little attention, however, by any field of biology except for Vannier & Abe (1993) who described the parasite in the viewpoint of functional morphology of the host's seventh limbs (cleaning appendages). The purpose of this study is to elucidate the reproductive strategy of *Onisocryptus ovalis* based on the description of its life cycle together with its adaptive morphology.

## Materials and methods

Parasites and hosts examined here were collected from Tateyama Bay (139° 51′ E, 34° 59′ N) in the Boso Peninsula, and Sotoura Bay (138° 59′ E, 34° 41′ N) and Nabeta Bay (138° 56′ E, 34° 39′ N) in the Izu Peninsula, central Japan. They were collected most efficiently by a baited trap (Vannier & Abe, 1993; Abe et al., 1995). *Vargula hilgendorfii*, with or without parasites, were transferred to laboratory in aerated sea water, examined under a microscope with a fibre illuminator, counted the number of parasites within the carapace and video recorded (for detailed procedure, see Vannier & Abe, 1993). The nearly transparent carapace of the host animal enabled easy detection and counting of the number of parasites without dissection. Hosts and parasites were processed by critical point drying methods (Vannier & Abe, 1993) or with hexamethyldisilazane (HMDS) (Nation, 1983) for observation by SEM (JEOL JSM-35CFIIA).

† Deceased

## Results

*Life cycle and ontogenetical change in morphology of the parasite*

The life of *Onisocryptus ovalis* after invading the host myodocopid (*Vargula hilgendorfii*) was divided into five major stages on the basis of its general morphology and mode of life. It has been known since the first description of the species (Shiino, 1942) that *Onisocryptus ovalis* is a protandrous (producing first sperm, and then eggs) hermaphroditic animal, as is the usual case of many other parasitic isopods (Sars, 1898). Since neither copulation nor spermary has been observed in our study, nor that of Shiino (1942), it may not be appropriate to refer to a certain developmental stage as 'male' or 'female'. But for convenience we briefly describe, using such terms, what was observed in each developing stage of the parasite.

*Larvae before infection*

Epicaridium and microniscus are the first and the second developing stages of the parasite (Figure 1). Each adult female which had grown up in the host carapace yielded hundreds of offspring (200–1500) at a time. They promptly came out of the host carapace and swam around. We could keep them alive only for several days in a petri-dish. The second stage (microniscan stage) of *Onisocryptus ovalis* has not been observed in nature nor in laboratory. But we could presume the existence of this stage based on the general biology of isopods, and because parasites had the size and shape quite different from the first stage when it was first found as a male stage in the host carapace.

*Male stage* (Figures 2 and 3)

The parasitism commenced at this stage. Usually one or two (max. of five in our study) males were observed in a single host. The hosts were mostly females. These male parasites were very active or walked around restlessly on the surface of the posterodorsal region of the host's trunk, as if searching for the best place to rest. When removed compulsorily by a needle from the host's carapace, they easily reinvaded and seemed to prefer staying at immediately posterior to the heart. The host seemed to attempt removing the invaders using a pair of cleaning appendages (7th limbs) (Vannier & Abe, 1993). The male stage lasted more than 2 weeks in some cases of culture experiment before developing to the next stage. Major dorsal surface ornamentation of this stage was characterized by a transverse wrinkle.

*Transformational stage A* (Figures 4 and 5)

The parasite fixed its body at the surface of dorsal region of the host's trunk with two pairs of thoracopods (1st and 2nd pereiopods) and it probably started, or had already started in the male stage, to suck the 'blood' of the host (Abe & Vannier, 1995). The body was expanded and elongated, as the joint of each segment was slackened. The animal lost the capability of walking around, but the posterior region was observed to move to some extent. All the other appendages were still clearly recognizable, though their function seemed lost.

*Transformational stage B*

All the appendages disappeared except for the thoracopods which served as an anchor onto the host's trunk (Figure 6). At the region where the antennae were observed in the last stage, two small protrusions appeared, which developed longer in the next stage. The whole body was not greatly inflated yet.

*Transformational stage C*

The parasite took four days to two weeks from invasion to this stage. During this stage, even the thoracopods which had functioned as an anchoring apparatus disappeared and the whole body looked just like a sandbag (Figures 7 and 8). It was in this stage that the parasite fed on the whole eggs of the host. The mouth part is shown in Figure 9. The parasite tightly held eggs, one by one, with two protrusions, which became larger posterior to the mouth, and a bifurcate protrusion developed at the forehead. The parasite ate one egg in about 3 min, the egg being gradually reduced in size until only a wrinkled membrane was left. On the surface of the two protrusions, rasp-like microstructures were observed, which could be interpreted to function as a stopper when holding an egg. In such a manner, most of the eggs were eaten during two to three days, resulting in the sandbag-like parasite occupying the entire free inner space of the host carapace. Four to eight days were required before attaining the next stage.

*Female stage* (Figure 10)

The nutrition exploited from the host eggs was used in the last stage (female) for the development of its own eggs. The bag-like body of a female parasite contained

200–1500 eggs at most. The two protrusions of the former stage (transformational stage C or transformation II of Shiino (1942)) were lost and instead two spatula-like structures or 'rabbit ears' structures appeared inside the anterior part of the body (Figure 11). These new structures, probably having originated from the two protrusions of the former stage, moved constantly and rythmically to stir the eggs and/or embryos. The first instars left the parasite body, breaking partly the ventral surface of the mother parasite, about one month and a half after the onset of the male stage, and 4–8 days after its completion of transformation. The instars (epicaridium) left the host carapace immediately after hatching. The host remained alive for several days after offspring of parasites had dispersed.

The female stage seemed to consist merely of the integument and the 'rabbit ears' structures without any other organs (see the last section of 'Results'). In this sense it may be inadequate to refer to this stage as female.

*Method of invasion and the rate of infection*

Parasites attached to the first or the second antennae of the host, which were often protruded from the front gape of carapace. On the withdrawal of the antennae by the host, parasites could enter the host carapace. In other cases, parasites invaded from along the free margin of the ostracod carapace. In whichever manner once they succeeded in invasion, they finally moved to the place posterior to the heart of the host animal.

The rate of hosts being parasitized attained more than 50% in the sample collected from 7 m deep bottom of the Nabeta Bay (July, 1995), while it remained less than 10% in the rest of samples from the shallower (2–3 m) bottom of the three sampling localities. In the samples of May–July, most parasites showed some stage of transformational development. When more than two parasites (max. five in this study) were found in a single host, all of them were males in most cases.

*Preference of parasites for the female host*

Parasites did not seem to obtain any benefit from staying in the carapace of male *Vargula hilgendorfii*. In the laboratory, male parasites found in a male host soon left there and moved to a nearby female host. After shifting to a female host, they did not leave there any more. When compulsorily introduced to a male host by a pipette, a male parasite deserted the host in a few minutes. This behaviour of the males was apparently related to the lower frequency of parasitization in host males in nature.

*Reversal of bodily orientation and feeding on host eggs*

It was for the first time video-observed that a parasite of transformational stage C ate eggs of the host. Before eating, the parasite reversed its bodily orientation during the moulting from the stage B, with the moulted integument of thoracopods remaining in the host and working as a pivot of the body rotation. After the completion of this moulting, the parasite became detached from the host trunk again, but it stayed there because of the lack of walking appendages and the shortage of the space for the inflated body. As a result of the turning around, the mouth part of the parasite was brought to the midst of host eggs. Eggs were eaten one after another continuously until nearly all eggs (8–56) were consumed. Females which ate the more host eggs produced more of their own offspring after about one month (Figure 12) (Horiuchi & Abe, in prep).

*Care of embryos by the parasite before hatching*

At the very last stage, the female parasite showed a bizarre appearance. It looked like a mere bag made of transparent integument, containing numerous eggs. Close observation found a pair of organs of 'rabbit ears' shape in the anterior region inside the female body (Figure 11). They were probably derived from the protrusions which had held the host eggs with a mouth in the previous stage, and in this stage functioned as the stirring/beating eggs apparatus. The two counterparts successively moved and stirred eggs, with one cycle of the motion taking about 10 s.

The hatching of the parasite eggs were also video-recorded and 200–1500 larvae (epicaridium) were observed coming out from the mother and then the female host. After releasing offspring, the female parasite died, but the host continued to live at least for several days. When the integument of female was artificially torn by a needle, the female continued a stirring motion for a while even after most of the eggs had escaped from the tear. No special organ was found which might be responsible to make the 'rabbit ears' beat. The energy source of this motion remained a complete mystery.

*Figures 1–11.*

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*Figures 1–11.* Epicaridium, the first larval stage of *Onisocryptus ovalis*. Scale bar, 10 $\mu$m. Male stage of *O. ovalis*, ventral view. Scale bar, 100 $\mu$m. Male stage of *O. ovalis*, dorsal view. Scale bar, 100 $\mu$m. Transformational stage A of *O. ovalis*. Joint region of each segment is elongated. Scale bar, 100 $\mu$m. Transformational stage A–B. The parasite anchored its body onto the dorsal region of host trunk with pairs of thoracopods (left side). Scale bar, 100 $\mu$m. Transformational stage B. The parasite has lost all the appendages except for pairs of thoracopods used for anchoring the body. Scale bar, 100 $\mu$m. Lateral view of the host (left valve removed) and the transformational stage C of the parasite. Note that the parasite is still connected with the host (but soon to be detached). During the previous moulting the parasite changed its bodily orientation, with the integument of thoracopods remained anchoring on the host. Now the mouth part is on the right. Scale bar, 1000 $\mu$m. Lateral view of the transformational stage C. Mouth part is on the left. Scale bar, 1000 $\mu$m. Mouth part of the transformational stage C. The parasite holds a host egg with two protrusions posterior to the mouth and one bi-furcate protrusion anterior to the mouth. Note that rasp-like microstructures are widely developed on the body surface and inner sides of the two protrusions which are considered functioning to tightly hold the egg. Scale bar, 100 $\mu$m. Female, appearing to be a mere bag full of eggs. Anterior to the left. Scale bar, 1000 $\mu$m. 'Rabbbit ears' structures of the female stage. It may have been derived from the two protrusions of stage C shown in Figure 9. It stirred eggs/embryos by the rate of 10 s for one cycle of the motion. Anterior to the bottom. Scale bar, 100 $\mu$m.

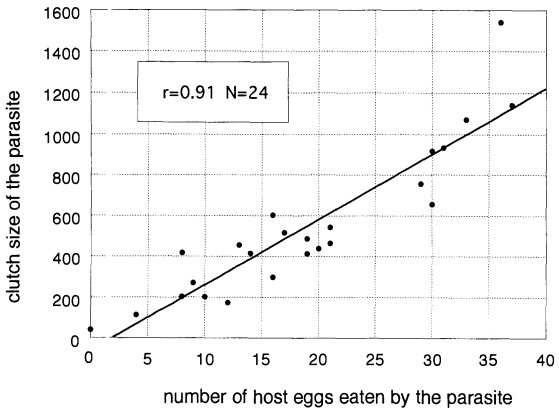


*Figure 12.* Clutch size of the parasite and the number of host eggs eaten by the parasite.

## Discussion

### *Synchronization in the ontogeny of hosts and parasites*

Since *Onisocryptus ovalis* obtains nutrition for its eggs exclusively from the eggs of the host, it is inevitable that it should have synchronized its life cycle to that of the host ostracod. Those parasites which infected earlier had to wait longer until the host laid eggs in the brood pouch. Too early or too delayed maturity of the ovary of the parasite would result in underdevelopment of offspring due to the lack of nutrition. On the other hand, a good timing should bring the parasite much profit, as is shown by the fact that the parasite which ate the more host eggs laid the more abundant epicaridium larvae (Figure 12).

In our study, parasite females with matured eggs were all found in host females which were usually carrying no, or only a small number of, eggs in the brood pouch, probably because the rest of eggs had been eaten by the parasite. This means that the parasites usually attained a mature female stage only when they fed on the host eggs (see Shiino, 1942). It is unknown, however, how the parasite knows the timing of maturity of host eggs.

The rate of infection may depend on the sex ratio of the host animal, because the parasites select the female host for the source of nutrition in eggs, but the annual change of the host sex ratio observed in the field (Yamada & Abe, in prep.) does not explain it well.

### *Functional morphology of the host and the parasite*

Recent shape analysis focusing upon the ontogenetical change (Abe et al., in press) revealed that the carapace of adult females of *Vargula hilgendorfii* become slightly inflated in the dorso-posterior region in contrast to that in males. This sexual dimorphism is best interpreted in terms of the functional morphology; female with an inflated carapace (marsupium) may be advantageous for bearing more and/or larger eggs and embryos at a time. The larger space for its own eggs and embryos, however, inevitably results in providing the parasitic isopod with more room for its offspring.

The drastic changes of the general morphology (transformation) of the parasitic isopod *O. ovalis*, including the loss of thoracic and abdominal appendages (no more need to walk), a sandbag-like structure in the last stage (capacity for eggs), appearance and microstructure on the surface of the new protrusions near the mouth (for steady holding of an egg) and 'rabbit ears' structures first found in the sandbag females (embryos need being stirred) are all explainable well with words of functional morphology as well.

*Reversal of bodily orientation and feeding on host eggs*

It was unknown whether or not the organism still defecated in the transformational stage C, and if it did, from where it excreted its wastes. But as a result of turning its body axis, the parasite obtained as much nutrition as possible at the cost of the loss of appendages and thus mobility in the limited space within the carapace of the host ostracod. We should, however, also seek a good explanation to the question of why the parasite does not fix its body reversely (mouth down) from the beginning of infection. Two reasons seem worthy to consider; 1. the region immediately posterior to the heart where the parasite anchors by thoracopods may be the best place to suck the blood (Abe & Vannier, 1995), 2. the active reciprocating motion of the host furcae, which is quite often observed, may prevent the parasite from anchoring its body at the posterior-end region (shaking off effect).

*Host specificity of the parasite-host system*

Field observations (rate of parasite-bearing host) and laboratory experiments (compulsory introduction of a parasite into a male host carapace) distinctly showed that *Onisocryptus ovalis* was capable to detect the sex of *Vargula hilgendorfii*. However, by what information (physical feature? chemical stimulus?) *Onisocryptus ovalis* knows the sex of host animals remains unknown. Among possible cues may be the sexually differentiated feature of the shape in the posterior edge of soft body. Dorsal muscles bands (see Cannon, 1940) are fewer but much more swollen in males than females, in probable correlation with the general enhanced activity of males (Vannier & Abe, 1993).

Comparison with non-luminous closely-related species may lead to better understanding of the host-parasite interaction. Several undescribed cypridinid species recently found in Shimoda have been provisionally surveyed and found to be seldom parasitized. In this sense, *O. ovalis* seems a host-specific parasite, but in the laboratory experiments (densely populated non-luminous species+several *O. ovalis* removed from *V. hilgendorfii*) a few of them infected the non-luminous species, too. Because the detailed ecology of this non-luminous species has not been clarified, it is unknown why *O. ovalis* show the host-specific infection in nature.

*V. hilgendorfii* attempts to remove the parasite using a pair of seventh limbs before being anchored, but there seems little promise of success. The reproductive strategy of *O. ovalis* may have also been responsible for the sexual dimorphism in host seventh limbs (more flexible in females) (Vannier & Abe, 1993), but the parasite always seems to evade the hosts efforts.

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