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Phylogenetic relationships of Darwin's "Mr. Arthrobalanus": The burrowing barnacles (Cirripedia: Acrothoracica)

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ABSTRACT

The barnacles of the superorder Acrothoracica are small, burrowing, epibiotic, and dioecious (large female with dwarf male) crustaceans largely found in the carbonate sediments and skeletons of marine invertebrates. The acrothoracicans represent the Cirripedia with the most plesiomorphic characters and have prominently featured in phylogenetic speculations concerning these crustaceans. Traditionally, Acrothoracica was divided into two main orders, Pygophora and Apygophora. The Apygophora had uniramus cirri and no anus. The Pygophora had biramus terminal cirri and an anus and was further divided into two families, Lithoglyptidae and Cryptophialidae. Kolbasov (2009) revised the superorder Acrothoracica on the basis of morphological examinations of females, dwarf males, and cyprids and rearranged the acrothoracican species into two new orders, Lithoglyptida and Cryptophialida. The present study is the first attempt to reconstruct the phylogenetic relationships of acrothoracican barnacles by sequencing two mitochondrial (cytochrome C oxidase I and 16S ribosomal DNA) and two nuclear (18S ribosomal DNA and histone H3) markers of 8 of the 11 genera comprising 23 acrothoracican species. All monophylies of the eight acrothoracican genera sampled in this study were strongly supported. The deep interfamilial relationship constructed is consistent with the recent morphological phylogenetic relationship proposed by Kolbasov, Newman, and Høeg (Kolbasov, 2009) that Cryptophialidae (order Cryptophialida) is the sister group to all other acrothoracicans (order Lithoglyptida). According to an ancestral character state reconstruction analysis, the posterior lobes of females; armament of opercular bars, attachment stalk, lateral projections of the body, and aperture slits in dwarf males; and habitat use appear to have phylogenetic importance.

1. Introduction

Cirripedes are a group of marine crustaceans that are sessile suspension feeders or specialized parasites as adults. Symbiosis is a widely adopted strategy for three superorders, Acrothoracica, Thoracica, and Rhizocephala. Thoracican barnacles are the most diverse group of species with different lifestyles; several genera are symbionts of corals, sponges, and other sea animals, including turtles and whales, and some species are parasites in crustaceans (Newman and Ross, 1976). Rhizocephalans are obligate parasites of malacostracan crustaceans (Høeg, 1992). The barnacles of the superorder Acrothoracica are largely miniaturized and bore into calcareous substrates in marine environments, including the shells of molluscs and thoracican barnacles, exoskeletons of corals and bryozoans, and calcareous rocks (Kolbasov, 2009; Newman, 1974; Stubbings, 1967; Tomlinson, 1969, 1987) (Fig. 1). The acrothoracicans represent an old group with an early divergence from remaining surviving cirripedes owing to their small size and burrowing habits, which protect them from predation. They are recognized as fossils primarily according to burrows or casts of burrows dating to the Lower Devonian (Baird et al., 1990) if not the Ordovician (Taylor and Wilson, 2003).

Although the Acrothoracica was first discovered at relatively high latitudes (Darwin, 1854; Hancock, 1849), the greatest diversity is now found in the tropical seas (Kolbasov, 2009; Tomlinson, 1969). During the voyage of HMS *Beagle*, Darwin discovered his first barnacle, an acrothoracican in a gastropod shell from Chile (Tomlinson, 1987), which he named "Mr. Arthrobalanus" and later described as *Cryptophialus minutus* (Darwin, 1854). The specialized morphology of this acrothoracican barnacle stimulated Darwin's interest in the diversity of the group of

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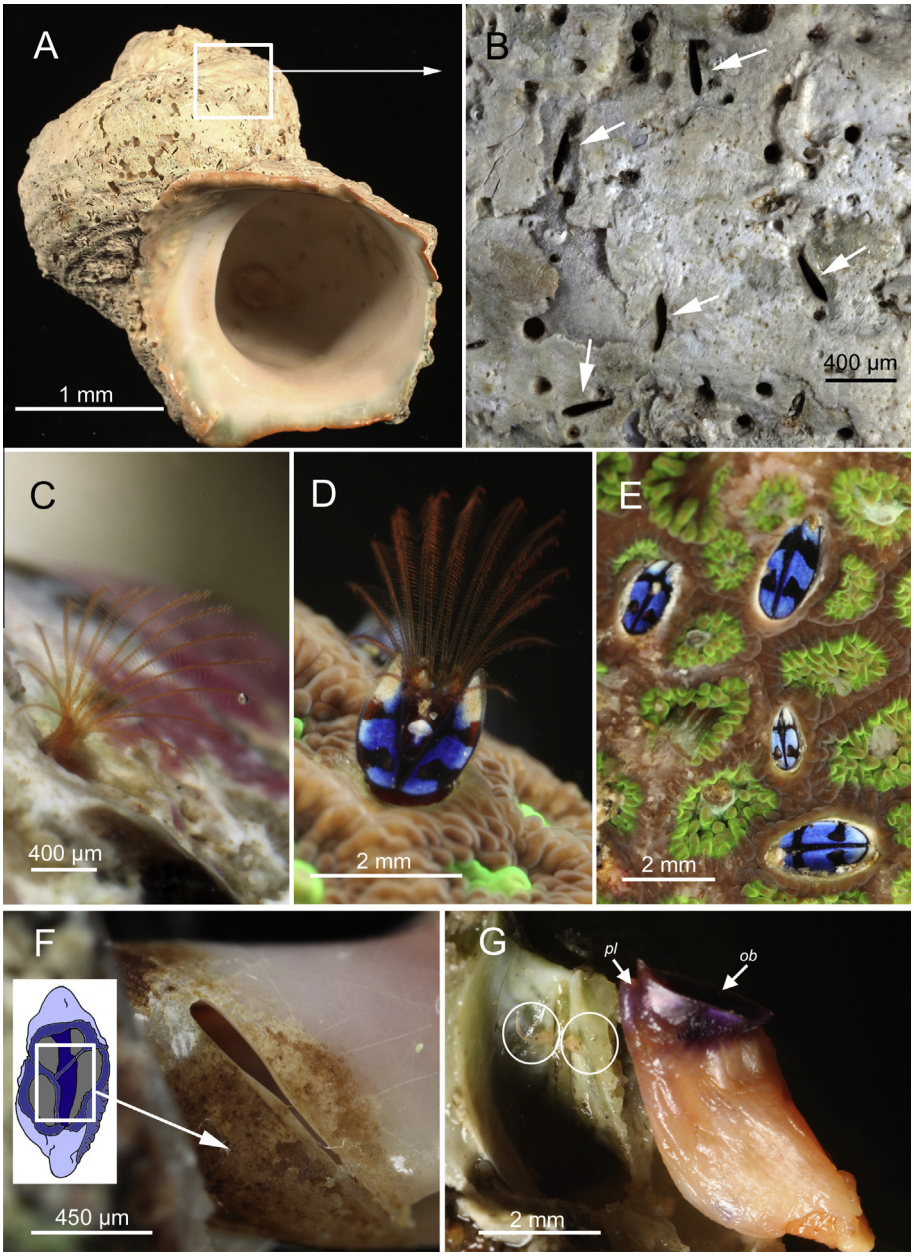

Fig. 1. (A) *Turbo* shell with burrows of acrothoracicans barnacles. (B) Magnified view of burrows of acrothoracicans (indicated by arrows) on *Turbo* shell. (C) Live *Balanodytes* extending terminal cirri out from burrow on *Thais* shell. (D) Live *Berndtia purpurea* extending terminal cirri out from burrow on *Lepastrea* coral. (E) Group of *Berndtia* with closed opercular bars in *Lepastrea* coral. (F) Burrow of specialized acrothoracian barnacle *Trypetesa* living in columella of gastropod shells occupied by hermit crabs. (G) Lateral view of cross section of burrow of *Berndtia purpurea* showing dwarf males on burrow walls (indicated by round outlines). All images from Chan et al., 2014b. Abbreviations: *ob* – opercular bars, *pl* – posterior lobes of operculum.

Cirripedia, leading to almost a decade of study into cirripede taxonomy and evolution (Newman, 1993). Since Berndt (1907), the Acrothoracica has been divided into two orders and three families, and almost 70 species have been identified (Chan et al., 2012; Kolbasov, 2009). However, molecular data have indicated that higher species diversity is expected on the basis of cryptic differentiation (Chan et al., 2012, 2013).

Acrothoracican barnacles are dioecious, with large females and dwarf males being attached on the mantle surfaces of females (Darwin, 1854; Kolbasov, 2009) (Figs. 1G and S1A, B). The taxonomy of acrothoracicans is based mostly on large and long-living females. The body proper (or prosoma) is sheathed in the mantle sac or carapace with the opercular opening (or aperture) along the ventral margin (Fig. S1A and B). Normally, the mantle sac lacks calcareous plates, except for a single basal attachment plate found in a few species of Lithoglyptida (Grygier and Newman, 1985; Kolbasov, 2009; Newman, 1971, 1974). The aperture is armed with a pair of chitinous opercular bars and a comb collar (Fig. S1A). Acrothoracicans possess a pair of mouth cirri and separated terminal cirri. The boring apparatus consists of spines and ctenoid multifid scales.

Berndt (1907) divided acrothoracicans into two orders, the relatively plesiomorphic Pygophora and the rather specialized Apygophora (Fig. 2). The apygophorans have three pairs of uniramus terminal cirri and lack an anus, whereas the pygophorans have three to five pairs of biramus terminal cirri and an anus (Berndt, 1907; Tomlinson, 1969). The pygophorans are divided into two families, Lithoglyptidae and Cryptophialidae, and the

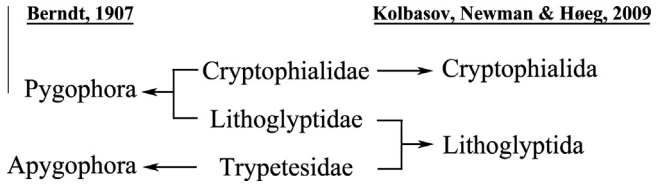

Fig. 2. Taxonomic classification of acrothoracican barnacles. Classification scheme of Berndt (1907) classified Acrothoracica into two orders Pygophora and Apygophora, based on presence/absence of anus. Classification scheme suggested by Kolbasov, Newman and Høeg (in Kolbasov, 2009), classifying the Acrothoracica into orders Lithoglyptida and Cryptophialida based on a number of morphological characters of females, dwarf males and cypris larvae.

apygophorans consist of a single family, Trypetesidae (Martin and Davis, 2001; Tomlinson, 1969). This systematics of acrothoracicans was hypothesized on the basis of a few morphological characters of females, and some of them represent symplesiomorphies (Kolbasov, 2009). Grygier and Newman (1985) commented on the plesiomorphic characters defining the Lithoglyptidae and suggested that this family is paraphyletic.

Recent studies on the morphology of acrothoracican females, dwarf males, and cypris larvae conducted using a scanning electron microscope (SEM) reported numerous ultrastructural characters (Kolbasov, 2009), and the evolutionary relationships suggested seemingly contradict Berndt's systematics. Kolbasov (2002) identified two morphological groups of dwarf males that do not correspond with Pygophora and Apygophora (Fig. S1C and D). The first group includes Lithoglyptidae and Trypetesidae, which are characterized by dense cuticular projections and complications of the body form (Figs. 2 and S1C). The second group comprises dwarf males of Cryptophialidae that have circular cuticular ribs and teeth (Kolbasov, 2002) (Figs. 2 and S1D). Furthermore, Kolbasov and Høeg (2007) investigated the morphology of the cypris larvae of all three acrothoracican families. Compared with the Lithoglyptidae and Trypetesidae (Fig. S1E), the cyprids of the Cryptophialidae (Fig. S1F) exhibit apomorphies including simplified and reduced carapaces, antennules, and thoraxes and the absence of swimming appendages (Kolbasov and Høeg, 2007). On the basis of a morphological data matrix consisting of 43 female, 6 male, and 16 cypris characters, Kolbasov, Newman, and Høeg (in Kolbasov, 2009) rearranged acrothoracicans into two new orders, Lithoglyptida (Lithoglyptidae and Trypetesidae) and Cryptophialida (Cryptophialidae) (Kolbasov, 2009) (Fig. 2). The females of the Cryptophialida can be distinguished from those of the Lithoglyptida according to the presence of a bottle-shaped mantle sac with a narrow-necked operculum, narrow crown-shaped opercular bars, an elongated and tongue-shaped labrum, and reduced mouth cirri.

Kolbasov and Newman (2005) reviewed the phylogenetic relationships of three lithoglyptid subfamilies, Weltneriinae, Lithoglyptinae, and Kochlorininae, which can be distinguished from each other by the number of terminal cirri in females. Moreover, they revised the largest and relatively plesiomorphic lithoglyptid genus *Lithoglyptes s.l.* Aurivillius, 1892 and divided it into three genera, *Lithoglyptes s.s.* Aurivillius, *Auritoglyptes* Kolbasov and Newman, 2005, and *Armatoglyptes* Kolbasov and Newman, 2005 (Kolbasov and Newman, 2005). *Armatoglyptes* was later identified as a *nomen nudum* and a junior synonym of *Balanodytes* (Chan et al., 2013; Utinomi, 1950). *Balanodytes* was suggested as the sister clade to *Lithoglyptes* and *Auritoglyptes* on the basis of 13 female and male characters but with limited node support (Kolbasov and Newman, 2005). Weltneriinae was recently considered a junior synonym to Berndtiinae (Chan et al., 2014b; Poore, 2012).

The validity of these taxonomic divisions remains untested through molecular approaches. The present study is the first attempt to reconstruct the phylogenetic relationships of acrothoracican barnacles on the basis of molecular data. We sequenced two mitochondrial [cytochrome C oxidase I (COI) and 16S ribosomal DNA (16S rDNA)] and two nuclear [18S ribosomal DNA (18S rDNA) and histone H3 (H3)] markers from 8 of the 11 genera comprising 23 acrothoracican species (Table 1). We aimed to provide a well-supported phylogenetic hypothesis at the genus level, test former hypotheses of relationships based only on morphology, and explore the evolution of females, dwarf males, and cypris larvae characters and habitat use of these specialized groups of barnacles.

2. Materials and methods

2.1. Taxon sampling

We collected the molecular and morphological data of 75 acrothoracican individuals (females): 66 in Lithoglypidae (19 species), 2 in Trypetesidae (2 species), and 7 in Cryptophialidae (2 species, Tables 1 and S1). The selection of taxa covered the major lineages (families and subfamilies) of Acrothoracica (Table 1). We excluded two low-diversity genera, *Kochlorinopsis* and *Tomlinsonia*, and *Australophialus* which is mostly distributed in the southern hemisphere. On the basis of previous hypotheses from molecular and morphological evidence, Acrothoracica is the sister clade to both rhizocephalan and thoracican barnacles (Kolbasov, 2009; Pérez-Losada et al., 2009) and Cirripedia is the sister clade to Ascothoracida (Pérez-Losada et al., 2009). According to these hypotheses, four rhizocephalan, two thoracican, and one ascothoracidan species were included as outgroups for analyses. The voucher ID, locality, and habitat information are provided in Table S1.

2.2. DNA sequence analysis

Total genomic DNA was extracted from the muscle tissue by using the Qiagen (Chatsworth, CA) QIAquick Tissue Kit according to the manufacturer's instructions. The DNA sequences of two mitochondrial DNA markers, COI and 16S rDNA, and two nuclear markers, 18S rDNA and H3, were obtained to reconstruct phylogenetic relationships. The sequences and primers used for amplifying the sequences in the polymerase chain reaction (PCR) were obtained from previous studies: COI (Chen et al., 2012; Folmer et al., 1994; Roman and Palumbi, 2004; Schubart and Huber, 2006), 16S rDNA (Crandall and Fitzpatrick, 1996), 18S rDNA (Lin et al., 2015), and H3 (Colgan et al., 1998). The forward primer 5′-GGCHCCMMGGAAGCAGCTGG-3′ and the reverse primer 5′-CTTG GCGTGRATDGCRCACA-3′ were newly designed for H3 in some basal groups. The PCR solution contained approximately 40 ng of template DNA, 5 μL of Taq DNA Polymerase Master Mix (1.5 mM $MgCl_2$; Ampliqon, Denmark), each primer at 1 μM, and ddH_2O with a final volume of 10 μL. The PCR reaction was conducted under the following conditions: 2 min at 95 °C for initial denaturation, 35 cycles of 30 s at 95 °C, 1 min at 50 °C, and 1 min at 72 °C, with a final extension for 5 min at 72 °C. The PCR products were then purified using the DNA Gel purification kit (Tri-I Biotech, Taipei, Taiwan). The direct sequencing of purified PCR products was performed on an ABI 3730XL Genetic Analyzer by using BigDye terminator cycle sequencing reagents (Applied Biosystems, Foster City, California, USA).

On the basis of translated amino acid sequences in Geneious, the sequences were assembled, edited, and aligned to ensure that no gaps interrupted reading frames (Drummond et al., 2011). For the two protein coding genes, COI and H3, the rapidly evolving third codon position was partitioned from the slowly evolving first and second positions in Bayesian and likelihood analyses (Brandley et al., 2005). For the two rDNA genes, nuclear 18S and

Table 1
Currently recognized taxa of the superorder Acrothoracica (Kolbasov, 2009). Numbers in parentheses are the sampled genera number for families and subfamilies, and species number for genera in this study in ratio to the total number identified in Kolbasov (2009) and Chan et al. (2012, 2013, 2014).

Order Lithoglyptida Kolbasov, Newman & Høeg 2009
Family Lithoglyptidae Aurivillius, 1892 (6/7)
Subfamily Berndtiinae Utinomi, 1950 (2/2)
Weltneria Berndt, 1907 (1/11)
Berndtia Utinomi, 1950 (4/6)
Subfamily Lithoglyptinae Aurivillius, 1892 (3/3)
Balanodytes Utinomi, 1950 (6/11)*
Lithoglyptes Aurivillius, 1892 (2/4)
Auritoglyptes Kolbasov and Newman, 2005 (4/1)
Subfamily Kochlorininae Gruvel, 1905 (1/2)
Kochlorine Noll, 1872 (2/7)
Kochlorinopsis Stubbings, 1967 (0/1)
Family Trypetesidae Stebbing, 1910 (1/2)
Trypetesa Norman, 1903 (2/5)
Tomlinsonia Turquier, 1985 (0/2)
Order Cryptophialida Kolbasov, Newman & Høeg 2009
Family Cryptophialidae Gerstaecker, 1866 (1/2)
Australophialus Tomlinson, 1969 (0/5)
Cryptophialus Darwin, 1854 (2/16)

* Sensu *Armatoglyptes* Kolbasov and Newman, 2005 (Chan et al., 2013). One additional species *Balanodyte flexuosus* was newly described from the Mozambique Channel in Chan et al. (2012), after Kolbasov (2009).

mitochondrial 16S, the proportion of ambiguity sites in the alignment was low (<3%). The Akaike information criterion (Akaike, 1974) implemented in jModelTest, Version 2.1.3 (Darriba et al., 2012; Guindon and Gascuel, 2003) was used to select the best-fit evolutionary model for each partition (Table 2). The likelihood was calculated for 88 models, including 11 substitution schemes, equal or unequal base frequencies, a proportion of invariant sites (I), and rate variation among sites for rate categories (G) on a maximum likelihood (ML) optimized tree.

2.3. Phylogenetic relationships of acrothoracican barnacles

We performed Bayesian inference (BI), ML, and maximum parsimony (MP) analyses to reconstruct phylogenetic relationships. The genetic markers that could not be amplified and sequenced were treated as missing data for all phylogenetic analyses. The Bayesian Metropolis-coupled Markov chain Monte Carlo (MCMC) estimation of phylogeny was performed using MrBayes, Version 3.1.2 (Huelsenbeck and Ronquist, 2001) through the CIPRES Science Portal, Version 2.2 (Miller et al., 2010). The best-fit evolutionary models selected by jModeltest were applied to six genetic partitions (two rDNA genes and two protein coding genes with two partitions each, the first and second codon positions, and the third codon position; Table 2). If a selected model could not be implemented in MrBayes, then the least complex model that included all the parameters of the selected model was used. The general time-reversible (GTR) model with gamma rate heterogeneity (G) was selected for the third codon position of COI, 16S, 18S, and the third codon position of H3; the GTR model with I was selected for the first and second codon positions of H3, and the GTR model with G and I (Tavaré, 1986) was selected for the first and second codon positions of COI. A partitioned mixed-model analysis was applied, and all model parameter values were "unlinked" among partitions (Ronquist and Huelsenbeck, 2003). In all analyses, average substitution rates (prset ratepr = variable) and model parameters, including branch lengths within a tree (unlink brlens), were allowed to vary among partitions. Two simulated independent runs were performed starting from different random trees. Each run consisted of four chains (one cold and three heated) and was sampled every 1000 generations. The sampled parameter values from Bayesian MCMC estimation were evaluated using Tracer, Version 1.4, and generations before reaching a plateau were discarded as burn-in. Trees from the stationary phase of two runs were pooled using LogCombiner, Version 1.5.4 (Drummond and Rambaut, 2007), and the posterior probability of each node and the mean branch length of the maximum clade credibility tree were calculated using TreeAnnotator, Version 1.5.4 (Drummond and Rambaut, 2007).

ML tree searching was conducted using RAxML, Version 7.2.8 BlackBox (Stamatakis et al., 2008). The data set was partitioned as in BI analyses and the G model was applied per partition. Ten replicates were run to find the tree topology with the best likelihood, and 1000 bootstrap replicates were performed to estimate node supports.

We conducted MP analyses by using PAUP 4.0b10. Heuristic searches were performed using tree-bisection-reconnection branch swapping from 1000 random addition sequence replicates to prevent entrapment at local optima. All nucleotide sites were equally weighted and gaps were treated as missing characters. Nonparametric node supports for trees were estimated through 1000 heuristic searches starting with 10 random addition sequence replicates with a maximum tree limit of 1000 for each.

The feasibility of combining phylogenetic information from multiple molecular markers was evaluated by searching strongly supported but conflicting clades among BI trees constructed using each gene data set (Wiens, 1998). Clades were considered strongly supported if the posterior probability was $\geqslant$95% (Leaché and Reeder, 2002). No significant conflict was observed among BI trees on the basis of individual genes.

2.4. Ancestral character state reconstruction

Of the eight sampled genera, we reconstructed the ancestral states and character evolutions of six characters (five morphological characters and one ecological character; Table 3). Recently, the morphological characters of acrothoracican females, dwarf males, and cypris larvae were thoroughly reviewed and summarized by Kolbasov (2009). We analyzed 5 of 65 morphological characters reported (Table 1 in Kolbasov, 2009) because they are Acrothoracica specific, have no missing data, and are parsimony informative. In addition, one ecological character, that is, the host type of acrothoracicans, was recorded and analyzed (Tables 3 and S2). The host type was classified as follows: (0) external surfaces of

Table 2
Phylogenetic information of datasets used in this study. The best-fit nucleotide substitution model, invariable sites (I) and rate variation among sites (G) were selected under the Akaike information criterion (AIC) by jModelTest.

	Codon position	Total characters	Variable characters	Parsimony informative characters	Best-fit Model	I	G
COI	1st + 2nd	380	153 (40.26%)	115 (30.26%)	TrN1 + I + G	0.3850	0.2560
	3rd	191	190 (99.48%)	188 (98.43%)	GTR + G	–	1.3030
16S		562	368 (65.48%)	325 (57.83%)	TIM2 + G	–	0.3010
18S		1826	571 (31.27%)	418 (22.90%)	TIM3 + G	–	0.0210
H3	1st + 2nd	184	26 (14.13%)	18 (9.78%)	TrNef + I	0.7920	–
	3rd	93	89 (95.70%)	80 (86.02%)	TPM2uf + G	–	1.8430

calcareous molluscan (including chiton) and barnacle shells, skeletons of dead corals, chalk, calcite rocks and conglomerates, and bryozoans (Chan et al., 2014b; Tomlinson, 1969); (1) live corals; and (2) columella or inner surface of the wall of gastropod shells occupied by hermit crabs.

The ancestral state and evolutionary history of the six characters were reconstructed through MP and ML methods by using Mesquite, Version 2.75 (Maddison and Maddison, 2011) and through the MCMC method by using BayesTraits, Version 2.0 beta (Pagel, 1994, 1997; Pagel et al., 2004; Pagel and Meade, 2006). In Mesquite, we assigned character states to each species in the final tree inferred from BI and collapsed the node that was not significantly supported by BI (Fig. 3). Any state change of characters was assumed equally probable and unordered. Although biological objections may be raised against this assumption, our knowledge is not adequate to suggest any alternative hypotheses concerning specific character changes. The MP method in Mesquite found ancestral states that minimized the number of character change steps provided in a tree and observed character distribution. The ML method in Mesquite found ancestral states that maximized the probability that observed states would evolve under a stochastic model of evolution (Pagel, 1999; Schluter et al., 1997). Because polymorphic taxa are currently unsupported by the ML method in Mesquite, the analyses were performed twice with the character state of these taxa assigned as either state. Ancestral states were reported as proportional likelihoods at each node for each character state. In addition, character states were estimated for selective nodes with a posterior probability of $\geqslant 0.95$ by using the MCMC method in BayesTraits. BayesTraits used the reversible-jump MCMC (RJ MCMC) approach to search among the possible models of character state evolution while sampling from a set of trees. To consider the phylogenetic uncertainty, the final 300 trees generated by MrBayes after 20% burn-in and subsampling of every 10 trees were applied. The RJ MCMC method automatically found the posterior distribution of evolution parameters that explained the data the most adequately. As recommended in the manual, ML methods with 25 optimization attempts were first applied to estimate optimal rate parameters. For the MCMC analyses, the number of iterations and whether reliable convergence stability was achieved using Tracer were determined. To test whether one ancestral state was supported over others at a particular node, the "fossil" command was applied to duplicate runs constraining the most recent common ancestor (MRCA) as one of the states. Twice the difference between these alternative hypotheses in the harmonic means of logarithm likelihood values of MCMC analyses was assessed for evidence of support (BF: Bayes factor, Kass and Raftery, 1995). A BF value between 2 and 5 indicated positive support and >5 indicated strong support.

Table 3
Six characters studied for ancestral state reconstruction in this study.

	Character	Reference
Female		
1	Small or long (auricles) posterior lobes of operculum 0: absent, 1: present	13,14 in Kolbasov, 2009
2	Armament of opercular bars 0: feeble, with small teeth, 1: developed, with big teeth of different forms	15 in Kolbasov, 2009
Dwarf male		
3	Morphology of attachment antennules 0: simple, 1: with attachment process or stalk	46 in Kolbasov, 2009
4	Lateral projections/wings of body 0: absent, 1: present	48 in Kolbasov, 2009
5	Apertural slit on posterior end 0: present, 1: absent	49 in Kolbasov, 2009
Habitat		
6	Host 0: external surface of calcareous substrate including dead corals and gastropod shells, 1: live coral, 2: columella or inner surface of wall of gastropod shell inhabited by hermit crab	Tomlinson, 1967, Kolbasov, 2009

3. Results

3.1. Sequence analysis

The sequence matrix comprised 75 acrothoracicans, 2 thoracicans, 4 rhizocephalans, and 1 ascothoracidan, representing 23, 2, 4, and 1 species, respectively (Tables 1 and S1). Because of the presence of cryptic and undescribed species in Acrothoracica, conspecies individuals were identified on the basis of their significantly shorter genetic distance than that in other congeneric species pairs as well as on the basis of two mitochondrial markers, COI and 16S, which have been proved useful in delineating the species boundaries of acrothoracican barnacles (Chan et al., 2013). No significant differences were observed in morphological characters among these conspecific individuals. Therefore, a total of 23 acrothoracican species, namely 19 lithoglyptid, 2 trypetesid, and 2 cryptophialid species, were included in analyses (Table 1). The concatenated sequence data set included 573 bp in COI, 562 bp in 16S, 1826 bp in 18S, and 279 bp in H3. The alignment of the two protein coding genes was confirmed using translated amino acid sequences and was thus unambiguous. In the final alignment of 3240 nucleotide sites, 1394 (43.02%) were variable. Of the 1394 variable nucleotide sites, 1144 (35.31%) were parsimony informative. The sequences of the six partitions had several parsimony informative characters (Table 2). For the two protein coding genes, COI and H3, the third codon position had more parsimony informative characters (86.02–98.43%) than did the first and second positions (9.78–30.26%). In the two rDNA genes, the number of parsimony informative sites was higher in the mitochondrial 16S rDNA (57.83%) than in the nuclear 18S rDNA (22.90%).

3.2. Phylogenetic relationships

A spectrum of phylogenetic information was provided by the four genetic markers. In general, the tree based on mitochondrial COI and 16S most effectively resolved relationships at the species level, whereas the tree based on nuclear 18S and H3 most effectively resolved relationships at the genus and family levels (Fig. S2). For phylogenies reconstructed on the basis of the concatenated data set of all four markers, overall similar topologies were observed using the BI, MP, and ML approaches, and only the topology of the maximum clade credibility BI tree is presented (Fig. 3). The optimization likelihood score of the best-fit ML tree was −25679.4137. Parsimony analysis yielded 96,904 equally parsimonious trees with 5289 steps. The BI posterior probability, ML bootstrap, and MP bootstrap percentage values are provided for monophyletic taxa in Fig. 3 and in parentheses in the following paragraph.

With one ascothoracidan species as the outgroup, the acrothoracican individuals formed a monophyletic group (100/100/100) and constituted the sister group to a clade composing the other two cirripede superorders, Rhizocephala (100/100/97) and Thoracica (100/100/100; Fig. 3). All monophylies of the eight acrothoracican genera sampled in this study were strongly supported. All three families of Acrothoracica were included in this study, and the monophyly of Lithoglyptidae with more than one genera included in this study was weakly supported in

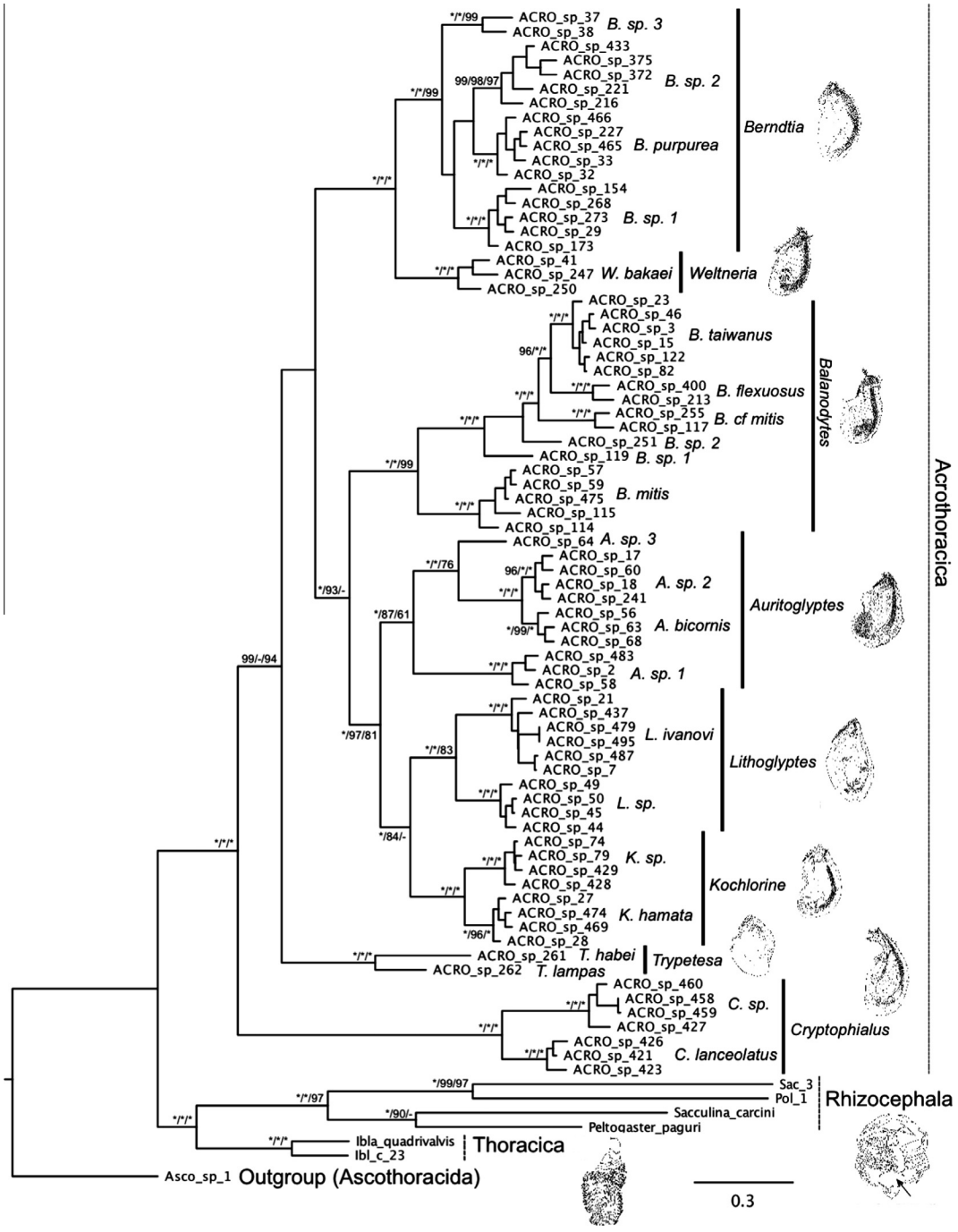

Fig. 3. Phylogenetic relationships of Acrothoracica based on Bayesian inference. Node supports are Bayesian posterior probability/Maximum Likelihood bootstrap/Maximum Parsimony bootstrap. * = 100. – = not supported. Representative drawings of the eight acrothoracican genera are *Berndtia nodosa*, *Weltneria hirsuta*, *Balanodytes balanodytes*, *Auritoglyptes bicornis*, *Lithoglyptes indicus*, *Kochlorine anchorella*, *Trypetesa habei*, *Cryptophialus gantsevichi* (from top to bottom). Scale bars indicate number of expected substitutions per site.

BI (posterior probability = 54) and MP (bootstrap = 73) analyses. In Lithoglyptidae, one (Berndtiinae) of the three subfamilies composing two genera (*Berndtia* and *Weltneria*) was monophyletic (100/100/100); however, the other two (Lithoglyptinae and Kochlorininae) were not. One of the two genera of Kochlorininae (*Kochlorine*) was the sister clade of *Lithoglyptes* in the BI (posterior probability = 100) and ML (bootstrap = 84) analyses and was thus nested within the three genera of Lithoglyptinae, making this subfamily nonmonophyletic (Figs. 3 and 4).

3.3. Character evolution

The evolution of six characters was investigated. Two characters were related to female morphology (characters 1 and 2), three were related to dwarf male morphology (characters 3–5), and one was related to host type (character 6; Table 3, Fig. 5). The molecular data of three acrothoracican genera (*Kochlorinopsis*, *Tomlinsonia*, and *Australophialus*) were not included in the phylogenetic analysis; therefore, their morphological character states were

not considered in the character evolution analysis. These missing data should have limited effects on final results because their respective sister relationships with *Kochlorine*, *Trypetesa*, and *Cryptophialus* were well defined with morphological evidence and each sister pair has the same state for the characters analyzed. The following results of the character evolution analysis should still be considered with caution because not all acrothoracican genera were sampled and monophylies of some genera (e.g., *Weltneria* and *Balanodytes*) are still in question (Kolbasov, 2009).

The evolutionary histories of the six characters were estimated on the basis of the results of the MP, ML, and MCMC methods (Fig. 5). In females, the MRCA of acrothoracicans likely did not have the small posterior lobes or long auricles of the operculum (character 1; "pl" in Fig. 1G) according to the MP and MCMC (BF = 5.39) methods (Fig. 5A). These small posterior lobes in *Lithoglyptes* and *Berndtia* species and long posterior auricles in *Auritoglyptes* may have developed independently. However, the ML method provided ambiguous results for all internal nodes (absence = 50%; presence = 50%). We could not clarify whether the MRCA of acrothoracicans had opercular bars with small or large teeth (character 2; "ob" in Fig. S1A and B) by using the ML and MCMC methods. However, using the MP and MCMC (BF = 2.69) methods, we observed that the large teeth of opercular bars in *Balanodytes*, *Auritoglyptes*, and *Kochlorine* likely shared the same origin. The ML method provided ambiguous results for all internal nodes (absence = 50%; presence = 50%). The ML and MCMC methods provided very similar results by assigning the polymorphic *Weltneria* as either state; therefore, only the one with small teeth (state 0) is shown (Fig. 5B). The dwarf males of the acrothoracican MRCA likely had simple attachment antennules (character 3; "ant" in Fig. S1D) and no lateral projections or lobes of the body (wings) (character 4; "wg" in Fig. S1C) according to the MP and MCMC (BF = 2.28) methods (Fig. 5C and D). An attachment stalk ("stl" in Fig. S1C) between the antennules and body and lateral projections of the body may have developed in the MRCA of *Lithoglyptes*, *Auritoglyptes*, and *Kochlorine* independently from that in Trypetesidae. However, the ML method provided ambiguous results on the MRCA of acrothoracicans (character 3: simple = 46%, stalk = 54%; character 4: absent = 46%, present = 54%) and MRCA of all noncryptophialid acrothoracicans (character 3: simple = 41%, stalk = 59%; character 4: absent = 41%, present = 59%). The acrothoracican MRCA likely had an apertural slit at the posterior end of the dwarf male body (character 5) according to the MP and MCMC (BF = 2.83) methods (Fig. 5E). This character was subsequently lost in *Trypetesa*, and *Kochlorine* and *Lithoglyptes*, independently. However, the ML method provided ambiguous results for all deeper internal nodes (absence = 50%; presence = 50%).

The MRCA of acrothoracicans likely lived in calcareous substrates excluding live corals and the columella or inner surface of wall of gastropod shells occupied by hermit crabs on the basis of the MP, ML (94%), and MCMC (BF = 5.81) methods (character 6; Fig. 5F). Colonization in the central columella or inner wall of gastropod shells occupied by hermit crabs was specialized in *Trypetesa*. *Berndtia* might have independently colonized live corals according to the ML (90%) and MCMC (BF = 4.93) methods.

4. Discussion

4.1. Phylogenetic relationships of the major clades of Acrothoracica

This is the first study to reconstruct the phylogenetic relationships of acrothoracican barnacles on the basis of molecular data, and the relationships are well resolved with significant support for most branches (Fig. 3). As reported by Pérez-Losada et al. (2009) and Kolbasov (2009), our results indicated that acrothoracican barnacles formed a monophyletic group and constitute a sister group to the other two cirripede superorders, the commonly known thoracican barnacles and the largely malacostracan crustacean-parasitic rhizocephalan barnacles (Fig. 3). These three superorders are well defined with distinct synapomorphic characters. The acrothoracicans are unique in having a pair of chitinous opercular bars, an orificial knob, an attachment disk, lateral bars, an elongated thorax, isolated mouth cirri, and a boring apparatus from spines and ctenoid scales.

The systematics and evolutionary relationships of acrothoracican barnacles were previously based on the morphological characters of adults (both female and dwarf male) and cypris larvae (Kolbasov, 2009; Kolbasov and Høeg, 2007; Kolbasov and Newman, 2005; Tomlinson, 1969). In this study, the molecular data of three families, 8 of the 11 acrothoracican genera, and a total of 23 species (including eight putative new species from the west Pacific region) (Chan et al., 2012, 2013, 2014a, 2014b) were collected and analyzed (Table S1, Fig. 3). The deep interfamilial relationship constructed is consistent with the recent morphological phylogenetic relationship proposed by Kolbasov, Newman, and Høeg (Kolbasov, 2009) with Cryptophialidae (order Cryptophialida) being the sister group of all other acrothoracicans (order Lithoglyptida); however, it is not consistent with the earlier hypothesis that Trypetesidae (Apyogophora) is the sister group of all other acrothoracicans (Pygophora) based on the presence of an anus and the branching number of terminal cirri in females (Berndt, 1907; Tomlinson, 1969) (Figs. 2 and 4).

Cryptophialida has several synapomorphies and consists of a single family, Cryptophialidae (Kolbasov, 2009), whereas Lithoglyptida is divided into two families, Trypetesidae and Lithoglyptidae. However, the monophyly of Lithoglyptidae was only weakly supported by our molecular data (Fig. 3). The majority of the shared characters of Lithoglyptida species are symplesiomorphic

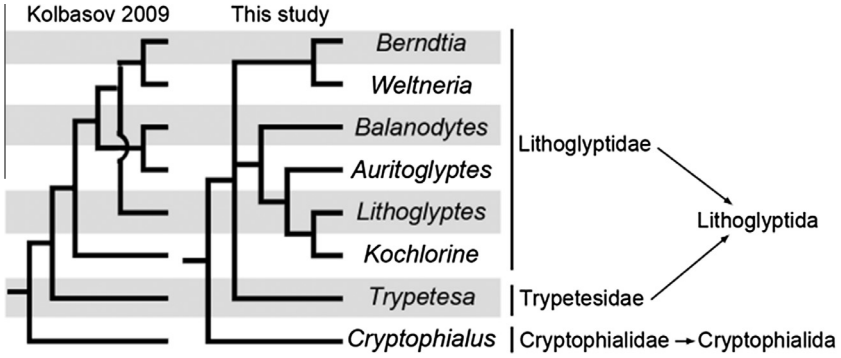

Fig. 4. Phylogenetic relationships of eight acrothoracican genera based on 65 morphological characters from Kolbasov, 2009 (left) and molecular data from this study (right).

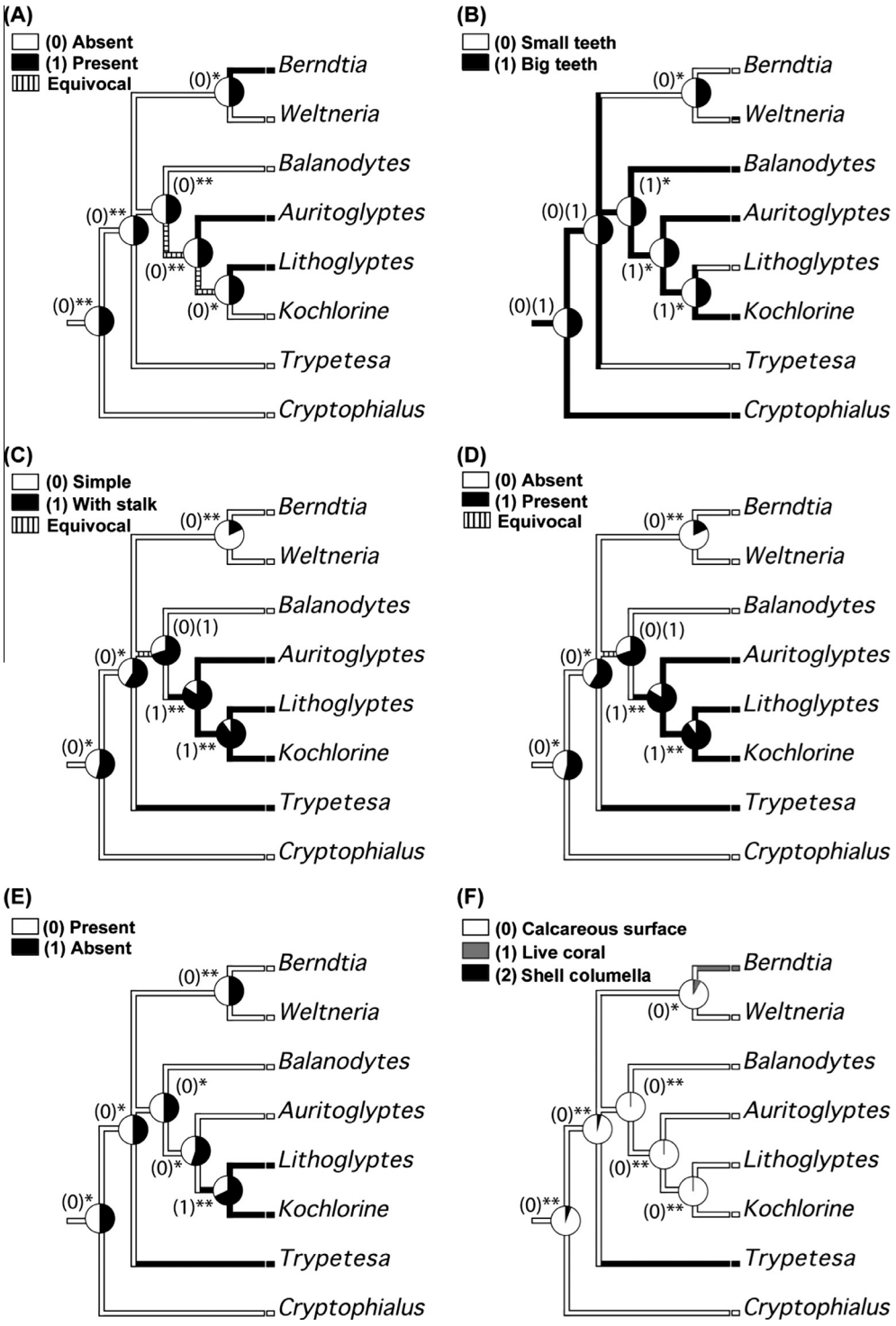

Fig. 5. Reconstructed ancestral states of (A) posterior lobes/auricles of operculum, (B) armament of opercular bars, (C) morphology of attachment antennules of dwarf males, (D) lateral projections/wings of body in dwarf males, (E) apertural slit on posterior end of dwarf male, and (F) substratum of hosts the Bayesian majority clade credibility tree. Branch shading indicates maximum parsimony reconstruction. The small pie charts indicate the proportional likelihoods of each characters state at each node. Numbers in parenthesis are the states with the highest harmonic mean likelihood values based on MCMC method and the support level is indicated (*: BF = 2–5, **: BF > 5), Two or more numbers in parenthesis indicate an ambiguous assignment on the ancestral states.

and do not indicate close phylogenetic relationships (Kolbasov, 2009). The basal calcareous attachment plates representing a plesiomorphic condition were found in a few deep sea refugial lithoglyptid species of the *Weltneria* and *Lithoglyptes* genera (Grygier and Newman, 1985; Newman, 1971, 1974) as well as later in shallow water *Balanodytes* (Kolbasov, 2009). Lithoglyptidae accommodates taxa that do not fit in the other two well-defined families, Trypetesidae and Cryptophialidae. In this study, the well-supported clades identified that Lithoglyptidae has unresolved relationships with Trypetesidae (Figs. 3 and 4). One lithoglyptidan clade is composed of two berndtiine genera (*Berndtia* and *Weltneria*), whereas the other is composed of three lithoglyptine genera

(*Balanodytes*, *Lithoglyptes*, and *Auritoglyptes*) and one kochlorinine genus (*Kochlorine*). However, that *Kochlorine* is nested within Lithoglyptinae is hypothesized for the first time and thus requires confirmation by the inclusion of another kochlorinine genus, *Kochlorinopsis* from a bryozoan in West Africa, in future analysis.

4.2. Cryptophialidae

Cryptophialidae is a monotypic family of the order Cryptophialida and includes two genera, *Cryptophialus* and *Australophialus*. *Australophialus* is mostly restricted to the southern hemisphere (only *A. pecorus* was found in the Strait of Gibraltar) (Turquier, 1985) and was not included in our study. Cryptophialidae has a set of apomorphic characters in adults and larvae (Fig. S1B, D, and F). Moreover, the burrow opening of Cryptophialidae is small and oval in shape, which contrasts the elongated shape in Lithoglyptida, and such a reduction in the burrow opening may reduce predation risks (Kolbasov, 2009). Cryptophialid females gained various apomorphic characters, and some are likely the results of adaptation to such a small burrow opening. These characters include a bottled-shaped mantle with a narrow-necked area for fitting into the small opening and reduced mouth cirri and a tongue-shaped labrum instead of fully developed mouth cirri and a large saddle-shaped labrum that is suitable for the narrow neck area. In addition, the reduction in the number of terminal cirri from five pairs in the MRCA of acrothoracicans to four in *Australophialus* and three in *Cryptophialus* is likely caused by the origination of a narrow neck area, which probably has limited space for the large number of cirri. The early-stage larvae (i.e. nauplii) are brooded as "nauplii-in-eggs" within the mantle of females and later released as cypris larvae for settlement (Batham and Tomlinson, 1965; Kolbasov et al., 1999; Kolbasov and Høeg, 2007; Tomlinson, 1969; Turquier, 1985). Unlike other acrothoracicans, cryptophialid cyprids lack swimming thoracopods; thus, they can only engage in an exploratory "walk" with antennules on the surface close to their parents and settle afterward (Darwin, 1854; Kolbasov and Høeg, 2007) or be dispersed passively with water currents. Consequently, the burrows of Cryptophialidae often occur in dense clusters on a single host. Other identified apomorphic characters in cyprids likely relate to this special mode of settlement (Kolbasov and Høeg, 2007). For instance, the reduction of the carapace size is probably because of the reduction of the thorax with thoracopods and the absence of a telson with a furca. A lack of active swimming and substrate exploration in cryptophialid cyprids leads to the reduction of several chemosensory structures, such as the absence of frontolateral pores on the carapace, modification of the structure of lattice organs, fusion of the setae of the fourth antennular segment in a single tuft, and presence of a simple morphology (Kolbasov et al., 2014).

4.3. Trypetesidae

Trypetesidae is specialized to inhabit mostly the columella and sometimes the inner side of the wall of gastropod shells occupied by hermit crabs (Larsen et al., 2016). Moreover, trypetesids can feed on the eggs of female hermit crabs that they infest (Murphy and Williams, 2013; Williams and Boyko, 2006; Williams et al., 2011). This substantial transition from being suspension feeders like other acrothoracican barnacles to egg predators and parasites of hermit crabs caused morphological adaptations, particularly related to burrowing and feeding. Critical characters in catching and processing food through filtering are found to be degenerated in Trypetesidae, including a decrease in the size of mouth legs and mandibular palps and shortened terminal cirri with only a single ramus and loss of the anus. The origination of special pads on the terminal cirri might have facilitated grasping and rasping the host's egg mass, and the absence of an anus (Tomlinson, 1987) suggests that undigested material is excreted into the environment through regurgitation.

4.4. Lithoglyptidae

In our phylogenetic tree, *Lithoglyptes*, *Auritoglyptes*, and *Balanodytes* are sister groups to *Kochlorine* (Figs. 3 and 4). Previously, this basal position of *Balanodytes* in Lithoglyptinae was based on 13 male and female morphological characters (Kolbasov and Newman, 2005). However, the inclusion of *Kochlorine*, which has three pairs of terminal cirri (likely the state for the entire Kochlorininae subfamily and for an additional monotypic genus *Kochloriopsis*) within Lithoglyptinae, which has four pairs of terminal cirri, was hypothesized for the first time. Despite the strong support based on our molecular data (Fig. 3), no firm synapomorphies have been reported before or were observed in the current study to support this newly hypothesized relationship. A few morphological characters provide the following potential evidence: (1) The dwarf males of most acrothoracicans are plesiomorphic and have a pear-shaped body with simple attachment antennules (Fig. S1D). In addition, the apomorphic attachment stalk between the body and antennules and wing-like lateral projections in *Lithoglyptes*, *Auritoglyptes*, and *Kochlorine* (Fig. S1C) may provide closer proximity with females and more space for long penises, thus increasing mating success. In *Lithoglyptes* and *Kochlorine*, the attachment stalk is further embedded in a deep pit of the female mantle (Kolbasov, 2009). (2) The cuticle of dwarf males in both *Lithoglyptes* and *Kochlorine* is covered by dense, large, and sharp denticles, whereas they are smaller in other lithoglyptid genera. (3) Both *Lithoglyptes* and *Kochlorine* do not have an apertural slit at the posterior end in dwarf males.

4.5. Habitat use of Acrothoracica

Acrothoracican fossils have been recorded from the Devonian to the Pliocene, and they have been found as burrows or casts of burrows (Baird et al., 1990; Newman et al., 1969; Petriconi, 1969; Rodriguez and Gutschick, 1977). All known extinct acrothoracicans were identified as Lithoglyptida. As an indication of habitat use, the burrows were found in a variety of biotic and abiotic calcareous substrates and a trend of colonizing thicker substrates was hypothesized (Tomlinson, 1969).

In this study, the external surface of calcareous molluscan (including chiton) and barnacle shells; skeletons of dead corals; chalk; calcite rocks, conglomerates, and clays; and bryozoans were estimated as the habitats of ancestral acrothoracicans (Fig. 1A–C), and are also the most common habitats of the current living acrothoracicans (Batham and Tomlinson, 1965; Grygier and Newman, 1985; Kolbasov, 2009; Tomlinson, 1969). The decedents further colonized the central columella or wall of gastropod shells in Trypetesidae (Fig. 1F) and the skeletons of live corals in *Berndtia* (Fig. 1D and E). *Trypetesa* is specialized to live mostly on the internal columella or sometimes the inner surface wall of gastropod shells occupied by hermit crabs (Tomlinson, 1969). This symbiotic relationship was recognized to occur as early as the Miocene and may have developed for providing a refuge for the ancestral endolithic mode of life (Baluk and Radwanski, 1967). The ancestors of *Berndtia* further localized in the skeletons of live corals, and species of this genus were exclusively observed in two coral genera, *Leptastrea* and *Psammocora* (Chan et al., 2014a; Utinomi, 1957). Coral polyps hide and defend individuals of *Berndtia* from predators (Utinomi, 1957).

The present study is the first to examine the molecular phylogeny of the whole superorder Acrothoracica. Results support the new taxonomic classification of the two orders proposed by

Kolbasov, Newman, and Høeg (in Kolbasov, 2009). However, because of sampling limitations, the species in the order Cryptophialida was represented by only one genus, *Cryptophialus*. Additional studies should examine the phylogeny within Cryptophialida with the inclusion of *Australophialus*, which has a highly relict distribution, first recognized in the southern hemisphere (Newman and Ross, 1971) and later observed to be amphitropical (Turquier, 1985).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.03.016.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723.
Baird, G.C., Brett, C.E., Tomlinson, J.T., 1990. Host-specific acrothoracid barnacles on Middle Devonian platyceratid gastropods. Hist. Biol. 4, 221–244.
Baluk, W., Radwanski, A., 1967. Miocene cirripeds domiciled in corals. Acta Palaeontol. Pol. 12, 457–513.
Batham, E.J., Tomlinson, J.T., 1965. On Cryptophialus melampygos Berndt, a small boring barnacle of the order Acrothoracica in some New Zealand molluscs. Trans. R. Soc. N.Z. 7, 141–154.
Berndt, W., 1907. Über das system der Acrothoracica. Archiv für Naturgeschichte 73, 287–289.
Brandley, M., Schmitz, A., Reeder, T., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54, 373–390.
Chan, B.K.K., Kolbasov, G.A., Cheang, C.C., 2012. Cryptic diversity of acrothoracican barnacle *Armatoglyptes taiwanus* in the Indo-Pacific waters, with description of a new species from the Mozambique Channel collected from the French MAINBAZA cruise. Zoosystema 34 (1), 5–20.
Chan, B.K.K., Cheang, C.C., Chen, I.-H., Kolbasov, G.A., 2013. Acrothorthoracican barnacles (Litoglyptida) in Taiwan, including the taxonomic status of *Balanodytes taiwanus* Utinomi, 1950 and cryptic diversity of *Auritoglyptes bicornis* (Aurivillius, 1892). Zootaxa 3694 (3), 221–239.
Chan, B.K.K., Kolbasov, G.A., Hirose, M., Mezaki, T., Suwae, R., 2014a. Biodiversity and biogeography of the coral boring barnacles of the genus *Berndtia* (Cirripedia: Acrothoracica) in the West Pacific, with description of three new species. J. Nat. Hist. 48 (25–26), 1503–1541.
Chan, B.K.K., Hsieh, W.P., Kolbasov, G.A., 2014b. Crustacean fauna of Taiwan: Barnacles, Vol III – Cirripedia: Acrothoracica. Biodiversity Research Center. Academia Sinica Press, 107 pp.
Chen, Y.-Y., Lin, H.-C., Chan, B.K.K., 2012. Description of a new species of coral-inhabiting barnacle, *Darwiniella angularis* sp. n. (Cirripedia, Pyrgomatidae) from Taiwan. ZooKeys 214, 43–74.
Crandall, K.A., Fitzpatrick Jr., J.F., 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. Syst. Biol. 45, 1–26.
Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust. J. Zool. 46, 419–437.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.
Darwin, C.R., 1854. A monograph on the sub-class Cirripedia, with figures of all the species. The Balanidae (or sessile cirripedes); the Verrucidae, etc., etc., etc. Ray Society, London, 684 pp. + pls. 1–30.
Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Drummond, A., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., et al., 2011. Geneious v5.4.—check reference.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
Grygier, M.J., Newman, W.A., 1985. Motility and calcareous parts in extant and fossil Acrothoracica (Crustacea: Cirripedia), based primarily upon new species burrowing in the deep-sea coral Enallopsammia. Trans. San Diego Soc. Nat. Hist. 21, 1–22.
Hancock, A., 1849. Notice of the occurrence on the British coast of a burrowing barnacle belonging to a new order of the class Cirripedia. Ann. Mag. Nat. Hist. 4, 305–314.
Høeg, J.T., 1992. The phylogenetic position of the Rhizocephala: are they truly barnacles? Acta Zool. 73, 323–326.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.
Kolbasov, G.A., 2002. Cuticular structures of some acrothoracican dwarf males (Crustacea: Thecostraca: Cirripedia: Acrothoracica). Zool. Anz. 241, 85–94.
Kolbasov, G.A., Høeg, J.T., Elfimov, A.S., 1999. Scanning electron microscopy of acrothoracican cypris larvae (Crustacea, Thecostraca, Cirripedia, Acrothoracica, Lithoglyptidae). Contrib. Zool. 68, 143–160.
Kolbasov, G.A., 2009. Acrothoracica, Burrowing Crustaceans. KMK Scientific Press Ltd., Moscow, p. 452.
Kolbasov, G.A., Newman, W.A., 2005. Revision of the Lithoglyptidae sensu Tomlinson, 1969 and *Lithoglyptes* Aurivillius, 1892 (Cirripedia, Acrothoracica), including a new species from Bermuda. Zootaxa 1013, 35–64.
Kolbasov, G.A., Høeg, J.T., 2007. Cypris larvae of acrothoracican barnacles (Thecostraca: Cirripedia: Acrothoracica). Zool. Anz. 246, 127–151.
Kolbasov, G.A., Chan, B.K.K., Høeg, J.T., 2014. Acrothoracica. In: Martin, J.W., Olesen, J., Høeg, J.T. (Eds.), Atlas of Crustacean Larvae. John Hopkins Press, pp. 107–110.
Larsen, S.K., Høeg, J.T., Yusa, Y., 2016. Host relation, size and reproduction in the burrowing barnacle Trypetesa lampas (Hancock) (Crustacea Cirripedia Acrothoracica). Zool. Stud. 55.
Leaché, A.D., Reeder, T.W., 2002. Molecular systematics of the eastern Fence Lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. Syst. Biol. 51, 44–68.
Lin, H.C., Høeg, J.T., Yusa, Y., Chan, B.K.K., 2015. The origins and evolution of dwarf males and habitat use in thoracican barnacles. Mol. Phylogenet. Evol. 91, 1–11. http://dx.doi.org/10.1016/j.ympev.04.026.
Maddison, W.P., Maddison, D.R., 2011. Mesquite: a modular system for evolutionary analysis. Version 3.04 <http://mesquiteproject.org>.
Martin, J.W., Davis, G.E., 2001. An updated Classification of the Recent Crustacea. Natural History Museum of Los Angeles County, Los Angeles, California.
Miller, M.A., Pfeiffer, W., Schwartz, T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, pp. 1–8, http://dx.doi.org/10.1109/GCE.2010.5676129.
Murphy, A.E., Williams, J.D., 2013. New records of two trypetesid burrowing barnacles (Crustacea: Cirripedia: Acrothoracica: Trypetesidae) and their predation on host hermit crab eggs. J. Mar. Biol. Assoc. U. K. 93, 107–133.
Newman, W.A., 1971. A deep-sea burrowing barnacle (Cirripedia: Acrothoracica) from Bermuda. J. Zool. Lond. 165, 423–429.
Newman, W.A., 1974. Two new deep-sea Cirripedia (Ascothoracica and Acrothoracica) from the Atlantic. J. Mar. Biol. Assoc. U.K. 54, 437–456.
Newman, W.A., 1993. Darwin and cirripedology. In: Truesdale, J. (Ed.), The History of Carcinology. Crustacean Issues 8, 349–434 (Balkema, Rotterdam).
Newman, W.A., Zullo, V.A., Withers, T.H., 1969. Cirripedia. Treatiese in Invertebrate Paleontology. Pt. R. Arthropoda. 4, Vol. 1. Geological Society of America, pp. 206–295.
Newman, W.A., Ross, A., 1971. Antarctic Cirripedia. Antarctic Res. Ser. 14, 1–257.
Newman, W.A., Ross, A., 1976. Revision of the Balanomorph barnacles; including a catalog of the species. San Diego Soc. Nat. Hist. Mem. 9, 1–108.
Pérez-Losada, M., Høeg, J.T., Crandall, K.A., 2009. Remarkable convergent evolution in specialized parasitic Thecostraca (Crustacea). BMC Biol. 7, 15.
Pagel, M., 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc. R. Soc. Lond. B Biol. Sci. 255, 37–45.
Pagel, M., 1997. Inferring evolutionary processes from phylogenies. Zool. Scr. 26, 331–348.
Pagel, M., 1999. Inferring the historical patterns of biological evolution. Nature 401, 877.
Pagel, M., Meade, A., 2006. Bayesian analysis of correlated evolution of discrete characters by Reversible-Jump Markov Chain Monte Carlo. Am. Nat. 167, 808–825.
Pagel, M., Meade, A., Barker, D., 2004. Bayesian estimation of ancestral character states on phylogenies. Syst. Biol. 53, 673–684.
Petriconi, V., 1969. Vergleichend anatomische Untersuchungen an Rankenfüssern (Crustacea, Cirripedia). I. Die Mundwerkzeuge von Lepas anatifera. Zool. Jb. Abt. Anat. Bd. 86, S67–S83.
Poore, G.C.B., 2012. Superorder Acrothoracica. Australian Faunal Directory. Australian Biological Resources Study, Canberra Available from: <http://www.

environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/mainchecklist> (Accessed date: 6 November 2012).
Rodriguez, J., Gutschick, R.C., 1977. Barnacle borings in live and dead hosts from the Louisiana Limestone (*Famennian*) of Missouri. J. Paleontol. 51, 718–724.
Roman, J., Palumbi, S.R., 2004. A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. Mol. Ecol. 13, 2891–2898.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Schluter, D., Price, T., Mooers, A.Ø., Ludwig, D., 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51, 1699–1711.
Schubart, C.D., Huber, M.G.J., 2006. Genetic comparisons of German populations of the stone crayfish, *Austropotamobius torrentium* (Crustacea: Astacidae). Bulletin Français de la Pêche et de la Pisciculture 380–381, 1019–1028.
Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML Web-servers. Syst. Biol. 75, 758–771.
Stubbings, H.G., 1967. The cirriped fauna of tropical West Africa. Bulletin of the British Museum (Natural History). Zoology 15 (6), 229–319, + pl. 1.
Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Am. Math. Soc. 17, 57–86.
Taylor, P.D., Wilson, M.A., 2003. Palaeoecology and evolution of marine hard substrate communities. Earth-Sci. Rev. 62, 1–103.
Tomlinson, J.T., 1969. The burrowing barnacles: (Cirripedia: order Acrothoracica). Bull. U.S. Natl. Mus. 296, 1–162.
Tomlinson, J.T., 1987. The burrowing barnacles (Acrothoracica). In: Southward, A.J. (Ed.), Barnacle Biology, Crustacean Issues 5. A. A. Balkema, Rotterdam, pp. 63–71.
Turquier, Y., 1985. Cirripedes Acrothoraciques des cotes occidentales de la Mediterranee et de l'Afrique du nord: I. Cryptophialidae. Bull. Soc. Zool. Fr. 110, 151–168.
Utinomi, H., 1950. On another form of Acrothoracica, newly found from Formosa. Mem. Coll. Sci., Univ. Koyoto, Series B 19 (3), 95–100.
Utinomi, H., 1957. Studies on the Cirripedia Acrothoracica I. biology and external morphology of the female of *Berndtia purpurea* Utinomi. Publ. Seto Mar. Biol. Lab. 6, 1–26.
Wiens, J.J., 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47, 568–581.
Williams, J.D., Boyko, C.B., 2006. A new species of *Tomlinsonia* Turquier, 1985 (Crustacea, Cirripedia, Trypetesidae) in hermit crab shells from the Philippines, and a new parasite species of *Hemioniscus* Buchholz, 1866 (Crustacea, Isopoda, Hemioniscidae). Zoosystema 28, 285–305.
Williams, J.D., Gallardo, A., Murphy, A.E., 2011. Crustacean parasites associated with hermit crabs from the western Mediterranean Sea, with first documentation of egg predation by the burrowing barnacle *Trypetesa lampas* (Cirripedia: Acrothoracica: Trypetesidae). Integr. Zool. 6, 13–27.