



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

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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, **113**, 162–179.

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## 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 & Aчитuv, 2007).

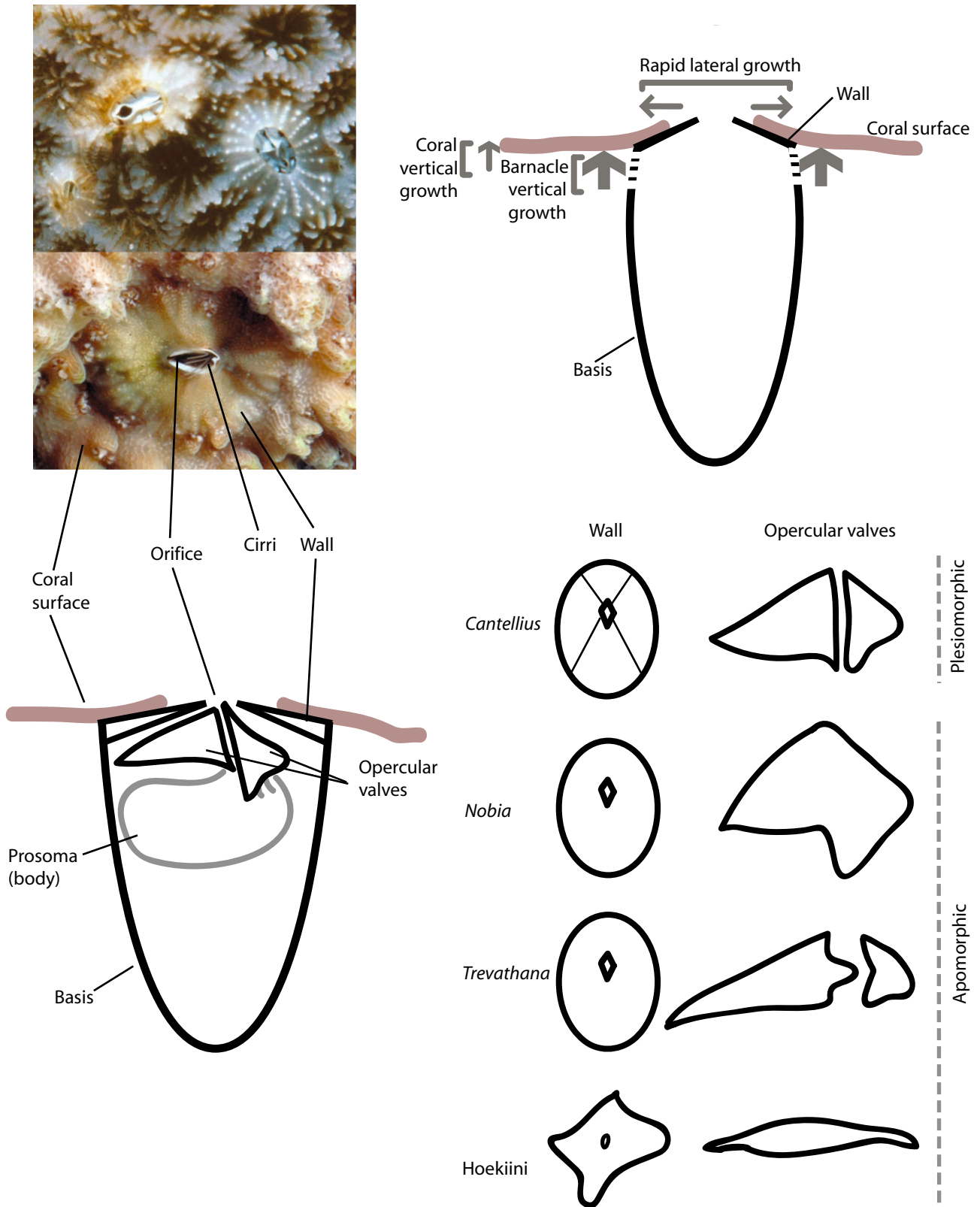
This study focuses on the phylogenetic systematics of the Pyrgomatidae, a morphologically and ecologically distinctive group that is obligately associated

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

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**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							
<b>Ceratoconcha Kramberger-Gorjanovic 1889</b>	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							
<b>Cantellius Ross and Newman 1973</b>	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)							
<b>Cionophorus</b> 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							
<b>Creusia</b> Leach 1817	IWP	4	f	b	ca	pk	zx
<i>spinulosa</i> * Leach 1818							
<b>Darwiniella</b> Anderson 1992	IWP	1	f	m	ca	pk	zx
<i>angularis</i> Chen <i>et al.</i> 2012							
<i>conjugatum</i> * (Darwin, 1854)							
<b>Galkinia</b> 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							
<b>Hiroa Ross and Newman 1973</b>	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)							
<b>Neotrevathana Ross 1999a</b>	IWP	1	f	m	ca	pk	zx
<i>elongatum</i> (Hiro, 1931)							
<b>Nobia Sowerby 1839</b>	IWP	1	f	m	ca	pk	zx
<i>grandis</i> * Sowerby 1839							
<i>halomitrae</i> (Kolosváry, 1948)							
<i>orbicellae</i> (Hiro, 1934)							
<b>Pyrgoma Leach 1817</b>	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)							
<b>Savignium Leach 1825</b>	IWP	1	uf	m	ca	pk	zx
<i>crenatum</i> * (Sowerby, 1823)							
<i>tuamotum</i> Achituv and Langsam 2005							
<b>Trevathana Anderson 1992</b>	IWP	1	uf	m	ca	pk	zx
<i>dentata</i> * (Darwin, 1854)							
<i>isfae</i> Achituv and Langsam 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 Langsam 2009							
<i>tureiae</i> Achituv and Langsam 2005							
<b>Wanella Anderson 1993</b>	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							
<b>Pyrgopsella Zullo 1967</b>	IWP	1	uf	m	me	pk	zx
<i>annandalei</i> * (Gravel, 1907)							
<i>youngi</i> Achituv and Simon-Blecher 2006							
<b>Tribe Hoekiini Ross and Newman 1995</b>							
<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							
<b><i>Adna</i> Sowerby 1823</b>	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 intrageneric 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  $\mu$ l M2 buffer and 150  $\mu$ 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/ $\kappa$  = 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 ( $\delta$ ) 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  $T_{MPT2}$ ).

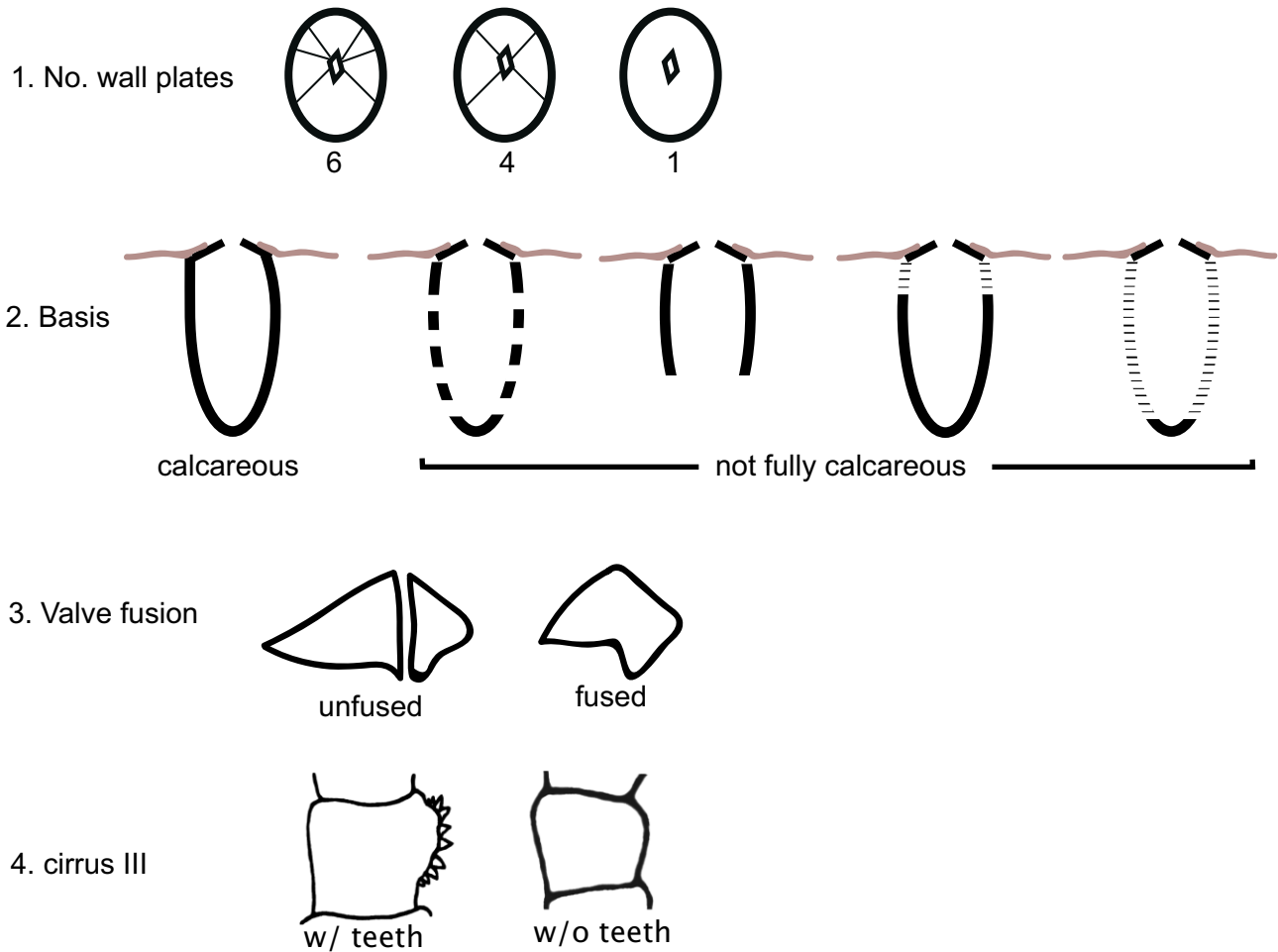
While the S-H test is easily implemented and is an appropriate test for the question at hand, it is highly conservative (Shimodaira, 2002). The SOWH test is a parametric bootstrapping technique that offers a more powerful and less conservative approach. The SOWH test was used to compare the difference in likelihoods ( $\delta_c = \text{likelihood}[T_{ML}] - \text{likelihood}[T_C]$ ) against a null distribution of  $\delta$  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](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 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

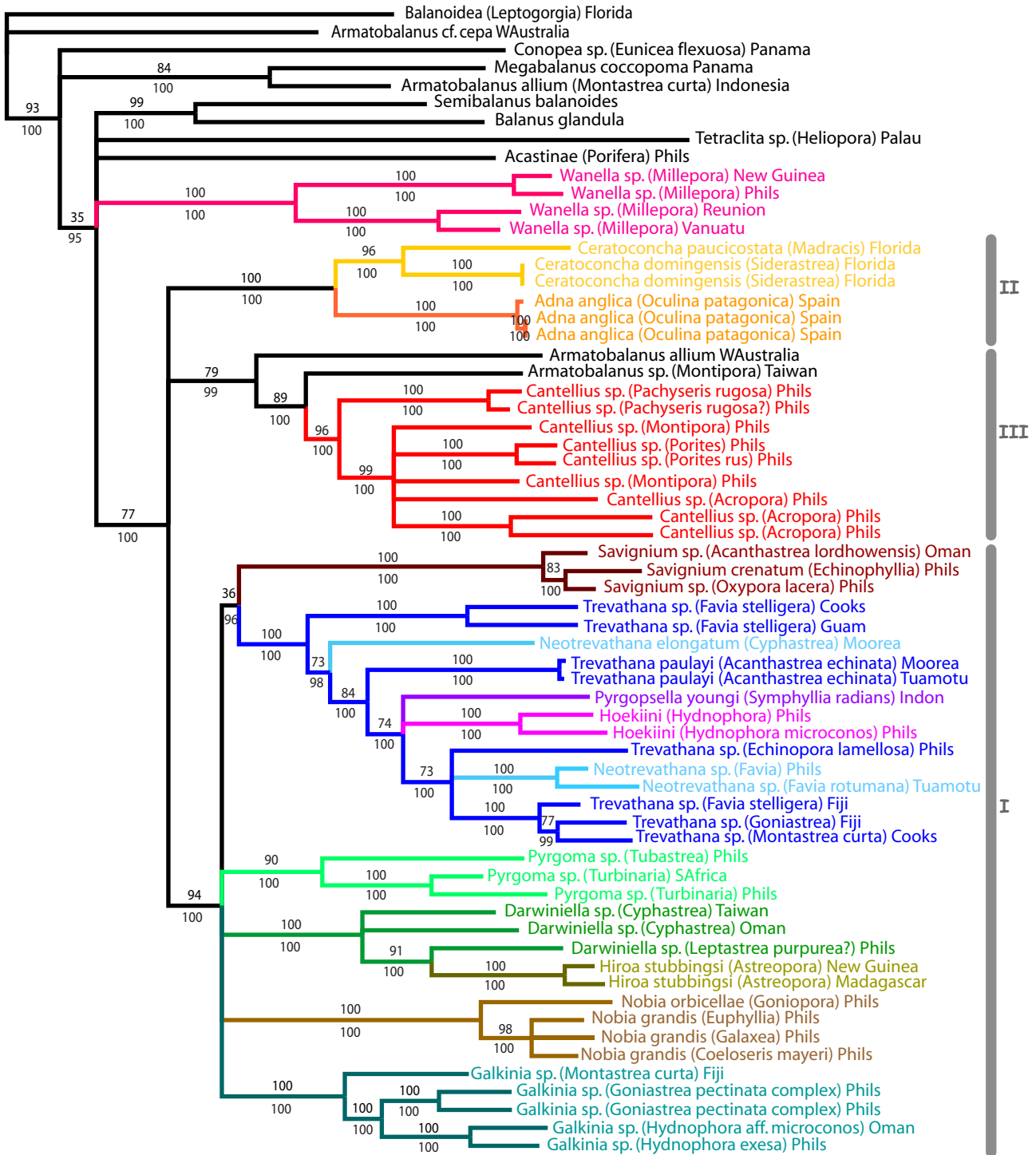
We used topological tests to investigate whether *Wanella* is monophyletic with the rest of the Pyrgomatidae. The S-H test (Table 3) rejected the hypothesis of a monophyletic Pyrgomatidae ( $P < 0.01$  for all three topologies); while the SOWH test rejected the constrained topology (with  $P < 0.002$ ) 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  $\delta$  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)	#Invariant 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



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**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.*

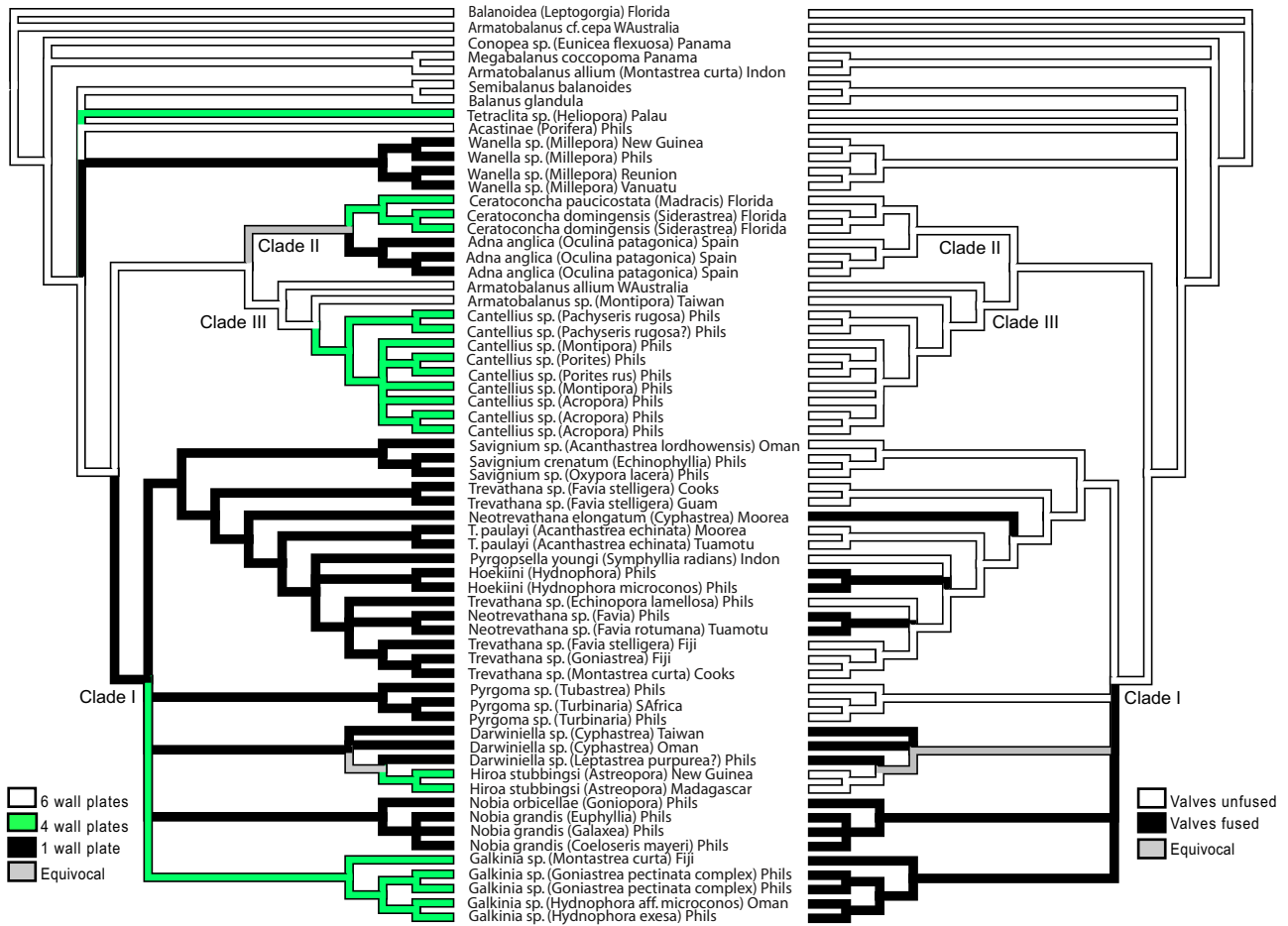
Clade II unites the only EA genus, *Adna* (Megatrematinae), with *Ceratoconcha* (Ceratoconchinae), one of two extant WA genera. It will be interesting to investigate the placement of other megatrematine genera (i.e., the WA/IWP *Megatrema* and the IWP *Memagreta* and *Pyrgomini*) to test whether the 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).

### SKELTAL 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
<b>Number of wall plates</b>				
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
<b>Basis calcareousness</b>				
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
<b>Opercular valve fusion</b>				
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
<b>Cirrus III teeth</b>				
AI	1.511 × 10 <sup>-9</sup> (0–2.728 × 10 <sup>-13</sup> )	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 3<sup>rd</sup> 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 it is 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.

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## 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.