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Stalked and acorn barnacles (Thoracica)

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Abstract

The Superorder Thoracica is the most diverse group of barnacles, Class Maxillopoda, and contains over 1000 species in nearly 30 families. Fossil Thoracica are known from the late Carboniferous and early Permian (310–290 million years ago, Ma), while the earliest cirripeds are known from the Silurian (443–416 Ma). Molecular divergence times place the divergence of the orders of Thoracica in the early Carboniferous (340 Ma). The suborders of the polyphyletic Order Pedunculata further diversified between the early Permian (287 Ma) and the early Jurassic (198 Ma). The Order Sessilia, excluding the Brachylepadomorpha, appeared in the late Jurassic (147 Ma).

Barnacles are a member of a non-monophyletic assemblage of crustaceans including (sometimes) ostracods, copepods, barnacles, and other assorted crustaceans. Within the Class Maxillopoda, the most recent classification places the barnacles into the Subclass Thecostraca (1). Within the Thecostraca, they are further subdivided into three Infraclasses, namely the Facetotecta (with a single family), the Ascothoracida (with six families), and the Cirripedia (containing the bulk of the barnacle diversity). Within the Cirripedia (e.g., Fig. 1), there are three superorders, the boring Acrothoracica, the parasitic Rhizocephala, and the stalked and sessile Thoracica. It is the Thoracica (the stalked and acorn barnacles) that we concentrate on in this chapter with representatives from the Rhizocephala as outgroups. The Thoracica contains two orders: the stalked Pedunculata (14 families) and the

sessile Sessilia (15 families). Note that this current classification is in the midst of some flux as new phylogenetic studies come to light that bare directly on the classification of barnacles (2, 3).

The Thoracica, ordinary or true barnacles, is the most diverse group of barnacles with over 1000 species found in virtually all marine and estuarine environments from intertidal pools to abyssal vents. They deviate from all other Crustacea in being permanently and irreversibly attached suspension feeders that have abandoned the normal arthropod growth pattern by being armed externally with mineralized plates that are never shed in molts but increase incrementally in size (4). Barnacles were in many respects the first model organism in evolutionary biology as reflected in Darwin's work (5–8). Their very specialized morphologies, diverse habitats,



Fig. 1 A goose barnacle (*Lepas anatifera*) from Taiwan showing the cirri or feeding legs that define the infraclass Cirripedia. Credit: J. T. Høeg, B. B. Chan, and C.-H. Hsu.

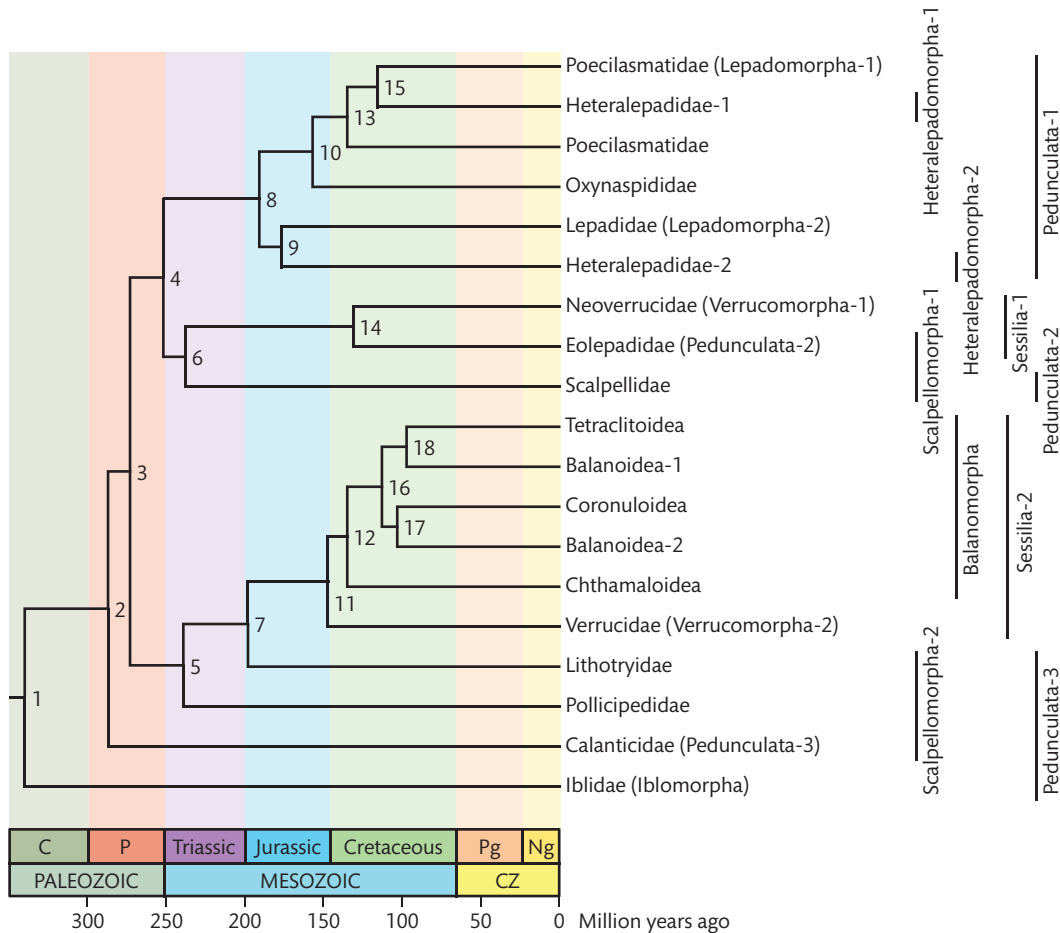


Fig. 2 A timetree of stalked and acorn barnacles (Thoracica). The divergence times are shown in Table 1. *Abbreviations:* C (Carboniferous), CZ (Cenozoic), Ng (Neogene), P (Permian), and Pg (Paleogene). Heteralepadidae-1 (Heteralepadomorpha), Heteralepadidae-2 (*Paralepas palinuri*, *Paralepas dannevigii*), Balanoidea-1 (*Austrominius modestus*, *Elminius kingie*), Balanoidea-2 (*Austromegabalanus psittacus*, *Balanus balanus*, *Balanus crenatus*, *Balanus glandula*, *Balanus perforates*, *Megabalanus californicus*, *Megabalanus tintinnabulum*, *Megabalanus spinosus*, *Menesiniella aquila*, *Semibalanus balanoides*, *Semibalanus cariosus*).

and reproductive systems made them excellent for testing and honing his ideas on biological evolution (9, 10). Barnacles have retained the attention of biologists ever since. They are important members of many marine habitats, such as the rocky intertidal zone, and their sessile mode of life makes them the primary fouling objects on man-made structures in the sea (11).

However, there is still a pervasive lack of phylogenetic information for a group that has been the focus of intense study for almost two centuries (12). A robust phylogeny is therefore pivotal in understanding how barnacles have evolved and diversified from a more conventional ancestor and also how experimental studies on single species, such as in antifouling research, can be extended to larger groups. Many hypotheses concerning barnacle evolution have been proposed (4, 13–21), but Glenner *et al.* (22)

were the first to apply cladistic approaches to determine their interrelationships. The morphology-based analysis of Glenner *et al.* (22) and the subsequent molecular studies (2, 3, 23–25) differed in their conclusions from each other and from existing taxonomies (1, 4, 14, 26). Pérez-Losada *et al.* used a more extensive taxon sampling and both molecular and morphological data sets for a thorough reassessment of evolutionary relationships (2).

Timing the radiation of the main barnacle groups based on their extinct relatives has always provoked great interest. Fossils and evolutionary hypotheses have been combined previously (22, 26, 27, 28), but only two studies (2, 3) have integrated both within a statistical framework. In this study, phylogenetic procedures of time estimation and three fossil calibration points were used to date the radiation of the main thoracican clades using 18S ribosomal

Table 1. Divergence times (Ma) and their credibility/confidence intervals (CI) among stalked and acorn barnacles (Thoracica), based on ref. (2).

Node	Timetree	
	Time	CI
1	340.0	415-310
2	287.0	351-241
3	273.0	333-228
4	252.0	310-207
5	239.0	295-195
6	238.0	294-194
7	198.0	242-162
8	187.0	247-139
9	177.0	236-130
10	157.0	215-109
11	147.0	153-130
12	135.0	150-114
13	135.0	190-91
14	131.0	196-73
15	116.0	170-73
16	113.0	137-89
17	103.0	128-78
18	97.0	122-73

Note: Calibrations ranged from the Carboniferous (M. Pennsylvanian, 312-307 Ma) to the Neogene (M.-L. Miocene, 23-12 Ma).

DNA sequences. However, divergence time estimates based on a single gene can be biased (29, 32). The time estimate will be biased if the phylogeny used is incorrect (33). Unbiased and accurate divergence time estimates can be obtained by integrating multiple gene loci and multiple fossil calibration points into a robust phylogeny (29, 30, 32). Therefore, Thoracica divergence times generated using the Bayesian method of Thorne and Kishino (T-K) (29) from multiple gene regions and multiple calibration points (including maximum and/or minimum age constraints) are expected to be the most reliable (2).

In that study (2), 14 fossil calibration points were used to anchor local molecular clocks (Table 1). Previous calibrations used in Pérez-Losada *et al.* (3) for the Heteralepadomorpha (*Priscansermarinus*, M. Cambrian) and the Scalpellomorpha (*Pabulum*, L. Carboniferous) were not used in this study, because their barnacle affinities have been questioned and because these groups were not monophyletic in our new phylogenetic analysis. *Pabulum* is now considered to be a

bivalve mollusk (Martin Whyte, personal communication) and there is only feeble evidence for a cirriped origin of *Priscansermarinus* (34, 35). Given that most fossils are dated to an age range, the midpoint of each geologic range was chosen in the divergence time estimation.

A likelihood ratio test significantly ($P < 0.001$) rejected the null hypothesis that all genes, separately or combined, were evolving with equal rates across all lineages, requiring the use of methods that relax the molecular clock hypothesis to estimate divergence times. The thoracican T-K chronogram was estimated using the Bayesian phylogeny, three genes, and 14 fossil calibrations (Fig. 2). Multiple independent Bayesian runs produced identical mean time estimates for all the major clades, including 95% credibility intervals (CI) (Table 1). The 95% CI were large for most clades, because we used only one upper limit (*Pycnolepas rigida*). However, as previously shown (32), incorporating both lower and upper constraints during time estimation can reduce the standard deviation of the estimates. This analysis places the origin of the Thoracica suborders in the early Carboniferous (340 Ma). The suborders of the polyphyletic Pedunculata radiated between the early Permian (287 Ma) and the early Jurassic (198 Ma). The Sessilia (excluding the Brachylepadomorpha) appeared in the late Jurassic (147 Ma). These estimates have reasonable corroboration with the fossil record. For example, the presumptive iblomorph *Illilepas damrowi* (36) from the Carboniferous (359-299 Ma) (although significantly later than previous calibrations, 28) would agree reasonably well with our molecular estimates. Clearly, this timetree holds many implications for the evolution of barnacle morphology (2).

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