

Unraveling the Evolutionary Radiation of the Thoracican Barnacles Using Molecular and Morphological Evidence: A Comparison of Several Divergence Time Estimation Approaches

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Abstract.—The Thoracica includes the ordinary barnacles found along the sea shore and is the most diverse and well-studied superorder of Cirripedia. However, although the literature abounds with scenarios explaining the evolution of these barnacles, very few studies have attempted to test these hypotheses in a phylogenetic context. The few attempts at phylogenetic analyses have suffered from a lack of phylogenetic signal and small numbers of taxa. We collected DNA sequences from the nuclear 18S, 28S, and histone H3 genes and the mitochondrial 12S and 16S genes (4,871 bp total) and data for 37 adult and 53 larval morphological characters from 43 taxa representing all the extant thoracican suborders (except the monospecific Brachylepadomorpha). Four Rhizocephala (highly modified parasitic barnacles) taxa and a Rhizocephala + Acrothoracica (burrowing barnacles) hypothetical ancestor were used as the outgroup for the molecular and morphological analyses, respectively. We analyzed these data separately and combined using maximum likelihood (ML) under “hill-climbing” and genetic algorithm heuristic searches, maximum parsimony procedures, and Bayesian inference coupled with Markov chain Monte Carlo techniques under mixed and homogeneous models of nucleotide substitution. The resulting phylogenetic trees answered key questions in barnacle evolution. The four-plated Iblomorpha were shown as the most primitive thoracican, and the plateless Heteralepadomorpha were placed as the sister group of the Lepadomorpha. These relationships suggest for the first time in an invertebrate that exoskeleton biomineralization may have evolved from phosphatic to calcitic. Sessilia (nonpedunculate) barnacles were depicted as monophyletic and appear to have evolved from a stalked (pedunculate) multiplated (5+) scalpelloidlike ancestor rather than a five-plated lepadomorphan ancestor. The Balanomorpha (symmetric sessile barnacles) appear to have the following relationship: (Chthamaloidea(Coronuloidea(Tetraclitoidae, Balanoidea))). Thoracican divergence times were estimated under ML-based local clock, Bayesian, and penalized likelihood approaches using an 18S data set and three calibration points: Heteralepadomorpha = 530 million years ago (MYA), Scalpellomorpha = 340 MYA, and Verrucomorpha = 120 MYA. Estimated dates varied considerably within and between approaches depending on the calibration point. Highly parameterized local clock models that assume independent rates ($r \geq 15$) for confamilial or congeneric species generated the most congruent estimates among calibrations and agreed more closely with the barnacle fossil record. Reasonable estimates were also obtained under the Bayesian procedure of Kishino et al. (2001, Mol. Biol. Evol. 18:352–361) but using multiple calibrations. Most of the dates estimated under the Bayesian procedure of Aris-Brosou and Yang (2002, Syst. Biol. 51:703–714) and the penalized likelihood method using single and/or multiple calibrations were inconsistent among calibrations and did not fit the fossil record. [Calibration point; Cirripedia; divergence times; DNA sequences; fossils; genetic algorithms; mixed models; phylogeny.]

The barnacles (Crustacea: Cirripedia: Thoracica) are among the most commonly encountered marine crustaceans in the world. They deviate from other crustaceans in having irreversibly sessile (nonmobile) adults, a habit that has been accompanied by dramatic changes in both morphology and biology. These animals have dispensed with the normal crustacean scheme of growing by molting the entire exoskeleton and are instead clad in a protective armor of mineralized shell plates. They are thoracopodal filter feeders, and the majority of them are hermaphrodites (Anderson, 1994). These drastic adaptations make barnacles excellent model organisms for evolutionary studies (Schram and Høeg, 1995).

Barnacles have attracted the attention of many prominent specialists, most notably Charles Darwin, whose seminal monographs (1851–1855), contain a prodigious body of information on barnacle biology, paleontology, and systematics. However, for a group that has been the focus of intense study for almost two centuries, there is still a pervasive lack of phylogenetic information (Schram and Høeg, 1995). Multiple hypotheses have been proposed concerning barnacle evo-

lution (see Newman and Ross, 1976; Newman, 1979, 1987; Newman and Hessler, 1989; Healy and Anderson, 1990; Yamaguchi and Newman, 1990; Buckeridge and Newman, 1992; Anderson, 1994; Newman and Yamaguchi, 1995; Kolbasov, 1996; Newman, 1996), but Glenner et al. (1995) were the first to apply phylogenetic approaches to determine their interrelationships. Until recently, evolutionary ideas have been presented in journals and books in the form of systematic diagrams, where extant taxa are shown as direct ancestors to present or extinct lineages. Authors followed the traditional approach of discussing every step in their drawings in terms of a limited set of “key” characters, without providing any formal matrix with which to reanalyze the hypotheses proposed or add new data. Even the most recent monograph on barnacle biology describes their phylogeny in terms of balloon diagrams replete with paraphyletic assemblages (Anderson, 1994).

Since the Glenner et al. (1995) analysis, which was based on 32 morphological characters from 26 fossils and extant taxa, only two other phylogenetic morphological studies have explored thoracican relationships; one of

these was limited to 10 stalked barnacles and 17 characters (Høeg et al., 1999), and the other (Newman and Ross, 2001) included an extensive number of thoracican genera (31) and larval features (53). Although the results of Newman and Ross fell short of expectations, morphological studies have accomplished some resolution (e.g., the monophyly of the Verrucomorpha and the monophyly of the Balanomorpha sensu stricto have been corroborated). However, these analyses revealed that several key issues in thoracican systematics remain unresolved, notably the position of the four-plated Iblomorpha and the plateless forms (Heteralepadomorpha), the transition from pedunculate (stalked) to sessile (hereinafter the term *sessile* will be used to refer to nonpedunculate forms unless otherwise stated) barnacles (see Fig. 1), the monophyly of the sessile barnacles, and the relationships among the major Balanomorpha groups (symmetric sessile barnacles).

Molecular evidence, mostly from 18S ribosomal DNA (rDNA) sequences, has supported Cirripedia (Thoracica, Rhizocephala, and Acrothoracia) monophyly (Pérez-Losada et al., 2002) and resolved sister relationships within the group (Spears et al., 1994). Using 18S rDNA data, Harris et al. (2000) and Perl-Treves et al. (2000) also examined thoracican relationships. However, some of the results from these studies are conflicting, presumably because of the different outgroups used in the analyses (Harris et al., 2000).

We used three nuclear and two mitochondrial genes and 37 adult and 53 larval (as described by Newman and Ross, 2001) morphological characters representing 47 Cirripedia species to explore thoracican evolutionary relationships using several maximum likelihood, maximum parsimony, and Bayesian phylogenetic methods. More specifically, we assessed the taxonomic positioning of the ibloids and the heteralepadoids, the acquisition of the sessile condition, the monophyly of the Sessilia and the radiation of the Balanomorpha.

Since the molecular clock hypothesis (i.e., the constancy of evolutionary rate over time) was proposed (Zuckermandl and Pauling, 1962, 1965), evidence accumulated over the last 30 years (e.g., Langley and Fitch, 1974; Britten, 1986; Gillespie, 1991; Li, 1997; Nei and Kumar, 2000) has indicated that the substitution process is overdispersed (i.e., the variance to mean ratio of the number of substitutions is generally >1) and the rates of substitution vary across lineages. Because of the serious bias in divergence time estimation that violation of the molecular clock can cause (Takezaki et al., 1995; Yoder and Yang, 2000; Soltis et al., 2002), several methods have been proposed for estimating divergence times in the absence of rate constancy. Some approaches involve selective removal of the lineages that deviate from a clocklike model (e.g., Takezaki et al., 1995), and then divergence times are calculated using the remaining lineages under the assumption of rate constancy. Other methods, however, incorporate rate variation into inference procedures using either local molecular clock models or a stochastic process to describe the evolutionary rate change over lineages.

Likelihood-based local divergence time estimation techniques allow different portions of a phylogeny to evolve at different rates but force all branches within a particular portion to evolve at the same rate (e.g., Rambaut and Broham, 1998; Yoder and Yang, 2000). The local molecular clock approach is straightforward to apply if the branches with different rates can easily be identified a priori. However, when such information is unavailable, date estimates might be sensitive to the assumptions about the rates (Aris-Brosou and Yang, 2002). In large data sets, the number of ways to assign different rates is large (Sanderson, 1998). Nevertheless, closely related lineages tend to have similar rates (Sanderson, 1997; Kishino et al., 2001). Some aid for identifying subtrees with different rates can be obtained from branch length estimates under the no-clock assumption (Yoder and Yang, 2000; Yang and Yoder, 2003).

The second approach to estimating divergence times is to use stochastic methods that do not assume globally or locally constant rates of molecular evolution. Instead, they have the attractive property of allowing the rate to evolve over time. Several parametric methods have been described within a Bayesian framework. These methods are based on different models for the rate of molecular evolution, such as the lognormal distribution and its recent variants (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002), the gamma, the exponential, or the Ornstein-Uhlenbeck processes (Aris-Brosou and Yang, 2002). However, given that little is known about the time scale for rate variation (Gillespie, 1991), estimation procedures that are less reliant on parametric assumptions might prove useful (Sanderson, 2002). A recently proposed semiparametric approach (Sanderson, 2002) attempts to combine the power and robustness of previous likelihood parametric and nonparametric (Sanderson, 1997) methods by the use of a penalized likelihood function that smoothes the rate differences across lineages.

Because of the presence of mineralized shell plates, thoracican barnacles have left a reasonably complete fossil record (Foster and Buckeridge, 1987). Hence, the study of their evolution has always been intimately linked to paleontological data (see Newman's studies). Fossils and evolutionary hypotheses have also been combined to obtain approximations of the timing of radiations within the group (e.g., Foster and Buckeridge, 1987; Glenner et al., 1995; Newman, 1996). This timing has been accomplished by superimposing a branching pattern (based or not in a phylogenetic analysis) over the time-stratigraphic distributions of the fossils and then matching the branching points (usually represented as polytomies) with the latest possible horizon for the event (see Schram and Høeg, 1995).

We compared the results of the likelihood-based local clock method of Yoder and Yang (2000), several Bayesian approaches (Kishino et al., 2001; Aris-Brosou and Yang, 2002), and the semiparametric penalized likelihood method (Sanderson, 2002) for estimating absolute divergence times within the Thoracica radiation under three fossil calibrations.

MATERIALS AND METHODS

Ingroup Taxa

Thirty-five thoracican and rhizocephalan species were collected by hand, otter trawl, or SCUBA from December 1999 to January 2002. Muscle and cirral tissues (Thoracica) or external tissues (Rhizocephala) from at least one individual from every species were dissected and preserved in 100% EtOH for DNA extraction. The remaining specimens were preserved in 70% EtOH and are housed in the crustacean collection at the Monte L. Bean Life Science Museum, Brigham Young University. In combination with these samples, 1 rhizocephalan and 15 thoracican 18S rDNA sequences from GenBank were included in the analysis (Table 1). Thus, our study combines representatives from six of the seven extant thoracican suborders, according to the most updated classification of the recent Crustacea (Martin and Davis, 2001). Only the Brachylepdomorpha are not represented here; this order consists of one extant abyssal species (*Neobrachylepas relictus*), described by Newman and Yamaguchi (1995).

Outgroup Choice

The Cirripedia are comprised of the boring Acrothoracica, the stalked and sessile Thoracica, and the parasitic Rhizocephala. Numerous larval features (reviewed by Høeg, 1992a, 1992b; Høeg et al., 2003) and sperm ultrastructure features (Healy and Anderson, 1990; Jamieson, 1991) strongly support Cirripedia monophyly. However, there is no general agreement about the relationships among these three cirripede taxa based on morphological analyses. Adult morphology is problematic for analyzing cirripede relationships because the parasitic Rhizocephala lack almost all of the traits normally found in arthropods (see Newman et al., 1969; Schram, 1986; Newman, 1987; Høeg, 1992a; Schram and Høeg, 1995; Høeg and Kolbasov, 2002). Several molecular studies (Spears et al., 1994; Billoud et al., 2000; Perl-Treves et al., 2000; Pérez-Losada et al., 2002), however, have produced strong support (100% bootstrap proportions) for the rhizocephalans as the sister group to the thoracicans, with the acrothoracicans clearly separated from them. A basal position of the Acrothoracica within the Cirripedia is also supported by larval (cyprid) characters (Høeg and Kolbasov, 2002) and by the plesiomorphic position of the mandibular palp on the mandible rather than on the labrum, as it is in all Thoracica. Hence, we have used the rhizocephalans *Loxothylacus texanus*, *Loxothylacus panopaei*, *Sacculina carcini*, and *Heterosaccus californicus* as the outgroup. For morphological analyses, however, the use of this outgroup presents a problem for polarizing thoracican characters because of the adaptation of rhizocephalans to a parasitic life. By including the Acrothoracica in the analysis as a second outgroup, we could unambiguously code a few more characters for the outgroup, but we also may have introduced more uncertainty because surviving members of this group either primarily lack or have secondarily lost most or all of their

hard parts (Kolbasov and Høeg, 2000). Therefore, we have combined Rhizocephala and Acrothoracica characters into a hypothetical ancestral taxon in an attempt to increase the phylogenetic signal of the outgroup for the morphological analyses.

Sequence Data

Barnacle DNA was extracted using methods described by Crandall and Fitzpatrick (1996). Polymerase chain reaction (PCR; Saiki et al., 1988) products for the complete 18S (1,848 bp) and partial 28S (1,823 bp), histone H3 (328 bp), 12S (345 bp), and 16S (527 bp) nuclear and mitochondrial genes were amplified using primers as described by Whiting et al. (1997), Whiting (2001), Colgar et al. (1998), Mokady et al. (1999), and Crandall and Fitzpatrick (1996), respectively. Standard PCR conditions (5 μ l 10 \times Taq buffer, 6–8 μ l 25 mM Mg₂Cl₂, 8 μ l 10 mM dNTPs, 5 μ l each of two 10 mM primers, 1.25 U Taq, \approx 20 μ l double-distilled water) were used on a Perkin-Elmer 9600 machine under the following conditions: an initial denaturation at 96°C for 3 min followed by 50 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 1 min, followed by an extension at 72°C for 5 min. PCR products were visualized by agarose (1.5%) gel electrophoresis and were purified using a GeneClean II kit (Bio 101). Automated sequences were generated in both directions from different runs on an Applied Biosystems (ABI) 377XL automated sequencer using the ABI Big-dye Ready-Reaction kit and following the standard cycle sequencing protocol but using a quarter of the suggested reaction volume.

Morphological Data

Thirty-seven adult and juvenile morphological characters covering hard and soft parts from the specimens (Appendix 1) were scored for each of the 24 thoracican genera and the outgroup (Appendix 2). In addition, 53 multistate larval characters covering the setation of the nauplii (from Newman and Ross, 2001) were also scored for 16 of the thoracican genera (see Appendix 2). The larval characters and their coding were extensively described by Newman and Ross (2001). All the adult and juvenile characters used in this study have been compiled from previous cladistic analyses or were available in the literature of cirripede systematics. Thirty of these characters were selected from previous studies developed by Glenner et al. (1995), Buckeridge (1995), and Høeg et al. (1999); the other seven (characters 5 and 32–37) were obtained from the specialized literature (e.g., Newman and Ross, 1976; Kolbasov and Høeg, 2000), although they have not been used before in phylogenetic studies. Several of the characters previously used by Glenner et al. (1995), Buckeridge (1995), and Høeg et al. (1999) were edited for our purpose, i.e., the definitions of states were adapted so that all characters are now binary, and some scorings were changed (Appendix 2). For example, *Verruca* was scored for characters 10, 13, 14, 15, 18, 20, and 30 using the scorings of Glenner et al. (1995) for the living fossil *Neoverruca brachylepadiformis* Newman and Hessler. The morphology of this verruciform species provides critical and now indisputable

TABLE 1. Collection locations and genes sequenced for the Thoracica and Rhizocephala cirripedes included in this study.

Taxon	Location	Gene ^a				
		18S	28S	H3	12S	16S
Thoracica						
Sessilia						
Balanomorpha						
Balanoidea						
<i>Austromegabalanus psittacus</i> (Molina)	Molinos Beach, Valdivia, Chile	y	y	y	y	y
<i>Balanus balanus</i> (Linnaeus)	Japan	y	y	y	y	y
<i>B. crenatus</i> Bruguière	Menai Straits, Wales, U.K.	y	y	y	y	y
<i>B. eburneus</i> Gould	GenBank	y	n	n	n	n
<i>B. glandula</i> Darwin	Monterey Bay, CA, USA	y	y	y	y	y
<i>B. nubilus</i> Darwin	GenBank	y	n	n	n	n
<i>B. perforatus</i> Bruguière	Vigo Bay, Galicia, Spain	y	y	y	y	y
<i>Elminius kingii</i> Gray	Molinos Beach, Valdivia, Chile	y	y	y	y	y
<i>E. modestus</i> Darwin	Menai Straits, Wales, U.K.	y	y	y	y	y
<i>M. californicus</i> (Pilsbry)	Monterey Bay, CA, USA	y	y	y	y	y
<i>M. tintinnabulum</i> (Linnaeus)	Monterey Bay, CA, USA	y	y	y	y	y
<i>Megabalanus spinosus</i> (Gmelin)	Annobón, Equatorial Guinea	y	y	y	y	y
<i>Menesiniella aquila</i> (Pilsbry)	Monterey Bay, CA, USA	y	y	y	y	y
<i>Semibalanus balanoides</i> (Linnaeus)	Isefjord, Denmark	y	y	y	y	y
<i>S. cariosus</i> (Pallas)	Monterey Bay, CA, USA	y	y	y	y	y
Chthamaloidea						
<i>Catomerus polymerus</i> (Darwin)	Pirates Bay, Tasmania, Australia	y	y	y	y	y
<i>Chamaesipho tasmanica</i> Foster and Anderson	Pirates Bay, Tasmania, Australia	y	y	y	y	y
<i>Chthamalus bisinuatus</i> Pilsbry	Tramandaí Beach, RGS, Brazil	y	y	y	y	y
<i>C. challengeri</i> Hoek	Japan	y	y	y	y	y
<i>C. fragilis</i> Darwin	GenBank	y	n	n	n	n
<i>C. montagui</i> Southward	Vigo Bay, Galicia, Spain	y	y	y	y	y
<i>C. stellatus</i> (Poli)	Vigo Bay, Galicia, Spain	y	y	y	y	y
<i>Jehlius cirratus</i> (Darwin)	Molinos Beach, Valdivia, Chile	y	y	y	y	y
<i>Notochthamalus scabrosus</i> Darwin	Molinos Beach, Valdivia, Chile	y	y	y	y	y
Coronuloidea						
<i>Chelonibia patula</i> (Ranzani)	GenBank	y	n	n	n	n
Tetraclitoidea						
<i>Tetraclita japonica</i> Pilsbry	Japan	y	y	y	y	y
<i>T. squamosa</i> (Bruguière)	Cooktown, Australia	y	y	y	y	y
<i>T. stalactifera</i> (Lamarck)	GenBank	y	n	n	n	n
<i>Tetraclitella divisa</i> (Nilsson-Cantell)	Annobón, Equatorial Guinea	y	y	y	y	n
<i>T. purpurascens</i> (Wood)	Eaglehawk Neck, Tasmania, Australia	y	y	y	y	y
Verrucomorpha						
<i>Verruca spengleri</i> Darwin	GenBank	y	n	n	n	n
<i>V. stroemia</i> (Müller)	Vigo Bay, Galicia, Spain	y	y	y	y	n
Pedunculata						
Heteralepadomorpha						
<i>Paralepas palinuri</i> Newman	GenBank	y	n	n	n	n
Iblomorpha						
<i>Ibla cumingi</i> Darwin	GenBank	y	n	n	n	n
<i>Ibla quadrivalvis</i> Cuvier	Kingston Beach, Tasmania, Australia	y	y	y	y	y
Lepadomorpha						
<i>Lepas anatifera</i> Linnaeus	GenBank	y	n	n	n	n
<i>Octolasmis lowei</i> (Darwin)	GenBank	y	n	n	n	n
<i>Poecilasma inaequilaterale</i> Pilsbry	Gulf of Mexico	y	y	y	y	y
Scalpellomorpha						
<i>Calantica villosa</i> (Leach)	GenBank	y	n	n	n	n
<i>Capitulum mitella</i> (Linnaeus)	Japan	y	y	y	y	y
<i>Litoscalpellum regina</i> Zevina	Gulf of Mexico	y	y	y	y	y
<i>Pollicipes pollicipes</i> Gmelin	Vigo Bay, Galicia, Spain	y	y	y	y	y
<i>P. polymerus</i> Sowerby	Monterey Bay, CA, USA	y	y	y	y	y
Rhizocephala						
<i>Loxothylacus texanus</i> Boschma	GenBank	y	n	n	n	n
<i>Loxothylacus panopaei</i> (Gissler)	GenBank	y	n	n	n	n
<i>Sacculina carcini</i> Thompson	Sweden	y	y	y	y	n
<i>Heterosaccus californicus</i> George	CA, USA	y	y	y	y	y

^ay = yes; n = no.

information on the ground pattern states of the Verucomorpha (see discussion of Glenner et al., 1995); by ignoring this information we would be reverting to the pre-1989 hypotheses of cirripede phylogeny (see Newman and Hessler, 1989). We believe our morphological matrix exhausts the presently available information in the cirripede literature for the taxa analyzed here.

Phylogenetic Analyses: Molecular Data Sets

Sequence alignment, incongruence, and model selection.—Nucleotide sequences were aligned using Clustal X (Thompson et al., 1997). Dynamic programming was used under the default settings for the gap opening (10) and gap extension (0.10), as suggested by the authors. Default settings were also used for the multiple alignment parameters gap opening (10), gap extension (0.20), and delay divergent sequence (30%), but a value of 0.3 was used for the DNA transition weight, as suggested by the authors for distantly related sequences. Multiple sequence alignments using gap-opening penalties of 7 and 13 and transition weights of 0.5 (default setting) were also examined, but better alignments were not obtained. Alignments were trivial for the protein-coding genes and conserved domains of the mitochondrial and nuclear rDNA gene regions. Nevertheless, Clustal rDNA alignments were refined by hand based on the most recent compilation for alignment and secondary structure analyses provided by the European Ribosomal RNA database (<http://oberon.rug.ac.be:8080/rRNA>).

Congruence between genes was addressed using the methodology proposed by Wiens (1998). Separate maximum parsimony (MP) and Bayesian phylogenetic analyses were conducted on the two mitochondrial genes combined (because all genes in the mitochondrial genome are linked and should therefore share the same phylogenetic history) and on the 18S, H3, and 28S genes to detect potential areas of strongly supported incongruence (where combined analyses may fail; Wiens, 1998), as indicated by conflicting nodes with bootstrap proportions (BP) \geq 70% or posterior probabilities (pP) \geq 95%, respectively. Hereinafter, unless otherwise specified we will refer to the aligned 18S, 28S, H3, 16S, and 12S data sets together as the molecular data set. We use the term combined data set for the combination of morphology and molecular data sets.

For model selection, we used the procedure outlined by Huelsenbeck and Crandall (1997). The likelihood scores from each model were compared using a likelihood ratio test as implemented in Modeltest 3.6 (Posada and Crandall, 1998). The general time reversible model with rate heterogeneity and invariable sites (GTR+ Γ +I) was selected as the best-fit model of nucleotide substitution for the molecular data set (see Table 2). Therefore, we used this model in our maximum likelihood (ML) hill-climbing analyses. Bayesian analyses were performed under a homogeneous GTR+ Γ +I model for the molecular data set and under mixed models for the combined data set (see Table 2). Variable rate priors among partitions were chosen for the latter Bayesian analyses. Unfortunately, the most complex genetic model currently implemented in MetaPIGA (Lemmon and Milinkovitch, 2002) is HKY85+ Γ +I; thus, we used this model for performing the ML genetic algorithm searches.

Phylogenetic inference.—Phylogenetic relationships were estimated using MP, ML under regular hill climbing (MLhc) and genetic algorithm (MLga) heuristic searches, and Bayesian methods coupled with Markov chain Monte Carlo (BMCMC) techniques. We conducted equally weighted MP heuristic searches with 100 random addition (RA) replicates and tree bisection-reconnection (TBR) branch swapping using PAUP* 4.0b10 (Swofford, 2002). Confidence in the resulting relationships was assessed using the nonparametric bootstrap procedure (Felsenstein, 1985) with 1,000 bootstrap replicates, TBR branch swapping, and 10 RA replicates.

MLhc searches (Felsenstein, 1981) were performed using PAUP*, with 10 RA replicates and TBR branch swapping. One hundred replications, TBR branch swapping, and one RA replicate were used for the bootstrap analysis. MLga searches were performed using the MetaGA algorithm implemented in MetaPIGA and under the optimal running conditions specified by the authors, but a population size of 16 and the strict group consensus option were chosen for better accuracy. Lemmon and Milinkovitch (2002) argued that a set of multiple genetic algorithm searches would produce trees and clades with frequencies that closely approximate their posterior probabilities. Hence, we performed 16 independent searches of 10 populations each (160 trees) to estimate nodal support.

TABLE 2. Parameter estimates for the best-fit models of nucleotide substitution. π_A , π_C , π_G , and π_T are the empirical base frequencies; r_{AC} , r_{AG} , r_{AT} , r_{CG} , and r_{CT} are the relative substitution rates among nucleotides ($r_{CT} = 1$); I is the proportion of invariable sites; and α is the shape parameter of the gamma distribution for the variation among sites under $I \neq 0$ and $I = 0$. Parsimony-informative sites for all the taxa (All) and for only the ingroup taxa (Ingroup) are also indicated.

Gene partition	Model	π_A	π_C	π_G	π_T	r_{AC}	r_{AG}	r_{AT}	r_{CG}	r_{CT}	α			No. informative sites	
											I = 0	I = 0	I	All	Ingroup
18S	TrN+ Γ +I	0.234	0.231	0.286	0.249	1.0	1.838	1.0	1.0	4.565	0.688	0.195	0.577	255	151
28S	TrN+ Γ +I	0.220	0.236	0.308	0.236	1.0	2.838	1.0	1.0	7.408	0.393	0.173	0.481	345	262
H3	GTR+ Γ +I	0.211	0.332	0.277	0.180	0.471	2.092	1.718	1.296	9.538	0.989	0.166	0.572	92	85
12S	GTR+ Γ	0.414	0.071	0.134	0.381	0.765	4.002	1.366	0.389	10.329	0.389	0.389	0.0	225	179
16S	TVM+ Γ	0.363	0.095	0.150	0.392	0.742	8.217	2.488	0.50	8.217	0.222	0.222	0.0	200	182
All genes	GTR+ Γ +I	0.242	0.222	0.267	0.269	0.545	3.263	2.891	0.817	4.845	0.409	0.191	0.474	1117	859

Bayesian phylogeny estimation was performed using MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). Each Markov chain was started from a random tree and run for 3.0×10^6 cycles, with every 1,000th cycle sampled from the chain in each case. Model parameters were treated as unknown variables with uniform default priors and were estimated as part of the analysis. We ran four chains simultaneously, three heated (temperature = 0.2) and one cold, using a Metropolis-coupled MCMC procedure to enhance the mixing capabilities of the Markov chains. To confirm that our Bayesian analyses converged and mixed well, we monitored the fluctuating value of the likelihood and all the phylogenetic parameters graphically, compared means and variances of all likelihood parameters and likelihood scores from independent runs, and repeated each simulation four times starting from different random trees. All sample points prior to reaching stationary were discarded as burn-in. The posterior probabilities for individual clades obtained from separate analyses were compared for congruence and then combined and summarized on a majority-rule consensus tree (Huelsenbeck and Imennov, 2002; Huelsenbeck et al., 2002).

Testing for alternative hypotheses.—Alternative a priori phylogenetic hypotheses from the literature were tested using our molecular data and the Kishino and Hasegawa (1989; KH) test. When more than two topologies were involved or when any of the hypotheses were obtained a posteriori based on our data, the Shimodaira and Hasegawa (1999; SH) test was used instead. Goldman et al. (2000), Buckley (2002), and Strimmer and Rambaut (2002) have pointed out that the SH test may be subject to a certain type of bias such that the number of trees included in the confidence set tends to be very large as the number of trees to be compared increases, which makes the test conservative. However, as these authors recognized and Shimodaira (2002) concluded, the SH test is still safe to use and is a good option when the number of candidate trees is not very large and more data are accumulated. Ten thousand replicates were performed for every topology test resampling the partial likelihoods for each site (RELL model). A two-tailed probability was estimated for the KH test. All the a priori hypotheses of barnacle evolution concern only specific taxa from our analysis (e.g., Iblomorpha are the most basal thoracican), and most of them are not expressed in a phylogenetic context (i.e., there are not real alternative topologies to compare). Therefore, we have used our MLhc tree to constrain the relationships among the taxa not involved in the conflict and have altered the topology of the clades of concern according to what the actual a priori hypotheses indicate. Although this approach may not be the best use of the SH test because it limits the number of possible a priori topologies, the alternative option of considering all the possible topologies for 47 taxa is completely impractical. We have used the same procedure for testing Archaeobalanidae monophyly (a priori hypothesis) versus Archaeobalanidae paraphyly (our a posteriori hypothesis) using the SH test. The KH and SH tests were carried out in PAUP*.

Phylogenetic Analyses: Morphological and Combined Data Sets

Morphological MP phylogenies were estimated with PAUP* using the adult–juvenile data set alone and combined with the larval data set. An extensive cladistic analysis of 31 thoracicans using the larval data set was performed by Newman and Ross (2001). Searches were heuristic, with 100 RA replicates and TBR branch swapping. One thousand bootstrap replicates with 10 RA replicates were run during the bootstrap analysis.

Our adult–juvenile and/or larval data sets were also combined with the molecular data set and then analyzed using MP (same conditions as above) and BMCMC under mixed models of evolution (same conditions as above).

Divergence Time Estimation

The divergence time estimation methods used here do not differ only in their underlying statistical approaches (ML, BMCMC, and penalized likelihood [PL]), they also differ in other characteristics related to their functionality, such as the peculiarities of their input data sets (e.g., single locus *vs.* multilocus, branch lengths *vs.* sequence data, and the ability to deal with missing taxa and data, gaps, and undetermined positions), the implemented models of evolution, and the way they accommodate external information from calibration points. Some methods require an extra outgroup to estimate the position of the tree root, whereas others do not. To account for all of these differences, we have used in all of our analyses the previously defined 18S rDNA data set (the only complete data set for all the barnacle species in this study) in combination with two new 18S sequences from GenBank corresponding to the acrothoracicans (Cirripedia) *Trypetesa lampas* (Hancock) and *Berndtia purpurea* Utinomi. Alignment gaps and undetermined nucleotide positions were removed, leaving 1,566 nucleotides in the “clean” sequences. These data were analyzed under the HKY85+ Γ model of nucleotide substitution (the most complex model implemented by all the methods), using the empirical base frequencies and with the transition:transversion rate ratio and the shape parameter estimated without a clock ($\kappa = 3.049$ and $\alpha = 0.2386$). Under these same conditions, we reestimated the branch lengths of the MLhc tree in Figure 1 with PAML 3.13.1 (Yang, 1997) and used them as the input data for the PL approach (Sanderson, 2002).

Calibration points and fossil uncertainty.—There are five possible calibration points representing the major stem thoracican lineages in Figure 1 for which we have fossil information: nodes 41, 37, 36, 30, and 21. The oldest fossil information for node 41 provides an age of 360 million years ago (MYA), and the oldest fossil information for node 37 dates it as 530 MYA; however, our phylogenetic tree shows that node 41 is older than node 37, so node 41 is not a reliable calibration point. The oldest fossil information for node 21 is from the late Cretaceous (dated to 82 MYA), but in preliminary analyses this and younger calibrations produced great discrepancies

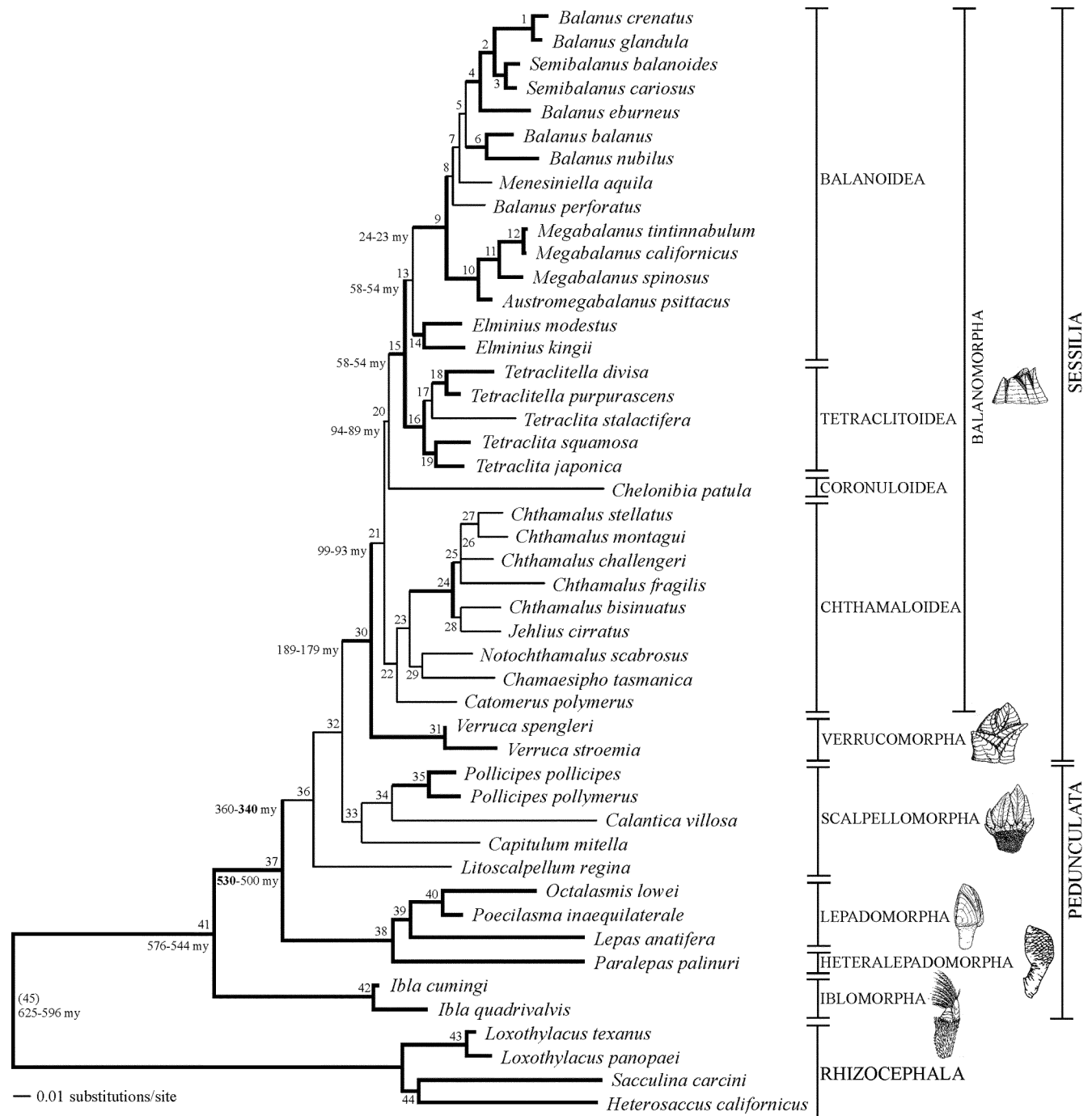


FIGURE 1. MLhc tree assuming the GTR+ Γ +I model of evolution. Branch lengths are shown proportional to the amount of change along the branches. All the BMCMC, MLga, and MP analyses resulted in the same topology. Clades supported by BP $\geq 70\%$ in the MLhc tree and $pP \geq 0.95$ in the BMCMC analyses are represented with thicker lines on the tree. Nodal support values are indicated in Table 4. Drawings from Buckeridge (1995), Newman (1987), and Høeg et al. (1999) are included for the six thoracican suborders. Divergence times estimated under the r_{gen} Yoder and Yang (2000) local clock model (Table 3) and using C_1 and C_2 calibration points (in bold) are represented for 10 stem lineages.

among approaches and reciprocal calibration estimates. Therefore, for our analyses we used the heteralepadomorph *Priscansermarinus*[†] from the Middle Cambrian (dated to 530 MYA), the scalpellomorph *Pabulum*[†] from the early Carboniferous (dated to 340 MYA), and the verrucomorphs *Eoverruca*[†] and *Proverruca*[†] from the early

Cretaceous (dated to 120 MYA) to fix the ages of the Heteralepadomorpha (node 37 in Fig. 1; C_1 in Table 3), Scalpellomorpha (node 36; C_2), and Verrucomorpha (node 30; C_3) stem lineages, respectively (see Foster and Buckeridge, 1987, and references therein). Barnacle radiation covers a period of at least 550 million years

(MY); hence, we believe these three calibration points adequately represent the time span of the Thoracica radiation based on the fossil record. We used the geologic time scale of Palmer (1983).

Any extant group has two ages: the age at which its stem lineage branched from the line leading to its extant sister group and the age of the most recent ancestor of all its living members or the crown group (Jefferies, 1979). Because of the limited representation of the suborders Heteralepadomorpha, scalpellomorpha, and Verrucomorpha in our analysis and considering that the three fossils are apparently deeply located in the stem lineages leading to their respective crown groups (see Whyte, 1976; Foster and Buckeridge, 1987; Newman and Hessler, 1989), we have used these fossils to fix the stem nodes of their respective lineages as minimum ages. We understand that forcing some internal nodes to have a certain age is not the most appropriate way to introduce fossil information into the analysis because it does not take into account the inaccuracy of the paleontological record. However, because some of the methods compared here do not allow for a better implementation, as a general procedure in this study and following Yang and Yoder (2003) we have assumed that the node age is known without error. How the error in fossil dating may affect the dates estimated here is outside the scope of this study. Nevertheless, when possible the more realistic approach of using lower and upper bounds to constraint node ages was also enforced. We also measured the degree of fit between the barnacle fossil record and the estimated phylogenetic tree by using the stratigraphic consistence index (SCI) proposed by Huelsenbeck (1994).

ML approach.—We used the ML-based local molecular clock approach of Yoder and Yang (2000) as implemented in PAML (the YY method). This method preassigns independent evolutionary rates to some lineages while all the other branches evolve at the same rate. However identification of the branches with independent rates is not easy because evolutionary rates and times are confounded in the branch length. Two different strategies were used to preassign independent rates, referred to here as rate categories and rank categories. We defined up to six rate categories including one to six independent rates based on the branch length estimates without the clock assumption. For example, the second rate category in Table 3 ($r_{c3} = 2$) includes two independent rates for the branches connecting nodes 44 and 45 (rate 1) and nodes 41 and 45 (rate 2) and the same rate for all the other branches (rate 0). Rank categories were devised because closely related evolutionary lineages tend to evolve at similar rates (Kishino et al., 2001; Thorne and Kishino, 2002). We divided the taxa into one to five groups according to the following taxonomic ranks: superorder, order, suborder, family, and genus. The number of groups minus 1 independent rates were assigned to the different classes, plus an extra independent rate for the branch connecting nodes 41 and 45. For example, the rank "order" in Table 3 ($r_{ord} = 3$) includes three independent rates for the subclades Rhizocephala and Pedunculata or Sessilia and for the branch connecting nodes 41 and 45 and a different

rate (rate 0) for the subclades Pedunculata or Sessilia. Divergence time estimations under a ML global molecular clock ($r = 0$) were also estimated under this approach for comparison.

Bayesian approach.—Two different sets of Bayesian methods have been applied that assume different MCMC approximations to estimate prior and posterior distributions and different priors for rates and times and incorporate external information (calibration points) differently into the analysis: the Kishino et al. (2001) method (KTB) and the Aris-Brosou and Yang (2002) method (A-BY). Both approaches are described in detail by these authors and by Thorne et al. (1998). Recently, Thorne and Kishino (2002) and Yang and Yoder (2003) extended the KTB and the ML-based YY methods for analyzing multilocus data sets. Unfortunately, these new implementations are not applicable to our molecular data set because of the missing taxa in all the individual gene data sets except that for 18S.

The KTB method is implemented in the programs *esbranches* and *multidivtime* (thorne@statgen.ncsu.edu). After inspecting the branch-length estimates from the 18S rDNA data set, the evolutionary rate at the root node was given a gamma prior distribution with mean (*rrate*) and SD (*rratesd*) both equal to 0.011 substitutions at the average site per 100 MY. We chose this prior to obtain a distribution for the root that was simultaneously both reasonable and relatively diffuse. The *rrate* and *rratesd* 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 10^4 samples were collected every 100 accepted states; posterior distributions (less diffuse) included a burn-in period of 10^5 steps, after which 10^4 samples were collected every 100 accepted states. Default options were chosen for all the other parameters of the prior distribution and the MCMC approach. Convergence was monitored by checking the proportion of successes (*psuc*) of times and rate changes proposed along the Markov chain. Multiple chains were run from different starting points.

The A-BY analyses, as implemented in *PhyBayes* (<http://abacus.gene.ucl.ac.uk/stephane/>), were conducted by choosing a birth and death process prior for times, with their hyperparameters drawn from the following uniform distributions, $\lambda \sim (0, 15)$, $\mu \sim U(0, 5)$, and $\rho \sim (0, 0.001)$. Five different prior distributions for rates of evolution were tested: the lognormal (LND), stationary lognormal (SLD), Ornstein-Uhlenbeck (OUP), gamma (GD) and exponential (ED). All of these models were evaluated using the empirical Bayes phase indicated by Aris-Brosou and Yang (2002) for the hyperparameters β (only for OUP) and σ^2 ($\beta = 0.01, 0.1$ and 1 ; $\sigma^2 = 1, 10, 20$, and 40), and the resulting $\log L_k^{\text{post}}$ were compared. MCMC runs included a burn-in period of 10^5 steps, after which 10^5 samples were collected every 100 accepted states. Convergence was monitored by plotting time series of times and rate variables. Multiple chains were run from different starting points.

PL approach.—The PL approach of Sanderson (2002) was applied as implemented in the computer program *r8sv1.6* developed by Sanderson. The smoothing parameter (λ) was estimated by cross validation (CV) using the truncated Newton (TN) algorithm and default options for the CV parameters. 10 initial starts and 10 perturbed restarts after the initial solution was found were executed under the TN algorithm (as recommended by Sanderson, 2002) for estimating divergence times.

RESULTS

Thoracican Phylogenetics

Molecular data set.—One hundred sixty-seven new 18S, 28S, H3, 12S, and 16S sequences from 35 species of thoracican and rhizocephalan barnacles were obtained (Table 1). The new sequences have been deposited in GenBank (accession numbers AY520590–AY520756). Two independent Bayesian analyses (10^6 generations, every 1000th cycle sampled) for each nuclear data set and for the combined mitochondrial data sets under the GTR+ Γ +I model converged on similar likelihood scores. No areas of strongly supported conflict were identified between either BMCMC or MP mitochondrial and nuclear gene trees. When the two mitochondrial and three nuclear data sets were combined, more nodes showed BP $\geq 70\%$ (7–25) and *pP* $\geq 95\%$ (9–22) than did any of the four data sets alone.

Ten MLhc searches and four independent Bayesian analyses of 11,896 combined samples (108 samples discarded) under the GTR+ Γ +I model of evolution resulted in the topology ($-\ln L = -31171.9$) shown in Figure 1. Accordingly, the MLga search under the HKY85+ Γ +I model and the MP analysis resulted in a best score ML and one most-parsimonious tree 4,070 steps long, respectively, with the same topology as depicted in Figure 1. Nodal support (BP and *pP*) for the previous analyses is shown in Figure 1 and numerically described in Table 4. Our phylogenetic hypothesis presented the Iblomorpha as the most basal suborder in Thoracica. The Pedunculata and the Scalpellomorpha are shown as paraphyletic, with the latter leading to the monophyletic Sessilia. Within the Sessilia, all of the Balanomorpha superfamilies included in this study constituted monophyletic assemblages, with the Chthamaloidea as the sister group to the remaining balanomorphs (coronuloids, tetracitoids, archaebalanids, and balanids). The Archaeobalanidae, represented here by *Elminius* and *Semibalanus*, did not form a monophyletic group, with the latter genus clustered with *Balanus* species.

Morphological data set.—The MP analysis of the 37 adult and juvenile barnacle morphological characters produced one island of 80 trees and a tree length of 50 steps. The addition of the 53 larval characters produced one island of 2,600 trees and a tree length of 176 steps. The 50% majority-rule consensus trees resulting from both analyses are shown in Figure 2. The topologies were different from each other and also from the larval phylogeny generated by Newman and Ross (2001:

Fig. 4). Greater bootstrap support was obtained from the analysis of the adult–juvenile morphological data. These results stress the differences between larval and adult classifications (Newman and Ross, 2001); both forms are living in completely different environments, so it might be expected they would have diverged in various independent ways. Both of our morphological trees agree closely with our molecular trees at the subordinal level, with the most notable differences in the positioning of the Heteralepadomorpha (*Paralepas*) and the Verrucomorpha (*Verruca*). The former is shown as a link between the Iblomorpha and the Lepadomorpha (BP $\leq 70\%$), and the latter is shown either as a sister clade to the scalpellomorphan *Pollicipes* (BP $< 50\%$) or as a link between the Scalpellomorpha and the Lepadomorpha (BP $< 50\%$). None of these topological differences represented a strong conflict with our molecular trees according to the criterion of BP $\geq 70\%$ suggested by Wiens (1998).

Combined data sets.—Four independent Bayesian analyses of the combined data set (11,917 samples, 87 samples discarded) under mixed models of evolution resulted in the same topology as shown in Figure 1, although with slightly different *pP* values (Table 4).

The combined analysis of the molecular and the adult–juvenile and/or larval data sets under the MP approach produced three sets of hypotheses of two or three trees, each 5,200, 5,271, and 5,330 steps long, respectively (trees not shown). No major topological differences between the combined and molecular phylogenies were found, and all the combined analyses gave greater support to the deeper nodes than did the MP molecular analysis (see Table 4).

Thoracican Divergence Times

Thirty-five of 45 internal nodes in our phylogenetic hypotheses were stratigraphically consistent and gave a significant ($P < 0.01$) SCI value of 0.778, meaning the trees fit the barnacle stratigraphic record quite well.

A likelihood ratio test significantly rejected ($P < 0.001$) the null hypothesis that all genes, separately and combined, were evolving with rate constancy across the thoracican barnacles, justifying the use of nonclocklike molecular methods to estimate divergence times. Time estimates under the ML-based local clock method of Yoder and Yang (2000), the Bayesian-based procedures of Aris-Brosou and Yang (2002) and Kishino et al. (2001), and the PL method of Sanderson (2002) are shown in Table 3 for 10 major stem lineages.

The maximum L_k^{post} for the models of rate change compared under the A-BY method was around $\beta = 1$ and $\sigma^2 = 10$. The OUP model explained the data better than did any other model (i.e., OUP $\log L_k^{\text{post}} = -6888.2$). The posterior Bayes factor was ≥ 13 when the OUP model was compared with any other model, so that the differences are “strong” (see Kass and Raftery, 1995:777). The smoothing parameter that minimized CV under the PL approach resulted in $\lambda = 2.0$.

TABLE 4. Posterior probability (BMCMC and MLga) and bootstrap (MLhc and MP) proportions. Node numbers correspond to those in Figure 1. Dashes represent nodes that show values <50%. All the BMCMC_{mixed} and MP_{combined} values resulted from the combination of the molecular and the adult and/or larval morphology data sets.

Node	BMCMC _{homo}	BMCMC _{mixed} (Adult + larval)	MLga	MLhc	MP _{molecular}	MP _{combined}		
						Adult	Larval	Adult + larval
1	100	100	100	100	100	100	100	100
2	100	100	100	98	81	79	53	—
3	100	100	100	100	94	96	100	100
4	100	100	100	76	70	59	—	—
5	99	100	81	—	—	—	—	—
6	100	100	99	85	94	95	97	95
7	100	100	69	54	67	61	—	50
8	99	100	100	67	—	—	—	—
9	100	100	100	91	77	72	—	52
10	100	100	100	97	98	99	99	100
11	100	100	100	100	99	100	100	100
12	100	100	100	100	100	100	100	100
13	99	98	—	55	—	—	—	—
14	100	100	93	88	92	95	99	98
15	99	100	74	70	57	87	99	100
16	100	98	85	82	70	79	81	82
17	69	75	82	66	—	—	82	81
18	100	99	100	93	93	96	—	—
19	84	84	100	84	77	66	99	99
20	—	—	—	—	—	—	—	—
21	81	100	96	63	76	99	98	100
22	54	100	78	77	70	73	97	82
23	50	—	78	71	70	81	88	92
24	98	100	99	93	93	95	100	100
25	54	77	77	—	—	—	51	—
26	—	—	—	—	—	—	54	—
27	86	95	100	80	88	91	97	96
28	60	60	—	—	—	—	—	—
29	96	59	—	66	57	57	51	66
30	99	100	99	80	70	84	71	72
31	100	100	99	100	100	100	100	100
32	96	95	100	50	58	53	64	—
33	89	87	99	—	—	—	—	—
34	94	92	99	58	—	—	—	—
35	100	100	100	93	99	100	100	100
36	93	100	100	65	92	100	93	99
37	100	100	100	99	98	99	100	100
38	100	100	100	100	99	98	100	100
39	92	87	—	87	74	74	84	69
40	100	100	84	89	97	98	97	98
41	100	100	100	100	100	100	100	100
42	100	100	100	100	100	100	100	100
43	100	100	98	100	100	100	100	100
44	62	70	100	66	87	87	83	83

DISCUSSION

Thoracican Phylogenetic Inferences

We addressed several key questions concerning thoracican evolution within a phylogenetic hypothesis testing framework. Compared hypotheses are summarized in Table 5. Supplementary information is provided in Appendix 3.

Phylogenetic position of the Iblomorpha and Heteralepadomorpha.—All of our trees based on genetic and/or morphological evidence placed the Iblomorpha at the base of the thoracican tree in agreement with recent morphological (Høeg et al., 1999) and molecular (Harris et al., 2000) phylogenetic analyses and previous nonphylogenetic studies (e.g., Newman et al., 1969; Anderson, 1994). Our molecular data also significantly

rejected (SH test; $P < 0.001$) the alternative hypotheses of Darwin (1851), Glenner et al. (1995), and Perl-Treves et al. (2000). Klepal's (1985) hypothesis could not be tested in this study, although it has not been supported by previous morphological (e.g., Glenner et al., 1995) or molecular (e.g., Pérez-Losada et al., 2002) analyses.

Our morphological trees (Fig. 2) placed the plateless Heteralepadomorpha (represented here by *Paralepas pallinuri*) between the Iblomorpha and the Lepadomorpha (BP $\approx 65\%$), in agreement with Høeg et al. (1999), but our better supported molecular and combined phylogenies (BP and pP = 98–100%) showed the Heteralepadomorpha as the sister group to the Lepadomorpha, confirming previous molecular results and some morphological evidence (Pilsbry, 1907; Nilsson-Cantell, 1921). Moreover, all of the alternative hypotheses that dissociate the sister

TABLE 5. Testing alternative thoracican phylogenetic hypotheses. KH and SH tests were used to compare two or more trees, respectively. All the H_1 hypotheses are represented by our single ML tree ($-\ln L = 31719.1$) in Figure 1. The number of trees compared, the likelihood scores, and the probabilities for the alternative hypotheses are also provided.

Taxon	H_1 (author)	H_2 (author)	No. trees	$-\ln L$	P
Iblomorpha	Most basal thoracican (Newman, 1987; and others)	Sister group to the Heteralepadomorpha (Darwin, 1851);	3	≥ 31763.8	<0.001
		Link between the Heterolepadomorpha and the Lepadomorpha (Glenner et al., 1995);	2	31767.2	<0.001
		Internal position within the Thoracica (Perl-Treves et al., 2000)	2	31777.4	<0.001
Heteralepadomorpha	Sister group to the Lepadomorpha (Harris et al., 2000; and others)	Bottom of the thoracican tree (Anderson, 1994; and Newman, 1996);	2	31762.6	<0.001
		Link between the Iblomorpha and the Lepadomorpha (Høeg et al., 1999)	2	31768.8	<0.001
Pedunculata ↔ Sessilia	Lepadomorpha → Scalpellomorpha → Sessilia (Newman, 1987; and others)	Scalpellomorpha → Lepadomorpha → Sessilia (Darwin, 1851; and others)	2	31745.5	0.006
Sessilia	Monophyletic (Newman, 1987)	Verrucomorpha and Balanomorpha polyphyletic (Pilsbry, 1907, 1916; and others);	8	≥ 31748.2	≤ 0.017
		Chthamaloidea and Balanoidea polyphyletic	8	≥ 31748.2	≤ 0.02
		(Zullo, 1963; and others)	8	≥ 31786.6	<0.001
Archaeobalanidae	Paraphyletic (this study)	Archaeobalanidae monophyletic (Newman and Ross, 1976; and others)	3	≥ 31842.4	<0.001
				≥ 31842.8	<0.001

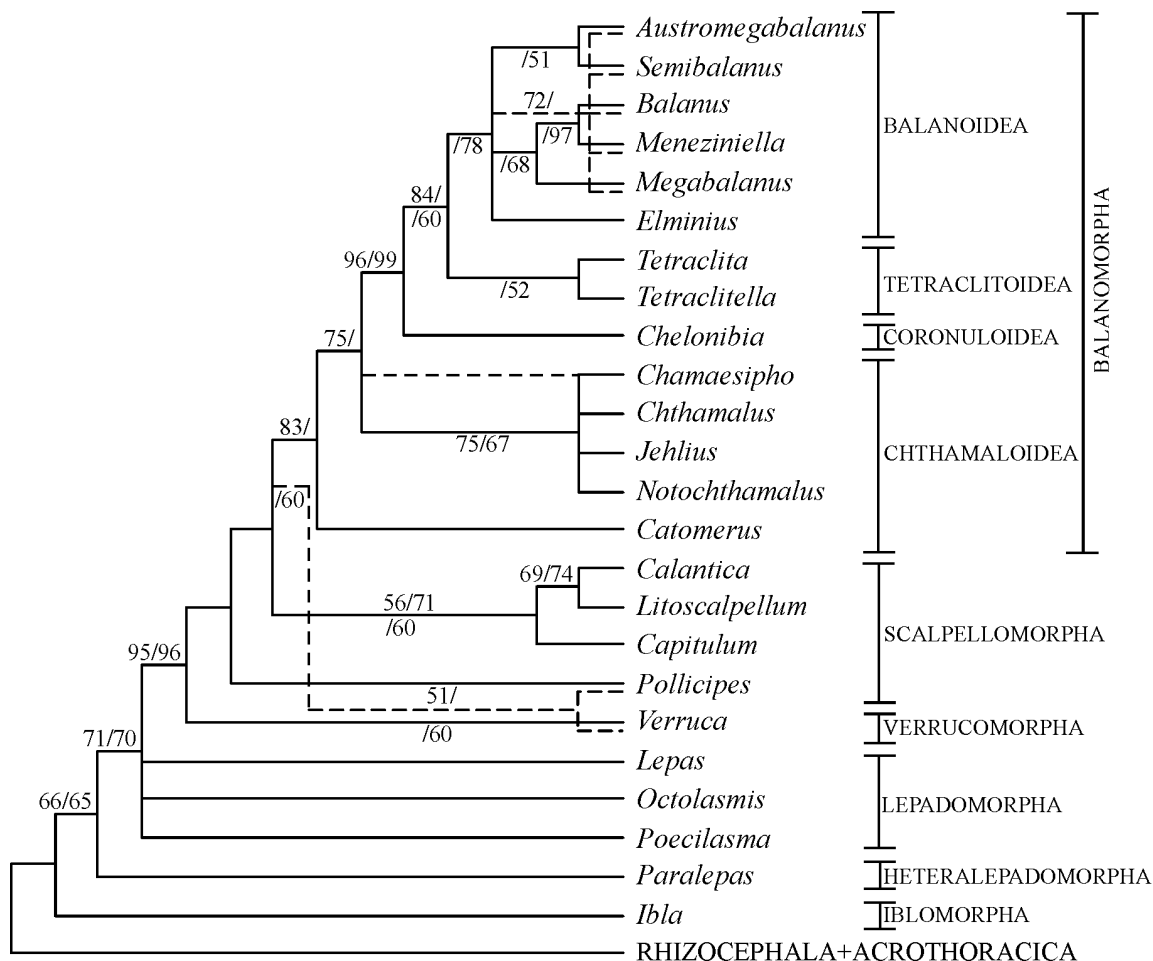


FIGURE 2. The 50% majority-rule consensus trees of 80 and 2,600 most-parsimonious trees using adult (solid lines) and adult/larval morphological data, respectively. Differences between both hypotheses are indicated by dashed lines. Consensus values (if $<100\%$) are shown below the branches. Bootstrap proportions (if $>50\%$) are shown above the branches and are based on 1,000 bootstrap replicates.

relationship Heteralepadomorpha + Lepadomorpha were significantly rejected ($P < 0.001$) by the KH test. In any case, it seems certain that the Heteralepadomorpha diverged after the Iblomorpha, which indicates that the absence of capitular plates in the former is indeed a secondary loss, as argued by Høeg et al. (1999). This scenario also suggests that there are no extant Thoracica with a primary absence of mineralized shell plates, whereas the absence of such plates in the other two cirripede superorders (Rhizocephala and Acrothoracica) is most parsimoniously considered primary. Mineralized plates, therefore, appear to be a true synapomorphy for the Thoracica but not for the Cirripedia as a whole. This finding is interesting because filter feeding occurs also in the most basal cirripedes (Acrothoracica) and therefore must have evolved in the cirripede stem line. Thus, the parasitic Rhizocephala (sister group to the Thoracica) must have originated from filter feeding forms, as argued by Newman (1987). Unlike the situation in another important filter-feeding group of invertebrates, the Bivalvia, our phylogenetic results in combination with previous phylogenetic analyses (e.g., Pérez-Losada et al., 2002) indicate that the protective armor in Cirripedia evolved separately from and after the change into a new feeding mode.

The previous phylogenetic results suggest that exoskeleton biomineralization in barnacles may have evolved from phosphatic (*Ibla*) to calcitic (other thoracicans except heteralepadomorphans). Such a change has not been documented in any other invertebrate (Whyte, 1988; Høeg et al., 1999). Crystalline phosphatic tissue is very rare in recent invertebrates (Löwenstam and Weiner, 1992) but was found in about half of the mineralized tissues from the early Cambrian, suggesting that phosphatic plates are a primary condition in cirripedes (Löwenstam et al., 1992; Høeg et al., 1999). Nevertheless, we cannot rule out a less parsimonious alternative hypothesis of biomineralization of the skeleton having evolved independently in *Ibla* and the other thoracicans.

Acquisition of the sessile condition.—All of our molecular and combined phylogenetic analyses support a character polarity as follows: five plated (as in Lepadomorpha) → 5+ multiplated (Scalpellomorpha) → 8+ multiplated, sessile (Verrucomorpha and Balanomorpha). This polarity suggests that the sessile condition evolved from a multiplated scalpellomorphan-like ancestor. Moreover, a scalpelloid → lepadoid → sessile hypothesis with the Heteralepadomorpha as the sister group to the Lepadomorpha is significantly rejected ($P = 0.006$) by the KH test.

These results dovetail with available information from both ontogeny and paleontology (see Newman, 1987; Newman and Hessler, 1989; Anderson, 1994) and previous morphological analyses (Glennner et al., 1995; Høeg et al., 1999). Hence, different sources of evidence indicate that additional capitular plates and peduncular scales (in Pedunculata) are evolutionary advancements. An alternative hypothetical lepadomorphan immediate ancestor of the Sessilia would imply secondary loss of plates and posterior regain and lacks the required capitular level of organization to be a satisfactory model (Newman, 1987).

The acquisition of the sessile condition from scalpelloid ancestors morphologically involved a gradual and complicated process of shortening and eventual elimination of the peduncle with concomitant changes in capitular organization (see Newman and Hessler, 1989, for a description of the morphological innovations acquired by the asymmetrical [Verrucomorpha] and symmetrical [Balanomorpha barnacles]). This amazing evolutionary transition is shown ontogenetically in the metamorphosis of the Sessilia larvae as a progressive reduction of the number of pedunculate stages from several to essentially one (Newman, 1989; Yamaguchi and Newman, 1990). The evolutionary pressures leading to this ontogenetic trend were likely similar to those that favored the evolution of the sessile mode in the first place, i.e., the dramatic increase in shell-crushing predators such as true crabs in the late Mesozoic and their significance to barnacles and interspecific competition between juveniles for settlement space (Moyses and Hui, 1981; Newman, 1989).

Thoracica also exemplify a most amazing variety of sexual systems, not only compared with other Crustacea but also with metazoans as a whole (Høeg, 1995). These systems range from dioecy (separate sexes; Iblomorpha and Scalpellomorpha), through androdioecy (hermaphrodites and separate male sex; Iblomorpha, Scalpellomorpha, and Balanomorpha), to pure hermaphroditism (no separate male sex, all the thoracican suborders). Based on our previous phylogenetic results, the most-parsimonious reconstructions for the sexual systems suggest that hermaphroditism evolved once along the lineage connecting the Rhizocephala to the Iblomorpha (i.e., a synapomorphy for the Thoracica; all the cirripede outgroups have separate sexes), whereas a free male sex was originally present in the cirripede stem line but reevolved at least twice (Scalpellomorpha and Balanomorpha) from pure hermaphroditism within the Thoracica.

Sessilia monophyly.—Our molecular and combined trees strongly supported (BP = 70–84%; pP = 99–100%) Sessilia monophyly, in agreement with Newman (1987), Newman and Hessler (1989), Buckeridge and Newman (1992), and Newman and Yamaguchi (1995). To test the a priori hypotheses that consider the Sessilia polyphyletic, we have used the Scalpellomorpha as the ancestor based on previous hypotheses and our own results. For the Verrucomorpha, Balanomorpha, Chthamaloidea, and Balanoidea clades, we have checked independently all of the possible topologies that establish sister relationships between them and the Scalpellomorpha genera. These alternative hypotheses were compared separately to the monophyletic hypothesis (four sets of eight topologies) using the SH test, and all of them were significantly different ($P \leq 0.02$; Table 5). Our results, therefore, represent the first well-supported phylogenetic evidence of Sessilia monophyly (as taxonomically represented in this study) after more than a century of discussion and agree with Newman's ideas of a brachylepadomorphan-like ancestor to the Verrucomorpha and Balanomorpha. Our efforts are currently focused on sampling this key suborder to test this hypothesis.

Balanomorpha.—All of our phylogenetic trees confirmed the pattern suggested by Newman and others about balanomorph evolution depicted as (Chthamaloidea(Coronuloidea(Tetraclitoidea, Balanoidea))), although only partial support ($BP \leq 70$ or $pP \leq 95$) was found for some of these assemblages. Our trees support a sister relationship between the Catophragmidae (*Catomerus*) and the other Chthamaloidea, in agreement with Buckeridge and Newman (1992), and showed the Archaeobalanidae (*Elminius* and *Semibalanus*) as paraphyletic (the monophyly of this group was significantly rejected by the SH test, $P < 0.001$). This result is not surprising because the taxonomic diagnosis of the Archaeobalanidae relies on plesiomorphic traits rather than on synapomorphies. We need more extensive taxon sampling for molecular analysis and we must develop many more morphological characters before the finer details of the Balanoidea phylogeny can be reliably analyzed.

Thoracican Divergence Time Estimation

ML-based YY approach.—Full molecular clock ($r = 0$) and local clock models with 1 or 2 independent rates (r_{c2} , r_{c3} , and r_{spord} ; Table 3) gave similar time estimates for each calibration point. However, the more parameterized local clock models gave different results depending on the number of implemented independent rates. This result suggests that local clock models with few parameters do not really account for most of the rate variation in our data set. Most of the local clock models inconsistently estimated the age of the most basal ingroup node (Thoracica; node 45), but better resolution (i.e., more plausible estimations and lower standard errors; data not shown) was achieved under the most highly parameterized model (r_{gen}) using 26 independent rate parameters. Time estimates under rate categories four to seven (r_{c4} – r_{c7}) did not differ very much (range: ≤ 38 MY for C_1 , ≤ 40 MY for C_2 , ≤ 32 MY for C_3) for each calibration point; but they did differ among order, suborder, and family/genus rank categories (range: ≤ 100 MY for C_1 , ≤ 106 MY for C_2 , ≤ 99 MY for C_3), presumably because of the greater differences in number of independent rates (see Table 3). Reciprocal calibrations did not match under local clock models with few parameters for any of the three possible combinations, but they came very close under highly parameterized local clocks (r_{fam} and r_{gen}) for C_1 and C_2 . Time estimates under these two calibrations agreed with the barnacle fossil record, suggesting that the method might be robust under the latter conditions (Yang and Yoder, 2003). Divergence times estimated under r_{fam} and r_{gen} and using C_3 were more recent than those estimated under C_1 and C_2 , but they also agreed reasonably well with most of the fossil ages for the shallow nodes (Table 4). The better performance of r_{fam} and r_{gen} can be explained based on the evolution of the rate of evolution: closely related evolutionary lineages are expected to evolve at similar rates because they are affected by biological factors that are also more similar between related lineages (Kishino et al., 2001). Thoracican and

rhizocephalan families and genera seem to share similar rates of evolution for the 18S gene. Although local clock models with 15 or 26 independent rates may look overparameterized, they actually include fewer parameters (rates) than the most highly parameterized local clock model that can be estimated for the tree in Figure 1, which would include 48 parameters. Because of the broad taxonomic rank of this phylogeny (from superorder to species) and the long evolutionary scale considered, the assumption of different rates for families and genera seems reasonable to us.

Bayesian-based A-BY and KTB approaches.—Estimates under the five different models of rate evolution specified under the A-BY method gave similar divergence estimation times for nodes 45–20, but lower estimates for nodes 15–9 were found under the OUP model (Table 3). These time estimates were also different from those calculated under the KTB approach using single calibrations (KTB₁, Table 3), which in general showed higher estimates for the deeper nodes (45–36) and lower estimates for the shallower nodes (except under the OUP model). Independent of the Bayesian method used, time span estimations between deep and shallow nodes were relatively narrow (especially under the A-BY approach), resulting in very incongruent reciprocal estimations between calibrations and notable disagreements with the ages of first occurrence of most of the groups (Table 3). These results, therefore, question either the reliability of this method under our particular conditions of analysis or the reliability of the barnacle fossil record. However, considering the good performance of the YY method under similar conditions, we believe the A-BY method may be problematic.

PL approach.—The PL technique requires at least one internal node in the tree to be fixed for estimating divergence times (r8sv1.6 manual). However, we found that unless the age of the root is fixed the method overestimates all the nodes above the calibration point (PL₁, Table 3). Even when several internal nodes are fixed or calibrated within an age interval and different algorithms are used, the problem persists. One possible way to get around this problem is to empirically fix the age of the root and then run the program until the known age of a specific node (neither fixed nor constrained) is estimated correctly. Using this procedure, we reestimated the ages of the stem clades in Table 3 (PL₂–PL₄). The resulting time estimates were in general very similar to those estimated under the KTB approach and so had the same inconsistencies.

The most important factor affecting time estimation is the calibration point, as has been pointed out before (Lee, 1999; Yoder and Yang, 2000; Thorne and Kishino, 2002; Yang and Yoder, 2003), although some methods seem to be more sensitive than others. 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 constraint nodes to lie within some interval rather than fix them to a particular time (Norell, 1992). However, only the KTB and the PL approaches allow this kind of refinement. Using these two methods,

we reestimated the divergence times for all the clades in Table 3 constraining the ages of the calibrations within the interval of their first occurrences. Estimated times under the KTB approach (KTB₂, Table 3) were now more consistent with the fossil record, although they were still slightly high for the shallow nodes 21–9. Estimates under the PL method (PL₅, Table 3) were similar to those of the KTB approach, but nodes 45 and 41 were still overestimated because the root was not fixed.

Divergence times.—Although the above discussion seems to leave open the question of whether this study has accurately estimated barnacle divergence dates, some reliable (i.e., congruent) information can be extracted from the comparison of the r_{fam} and r_{gen} YY local clock models under the C₁, C₂, and C₃ (only for the shallow nodes) and the KTB and PL (only for the shallow nodes) methods under multiple calibrations. A late Precambrian origin is suggested for the Rhizocephala and the Thoracica (node 45), as Collins and Rudkin (1981) proposed based on the discovery of *Priscansermarinus*[†]. Considering the conservative nature of our calibration points and the high degree of modification of barnacles for a permanently attached existence, an older origin might be postulated for the basic crustacean radiation, which agrees with a late Precambrian arthropod radiation as postulated by Whittington (1979). The oldest known ibloid (*Illilepas*[†]) fossil is from the Carboniferous (360–285 MAY), but both the phylogenetic position of *Ibla* and our time estimates suggest that the range of Iblomorpha (node 41) must be extended back to the early Cambrian, as postulated based on the phosphatic composition of their valves (Lowenstam et al., 1992). Reciprocal calibrations under the YY approach for the Heteralepadoromorpha + Lepadoromorpha (node 37) and Scalpellomorpha (node 36) stems are congruent, so they match their fossil records. The local clock models under C₁ and C₂ suggest a Middle Jurassic origin for the Verucomorpha (node 30), the oldest Sessilia representative in our analysis. Brachylepadoromorpha fossils (the most primitive Sessilia known) are represented since the late Jurassic. Thus, our local clock estimates suggest that the Sessilia radiation might have taken place earlier. Our local clock estimates also suggest a late Cretaceous–late Eocene radiation for the Balanomorpha groups Chthamaloidea (node 21), Coronuloidea (node 20), and Tetracitoidea and Balanoidea (node 15), but an older diversification (early Cretaceous) is indicated by the KTB and PL approaches. The balanomorph fossil record begins in the late Cretaceous with the polemic *Pachydiadema*[†] (Foster and Buckeridge, 1987) and expands through the Tertiary; hence, overall the ages suggested by the YY approach fit the fossil record better. The discrepancies among these three approaches are accentuated when estimating the age of the Balanidae (node 13) and *Balanus* (node 9) radiations (Table 3). Nevertheless, because fossil morphological data identify crown clades whereas genetic data identify the split between lineages (i.e., stem clades) before they developed crown-group features, ages estimated by the KTB and PL approaches cannot be completely rejected.

SUMMARY

Thoracican Phylogenetics

ML, MP, and Bayesian phylogenetic analyses of five genes (4,871 bp) and 90 morphological characters placed the four-plated Iblomorpha as the most primitive thoracican and the plateless Heteralepadoromorpha as the sister group of the Lepadoromorpha. The Sessilia (nonpedunculate) barnacles are monophyletic and have evolved from a stalked (pedunculate) multiplated (5+plates) scalpelloid ancestor. The Balanomorpha (symmetric sessile barnacles) relationships are (Chthamaloidea(Coronuloidea(Tetracitoidea, Balanoidea))). These phylogenetic hypotheses have relevant implications for the understanding of evolutionary processes in barnacles, such as the acquisition of shell plates, biomineralization, filter feeding mode, and sexual systems.

Divergence Time Estimation

Although some possible biases (e.g., single locus estimates, model of evolution, and calibration points) might affect our absolute barnacle time estimates, these biases apply to all of the procedures compared, so the best we can do is to compare estimates from different methods and discuss their limitations. First, estimated dates varied considerably within and between approaches depending on the calibration points. Second, times estimated via local clock models that assume independent rates for close taxonomic lineages (i.e., confamilial or congeneric species) showed the highest congruence between calibrations and were a better fit to the known barnacle fossil record. If that fit is interpreted as an indication of accuracy, it seems reasonable to conclude that the YY local clock approach under highly parameterized models ($r \geq 15$) outperforms all the other methods. Third, sequence data cannot completely surmount the inability of the fossil record to perfectly date internal nodes; thus, methods such as the KTB that can constrain several nodes to certain intervals and can accommodate multi-locus data sets may overcome that intrinsic inaccuracy (see also Yang and Yoder, 2003). Fourth, with respect to computational speed, all the previous analyses were run on an Intel Pentium 4 with 1.8 GHz and 1.0 GB of RAM or on a Macintosh OS X PowerPC G4 with 0.933 GHz and 1.024 GB of RAM. On a coarse grain scale, the computer programs that implement the PL, YY, KTB, and A-BY approaches needed seconds, minutes, hours, and days, respectively, to calculate time estimates under our conditions.

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REFERENCES

- Anderson, D. T. 1994. Barnacles—Structure, function, development and evolution. Chapman and Hall, London.
- Aris-Brosou, S., and Z. Yang. 2002. Effects of models of rate evolution on estimation of divergence dates with special reference to the metazoan 18S ribosomal RNA phylogeny. *Syst. Biol.* 51:703–714.
- Billoud, B., M.-A. Guerrucci, M. Masselot, and J. S. Deusch. 2000. Cirripede phylogeny using a novel approach: Molecular morphometrics. *Mol. Biol. Evol.* 17:1435–1445.
- Britten, R. J. 1986. Rates of DNA sequence evolution differ between taxonomic groups. *Science* 231:1393–1398.
- Broch, H. 1922. Studies on Pacific cirripeds. Papers from Dr. Th. Mortensen's Pacific Expedition 1914–1916, No. X. *Vidensk. Medd. Dan. Naturhist. Foren.* 73:215–358.
- Buckeridge, J. S. 1995. Phylogeny and biogeography of the primitive Sessilia and a consideration of a Tethyan origin for the group. Pages 255–268 in *Crustacean issues, Volume 10* (F. R. Schram, gen. ed.). New frontiers in barnacle evolution (F. R. Schram and J. T. Høeg, eds.). A. A. Balkema, Rotterdam, The Netherlands.
- Buckeridge, J. S., and W. A. Newman. 1992. A reexamination of *Waikalasma* (Cirripedia: Thoracica) and its significance in balanomorphy phylogeny. *J. Paleontol.* 66:341–345.
- Buckley, T. R. 2002. Model misspecification and probabilistic test of topology: Evidence from empirical data sets. *Syst. Biol.* 51:509–523.
- Colgar, D. J., A. McLauchlan, G. D. F. Wilson, S. P. Livingston, G. D. Edgecombe, J. Macaranas, G. Cassis, and M. R. Gray. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust. J. Zool.* 46:419–437.
- Collins, D., and D. M. Rudkin. 1981. *Priscansermarinus barnetti*, a probable lepadomorph barnacle from the Middle Cambrian Burgess Shale of British Columbia. *J. Paleontol.* 55:1006–1015.
- Crandall, K. A., and J. F. Fitzpatrick, Jr. 1996. Crayfish molecular systematics: Using a combination of procedures to estimate phylogeny. *Syst. Biol.* 45:1–26.
- Darwin, C. 1851. A monograph of the fossil Lepadidae or, pedunculated cirripedes of Great Britain. Paleontological Society, London.
- Darwin, C. 1852. A monograph of the sub-class Cirripedia, with figures of all the species. The Lepadidae: Or, pedunculated cirripedes. Ray Society, London.
- Darwin, C. 1854. A monograph of the sub-class Cirripedia, with figures of all the species. The Balanidae (or sessile cirripedes); the Verrucidae, etc., etc., etc. Ray Society, London.
- Darwin, C. 1855. A monograph of the fossil Balanidae and Verrucidae of Great Britain. Paleontological Society, London.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* 17:368–376.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783–791.
- Foster, B. A. 1978. The marine fauna of New Zealand: Barnacles (Cirripedia: Thoracica). *Mem. N.Z.J. Zool. Oceanogr. Inst.* 69:1–16.
- Foster, B. A., and J. S. Buckeridge. 1987. Barnacle paleontology. Pages 43–61 in *Crustacean issues, Volume 5* (F. R. Schram, gen. ed.). Barnacle biology (A. J. Southward, ed.). A. A. Balkema, Rotterdam, The Netherlands.
- Gillespie, J. H. 1991. The causes of molecular evolution. Oxford Univ. Press, Oxford, U.K.
- Glenner, H., M. J. Grygier, J. T. Høeg, P. G. Jensen, and F. R. Schram. 1995. Cladistic analysis of the Cirripedia Thoracica (Crustacea: Thecostraca). *Zool. J. Linn. Soc.* 114:365–404.
- Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.* 49:652–670.
- Harris, D. J., L. S. Maxson, L. F. Braithwaite, and K. A. Crandall. 2000. Phylogeny of the thoracican barnacles based on 18S rDNA sequences. *J. Crustacean Biol.* 20:393–398.
- Healy, J. M., and D. T. Anderson. 1990. Sperm ultrastructure in the Cirripedia and its phylogenetic significance. *Rec. Aust. Mus.* 42:1–26.
- Høeg, J. T. 1992a. The phylogenetic position of Rhizocephala: Are they truly barnacles? *Acta Zool.* 73:323–326.
- Høeg, J. T. 1992b. Rhizocephala. Pages 313–345 in *Microscopic anatomy of invertebrates* (F. W. Harrison and A. G. Humes, eds.). Wiley-Liss, New York.
- Høeg, J. T. 1995. Sex and the single cirripede: A phylogenetic perspective. Pages 195–207 in *Crustacean issues, Volume 10* (F. R. Schram, gen. ed.). New frontiers in barnacle evolution (F. R. Schram and J. T. Høeg, eds.). A. A. Balkema, Rotterdam, The Netherlands.
- Høeg, J. T., and G. A. Kolbasov. 2002. Lattice organs in y-cyprids of the Facetotecta and their significance in the phylogeny of the Crustacea Thecostraca. *Acta Zool.* 83:67–79.
- Høeg, J. T., N. C. Lagerström, and H. Glenner. 2003. The complete cypris larva and its significance in thecostracan phylogeny. Pages 197–215 in *Evolutionary and developmental biology of Crustacea* (G. Scholtz, ed.). A. A. Balkema, Lisse, Netherlands.
- Høeg, J. T., M. A. Whyte, H. Glenner, and F. R. Schram. 1999. New evidence on the basic phylogeny of the Cirripedia Thoracica. Crustaceans and the biodiversity crisis. *Proc. IV Int. Crust. Cong.* 1:101–114.
- Huelsenbeck, J. P. 1994. Comparing the stratigraphic record to estimates of phylogeny. *Paleobiology* 20:470–483.
- Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annu. Rev. Ecol. Syst.* 28:437–466.
- Huelsenbeck, J. P., and N. S. Imennov. 2002. Geographic origin of human mitochondrial DNA: Accommodating phylogenetic uncertainty and model comparison. *Syst. Biol.* 51:155–165.
- Huelsenbeck, J. P., B. Larget, R. E. Miller, and F. Ronquist. 2002. Potential applications and pitfalls of Bayesian inference phylogeny. *Syst. Biol.* 51:673–688.
- Jamieson, B. G. M. 1991. Ultrastructure and phylogeny of crustacean spermatozoa. *Mem. Queensl. Mus.* 31:109–142.
- Jefferies, R. P. S. 1979. The origin of chordates—A methodological essay. Pages 443–477 in *The origin of major invertebrate groups* (M. R. House, ed.). Academic Press, London.
- Kass, R. E., and A. E. Raftery. 1995. Bayes factors. *J. Am. Stat. Assoc.* 90:773–795.
- Kishino, H., and M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* 29:170–179.
- Kishino, H., J. L. Thorne, and W. J. Bruno. 2001. Performance of a divergence time estimation method under a probabilistic model of rate evolution. *Mol. Biol. Evol.* 18:352–361.
- Klepal, W. 1985. *Ibla cumingi* (Crustacea, Cirripedia)—a gonochoristic species (anatomy, dwarfing and systematic implications). *Mar. Ecol.* 6:47–119.
- Kolbasov, G. A. 1996. The significance of symbiosis in the evolution of sessile barnacles (Cirripedia Balanoidea). *Arthropoda Selecta* 5:3–16.
- Kolbasov, G. A., and J. T. Høeg. 2000. External morphology of females in the burrowing barnacles *Lithoglyptes mitis* and *L. habeii* (Lithoglyptidae) and the phylogenetic position of the Cirripedia: Acrothoracica (Crustacea, Thecostraca). *Arthropoda Selecta* 9:13–27.
- Korn, O. M. 1995. Naupliar evidence for cirripede taxonomy and phylogeny. Pages 87–121 in *Crustacean issues, Volume 10* (F. R. Schram, gen. ed.). New frontiers in barnacle evolution (F. R. Schram and J. T. Høeg, eds.). A. A. Balkema, Rotterdam, The Netherlands.
- Langley, C. H., and W. M. Fitch. 1974. An estimation of the constancy of rate of molecular evolution. *J. Mol. Evol.* 3:161–177.
- Lee, M. S. Y. 1999. Molecular clock calibrations and metazoan divergence dates. *J. Mol. Evol.* 49:385–391.
- Lemmon, A. R., and M. C. Milinkovitch. 2002. The metapopulation genetic algorithm: An efficient solution for the problem of large phylogeny estimation. *Proc. Natl. Acad. Sci. USA* 99:10516–10521.
- Li, W.-H. 1997. Molecular evolution. Sinauer, Sunderland, Massachusetts.
- Löwenstam, H. A., and S. Weiner. 1992. Phosphatic shell plate of the barnacle *Ibla* (Cirripedia): A bone-like structure. *Proc. Natl. Acad. Sci. USA* 89:10573–10577.

- Löwenstam, H. A., S. Weiner, and W. A. Newman. 1992. Carbonate apatite-containing plates of a barnacle (Cirripedia). Pages 73–84 in *Chemistry and biology of mineralized tissues* (H. Slavkin and P. Priece, eds.). Elsevier Science, Amsterdam.
- Martin, J. W., and G. E. Davis. 2001. An updated classification of the recent Crustacea. *Nat. Hist. Mus. Los Angel. Cy. Sci. Ser.* 39:1–124.
- Mokady, O., Y. Loya, Y. Achituv, E. Geffen, D. Graur, S. Rozenblatt, and I. Brickner. 1999. Speciation versus phenotypic plasticity in coral-inhabiting barnacles: Darwin's observation in a phylogenetic context. *J. Mol. Evol.* 49:367–375.
- Moyse, J. P. 1987. Larvae of lepadomorph barnacles. Pages 329–362 in *Crustacean issues, Volume 5* (F. R. Schram, gen. ed.). Barnacle biology (A. J. Southward, ed.). A. A. Balkema, Rotterdam, The Netherlands.
- Moyse, J. P., and E. Hui. 1981. Avoidance by *Balanus balanoides* cyprids of settlement on conspecific adults. *J. Mar. Biol. Assoc. U.K.* 61:449–460.
- Nei, M., and S. Kumar. 2000. *Molecular evolution and phylogenetics*. Oxford Univ. Press, Oxford, U.K.
- Newman, W. A. 1979. A new scalpellid (Cirripedia); a Mesozoic relic living near an abyssal hydrothermal spring. *Trans. San Diego Soc. Nat. Hist.* 19:153–167.
- Newman, W. A. 1987. Evolution of cirripedes and their major groups. Pages 3–42 in *Crustacean issues, Volume 5* (F. R. Schram, gen. ed.). Barnacle biology (A. J. Southward, ed.). A. A. Balkema, Rotterdam, The Netherlands.
- Newman, W. A. 1989. Juvenile ontogeny and metamorphosis in the most primitive living sessile barnacle, *Neoverruca*, from abyssal hydrothermal springs. *Bull. Mar. Sci.* 45:467–477.
- Newman, W. A. 1996. Cirripedia; suborders Thoracica and Acrothoracica. Pages 453–540 in *Traité de Zoologie* (J. Forest, ed.), Volume 7. Crustacés 2. Masson, Paris.
- Newman, W. A., and R. R. Hessler. 1989. A new abyssal hydrothermal verrucomorphan (Cirripedia; Sessilia): The most primitive living sessile barnacle. *Trans. San Diego Soc. Nat. Hist.* 21:259–273.
- Newman, W. A., and A. Ross. 1976. Revision of the balanomorph barnacles; including a catalog of the species. *Mem. San Diego Soc. Nat. Hist.* 9:1–108.
- Newman, W. A., and A. Ross. 2001. Prospectus on larval cirriped setation formulae, revisited. *J. Crustacean Biol.* 21:56–77.
- Newman, W. A., and T. Yamaguchi. 1995. A new sessile barnacle (Cirripedia, Brachylepadomorpha) from the Lau Back-Arc Basin, Tonga; first record of a living representative since the Miocene. *Bull. Mus. Natl. Hist. Nat.* 4 Ser. A 17:211–243.
- Newman, W. A., V. A. Zullo, and T. H. Withers. 1969. Cirripedia. Pages 206–295 in *Treatise of invertebrate paleontology, Part R, Arthropoda 4, Volume 1* (R. C. Moore, ed.). Geological Society of America and Univ. Kansas Press, Lawrence.
- Nilsson-Cantell, C. A. 1921. Cirripeden-Studien. Zur Kenntnis der Biologie, Anatomie, und Systematik dieser Gruppe. *Zool. Bidr. Upps.* 7:75–390.
- Norell, M. 1992. Taxic origin and temporal diversity: The effect of phylogeny. Pages 89–118 in *Extinction and phylogeny* (N. J. Novacek and Q. D. Wheeler, eds.). Columbia Univ. Press, New York.
- Palmer, A. R. 1983. The decade of North American geology: 1983 geologic time scale. *Geology* 11:504–505.
- Pérez-Losada, M., J. T. Høeg, G. A. Kolbasov, and K. A. Crandall. 2002. Reanalysis of the relationships among the Cirripedia and the Ascothoracida and the phylogenetic position of the Facetotecta (Maxillopoda: Thecostraca) using 18S rDNA sequences. *J. Crustacean Biol.* 22:661–669.
- Perl-Treves, R., L. Mizrahi, D. J. Katcoff, and Y. Achituv. 2000. Elucidation of the phylogenetic relationships of three thecostracans, *Verruca*, *Paralepas*, and *Dendrogaster* based on 18S rDNA sequences. *J. Crustacean Biol.* 20:385–392.
- Pilsbry, H. A. 1907. The barnacles (Cirripedia) contained in the collection of the US National Museum. *Bull. U.S. Natl. Mus.* 60:1–122.
- Pilsbry, H. A. 1916. The sessile barnacles (Cirripedia) contained in the collection of the US National Museum; including a monograph of the American species. *Bull. U.S. Natl. Mus.* 60:1–122.
- Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- Rambaut, A., and L. Broham. 1998. Estimating divergence data from molecular sequences. *Mol. Biol. Evol.* 15:442–448.
- Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- Saiki, R., D. H. Gelfand, S. Stofell, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487–491.
- Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* 19:101–109.
- Sanderson, M. J. 1998. Estimating rate and time in molecular phylogenies: Beyond the molecular clock? Pages 242–264 in *Plant molecular systematics* (P. Soltis, D. Soltis, and J. Doyle, eds.). Chapman and Hall, London.
- Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Mol. Biol. Evol.* 14:1218–1231.
- Schram, F. R. 1986. *Crustacea*. Oxford Univ. Press, New York.
- Schram, F. R., and J. T. Høeg. 1995. New frontiers in barnacle evolution. Pages 297–312 in *Crustacean issues, Volume 10* (F. R. Schram, gen. ed.). New frontiers in barnacle evolution (F. R. Schram and J. T. Høeg, eds.). A. A. Balkema, Rotterdam, The Netherlands.
- Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.* 51:492–508.
- Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16:1114–1116.
- Smith, A. B. 1994. *Systematics and the fossil record*. Blackwell, Oxford, U.K.
- Soltis, P. S., D. E. Soltis, V. Savolainen, P. R. Crane, and T. G. Barraclough. 2002. Rate heterogeneity among lineages of tracheophytes: Integration of molecular and fossil data and evidence for molecular living fossils. *Proc. Natl. Acad. Sci. USA* 99:4430–4435.
- Spears, T., L. G. Abele, and M. A. Applegate. 1994. Phylogenetic study of cirripedes and selected relatives (Thecostraca) based on 18S rDNA sequence analysis. *J. Crustacean Biol.* 14:641–656.
- Strimmer, K., and A. Rambaut. 2002. Inferring confidence sets of possible misspecified gene trees. *Proc. R. Soc. Lond. B* 269:137–142.
- Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b8. Sinauer, Sunderland, Massachusetts.
- Takezaki, N., A. Rzhetsky, and M. Nei. 1995. Phylogenetic test of the molecular clock and linearized trees. *Mol. Biol. Evol.* 12:823–833.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24:4876–4882.
- Thorne, J. L., and H. Kishino. 2002. Divergence time and evolutionary rate estimation with multilocus data. *Syst. Biol.* 51:689–702.
- Thorne, J. L., H. Kishino, and I. S. Painter. 1998. Estimating the rate of evolution of the rate of molecular evolution. *Mol. Biol. Evol.* 15:1647–1657.
- Tomlinson, J. T. 1987. The burrowing barnacles (Acrothoracica). Pages 63–71 in *Crustacean issues, Volume 5* (F. R. Schram, gen. ed.). Barnacle biology (A. J. Southward, ed.). A. A. Balkema, Rotterdam, The Netherlands.
- Utinomi, H. 1968. A revision of the deep sea barnacles *Pachylasma* and *Hexelasma* from Japan, with a proposal of new classification of the Chthamalidae (Cirripedia: Thoracica). *Publ. Seto Mar. Biol. Lab.* 16:21–39.
- Whiting, M. F. 2001. Mecoptera is paraphyletic: Multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zool. Scr.* 31:93–104.
- Whiting, M. F., J. C. Carpenter, Q. D. Wheeler, and W. C. Wheeler. 1997. The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Syst. Biol.* 46:1–68.
- Whittington, H. B. 1979. Early arthropods, their appendages and relationships. Pages 253–268 in *The origin of major invertebrate groups* (M. R. House, ed.). Academic Press, London.
- Whyte, M. A. 1976. A carboniferous pedunculate barnacle. *Proc. Yorkshire Geol. Soc.* 41:1–12.
- Whyte, M. A. 1988. The mineral composition of the valves and peduncle scales of *Ibla quadricostis* (Cuvier) (Cirripedia, Thoracica). *Crustaceana* 55:219–224.

- Wiens, J. J. 1998. Combining data sets with different phylogenetic histories. *Syst. Biol.* 47:568–581.
- Woodward, H. 1901. On "*Pyrgoma cretacea*," a cirripede from the Upper Chalk of Norwich and Margate. *Geol. Mag.* 8:145–152.
- Yamaguchi, T., and W. A. Newman. 1990. A new and primitive barnacle (Cirripedia: Balanomorphia) from the North Fiji basin abyssal hydrothermal field, and its evolutionary implications. *Pac. Sci.* 44:135–155.
- Yang, Z. 1997. PAML: A program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Bioci.* 13:555–556.
- Yang, Z., and A. D. Yoder. 2003. Comparison of likelihood and Bayesian methods for estimating divergence times using multiple gene loci and calibration points, with application to a radiation of cute-looking mouse lemur species. *Syst. Biol.* 52:705–716.
- Yoder, A. D., and Z. Yang. 2000. Estimation of primate speciation dates using local molecular clocks. *Mol. Biol. Evol.* 17:1081–1090.
- Zevina, G. B. 1982. Cirripede crustaceans of the suborder Lepadomorpha (Cirripedia, Thoracica) of the world ocean, Part 2. Family Lepadidae. *Opredeliteli Faune SSSR* 133:1–223. (In Russian.)
- Zuckermandl, E., and L. Pauling. 1962. Molecular disease, evolution, and genetic heterogeneity. Pages 189–225 in *Horizons in biochemistry* (M. Kasha and B. Pullman, eds.). Academic Press, New York.
- Zuckermandl, E., and L. Pauling. 1965. Evolutionary divergence and convergence in proteins. Pages 97–166 in *Evolving genes and proteins* (V. Bryson and H. J. Vogel, eds.). Academic Press, New York.
- Zullo, V. A. 1963. A classification and phylogeny of the Chthamulidae (Cirripedia: Thoracica). *Proc. XVI Int. Cong. Zool.* 1:190.
4. Musculus adductor scutorum (MAS): 0 = postesophageal; 1 = preesophageal. (27)
5. Comb collar: 0 = present; 1 = absent.
6. Scuta and terga: 0 = absent; 1 = present.
7. Carina (C): 0 = absent; 1 = present. (7)
8. Rostrum (R): 0 = absent; 1 = present. (8)
9. Size of rostrum: 0 = rostrum length \geq carina length; 1 = rostrum length $<$ carina length. (9)
10. Median latus (L): 0 = absent; 1 = present. (10)
11. Carinolatus (CL): 0 = absent; 1 = present. (12)
12. Rostrulatus (RL): 0 = absent; 1 = present. (13)
13. Compound rostrum (RL + R + RL): 0 = not fused; 1 = imperceptibly fused (no sulcus). (14) [6]
14. CL2 (duplication of CL1): 0 = absent; 1 = present. (15) [9]
15. Wall of shell plates: 0 = not in contact with substratum; 1 = in contact with substratum. (16)
16. Sheath: 0 = absent; 1 = present. (17)
17. Separation of opercular plates from wall plates: 0 = no operculum; 1 = operculum present. (19)
18. Articulation tergum–scutum (hinge): 0 = no hinge; 1 = hinge present. (20)
19. Complexity of hinge (tergum–scutum): 0 = hinges movably interlocked; 1 = highly complex hinge. (20)
20. Imbricating whorls: 0 = absent; 1 = present. (21) [8]
21. Small isolated plates below carina–rostrum tier: 0 = absent; 1 = present. (23)
22. Symmetry of scutum and tergum: 0 = free and identical on both sides; 1 = fixed on one side, movable on the other. (24)
23. Symmetry of carina and rostrum: 0 = both symmetrical, 1 = asymmetric and meet on one side. (25)
24. Tubiferous radii: 0 = absent; 1 = present. [1]
25. Parietal chitin: 0 = absent; 1 = present. [3]
26. Radii: 0 = absent; 1 = present. [4]
27. Tubiferous paries: 0 = absent; 1 = present. [5]
28. Ovigerous frena: 0 = absent; 1 = present. (29)
29. Branchiae: 0 = absent; 1 = present. (30)
30. Filamentary appendages: 0 = absent; 1 = present. (31)
31. Caudal filaments: 0 = absent; 1 = present. (32)
32. Shape of labrum: 0 = strongly bullate; 1 = weakly bullate or thin.
33. Cirrus III: 0 = resembling cirrus IV more than II; 1 = resembling cirrus II more than IV or intermediate
34. Fusion CL1 + CL2: 0 = not fused; 1 = fused.
35. Fusion all parietes (RL + R + RL) + CL1 + CL2 + C: 0 = not fused; 1 = fused.
36. Labrum crest: 0 = never deeply incised; 1 = deeply incised.
37. Penis shape: 0 = without basidorsal point; 1 = with basidorsal point.

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APPENDIX 1

Adult morphological characters and states used for the maximum parsimony and Bayesian Markov chain Monte Carlo phylogenetic analyses. Numbers in parentheses refer to character numbers of Glenner et al. (1995) and those in brackets refer to character numbers of Buckeridge (1995). Character states and scorings have been revised from these sources, and all of them are now binary. Character 5 is from Kolbasov and Høeg (2000); the other new characters are principally from Newman and Ross (1976).

1. Peduncle in adult: 0 = absent; 1 = present. (1)
2. Peduncular scales: 0 = absent; 1 = present. (1)
3. Primordial valves: 0 = absent; 1 = present. (4)

APPENDIX 3

Phylogenetic Position of the Iblomorpha

The systematic position of *Ibla* has been discussed since it was first described by Darwin (1851), and it remains problematic. Both adults and larvae of *Ibla* exhibit several morphological features that suggest the taxon is primitive: in the adult a poorly armed capitulum with largely chitinous plates, the lack of a sharp distinction between the capitulum and the peduncle, the lack of a carina, the simplest and most primitive arrangement of cirri, multiarticulated caudal appendages, a postoral scutal adductor muscle, and opercular plates impregnated with calcium phosphate, and in the nauplius, total absence of the dorsal thoracic spine and characteristic sculpturing of the upper surface in the cephalic shield (for more details, see Darwin, 1851; Newman et al., 1969; Klepal, 1985; Tomlinson, 1987; Newman, 1987; Korn, 1995). However, the exact systematic position of *Ibla* within the Cirripedia has been challenged several times. Initial morphology-based studies by Darwin (1851) suggested an affinity between *Ibla* and the Heteralepadomorpha, although subsequent morphological studies have shown *Ibla* as (1) an early offshoot of the thoracican tree (Newman et al., 1969; Newman, 1987; Høeg et al., 1999), (2) part of a new superorder Prothoracica (=Iblomorpha), from which the Thoracica might have evolved (Anderson, 1994; this hypothesis is topologically identical to the previous hypothesis), (3) an early bifurcation of the Cirripedia stock before the separation of the Thoracica and the Acrothoracica or a missing link between both (Klepal, 1985), and (4) an intermediate link between the Heteralepadomorpha and the Lepadomorpha (Glennner et al., 1995). The few published molecular studies including this taxon have produced controversial results, although all of them positioned the ibloids within the Thoracica. Perl-Treves et al. (2000) placed *Ibla* in a more internal position, forming a polytomy with the Verrucomorpha, Scalpellomorpha, and Balanomorpha; but reanalysis of the same molecular data by Pérez-Losada et al. (2002) resulted separation of the ibloids from the Balanomorpha, in agreement with Harris et al. (2000) who placed them at the most basal position of the thoracican tree.

Phylogenetic Position of the Heteralepadomorpha

The plateless Heteralepadomorpha have several morphological features (e.g., capitulum not covered with calcareous plates, a pair of small chitinous thickenings for the attachment of the adductor muscle, six pairs of cirri of which five or six may be modified, and articulated caudal appendages) that make their phylogenetic positioning problematic. Anderson (1994) and Newman (1996) regarded most of these morphological features as plesiomorphic, and the Newman, following Foster (1978), placed the group at the most basal position of the thoracican tree, in agreement with the Glennner et al. (1995) analysis. However, in the traditional view (Pilsbry, 1907; Nilsson-Cantell, 1921) the lack of plates in heteralepadids is a secondary loss (i.e. an apomorphic feature) that positions them within the Lepadomorpha sensu lato (Zevina, 1982). Høeg et al. (1999) argued that the loss of the shell plates required only one evolutionary step (instead of five as usually accepted) and placed the Heteralepadomorpha between the Iblomorpha and the Lepadomorpha sensu stricto, as did Newman (1987). The presence of a preoral scutal adductor muscle, as in all Cirripedia except for the Acrothoracica and *Ibla*, also argues that the Heteralepadidae diverged somewhat beyond the root of the thoracican lineage (Høeg et al., 1999). Molecular studies by Harris et al. (2000), Perl-Treves et al. (2000), and Pérez-Losada et al. (2002) indicated that the heteralepadids are indeed near the root of the thoracican tree, as a sister group to the Lepadomorpha sensu stricto, a position consistent with the traditional view and supported by the morphology-based cladistic analysis of Høeg et al. (1999).

The Acquisition of the Sessile Condition

Since Darwin (1851, 1852, 1854, 1855), cirripedologists have unanimously agreed that barnacles with a peduncle represent a more plesiomorphic condition than those without (=the "sessile condition" as seen in both the Verrucomorpha and the Balanomorpha). In contrast, there was no consensus of opinion on which pedunculated forms are the more ancient, those possessing numerous capitular plates and peduncular scales (scalpellomorphs) or those with few capitular plates and a naked peduncle (lepadomorphs). Based on the limited fossil

record, Darwin (1851) was compelled to conclude that the scalpellomorphs were primitive and gave rise to the lepadomorphs. Later Broch (1922), studying cyprid metamorphosis in scalpelloids, inferred that the five-plate rather than the six-plate condition was primitive, suggesting that the lepadoids are primitive compared with the scalpelloids. Several lines of evidence, including the fossil record, also suggest that a five-plated state is more plesiomorphic than a multiplated (5+ plates) state and that the sessile condition evolved from the latter (see Newman, 1987; Newman and Hessler, 1989; Anderson, 1994). Phylogenetic analyses of adult morphology (Glennner et al., 1995; Høeg et al., 1999) have shown a lepadoid → scalpelloid → sessile evolutionary trend. However, some larval character states in the Scalpellomorpha seem to be more plesiomorphic than those in the Lepadomorpha (Korn, 1995), which generates a disagreement between larval and adult morphology-based trees when both features are subject to phylogenetic analysis (see Newman and Ross, 2001). However, the polarization of larval characters is fraught with uncertainty because of the lecithotrophic nauplii in the outgroups. Previous molecular results from Perl-Treves et al. (2000) and Harris et al. (2000) placed the Lepadomorpha as primitive but were ambiguous about the order of the next two links; the Sessilia are placed either below or above the Scalpellomorpha.

Sessilia Monophyly

Darwin (1854) considered the asymmetrical (Verrucomorpha) and symmetrical (Balanomorpha) sessile barnacles to be sufficiently distinct to be placed in separate families coordinate with the Pedunculata. Nonetheless, with much hesitation, he considered these sessile groups to be more closely related to each other than to the pedunculates. Woodward (1901) resolved this problem by grouping Darwin's sessile families under the order Operculata (=Sessilia). But following Pilsbry (1907, 1916), subsequent authors treated the sessile barnacles (Verrucomorpha, Balanomorpha, and Brachylepadomorpha) as having descended independently from similar pedunculate stocks (Nilsson-Cantell, 1921; Newman et al., 1969; Newman and Ross, 1976). The situation became more complicated with the studies of Zullo (1963), Utinomi (1968), Moyses (1987), Anderson (1994), and Korn (1995), who found the Balanomorpha diphyletic, with chthamalooids and balanoids evolving independently from different scalpelloid ancestors. Thus, if these notions were correct, sessile barnacles would have arisen from pedunculate stocks three to five times. The situation changed when Newman, first in 1987 and then in successive studies on several living fossils from hydrothermal vents (Newman and Hessler, 1989; Buckeridge and Newman, 1992; Newman and Yamaguchi, 1995), suggested that the Verrucomorpha and the Balanomorpha have evolved from a brachylepadomorphanlike ancestor (i.e., the Sessilia are monophyletic in a strict sense). This statement revived Woodward's hypothesis with new significant morphological support. Another major step was gained by Glennner et al. (1995), who by using new characters and taxa confirmed the monophyly of the balanoids, but until now no phylogenetic analysis has clearly confirmed or rejected the monophyly of the Sessilia.

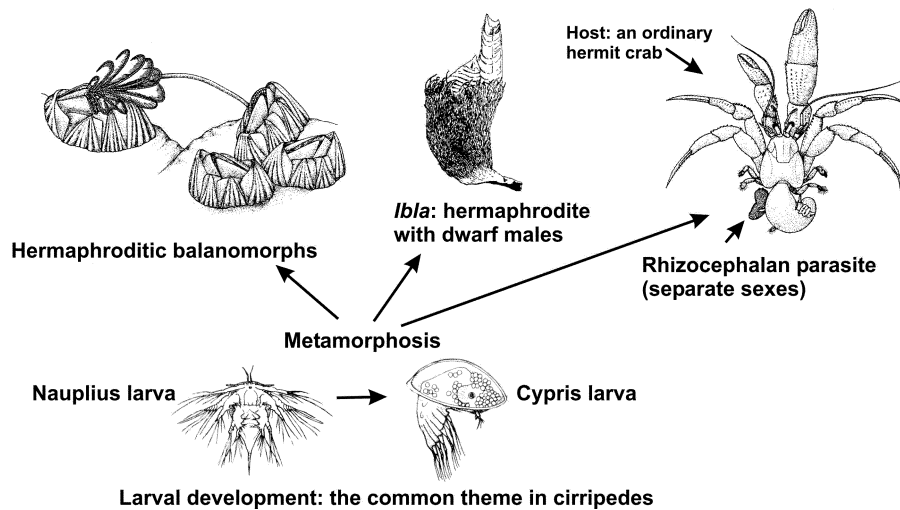
Balanomorpha

Resolving the intrinsic relationships between the chthamalooids and the remaining Balanomorpha has always been a main issue in thoracican systematics. Darwin (1854) and subsequent authors considered the Chthamaloidea the oldest balanomorph group. Many adult (e.g., trophi, tridentoid mandibles) and larval (e.g., labrum, number of abdominal spines) morphological characters clearly separate the chthamalooids from their relatives (see Newman and Ross, 1976; Korn, 1995), but few synapomorphies reveal the branching pattern within the other balanomorphs. Based on the ideas proposed by Newman and Ross (1976), Newman (1987), Yamaguchi and Newman (1990), Buckeridge and Newman (1992), Anderson (1994), and Buckeridge (1995), the evolution of the major balanomorph groups could be represented as (Chthamaloidea (Coronuloidea (Tetraclitoidea, Balanoidea))). However the phylogenetic studies developed by Glennner et al. (1995) and Newman and Ross (2001) failed to confirm or reject the previous branching pattern. The selection, number, reliability, and manipulation of the data, and the potential (i.e., phylogenetic signal) of the larval characters to reflect adult relationships were pointed out by the

authors as the main problems in their analyses. Phylogenetic analyses of 18S rDNA sequences by Harris et al. (2000) and Perl-Treves et al. (2000) did not achieve better resolution because of the limited number of species used. Therefore, the evolution of the main Balanomorpha lineages is still an open question in Thoracica systematics.

Within the Chthamaloidea, the Catophragmidae (e.g., *Catomerus*) have been considered the oldest balanomorph and the transitional

link between the pedunculates and the Balanomorpha (Darwin, 1854; Newman and Ross, 1976). However, the discovery and interpretations of *Eochionelasmus* Yamaguchi and Newman and the reexamination of the taxonomic status of the fossil *Waikalasma* Buckeridge revoked these assessments and moved the Catophragmidae up to a sister relationship with the other members of the Chthamaloidea (Buckeridge and Newman, 1992).



Barnacles (Cirripedia) exhibit a diversity of morphologies ranging from parasitic forms devoid of all conventional arthropod traits (Rhizocephala) to shell clad filter feeders (Thoracica). In addition, their sexual systems vary from separate sexes with dwarf males, through androodioecy (dwarf males with hermaphrodites) to pure hermaphroditism. A robust phylogeny of all Cirripedia is needed to arrive at an understanding of the evolution that have occurred at the morphological and ecological levels in this specialized group of crustacean arthropods.