



Phylogenetics and morphological evolution of coral-dwelling barnacles (Balanomorpha: Pyrgomatidae)

MARIA CELIA (MACHEL) DEFRANCE MALAY^{1,2*} and FRANÇOIS MICHONNEAU²

¹*University of Guam Marine Laboratory, University of Guam*

²*Florida Museum of Natural History, University of Florida*

Received 23 June 2013; revised 15 March 2014; accepted for publication 15 March 2014

Pyrgomatid barnacles are a family of balanomorphs uniquely adapted to symbiosis on corals. The evolution of the coral-dwelling barnacles is explored using a multi-gene phylogeny (COI, 16S, 12S, 18S, and H3) and phenotypic trait-mapping. We found that the hydrocoral associate *Wanella* should be excluded, while some archaeobalanids in the genus *Armatobalanus* should be included in the Pyrgomatidae. Three well supported clades were recovered: clade I is the largest group and is exclusively Indo-West Pacific, clade II contains two plesiomorphic Indo-West Pacific genera, while clade III is comprised of East and West Atlantic taxa. Some genera did not form reciprocally monophyletic groups, while the genus *Trevathana* was found to be paraphyletic and to include members of three other apomorphic genera/tribes. The highly unusual coral-parasitic hoekiines appear to be of recent origin and rapidly evolving from *Trevathana sensu lato*. Pyrgomatids include six-, four-, and one-plated forms, and exhibit convergent evolutionary tendencies towards skeletal reduction and fusion, loss of cirral armature, and increased host specificity. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, ••, ••–••.

ADDITIONAL KEYWORDS: Cirripedia – coral barnacles – host specificity – parasitism – phylogeny – Pyrgomatidae – skeletal fusion – systematics – taxonomy.

INTRODUCTION

The Balanoidea is a large superfamily of free-living and symbiotic acorn barnacles. Newman & Ross (1976) recognized three balanoid families: the Balanidae Leach 1817, Archaeobalanidae Newman and Ross 1976, and Pyrgomatidae Gray 1825. Recent molecular and morphological studies (Healy & Anderson, 1990; Perez-Losada, Hoeg & Crandall, 2004; Perez-Losada *et al.*, 2008) have shown that balanids and archaeobalanids are mutually paraphyletic. The position of the Pyrgomatidae vis-à-vis the other two families is less well understood, however early results suggest possible paraphyly (Simon-Blecher, Huchon & Achituv, 2007).

This study focuses on the phylogenetic systematics of the Pyrgomatidae, a morphologically and ecologically distinctive group that is obligately associated

with hard corals (mostly scleractinians as well as a few hydrocorals). There are over 100 extant and extinct species of pyrgomatid barnacles (Ross & Newman, 2002a), making the Pyrgomatidae one of the larger families of balanomorph barnacles.

Pyrgomatid cyprid larvae settle and metamorphose on the surface of the host coral (Brickner & Hoeg, 2010). Post-settlement growth is rapid and mostly lateral, while growth of the adult barnacle is primarily vertical and confined to the margins of the basis (Hiro, 1938). This lifetime vertical growth is what allows the barnacle to avoid being overgrown by its coral host (Anderson, 1992; Fig. 1). Pyrgomatids also prevent entombment through abrasive action of the opercular valves and cirri, and suppression of coral skeleton deposition over their orifice and wall growth zones through an unknown, possibly chemically-mediated process (Anderson, 1992).

Morphological tendencies characterizing the pyrgomatid barnacles include a cup-shaped basis, a flattened wall, and reduced and fused wall plates and

*Corresponding author. E-mail: machel.malay@gmail.com

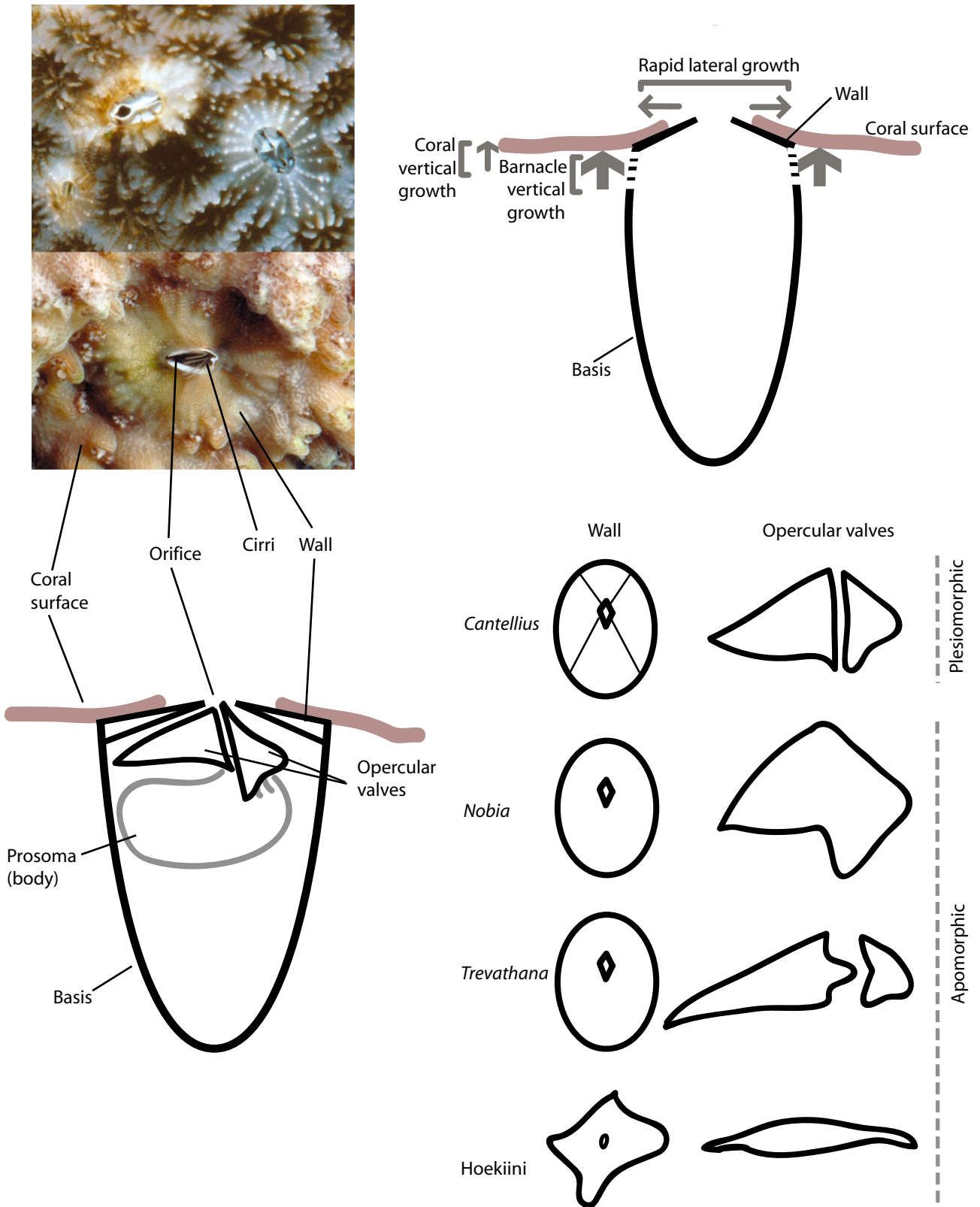


Figure 1. Schematic illustrations of pyrgomatid anatomy and growth process, and diversity in shell morphology.

opercular valves (Fig. 1). However these morphological tendencies vary considerably across the family. While most balanoids have six-plated walls, extant pyrgomatids either have four-plated walls or a single, fused wall plate (however one extinct pyrgomatid, genus *Eoceratoconcha*, possessed six wall plates; Newman & Ladd, 1974). Among balanomorphs, the fused, single wall plate and fully concrescent opercular valves are characters believed to be unique to the pyrgomatids (Fig. 1). Fusion of skeletal elements has traditionally been the most important characters used in delineating pyrgomatid genera and species, and is considered an apomorphic character (e.g., Darwin, 1854; Ross & Newman, 1973).

CURRENT STATE OF PYRGOMATID SYSTEMATICS

Current taxonomy divides the Pyrgomatidae into three subfamilies. The subfamily Ceratoconchinae Ross and Newman 1976 contains the earliest known records of pyrgomatids. It is comprised of two genera. The extinct six-plated genus *Eoceratoconcha* is known from the early Miocene to the Pliocene in the Caribbean. The four-plated *Ceratoconcha* is known from an earlier epoch, the late Oligocene in the Caribbean, and attained its peak diversity and distribution during the Miocene, ranging across the tropical Atlantic, Eastern Pacific (EP), and Paratethyan region (Ross & Newman, 2002a; Santos *et al.*, 2012). The four extant *Ceratoconcha* species are limited to the tropical West Atlantic (WA; Table 1).

The Pyrgomatinae (Gray, 1825) is the largest subfamily with 20 genera and 82 extant described species (Table 1), all exclusively Indo-West Pacific (IWP). The pyrgomatines show the highest morphological and ecological diversity among coral-dwelling barnacles, ranging from four-plated to single-plated taxa, and from typically balanoid opercular valves to highly derived valve morphologies (see Table 1; reviewed in Ross & Newman, 1973; Anderson, 1992). Two tribes were erected within the Pyrgomatinae for its most distinctive members: Hoekiini Ross and Newman 1995 (five genera, 11 species) and Pyrgopsellini Ross and Newman 1995 (one genus, two species). Hoekiines are true coral parasites, feeding exclusively on coral tissue with enlarged biting mouthparts and possessing non-functional, degenerate cirri (Ross & Newman, 1995). Hoekiines also have highly modified opercular valves, an irregularly-shaped wall, and a partly membranous basis. Pyrgopsellini resemble other pyrgomatines in opercular valve and wall morphology, but have almost entirely membranous bases. Previously thought to be sponge dwellers (Rosell, 1975), pyrgopsellines are now known to live suspended in the tissue of scleractinian corals (Achituv & Simon-Blecher, 2006). A third tribe, the Pyrgomatini

Gray 1825, contains the remaining 14 pyrgomatine genera. Members of this catch-all tribe range from plesiomorphic genera that resemble free-living balanoids in skeletal characters (e.g., *Cantellius*) to apomorphic taxa possessing fused and modified skeletal parts, but not distinct enough to warrant a separate tribe (Table 1). Fossil pyrgomatines are known from the late Miocene, Pleistocene, and Holocene of the IWP. All pyrgomatine fossils have been classed into extant species of Pyrgomatini (Asami & Yamaguchi, 1997; Ross & Newman, 2002a).

The Megatrematinae Holthuis 1982 is a small subfamily (4 genera, 6 extant species; Table 1) distributed in the WA, IWP, and East Atlantic (EA). It includes shallow-water genera on hermatypic corals as well as deeper-water taxa on ahermatypic corals. Megatrematines are known as fossils from the Pliocene of the Mediterranean and the Pleistocene of the Caribbean. Despite their wide distribution, megatrematines have never attained high species diversity (Ross & Newman, 2002a). The subfamily is divided into two tribes: the Pyrgominini Ross and Pitombo 2002 (with a tall conical wall) and the Megatrematini Holthuis 1982 (with a trapezoidal beaked tergum; Table 1).

The current classification of Pyrgomatidae is based on a few morphological characters, especially the fusion and form of the wall and opercular valves. A fused and flattened wall, and fused and modified opercular valves, are considered apomorphic; while 6-plated or 4-plated conical walls, and unfused and typically balanoid opercular valves, are considered plesiomorphic (Fig. 1). Given the paucity of characters utilized and diversity of opinion regarding the origins of coral barnacles (see below), it is not surprising that the systematics of the pyrgomatids has remained unstable. The fact that numerous new pyrgomatids are still being described (31 new species since 2000) is further evidence of our imperfect knowledge of the group.

PHYLOGENETIC HYPOTHESES REGARDING THE PYRGOMATIDS

Earlier taxonomists essentially espoused that pyrgomatids evolved from other currently extant, less morphologically specialized coral associates. There are several non-pyrgomatid balanoids that live obligately on corals, such as *Megabalanus ajax* and *M. stultus* (Megabalanidae) on the stinging hydrocoral *Millepora* (Ross, 1999b); *Tetraclita* sp. (Tetraclitidae) on the blue coral *Heliopora* (Newman & Ladd, 1974); *Hexacreusia* spp. (Archaeobalanidae) on the scleractinian genus *Porites* (Pitombo & Ross, 2002); and *Armatobalanus* spp. (Archaeobalanidae) on various scleractinians (Zullo, 1963; Anderson, 1992). Of these, the

Table 1. Extant pyrgomatid species, with summary of morphological and ecological characters at the genus level. Genera represented in the phylogeny are bolded. Regions: Indo-West Pacific (IWP), West Atlantic (WA), or East Atlantic (EA). Opercular valve fusion: unfused (uf) or fused (f). Valve appearance: balanoid (b) or modified (m). Basis: fully calcareous (ca), calcareous with passageways (cp), calcareous with narrow membranous zone (mz), or mostly membranous (me). Trophism: planktotrophic (pk) or parasitic (pr). Host: zooxanthellate scleractinian (zx), azooxanthellate scleractinian (az), or hydrocoral (h)

| | Region | #Wall plates | Valve fusion | Valve appearance | Basis | Trophism | Host |
|---|--------|--------------|--------------|------------------|-------|----------|--------|
| Family Pyrgomatidae Gray 1825 | | | | | | | |
| Subfamily Ceratoconchinae Newman and Ross 1976 | | | | | | | |
| Ceratoconcha Kramberger-Gorjanovic 1889 | WA | 4 | uf | b | ca | pk | zx |
| <i>domingensis</i> (Des Moulins, 1866) | | | | | | | |
| <i>floridanum</i> (Pilsbry, 1931) | | | | | | | |
| <i>paucicostata</i> Young 1989 | | | | | | | |
| Subfamily Pyrgomatinae (Gray, 1825) | | | | | | | |
| Tribe Pyrgomatini Gray 1825 | | | | | | | |
| <i>Arossella</i> (Anderson, 1993) | IWP | 4 | uf | m | ca | pk | zx |
| <i>lynnae</i> * Ross 2000a | | | | | | | |
| Cantellius Ross and Newman 1973 | IWP | 4 | uf | m | ca | pk | zx / h |
| <i>acutum</i> (Hiro, 1938) | | | | | | | |
| <i>albus</i> Ren 1986 | | | | | | | |
| <i>alphonsei</i> Achituv 2001 | | | | | | | |
| <i>arcuatum</i> (Hiro, 1938) | | | | | | | |
| <i>brevitergum</i> (Hiro, 1938) | | | | | | | |
| <i>cardenae</i> Achituv and Hoeksema 2003 | | | | | | | |
| <i>euspinulosum</i> (Broch, 1931) | | | | | | | |
| <i>gregarius</i> (Sowerby, 1823) | | | | | | | |
| <i>hiroii</i> Galkin 1982 | | | | | | | |
| <i>hoegi</i> Achituv, Tsang, and Chan 2009 | | | | | | | |
| <i>iwayama</i> (Hiro, 1938) | | | | | | | |
| <i>madreporae</i> (Borradaile, 1903) | | | | | | | |
| <i>maldiviensis</i> Galkin 1982 | | | | | | | |
| <i>octavus</i> Ross and Newman 1973 | | | | | | | |
| <i>pallidus</i> (Broch, 1931) | | | | | | | |
| <i>preobrazhenskyi</i> Galkin 1982 | | | | | | | |
| <i>pseudopallidum</i> (Kolosváry, 1947) | | | | | | | |
| <i>quintus</i> Ross and Newman 1973 | | | | | | | |
| <i>secundus</i> (Broch, 1931) | | | | | | | |
| <i>septimus</i> (Hiro, 1938) | | | | | | | |
| <i>sextus</i> (Hiro, 1938) | | | | | | | |
| <i>sinensis</i> Ren 1986 | | | | | | | |
| <i>sumbawae</i> (Hoek, 1913) | | | | | | | |
| <i>transversalis</i> * (Nilsson-Cantell, 1938) | | | | | | | |
| <i>tredecimus</i> (Kolosváry, 1947) | | | | | | | |
| <i>Cionophorus</i> Ross and Newman 1999 | IWP | 1 | f | m | ca | pk | zx |
| <i>guillaumae</i> Achituv and Newman 2002 | | | | | | | |
| <i>kushimotoensis</i> Ogawa and Nomura 2010 | | | | | | | |
| <i>soongi</i> * Ross and Newman 1999 | | | | | | | |
| <i>Creusia</i> Leach 1817 | IWP | 4 | f | b | ca | pk | zx |
| <i>spinulosa</i> * Leach 1818 | | | | | | | |
| Darwiniella Anderson 1992 | IWP | 1 | f | m | ca | pk | zx |
| <i>angularis</i> Chen <i>et al.</i> 2012 | | | | | | | |
| <i>conjugatum</i> * (Darwin, 1854) | | | | | | | |
| Galkinia Ross and Newman 1995 | IWP | 4 | f | b | ca | pk | zx |
| <i>adamanteus</i> Chan <i>et al.</i> 2013 | | | | | | | |
| <i>altiapiculus</i> Chan <i>et al.</i> 2013 | | | | | | | |
| <i>angustiradiata</i> (Broch, 1931) | | | | | | | |
| <i>decima</i> (Ross & Newman, 1973) | | | | | | | |
| <i>depressa</i> Chan <i>et al.</i> 2013 | | | | | | | |

Table 1. Continued

| | Region | #Wall plates | Valve fusion | Valve appearance | Basis | Trophism | Host |
|---|--------|--------------|--------------|------------------|-------|----------|---------|
| <i>equus</i> Chan <i>et al.</i> 2013 | | | | | | | |
| <i>indica</i> * (Annandale, 1924) | | | | | | | |
| <i>supraspinulosa</i> Ogawa 2000 | | | | | | | |
| <i>tabulatus</i> Chan <i>et al.</i> 2013 | | | | | | | |
| <i>trimegadonta</i> Chan <i>et al.</i> 2013 | | | | | | | |
| Hiroa Ross and Newman 1973 | IWP | 4 | uf | m | ca | pk | zx |
| <i>stubbingsi</i> * Ross and Newman 1973 | | | | | | | |
| <i>Neopyrgoma</i> Ross and Newman 2002b | IWP | 1 | unk. | unk. | cp | pk | zx |
| <i>lobata</i> * (Gray, 1825) | | | | | | | |
| Neotrevathana Ross 1999a | IWP | 1 | f | m | ca | pk | zx |
| <i>elongatum</i> (Hiro, 1931) | | | | | | | |
| Nobia Sowerby 1839 | IWP | 1 | f | m | ca | pk | zx |
| <i>grandis</i> * Sowerby 1839 | | | | | | | |
| <i>halomitrae</i> (Kolosváry, 1948) | | | | | | | |
| <i>orbicellae</i> (Hiro, 1934) | | | | | | | |
| Pyrgoma Leach 1817 | IWP | 1 | uf | m | cp | pk | zx / az |
| <i>cancellatum</i> * Leach 1818 | | | | | | | |
| <i>japonica</i> Weltner 1897 | | | | | | | |
| <i>kuri</i> Hoek 1913 | | | | | | | |
| <i>projectum</i> Nilsson-Cantell 1938 | | | | | | | |
| <i>sinica</i> (Ren, 1986) | | | | | | | |
| Savignium Leach 1825 | IWP | 1 | uf | m | ca | pk | zx |
| <i>crenatum</i> * (Sowerby, 1823) | | | | | | | |
| <i>tuamotum</i> Achituv and Langsam 2005 | | | | | | | |
| Trevathana Anderson 1992 | IWP | 1 | uf | m | ca | pk | zx |
| <i>dentata</i> * (Darwin, 1854) | | | | | | | |
| <i>isfae</i> Achituv and Langzam 2009 | | | | | | | |
| <i>jensi</i> Brickner <i>et al.</i> 2010 | | | | | | | |
| <i>margaretae</i> Brickner <i>et al.</i> 2010 | | | | | | | |
| <i>mizrachae</i> Brickner <i>et al.</i> 2010 | | | | | | | |
| <i>niuea</i> Achituv 2004 | | | | | | | |
| <i>orientale</i> (Ren, 1986) | | | | | | | |
| <i>paulayi</i> Asami and Yamaguchi 2001 | | | | | | | |
| <i>sarae</i> Brickner <i>et al.</i> 2010 | | | | | | | |
| <i>synthesysae</i> Achituv and Langzam 2009 | | | | | | | |
| <i>tureiae</i> Achituv and Langsam 2005 | | | | | | | |
| Wanella Anderson 1993 | IWP | 1 | uf | m | ca | pk | h |
| <i>andersonorum</i> (Ross, 1999a) | | | | | | | |
| <i>milleporae</i> * (Darwin, 1854) | | | | | | | |
| <i>snelliusi</i> (Kolosváry, 1950) | | | | | | | |
| Tribe Pyrgopsellini Ross and Newman 1995 | | | | | | | |
| Pyrgopsella Zullo 1967 | IWP | 1 | uf | m | me | pk | zx |
| <i>annandalei</i> * (Gravel, 1907) | | | | | | | |
| <i>youngi</i> Achituv and Simon-Blecher 2006 | | | | | | | |
| Tribe Hoekiini Ross and Newman 1995 | | | | | | | |
| <i>Ahoekia</i> Ross and Newman 1995 | IWP | 1 | f | m | mz | pr | zx |
| <i>chuangi</i> Ross and Newman 1995 | | | | | | | |
| <i>microtrema</i> Ross 2000b | | | | | | | |
| <i>tanabensis</i> * Ross and Newman 1995 | | | | | | | |
| <i>Australhoekia</i> Ross and Newman 2000b | IWP | 1 | f | m | mz | pr | zx |
| <i>cardenae</i> * Ross and Newman 2000b | | | | | | | |
| <i>Eohoekia</i> Ross and Newman 1995 | IWP | 1 | f | m | mz | pr | zx |
| <i>chaos</i> * Ross and Newman 1995 | | | | | | | |
| <i>nyx</i> Ross and Newman 1995 | | | | | | | |
| <i>Hoekia</i> Ross and Newman 1973 | IWP | 1 | f | m | mz | pr | zx |
| <i>fornix</i> Ross and Newman 1995 | | | | | | | |

Table 1. Continued

| | Region | #Wall plates | Valve fusion | Valve appearance | Basis | Trophism | Host |
|---|-----------|--------------|--------------|------------------|-------|----------|---------|
| <i>monticulariae</i> * (Gray, 1831) | | | | | | | |
| <i>mortensi</i> Ross and Newman 1995 | | | | | | | |
| <i>philippinensis</i> Ross 2000b | | | | | | | |
| <i>Parahoekia</i> Ross and Newman 1995 | IWP | 1 | f | m | mz | pr | zx |
| <i>aster</i> * Ross and Newman 1995 | | | | | | | |
| Subfamily Megatrematinae Holthuis 1982 | | | | | | | |
| Tribe Megatrematini Holthuis 1982 | | | | | | | |
| <i>Megatrema</i> Sowerby 1823 | WA, IWP | 1 | uf | b | ca | pk | zx |
| <i>madreporarum</i> * (Bosc, 1801) | | | | | | | |
| <i>youngi</i> Ross and Pitombo 2002 | | | | | | | |
| <i>Memagreta</i> Ross and Pitombo 2002 | IWP | 1 | uf | b | ca | pk | zx |
| <i>pandorae</i> * Ross and Pitombo 2002 | | | | | | | |
| Tribe Pyrgominini Ross and Pitombo 2002 | | | | | | | |
| Adna Sowerby 1823 | EA, IWP?† | 1 | uf | b | ca | pk | zx / az |
| <i>anglica</i> * Sowerby 1823 | | | | | | | |
| <i>Pyrgomina</i> Baluk and Radwanski 1967 | IWP | 1 | uf | b | ca | pk | zx / az |
| <i>djanae</i> Ross and Pitombo 2002 | | | | | | | |
| <i>oulastreae</i> (Utinomi, 1962) | | | | | | | |

*Type species.

†IWP records of *Adna* appear to be mis-identifications (Ross & Pitombo, 2002).

archaeobalanid genus *Armatobalanus* is considered to be morphologically closest to the pyrgomatids, and thus it is thought to represent either the ancestor of, or the sister clade to the pyrgomatids (Hiro, 1938; Ross & Newman, 1973; Healy & Anderson, 1990; Anderson 1992; Ross & Newman, 2000a; Simon-Blecher *et al.*, 2007).

Armatobalanus (~12 species) is comprised of both obligate coral associates and free-living species, and is diagnosed by six tall, conical wall plates and the presence of teeth on the third or fourth pair of cirri (Zullo, 1963). However, at least two *Armatobalanus* species lack cirral armature (Broch, 1931; Zullo, 1963). Neither is cirral armature a character exclusive to *Armatobalanus* (e.g., the sponge-dwelling Acastinae, Kolbasov 1993 and the gorgonian- or antipatharian-associated archaeobalanid *Conopea cymbiformis* (= *Balanus proripiens*), Hoek 1913).

Within the Pyrgomatidae, the plesiomorphic genus *Cantellius* was proposed to represent the 'ancestral stock, and a number of different extant *Cantellius* species were suggested to have independently given rise to other pyrgomatine genera (Ross & Newman, 1973; Galkin, 1989; Anderson, 1992). While the concept of extant species giving rise to other genera is problematic, the prevailing hypothesis that pyrgomatids are derived from a morphologically generalized, six-plated ancestor (i.e., *Armatobalanus*), which evolved into a four-plated form (i.e., *Cantellius*), and then into more derived single-plated forms, remains

prevalent (e.g. Healy & Anderson, 1990, Anderson 1992).

In their review of the Pyrgomatidae, Ross & Newman (1973) proposed that the three subfamilies represent balanoid lineages that independently colonized corals (see also Hiro, 1938; Baluk & Radwanski, 1967). In contrast, monophyly of the family was supported by Healy & Anderson (1990) and Anderson (1992) on the basis of sperm structure and skeletal and cirral morphology, respectively. Later Ross and Newman (2002a) likewise espoused pyrgomatid monophyly, and proposed that the family may have arisen in the western Tethys during the Palaeogene.

Evolutionary hypotheses regarding the Pyrgomatidae were recently reviewed and evaluated by Simon-Blecher *et al.* (2007). Using three genes (16S, 12S, and 18S), Simon-Blecher *et al.* (2007) they found that the pyrgomatid *Wanella* fell outside while *Armatobalanus* fell within the pyrgomatid clade, although with weak branch support values; moreover the authors were not able to reject the alternative hypothesis of a monophyletic Pyrgomatidae. The objective of this study is to investigate pyrgomatid evolution using molecular and morphological characters, and to specifically evaluate: (1) the monophyly of the family Pyrgomatidae, as well as of the three subfamilies and various genera; (2) the relationship of *Armatobalanus* to pyrgomatids; (3) the evolution of skeletal fusion and reduction; and (4) the evolution of the interaction between pyrgomatids and their coral hosts.

MATERIAL AND METHODS

TAXON SELECTION AND IDENTIFICATION

Specimens were collected by SCUBA on reefs spanning the IWP and Atlantic. Sixty-four specimens were selected for inclusion in this study from among > 350 specimens sequenced; these represent 14 ingroup genera as well as eight outgroup taxa chosen to represent a broad range of both free-living and symbiotic balanoids. The majority of the specimens are deposited in the Invertebrate Zoology collections of the Florida Museum of Natural History, University of Florida (UF). Additional specimens or tissue samples were borrowed from other institutions (institution codes: WA = Western Australian Museum; ORI = Oceanographic Research Institute, Durban South Africa; TAU = Zoological Museum, Tel Aviv University) and further supplemented by sequences accessed from GenBank (Supporting Information Table S1). All of the sequenced coral barnacles were collected from separate coral colonies.

Specimens were removed from the host coral and examined microscopically. Whenever possible, we studied both the sequenced individual as well as other conspecific barnacles co-occurring on the same coral colony. Any differences between conspecifics from the same coral colony was scored as an ambiguous character state.

Most pyrgomatid specimens were identified using the primary taxonomic literature. Because the species level taxonomy of coral-dwelling barnacles is problematic, and because sampling appears to have included many yet-undescribed species, many samples could not be identified to the species level with full confidence. Thus for the purposes of this study many identifications were limited to the genus level. Three cirripede taxonomists independently identified three samples of the archaeobalanid genus *Armatobalanus* (see acknowledgements); while the *Pyrgopsella youngi* sample was identified by Yair Achituv (Achituv & Simon-Blecher, 2006).

MOLECULAR METHODS

Over 350 specimens were initially sequenced for the mitochondrial gene COI. Based on the initial COI tree (not shown), representatives of each genus were selected to cover as much intragenetic genetic diversity as possible. These representative taxa were sequenced for four additional genes (mitochondrial 16S ribosomal and 12S ribosomal DNA; nuclear 18S ribosomal DNA and Histone 3; see Supporting Information Table S1).

Extraction and amplification of DNA (COI only) from some specimens were performed at the Smithsonian Institution's Laboratories of Analytical

Biology (LAB). Tissue was digested overnight in 150 μ l M2 buffer and 150 μ l M1 + proteinase K buffer at 56.5 °C and 50 revolutions per minute (rpm). DNA was extracted using an automated phenol–chloroform extraction (Autogen AutoGenprep 965 Automated DNA Isolation System). Polymerase chain reactions (PCRs) were performed on an ABI 2720 Thermal Cycler or MJ Research PTC-225 Peltier Cycler. A standard PCR profile was used for amplifying COI (initial denaturation: 95 °C/5 min; 35 cycles of denaturation: 95 °C/30 s, annealing: 48 °C/30 s, and elongation: 72 °C/5 s; and a terminal elongation step: 72 °C/5 min). PCR products were cleaned using ExosapIT (from USB). Sequencing was done in a 96-well format using ABI BigDyeTerminator cycle sequencing reactions. The reactions were cleaned using Sephadex G-50 (Sigma Aldrich), and run on an ABI-3730-XL DNA analyzer. All PCR products were sequenced along both directions. Only COI was sequenced at the LAB.

Remaining specimens were extracted at UF using DNazol and proteinase K following the protocol in Meyer (2003). DNA extracts were purified using QIAGEN cleanup kits. Primers and PCR protocols followed Perez-Losada *et al.* (2004; for H3 and 18S), Meyer (2003; for COI and 16S), and Simon-Blecher *et al.* (2007; for 12S). PCR products were cleaned using ExoSAP (USB) and sequenced in a 96-well format using BigDyeTerminator cycle sequencing reactions and employing an ABI-3730-XL for electrophoresis. All PCR products were sequenced along both directions. All new sequences are deposited in EMBL (accession nos. HG970336–HG970632).

SEQUENCE ANALYSES AND PHYLOGENY CONSTRUCTION

Chromatograms were checked and manually edited using the software Geneious Pro 4.9.2 (Drummond *et al.*, 2009). Sequence alignment was done in 2 ways: (a) manually for all gene regions, using Se-Al v2.0a11 (Rambaut, 2002); and (b) using MAFFT v.6.717 (Kato *et al.*, 2002) for the three non-coding gene fragments (16S, 18S, and 12S) and manually for the two coding genes (COI, H3). In the MAFFT alignments, the L-INS-i search strategy and the following parameters were used: scoring matrix for nucleotide sequences = 1PAM/ κ = 2; gap opening penalty = 1.53; offset value = 0.1. Trees resulting from both manually aligned and software-aligned sequences were compared for topological congruence. As no incongruences were found, and automated alignment is more objective, the MAFFT analysis was used for all downstream analyses. Higher branch support values were obtained from the MAFFT-aligned dataset (data not shown) than from the manual alignment.

Sites were weighted equally, characters were unordered, and gaps were treated as missing data. Phylogenetic interpretations were confined to reciprocally monophyletic clades with strong branch support, herein defined as clades with $\geq 80\%$ bootstrap values and $\geq 95\%$ Bayesian posterior probability values (note that bootstrap values are assumed to be overly conservative while posterior probability values have been shown to overestimate phylogenetic support; Soltis & Soltis, 2003; Taylor & Piel, 2004). To determine the appropriateness of concatenating gene regions into a single analysis, Bayesian and maximum likelihood (ML) tree topologies were compared from independent searches for each of the five gene fragments. No strongly supported incongruences were found, thus all downstream analyses were performed on the concatenated dataset.

Model selection for the five-gene dataset used the Akaike Information Criterion (AIC) as implemented by the program Modeltest 3.7 (Posada & Crandall, 1998). ML analyses were implemented using RAxML 8.0.0 on the CIPRES portal and at the University of Florida High-Performance Computing Center (Miller, Pfeiffer & Schwartz, 2010; Stamatakis, 2014). Analyses were partitioned into the gene regions, with model parameters unlinked between partitions. One thousand rapid bootstrap inferences were performed, followed by a thorough ML tree search. A GTRGAMMAI model and a random starting tree were utilized.

Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) using GTR+I+GAMMA models and flat priors. The dataset was partitioned by gene region prior to analysis, and parameters and models of evolution in each partition were unlinked. Four independent chains were run for ten million generations each; each chain was sampled every 100 generations. Convergence of runs was assessed using Tracer (Rambaut & Drummond, 2007) and AWTY (Nylander *et al.*, 2008). The initial 25% of the trees were discarded as the burn-in phase, and posterior probabilities were calculated based on the remaining 75% of the trees.

PHYLOGENETIC PLACEMENT OF *WANELLA*

The position of the hydrocoral-dwelling *Wanella* was not well resolved in the highest-scoring ML and Bayesian trees (see Results section). To test the placement of *Wanella* within the Pyrgomatidae, topological tests were used to determine whether the best-scoring ML tree (topology T_{ML}) is significantly better than the best ML topology where *Wanella* is constrained to be monophyletic with the rest of the Pyrgomatidae (T_C). Two tests were employed: the non-parametric Shimodaira-Hasegawa test (S-H test; Shimodaira &

Hasegawa, 1999; Goldman, Anderson & Rodrigo, 2002) and the parametric Swofford–Olson–Waddell–Hillis test (SOWH test; Swofford *et al.*, 1996; Goldman, Anderson & Rodrigo, 2002). Both tests use the difference in likelihood values (δ) of the T_{ML} and some alternative topology T_x ($\delta = \text{likelihood}[T_{ML}] - \text{likelihood}[T_x]$) as the test statistic. The S-H test simultaneously compares a set of potentially correct topologies (chosen *a priori*) to the best ML tree. It was implemented in RAxML 8.0.0 by comparing T_{ML} to T_C , as well as to two additional best-scoring trees obtained using the maximum parsimony algorithm (T_{MPT1} and T_{MPT2}).

While the S-H test is easily implemented and is an appropriate test for the question at hand, it is highly conservative (Shimodaira, 2002). The SOWH test is a parametric bootstrapping technique that offers a more powerful and less conservative approach. The SOWH test was used to compare the difference in likelihoods ($\delta_C = \text{likelihood}[T_{ML}] - \text{likelihood}[T_C]$) against a null distribution of δ values obtained from 500 simulated replicate data sets generated under the null hypothesis T_C . We followed guidelines outlined by Anderson, Goldman & Rodrigo (2000), and used RAxML 8.0.0 to perform the likelihood analyses and Seq-Gen v.1.3.3 (Rambaut & Grassly, 1997) for the sequence simulations. Since the best tree (T_{ML}) was obtained from a partitioned analysis of five genes, each gene partition was simulated in Seq-Gen independently according to its characteristics optimized on the topology of T_C . For each replicate, loci were concatenated and the difference in likelihood between the best unconstrained and constrained trees was used to generate the null distribution.

Lastly, the percentage of the post-stationarity Bayesian trees (from the five-gene concatenated analysis) that conformed with the topological constraint tested was computed. This percentage, divided by the total number of post-stationarity trees, gives the posterior probability of the hypothesis being tested (following http://insects.oeb.harvard.edu/farrell_lab/techniques/pa_hypothesis.html).

CHARACTER MAPPING AND MORPHOLOGICAL EVOLUTION

In studying morphological evolution in pyrgomatids, particular attention was paid to phenotypic features related to fusion and simplification of shell structures, as well characters related to the interactions between the barnacle and its coral host. The following characters were examined: (1) number of wall plates; (2) calcareousness of basis; (3) fusion of opercular valves; and (4) presence of teeth on the anterior margin of the third pair of cirri (Fig. 2).

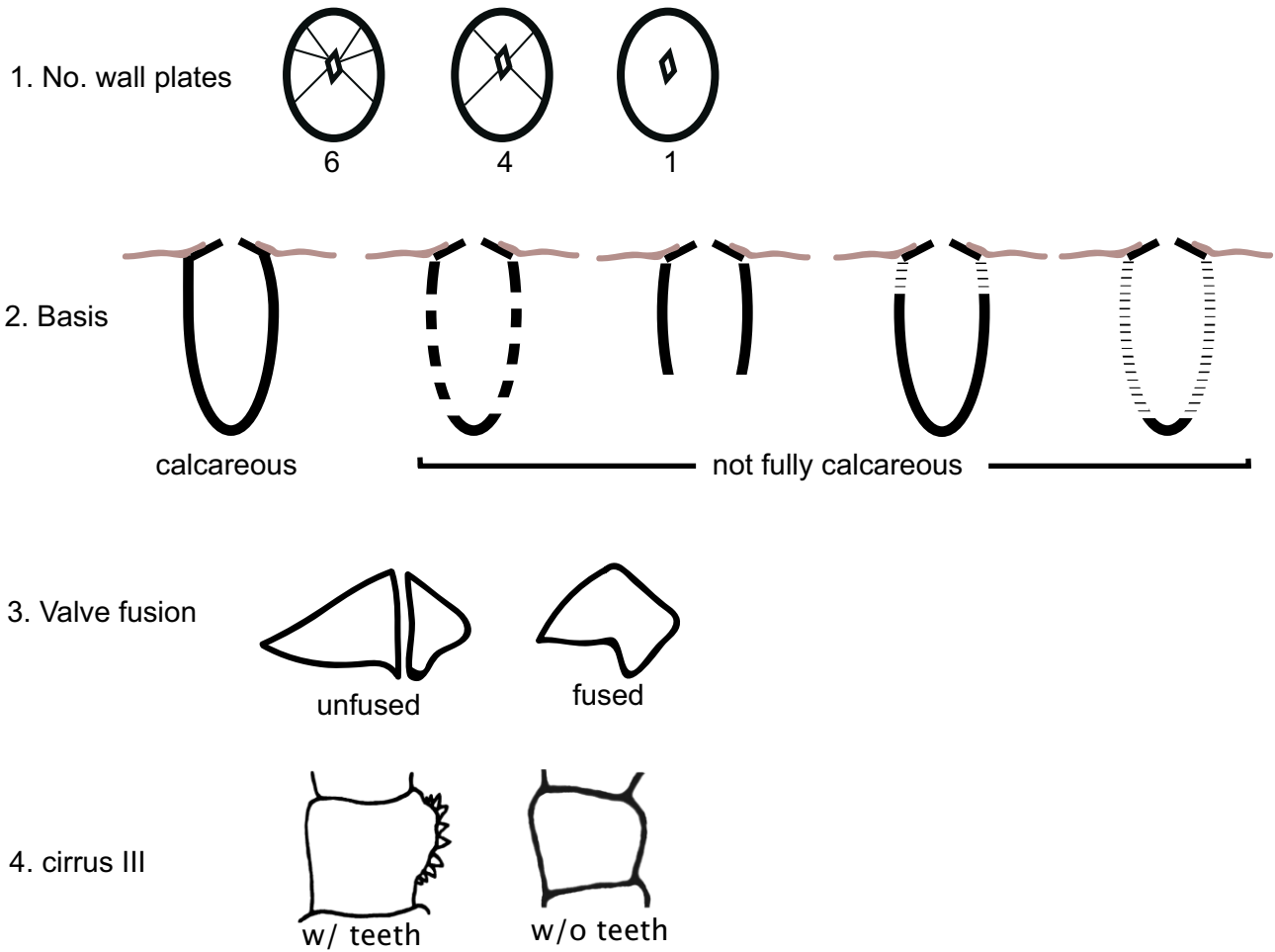


Figure 2. Range of variation in four pyrgomatid phenotypic characters.

The character history was mapped onto the best ML phylogeny generated by RAxML using the software package Mesquite 2.72 (Maddison & Maddison, 2009). Prior to character tracing, nodes on the tree with < 85% bootstrap support and < 95% Bayesian posterior probability were collapsed. Characters were specified as unordered, and character gains and losses were weighted equally. While this may not be a realistic model of character state evolution (character gains and losses may not be equally probable), given the general paucity of fossil information for the majority of these characters, a weighted model applied to these characters would be purely conjectural. However, for two of the characters, ‘number of wall plates’ and ‘opercular valve fusion’, a second analysis was performed where plate or valve fission (i.e., from fused to multiple wall plates or opercular valves) cost one more step than plate or valve fusion. Weighted step matrices were applied because fusion of skeletal elements is well established in the fossil

record as a prevalent and recurrent theme in barnacle evolution (e.g., Newman, 1987).

To determine whether a phenotypic trait is phylogenetically structured, the parsimony score (PS, Fitch, 1971) and association index (AI, Wang *et al.*, 2001) were calculated using BaTS v1.0 (Parker, Rambaut & Pybus, 2008), with no phylogenetic structure as the null hypothesis. BaTS explicitly accounts for phylogenetic uncertainty by calculating and averaging phylogeny–trait association statistics across a posterior sample of trees (PST) generated by Bayesian Markov chain Monte Carlo (MCMC) programs. The same post-stationarity five-gene Bayesian trees used to reconstruct the pyrgomatid phylogeny were used as the input trees, but outgroup taxa and taxa with missing character state data were pruned from the trees using PAUP* ver. 4.0b10 (Swofford, 2002) prior to the BaTS analyses. One hundred replicates of state randomizations were used to calculate the null distributions of the statistics.

RESULTS

PHYLOGENETIC RELATIONSHIPS WITHIN THE PYRGOMATIDAE AND CONGRUENCE OF GENE TREES

Sequence attributes and best likelihood models for each gene fragment are given in Table 2. Gene trees from each of the five sequenced markers (Supporting Information Figs S1–S5) showed that individual gene trees were largely congruent (the only incongruency noted concerned the placement of one *Armatobalanus* specimen in the 18S tree, of which only 4% of the sequence data is parsimony-informative). Gene trees were also generally in agreement with analyses of the five concatenated markers, presented in Figure 3.

All analyses recovered three well supported clades of pyrgomatids (Fig. 3). Most members of the Pyrgomatinae – including the most apomorphic groups – clustered in clade I. *Adna* and *Ceratoconcha* (Megatrematinae and Ceratoconchinae, respectively), were recovered as sister taxa (clade II). Clade III was comprised of the plesiomorphic genus *Cantellius*, and two specimens of the archaeobalanid ‘outgroup’ *Armatobalanus*. Lastly, *Wanella* did not cluster with other pyrgomatids in any of the topologies. Relationships between the three clades were unresolved.

Most pyrgomatid genera were recovered as reciprocally monophyletic units with high branch support, except *Neotrevathana*, *Trevathana*, and *Darwiniella*. Within clade I, the phylogeny recovered a subclade, hereafter referred to as the *Trevathana sensu lato* subclade, comprised of *Trevathana*, *Neotrevathana*, *Pyrgopsella*, and the Hoekiini. *Neotrevathana* was found to be polyphyletic (with one putative new species), *Trevathana* was paraphyletic, and *Pyrgopsella* and the Hoekiini also came out in the middle of the subclade.

PLACEMENT OF OUTGROUP TAXA

Four different sequences identified as *Armatobalanus* were included in the analyses. None of the gene trees nor any of the concatenated analyses recovered a well supported monophyletic *Armatobalanus* clade (Fig. 3, Supporting Information Figs S1–S5). ‘*Armatobalanus*

allium’ (KACb154) and ‘*Armatobalanus* sp.’ (UF 11887) were recovered as sister to the pyrgomatid genus *Cantellius*, while the two other *Armatobalanus* sequences (‘*A. allium*’ TAU Ar27835 and ‘*Armatobalanus* sp.’ KACb163) were both resolved as outgroups to the pyrgomatids, yet did not cluster together. Because specimens were identified by different experts (see Methods), inconsistencies in identifications cannot be ruled out. However, all specimens identified as ‘*Armatobalanus*’ possess six wall plates, which means that the sister group to *Cantellius* is indisputably a six-plated barnacle.

PHYLOGENETIC PLACEMENT OF WANELLA

We used topological tests to investigate whether *Wanella* is monophyletic with the rest of the Pyrgomatidae. The S-H test (Table 3) rejected the hypothesis of a monophyletic Pyrgomatidae ($P < 0.01$ for all three topologies); while the SOWH test rejected the constrained topology (with $P < 0.002$) in favor of an unconstrained topology where *Wanella* is not monophyletic with other pyrgomatids. The observed difference in likelihood scores ($\delta_c = 93.11$) is much greater than the simulated null distribution of δ values, as the highest difference in likelihoods in the null distribution was merely 0.3. Lastly, we found that of the 15 000 post-stationarity trees obtained from Bayesian analysis, not one of the trees conformed to the hypothesis of a monophyletic Pyrgomatidae. The pyrgomatids with *Wanella* excluded is hereinafter referred to as Pyrgomatidae *s.s.*

CHARACTER MAPPING AND PHYLOGENY–TRAIT CORRELATION

Fusion of four wall plates into a single plate is estimated to have evolved three times in clade I, clade II, and in *Wanella* (Fig. 4). The ancestral state of clade I was reconstructed as single-plated, with two reversals to four wall plates (*Hiroa* and *Galkinia*). Clade II is comprised of the four-plated *Ceratoconcha* and the

Table 2. Sequence attributes for the five gene fragments. GTR = general time-reversible model, TVM = transversional model, TrN = Tamura-Nei model; I = invariant sites; G = gamma shape parameter for rate variation among sites

| Gene | Sequence length (bp) | #Parsimony-informative sites (bp) | #Invariable sites (bp) | A-T bias | Best fit model |
|------|----------------------|-----------------------------------|------------------------|----------|----------------|
| COI | 599 | 228 | 360 | 66% | GTR + I + G |
| 16S | 458 | 126 | 296 | 72% | TVM + I + G |
| 12S | 361 | 132 | 193 | 69% | TVM + I + G |
| 18S | 1766 | 74 | 1633 | 47% | GTR + I + G |
| H3 | 324 | 96 | 212 | 37% | TrN + I + G |

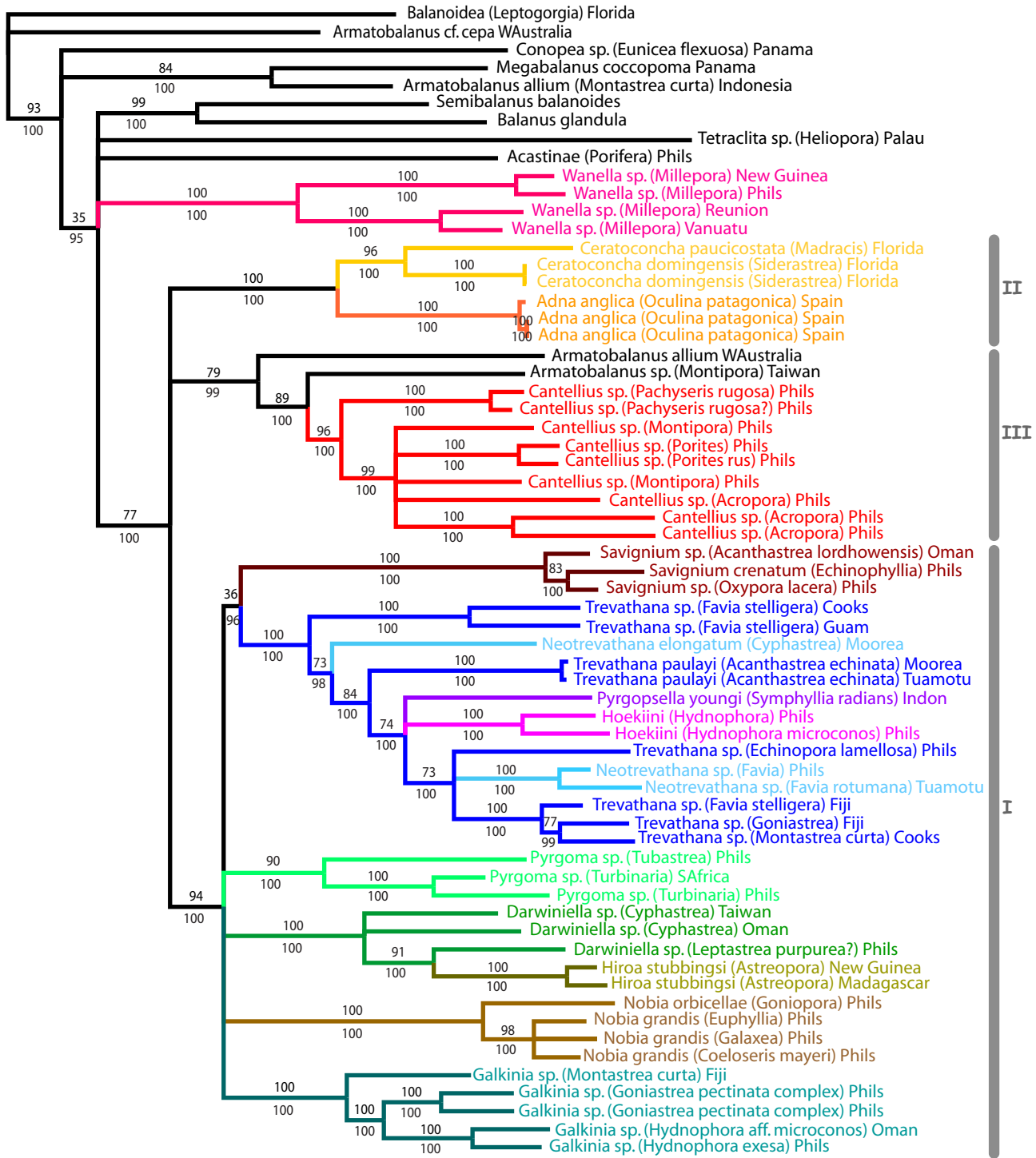


Figure 3. ML phylogeny for all five sequenced genes, as computed using RAxML. Taxa and branches are colored according to genera; taxa in black represent non-pyrgomatid samples. Bootstrap values and Bayesian posterior probabilities are indicated above and below the branches, respectively. Nodes with <85% bootstrap support and <90% posterior probability were collapsed. Taxon labels include host information in parentheses, when available, and collection locality.

Table 3. P-values obtained from Shimodaira-Hasegawa test. MPT = most parsimonious tree

| Hypothesis tested | Difference in likelihood (SD) |
|---|-------------------------------|
| Pyrgomatidae (incl. <i>Wanella</i>) monophyletic | -92.344 (23.76)* |
| MPT 1 | -91.374 (28.97)* |
| MPT 2 | -97.678 (29.456)* |

*Significant at $P \leq 0.05$.

single-plated *Adna*. Clade III has six-plated basal members (*Armatobalanus*) and a six-plated ancestral state, with fusion to four plates in *Cantellius*. The Pyrgomatidae *sensu stricto* ancestor is reconstructed as six-plated. Results for the weighted (Fig. 4) and unweighted analyses are mostly identical, however in the unweighted analysis the ancestral state for the Pyrgomatidae is reconstructed as equivocal.

Most pyrgomatids have a solid, calcareous basis, with four exceptions; all in clade I (*Pyrgopsella*, Hoekiinae, *Pyrgoma*, and some members of *Savignium*; Supporting Information Fig. S6). *Pyrgoma* has a perforated basis, while the *Pyrgopsella* + Hoekiini clade have partially (*Pyrgopsella*) to fully (Hoekiinae) membranous bases. Furthermore we found that a species of *Savignium* on the coral *Oxypora lacera* possesses a calcareous cylindrical 'sleeve' that supports the barnacle wall on top, while the bottom half of the basis is non-calcareous. This previously unreported feature was not found in specimens of *Savignium* inhabiting other coral hosts.

Fusion of the opercular valves occurs exclusively in members of clade I, but is estimated to have occurred four times within the clade (Fig. 4); thrice in the *Trevathana s.l.* subclade alone. The ancestor of pyrgomatids is reconstructed with separate valves when the analysis is weighted, as were the ancestors of each of the three clades (however, when the weighted stepmatrix is not applied the ancestor of clade I is reconstructed as equivocal). Regardless of use of character weighting, the opercular valves revert from fused to the unfused condition twice in clade I (i.e., *Hiroa* and *Pyrgoma*).

All examined members of clades II and III had cirral teeth, while cirral armature was absent from clade I (Supporting Information Fig. S6). Cirrus III teeth were present in all four specimens identified as *Armatobalanus* spp.

The BaTS analyses (Table 4) rejected the null hypothesis of no phylogenetic structure for all of the phenotypic traits analyzed.

DISCUSSION

SYSTEMATICS

Results contradict classical hypotheses of pyrgomatid evolution in several fundamental ways. First, the hydrocoral associate *Wanella* was not recovered within the Pyrgomatidae. Second, while three main clades were recovered, these do not correspond to the recognized subfamilies (Ceratoconchinae, Megatrematinae, Pyrgomatinae); rather the first two form one clade, while the latter comprises two major clades (Clades I and III). Third, two of the tribes of the Pyrgomatinae (Pyrgopsellini and Hoekiini) are sister taxa, deeply nested within a paraphyletic Pyrgomatini. Fourth, some species of the 6-plated archaeobalanid 'outgroup' *Armatobalanus* are recovered as sister to the plesiomorphic pyrgomatid *Cantellius*. Phylogenetic evidence from this study and from Simon-Blecher *et al.* (2007) indicate a need to revise the classification of the Pyrgomatidae.

Within the *Trevathana s.l.* subclade, *Neotrevathana* was recovered as polyphyletic, while Pyrgopsellini and Hoekiini were nested as sister taxa within *Trevathana s.l.*

Clade II unites the only EA genus, *Adna* (Megatrematinae), with *Ceratoconcha* (Ceratoconchinae), one of two extant WA genera. It will be interesting to investigate the placement of other megatrematine genera (i.e., the WA/IWP *Megatrema* and the IWP *Memagreta* and *Pyrgomini*) to test whether the subfamily is monophyletic or whether relationships follow biogeographic lines. A monophyletic Megatrematinae would indicate that diversification occurred across three biogeographic regions. An alternative hypothesis might be that the two WA genera, *Ceratoconcha* and *Megatrema*, are sister taxa, which would mean that diversification occurred within the WA.

Close relationships of some '*Armatobalanus*' with *Cantellius* implies that: (1) the Pyrgomatidae needs to be re-circumscribed to include some of the 6-plated coral-dwelling balanomorphs; and (2) *Armatobalanus* as presently defined is polyphyletic. There are also indications that clade III may be sister to clade II. Although branch support for this relationship is low, a new morphological character supports such a grouping (see next section).

SKELETAL FUSION AND REDUCTION

Wall and opercular fusion and reduction of calcification of the basis evolved multiple times in pyrgomatids, with reversals in these character states. The degree of fusion of opercular valves and wall plates is homoplasious in pyrgomatids, and should be used with caution for defining clades.

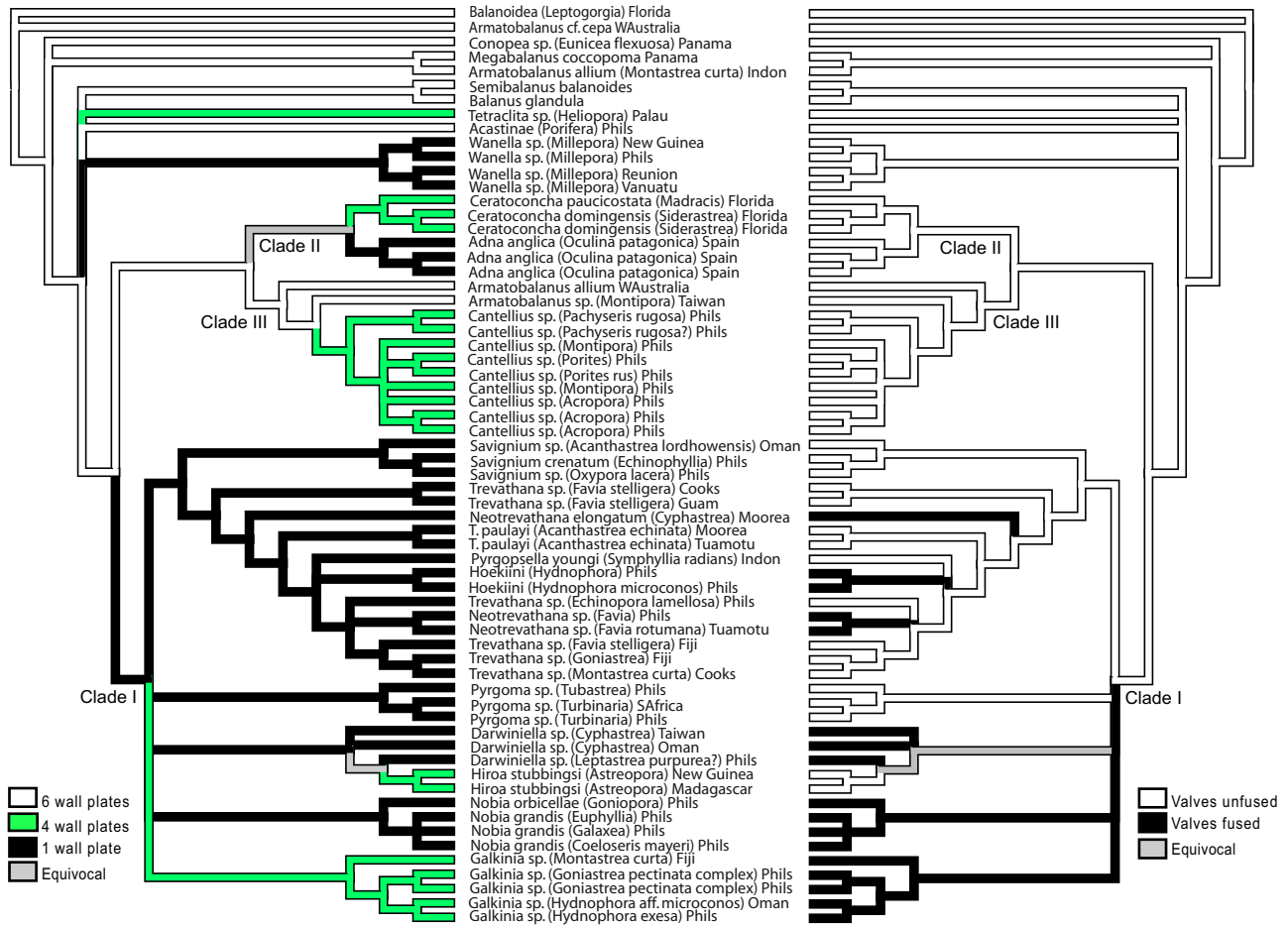


Figure 4. Number of wall plates (left) and opercular valve fusion (right) plotted on the multi-gene ML phylogeny using Mesquite. Colors on the interior branches are ancestral character state reconstructions, terminal branches were scored from actual specimens. Characters were weighted (see text for alternate results without character weighting).

Table 4. Results of analyses of phylogenetic structuring for four phenotypic characters. AI = association index (Wang *et al.*, 2001); PS = parsimony score (Fitch, 1971). Values in parentheses are the 95% confidence intervals for both observed and expected scores

| | Observed value (95% CI) | Expected value (95% CI) | Obs.& exp. overlap? | P-value |
|-------------------------------|---|-------------------------|---------------------|---------|
| Number of wall plates | | | | |
| AI | 0.174 (0.125–0.190) | 2.98 (2.157–3.828) | no | << 0.01 |
| PS | 5.006 (5.000–5.000) | 17.065 (14.878–19.880) | no | << 0.01 |
| Basis calcareousness | | | | |
| AI | 0.650 (0.429–0.679) | 1.535 (0.903–2.154) | no | < 0.02 |
| PS | 4.906 (4.000–5.000) | 7.577 (6.382–8.000) | no | < 0.01 |
| Opercular valve fusion | | | | |
| AI | 0.243 (0.153–0.351) | 2.605 (1.868–3.502) | no | << 0.01 |
| PS | 6.342 (6.000–7.000) | 14.440 (12.000–16.884) | no | << 0.01 |
| Cirrus III teeth | | | | |
| AI | 1.511 × 10 ⁻⁹ (0–2.728 × 10 ⁻¹³) | 1.774 (1.080–2.442) | no | << 0.01 |
| PS | 1.000 (1.000–1.000) | 9.101 (7.749–10.000) | no | << 0.01 |

All known instances of opercular valve fusion and reduction in basis calcification occurred in clade I. Single-plated walls, on the other hand, evolved in both clades I and II. Within clade I, the extreme in skeletal reduction is attained by the parasitic Hoekiinae, which have highly reduced, thin, and fused opercular valves as well as membranous bases. Uncalcified or less-calcified bases could be energetically less costly to produce, and thus may permit more rapid growth (Ross & Newman, 1995). Alternatively, passageways or membranous zones in the basis may function in barnacle-coral communication (Ross & Newman, 1973; Ross & Newman, 2000a) or in uptake of dissolved nutrients from the host (Anderson, 1992; Ross & Newman, 1995). A similar reduction in shell calcification has been noted in sponge barnacles, with ‘windows’ between wall plates suggested to be involved in barnacle-host communication (Kolbasov, 1993).

A single wall plate evolved independently in clades I, II, and *Wanella*. Clade III includes six-plated and four-plated morphs, and the six-plated forms gave rise to four-plated species through valve fusion. The only previously known six-plated pyrgomatid is *Eoceratoconcha* from the Miocene and Pliocene of the WA. Since *Cantellius* is sister to another six-plated barnacle (*Armatobalanus*), the implication is that fusion from six to four -wall plates may have occurred more than once. The fusion of wall plates, particularly into a single plate, has been proposed to be an adaptation allowing pyrgomatids to better withstand the lateral pressure exerted by the coral as it grows (Simon-Blecher *et al.*, 2007).

BARNACLE–CORAL INTERACTIONS

Cirral teeth are used to rasp away coral overgrowing the barnacle aperture (Anderson, 1992). Teeth on the anterior margins of the 3rd pair of cirri was believed to be characteristic of *Armatobalanus*; its presence in *Cantellius* was considered to be ‘vestigial’ and limited to only two species (Anderson, 1992). We found cirral teeth to be more widespread in *Cantellius*, but also to vary in size; i.e., some species had prominent cirral teeth resembling those in *Armatobalanus* while others had poorly developed teeth. Cirral armature is also prevalent in clade II, but is absent in clade I. Interestingly, clades II and III are recovered as sister-groups in most phylogenetic reconstructions, albeit with low branch support. Cirral armature may be a synapomorphy uniting these clades. Cirral armature is also present among non-pyrgomatids: for instance in the two ‘*Armatobalanus*’ specimens that are recovered as outgroups to the phylogeny; as well as in other groups (e.g., *Conopea*, acastines). The broad phylogenetic distribution of rasping cirral teeth

suggest that it may be an important adaptive feature in symbiotic barnacles.

EVOLUTION OF PARASITIC HOEKIINES AND OF HOST SPECIFICITY

The Hoekiini, or ‘coral-eating barnacles’, are a bizarre group with reduced skeletons, non-functional cirral nets and large biting mouthparts for feeding on coral tissue that completely covers the wall and orifice of the barnacle (Ross & Newman, 1995). Despite their extreme morphological and ecological divergence, hoekiines are not a long branch on the phylogeny, suggesting that they may have evolved quite recently and that a parasitic lifestyle led to rapid morphological evolution.

Hoekiines are among the most host specific of all coral barnacles, and are found only on *Hydnophora* (Merulinidae in the new sense; Budd *et al.*, 2012). The entire *Trevathana sensu lato* subclade and its sister group *Savignium* are overwhelmingly found in merulinid corals (clade XVII of Fukami *et al.*, 2008). This contrasts with the broad host range of the highly plesiomorphic *Cantellius*, which occupies 11 different clades of corals (as defined by Fukami *et al.*, 2008; including the non-scleractinian stylasterids); and *Armatobalanus* recorded from eight coral clades (again, including stylasterids). Such an evolutionary tendency towards host specialization in morphologically more derived pyrgomatids has been previously noted by Hiro (1935, 1938).

CONCLUSIONS

Our analyses generated a well supported phylogeny that is at odds with the current classification of the Pyrgomatidae. Nonetheless, the coral-dwelling barnacles remain a natural clade of balanomorphs highly adapted to coexisting with their cnidarian hosts. The evolutionary innovations within the Pyrgomatidae include fusion of wall plates (from an original six, to four, and from four to a single plate), fusion of opercular valves, reduction or loss of basis calcification, use of either mechanical abrasion or other means (presumably chemical secretions) to deter coral overgrowth, and evolution of parasitism. Apomorphic taxa tend to be more host specific than plesiomorphic genera. Extant species diversity is highest in the IWP, which parallels the diversity of reef corals.

Polyphyly of all three balanoid families – Balanidae, Archaeobalanidae, and Pyrgomatidae – has now been demonstrated. There is a need for a rigorous phylogenetic re-assessment of the superfamily Balanoidea, with more complete taxon sampling and the use of additional genetic markers and

morphological characters (e.g. sperm morphology, following Healy & Anderson, 1990). An important future goal would be to re-evaluate the status of the genera *Hexacreusia* and *Zulloana*, exclusively coral-dwelling archaeobalanids from the East Pacific. Zullo (in litt. in Newman, 1996) suggested that these taxa be placed in the Pyrgomatidae, however Ross & Newman (2000a) did not agree because of their six-plated walls and use of mechanical means to break coral overgrowth at the wall-basis suture – characteristics now known to exist within the Pyrgomatidae *sensu stricto*. The pyrgomatine *Cionophorus* also needs reevaluation, because its genus is highly host specific, shows skeletal reduction, and exhibits incidental parasitism on its host coral (Ogawa & Nomura, 2010). These characteristics suggest it may be a transitional form between planktotrophic coral-dwelling barnacles and parasitic forms.

ACKNOWLEDGEMENTS

We wish to thank G Paulay, the late A Ross, and WA Newman for advice, encouragement, and references provided at various stages of this research. For fieldwork, we acknowledge the support of colleagues across the globe: K Soong, the late M Chen, W Licuanan, MA Juinio-Meñez, P Aliño, E Gomez, T Rongo, J Rongo, E Emmanuelli, V Bonito, M Claereboudt, C Bourmaud, M Guillaume, and H Bruggemann. For help obtaining key specimens and sequences, we thank P Cabezas-Padilla, M Perez-Losada, M Finley, Y Achituv, A Hosie, and C Floros. For identifications of *Armatobalanus* specimens, Y Achituv (Bar-Ilan University, Israel), G Kolbasov (Moscow State University, Russia), and A Hosie (Western Australian Museum, Australia) are acknowledged. For advice with phylogenetic analyses, we thank E Braun, M Miyamoto, F Qiu, R Kimball, P Soltis, D Reed, and N Evans. For curatorial support, we are grateful to J Slapcinsky, A Bemis, S McPherson, J Moore, C Campbell, K Kemppanion, and the rest of the Invertebrate Zoology team at the Florida Museum of Natural History. For logistical support with sequencing and phylogenetic analyses, we thank C Meyer, M Fagan, and M Gitzendanner. Two anonymous reviewers are acknowledged for their comments to the manuscripts. The University of Florida Phyloinformatics Clusters for High-Performance Computing in the Life Sciences is acknowledged for computational support. Grants from the American Microscopical Society, the Carcinological Society of Japan, the National Sun Yat-Sen University, the French-American Cultural Exchange (FACE) Ocean Bridges program, the American Museum of Natural History, and the Department of Zoology at the University of Florida helped further this research project.

REFERENCES

- Achituv Y. 2001.** *Cantelliuss alphonsei* n. sp., a new coral-inhabiting barnacle (Cirripedia, Pyrgomatinae) of Montipora. *Crustaceana* **74**: 617–626.
- Achituv Y. 2004.** Coral-inhabiting barnacles (Cirripedia: Balanomorpha: Pyrgomatinae) from the Kermadec Islands and Niue Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **38**: 43–49.
- Achituv Y, Hoeksema BW. 2003.** *Cantelliuss cardenae* spec. nov. (Cirripedia: Pyrgomatinae) from *Acropora* (*Isopora*) *brueggemanni* (Brook, 1893) (Anthozoa: Acroporidae), a case of host specificity in a generalist genus. *Zoologische Mededelingen* **77**: 1–8.
- Achituv Y, Langsam Y. 2005.** Coral-inhabiting barnacles (Cirripedia; Thoracica; Balanomorpha; Pyrgomatinae) from east of 150° W. *Journal of Natural History* **39**: 1705–1717.
- Achituv Y, Langsam Y. 2009.** Two new species of *Trevathana* (Crustacea, Cirripedia, Balanomorpha, Pyrgomatidae) from the Western Indian Ocean and French Polynesia. *Zootaxa* **2116**: 46–52.
- Achituv Y, Newman WA. 2002.** The barnacles of *Astreopora* (Cirripedia, Pyrgomatini/Scleractinia, Acroporidae): organization plans, host specificity, species-richness and geographic range. *Journal of Natural History* **36**: 391–406.
- Achituv Y, Simon-Blecher N. 2006.** *Pyrgopsella* (Cirripedia: Balanomorpha: Pyrgomatidae) is not a sponge-inhabiting barnacle. *Zootaxa* **1319**: 29–42.
- Achituv Y, Tsang LM, Chan BKK. 2009.** A new species of *Cantelliuss* and a redescription of *C. sextus* (Hiro, 1938) (Cirripedia, Balanomorpha, Pyrgomatidae) from the elephant skin coral, *Pachyseris speciosa* (Dana, 1846) (Scleractinia, Agariciidae) from Taiwan. *Zootaxa* **2022**: 15–28.
- Anderson DT. 1992.** Structure, function, and phylogeny of coral-inhabiting barnacles (Cirripedia, Balanoidea). *Zoological Journal of the Linnean Society* **106**: 277–339.
- Anderson DT. 1993.** Addendum/Corrigendum. *Zoological Journal of the Linnean Society* **105**: 337.
- Anderson J, Goldman N, Rodrigo A. 2000.** *Guidelines for performing the SOWH test*. Available at: <http://www.ebi.ac.uk/goldman/tests/SOWHinstr.html>
- Annandale H. 1924.** Cirripeds associated with Indian corals of the families Astreaeidae and Fungidae. *Memoirs of the Indian Museum* **8**: 61–68.
- Asami K, Yamaguchi T. 1997.** Distribution of living and fossil coral barnacles (Cirripedia; Pyrgomatidae) in Japan. *Sessile Organisms* **14**: 9–16.
- Asami K, Yamaguchi T. 2001.** A new coral barnacle, *Trevathana paulayi* (Cirripedia: Pyrgomatidae), from Guam. *Sessile Organisms* **18**: 19–26.
- Baluk W, Radwanski A. 1967.** Miocene cirripeds domiciled in corals. *Acta Palaeontologica Polonica* **12**: 457–521.
- Borradaile LA. 1903.** Marine Crustaceans. In: Gardiner JS, ed. *The fauna and geography of the Maldives and Laccadive Archipelagoes, Part 7. The barnacles (Cirripedia)*. Cambridge: Cambridge University Press, 440–443.

- Bosc LAG. 1801.** Observation et description d'une espèce de Balanite qui se fixe dans les madrépores. *Bulletin des Sciences, par la Société philomathique de Paris* **3**: 66, pl.3.
- Brickner I, Hoeg JT. 2010.** Antennular specialization in cyprids of coral-associated barnacles. *Journal of Experimental Marine Biology and Ecology* **392**: 115–124.
- Brickner I, Simon-Blecher N, Achituv Y. 2010.** Darwin's *Pyrgoma* (Cirripedia) revisited: revision of the *Savignium* group, molecular analysis and description of new species. *Journal of Crustacean Biology* **30**: 266–291.
- Broch H. 1931.** Papers from Th. Mortensen's Pacific expedition 1914-1916, LVI Indomalayan Cirripedia. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kobenhavn* **91**: 1–146.
- Budd AF, Fukami H, Smith ND, Knowlton N. 2012.** Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). *Zoological Journal of the Linnean Society* **166**: 465–529.
- Chan BKK, Chen YY, Lin HC. 2013.** Biodiversity and host specificity of coral barnacles of *Galkinia* (Cirripedia: Pyrgomatidae) in Taiwan, with descriptions of six new species. *Journal of Crustacean Biology* **33**: 392–431.
- Chen YY, Lin HC, Chan BK. 2012.** Description of a new species of coral-inhabiting barnacle, *Darwiniella angularis* sp. n. (Cirripedia, Pyrgomatidae) from Taiwan. *ZooKeys* **214**: 43.
- Darwin C. 1854.** *A monograph on the sub-class cirripedia: balanidae, verrucidae, etc.* London: Ray Society.
- Des Moulins C. 1866.** Liste des principaux fossiles recueillies par les membres de la société à Cazeneuve dans le Calcaire de Bazas pendant l'excursion de la 50^e Fête Linnéenne. *Actes Société Linné Bordeaux* **26** (ser. 3, vol. 6): 293–344.
- Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A. 2009.** *Geneious v4.7*. Available at: <http://www.geneious.com/>
- Fitch WM. 1971.** Toward defining the course of evolution: minimal change for a specific tree topology. *Systematic Zoology* **20**: 406–416.
- Fukami H, Chen CA, Budd AF, Collins A, Wallace C, Chuang Y-Y, Chen C, Dai C-F, Iwao K, Sheppard C, Knowlton N. 2008.** Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). *PLoS ONE* **3**: e3222. doi:10.1371/journal.pone.0003222.
- Galkin SV. 1982.** A new species of the genus *Cantellius* (Balanidae, Pyrgomatinae). *Akademii nauk SSSR, Zoologicheskii Zhurnal* **61**: 1259–1262.
- Galkin SV. 1989.** The specificity of barnacle coralobionts to their hosts. In: Sveshnikov VA, ed. *Symbiosis in marine animals*. Moscow: A.N. Severtosov Institute of Evolutionary Morphology and Animal Ecology, Academy of Sciences of the USSR, 89–101. [Translated by MJ Grygier].
- Goldman N, Anderson JP, Rodrigo AG. 2002.** Likelihood-based tests of topologies in phylogenetics. *Systematic Biology* **49**: 652–670.
- Gray JE. 1825.** A synopsis of the genera of cirripedes arranged in natural families, with a description of some new species. *Annals of Philosophy new ser.* **10**: 97–107.
- Gray JE. 1831.** Description of two new species of *Serpula*, and an undescribed coral barnacle, discovered by Samuel Pearce Pratt, Esq. *The zoological miscellany. To be continued occasionally.* Treuttel, Wurtz and Co., London, no. 1, 5–6.
- Gruvel A. 1907.** Cirrhipèdes operculés de l'Indian Muséum de Calcutta. *Memoirs of the Asiatic Society of Bengal* **2**: 1–10.
- Healy JM, Anderson DT. 1990.** Sperm ultrastructure in the Cirripedia and its phylogenetic significance. *Records of the Australian Museum* **42**: 1–26.
- Hiro F. 1931.** Notes on some new Cirripedia from Japan. *Memoirs of the College Science, Kyoto Imperial University, Series B* **7**: 143–158.
- Hiro F. 1934.** A new coral-inhabiting barnacle *Pyrgoma orbicellae* n. sp. *Proceedings of the Imperial Academy (Japan)* **10**: 367–369.
- Hiro F. 1935.** A study of cirripeds associated with corals occurring in Tanabe Bay. *Records of Oceanographic Works in Japan* **7**: 1–28.
- Hiro F. 1938.** Studies on animals inhabiting reef corals II. Cirripeds of the genera *Creusia* and *Pyrgoma*. *Palao Tropical Biological Station Studies* **3**: 391–416.
- Hoek PP. 1913.** The cirripedia of the siboga expedition. B. Cirripedia sessilia. In: Weber MCW. *Siboga Expeditie* **31b**: 129–275.
- Holthuis LB. 1982.** The nomenclature of some coral inhabiting barnacles of the family Pyrgomatidae (Cirripedia, Balanomorpha). *Crustaceana* **43**: 316–320.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002.** MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.
- Kolbasov GA. 1993.** Revision of the genus *Acasta* Leach (Cirripedia: Balanoidea). *Zoological Journal of the Linnean Society* **109**: 395–427.
- Kolosváry G. 1947.** A study of the cirripedes associated with corals in the collection of the Hungarian National Museum. *Proceedings of the Zoological Society of London* **117**: 425–428.
- Kolosváry G. 1948.** New data of cirripedes associated with corals. *The Annals and Magazine of Natural History* **14**: 358–368.
- Kolosváry G. 1950.** On some balanids living in corals, collected by the Snellius Expedition in 1930. *Zoologische Mededelingen Leiden* **30**: 289–296.
- Kramberger-Gorjanovic D. 1889.** Über einem tertiären Rudisten aus Podused bei Agram. *Glasnik Hrvatskoga narodnoslovnoga Drustva, Zagreb* **4**: 48–55.
- Leach WE. 1817.** Distribution systématique de la classe des Cirripèdes; par le même. *Journal de Physique, de Chimie, et d'Histoire naturelle* **85**: 67–69.
- Leach WE. 1818.** Cirripedes. In: *Supplement to the fourth, fifth, and sixth editions of the Encyclopaedia Britannica. With preliminary dissertations on the history of the sciences.* Edinburgh: Archibald Constable and Co., 168–171.

- Leach WE. 1825.** A tabular view of the genera comprising the class Cirripedes, with descriptions of the species of *Otion*, *Cineras*, and *Clyptra*. *Zoological Journal of London* **2**: 208–215.
- Maddison WP, Maddison DR. 2009.** *Mesquite: a modular system for evolutionary analysis, Version 2.72*. Available at: <http://mesquiteproject.org>
- Meyer CP. 2003.** Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society* **79**: 401–459.
- Miller MA, Pfeiffer W, Schwartz T. 2010.** Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop*, 14 Nov 2010. New Orleans LA, 1–8.
- Newman WA. 1987.** Evolution of cirripedes and their major groups. In: Southward AJ, ed. *Crustacean issues 5: barnacle biology*. Rotterdam: Balkema, 3–42.
- Newman WA. 1996.** Cirripedia; Suborders Thoracica and Acrothoracica. In: Forest J, ed. *Traité de Zoologie, tome VII, Crustacés*, fascicule. 2: Paris: Masson, 453–540.
- Newman WA, Ladd HS. 1974.** Origin of coral inhabiting balanids (Cirripedia, Thoracica). *Verhandlungen der Naturforschenden Gesellschaft in Basel* **84**: 381–396.
- Newman WA, Ross A. 1976.** Revision of the balanomorph barnacles; including a catalog of the species. *Memoirs of the San Diego Society of Natural History* **9**: 1–108.
- Nilsson-Cantell CA. 1938.** Cirripedes from the Indian Ocean in the collections of the Indian Museum, Calcutta. *Memoirs of the Indian Museum* **13**: 1–81.
- Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL. 2008.** AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24**: 581–583.
- Ogawa K. 2000.** Coral-inhabiting barnacles (Cirripedia; Pyrgomatidae) from west coast of Babeldaob Island of the Republic of Palau. *Biogeography* **2**: 29–43.
- Ogawa K, Nomura K. 2010.** A new coral-inhabiting barnacle, *Cinophorus* [sic] *kushimotoensis* n. sp. (Cirripedia, Pyrgomatidae), with complete larval stages. *Biogeography* **12**: 83–104.
- Parker J, Rambaut A, Pybus OG. 2008.** Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. *Infection, Genetics, and Evolution* **8**: 239–246.
- Perez-Losada M, Harp M, Hoeg JT, Achituv Y, Jones D, Watanabe H, Crandall KA. 2008.** The tempo and mode of barnacle evolution. *Molecular Phylogenetics and Evolution* **46**: 328–346.
- Perez-Losada M, Hoeg JT, Crandall KA. 2004.** Unraveling the evolutionary radiation of the thoracican barnacles using molecular and morphological evidence: a comparison of several divergence time estimation approaches. *Systematic Biology* **53**: 244–264.
- Pilsbry HA. 1931.** The cirriped genus *Pyrgoma* in American waters. *Proceedings of the Academy of Natural Sciences of Philadelphia* **83**: 81–83.
- Pitombo FB, Ross A. 2002.** A review of the *Hexacreusia* species complex: Eastern Pacific coral barnacles (Cirripedia, Balanomorpha). *Arquivos do Museu Nacional, Rio de Janeiro* **60**: 89–94.
- Posada D, Crandall KA. 1998.** Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Rambaut A. 2002.** *Se-Al v. 2.0a11: sequence alignment program*. Available at: <http://tree.bio.ed.ac.uk/software/seal/>
- Rambaut A, Drummond AJ. 2007.** *Tracer v1.4*. Available at: <http://beast.bio.ed.ac.uk/Tracer>
- Rambaut A, Grassly NC. 1997.** Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Computer Applications in the Biosciences* **13**: 235–238.
- Ren X. 1986.** Studies on Chinese Cirripedia (Crustacea), VII. Family Pyrgomatidae. *Studia Marina Sinica* **26**: 129–153.
- Ronquist F, Huelsenbeck JP. 2003.** MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Rosell NC. 1975.** On two noteworthy balanids (Cirripedia Thoracica) from the Sulu Archipelago, Philippines. *Crustaceana* **29**: 206–214.
- Ross A. 1999a.** Notes on the coral-inhabiting barnacles of the Great Barrier Reef, Australia (Cirripedia: Pyrgomatidae). *Memoirs of the Queensland Museum* **43**: 833–836.
- Ross A. 1999b.** On the occurrence of *Megabalanus stultus* (Darwin), 1854 (Cirripedia: Balanomorpha) in Taiwan: a reappraisal. *Zoological Studies* **38**: 275–278.
- Ross A. 2000a.** *Arossella* Anderson, 1993: what is the type species (Cirripedia: Balanomorpha)? *Sessile Organisms* **16**: 15–20.
- Ross A. 2000b.** Coral-eating barnacles: wall morphology and the description of two new species. *Sessile Organisms* **17**: 45–56.
- Ross A, Newman WA. 1973.** Revision of the coral-inhabiting barnacles (Cirripedia, Balanidae). *Transactions of the San Diego Society of Natural History* **17**: 137–174.
- Ross A, Newman WA. 1995.** A coral-eating barnacle, revisited (Cirripedia, Pyrgomatidae). *Contributions to Zoology* **65**: 129–175.
- Ross A, Newman WA. 1999.** A new coral-inhabiting barnacle from Taiwan (Cirripedia:Pyrgomatidae). *Zoological Studies* **38**: 387–390.
- Ross A, Newman WA. 2000a.** *Pyrgoma kuri* Hoek, 1913: a case study in morphology and systematics of a symbiotic barnacle (Cirripedia: Balanomorpha). *Contributions to Zoology* **68**: 65–80.
- Ross A, Newman WA. 2000b.** A new coral-eating barnacle; the first record from the Great Barrier Reef, Australia. *Memoirs of the Queensland Museum, Brisbane* **45**: 585–591.
- Ross A, Newman WA. 2002a.** Coral barnacles: cenozoic decline and extinction in the Atlantic/East Pacific versus diversification in the Indo-West Pacific. *Proceedings of the 9th Int. Coral Reef Symposium, Bali, Indonesia* **1**: 135–139.
- Ross A, Newman WA. 2002b.** A review of the *Pyrgoma cancellatum* species complex (Cirripedia: Pyrgomatidae). *Journal of Natural History* **36**: 407–421.
- Ross A, Pitombo FB. 2002.** Notes on the coral-inhabiting Megatrematinae and the description of a new tribe,

- new genus and three new species (Cirripedia: Sessilia: Pyrgomatidae). *Sessile Organisms* **19**: 57–68.
- Santos A, Mayoral E, Gudveig Baarli B, Da Silva CM, Cachao M, Johnson ME. 2012.** Symbiotic association of a pyrgomatid barnacle with a coral from a volcanic middle Miocene shoreline (Porto Santo, Madeira Archipelago, Portugal). *Paleontology* **55**: 173–182.
- Shimodaira H. 2002.** An approximately unbiased test of phylogenetic tree selection. *Systematic biology* **51**: 492–508.
- Shimodaira H, Hasegawa M. 1999.** Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.
- Simon-Blecher N, Huchon D, Achituv Y. 2007.** Phylogeny of coral-inhabiting barnacles (Cirripedia; Thoracica; Pyrgomatidae) based on 12S, 16S and 18S rDNA analysis. *Molecular Phylogenetics and Evolution* **44**: 1333–1341.
- Soltis PS, Soltis DE. 2003.** Applying the bootstrap in phylogeny reconstruction. *Statistical Science* **18**: 1–12.
- Sowerby GB II. 1839.** *A conchological manual*. London: GB Sowerby, 1–130.
- Sowerby JD. 1823.** [1820–1834]. *The genera of fossil and recent shells, for the use of students in conchology and geology*. London: GB Sowerby.
- Stamatakis A. 2014.** RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
- Swofford DL. 2002.** *Phylogenetic analysis using parsimony (*and other methods), version 4*. Sunderland, MA: Sinauer Associates, Inc.
- Swofford DL, Olsen GJ, Waddell PJ, Hillis DM. 1996.** Phylogenetic Inference. In: Hillis DM, Moritz C, Mable BK, eds. *Molecular systematics*. Sunderland, MA: Sinauer, 407–514.
- Taylor DJ, Piel WH. 2004.** An assessment of accuracy, error, and conflict with support values from genome-scale phylogenetic data. *Molecular Biology and Evolution* **21**: 1534–1537.
- Utinomi H. 1962.** Studies on the cirripedian fauna of Japan. VIII. Thoracic cirripeds from the western Kyusyu. *Publications of the Seto Marine Biological Laboratory* **10**: 211–239.
- Wang TH, Donaldson YK, Brettle RP, Bell JE, Simmonds P. 2001.** Identification of shared populations of human immunodeficiency Virus Type I infecting microglia and tissue macrophages outside the central nervous system. *Journal of Virology* **75**: 11686–11699.
- Weltner W. 1897.** Verzeichnis der bisher beschriebenen receten Cirripedenarten. Mit Angabe der im Berliner Museum vorhandenen. *Archiv für Naturgeschichte* **63**: 227–280.
- Young PS. 1989.** *Ceratoconcha paucicostata*, a new species of coral inhabiting barnacle (Cirripedia, Pyrgomatidae) from the western Atlantic. *Crustaceana* **56**: 193–199.
- Zullo VA. 1963.** A review of the subgenus *Armatobalanus* Hoek (Cirripedia: Thoracica), with the description of a new species from the California coast. *Annals and Magazine of Natural History* **6**: 587–594.
- Zullo VA. 1967.** *Pyrgopsella*, new name for *Pyrgopsis* Gruvel, 1907 (Cirripedia, Thoracica), non-*Pyrgopsis* de Rochebrune, 1884. *Crustaceana* **13**: 123.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Maximum likelihood phylogram for COI computed using RAxML. Values above the branches are bootstraps. Branches with < 85% bootstrap support are collapsed.

Figure S2. Maximum likelihood phylogram for 16S computed using RAxML. Values above the branches are bootstraps. Branches with < 85% bootstrap support are collapsed.

Figure S3. Maximum likelihood phylogram for 12S computed using RAxML. Values above the branches are bootstraps. Branches with < 85% bootstrap support are collapsed.

Figure S4. Maximum likelihood phylogram for 18S computed using RAxML. Values above the branches are bootstraps. Branches with < 85% bootstrap support are collapsed.

Figure S5. Maximum likelihood phylogram for H3 computed using RAxML. Values above the branches are bootstraps. Branches with < 85% bootstrap support are collapsed.

Figure S6. Basis calcareousness (left) and cirrus III armature (right) plotted on the multi-gene ML phylogeny using Mesquite. Colors on the interior branches are ancestral character state reconstructions, terminal branches were scored from actual specimens.

Table S1. List of sequenced specimens, and gene sequences available. Specimen repository code: UF = Florida Museum of Natural History, WA = Western Australian Museum, ORI = Oceanographic Research Institute, Durban, South Africa, TAU = Zoological Museum, Tel Aviv University.