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ScienceDirect
Molecular Phylogenetics and Evolution 46 (2008) 328–346
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

The tempo and mode of barnacle evolution

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Received 4 May 2007; revised 26 September 2007; accepted 6 October 2007
Available online 13 October 2007

Abstract

Previous phylogenetic attempts at resolving barnacle evolutionary relationships are few and have relied on limited taxon sampling. Here we combine DNA sequences from three nuclear genes (18S, 28S and H3) and 44 morphological characters collected from 76 thoracican (ingroup) and 15 rhizocephalan (outgroup) species representing almost all the Thoracica families to assess the tempo and mode of barnacle evolution. Using phylogenetic methods of maximum parsimony, maximum likelihood, and Bayesian inference and 14 fossil calibrations, we found that: (1) Iblomorpha form a monophyletic group; (2) pedunculated barnacles without shell plates (Heteralepadomorpha) are not ancestral, but have evolved, at least twice, from plated forms; (3) the ontogenetic pattern with $5 \rightarrow 6 \rightarrow 8 \rightarrow 12+$ plates does not reflect Thoracica shell evolution; (4) the traditional asymmetric barnacles (Verrucidae) and the Balanomorpha are each monophyletic and together they form a monophyletic group; (5) asymmetry and loss of a peduncle have evolved twice in the Thoracica, resulting in neither the Verrucomorpha nor the Sessilia forming monophyletic groups in their present definitions; (6) the Scalpellomorpha are not monophyletic; (7) the Thoracica suborders evolved since the Early Carboniferous (340 mya) with the final radiation of the Sessilia in the Upper Jurassic (147 mya). These results, therefore, reject many of the underlying hypotheses about character evolution in the Cirripedia Thoracica, stimulate a variety of new thoughts on thoracican radiation, and suggest the need for a major rearrangement in thoracican classification based on estimated phylogenetic relationships.
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Keywords: Barnacle; Divergence times; DNA sequences; Fossils; Morphology; Rhizocephala; Systematics; Taxonomy; Thoracica

1. Introduction

Thoracica (Maxillopoda: Cirripedia) barnacles are found in virtually all marine and estuarine environments from intertidal pools to abyssal vents. They deviate from all other Crustacea in being permanently and irreversibly attached suspension feeders that have abandoned the normal arthropod growth pattern by being armed externally with mineralized plates that are never shed in molts but increase incrementally in size (Anderson, 1994). Barnacles were in many respects the first model organism in evolutionary biology as reflected in Darwin's work (Darwin, 1851, 1852, 1854, 1855). Their very specialized morphologies, diverse habitats, and reproductive systems made them excellent for testing and honing his ideas on biological evolution (Ghiselin, 1969; Høeg and Møller, 2006). Barnacles have retained the attention of biologists ever since. They are important members of many marine habitats such as the rocky intertidal and their sessile mode of life leaves them the primary fouling objects on man made structures in the sea (Thompson and Nagabhushanam, 1999). A

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doi:10.1016/j.ympev.2007.10.004

robust phylogeny is therefore pivotal in understanding how barnacles have evolved and diversified from a more conventional ancestor and also how experimental studies on single species, such as in antifouling research, can be extended to larger groups.

The adult thoracicans are either "goose neck barnacles" with a muscular "peduncle" attached to the substrate topped by a "capitulum" housing the brood chamber and the feeding appendages, or "acorn barnacles" that lack the peduncle and have the capitulum cemented directly to the substratum (Anderson, 1994). The precise number, shape and disposition of mineralized shell plates covering the body have been the principle characters in thoracican taxonomy, in part because they have left an impressive fossil record. But reliance on these characters has greatly impeded the study of basal thoracican phylogenetic relationships since the two relevant outgroups, the parasitic barnacles (Rhizocephala) and the burrowing barnacles (Acrothoracica) lack shell plates altogether. Adult Rhizocephala cannot at all be compared with the Thoracica, and while adult Acrothoracica are suspension feeders with cirri, there are few useful characters for comparison (Kolbasov et al., 1999; Kolbasov and Høeg, 2000). The lack of reasonable homology statements between the thoracicans and reasonable outgroups has left ingroup comparisons as the only means of analyzing thoracican evolution and polarizing characters states (through the ontogeny of shell plates or, to some extent, based on the stratigraphy of fossil forms). Larval morphology can be more easily compared to outgroups, but the data are time consuming to compile and the only comprehensive study to date did in fact polarize the characters using a hypothetical ancestor derived from the ingroup (Newman and Ross, 2001). Therefore, DNA sequences offer not only an alternative character set for analyzing thoracican evolution, but also a framework for testing the value of ontogenetic and fossil data in phylogenetic studies in general.

Previous cladistic studies on thoracican phylogeny are few and relied on limited taxon sampling. The morphology-based analysis of Glenner et al. (1995) and the few later studies using DNA sequences (Harris et al., 2000; Pérez-Losada et al., 2004; Perl-Treves et al., 2000; Spears et al., 1994) deviated not only among themselves but also from existing taxonomies (Anderson, 1994; Martin and Davis, 2001; Newman, 1987, 1996). We therefore decided to perform a new analysis on a much more extensive taxon sampling and use both molecular and morphological datasets for a more thorough assessment of evolutionary relationships.

Since Darwin, timing the radiation of the main barnacle groups based on their extinct relatives has always provoked great interest. Fossils and evolutionary hypotheses have been combined previously (Buckeridge and Newman, 2006; Foster and Buckeridge, 1987; Glenner et al., 1995; Newman, 1996), but only one study (Pérez-Losada et al., 2004) has actually integrated both within a statistical framework. Using phylogenetic procedures of time estimation and three fossil calibration points, the authors dated the radiation of the main thoracican clades in an 18S rRNA gene tree. Unfortunately, divergence time estimates based on a single locus and single calibrations can be severely biased (Porter et al., 2005; Thorne and Kishino, 2002; Yang, 2004; Yang and Yoder, 2003). The bias is even more acute if there is considerable uncertainty in the phylogenetic relationships among the taxa (Drummond et al., 2006). More accurate divergence time estimates can be obtained by integrating multiple gene loci and multiple fossil calibration points into a robust phylogeny (Thorne and Kishino, 2002; Yang, 2004).

In this study we will combine new molecular and morphological data to re-examine both the morphological and phylogenetic mode and evolutionary tempo in thoracican radiation. We use sophisticated phylogenetic methods to assess key questions in thoracican evolution such as: are forms without shell plates (i.e., Heteralepadomorpha) ancestral or derived? Did thoracicans evolve by an incremental addition of shell plates as indicated by their ontogeny? Are the "sessilian" barnacles without a peduncle monophyletic? And what is the phylogenetic position of the asymmetric barnacles (Verrucomorpha). Then we will use our most robust phylogeny, a multi-loci data set, and 14 fossil calibrations to estimate a Bayesian chronogram of barnacle radiation.

2. Methods

2.1. Taxon sampling

Seventy-six thoracican (ingroup) and fifteen rhizocephalan (outgroup) species were analyzed in this study (Table 1). Molecular and morphological evidence support our outgroup choice (Pérez-Losada et al., 2002, 2004). All the orders and suborders within the superorder Thoracica are represented in this study, except the Brachylepadomorpha, which consists of one extant abyssal species (*Neobrachylepas relicta*). As for the pedunculates (stalked barnacles), we include representatives from each family except for those in the Heteralepadomorpha, which are represented by only one or two families out of six described. As for the acorn barnacles (Sessilia), we have included an assorted representation of the highly-evolved symmetrical balanomorphs (see Table 1) and several species of the more basal asymmetrical verrucomorph families Neoverrucidae and Verrucidae. Specimens were preserved in 70% EtOH and are housed in the crustacean collection at the Monte L. Bean Life Science Museum, Brigham Young University.

2.2. DNA extraction, PCR, and sequencing

Barnacle DNA extraction, amplification, and sequencing were performed as described in Pérez-Losada et al. (2004). Because this study attempts to resolve the evolution of backbone lineages in the thoracican tree, we selected the three more informative genes in Pérez-Losada et al. (2004)

Table 1
Thoracica (ingroup) and Rhizocephala (outgroup) cirripedes included in this study

Species	Location
THORACICA	
SESSILIA	
BALANOMORPHA	
Balanoidea	
Austromegabalanus psittacus (Molina)	Molinos Beach, Valdivia, Chile
Austrominius modestus (Darwin)	Menai Straits, Wales, UK
Balanus balanus (Linnaeus)	Japan
Balanus crenatus (Bruguière)	Menai Straits, Wales, UK
Balanus glandula (Darwin)	Monterey Bay, CA, USA
Balanus perforatus (Bruguière)	Vigo Bay, Galicia, Spain
Elminius kingii (Gray)	Molinos Beach, Valdivia, Chile
Megabalanus californicus (Pilsbry)	Monterey Bay, CA, USA
Megabalanus tintinnabulum (Linnaeus)	Monterey Bay, CA, USA
Megabalanus spinosus (Gmelin)	Annobón, Equatorial Guinea
Menesiniella aquila (Pilsbry)	Monterey Bay, CA, USA
Semibalanus balanoides (Linnaeus)	Isefjord, Denmark
Semibalanus cariosus (Pallas)	Monterey Bay, CA, USA
Chthamaloidea	
Catomerus polymerus (Darwin)	Pirates Bay, Tasmania, Australia
Chamaesipho tasmanica (Foster and Anderson)	Pirates Bay, Tasmania, Australia
Chthamalus bisinuatus (Pilsbry)	Tramandaí Beach, RGS, Brazil
Chthamalus challengeri (Hoek)	Japan
Chthamalus montagui (Southward)	Vigo Bay, Galicia, Spain
Chthamalus stellatus (Poli)	Vigo Bay, Galicia, Spain
Jehlius cirratus (Darwin)	Molinos Beach, Valdivia, Chile
Notochthamalus scabrosus Darwin	Molinos Beach, Valdivia, Chile
Coronuloidea	
Chelonibia patula (Ranzani)	Israel
Tetraclitoidea	
Tetraclita japonica (Pilsbry)	Japan
Tetraclita squamosa (Bruguière)	Cooktown, Australia
Tetraclitella divisa (Nilsson-Cantell)	Annobón, Equatorial Guinea
Tetraclitella purpurascens (Wood)	Eaglehawk Neck, Tasmania, Australia
VERRUCOMORPHA	
Neoverrucidae	
Neoverruca sp. 1	Okinawa Trough, Japan (vent)
Neoverruca sp. 2	Ogasawara Arc, Japan (vent)
Neoverruca brachylepadoformis (Newman and Hessler)	Mariana Trough (vent)
Verrucidae	
Altiverruca sp.	Nansei Islands, Japan
Metaverruca recta (Aurivillius)	Ogasawara Islands, Japan
Rostratoverruca sp.	Gulf of Mexico, USA
Rostratoverruca krugeri (Broch)	Nansei Islands, Japan
Verruca laevigata (Sowerby)	Chile
Verruca spengleri (Darwin)	GenBank
Verruca stroemia (Müller)	Vigo Bay, Galicia, Spain
PEDUNCULATA	
HETERALEPADOMORPHA	
Heteralepadidae	
Paralepas palinuri (Newman)	GenBank
Paralepas dannevigi (Broch)	Australia
Heteralepadomorpha (unclassified species)	Australia
IBLOMORPHA	
Iblidae	
Ibla cumingi (Darwin)	Gulf of Aqaba, Israel
Ibla quadrivalvis (Cuvier)	Kingston Beach, Tasmania, Australia
LEPADOMORPHA	
Lepadidae	
Conchoderma auritum (Linnaeus)	Japan
Conchoderma virgatum (Spengler)	Hobart, Australia
Lepas anatifera (Linnaeus)	GenBank
Lepas anserifera (Linnaeus)	Queensland, Australia
Lepas australis (Darwin)	Wellington, New Zealand
Lepas pectinata (Spengler)	Rottnest Island, Australia
Lepas testudinata (Aurivillius)	Cottesloe, Australia
Oxynaspididae	
Oxynaspis celata (Darwin)	Azores Islands
Poecilasmatidae	
Megalasma striatum (Hoek)	Nansei Islands, Japan
Octolasmis sp.	Dongara, Australia
Octolasmis cor (Aurivillius)	Noumea, New Caledonia
Octolasmis lowei (Darwin)	GenBank
Octolasmis warwickii (Gray)	Moreton Bay, Australia
Poecilasma inaequilaterale (Pilsbry)	Gulf of Mexico
Poecilasma kaempferi (Darwin)	Albany, Australia
SCALPELLOMORPHA	
Calanticidae	
Calantica sp.	Bedwell Island, Australia
Calantica spinosa (Quoy and Gaimard)	Otago, New Zealand
Calantica villosa (Leach)	GenBank
Smilium peroni (Gray)	Marmion, Australia
Eolepadidae	
Ashinkailepas seepiophila (Yamaguchi, Newman and Hashimoto)	Hatsushima Island, Japan (vent)
Leucolepas longa (Southward and Jones)	S. Edison Field, W Pacific Ocean (vent)
Neolepas rapanuii (Jones)	Eastern Island, East Pacific Rise (vent)
Neolepas zevinae (Newman)	East Pacific Rise (vent)
Volcanolepas sp.	Hine Hina, Lau Basin (vent)
Volcanolepas osheai (Buckeridge)	Brothers Caldera, New Zealand (vent)
Lithotryidae	
Lithotrya sp.	Christmas Island, Indian Ocean
Lithotrya valentiana (Gray)	Gulf of Aqaba, Egypt
Pollicipedidae	
Capitulum mitella (Linnaeus)	Japan
Pollicipes pollicipes (Gmelin)	Vigo Bay, Galicia, Spain
Pollicipes polymerus (Sowerby)	Monterey Bay, CA, USA
Scalpellidae	
Arcoscalpellum sp.	GenBank
Litoscalpellum regina (Pilsbry)	Gulf of Mexico

Table 1 (*continued*)

Species	Location
Ornatoscalpellum stroemi (Sars)	Canada
Scalpellum scalpellum (Linnaeus)	Sweden
Trianguloscalpellum regium (Thomson)	Gulf of Mexico, USA
RHIZOCEPHALA	
KENTROGONIDA	
Peltogastridae	
Peltogaster paguri (Rathke)	Sweden
Peltogasterella sulcata (Lilljeborg)	Sweden
Sacculinidae	
Heterosaccus californicus (George)	California, USA
Heterosaccus dollfusi (Boschma)	Israel
Heterosaccus lunatus (Phillips)	Queensland, Australia
Loxothylacus panopaei (Gissler)	North Carolina, USA
Loxothylacus texanus Boschma	GenBank
Polyascus gregaria (Okada and Miyashita)	GenBank
Polyascus plana (Boschma)	GenBank
Polyascus polygenea (Lützen et Takahashi)	GenBank
Sacculina carcini (Thompson)	Sweden
Sacculina confragosa (Boschma)	GenBank
Sacculina leptodiae (Guerin-Ganivet)	GenBank
Sacculina oblonga (Lützen and Yamaguchi)	GenBank
Sacculina sinensis (Boschma)	GenBank

for inferring deep relationships: 18S rRNA (1892 bp), 28S rRNA (1820 bp), and histone H3 (328 bp). We have increased our previous sequencing efforts by adding 128 new sequences resulting in a combined data set of 243 sequences total: 91 18S rRNA, 77 28S rRNA, and 75 H3 sequnces. The new sequences have been deposited in GenBank under the Accession Nos. EU082295–EU082416.

2.3. Morphological data

A total of 44 adult and juvenile morphological characters taken from Pérez-Losada et al. (2004) were scored for each of the 76 Thoracica taxa (Appendix A). Some characters were slightly redefined or rescored from our previous matrix and a few new ones were added. We have not made any unsupported evolutionary assumptions in the taxa, so many characters are scored as unknown in the outgroup, which consists of morphologically reduced parasites (Høeg and Lützen, 1995). As in Glenner et al. (1995), we do not *a priori* distinguish between primary absence and secondary loss. Therefore, we score a character as absent whenever it is not present in the taxon. All the morphological characters were mapped onto our best hypothesis of phylogenetic relationships using MacClade (Maddison and Maddison, 2000) to trace their evolution. While we do not show those character tracings, they form the basis of the discussion below on the evolution of thoracican morphology.

2.4. Phylogenetic analyses

Nucleotide sequences were aligned using MAFFT v5.7 (Katoh et al., 2005) under iterative refinement methods incorporating the most accurate local (L-INS-i and E-INS-i) and global (G-INS-i) pairwise alignment information. Default settings were chosen for all the parameters involved under each algorithm. Multiple sequence alignments (MSA) for each gene resulting from these three methods were concatenated and maximum likelihood (ML) trees were estimated using PhyML (Guindon and Gascuel, 2003). G-INS-i (4289 sites) generated the trees with the best likelihood scores; hence, we used this MSA for our subsequent phylogenetic analyses. Congruence among gene regions was addressed using the Wiens's (1998) protocol. Separate bootstrap ML analyses (PhyML) were conducted on three genes to detect potential areas of strongly supported incongruence as indicated by conflicting nodes with bootstrap proportions (BP) $\geqslant$ 70%. No such areas of incongruence were observed in our alignment.

DNA sequence and morphological data were analyzed under different phylogenetic approaches. Molecular phylogenies were inferred using ML, as implemented in Treefinder (Jobb, 2006). Molecular and morphological data were analyzed under maximum parsimony (MP), as implemented in PAUP* v4b10 (Swofford, 2002), and Bayesian methods coupled with Markov chain Monte Carlo (BMCMC) inference, as implemented in MrBayes v3.04b (Ronquist and Huelsenbeck, 2003). Model selection under the ML and BMCMC approaches followed the procedure outlined by Posada and Buckley (2004) as implemented in ModelTest v3.6 (Posada and Crandall, 1998). Mix-model analyses were performed under both ML and BMCMC procedures. The GTR + Γ + I model was selected for 18S and 28S and the TrN + Γ + I model was chosen for H3. ML (Felsenstein, 1981) heuristic searches in Treefinder were conducted under optimum mixed models. Model parameters were estimated as part of the analysis. A global tree search started from 10 random trees generated from an initial neighbor-joining tree (center tree); only the tree with the best likelihood score was saved. MP heuristic searches were performed in PAUP* using 1000 random addition replicates and TBR branch swapping. Clade support was assessed using the nonparametric bootstrap procedure (Felsenstein, 1985) with 500 bootstrap replicates for the ML trees and 1000 bootstrap replicates and 10 random addition searches with TBR branch swapping per replicate for the MP trees. Four independent BMCMC analyses were run in MrBayes with each consisting of four chains. Each Markov chain was started from a random tree and run for 10^7 cycles, sampling every 2000th generation. Model parameters were unlinked and treated as unknown variables with uniform default priors; they were estimated as part of the analysis. Convergence and mixing were monitored using Tracer v1.2 (Rambaut and Drummond, 2003). All sample points prior to reaching stationary were discarded as burn-in. The posterior probabilities (PP) for individual clades obtained from separate analyses were compared for congruence and then combined and summarized on a 50% majority-rule consensus tree (Huelsenbeck and Imennov, 2002; Huelsenbeck et al., 2002).

Alternative phylogenetic hypotheses were built in MacClade as indicated in Pérez-Losada et al. (2004) and

tested under both likelihood and Bayesian frameworks. Likelihood topological tests were conducted using our molecular data and the Shimodaira and Hasegawa (S–H) (1999) test as implemented in Treefinder. Ten thousand replicates were performed for every topology test resampling the partial likelihoods for each site (RELL model). Bayesian topological tests were conducted using our combined molecular and morphological data sets and performed as described in Huelsenbeck et al. (2002).

2.5. Divergence time estimation

Thoracica divergence times were estimated using the Bayesian method of Thorne and Kishino (T–K) (2002). This method can accommodate multiple fossil calibration points (maximum or minimum ages) and multiple genes and allows for missing taxa. As previously shown, simultaneous analysis of gene sequences from multiple loci and multiple calibrations is expected to improve estimates of divergence times and rate estimates (Porter et al., 2005; Thorne and Kishino, 2002; Yang, 2004). T–K is implemented in the multidivtime package (http://statgen.ncsu.edu/thorne/multidivtime.html). The mean of the prior distribution for the time separating the ingroup root from the present (rttm) and the standard deviation (SD) of this prior distribution (rttmsd) were set to 6 (600 mya). Alternative values ranging from 5 to 7 were also tried but final estimates did not change much (±10 my). After inspecting the branch lengths estimated by est branches for each gene, the evolutionary rate of the root node was given a gamma prior distribution with mean (rtrate) and SD (rtratesd) both equal to 0.029 substitutions at the average site per 100 my. We chose this prior to obtain a distribution for the root that was simultaneously reasonable and relatively diffuse. The rtrate and rtratesd were estimated as suggested in the multidivtime manual. Prior distributions approximated under the MCMC approach included a burn-in period of 10^6 steps, after which 5×10^5 samples were collected every 100 accepted states; posterior distributions (less diffuse) included a burn-in period of 10^5 steps, after which 5×10^5 samples were collected every 100 accepted states. Default options were chosen for all the other parameters of the prior distribution and the MCMC procedure. Convergence was monitored by checking the proportion of successes (psuc) of times and rate changes proposed along the Markov chain. Three independent chains were run from different starting points. Parameters of the evolutionary model were estimated under the HKY + Γ model (Hasegawa et al., 1985), the most complex model implemented in this software. This model is less parameterized than the best-fit models selected by ModelTest, however, previous studies (Yang and Yoder (2003) and references therein) have shown that it is actually the rate variation among sites parameter that has the greatest effect on divergence time estimation. All the parameters within the model, as well as the branch lengths, were estimated separately for every gene.

2.5.1. Fossils calibrations

We used the 14 fossil calibrations, ranging from the Carboniferous (M. Pennsylvanian, 306.5–311.7 mya) to the Neogene (M.-L. Miocene, 11.6–23 mya), to anchor our divergence time estimates (Table 2). Previous calibrations used in Pérez-Losada et al. (2004) for the Heteralepadomorpha (*Priscansermarinus*, M. Cambrian) and the Scalpellomorpha (*Pabulum*, L. Carboniferous) were not used in this study because their barnacle affinities were questioned and because these groups were not monophyletic in our new phylogenetic analysis: *Pabulum* is now considered to be a bivalve mollusc (Martin Whyte personal communication); we also find the evidence for a cirripede origin of *Priscansermarinus* very feeble (Briggs, 1983; Briggs et al., 2005). Given that most fossils are dated to an age range, the midpoint of each geologic range was chosen for diver-

Table 2
Species and ages of fossils used as calibrations for divergence time estimations

Species	Reference	Geologic age (mya)	Node
Praelepas jaworskii	Newman et al. (1969)	Carboniferous–M. Pennsylvanian (306.5–311.7)	C1
Calantica (*Scillaelepas*) *ginginensis*	Buckeridge (1983)	U. Cretaceous (Santonian) (83.5–85.8)	C2
Cretiscalpellum glabrum	Buckeridge (1983)	L. Cretaceous (Aptian) (112–125)	C3
Arcoscalpellum fossula	Newman et al. (1969)	U. Cretaceous (Senonian) (70.6–89.3)	C4
Pollicipes aboriginalis	Buckeridge (1983)	U. Cretaceous (Santonian) (83.5–85.8)	C5
Proverruca vinculum	Newman et al. (1969)	U. Creteceous (Senonian) (70.6–89.3)	C6
Pynolepas rigida	Newman et al. (1969)	U. Jurassic (145.5–161.2)	C6
Verruca tasmanica	Buckeridge (1983)	U. Cretaceous (Santonian–Campanian) (70.6–85.8)	C7
Metaverruca sculpta	Buckeridge (1983)	Neogene–L. Miocene (Aquitanian) (20.4–23.0)	C8
Pachydiadema (*Catophragmus*) *cretacea*	Buckeridge (1983)	U. Creteceous (Senonian) (70.6–89.3)	C9
Chamaesipho brunnea	Buckeridge (1983)	Neogene–L. Miocene (16–23)	C10
Tetraclitella sp. cf. *purpurascens*	Buckeridge (1983)	Neogene–L. Miocene (Aquitanian) (20.4–23.0)	C11
Palaeobalanus lindsayi	Buckeridge (1983)	Paleogene–M. Eocene (37.2–48.6)	C12
Austromegabalanus victoriensis	Buckeridge (1983)	Neogene–M.–L. Miocene (11.6–23)	C13

L, lower; M, middle; U, upper. Calibrated nodes are indicated in Fig. 3. All calibrations were introduced as minimum ages, except C6, which was introduced as an interval (minimum and maximum ages).

gence time estimation. We used the 2004 GSA Geologic Time Scale in Gradstein et al. (2004). All calibrations were introduced as minimum ages except node C6, which was introduced as an interval based on two fossils (Table 2). In general, fossils do not fix the ages of internal nodes, they merely constrain them to be minimum ages (Smith, 1994). Hence, it seems more appropriate to constrain nodes to lie within some interval rather than fix them to a particular time (Norell, 1992). All calibrations were mapped to the node prior to the basal node of the clade of interest.

2.6. Comparison with existing taxonomy

The cladistic analysis of all Thoracica including some fossil groups by Glenner et al. (1995) were not accompanied by a formal taxonomy. The most recent, exhaustive taxonomic account is by Martin and Davis (2001), which is based on Newman (1987) but apparently by mistake omitting the family Eolepadidae (containing the subfamily Neolepadinae with several extant species) under the Scalpellomorpha. Buckeridge and Newman (2006) raised some of the suborders in Martin and Davis (2001) to ordinal level and acknowledged the polyphyly of the existing Heteralepadomorpha by including *Paralepas* in the Lepadomorpha (their Lepadiformes). For comparison, we use Martin and Davis (2001) with the addition of the Eolepadidae, but a resolved cladogram cannot be retrieved taken from their taxonomy, because more than two taxa are listed in several places at the same taxonomic level.

3. Results

3.1. Barnacle phylogenetics and taxonomy

In the ML phylogeny of barnacle DNA sequences inferred under mix-models and including clade bootstrap proportions (Fig. 1), the superorder Pedunculata is not monophyletic because it contains the Sessilia. But also the superorder Sessilia is itself not monophyletic sensu Newman (1996) and Martin and Davis (2001), because *Neoverruca* (Neoverrucidae) is not clustered with the other Verrucomorpha of the family Verrucidae (*Verruca*, *Rostratoverruca*, *Metaverruca*, and *Altiverruca*), but assembled with some of the Scalpellomorpha. An alternative topology forcing sessilian monophyly was significantly rejected by our topological tests (P and PP < 0.001).

Among the pedunculated taxa, only the suborder Iblomorpha was monophyletic, whereas the Scalpellomorpha, Heteralepadomorpha, and Lepadomorpha were all polyphyletic. At lower taxonomic levels (see Table 1), the taxa Poecilasmatidae (*Megalasma*, *Poecilasma*, and *Octolasmis*) from the Lepadomorpha, and the Tetraclitidae (*Tetraclita*, *Tetraclitella*) and Balanoidea (*Austrominius, Elminius*, *Semibalanus*, *Balanus*, *Menesinella*, *Austromegabalanus*, and *Megabalanus*) from the Sessilia were similarly polyphyletic. Within the Rhizocephala (outgroup), the Peltogastridae did not form a monophyletic group. All the other families included in the analysis resulted in monophyletic assemblages. At the genus level, only *Volcanolepas*, *Tetraclita*, *Balanus* (Thoracica) and, in the outgroup, *Sacculina* and *Heterosaccus* (Rhizocephala), did not form monophyetic taxa. Alternative topological hypotheses forcing all the polyphyletic groups to be monophyletic were independently compared to our ML tree (Table 3). The S–H test significantly rejected ($P < 0.05$) the following monophyletic assemblages: Scalpellomorpha, Heteralepadomorpha, Lepadomorpha, Verrucomorpha, *Balanus*, and *Sacculina*.

The Iblomorpha were shown to be the sistergroup to all remaining Thoracica. The latter clade (Thoracica excl. Iblomorpha) consists of the Calanticidae (''Scalpellomorpha'') (BP = 88%) as sistergroup to a clade containing all remaining ''pedunculated'' and ''sessilian'' taxa. The latter monophyletic assemblage is again divided into two large sistergroups. The first group (BP = 81%) contains a clade with most ''Scalpellomorpha'' and *Neoverruca* (''Verrucomorpha'') (BP = 92) and another with the Heteralepadomorpha + Lepadomorpha (BP = 100). The other group, supported by a low BP in the ML tree, contains the scalpellomorphan families Pollicipidae (*Pollicipes*, *Capitulum*) and Lithotryidae (*Lithotrya*), the Verrucidae and all Balanomorpha. Within that large clade, the Verrucidae (BP = 100) and the Balanomorpha (BP = 93) are highly supported whereas most other relationships have rather low support values (BP < 70%).

The MP analysis of concatenated DNA sequence and morphological data (1185 parsimony informative characters) generated 96 most parsimonious trees (L = 7180). The strict consensus tree results in the same non-monophyletic assemblages as in the ML analysis of DNA sequence data (Fig. 2). The relationships among the ''Pedunculata'' suborders were, however, depicted differently with the Heteralepadomorpha + Lepadomorpha estimated as the first derived clade after the Iblomorpha in the MP tree whereas a polyphyletic assemblage of the Scalpellomorpha are the first to branch off after the Iblomorpha in the ML tree. Similarly, the Scalpellidae–Neolepadinae clade in the MP analysis is sister to the clade containing the Pollicipidae, Lithotryidae, Verrucidae and Balanomorpha whereas the ML analysis shows the former clade sister to the Heteralepadomorpha + Lepadomorpha. Nevertheless, the MP hypothesis (Fig. 2), as indicated by the S–H test, was significantly worst ($P < 0.05$) than the ML hypothesis (Fig. 1). As in the ML analysis, all these clades (except the Pollicipedidae) were strongly supported by BP $\geqslant$ 95%, but with weak support (BP < 70%) for the relationships among them (except the Iblomorpha + other Thoracica).

The BMCMC mix-model analysis of DNA sequences and morphological data gave relationships essentially as in those estimated from the ML analysis, but support between and within clades was higher in the BMCMC tree (Fig. 3). All the main clades showed PP > 90%, with the majority having PP of 100%. The tree showed the same non-monophyletic groups described above, except for a

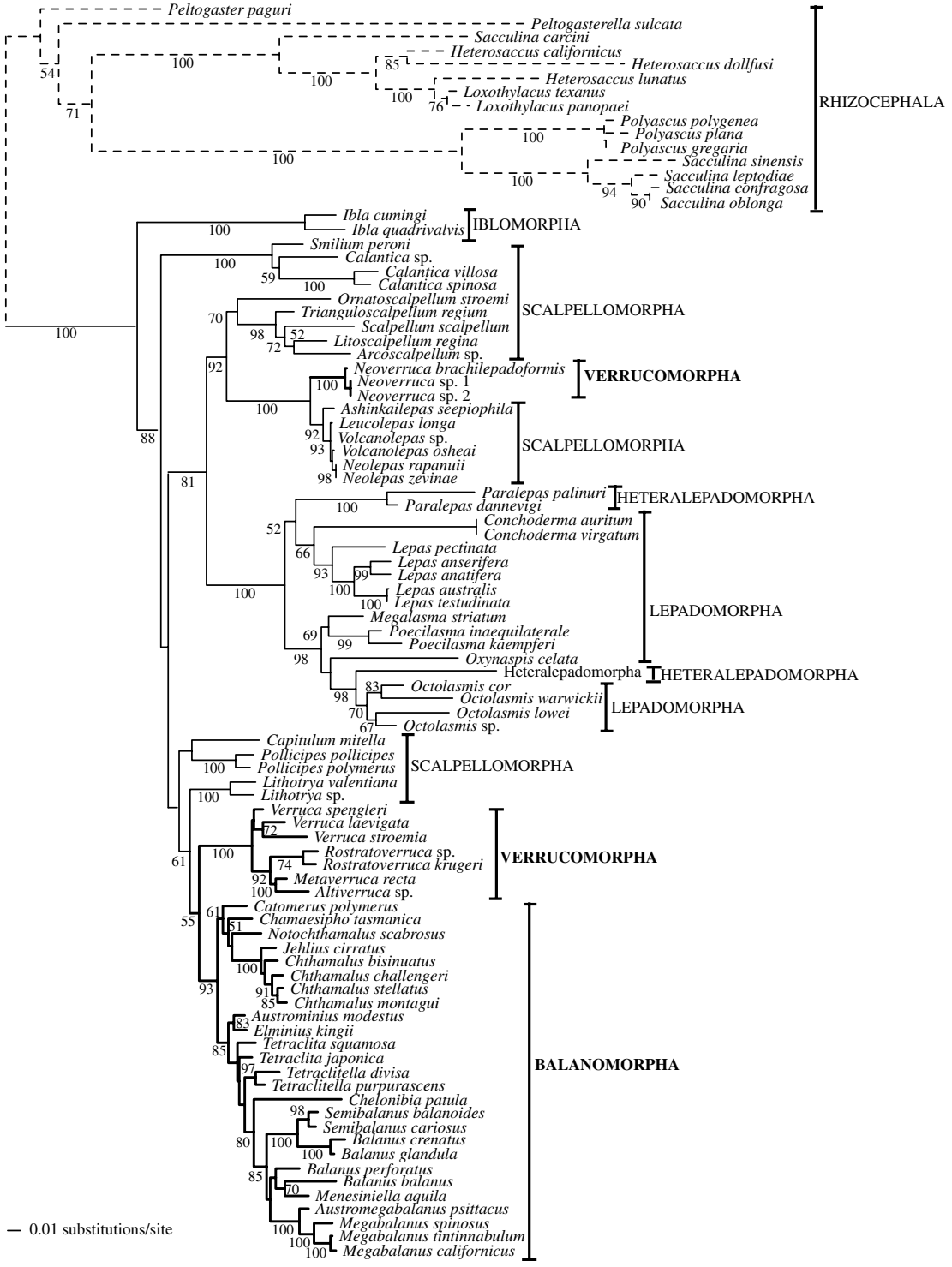

Fig. 1. Maximum likelihood mix-model tree of DNA sequence data. Branch lengths are shown proportional to the amount of change along the branches. Clades supported by BP $\geqslant$ 50% are indicated under the branches. Dashed, thin, and thick lines are used for the Rhizocephala (outgroup), Pedunculata, and Sessilia clades, respectively.

now monophyletic Tetraclitidae. Alternative topological hypotheses forcing all these groups to be monophyletic were significantly rejected (PP < 0.05) in all cases (Table 3).

The MP heuristic search of the morphological data alone resulted in a 50% majority-rule consensus tree (10^7 most parsimonious trees of L = 74; 10,000 maximum trees saved per replicate) with little resolution (Fig. 4). The Sessilia were again presented as polyphyletic because the Neoverrucidae were not clustered with the other Verrucomorpha (Verrucidae). Pedunculata suborders were assembled as follows: Iblomorpha + Heteralepadomorpha + Lepadomorpha → Scalpellomorpha + Verrucomor-

Table 3
Probabilities (*P*), as indicated by the Shimodaira and Hasegawa (1999) test, and Bayesian posterior probabilities (PP) of alternative monophyletic hypotheses to those depicted in the ML (Fig. 1) and BMCMC (Fig. 3) trees, respectively

Taxon	*P*	PP
Pedunculata		
Scalpellomorpha	<0.001	<0.001
Heteralepadomorpha	0.022	<0.001
Lepadomorpha	0.003	<0.001
Poecilasmatidae	0.101	<0.001
Volcanolepas	0.918	0.007
Sessilia		
Verrucomorpha	<0.001	<0.001
Balanoidea	0.202	<0.001
Tetraclitoidea	0.642	0.916
Balanus	<0.001	<0.001
Tetraclita	0.315	0.926
Rhizocephala		
Peltogastridae	0.960	0.023
Heterosaccus	0.307	<0.001
Sacculina	<0.001	<0.001

pha → Verrucomorpha → Balanomorpha, although this trend was supported by low BP.

3.2. Barnacle divergence times

A likelihood ratio test significantly ($P < 0.001$) rejected the null hypothesis that all genes, separately or combined, were evolving with rate constancy across our molecular ML tree, requiring the use of methods that relax the molecular clock hypothesis to estimate divergence times. The thoracican T–K chronogram was estimated using the Bayesian phylogeny, three genes, and 14 fossil calibrations (Fig. 3). Multiple independent Bayesian runs produced identical mean time estimates for all the major clades including 95% confidence intervials (CI) (Table 4). The 95% CI were large for most clades because we used only one upper limit (*Pycnolepas rigida*). However, as previously shown (Porter et al., 2005), incorporating both lower and upper limits can reduce the standard deviation. This analysis places the origin (crown age) of the Thoracica suborders in the Early Carboniferous (Mississippian—340 mya). The polyphyletic Pedunculata suborders radiated over the next 142 my, between the Early Permian (Cisuralian—287 mya) and the Lower Jurassic (198 mya). The Sessilia (excluding the Brachylepadomorpha) appeared in the Upper Jurassic (147 mya).

4. Discussion

4.1. Barnacle phylogeny and systematics

All our molecular and combined analyses (Figs. 1–3) resulted in trees with a very similar topology, which deviates extensively from any previously published suggestions for Thoracican phylogeny. These results show that many of the presently recognized thoracican taxa, from order to family levels, are not monophyletic and stimulate a need for a revision of both the taxonomy and the underlying hypotheses on character evolution.

The Pedunculata, as a group, the Sessilia and four out of six pedunculated suborders (Heteralepadomorpha, Lepadomorpha, Scalpellomorpha, and Verrucomorpha) formed polyphyletic assemblages and their alternative monophyletic topologies were significantly rejected (Table 3). While most families and genera were monophyletic, this also was not the case for the Poecilasmatidae (Lepadomorpha) and such a hypothesis was significantly rejected by the Bayesian analysis ($PP < 0.001$). Not surprisingly, the MP analysis of the purely morphological matrix returned a tree that is more consistent with the results in Glenner et al. (1995), because there is a large overlap in the characters used. Although it contained many unresolved nodes, our morphology-based MP tree agreed with the molecular and combined analyses in failing to demonstrate the monophyly of many currently recognized taxa such as the Verrucomorpha. Henceforth we shall base the discussion on the results from the trees in the molecular and combined analyses. In the outgroup, our results on the Rhizocephala largely match those recently found by Glenner and Hebsgaard (2006). Since that study used somewhat different methods and genes and used another outgroup, the consistency with Glenner and Hebsgaard (2006) puts confidence in our ingroup results.

4.2. Mode of barnacle evolution

Because a formalized outgroup analyses is very difficult, the current taxonomy of Thoracica is based on a set of hypotheses concerning homologies and character polarities that derive largely from ingroup comparisons. Below we use our analysis to test those hypotheses on Thoracica character evolution that have been pivotal in establishing the current taxonomy.

4.2.1. Are naked thoracicans ancestral? The origin of shell plates

The position of the Heteralepadomorpha is pivotal to understanding early thoracican evolution because the total absence of shell plates in this group could represent either the plesiomorphic state for all Thoracica (i.e., Heteralepadomorpha are the most basal thoracicans) or a secondary loss (i.e., Heteralepadomorpha falls at any other position) (Høeg et al., 1999; Newman, 1987). All possible outgroups to the Thoracica lack shell plates, so, seen in isolation, the absence of shell plates must indeed be interpreted as a plesiomorphy (Foster, 1978). Glenner et al. (1995) and our morphological analysis were unable to phylogenetically discriminate between the two hypotheses and the heteralepadomorphans were always part of an unresolved polytomy at the base of the Thoracica. In contrast, our molecular and combined analyses place all the heteralepadomorphs deep within

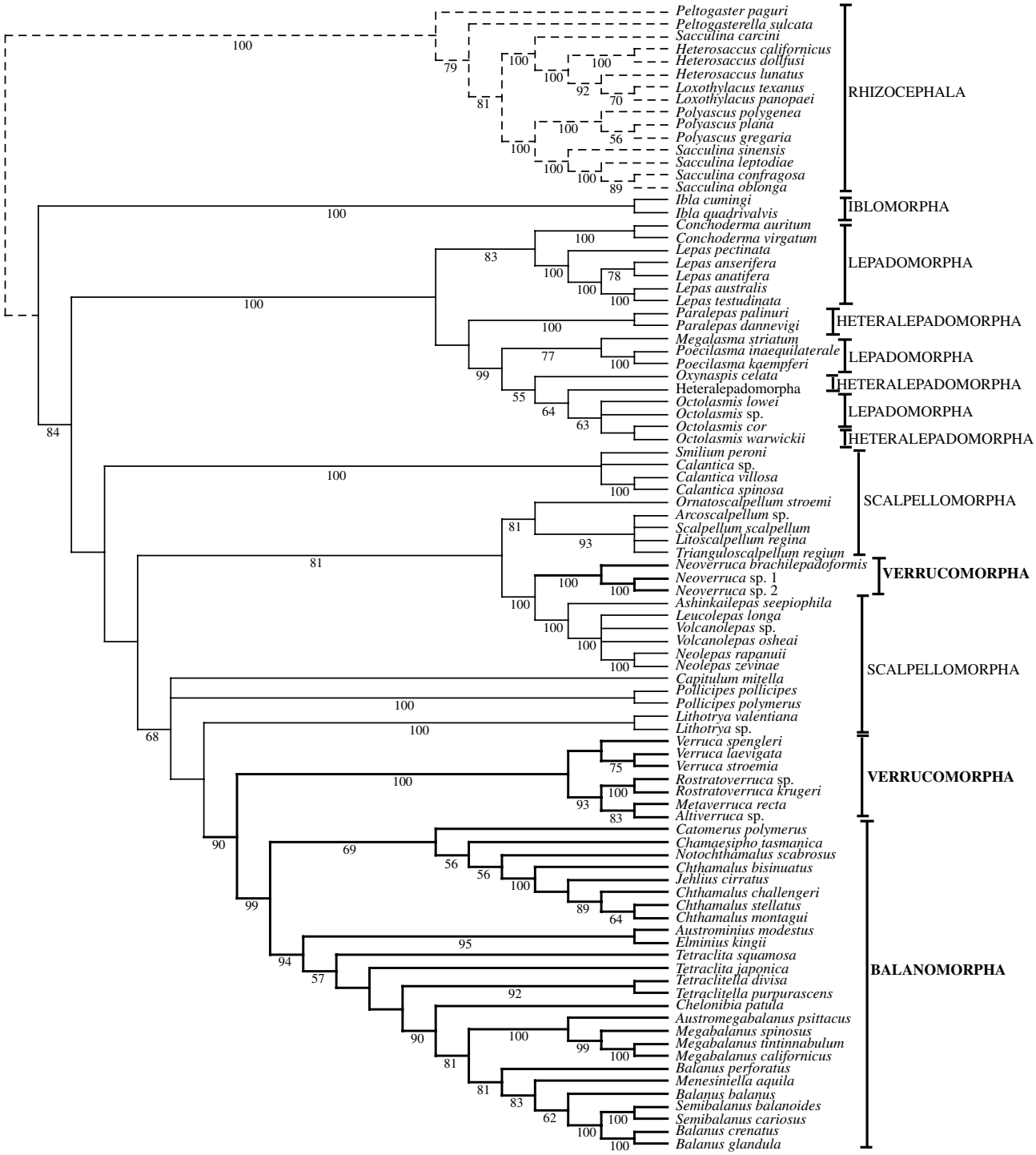

Fig. 2. Strict consensus tree of 96 most parsimonious trees using morphological and molecular data. Bootstrap proportions (if $\geqslant$50%) are shown under the branches. Dashed, thin, and thick lines are used for the Rhizocephala (outgroup), Pedunculata, and Sessilia clades, respectively.

pedunculated Thoracica, but they do not form a monophyletic group. This clearly shows that shell plates were present in the last common ancestor to all extant Thoracica but were later lost at least twice in thoracican evolution. Our position of the heteralepadomorphans dovetails with soft part morphology such as the anterior position of the carapace adductor in all thoracicans other than the Iblomorpha (see Glenner and Høeg, 1998; Klepal, 1985). This and other results in our analysis suggest that soft part characters have been much underused in thoracican systematics compared to those from shell plates. Buckeridge and Newman (2006) subsumed the ''Heteralepadomorpha'' in the Lepadomorpha and so in principle agree with our result on shell plate evolution, but this does not solve the taxonomy, since the undetermined heteralepadomorphan is the sister group to

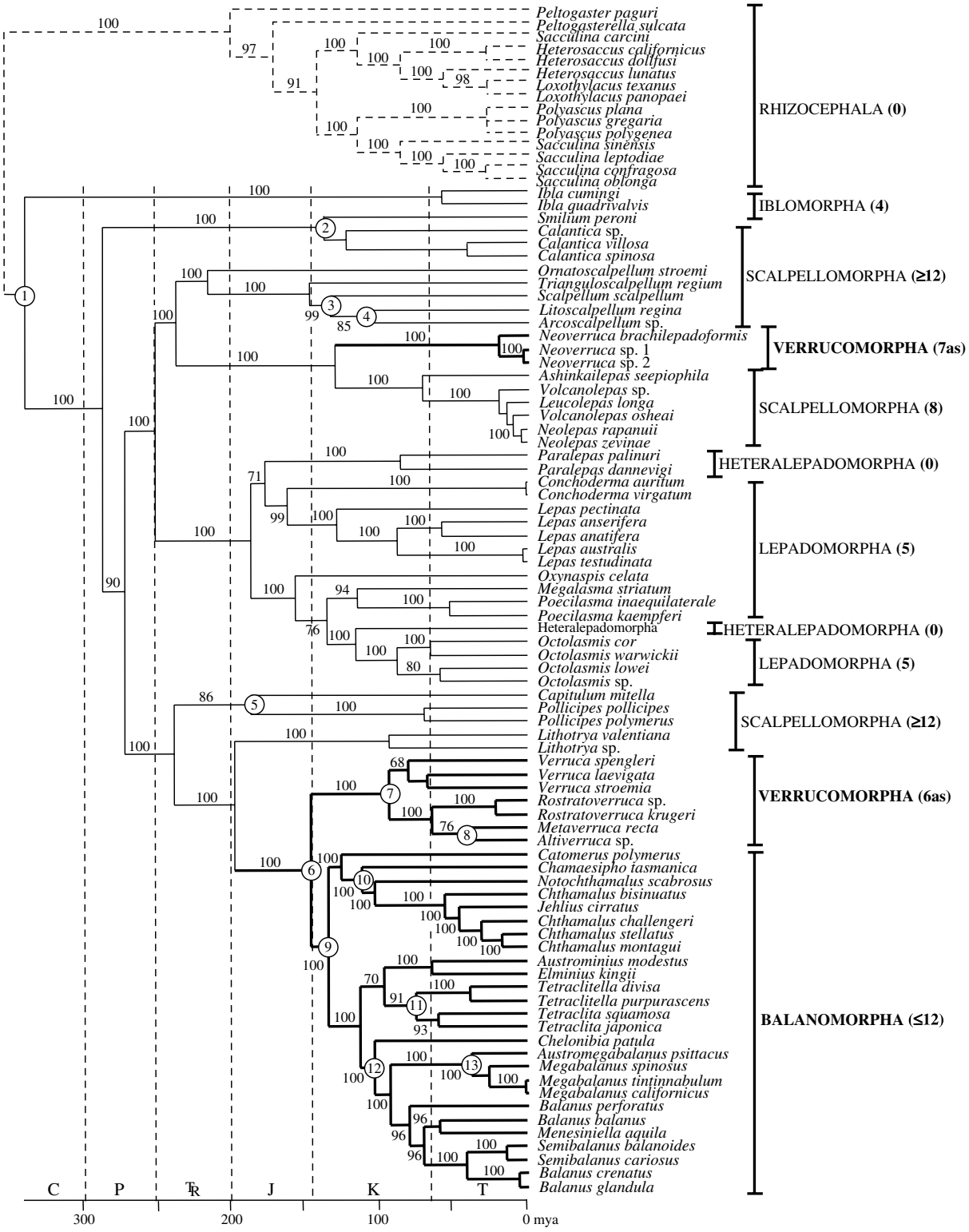

Fig. 3. Thoracican divergence time chronogram estimated using the 50% majority-rule consensus BMCMC tree of morphological and molecular data under mix-models. Clade posterior probabilities (if ⩾50%) are shown for each node. Fossil calibration nodes are indicated by C1–C14, corresponding with Table 2. Numbers in parentheses after taxon names indicate the number of shell plates, as = assymmetric disposition of plates. The major geologic periods are also mapped onto the phylogeny using the following standard symbols: D, Devonian; C, Carboniferous; P, Permian; TR, Triassic; J, Jurassic; K, Cretaceous, T, Tertiary. Dashed, thin, and thick lines are used for the Rhizocephala (outgroup), Pedunculata, and Sessilia clades, respectively.

Octolasmis, while *Paralepas* is the sistergroup to the clade comprising *Conchoderma* and *Lepas*. We surmise that also the five heteralepadomorphan families not represented here (Martin and Davis, 2001) have secondarily lost their plates, but, in view of the polyphyly of the Heteralepadomorpha, only future studies can decide on

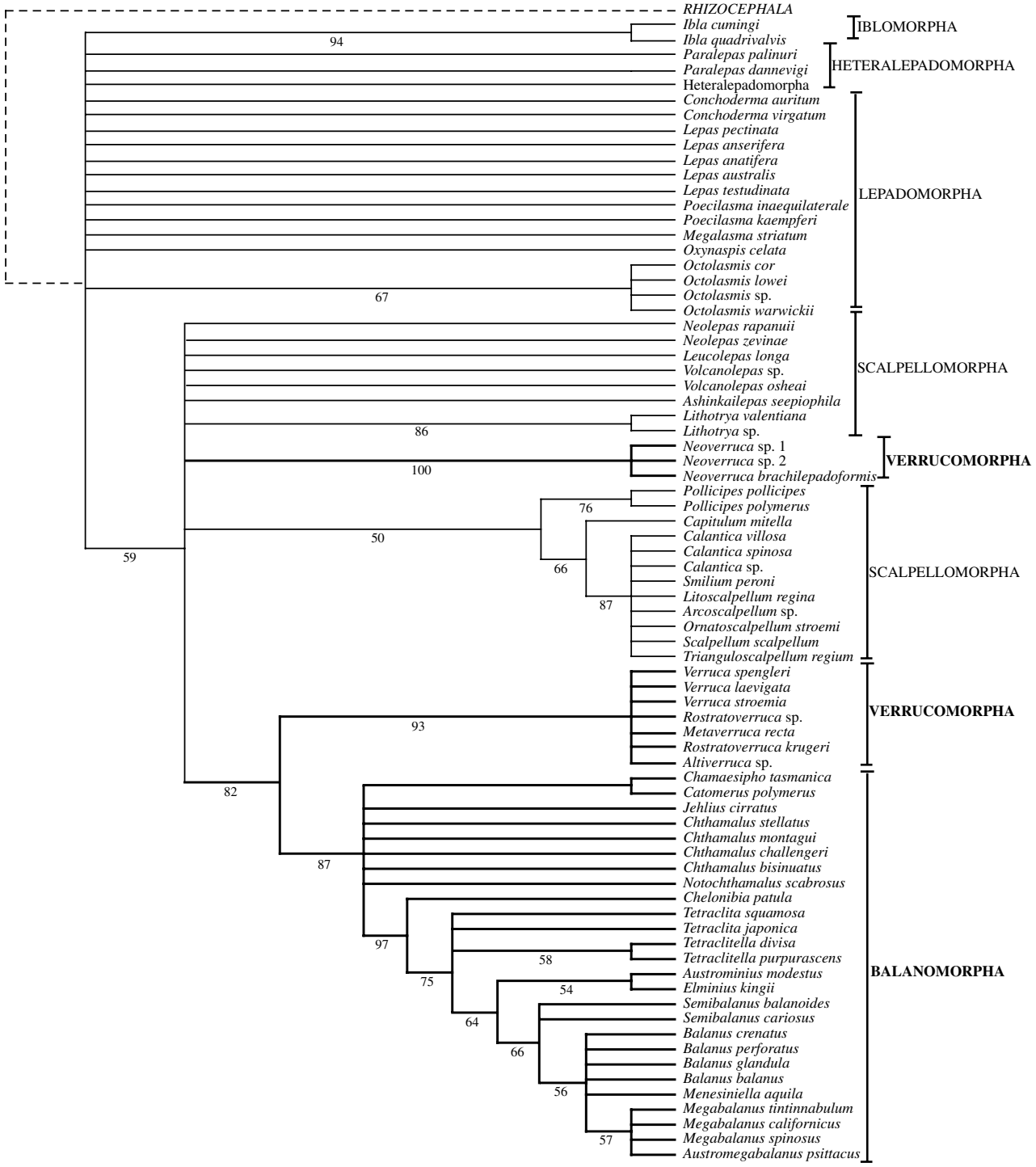

Fig. 4. Strict consensus tree of 10^7 (10,000 maximum trees saved per replicate) most parsimonious trees using morphological data. Bootstrap proportions (if $\geqslant$50%) are shown under the branches. Dashed, thin, and thick lines are used for the Rhizocephala (outgroup), Pedunculata, and Sessilia clades, respectively.

the phylogenetic position of these highly specialized thoracicans (see also Grygier and Newman, 1991; Yusa et al., 2001).

4.2.2. Shell plate mineralization

Growth in thoracican cirripedes is unlike anything seen elsewhere in arthropods. The shell plates are mineralized areas of the general cuticle, but they are not shed in a series of moults. Instead they are retained and increase gradually in size along their circumference (Anderson, 1994; Blomsterberg et al., 2004). Tracing the origin and evolution of shell plates is therefore pivotal not only for understanding the success of thoracican barnacles but also in terms of the potential contained in arthropod cuticle in general. In our

Table 4
Divergence times (and 95% CI) for major thoracican lineages as estimated from the T–K method based on three genes and 14 fossil calibrations and using the Bayesian tree (Fig. 3)

Taxon	Divergence time (95% CI) (mya)	
	Stem node	Crown node
Pedunculata		
Iblomorpha	340 (310–414)	58 (21–127)
Scalpellomorpha		
Calanticidae	287 (241–351)	138 (91–202)
Scalpellidae + Eolepadidae	252 (207–310)	238 (194–294)
Scalpellidae	238 (194–294)	216 (170–270)
Eolepadidae	131 (73–196)	72 (29–129)
Pollicipedidae	239 (195–294)	188 (132–249)
Lithotryidae	198 (162–242)	94 (46–152)
Heteralepadomorpha	252 (207–310)	187 (139–247)
Heteralepadidae	177 (130–236)	86 (23–156)
Unknown species	116 (73–170)	—
Lepadomorpha	252 (207–310)	187 (139–247)
Sessilia		
Verrucomorpha	147 (131–153)	94 (79–129)
Balanomorpha	147 (131–153)	135 (114–150)

Divergence times are both provided for the stem and crown nodes, when possible, in each clade.

analysis, the basal position of the Iblomorpha indicates that already the last common ancestor to all extant thoracican possessed mineralized plates. The Iblomorpha have four plates stated to be phosphatic, while all other armed thoracicans have at least five calcitic plates (Høeg et al., 1999; Whyte, 1988). This difference has prompted speculations that mineralization evolved independently in the two clades (Høeg et al., 1999). But recently, Buckeridge and Newman (2006) noted that the Iblomorpha seem to have some calcium carbonate in their plates. If true, this would further cement the concept of a basic homology of shell plates in all Thoracica and confirm Pérez-Losada et al. (2004) that phosphatized plates can change into calcitic ones.

4.2.3. Can ontogeny explain shell plate numbers?

Our analyses indicate that the dominant theory on shell plate evolution (Newman, 1987, 1989), largely derived from the study of ontogeny, cannot adequately explain thoracican phylogeny. Almost all recent analyses of thoracican evolution, whether cladistically framed or not, have accepted that the Thoracica originated from forms with four or five plates around the capitulum as in extant Iblomorpha and Lepadomorpha and evolved by the gradual addition of plates ($6 \rightarrow 8 \rightarrow 12$ plates) and culminating in multiplated forms (Anderson, 1994; Buckeridge and Newman, 2006; Glenner et al., 1995; Newman, 1987; Newman et al., 1969). Some larval evidence, however, has suggested that some multiplated forms could be basal in the Thoracica (Korn, 1995; Moyse, 1987). The low number of plates found in most Verrucomorpha and Balanomorpha are assumed to result from loss of the imbricating plates around the base of wall and, for the Balanomorpha, also fusions of wall plates themselves (characters 14 and 15). The outgroups lack shell plates altogether, so the hypothesis of a gradual increase in plate number stems primarily from the study of ontogeny and, to a lesser extent, on the stratigraphical occurrence of fossil forms. After settlement of the cypris larva, pedunculated thoracicans, except the Iblomorpha, start juvenile growth with the simultaneous appearance of five plates; thereafter, additional plates appear sequentially in a $6 \rightarrow 8 \rightarrow 12+$ pattern. The original five plates are preceded in the metamorphosing cyprid by 5 so-called primordial plates made of cuticle only, indicating that this number is a basal one. The theory also dovetails with the presence of five unmineralized plates in the Carboniferous *Praelepas jaworskii*, the earliest fossil that can be relegated to the Thoracica with certainty, but it confounds the pattern considerably that some scalpellomorphan forms actually have more than five primordial plates (Newman et al., 1969).

A sequential addition of shell plates was largely compatible with the cladograms in Glenner et al. (1995), but could not be confirmed by our molecular or combined dataset. In the MP tree the Calanticidae diverge after the Heteralepadomorpha–Lepadomorpha clade formation, but in our best-fit model based ML and well-supported Bayesian analyses, the multiplated Calanticidae diverge basal to most Thoracica as the first group to diverge after the Iblomorpha. Moreover, in all of our trees, all 5-plated (*Lepas, Octolasmis, Oxynaspis, Megalasma*) and 8-plated (*Neolepas, Ashinkailepas, Volcanolepas*) taxa are nested within multiplated forms. Even when used alone, our morphological characters did indicate that shell plates accumulated gradually in number probably because our taxon sampling is much more extensive and include many additional traits from both hard and soft parts than in Glenner et al. (1995). We hence conclude that extant 5- and 8-plated forms evolved secondarily from a multiplated condition. Five-plated forms that can securely be relegated to extant taxa are not found below the lower Tertiary, while multiplated forms extend deep into the Mesozoic. In the Palaeozoic (Carboniferous), we find a few forms with five plates (*Praelepas, Illilepas*), but they have uncertain systematic affiliation (Glenner et al., 1995; Schram, 1975), and the stratigraphical evidence is all too weak to be used alone to polarise the evolution of numbers of shell plates. Further complicating the issue, the basal Iblomorpha show no evidence of having ever had more than four plates (Buckeridge and Newman, 2006). It remains possible, that 5- and 6-plated thoracicans appeared before multiplated forms, but, if so, they are most likely not related to extant species with five plates. Thus, future studies should be very cautious in uncritically using ontogenetic or fossil data to establish character polarities and phylogenies in the Thoracica.

4.2.4. Asymmetric barnacles and sessilian monophyly

One of the most controversial issues in thoracican phylogeny concerns the sessilian forms (i.e., those that lack a

peduncle altogether). Competing polyphyletic (Anderson, 1994; Korn, 1995; Moyse, 1987; Newman and Ross, 1976; Newman et al., 1969; Nilsson-Cantell, 1921; Pilsbry, 1907, 1916; Utinomi, 1968; Zullo, 1963) and monophyletic (Buckeridge and Newman, 1992; Newman, 1987; Newman and Yamaguchi, 1995; Newman and Hessler, 1989) hypotheses have been proposed, but until recently none of them had been properly subjected to a phylogenetic test. The polyphyletic hypothesis states that the Verrucomorpha and Balanomorpha are related to different pedunculated groups, whence the peduncle has been convergently lost. In its extreme version, it has even been suggested that the Balanomorpha evolved polyphyletically (Newman and Ross, 1976). The monophyletic hypothesis argues that the absence of a peduncle and other putative similarities represent synapomorphies between the Verrucomorpha and Balanomorpha (Newman, 1987). Our new molecular and morphological analyses reject both the Verrucomorpha and the Sessilia as being monophyletic in the taxon definitions of Newman (1996) and Martin and Davis (2001). Instead, our two species of *Neoverruca* (Verrucomorpha, Neoverrucidae) were clustered with the pedunculated Neolepadinae with high confidence. On the other hand, we observed strong support for a Verrucidae–Balanomorpha relationship and therefore of sessilian monophyly in the formulation of Newman (1987).

In the Balanomorpha, the shell plates are divided into a fixed wall encircling the animal and a movable operculum, which, when closed, seals off the mantle cavity as a completely closed chamber. The resulting closed chamber around the barnacle body is virtually water tight, and this very specialized morphology offers not only protection against predators, but enables balanomorphans to endure exposure at low tides. Hence the balanomorphan morphology is crucial in explaining the tremendous success of these barnacles. The Verrucomorpha is a moderately successful group and never inhabits the intertidal. They have a distinctly asymmetric body shape, because the four opercular plates have separated to become part of the fixed wall on one side and a movable lid on the other. Arguing for a monophyletic Sessilia based on morphology alone as in Newman (1987) poses many obstacles. Some putatively basal balanomorphans show considerable affinity to some pedunculated forms. Chthamaloids, such as *Catomerus*, have one or several tiers of small plates encircling the body below the true wall plates (character 17), and this resembles the situation in the Pollicipedidae and Lithotryidae. There are also other assumed plesiomorphic traits that link the morphology of balanomorphans to that in pedunculated thoracicans (Buckeridge, 1995; Buckeridge and Newman, 1992; Newman, 1996; Yamaguchi and Newman, 1990). In contrast, little in the morphology of extant Verrucidae links them to a pedunculated ancestry. It was therefore exciting when Newman (1989) and Newman and Hessler (1989) described *Neoverruca* (Neoverrucidae) from hydrothermal vent habitats as having an asymmetrical sessilian morphology that is seemingly intermediate between the Verrucidae and the assumed ground pattern morphology for the Balanomorpha (Buckeridge, 1995; Buckeridge and Newman, 1992; Newman and Yamaguchi, 1995). *Neoverruca* has a transient peduncle during its early ontogeny (character 2), tiers of imbricating plates around the base of the wall (character 17), and what is claimed to be a true lateral plate (character 10), asymmetrically placed on one side of the body only. However, it severely complicates the picture that neither the Verrucidae, their fossil relatives (Proverrucidae) or even the Balanomorpha are supposed to have true Median Latera (character 10) (see Glenner et al., 1995; Newman, 1996).

The morphology-based analyses of Glenner et al. (1995), Høeg et al. (1999), and Newman and Ross (1976) also found a sistergroup relationship between the traditional verrucomorphans (Verrucidae) and the Balanomorpha and therefore, like the molecular analysis of Pérez-Losada et al. (2004), were in support of a monophyletic Sessilia as defined by Newman (1987). But Glenner et al. (1995) could not confirm a monophyletic Verrucomorpha including both the Verrucidae and *Neoverruca* (Neoverrucidae). Instead, *Neoverruca* formed part of a polytomy below the base of the Sessilia, and in some trees it clustered with the Brachylepadomorpha.

We must therefore conclude that an asymmetric disposition of shell plates evolved independently in the Neoverrucidae and in the Verrucidae–Balanomorpha clade. The morphological similarity between *Neoverruca* and the other Verrucomorpha, including the fossil Proverrucidae is somewhat tenuous. Neither the Proverrucidae nor the Verrucidae have tiers of imbricating plates. *Neoverruca* has a single asymmetrically placed Upper Latus (character 10), but the two lateral plates in the Proverrucidae have very uncertain homologies and the Balanomorpha seem not to possess a true Median Latus (Yamaguchi and Newman, 1990). In our analysis, the presence of tiers of imbricating plates (character 17, state 1) in *Neoverruca*, basal Balanomorpha, the Pollicipedidae and the Lithotryidae becomes an old character rather than one characterizing only the Balanomorpha and their closest pedunculated relatives, as in Glenner et al. (1995). In this context, it would be interesting to see where *Neobrachylepas*, the only extant member of the Brachylepadomorpha, would fit when analyzed from DNA sequences. All species of the Neoverrucidae–Neolepadinae clade inhabit hydrothermal vents. This points to a monophyletic origin of these vent forms, although they obviously have not at all retained a consistently plesiomorphic morphology as is sometimes assumed for the vent fauna (Newman, 1979, 1985; Svane, 1986). If thoracicans did indeed evolve from forms with few plates, the Neolepadinae would actually exemplify a secondary reversal to this state. The alternative hypothesis, that multiplated forms evolved convergently, is not supported by our trees. Again, our analyses put caution in a heavy reliance on shell plates and their ontogenetic pattern when analyzing thoracican phylogeny.

4.2.5. Males and reproductive systems

An extensive literature has dealt with the evolution of sexual systems in barnacles (Buckeridge and Newman, 2006; Høeg, 1995; Newman, 1996). A separate male sex occurs in the outgroups and also in some thoracicans, which can have either truly separate sexes (dioecy) or males combined with hermaphrodites (androdioecy) (Buhl-Mortensen and Høeg, 2006; Svane, 1986). In our phylogeny the presence of males becomes a plesiomorphy taken over from the cirripede ground pattern and retained in both the Iblomorpha and the Calanticidae, but those in the Scalpellidae seem to have evolved secondarily from a purely hermaphroditic ancestor. This is just one example of how a robust phylogeny can be used to trace the evolution of complex life history traits.

4.3. Tempo of barnacle evolution

The divergence time estimates presented here (Table 4) are considerably younger than previous estimates in Pérez-Losada et al. (2004), which were as follows: Iblomorpha = 576 to 544 mya, Heteralepadomorpha + Lepadomorpha = 530 to 500 mya, Scalpellomorpha = 360 to 340 mya, Verrucomorpha and Balanomorpha = 189 to 179 mya. Three main factors have contributed to this discrepancy: here we accommodated many more and different fossil calibrations and none of them were used to fix node ages; we added two more loci (28S and H3) to our 18S sequence anlaysis, and our Bayesian T–K analysis is based on a different and more robust phylogeny. The ancient thoracican estimates shown in Pérez-Losada et al. (2004) were mainly a consequence of using two fossil calibrations, the heteralepadomorphan *Priscansermarinus* (dated to 530 mya) and the scalpellomorph *Pabulum* (dated to 340 mya). The affiliation of these two fossils has been recently questioned (Buckeridge and Newman, 2006). *Priscansermarinus* is very uncertain as a thoracican (Briggs, 1983; Briggs et al., 2005) and *Pabulum* is now considered a bivalve (Martin Whyte, personal communication); therefore, we opted for more reliable calibrations. Our oldest fossil calibration was *Praelepas jaworskii* from the Carboniferous (M. Pennsylvanian—306.5–311.7 mya), which was used to date the minimum age of the Thoracica. The second factor contributing to the decrease of the time estimates presented here is the topological positions of the Scalpellomorpha and Lepadomorpha + Heteralepadomorpha. Contrary to Pérez-Losada et al. (2004), the Scalpellomorpha (Calanticidae) were basal to the Lepadomorpha + Heteralepadomorpha, which results in younger estimates for the latter. Finally our new analyses included two new genes and 14 fossil calibrations, which were used to constrain nodes to minimum or maximum ages; such an improvement has probably contributed to reduce the intrinsic inaccuracy of the fossil record associated to our estimates (Porter et al., 2005; Yang and Yoder, 2003). In conclusion, we find our new time estimates more accurate and reliable than those previously published by our group and until new barnacle fossils and molecular data are collected, we recommend using this chronogram (Fig. 3) for further studies on barnacle radiation. But how realistic are these estimates? Some corroboration can be obtained from the fossil record. For example, the presumptive iblomorph *Illilepas damrowi* (Schram, 1986) from the Carboniferous (299–359 mya), if confirmed [although see Buckeridge and Newman (2006) for a different opinion], would agree reasonably well with our molecular estimates.

5. Conclusions

Our analyses yielded a well-supported phylogeny with new estimates of divergence times among the major radiations of barnacles. We confirm some traditional taxonomic groups but reject many others and therefore also many of the underlying hypotheses about character evolution in the Cirripedia Thoracica, which are now reinterpreted based on our findings. A major rearrangement in thoracican systematics is needed to better reflect the evolutionary history of the group and the traditional suite of mainly shell plate characters was not sufficient to resolve basic patterns of phylogenetic relationships.

Acknowledgments

We are very grateful to William Newman for his advise, samples, discussions, friendship, and enthusiasm. We also thank Janet Voight, Robert Vrijenhoek, Toshiyuki Yamaguchi, Ryusuke Kado, Armand Kuris, and Verena Tunnicliffe for sampling specimens crucial to this study. This research was supported by the Grants NSF DEB-0236135 to K.A.C. and MP-L; the US–Israel Binational, Scientific Fund (BSF) Grant 2004-239, to Y.A. and K.A.C.; and the Danish Natural Science Research Council and European Union Framework 5 and six Grants under the COBICE and SYNTHESYS programs to J.T.H. Finally, we are all indebted to three excellent referees for numerous helpful comments on earlier drafts of this manuscript.

Appendix A

Morphological character matrix for the Thoracica (ingroup) and Rhizocephala (outgroup)

Pérez-Losada et al. (2004) character numbers	1		2	3	6		7	8	9	10	11	12	14	13	34		20	21	15		26	24	25	27	16		17	18	19	22	23		32	36		33	31	37	28	29	30	5	4	
New character numbers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Altiverruca sp.	0	0	?	1	1	0	1	1	0	0	0	0	0	?	?	0	0	0	1	0	0	?	0	0	0	1	1	1	1	1	1	?	0	0	1	1	1	0	0	0	0	0	1	0
Arcoscalpellum sp.	1	1	1	1	1	0	1	1	1	1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1
Ashinkailepas seepiophila	1	1	1	1	1	0	1	1	0	1	0	0	0	0	?	0	0	0	0	0	0	?	?	0	0	1	0	0	?	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0
Austromegabalanus psittacus	0	0	?	?	1	0	1	1	0	0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	?	1	1	1	1	1	1	0	1	0	0	1	0
Austrominius modestus	0	0	?	1	1	0	1	1	0	0	1	1	1	1	1	0	0	0	1	0	1	0	1	0	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Balanus balanus	0	0	?	?	1	0	1	1	0	0	1	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Balanus crenatus	0	0	?	?	1	0	1	1	0	0	1	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Balanus glandula	0	0	?	?	1	0	1	1	0	0	1	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Balanus perforatus	0	0	?	?	1	0	1	1	0	0	1	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Calantica sp.	1	1	1	1	1	0	1	1	1	1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	?	0	1	1
Calantica spinosa	1	1	1	1	1	0	1	1	1	1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	?	0	1	1
Calantica villosa	1	1	1	1	1	0	1	1	1	1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1
Capitulum mitella	1	1	1	1	1	0	1	1	1	1	1	1	0	0	?	1	1	0	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0
Catomerus polymerus	0	0	?	?	1	0	1	1	0	0	1	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	1	0	1	1	0	0	1	0
Chamaesipho tasmanica	0	0	?	?	1	0	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	1	0	1	1	0	0	1	0
Chelonibia patula	0	0	?	?	1	0	1	1	0	0	1	1	1	1	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	1	0	0	?	1	0	1	1	0	0	0	1	0	0	1	0
Chthamalus bisinuatus	0	0	?	?	1	0	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	0	0	0	1	0	0	1	0
Chthamalus challengeri	0	0	?	?	1	0	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	0	0	0	1	0	0	1	0
Chthamalus montagui	0	0	?	?	1	0	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	0	0	0	1	0	0	1	0
Chthamalus stellatus	0	0	?	?	1	0	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	0	0	0	1	0	0	1	0
Conchoderma auritum	1	1	0	?	1	0	1	0	?	0	0	0	0	?	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	1	0	0	0	1	0	1	0	1	0	1	0
Conchoderma virgatum	1	1	0	?	1	0	1	0	?	0	0	0	0	?	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	?	0	0	0	1	0	1	0	1	0	1	0
Elminius kingii	0	0	?	?	1	0	1	1	0	0	1	1	1	1	1	0	0	0	1	0	1	0	1	0	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Heteralepadomorpha (unclassified species)	1	1	0	?	0	?	0	0	?	0	0	0	0	?	?	?	0	0	?	0	?	?	?	?	?	1	?	?	?	?	?	?	0	0	0	0	1	0	0	0	1	0	1	0
Ibla cumingi	1	1	0	1	1	0	0	0	?	0	0	0	0	?	?	0	0	0	?	0	?	?	?	?	?	0	?	0	?	0	?	?	0	0	0	0	1	0	1	0	1	1	0	1
Ibla quadrivalvis	1	1	0	1	1	0	0	0	?	0	0	0	0	?	?	0	0	0	?	0	?	?	?	?	?	0	?	0	?	0	?	?	0	0	0	0	1	0	1	0	1	1	0	1
Jehlius cirratus	0	0	?	?	1	0	1	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	?	0	0	1	0	0	1	0
Lepas anatifera	1	1	0	1	1	0	1	0	?	0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Lepas anserifera	1	1	0	1	1	0	1	0	?	0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Lepas australis	1	1	0	1	1	0	1	0	?	0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Lepas pectinata	1	1	0	1	1	0	1	0	?	0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Lepas testudinata	1	1	0	1	1	0	1	0	?	0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Leucolepas longa	1	1	1	1	1	0	1	1	0	1	0	0	0	0	?	0	0	0	0	0	0	?	?	0	0	1	0	0	?	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0
Lithotrya sp.	1	1	1	1	1	0	1	1	0	1	0	0	0	0	?	1	1	0	0	1	0	?	?	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0
Lithotrya valentiana	1	1	1	1	1	0	1	1	0	1	0	0	0	0	?	1	1	0	0	1	0	?	?	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0
Litoscalpellum regina	1	1	1	1	1	0	1	1	1	1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1

Megabalanus californicus	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Megabalanus spinosus	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Megabalanus tintinnabulum	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Megalasma striatum	1 1 ? 1 1 0 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	?	0	0	0	1	0	1	0	?	0	1	0
Menesiniella aquila	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Metaverruca recta	0 0 ? 1 1 0 1 1 0 0	0	0	0	?	?	0	0	0	1	0	0	?	0	0	0	1	1	1	1	1	1	?	0	0	1	1	1	0	0	0	0	0	1	0
Neolepas rapanuii	1 1 1 1 1 0 1 1 0 1	0	0	0	0	?	0	0	0	0	0	0	?	?	0	0	1	0	0	?	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0
Neolepas zevinae	1 1 1 1 1 0 1 1 0 1	0	0	0	0	?	0	0	0	0	0	0	?	?	0	0	1	0	0	?	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0
Neoverruca brachilepadoformis	0 1 ? 1 1 0 1 1 0 1	0	0	0	0	?	1	1	0	0	0	0	?	?	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	1	0	1	0	1	0
Neoverruca sp. 1	0 1 ? 1 1 0 1 1 0 1	0	0	0	0	?	1	1	0	0	0	0	?	?	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	1	0	1	0	1	0
Neoverruca sp. 2	0 1 ? 1 1 0 1 1 0 1	0	0	0	0	?	1	1	0	0	0	0	?	?	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	1	0	1	0	1	0
Notochthamalus scabrosus	0 0 ? ? 1 0 1 1 0 0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	?	0	0	1	0	0	0	0	1	0	0	1	0
Octolasmis cor	1 1 0 1 1 1 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Octolasmis lowei	1 1 0 1 1 1 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Octolasmis sp.	1 1 0 1 1 1 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Octolasmis warwickii	1 1 0 1 1 1 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Ornatoscalpellum stroemi	1 1 1 1 1 0 1 1 1 1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1
Oxynaspis celata	1 1 0 1 1 0 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	?	0	0	0	1	0	0	0	?	0	1	0
Paralepas dannevigi	1 1 0 ? 0 ? 0 0 ? 0	0	0	0	?	?	?	0	0	?	0	?	?	?	?	?	1	?	?	?	?	?	?	0	0	0	0	1	0	0	0	1	0	1	0
Paralepas palinuri	1 1 0 ? 0 ? 0 0 ? 0	0	0	0	?	?	?	0	0	?	0	?	?	?	?	?	1	?	?	?	?	?	?	0	0	0	0	1	0	0	0	1	0	1	0
Poecilasma inaequilaterale	1 1 0 1 1 0 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Poecilasma kaempferi	1 1 0 1 1 0 1 0 ? 0	0	0	0	?	?	0	0	0	0	0	?	?	?	?	?	1	0	0	?	0	?	?	0	0	0	0	1	0	1	0	1	0	1	0
Pollicipes pollicipes	1 1 1 1 1 0 1 1 0 1	1	1	0	0	?	1	1	0	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0
Pollicipes polymerus	1 1 1 1 1 0 1 1 0 1	1	1	0	0	?	1	1	0	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0
Rostratoverruca sp.	0 0 ? 1 1 0 1 1 0 0	0	0	0	?	?	0	0	0	1	0	0	?	0	0	0	1	1	1	1	1	1	?	0	0	1	1	1	0	0	0	0	0	1	0
Rostratoverruca krugeri	0 0 ? 1 1 0 1 1 0 0	0	0	0	?	?	0	0	0	1	0	0	?	0	0	0	1	1	1	1	1	1	?	0	0	1	1	1	0	0	0	0	0	1	0
Scalpellum scalpellum	1 1 1 1 1 0 1 1 1 1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1
Semibalanus balanoides	0 0 ? 1 1 0 1 1 0 0	1	1	1	1	0	0	0	0	1	0	1	0	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Semibalanus cariosus	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	0	0	0	0	1	0	1	0	1	1	1	1	1	1	1	0	0	?	1	1	1	1	0	1	0	1	0	0	1	0
Smilium peroni	1 1 1 1 1 0 1 1 1 1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1
Tetraclita japonica	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	1	0	0	0	1	0	1	0	1	1	1	1	1	1	1	0	0	?	1	0	1	1	0	0	0	1	0	0	1	0
Tetraclita squamosa	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	1	0	0	0	1	0	1	0	1	1	1	1	1	1	1	0	0	?	1	0	1	1	0	0	0	1	0	0	1	0
Tetraclitella divisa	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	1	0	0	?	1	0	1	1	0	0	0	1	0	0	1	0
Tetraclitella purpurascens	0 0 ? ? 1 0 1 1 0 0	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	1	0	0	?	1	0	1	1	0	0	0	1	0	0	1	0
Trianguloscalpellum regium	1 1 1 1 1 0 1 1 1 1	1	1	0	0	?	1	0	1	0	0	0	?	?	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1
Verruca laevigata	0 0 ? 1 1 0 1 1 0 0	0	0	0	?	?	0	0	0	1	0	0	?	0	0	0	1	1	1	1	1	1	?	0	0	1	1	1	0	0	0	0	0	1	0
Verruca spengleri	0 0 ? 1 1 0 1 1 0 0	0	0	0	?	?	0	0	0	1	0	0	?	0	0	0	1	1	1	1	1	1	?	0	0	1	1	1	0	0	0	0	0	1	0
Verruca stroemia	0 0 ? 1 1 0 1 1 0 0	0	0	0	?	?	0	0	0	1	0	0	?	0	0	0	1	1	1	1	1	1	?	0	0	1	1	1	0	0	0	0	0	1	0
Volcanolepas osheai	1 1 1 1 1 0 1 1 0 1	0	0	0	0	?	0	0	0	0	0	0	?	?	0	0	1	0	0	?	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0
Volcanolepas sp.	1 1 1 1 1 0 1 1 0 1	0	0	0	0	?	0	0	0	0	0	0	?	?	0	0	1	0	0	?	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0
Rhizocephala	? ? ? 0 0 ? 0 0 ? 0	0	0	0	?	?	?	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	1

Characters are described in Appendix B. 1 = present, 0 = absent, ? = unknown or inapplicable.

Appendix B

List of morphological characters. A more detailed description of the characters is provided in Newman and Ross (1976), Buckeridge (1995), Glenner et al. (1995), and Høeg et al. (1999)

(1) Peduncle in adult: 0 = absent; 1 = present
(2) Peduncle during ontogeny: 0 = no; 1 = yes
(3) Peduncular scales: 0 = absent; 1 = present
(4) Primordial valves (plates): 0 = absent; 1 = present
(5) Scuta/terga (S/T): 0 = absent: 1 = present
(6) Division of S: 0 = not sub-divided; 1 = divided in two parts, contiguous at least by chitinous parts
(7) Carina (C): 0 = absent; 1 = present
(8) rostrum (R): 0 = absent; 1 = present
(9) Relative size of R/C: (0) C $\leqslant$ 2 times length of R; (1) C $>$ 2 times length of R
(10) Medial latus (L): 0 = absent; 1 = present
(11) Carinolatus (CL): 0 = absent; 1 = present
(12) Rostrolatus (RL): 0 = absent; 1 = present
(13) CL2 (duplication of CL): 0 = absent; 1 = present
(14) Compound rostrum, (RL + R + RL) or (RL + RL): 0 = not fused; 1 = fused
(15) Fusion CL1 + CL2: 0 = not fused; 1 = fused
(16) Number of shell plates: 0 = 8 or less; 1 = 12 or more
(17) Imbricating whorls of overlapping, small plates: 0 = absent; 1 = present
(18) Small isolated plates beneath C–R tier: 0 = absent; 1 = present
(19) Wall of shell plates: 0 = not in contact with substratum; 1 = in contact with substratum
(20) Base (contact to substratum): 0 = membranous: 1 = calcareous
(21) Radii: 0 = absent; 1 = present
(22) Tubiferous radii: 0 = absent; 1 = present
(23) Parietal chitin: 0 = absent; 1 = present
(24) Tubiferous parietal (wall) plates: 0 = absent; 1 = present
(25) Sheath: 0 = absent; 1 = present
(26) Body (soma) long axis: 0 = at right angles to substratum; 1 = semi-parallel with substratum
(27) Opercular plates (S–T) separation from wall plates (C-Latera–R). 0 = no operculum (not separated); 1 = Operculum present (S–T on at least one side separated from wall plates)
(28) Hinge (articulation S–T): 0 = no hinge; 1 = hinge present
(29) Hinge complexity: 0 = hinge plates movably interlocked; 1 = highly complex hinge
(30) Symmetry of S–T: 0 = free and identical on both sides; 1 = fixed on one side, movable on the other
(31) Symmetry of C–R: 0 = both symmetrical; 1 = asymmetrical and meet on one side
(32) Symmetry of L: 0 = L present on both sides and symmetrical; 1 = present on one side only
(33) Labrum shape: 0 = strongly bullate; 1 = weakly bullate or thin
(34) Labrum crest: 0 = not deeply incised; 1 = deeply incised
(35) Cirrus II mouth cirrus (resembles Cirrus I and does not form part of the feeding basket): 0 = no; 1 = yes
(36) Cirrus III mouth cirrus: 0 = no, 1 = yes
(37) Caudal filaments: 0 = absent; 1 = present
(38) Basi-dorsal joint in penis: 0 = absent; 1 = present
(39) Ovigerous frenae: 0 = absent; 1 = present
(40) Branchiae: 0 = absent; 1 = present
(41) Filamentary appendages: 0 = absent; 1 = present
(42) Comb collar: 0 = absent; 1 = present
(43) Musculus adductor scutorum: 0 = postoral; 1 = preoral
(44) Dwarf (or complemental) males: 0 = absent; 1 = present

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