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Molecular Phylogenetics and Evolution 31 (2004) 4–15
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

A preliminary molecular and morphological phylogeny of the Antarctic Epimeriidae and Iphimediidae (Crustacea, Amphipoda)

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Received 18 October 2002; revised 6 July 2003

Abstract

The phylogenetic relationships of 14 species of the Antarctic amphipod families Epimeriidae and Iphimediidae were investigated using 553 bp of the gene for the mitochondrial cytochrome oxidase subunit I (COI) and 98 morphological characters. Both families are dominant members of the Antarctic benthic amphipod community. In contrast to previous studies, our molecular and morphological data suggest that the families Epimeriidae and Iphimediidae may not be sister taxa. Our study suggests that Iphimediidae are more closely related to *Eusirus* (Eusiridae) than to *Epimeria* (Epimeriidae). Phylogenetic analyses based on maximum parsimony (MP) and maximum likelihood (ML) indicate that the genera *Iphimediella* and *Gnathiphimedia* are not monophyletic.

Keywords: Antarctica; Benthic amphipods; Cytochrome oxidase I; Cladistics; Eusiridae; Epimeriidae; Iphimediidae

1. Introduction

The families Epimeriidae and Iphimediidae are dominant members of the Antarctic benthic amphipod community (Coleman, 1996; De Broyer et al., 2001). Both families occur worldwide although their main occurrence is in polar waters. Currently 25 species in six genera of Epimeriidae are known from the Southern Ocean, 17 of them from the genus *Epimeria*. The Iphimediidae consist of 48 species belonging to 13 genera. Both families are found throughout the Antarctic. Epimeriidae as well as Iphimediidae belong to the superfamily Iphimedoidea, also including, e.g., Dikwidae, Ochlesidae, Odiidae, and Acanthonotozomellidae.

Very little is known about the evolution and phylogeny of these two families. Watling and Thurston (1989) considered the Epimeriidae (former Paramphithoidae Stebbing 1906) as the sister taxon to the Iphimediidae, but the cladistic biogeography of Antarctic Iphimediidae was based on only six morphological characters. In addition the relatively small phylogenetic analysis, which was carried out before programs such as PAUP were readily available, has proved to be a powerful tool for biogeography. These authors suggested that the retraction of species from a former cosmopolitan distribution occurred before the thermal isolation of Antarctica.

This present study, presents the first molecular analysis of phylogeny of a subset of Antarctic Epimeriidae and Iphimediidae. It is not intended to represent a complete phylogeny of the two families because the number of species from previous expeditions was limited due to formaldehyde fixation of specimens. The Epimeriidae are represented by six species of *Epimeria*, while the Iphimediidae genera *Echiniphimedia*, *Gnathiphimedia*, and *Iphimediella* are represented by a total of eight species. The phylogenetic analysis presented here used morphological and mtDNA evidence, testing them for congruence.

For molecular study a mitochondrial DNA region was chosen to provide resolution at the intergeneric level. Among the mitochondrial genes investigated in Crustacea, the cytochrome oxidase I subunit (COI) gene has proved to be a very useful taxonomic and phylogenetic marker at the intergeneric level (e.g., Meyran et al., 1997; Wares, 2001).

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doi:10.1016/j.ympev.2003.07.019

The molecular study is compared with a phylogenetic approach based on morphological characters.

2. Material and methods

Amphipods were collected during the cruise ANT XVII-3 by the RV "Polarstern" (Arntz and Brey, 2001). The animals were hand-sorted from towed gear (bottom trawl and Rauschert dredge). In order to minimize degradation of DNA, live animals were briefly rinsed with pre-chilled freshwater and preserved in 96% ethanol at −30 °C (following Held, 2000). Muscle tissue of the first pleopods was isolated while keeping the animals on ice. The tissue was kept refrigerated in 96% ethanol until DNA extraction took place. Species names, sampling locality and depth, as well as accession numbers and collection numbers in the Zoological Institute and Zoological Museum Hamburg are listed in Appendix A.

Eusirus cf. *perdentatus* (Eusiridae) and *Monoculodes* sp. (Oedicerotidae) were chosen as outgroups for the analysis of morphological characters. While *Eusirus* is considered to be closely related to Epimeriidae and Iphimediidae (Englisch, 2001), the Oedicerotidae are believed to be distantly related to Epimeriidae or Iphimediidae (Berge et al., 2001). Specimens of the morphological outgroup species were collected on the same cruise and treated the same way as the epimeriid and iphimediid specimens.

For molecular analyses, five additional outgroup sequences were obtained from GenBank. The genera *Scopelocheirus* and *Hirondellea* belong to the family Lysianassidae whereas *Pontogammarus*, *Euxinia*, and *Obesogammarus* are members of the family Gammaridae.

Two morphological and five molecular outgroup taxa were chosen because multiple outgroup taxa can increase resolution and support for basal ingroup nodes (Maddison et al., 1984).

2.1. DNA amplification and sequencing

Genomic DNA was isolated using the method of Held (2000) from small pieces of muscle tissue using a QIAamp DNA Mini Kit. PCRs were carried out in 50-μl volumes; with 2 U Qiagen *Taq* polymerase, 5 μl 10 × PCR buffer including 1.5 mM $MgCl_2$, 250 μM of each dNTP, 60 pmol of each amplification primer, and 0.5–1.2 μl DNA template. Sterile water was added to a total of 50 μl. The COI fragment was amplified using primer sequences developed by Folmer et al. (1994). For amplification modified versions of the primers carrying a sequence tag on their 5′ tails were used (Held, 2003): HCO 5′-AGC GGA TAA CAA TTT CAC ACA GGT AAA CTT CAG GGT GAC CAA AAA ATC-3′ and LCO 5′-CCC AGT CAC GAC GTT GTA AAA CGG TCA ACA AAT CAT AAA GAT ATT GG-3′, both primers were provided by MWG-Biotech GmBH. The amplification profile was 3 min at 94 °C for denaturation, 36 cycles of 1 min at 94 °C, 1 min at 42 °C, 1.5 min at 72 °C, and last 7 min at 72 °C for final extension.

PCR products were purified with Qiagen spin columns (PCR purification kit) and run on an 1% ethidium bromide stained agarose minigel to evaluate purity and DNA content. Purified PCR product (1–3 μl) was used for dideoxy cycle sequencing using the manufacturer's protocols (Amersham and Biozym). The sequencing amplification protocol was 94 °C for 2 min, 30 cycles of 94 °C for 25 s, 52 °C for 25 s, and 70 °C for 35 s and stored at 4 °C.

For sequencing the COI amplification products the fluorescent labelled primers PFS: 5′-CCC AGT CAC GAC GTT GTA AAA C-3′ and PRS: 5′-AGC GGA TAA CAA TTT CAC ACA GG-3′ were used. Depending on the concentration of the COI amplification products 0.5–3 μl of the cycle sequencing reaction was loaded onto an automated sequencer (Li-Cor, models 4000 and 4200).

Gels were proofread using the image analysis software of the automated sequencer. Double stranded sequences were assembled with AlignIR v1.2.

2.2. Phylogenetic analysis

The proof-read sequences of the 16 species were aligned with Clustal W version 1.4 (Thompson et al., 1994) as included in BioEdit (Hall, 1999) using default parameters. The alignment was truncated to avoid excessive gaps at either end of the alignment. Minor corrections of the alignment were carried out in order to preserve a contiguous reading frame. One indel of serine occurred in five species of Iphimediidae which will be discussed below.

After exclusion of uninformative positions (Cunningham, 1997), an incongruence length difference test as implemented in PAUP was performed in order to test the combinability of different codon positions (codon positions 1 and 2 versus position 3). This test was repeated using different weighting schemes for transition/transversion substitutions.

Phylogenetic trees under the maximum parsimony (MP) optimality criterion were inferred using PAUP 4.10 beta (Swofford, 2002). χ^2 tests of homogeneity of base frequencies were also calculated in PAUP. The effect of different weighting schemes of substitution types and codon positions on the inferred tree topology was tested. Bootstrap tests with 1000 replicates were used to assess support of various phylogenetic groups.

Trees under the maximum likelihood (ML) optimality criterion were calculated using Paup 4b10, MrBayes 3.0 (Huelsenbeck and Ronquist, 2001) and for protein data also Tree-Puzzle 5.0 (Strimmer and von Haeseler,

1996). Models of sequence change over time were chosen based on a hierarchical likelihood ratio test (LRT) (Huelsenbeck and Crandall, 1997) as implemented in Modeltest version 3.06 (Posada and Crandall, 1998). This model was then used to calculate pairwise genetic distances and the ML tree.

Bayesian inference of phylogeny was carried out running four parallel chains in MrBayes3.0 for 100,000 generations, sampling trees every 100 generations. Six substitution types were allowed corresponding to the GTR model. Site specific rates were used, unlinking rate estimation of the third codon position from the rate estimate for the first two codon positions. The log likelihood reached stationarity after 5000 generations thus 50 trees were discarded as the "burnin."

For maximum likelihood analysis of protein data, the model proposed for mitochondrial genes proposed by Adachi and Hasegawa (1996) was used with gamma distributed rates.

All morphological characters coded in the matrix were examined on several individuals of each species deposited in the Zoological Museum Hamburg and through descriptions in the literature. One specimen of *Iphimediella georgei* Watling and Holman, 1980 was borrowed from the Museum für Naturkunde in Berlin, Germany. A database of 98 morphological characters was assembled using the software DELTA (Dallwitz et al., 1997). We primarily used binary rather than multistate characters (Appendix B). A data matrix (nexus file) was generated for input in PAUP 4.10 beta. All characters were unordered and treated as having equal weight. The list of characters is presented in Appendix B, the matrix is shown in Appendix C.

3. Results

3.1. Analysis of the nucleotide sequences

Among the remaining 553 aligned nucleotide sites in the mitochondrial COI gene fragment, 302 are variable of which 274 bases are parsimony-informative including the outgroup species (284 and 257 bp for the ingroup, respectively). As expected the majority of variable sites occurred in the third codon position (171 out of 274 bp).

When all taxa were included, their base composition was found to be significantly different ($p = 0.01893$). There was insufficient evidence for base composition differences, however, when the most divergent sequence (*Monoculodes*) was excluded ($p = 0.2005$).

A partition-homogeneity test revealed significant differences between first and second versus third codon positions ($p = 0.02$), whereas no significant difference was found between first and second codon positions ($p = 0.98$). This difference dissapeared when only ingroup sequences were compared ($p = 0.62$). For this reason all third codon positions were re-coded as missing information in the outgroup whereas they were retained for the ingroup. The rooting of the tree was thus based on the more conserved first two codon positions while the third codon position still contributes to the relation within the ingroup species (Whiting, 2002).

A heuristic search found a single most parsimonious tree when transitions and transversions are weighted equally (length 959 steps, CI = 0.5193, RI = 0.6174, RC = 0.3206).

Applying different weighting schemes for codon positions and substitution types mostly affected the branching pattern in the outgroup which is outside the scope of this paper.

Some points regarding the ingroup relationships are worth mentioning:

A sistergroup relationship between *E. hodgsoni* and *E. echinata* is parismonious only when substitution types and codon positions are weighted equally (ti = tv, codon weighting 111). When either transitions or third codon positions are downweighted, a sistergroup relation between *E. hodgsoni* and *E. waegeli* is favoured (see Figs. 1 and 2).

Similarly, the resolution between *Epimeria reoproi*, *Epimeria similis*, and *Epimeria macrodonta* is ambiguous when equal weighting is applied. When transitions or third codon positions are downweighted, a sistergroup relationship between *E. reoproi* and *E. macrodonta* becomes more parsimonious.

Equal weighting results in ambiguous support concerning the relationship of *Iphimediella cyclogena* and the two *Gnathiphimedia* species. Downweighting transitions and third codon positions consistently groups them as in Fig. 4. Taking into account the high variability of the third codon position, the nodes that can only be obtained with equal weighting of substitution types are considered unreliable and therefore a tree based on a weighted analysis is preferred. Trees based on various weighting combinations can be made available by the second author.

The LRT revealed the TvM model with gamma distributed rates (alpha = 0.8611) and invariant sites (pinvar = 0.4175) as the model with the best fit to the data. The ML tree is identical to the MP tree inside the Epimeriidae and Iphimediidae and with only insignificant variation in the placement among some outgroup sequences (Shimodaira–Hasegawa test, $p > 0.20$).

3.2. Analysis of the amino acid sequences

No conflicting signal can be identified between the protein and DNA data partitions under standard maximum parsimony (partition-homogeneity test, $p = 0.93$). Of 185 amino acids only 56 were parsimony-informative (tree length = 159, CI = 0.824, RI = 0.885, RC = 0.729). The ML tree based on the Adachi and Hasegawa (1996)

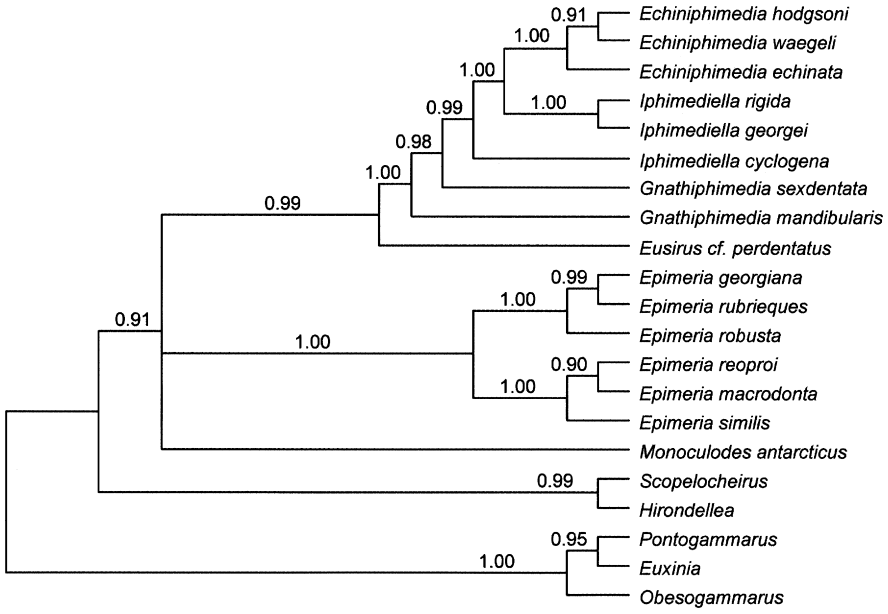

Fig. 1. Bayesian inference of phylogenetic relations based on 553 bp of the COI gene. Bayesian support values are indicated on the branches. Six substitution types with gamma distributes rates and rate estimates for the third codon position unlinked from the first two codon positions.

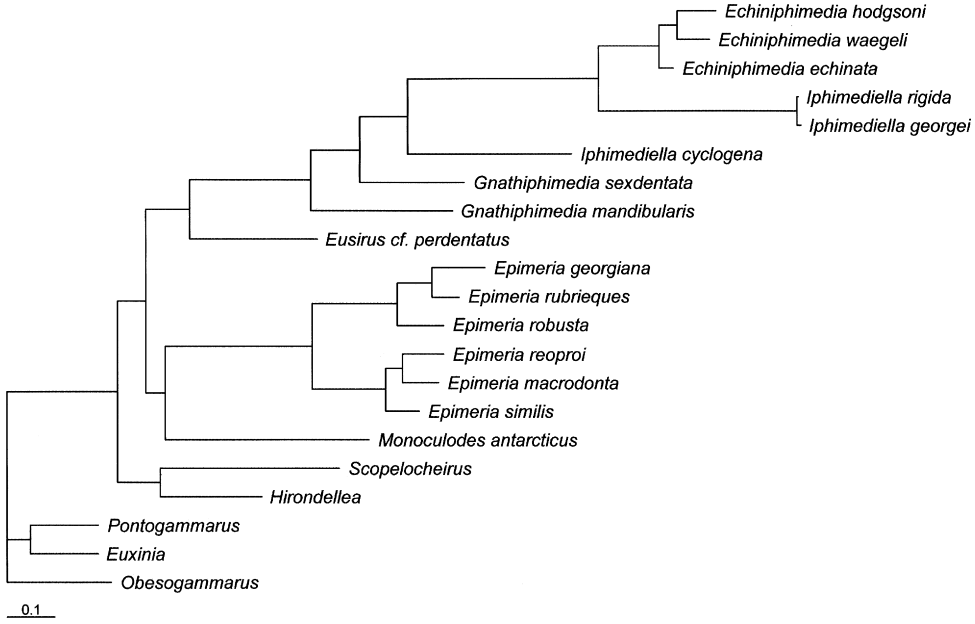

Fig. 2. Maximum likelihood (ML) phylogram based on 553 nucleotides from the COI gene. Model choice based on a hierarchical LRT (six substitution types with gamma distributed rates (alpha = 0.8611) and invariant positions (pinvar = 0.4175). A heuristic search with random addition of taxa (5 replicates each) and TBR branch swapping was conducted.

model for mitochondrially encoded genes is less well resolved than the ML tree based on nucleotide data of the same gene fragment with the subtrees for Iphimediidae and Epimeriidae being compatible with the tree inferred from DNA data (Fig. 3). Conflicting resolution between the ML trees based on DNA and protein data is confined to the outgroup (SH test, $p < 0.05$).

3.3. Analysis of the morphological characters

The tree for the morphological characters was rooted with *Monoculodes* based on its position in the molecular tree. The branch-and-bound search using unweighted characters resulted in one tree (tree length = 205, CI = 0.532, RI = 0.713, RC = 0.379), Fig. 5. Of 98

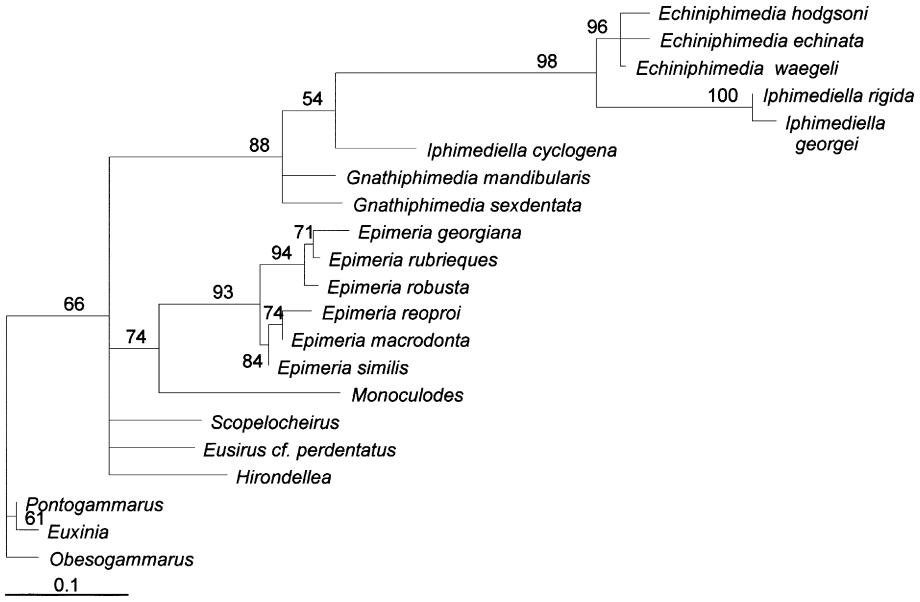

Fig. 3. An ML estimate of the phylogeny of 14 species of Antarctic Epimeriidae and Iphimediidae based on 185 aminoacids. The mtREV24 model for mitochondrially encoded genes was used with gamma distributed rates (Adachi and Hasegawa, 1996). Shape parameter estimated from the dataset (alpha = 0.27).

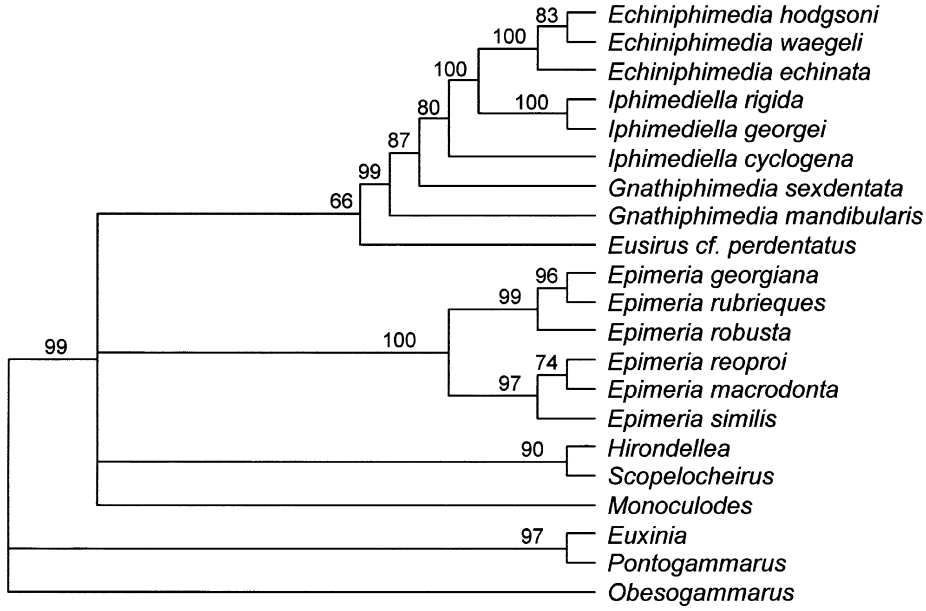

Fig. 4. Maximum parsimony (MP) 50% majority rule consensus tree. Numbers on branches are bootstrap values of 1000 replicates (higher than 50% shown). Third codon positions are downweighted by factor 3 and transversions are weighted 3 times over transitions. Third codon positions for the outgroup species and gaps are treated as missing information (see text for details).

unordered characters 9 are constant and 6 are parsimony-uninformative, 83 characters are parsimony-informative.

The tree based on morphological characters differs significantly from those based on the COI fragment (SH test, $p < 0.05$).

There is little doubt that *Gnathiphimedia* is paraphyletic with the two included representatives branching off sequentially at the base of the Iphimediidae subtree.

In no analysis the two families Iphimediidae and Epimeriidae are sistergroups. Instead, at least one of the outgroup sequences (*Eusirus* cf. *perdentatus*)

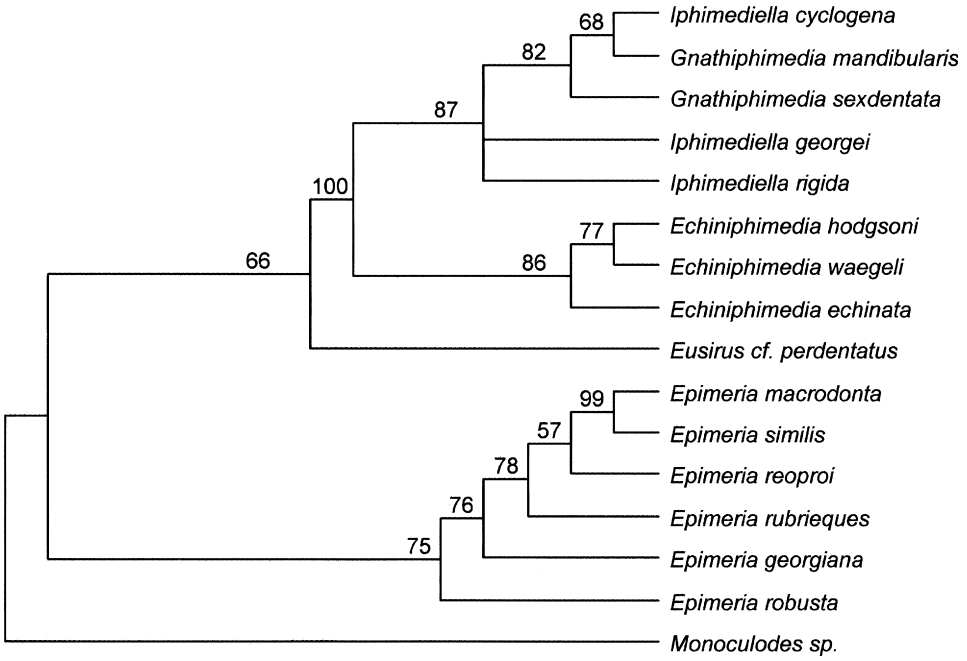

Fig. 5. Phylogenetic tree of 16 Antarctic Amphipoda based on 83 phylogenetic informative morphological characters, bootstrap values of 1000 replicates higher than 50% shown. For five species taken from GenBank no morphological information was available.

clustered consistently as sister to the Iphimediidae, in some analyses *Monoculodes* was sister to the Epimeriidae. Our analysis therefore provides no evidence of Epimeriidae and Iphimediidae being sister taxa as stated by Watling and Thurston (1989).

4. Discussion

Although only a relative small number of taxa were used in this study, our results show that analysis of the COI sequence is suitable for revealing differences at the interspecific level and family level for two Antarctic amphipod families. The higher classification of iphimedioid amphipods has frequently been revised in recent years (Berge et al., 1998; Coleman and Barnard, 1991; Watling and Thurston, 1989). The magnitude of the genetic differences observed between species of *Epimeria* and between species of Iphimediidae is not correlated with spatial differentiation. According to the zoogeographical zonation of the Southern Ocean (De Broyer and Jazdzewski, 1993), the outgroup taxa used in this analysis (*Eusirus* cf. *perdentatus*, *Monoculodes* sp.) and *Epimeria georgiana* and *E. reoproi* are from West Antarctica, while all the other species are from the East Antarctic. Since the two West Antarctic species showed the highest nucleotide divergence within the species of *Epimeria*, the geographic distance apparently does not influence the genetic differentiation.

All analyses indicated the monophyly of *Epimeria* and the Iphimediidae included in this study, supported by a bootstrap values of over 90 at the basal branch. There is a strongly supported monophylum consisting of all *Echiniphimedia* species and two of the three *Iphimediella* species. This clade lacks three nucleotides coding for the aminoacid serine that are uniformly present in all other species in this study including all outgroup sequences. The absence of this serine is therefore most likely a deletion which occurred in the most recent ancestor of *Echiniphimedia* and *Iphimediella rigida* and *I. georgei*. The genus *Iphimediella* in its current state is therefore clearly paraphyletic as *Iphimediella cyclogena* lacks this apomorphic deletion.

One of our aims was to gain an independent assessment of morphological and molecular characteristics that are thought to be of phylogenetic importance. Our molecular and morphological analyses result in phylogenies of the tested species that provide some new insights into character evolution that partly contradict previous interpretations (e.g., Watling and Thurston, 1989). Some characters and difficulties are discussed in more detail below.

Coleman and Barnard (1991) defined two characters for differentiation between the families Epimeriidae and Iphimediidae; the Iphimediidae do not have raker spines, but possess at least one pair of chelate gnathopods. Upon examination of 14 species of these families only certain characters turned out to be restricted to the family Iphimediidae or the genus *Epimeria*. Only *Epimeria* bear spines on the inner curvature of the dactyli of their gnathopods. The examined iphimediids as well as *Eusirus* cf. *perdentatus* have pointed posteroventral corners of

pereonite 5 and 6 while those of the examined *Epimeria* are rounded. The posteroventral corner of pereopod 7 is also pointed in *Epimeria*, except in *E. robusta*.

Referring to the compared mitochondrial sequences *Epimeria georgiana*, *E. rubrieques*, and *E. robusta* form a monophyletic clade. In contrast these taxa seem to be paraphyletic when comparing the morphological characters. In the present analysis all morphological characters have the same weight. Of the six *Epimeria* species studied only *E. georgiana*, *E. rubrieques*, and *E. robusta* have produced and pointed posteroventral angles on the basis of pereopods 5–7 (characters 55 and 56, see Appendix B). Most likely this morphological feature proves to be phylogenetically more informative than others tested in this study.

The monophyly of *Iphimediella* is questionable because characters such as the incisor show both toothed (*I. georgiana*, *I. rigida*) and smooth states (*I. cyclogena*). *I. cyclogena*, which bears a smooth incisor, clades with the genus *Gnathiphimedia*, which also bears a smooth incisor. In addition *Gnathiphimedia* and *Iphimediella* both have paired teeth on pereonite 7.

One main character used in species keys of *Epimeria* is the presence of dorsal carinae on the pereon (e.g., Wakabara and Serejo, 1999). This obvious character is not supported by our molecular analyses, since *E. robusta* with all pereon segments lacking dorsal carinae, appears to be closely related to *E. rubrieques* (Fig. 1), a species with carinae on all pereon segments. A morphological character shared by *E. robusta* and *E. georgiana* is the sharply notched basis of pereopod 5.

In view of the size of the molecular dataset the differences between the trees inferred from molecular and morphological data should not be overinterpreted.

4.1. Speciaton times

Wares (2001) estimated the substitution rate of the same region of COI for Cirripedia 3.1 percent divergence per million years under the general time reversible model. The two monophyletic groups within the genus *Epimeria* are separated by a mean genetic distance of 0.4891. When using the cirriped rate of substitution in this gene fragement the last common ancestor of the *Epimeria* species in this study can be estimated to have lived approximately 15.7 million years ago. Since the cooling of Antarctica took place about 40 million years ago (Crame, 1999) the divergence between *Epimerias* occurred after the cooling of the Southern Ocean. Even when the most conservative rate estimate for the corresponding fragment of the COI gene is applied (Knowlton and Weigt, 1998), the estimated age of the most recent common ancestor for the *Epimeria* spp. increases to 34.9 milllion years.

Similarly, all iphimediid species which are related through the supposedly oldest node in Fig. 2 are separated by an average distance around 1.0043 under the TvM model from *Gnathiphimedia mandibularis*. The inferred age of the last common ancestor of the iphimediid species is thus 34.4 million years using the cirriped rate, only when the snapping shrimp rate is applied this estimate increases to 71.7 million years.

The timeline of speciation as well as the endemicity to Antarctic waters are consistent with the view that the epimeriid and potentially also the iphimediid species in this study evolved in the Southern Ocean when it was already isolated from other fragments of Gondwanaland and cold.

Acknowledgments

Thanks are due to A. Brandt, J.-W. Wägele, K. Linse, and S. Gerken for helpful advice. During the cruise ANT XVII-3 following colleagues especially helped sorting the material: O. Ziemer, M. Schrödl, J. Bohn, and M. Rauschert. C.O. Coleman checked the determination of the amphipod species and kindly lent us specimens from the collection of the Museum für Naturkunde in Berlin. B. Hackethal is thanked for her technical assistance. Thanks are also due to U. Englisch for helpful discussions. This article is based on a doctoral study by Anne-Nina Lörz in the faculty of Biology, University of Hamburg, supported by the Studienstiftung des deutschen Volkes and by a grant to A. Brandt from the Deutsche Forschungsgemeinschaft (Br 1121/18-1).

Appendix A

Species, availability of sequences, collection localities, and specimen-deposition number of the Zoological Institute and Zoological Museum Hamburg (ZIM) and five outgroups (Lysianassidae and Gammaridae) from the GenBank

Taxa	Sequence Accession No.	Depth (m)	Latitude	Longitude	ZIM collection number
Epimeriidae					
Epimeria georgiana Schellenberg, 1931	AF451341	202	62°49.50′ S	060°49.30′ W	39888
Epimeria reoproi Lörz and Coleman, 2001	AF451342	48	63°00.10′ S	060°31.00′ W	39876

Appendix A (*continued*)

Taxa	Sequence Accession No.	Depth (m)	Latitude	Longitude	ZIM collection number
Epimeria robusta K.H. Barnard, 1930	AF451344	323	71°11.90′ S	012°21.70′ W	39902
Epimeria macrodonta Walker, 1906	AF451343	316	71°11.90′ S	012°20.70′ W	39889
Epimeria rubrieques De Broyer and Klages, 1991	AF451345	648	71°16.67′ S	013°45.79′ W	39890
Epimeria similis Chevreux, 1912	AF451346	648	71°16.67′ S	013°45.79′ W	39891
Iphimediidae					
Iphimediella georgei Watling and Holman, 1980	AF451349	316	71°11.90′ S	012°20.70′ W	39892
Iphimediella rigida K.H. Barnard, 1930	AF451347	323	71°11.90′ S	012°21.70′ W	39893
Iphimediella cyclogena K.H. Barnard, 1930	AF451348	323	71°11.90′ S	012°21.70′ W	39894
Eciniphimedia echinata Walker, 1906	AF451352	266	70°50.40′ S	010°35.20′ W	39895
Echiniphimedia hodgsoni Walker, 1906	AF451350	323	71°11.90′ S	012°21.70′ W	39896
Echiniphimedia waegeli Coleman and Andres, 1988	AF451351	266	70°50.40′ S	010°35.20′ W	39897
Gnathiphimedia mandibularis K.H. Barnard, 1930	AF451353	269	70°50.20′ S	010°34.89′ W	39898
Gnathiphimedia sexdentata (Schellenberg, 1926)	AF451354	318	71°12.19′ S	012°19.01′ W	39899
Eusiridae					
Eusirus cf. perdentatus Chevreux, 1912	AF451355	673	63°01.20′ S	059°09.20′ W	39900
Oedicerotidae					
Monoculodes sp.	AF451356	48	63°00.10′s	060°31.00′ W	39901
Lysianassidae					
Scopelocheirus schellenbergi	AY256968				
Hirondellea dubia	AY183359				
Gammaridae					
Pontogammarus robustoides	AY189523				
Euxinia maeoticus	AY189504				
Obesogammarus crassus	AY189482				

Appendix B

Character list for the morphological analysis of 16 Antarctic Amphipoda

1. Telson apically: (1) rounded; (2) pointed
2. Telson, setae on lobe: (1) absent; (2) present
3. Telson excavation: (1) wide, shallow or absent; (2) narrow
4. Telson: (1) entire or cleft u-shaped; (2) cleft v-shaped
5. Telson elongation: (1) absent; (2) present (clearly longer than broad)
6. Uropod 3 outer ramus: (1) at least twice the length of peduncle; (2) less than twice the length of peduncle
7. Uropod 3 pointed process on apical margin: (1) absent; (2) present

Appendix B (*continued*)

8. Uropod 2 outer ramus: (1) same length or longer than peduncle; (2) shorter than peduncle
9. Uropod 1outer ramus: (1) same length or longer than peduncle; (2) shorter than peduncle
10. Urosomit 1 dorsally: (1) smooth; (2) small projection; (3) long pointed projection; (4) multidentate carinae
11. Urosomite 1: (1) longer than urosomites 2 and 3 comined; (2) shorter than urosomites 2 and 3 combined
12. Urosomites 2 and 3 dorsally: (1) smooth; (2) articulated 13. Urosomite 1 posterolateral margin pointed process: (1) absent; (2) present
14. Urosomite 2 posterolateral margin pointed process: (1) absent; (2) present
15. Urosomite 3 posterolateral margin pointed process: (1) absent; (2) present
16. Urosomite 1 middorsal keel: (1) absent; (2) present
17. Urosomite 2 middorsal keel: (1) absent; (2) present
18. Urosomite 3 middorsal keel: (1) absent; (2) present
19. Pleon spinose cuticula: (1) absent; (2) present
20. Epimeral plate 3 posteroventral corner: (1) not produced; (2) slightly produced; (3) strongly produced and pointed
21. Epimeral plate 2 posteroventral corner: (1) not produced; (2) slightly produced; (3) strongly produced and pointed
22. Epimeral plate 1 posteroventral corner: (1) not produced; (2) slightly produced; (3) strongly produced
23. Epimeral plates 1–3 midlaterally: (1) not produced; (2) strongly produced
24. Epimeral plate 3 posterolateral margin: (1) not produced; (2) slightly produced; (3) strongly produced and pointed
25. Epimeral plate 2 posterolateral margin: (1) not produced; (2) produced
26. Epimeral plate 1 posterolateral margin: (1) not produced; (2) produced
27. Epimeral plates 1, 2, and 3 middorsally: (1) projection absent; (2) small projection; (3) long pointed projection
28. Epimeral plate 3 paired teeth on dorsal amature: (1) absent; (2) present
29. Epimeral plates 1 and 2 paired teeth on dorsal amature: (1) absent; (2) present
30. Epimeral plate 1 carinae: (1) absent; (2) present
31. Epimeral plates 2 and 3 carinae: (1) absent; (2) present
32. Pereon 1 carina: (1) absent; (2) small; (3) long and pointed
33. Pereon 2 carina: (1) absent; (2) small; (3) long and pointed
34. Pereon 3 carina: (1) absent; (2) small; (3) long and pointed
35. Pereon 4 carina: (1) absent; (2) small; (3) long and pointed
36. Pereon 5–7 carina: (1) absent; (2) small; (3) long and pointed
37. Pereon 1 dominant midlateral protrusion: (1) absent; (2) present
38. Pereon 3 and 4 dominant midlateral protrusion: (1) absent; (2) present
39. Pereon 5–7 dominant midlateral protrusion: (1) absent; (2) small; (3) long and pointed
40. Pereonite 6 spines on posterolateral margin: (1) absent; (2) present
41. Pereonite 7 spines on posterolateral margin: (1) absent; (2) present
42. Pereonites 1–4 posteroventral corner: (1) rounded; (2) pointed
43. Pereonite 5 posteroventral corner: (1) rounded; (2) pointed
44. Pereonite 6 posteroventral corner: (1) rounded; (2) pointed
45. Pereonite 7 posteroventral corner: (1) rounded; (2) pointed
46. Pereonite 2: (1) shorter than pereonite 1; (2) same length or longer than pereonite 1
47. Pereonite 7 paired teeth: (1) absent; (2) present
48. Coxal plate 1–3 dorsoventral ridge on lateral surface: (1) absent; (2) present
49. Coxa 4 dorsoventral ridge on lateral surface: (1) absent; (2) present
50. Coxal plates 5 and 6 anteriodorsal ridge on lateral surface: (1) absent; (2) present
51. Coxa 7 aterioposterior ridge on lateral surface: (1) absent; (2) present
52. Coxa 5 posteroventral angle: (1) rounded; (2) pointed not produced; (3) produced and pointed
53. Coxa 6 posteroventral angle: (1) rounded; (2) pointed not produced; (3) produced and pointed
54. Coxa 7 posteroventral angle: (1) rounded; (2) pointed
55. Basis 5 posteroventral angle: (1) rounded; (2) pointed not produced; (3) pointed and produced
56. Basis 6 and 7 posteroventral angles: (1) rounded; (2) pointed not produced; (3) produced and pointed
57. Basis 5–7 posterior margin: (1) smooth; (2) sinous
58. Coxa 5 winglike acute process: (1) absent; (2) present
59. Coxal plates 1–3 lateral face: (1) smooth; (2) acute teeth present

Appendix B (*continued*)

60. Coxa 4 laterally: (1) smooth; (2) acute teeth present
61. Coxa 5 and 6 lateral face: (1) smooth; (2) with acute teeth
62. Coxa 7 laterally: (1) smooth; (2) with acute teeth
63. Coxa 4 anteroventrally: (1) not produced; (2) produced
64. Coxa 4 margin midventrally: (1) rounded; (2) pointed
65. Coxa 4 posteroventral margin: (1) concav; (2) straight or convex
66. Coxa 4 posterolateral corner: (1) rounded; (2) pointed
67. Rostrum: (1) shorter than first article of Antenna 1; (2) at least reaching distal margin of first article of Antenna 1
68. Rostrum shape: (1) straight; (2) flexed
69. Antenna 1 peduncle article 1 number of processes: (1) 0; (2) 1; (3) 2; (4) 3; (5) 4; (6) 5
70. Antenna 1 peduncle article 2 number of processes: (1) 0; (2) 1; (3) 2; (4) 3; (5) 4
71. Antenna 2 peduncle article 3 number of processes: (1) 0 or 1; (2) 2 or more
72. Antenna 2 peduncle article 4 number of processes: (1) 0 or 1; (2) at least 2
73. Antenna 2 peduncle article 5 number of processes: (1) 0 or 1; (2) at least 2
74. Labrum: (1) entire; (2) incised
75. Mandible molar: (1) absent or reduced; (2) well developed
76. Mandibular rakers: (1) absent; (2) present
77. Mandibular body: (1) bulky; (2) elongate
78. Mandible incisor: (1) smooth; (2) toothed
79. Maxilla 1 palp: (1) two articulate; (2) three articulate
80. Maxilla 1 palp short robust setae: (1) absent; (2) present
81. Maxilla 1 palp long setae: (1) absent; (2) present
82. Maxilliped palp article 2 distally: (1) not produced; (2) produced
83. Maxille 1 palp: (1) larger than outer plate; (2) smaller than outer plate
84. Maxilla 2 outer plate: (1) broad; (2) narrow, less than 1 2 of inner plate
85. Maxilliped palp article 4: (1) absent or weakly developed; (2) well developed
86. Gnathopod 1 palm shape: (1) narrow; (2) wide
87. Gnathopod 2 palm shape: (1) narrow; (2) wide
88. Gnathopod 1 palm length: (1) shorter than dactylus; (2) same or longer than dactylus
89. Gnathopod 2 palm length: (1) shorter than dactylus; (2) same or longer than dactylus
90. Gnathopod 1 spines on inner curvature of dactylus: (1) absent; (2) present
91. Gnathopod 2 spines on inner curvature of dactylus: (1) absent; (2) present
92. Gnathopod 1: (1) simple or subchelat; (2) chelat
93. Gnathopod 2: (1) simple or subchelat; (2) chelat
94. Pereopod 3 and 4 merus: (1) not produced; (2) produced
95. Pereopod 5 merus: (1) not produced; (2) produced
96. Pereopod 6 merus: (1) not produced; (2) produced
97. Pereopod 7 merus: (1) not produced; (2) produced
98. Subantennal sinus: (1) absent; (2) present

The data set was prepared in DELTA, which labels the characters in binary states 1 and 2, therefore 0 is not used. The order of states does not reflect any assumptions on which state is plesiomorphic and apomorphic.

Appendix C

Character matrix of 16 Antarctic Amphipoda and 98 characters, character states shaded are variable

Character	10	20	30	40	50	60
Epimeria georgia	1 2 2 2 1 1 2 1 1 2	1 1 1 1 2 1 1 2 1 3	2 1 1 1 1 1 2 1 1 2	2 1 1 1 2 2 1 1 1 1	1 1 1 1 1 1 1 1 2 1	1 1 1 1 1 1 1 1 1 1
Epimeria reoproi	2 2 2 2 1 2 2 1 1 2	1 1 1 1 2 1 1 2 1 3	2 2 2 1 1 1 2 1 1 2	2 1 1 1 1 2 1 1 2 1	1 1 1 1 1 1 1 1 2 1	1 1 1 1 3 3 1 1 1 1
Epimeria robusta	1 2 1 2 1 2 2 1 1 3	1 1 1 2 2 1 1 2 1 1	3 3 1 1 1 1 2 1 1 2	2 1 1 1 1 1 1 1 1 1	1 1 1 1 2 1 1 1 1 1	1 3 3 1 1 1 1 1 1 1
Epimeria macrodonta	2 1 2 2 1 2 2 1 1 3	2 1 1 2 2 1 1 2 1 3	3 3 2 1 1 1 3 1 1 2	2 2 1 2 3 3 2 2 3 1	1 1 1 1 1 1 1 2 2 1	1 1 1 1 3 3 1 2 1 2
Epimeria rubrieques	2 1 2 2 1 1 2 1 1 2	1 1 1 1 2 1 1 2 1 3	2 1 1 1 1 1 3 1 1 2	2 3 3 3 3 3 1 1 2 1	1 1 1 1 1 1 1 2 2 2	2 3 1 1 1 1 1 2 1 1
Epimeria similis	2 1 2 2 1 2 2 1 1 3	1 1 1 ½ 2 1 1 2 1 3	3 3 2 1 1 1 3 1 1 2	2 1 1 2 3 3 1 2 3 1	1 1 1 1 1 1 1 2 2 1	1 3 1 1 3 3 1 2 1 2
Iphimediella georgia	2 1 1 2 2 1 2 2 2 2	1 1 1 1 2 2 1 1 1 2	2 1 1 3 2 2 1 1 2 1	2 1 1 1 1 1 1 1 1 1	1 1 2 2 2 1 2 1 1 1	1 2 2 2 2 2 2 1 1 1
Iphimediella rigida	2 1 1 1 2 1 2 2 2 2	1 1 1 1 2 2 1 1 1 3	2 1 1 3 2 2 1 2 2 2	2 1 1 1 1 1 1 1 1 1	1 1 2 2 2 1 2 1 1 1	1 2 2 2 1 2 1 1 1 1
Iphimediella cyclgena	1 2 2 1 2 2 2 2 2 1	1 1 1 1 2 1 2 2 1 3	3 1 1 3 2 1 1 2 2 1	1 1 1 1 1 1 1 1 1 1	1 1 2 2 2 1 2 1 1 1	1 2 2 2 2 2 2 1 1 1
Echiniphimedia echinata	1 1 1 1 1 1 2 2 2 4	1 1 1 1 2 2 2 1 1 3	2 1 2 3 1 1 1 1 1 2	2 1 1 1 1 1 1 1 1 1	2 2 2 2 2 1 1 1 1 2	1 3 3 1 2 2 2 1 1 1
Echiniphimedia hodgsoni	2 1 1 1 2 1 2 2 2 4	1 1 1 2 2 2 1 2 1 3	3 1 2 3 1 1 1 1 1 2	2 1 1 1 1 1 1 1 1 2	2 2 2 2 2 1 1 1 2 1	1 1 1 1 2 2 2 1 2 2
Echiniphimedia waegeli	1 1 1 1 1 1 2 2 2 4	1 1 1 1 2 2 1 2 1 3	3 1 2 3 1 1 1 1 1 2	2 1 1 1 1 1 1 1 1 2	2 2 2 2 2 1 1 2 1 2	2 2 2 2 2 2 2 2 1 2 2
Gnathiphimedia mandibularis	2 2 1 1 2 2 2 2 2 1	1 1 1 1 2 1 2 2 1 3	2 1 1 3 2 2 1 2 2 1	1 1 1 1 1 1 1 1 1 1	1 1 2 2 2 1 2 1 1 1	1 2 2 2 2 2 2 1 1 1
Gnathiphimedia sexdentata	2 2 2 1 2 1 2 2 2 1	2 1 1 1 2 1 1 1 1 3	2 2 1 3 2 1 1 1 2 1	1 1 1 1 1 1 1 1 1 1	1 1 2 2 2 1 2 1 1 1	1 2 2 2 2 2 2 1 1 1
Eusirus cf. *perdentatus*	2 1 1 2 2 1 1 1 1 2	1 1 1 1 1 1 1 1 1 1	2 1 1 1 1 1 3 1 1 2	2 1 1 1 1 3 1 1 1 1	1 1 2 2 2 2 2 1 1 1	1 1 1 1 2 2 1 1 1 1
Monoculodes sp.	1 2 1 1 1 2 1 1 2 1	1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 2	2 1 1 1 1 1 1 1 1 1	1 1 1 1 1 2 1 1 1 1	1 1 1 1 1 1 1 1 1 1

Character	70	80	90
Epimeria georgia	2 1 2 1 2 2 2 2 3 1	1 1 1 2 2 2 2 2 1 2	2 1 1 1 2 2 2 1 1 2	2 1 1 2 2 2 2 1
Epimeria reoproi	2 1 2 2 1 2 2 2 4 3	2 2 1 2 2 2 2 2 1 2	2 1 1 1 2 1 1 1 1 2	2 1 1 2 2 2 2 1
Epimeria robusta	1 1 2 1 2 2 2 1 1 1	1 1 1 2 2 2 2 2 1 2	2 1 1 1 2 2 2 2 1 2	2 1 1 2 2 2 2 1
Epimeria macrodonta	2 1 2 2 1 2 2 2 4 4	1 2 1 2 2 2 2 2 1 2	2 1 1 1 2 1 1 1 1 2	2 1 1 2 2 2 2 1
Epimeria rubrieques	2 1 2 2 1 2 2 2 3 3	1 1 1 2 2 2 2 2 1 2	2 1 1 1 2 2 2 1 1 2	2 1 1 2 2 2 2 1
Epimeria similis	2 1 2 2 1 2 ½ 2 4 3	1 2 1 2 2 2 2 2 1 2	2 1 1 1 2 1 1 1 1 2	2 1 1 2 2 2 2 1
Iphimediella georgia	1 1 1 2 1 1 1 2 4 3	1 2 1 2 1 1 2 2 1 1	2 1 1 1 1 1 1 2 2 1	1 2 2 2 2 2 2 2
Iphimediella rigida	1 1 1 2 1 2 2 2 3 2	1 2 1 2 1 1 2 2 1 1	2 1 1 1 1 1 1 1 2 1	1 2 2 2 2 2 2 2
Iphimediella cyclogena	1 1 1 2 1 1 1 2 3 3	1 2 1 2 1 1 2 1 1 1	2 1 1 1 1 1 1 2 2 1	1 2 2 2 2 2 2 2
Echiniphimedia echinata	1 1 1 2 1 2 2 2 4 3	1 1 1 1 1 1 2 ½ 1 1	2 1 1 1 1 1 1 2 2 1	1 2 2 2 2 2 2 2
Echiniphimedia hodgsoni	2 2 1 2 2 1 1 2 6 4	2 2 2 1 1 1 2 2 1 1	2 1 1 1 1 1 1 2 2 1	1 2 2 2 2 2 2 2
Echiniphimedia waegeli	2 2 1 2 1 2 2 2 4 3	1 2 1 1 1 1 2 2 1 2	1 1 1 1 1 1 1 2 2 1	1 2 2 2 2 2 2 2
Gnathiphimedia mandibularis	1 1 1 2 1 2 2 2 3 3	1 1 1 1 1 1 1 1 1 2	2 1 1 1 1 1 1 2 2 1	1 2 2 2 2 2 2 2
Gnathiphimedia sexdentata	1 1 1 2 1 2 1 2 4 2	1 1 1 1 1 1 1 1 1 1	2 1 1 1 1 1 1 2 2 1	1 2 2 2 2 2 2 2
Eusirus cf. *perdentatus*	1 1 2 1 2 1 1 1 4 5	2 2 1 1 2 2 2 2 1 2	2 ½ 1 1 2 2 2 2 2 1	1 1 1 1 2 2 2 2
Monoculodes sp.	1 1 1 1 2 1 1 1 1 1	1 1 1 1 2 2 2 2 1 2	2 1 1 1 2 2 2 1 2 1	1 1 1 1 1 2 2 1

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