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# Morphological and host specificity evolution in coral symbiont barnacles (Balanomorpha: Pyrgomatidae) inferred from a multi-locus phylogeny

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ABSTRACT

Coral-inhabiting barnacles (Thoracica: Pyrgomatidae) are obligatory symbionts of scleractinian and fire corals. We attempted to reconstruct the phylogeny of coral-inhabiting barnacles using a multi-locus approach (mitochondrial 12S and 16S rRNA, and nuclear EF1, H3 and RP gene sequences, total 3532 bp), which recovered a paraphyletic pattern. The fire-coral inhabiting barnacle *Wanella milleporae* occupied a basal position with respect to the other coral inhabiting barnacles. Pyrgomatids along with the coral-inhabiting archaeobalanid *Armatobalanus* nested within the same clade and this clade was subdivided into two major lineages: *Armatobalanus* + *Cantellius* with species proposed to be the ancestral stock of extant coral barnacles, and the other comprising the remaining genera studied. Ancestral state reconstruction (ASR) suggested multiple independent fusions and separations of shell plates and opercular valves in coral barnacle evolution, which counters the traditional hypothesis founded on a scheme of morphological similarities. Most of the coral barnacles are restricted to one or two coral host families only, suggesting a trend toward narrow host range and more specific adaptation. Furthermore, there is a close linkage between coral host usage and phylogenetic relationships with sister taxa usually being found on the same coral host family. This suggests that symbiotic relationships in coral-inhabiting barnacles are phylogenetically conserved and that host associated specialization plays an important role in their diversification.



## 1. Introduction

Coral-inhabiting barnacles (Thoracica: Balanomorpha: Pyrgomatidae) are obligatory symbionts of scleractinian and fire corals (Ross and Newman, 1973). Stable isotope ratios of $C^{12}$ and $C^{13}$ on corals and coral barnacles (Achituv et al., 1997) suggest that corals contribute one of the carbon sources to the barnacles as the barnacles feed on the coral organic matter and zooxanthellae expelled by the corals. On the other hand, the ammonium excreted by the coral barnacles is absorbed by the coral zooxanthellae contributing to coral growth (Achituv and Mizrahi, 1996). Evolution and adaptation to an obligate symbiotic relationship with other marine fauna including corals and sponges have contributed to an increased diversity of marine fauna (e.g. Duffy, 1996; Munday et al., 2004; Sotka, 2005; Macdonald et al., 2006; Faucci et al., 2007). Therefore elucidating the evolutionary history of this obligate symbiotic life style would provide valuable insights into the evolution of symbiotic relationships not only in barnacles, but also in marine fauna in general (Sotka, 2005).

The pyrgomatid barnacles have cup-shaped or tube-shaped bases that are embedded deeply in the skeleton of host corals. Other than pyrgomatid barnacles, balanomorph species of the genus *Armatobalanus* (family Archaeobalanidae, e.g. *Armatobalanus allium*) and *Megabalanus* (family Balanidae, e.g. *Megabalanus ajax* and *M. stultus*) also live symbiotically with corals (Liu and Ren, 2007; Ross, 1999). These species bear six-plated shells that are distinguished from the four-plated shells or fused shells found in pyrgomatids (Fig. 1; Ross and Newman, 1973; Anderson, 1992). The fused shell is unique to pyrgomatids and believed to be an adaptation to a symbiotic lifestyle (Anderson, 1992), and in response to overgrowth of corals over their surface (Barnes et al., 1970). In pyrgomatid barnacles, adaptations include wall plates that have flattened to form a discoidal-shaped apex and a base that has become cup-shaped. Moreover, their shell plates are reduced from six (a plesiomorphic condition in most of the balanomorph barnacles) to four (carina, rostrum and paired latera) or even fused into a single shell (Fig. 1A; Anderson, 1992) to provide improved

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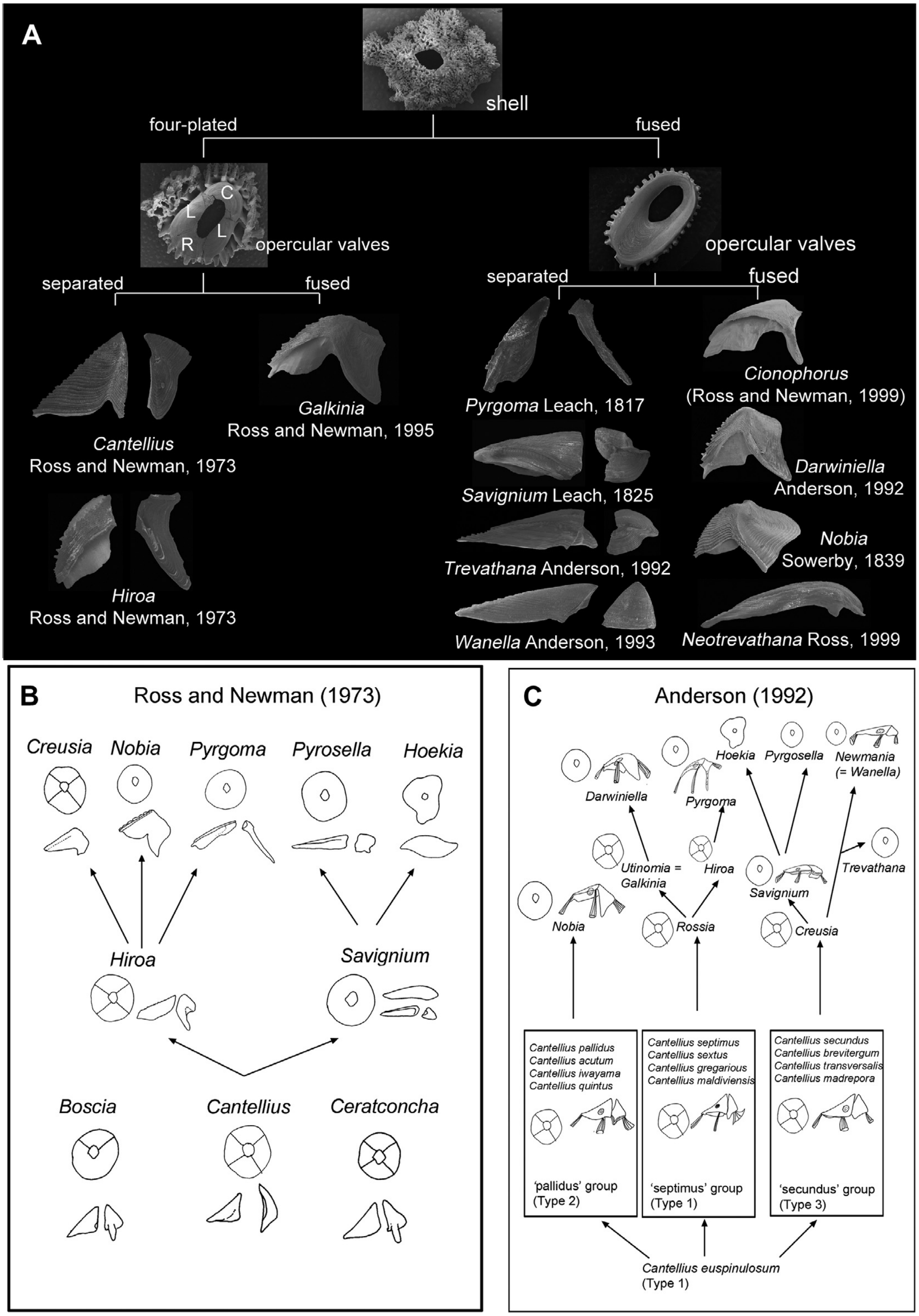


**Fig. 1.** (A) Shell structure and opercular valve morphology of various Pyrgomatidae genera (Carina [C], Rostrum [R], and two Laterals [L]). Hypotheses of phylogenetic relationship among pyrgomatid genera based on morphological similarities as proposed by (B) Ross and Newman (1973); and (C) Anderson (1992).

strength between apex and base. In addition to strengthened shells, pyrgomatid barnacles erect an aperture frill over the orifice which is believed to be a glandular fold that inhibits the growth of corals. Secretions from the frill act to chemically inhibit coral overgrowth (Anderson, 1992).

The morphology of the shell walls, opercular plates and aperture frills of coral-inhabiting barnacles have, therefore been considered key characters in traditional phylogenies given their important role in adaptation to a symbiotic lifestyle. Ross and Newman (1973) presented a non-cladistic phylogenetic tree of Pyrgomatidae (Fig. 1B). They suggested Pyrgomatidae evolved from an *Armatobalanus*-like (family Archaeobalanidae) ancestor that had a six-plated shell and separate opercular valves. The four-plated shell and separated opercular valves observed in