

# Molecular phylogeny of the lower acorn barnacle families (Bathylasmatidae, Chionelasmatidae, Pachylasmatidae and Waikalasmatidae) (Cirripedia: Balanomorpha) with evidence for revisions in family classification

BENNY KWOK KAN CHAN<sup>1</sup>, LAURE CORBART<sup>2</sup>, PAULA A. RODRIGUEZ MORENO<sup>3</sup> and LING MING TSANG<sup>4\*</sup>

<sup>1</sup>*Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan*

<sup>2</sup>*UMR7205 ISyEB, Muséum National d'Histoire Naturelle MNHN, Dept. Systematique and Evolution, 43, rue Cuvier, CP 26, 75005 Paris, France*

<sup>3</sup>*Direction des Collections – Invertébrés, Muséum National d'Histoire Naturelle MNHN CP 53, 61, rue Buffon, 75231 Paris Cedex 05, France*

<sup>4</sup>*Institute of Marine Biology, National Taiwan Ocean University, Keelung 20224, Taiwan*

Received 10 July 2015; revised 18 August 2016

The evolutionary history of the lower balanomorphan barnacle has remained controversial because of the complexities in shell wall ontogeny and the prevalence of plesiomorphic characteristics. *Catophragmus* Sowerby, 1826, *Eochionelasmus* Yamaguchi, 1990, *Pachylasma* Darwin, 1854 and *Waikalasma* Buckeridge, 1983 have been proposed as potentially the most primitive extant balanomorphans. In this study, we present the first molecular phylogenetic hypothesis on the evolution of lower Balanomorpha Pilsbry, 1916, based on 89 species and six molecular markers (mitochondrial *12S* and *16S*, nuclear *18S* rRNA, histone 3, elongation factor 1 $\alpha$  subunit and RNA polymerase subunit II). Chionelasmatoida Buckeridge, 1983, Chthamaloidea Darwin, 1854 and Pachylasmatoida Utinomi, 1968 intermingled, thus forming the earliest diverged lineage within the monophyletic Balanomorpha in the inferred phylogeny. Five major lineages (corresponding closely to Catophragmidae Utinomi, 1968, Chionelasmatidae Buckeridge, 1983, Chthamalidae Darwin, 1854, Pachylasmatidae Utinomi, 1968 and Waikalasmatidae Ross & Newman, 2001) were identified from this lower clade; however, the phylogenetic relationships amongst the five lineages could not be resolved in the present study. *Pseudoctomeris* Poltarukha, 1996 was previously considered Chthamalidae because it is a shallow-water inhabitant. However, it is nested within Pachylasmatidae in the phylogenetic analysis of the present study, and its opercular plates and rostrum are highly similar to those of *Pachylasma*. Bathylasmatidae Newman & Ross, 1971 and Tetraclitidae Gruvel, 1903 are sister families, whereas *Austrobalanus* Pilsbry, 1916 is highly diverged from the Bathylasmatidae and Tetraclitidae, supporting Buckeridge & Newman's (2010, in A review of the subfamily Elminiinae (Cirripedia: Thoracica: Austrobalanidae) including a new genus, *Protelminius* nov., from the Oligocene of New Zealand. *Zootaxa* 2349: 39–54.) proposal of full family ranking for Austrobalanidae Newman & Ross, 1976. However, *Austrominius* Buckeridge, 1983 and *Epopella* Ross, 1970 inclusions in Austrobalanidae are not supported because they are more closely related to Balanoidea and Tetraclitidae, respectively. On the basis of the molecular phylogenetic analyses in the present study and morphological evidence from previous analyses, a revised family-level classification in Pachylasmatoida and Tetraclitoida is proposed.

**ADDITIONAL KEYWORDS:** molecular phylogeny – *Austrobalanus* – Austrobalanidae – *Waikalasma* – Waikalasmatidae – Chionelasmatoida – Chthamaloidea – Pachylasmatoida – Tetraclitoida.

## INTRODUCTION

Balanomorpha Pilsbry, 1916 (Cirripeda: Sessilia) is composed of all acorn barnacles, exhibits extremely

diverse morphological forms, and is the most species rich group of barnacles. Shells of balanomorph species may be composed of eight, six or four plates or the whole shell may be solid and concrescent (e.g. pyrgomatid coral barnacles). Moreover, the shell bases can be membranous or calcareous. Balanomorph barnacles

\*Corresponding author. E-mail: [lmtsang@mail.ntou.edu.tw](mailto:lmtsang@mail.ntou.edu.tw)

can be found in a great variety of habitats including intertidal, corals, sponges, whales, marine turtles, decapods, deep-sea and hydrothermal vents (Chan & Høeg, 2015). Evolution of Balanomorpha barnacles is, however, poorly understood and there are several controversial hypotheses in balanomorph evolution. In an early review on the origin of Balanomorpha, Newman & Ross (1976) and later Ross & Newman (2001) summarized the evidence and suggested that Catophragmidae Utinomi, 1968 (Chthamaloidea) evolved into balanomorphans. This family possesses many primitive features, including lepadomorph-like trophi and imbricating plate whorls in the shell, which indicate a plesiomorphic character of the stalked barnacle ancestor. These observations are consistent with palaeontological evidence of the oldest known balanomorph fossil belonging to *Catophragmus* Sowerby, 1826. However, the discovery of the waikalasmatid fossil, *Waikalasma juneae* Buckeridge, 1983, provides new and crucial insights into the evolution of the lower balanomorphs (Buckeridge, 1983). In-depth examination of the species and comparison of the wall structure with extant *Chionelasmus* Pilsbry, 1911, suggested that *Waikalasma* Buckeridge, 1983, should be placed at the most basal position of the balanomorph barnacles (Buckeridge & Newman, 1992). Subsequent description of the first-known extant *Waikalasma*, *W. boucheti* Buckeridge, 1996 (pachylasmatidae: Eolasmatinae), revealed detailed characteristics including the presence of two or more whorls of small imbricating plates in *Waikalasma*, initially overlooked in the *W. juneae* Buckeridge, 1983 fossil, and provided further evidence for the phylogenetic position of Waikalasmatidae proposed by Buckeridge & Newman (1992) and Buckeridge (1995).

Newman & Ross (1971) erected the family Bathylasmatidae Newman & Ross, 1976, within Balanomorpha, to accommodate *Bathylasma* Newman & Ross, 1971 and *Hexelasma* Hoek, 1913, which inhabit deep-sea environments. Bathylasmatidae exhibit both balanid and chthamalid barnacle features (Hoek, 1913; Pilsbry, 1916; Bage, 1938; Utinomi, 1967; Newman & Ross, 1976), thus are considered to provide crucial information concerning higher balanomorph evolution. Newman & Ross (1976) grouped Bathylasmatidae and Tetracitidae Gruvel, 1903 under the superfamily Coronuloidea Leach, 1817, and suggested that Bathylasmatidae evolved into Tetracitidae. This proposed relationship was supported by Buckeridge & Newman (2010) and Martin & Davis (2001) who placed Bathylasmatidae and Tetracitidae under the superfamily Tetracitoida Gruvel, 1903. However, Jones (2000, 2007) classified Bathylasmatinae Newman & Ross, 1971 in Pachylasmatoida Utinomi, 1968, under superfamily Pachylasmatoida Utinomi, 1968, which contradicts the proposal by Newman & Ross (1976).

These phylogenetic hypotheses have not yet been tested using a molecular phylogenetic approach.

Pérez-Losada *et al.* (2014) conducted the first molecular phylogenetic analysis (on the basis of sequences of 18S, 28S, 12S, 16S rRNA and COI) of Balanomorpha, including several Pachylasmatoida and Chionelasmatoidea Buckeridge, 1983 exemplars and one Bathylasmatidae exemplar. Unexpectedly, Chthamaloidea Darwin, 1854 was the earliest diverged taxon in Balanomorpha, whereas Chionelasmatoidea and Pachylasmatoida formed an unsupported clade with Catophragmidae Utinomi, 1968 in their gene tree (Pérez-Losada *et al.*, 2014). Both Pérez-Losada *et al.* (2014) and Tsang *et al.* (2015) found that Bathylasmatidae nested within Tetracitoida, which was partially supported by Newman & Ross (1976). These arrangements, if proven, will have major implications for our understanding of balanomorph morphological evolution. However, most nodes amongst the lower clades are poorly supported, and taxon coverage was limited in the work by Pérez-Losada *et al.* (2014). Hence, additional studies are required to verify the proposed hypotheses.

In the present study, we attempted to reconstruct a multilocus-based molecular phylogeny for Balanomorpha to study the phylogenetic relationships among Chionelasmatoidea, Pachylasmatidae, Waikalasmatidae and Bathylasmatidae and other balanomorphans. Specifically, we attempt to examine: (1) whether Catophragmatidae, Chionelasmatoidea or Waikalasmatidae represent the earliest diverged balanomorphans; (2) whether Tetracitoida derived from a *Bathylasma*-like ancestor as suggested by Newman & Ross (1976); and (3) whether Pachylasmatidae and Bathylasmatidae comprise a monophyletic group as suggested by Jones (2000, 2007) or diphyletic assemblages as suggested by Newman & Ross (1976). To answer these questions, we included a broad range of taxa from four families: Chionelasmatoidea, Pachylasmatidae, Bathylasmatidae and Waikalasmatidae. In addition, the evolution of key morphological features was evaluated, and taxonomic revisions were proposed on the basis of the inferred phylogeny.

## MATERIAL AND METHODS

We obtained 49 samples from 30 species of the Chionelasmatoidea, Pachylasmatidae, Waikalasmatidae and Bathylasmatidae families from the Muséum National d'Histoire Naturelle (MNHN). These samples were collected during recent deep-sea MNHN expeditions (Table 1; Bouchet *et al.*, 2008; Richer de Forges *et al.*, 2013) off several localities in the Indo Pacific region, that is New Caledonia (EBISCO, EXBODI, NORFOLK 1 & 2 and SMIB 3 cruises); French Polynesia

**Table 1.** Information of the taxa included in the present study and GenBank accession number of the sequences analysed

Superfamily	Family	Voucher number	Species	Gene						
				12S	16S	18S	EF1	H3	RPII	
Chionelasmatoidea	Chionelasmatidae	MNHN-IU-2013-7438	<i>Chionelasma darwini</i>	KX230850	KX230926	KX230999	KX231081	KX231144	KX231216	
Chionelasmatoidea	Chionelasmatidae	MNHN-IU-2013-19201	<i>C. darwini</i>	KX230851	KX230927	KX231000	KX231082	KX231145	KX231217	
Chionelasmatoidea	Chionelasmatidae	MNHN-IU-2013-19202	<i>C. darwini</i>	KX230852	KX230928	KX231001	KX231118	KX231184	KX231252	
Chionelasmatoidea	Chionelasmatidae	MNHN-IU-2009-4019	<i>C. darwini</i>	KX230853	KX230929	KX231002	Na	KX231185	Na	
Chionelasmatoidea	Waikalasmatidae	MNHN-IU-2013-7721	<i>Waikalasma dianajonesae</i> <sup>^</sup>	KX230876	KX230952	KX231023	KX231100	KX231164	KX231232	
Chionelasmatoidea	Waikalasmatidae	MNHN-IU-2013-17872	<i>W. dianajonesae</i>	KX230877	KX230953	KX231024	KX231101	KX231166	KX231233	
Chionelasmatoidea	Waikalasmatidae	MNHN-IU-2013-7587	<i>Waikalasma boucheti</i>	KX230878	Na	KX231025	KX231084	KX231147	Na	
Chionelasmatoidea	Waikalasmatidae	MNHN-IU-2013-19208	<i>W. boucheti</i>	KX230879	KX230954	KX231026	KX231085	KX231148	KX231219	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7729	<i>Eutomolasma maclaughlinae</i>	KX230854	KX230930	KX231003	KX231102	KX231167	KX231234	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7792	<i>E. maclaughlinae</i>	KX230855	KX230931	KX231004	KX231108	KX231173	KX231241	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-19203	<i>E. maclaughlinae</i>	KX230856	KX230932	KX231005	KX231109	KX231174	KX231242	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7795	<i>E. maclaughlinae</i>	KX230857	KX230933	KX231006	Na	KX231175	KX231243	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7598	<i>Eutomolasma</i> sp.1	KX230858	KX230934	KX231008	KX231086	KX231149	KX231220	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-19204	<i>Eutomolasma</i> sp.1	KX230860	KX230935	KX231009	KX231087	KX231150	KX231221	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7701	<i>Eutomolasma</i> sp.2	KX230862	KX230936	KX231010	KX231097	KX231161	KX231230	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2009-4028	<i>Eurylasma pyramidale</i>	KX230859	KX230937	KX231007	Na	KX231186	Na	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7600	<i>Eurylasma</i> sp.1	KX230861	KX230938	Na	Na	KX231151	Na	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2011-6456	<i>Eurylasma</i> sp.2	KX230863	KX230939	KX231011	KX231077	KX231140	KX231212	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-19205	<i>Eurylasma</i> sp.2	KX230864	KX230940	KX231012	KX231078	KX231141	KX231213	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2011-6673	<i>Eurylasma</i> sp.2	KX230865	KX230941	KX231013	KX231080	KX231143	KX231215	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7736	<i>Pachylasma</i> sp.1	KX230866	KX230942	KX231014	KX231103	KX231168	KX231235	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-19206	<i>Pachylasma</i> sp.1	KX230867	KX230943	KX231015	KX231104	KX231169	KX231236	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2011-4435	<i>Pachylasma</i> sp.2	KX230868	KX230944	KX231016	KX231075	KX231139	KX231210	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7752	<i>Pachylasma</i> sp.2	KX230869	KX230945	KX231017	KX231105	KX231170	KX231237	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7683	<i>Pachylasma bacum</i>	KX230870	KX230946	KX231018	KX231096	KX231160	KX231229	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7706	<i>Pachylasma</i> cf. <i>bacum</i>	KX230871	KX230947	Na	KX231098	KX231162	Na	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-19207	<i>Pachylasma</i> cf. <i>bacum</i>	KX230872	KX230948	KX231019	KX231099	KX231163	KX231231	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7605	<i>Tetrapachylasma arcuatum</i>	KX230873	KX230949	KX231020	KX231089	KX231153	Na	
Pachylasmatoidea	Pachylasmatidae	MNHN-IU-2013-7665	<i>T. arcuatum</i>	KX230874	KX230950	KX231021	Na	KX231157	KX231227	

Table 1. Continued

		Gene							
Superfamily	Family	Voucher number	Species	12S	16S	18S	EF1	H3	RPII
Pachylasmatoidae	Pachylasmatidae	MNHN-IU-2014-12852	<i>Tetrapachylasma</i> sp.	<b>KX230875</b>	<b>KX230951</b>	<b>KX231022</b>	<b>KX231076</b>	Na	<b>KX231211</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-19210	<i>Bathylasma alearum</i>	<b>KX230896</b>	<b>KX230970</b>	<b>KX231040</b>	Na	<b>KX231183</b>	Na
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2011-1980	<i>Bathylasma</i> sp.*	<b>KX230895</b>	<b>KX230969</b>	Na	Na	<b>KX231136</b>	Na
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7602	<i>Hexelasma aureolum</i>	<b>KX230892</b>	<b>KX230966</b>	<b>KX231036</b>	<b>KX231088</b>	<b>KX231152</b>	<b>KX231222</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7786	<i>H. aureolum</i>	<b>KX230893</b>	<b>KX230967</b>	<b>KX231037</b>	<b>KX231107</b>	<b>KX231172</b>	<b>KX231240</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7783	<i>Hexelasma persicum</i>	<b>KX230888</b>	<b>KX230962</b>	<b>KX231033</b>	Na	Na	<b>KX231239</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7652	<i>Hexelasma sandaracum</i>	<b>KX230889</b>	<b>KX230963</b>	<b>KX231034</b>	<b>KX231092</b>	<b>KX231155</b>	<b>KX231225</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-19209	<i>H. sandaracum</i>	<b>KX230890</b>	<b>KX230964</b>	<b>KX231035</b>	<b>KX231093</b>	<b>KX231156</b>	<b>KX231226</b>
Tetraclitoidae	Bathylasmatidae	CEL-BB-102	<i>Hexelasma velutinum</i>	<b>KX230891</b>	<b>KX230965</b>	<b>KX231038</b>	<b>KX231122</b>	<b>KX231190</b>	<b>KX231256</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2014-12853	<i>Hexelasma</i> sp.1	<b>KX230880</b>	<b>KX230955</b>	<b>KX231027</b>	<b>KX231095</b>	<b>KX231159</b>	<b>KX231228</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7651	<i>Hexelasma</i> sp.2	<b>KX230881</b>	<b>KX230956</b>	<b>KX231028</b>	<b>KX231091</b>	<b>KX231154</b>	<b>KX231224</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7779	<i>Hexelasma</i> sp.2	<b>KX230882</b>	<b>KX230957</b>	<b>KX231029</b>	<b>KX231106</b>	<b>KX231171</b>	<b>KX231238</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2011-6519	<i>Hexelasma</i> sp.3	<b>KX230883</b>	<b>KX230958</b>	<b>KX231030</b>	<b>KX231079</b>	<b>KX231142</b>	<b>KX231214</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7605	<i>Hexelasma</i> sp.4	<b>KX230884</b>	Na	<b>KX231031</b>	<b>KX231090</b>	Na	<b>KX231223</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2011-2762	<i>Hexelasma</i> sp.5	<b>KX230885</b>	<b>KX230959</b>	Na	Na	<b>KX231137</b>	<b>KX231208</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7559	<i>Hexelasma</i> sp.5	<b>KX230886</b>	<b>KX230960</b>	<b>KX231032</b>	<b>KX231083</b>	<b>KX231146</b>	<b>KX231218</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2013-7666	<i>Hexelasma</i> sp.6	<b>KX230887</b>	<b>KX230961</b>	Na	<b>KX231094</b>	<b>KX231158</b>	Na
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2011-2772	<i>Mesolasma</i> sp.1	<b>KX230894</b>	<b>KX230968</b>	<b>KX231039</b>	<b>KX231074</b>	<b>KX231138</b>	<b>KX231209</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2011-1904	<i>Mesolasma</i> sp.2	<b>KX230897</b>	Na	<b>KX231041</b>	<b>KX231073</b>	Na	<b>KX231207</b>
Tetraclitoidae	Bathylasmatidae	MNHN-IU-2014-12854	<i>Mesolasma</i> sp.3	<b>KX230898</b>	<b>KX230971</b>	Na	Na	<b>KX231165</b>	Na
Balanoidea	Archaeobalanidae	MSL-AR03-acsul	<i>Acasta sulcata</i>	<b>KX230899</b>	<b>KX230972</b>	<b>KX231044</b>	<b>KX231110</b>	<b>KX231176</b>	<b>KX231244</b>
Balanoidea	Archaeobalanidae	MSL-BA13-emod	<i>Eliminus modestus</i>	KF776184	KF776233	<b>KX231042</b>	KF776282	KF776331	KF776383
Balanoidea	Archaeobalanidae	MSL-AR01-sama	<i>Striatobalanus amaryllus</i>	KF776186	KF776235	<b>KX231043</b>	KF776285	KF776334	KF776386
Balanoidea	Archaeobalanidae	MSL-BA10-sbal	<i>Semibalanus balanoides</i>	<b>KX230900</b>	<b>KX230974</b>	DQ777622	AF063404	<b>KX231196</b>	<b>KX231261</b>
Balanoidea	Balanidae	MSL-Amamp	<i>Amphibalanus amphitrite</i>	KF776181	KF776230	<b>KX231045</b>	KF776279	KF776328	KF776380
Balanoidea	Balanidae	CEL-BA12-bgral	<i>Balanus glandula</i>	KF776183	KF776232	AF201663	KF776281	KF776330	KF776382
Balanoidea	Balanidae	MSL-BA11-btri	<i>Balanus trigonus</i>	<b>KX230901</b>	<b>KX230973</b>	<b>KX231046</b>	<b>KX231111</b>	n.a.	<b>KX231245</b>

Table 1. Continued

		Gene							
Superfamily	Family	Voucher number	Species	12S	16S	18S	EF1	H3	RPII
Balanoidea	Balanidae	MSL-Mevol	<i>Megabalanus volcano</i>	NC006293	NC006293	<b>KX231048</b>	KF776284	KF776333	KF776385
Balanoidea	Pyrgomatidae	CEL-TI6_3	<i>Cantellius secundus</i>	KF776153	KF776202	<b>KX231047</b>	KF776251	KF776301	KF776352
Balanoidea	Pyrgomatidae	CEL-SU46_1	<i>Cantellius transversalis</i>	KF776154	KF776203	<b>KX231049</b>	KF776252	KF776302	KF776353
Balanoidea	Pyrgomatidae	CEL-TI1_4	<i>Darwinella angularis</i>	KF776155	KF776204	<b>KX231050</b>	KF776253	KF776303	KF776354
Balanoidea	Pyrgomatidae	CEL-KT78_4	<i>Pyrgoma cancellatum</i>	KF776168	KF776217	<b>KX231051</b>	KF776266	KF776316	KF776367
Balanoidea	Pyrgomatidae	CEL-KT102_1	<i>Savignium rossi</i>	KF776170	KF776219	<b>KX231052</b>	KF776268	KF776318	KF776369
Balanoidea	Pyrgomatidae	CEL-RYU166_1	<i>Trevathana dentata</i>	KF776177	KF776226	<b>KX231053</b>	KF776275	KF776325	KF776376
Chthamaloidea	Catophragmidae	CEL-Cata1	<i>Catophragmus imbricatus</i>	<b>KX230902</b>	<b>KX230975</b>	JX083887	<b>KX231112</b>	<b>KX231177</b>	<b>KX231246</b>
Chthamaloidea	Catophragmidae	GenBank	<i>Catomerus polymerus</i>	AY520682	AY520749	AY520648	Na	AY520716	Na
Chthamaloidea	Chthamalidae	MSL-CH3-Cscu-3	<i>Chinochthamalus scutelliformis</i>	<b>KX230905</b>	<b>KX230978</b>	<b>KX231055</b>	<b>KX231115</b>	<b>KX231180</b>	<b>KX231249</b>
Chthamaloidea	Chthamalidae	MSL-Cdal1	<i>Chthamalus dalli</i>	<b>KX230903</b>	<b>KX230976</b>	<b>KX231054</b>	<b>KX231113</b>	<b>KX231178</b>	<b>KX231247</b>
Chthamaloidea	Chthamalidae	MSL-CH13-Cpro3	<i>Chthamalus proteus</i>	<b>KX230904</b>	<b>KX230977</b>	JX083899	<b>KX231114</b>	<b>KX231179</b>	<b>KX231248</b>
Chthamaloidea	Chthamalidae	MSL-CH10-Cste1	<i>Chthamalus stellatus</i>	<b>KX230906</b>	<b>KX230979</b>	AY520641	<b>KX231116</b>	<b>KX231181</b>	<b>KX231250</b>
Chthamaloidea	Chthamalidae	MSL-CH9-Ecau	<i>Caudocuraphia caudata</i>	<b>KX230907</b>	<b>KX230980</b>	JX083903	<b>KX231119</b>	<b>KX231187</b>	<b>KX231253</b>
Chthamaloidea	Chthamalidae	MSL-CH14-Ehem	<i>Euraphia hembeli</i>	<b>KX230908</b>	<b>KX230981</b>	<b>KX231059</b>	<b>KX231120</b>	<b>KX231188</b>	<b>KX231254</b>
Chthamaloidea	Chthamalidae	MSL-CH2-Nsca	<i>Notochthamalus scabrosus</i>	<b>KX230909</b>	<b>KX230983</b>	<b>KX231060</b>	Na	<b>KX231192</b>	<b>KX231258</b>
Chthamaloidea	Chthamalidae	MSL-CH6-Octsp	<i>Octomeris</i> sp.	<b>KX230911</b>	<b>KX230984</b>	<b>KX231061</b>	<b>KX231124</b>	<b>KX231193</b>	Na
Chthamaloidea	Chthamalidae	MSL-CH1-Psul	<i>Pseudooctomeris sulcata</i> <sup>#</sup>	<b>KX230912</b>	<b>KX230985</b>	<b>KX231063</b>	<b>KX231125</b>	<b>KX231194</b>	<b>KX231259</b>
Coronuloidea	Chelonibiidae	CEL-BB-45	<i>Chelonibia testudinaria</i>	<b>KX230910</b>	<b>KX230982</b>	<b>KX231062</b>	<b>KX231117</b>	<b>KX231182</b>	<b>KX231251</b>
Tetracitoidea	Tetracitidae	GenBank	<i>Austrobalanus imperator</i>	AB723876	AB723894	AB723912	Na	AB723948	Na

Table 1. Continued

Superfamily	Family	Voucher number	Species	Gene						RPII
				12S	16S	18S	EFI	H3		
Tetraclitoidea	Tetraclitidae	CEL-Tetra-13-1	<i>Epopella plicata</i>	KX230913	KX230986	KX231064	KX231121	KX231189	KX231255	
Tetraclitoidea	Tetraclitidae	CEL-Tetra-9	<i>Tesseropora rosea</i>	KX230915	KX230988	KX231066	KX231131	KX231202	KX231267	
Tetraclitoidea	Tetraclitidae	CEL-Tetra-1-1	<i>Tetraclita achituvii</i>	KX230914	KX230987	KX231065	KX231126	KX231197	KX231262	
Tetraclitoidea	Tetraclitidae	CEL-BB-118	<i>Tetraclita japonica</i>	KX230916	KX230989	KX231067	KX231128	KX231199	KX231264	
Tetraclitoidea	Tetraclitidae	CEL-Tetra-3	<i>Tetraclita rufotincta</i>	KX230917	KX230990	KX231068	KX231132	KX231203	KX231268	
Tetraclitoidea	Tetraclitidae	CEL-Tetra-5	<i>Tetraclita singaporensis</i>	KX230918	KX230991	KX231069	KX231133	KX231204	KX231269	
Tetraclitoidea	Tetraclitidae	CEL-BB-117	<i>Tetraclita squamosa</i>	KX230922	KX230994	KX231072	KX231134	KX231205	KX231270	
Tetraclitoidea	Tetraclitidae	CEL-BB-6A	<i>Tetraclitella divisa</i>	KX230919	KX230992	KX231070	KX231127	KX231198	KX231263	
Tetraclitoidea	Tetraclitidae	MSL-TpilHK1	<i>Tetraclitella pilsbryi</i>	KX230920	KX230993	KX231071	KX231129	KX231200	KX231265	
Tetraclitoidea	Tetraclitidae	Unvoucher	<i>Tetraclitella purpurascens</i>	KX230921	KX230997	AY520638	KX231130	KX231201	KX231266	
Verrucomorpha (suborder)	Verrucidae	CEL_Mdef4	<i>Metaverruca defayae</i>	KX230923	KX230995	KX231056	KX231123	KX231191	KX231257	
Verrucomorpha (suborder)	Verrucidae	CEL_Rint3	<i>Rostratoverruca intexta</i>	KX230924	KX230996	KX231057	Na	KX231195	KX231260	
Verrucomorpha (suborder)	Verrucidae	CEL_Vstr3	<i>Verruca stroemia</i>	KX230925	KX230998	KX231058	KX231135	KX231206	KX231271	

Note: Na = sequence not available. New sequences obtained in the present study are shown in bold.

\**Bathylasma* sp. shared morphological features of both *Bathylasma* and *Hexelasma*. It has membranous bases and its shell lacks chitinous tubes which are characteristics of *Bathylasma*. However, the basal margin of scutum is concave, which is often seen in *Hexelasma*. In this study, we identified this species as *Bathylasma*, yet further detailed morphological examination is needed to confirm its taxonomic status.

^ *Waikatalasma dianaionesae* is a new species recently described in Chan et al. (2016).

#*Pseudotoomeris sulcata* is assigned to the family Pachylasmatidae in the discussion of the present study, after the present molecular analysis

(TARASOC cruise), Tonga (BORDAU 2 cruise), Fiji (BORDAU 1, MUSORSTOM 10 cruises), Solomon archipelago (SALOMON 1 & 2 cruises), Vanuatu (MUSORSOTOM 8, SANTO expeditions), Papua New Guinea (BIOPAPUA cruise) and Madagascar (ATIMO VATAE expedition). These cruises are part of a long-term research programme: Tropical Deep-Sea Benthos (TDSB) programme (details of the cruises are available at <http://expeditions.mnhn.fr/program/tropicaldeep-seabenthos>).

Exemplars from all of the selected balanomorphan families, except two (11 out of the 13 families, except Coronulidae and Platylepadidae of the superfamily Coronuloidea) were included to determine the phylogenetic position of the four target families. Sequences were obtained from previous studies (Pérez-Losada *et al.*, 2008, 2014; Tsang *et al.*, 2014, 2015) or generated in the present study (Table 1). Furthermore, three species from Verrucumorpha, the sister group of Balanomorpha (Newman & Ross, 1976; Pérez-Losada *et al.*, 2008, 2014) were analysed for distant outgroup comparison. The new samples were collected in the field and stored in ethanol ( $\geq 75\%$ ) before laboratory analysis.

#### LABORATORY PROTOCOL AND PHYLOGENETIC ANALYSES

Total genomic DNA was extracted from the adductor or abdominal muscle tissue using the commercial QIAamp Tissue Kit (QIAGEN). Six molecular markers were analysed, namely the mitochondrial *12S* and *16S rRNA* genes, nuclear *18S rRNA* genes, the *nuclear elongation factor 1 $\alpha$  subunit (EF1)*, *RNA polymerase subunit II (RPII)* and *histone 3 (H3)*. These markers have been widely applied in barnacle phylogenetic analyses (Pérez-Losada *et al.*, 2008, 2014; Tsang *et al.*, 2014, 2015) and are informative at different genetic divergence levels. Polymerase chain reaction (PCR) profiles and primers for the six loci were designed as described previously (Colgan *et al.*, 1998; Whiting, 2002; Tsang *et al.*, 2009, 2014, 2015) and listed in Table 2. Successful amplicons were then purified using the QIAquick gel purification kit (QIAGEN) or QIAquick PCR purification kit (QIAGEN) according to the manufacturer's instructions. Sequencing reactions were performed using the same sets of primers and the ABI Big-dye Ready-Reaction mix kit according to the standard cycle sequencing protocol on an ABI3700 automated sequencer.

**Table 2.** Primer sequences used for PCR amplification, annealing temperature and their sources

Primer	Direction	Sequence (5' to 3')	Annealing temperature (°C)	Sources
<i>12S</i>			50	
FB	Forward	GTGCCAGCAGCTGCGGTTA		Tsang <i>et al.</i> (2009)
R2	Reverse	CCTACTTTGTTACGACTTATCTC		Tsang <i>et al.</i> (2009)
<i>16S</i>			50–55	
Val-F	Forward	CTGTTTTAGCATTTTCATTTACACTG		Tsang <i>et al.</i> (2009)
16S-CR	Forward	TTACGGTACCTTTTGTATTAG		Tsang <i>et al.</i> (2014)
16S-SR	Reverse	CCGGTCTGAACTCAAATCGTG		Tsang <i>et al.</i> (2009)
1472	Reverse	AGATAGAAACCAACCTGG		Crandall & Fitzpatrick (1996)
<i>18S</i>			53–56	
18S 1.2F	Forward	TGCTTGTCTCAAAGATTAAGC		Whiting (2002)
18S ai	Forward	CCTGAGAAAACGGCTACCACATC		Whiting (2002)
18S 7R	Reverse	GCATCACAGACCTGTTATTGC		Whiting (2002)
18S 9R	Reverse	GATCCTTCCGCAGGTTACCTAC		Whiting (2002)
<i>EF1</i>			57–60	
EF1-for	Forward	GATTTTCATCAAGAACATGATCAC		Tsang <i>et al.</i> (2014)
EF1-rev	Reverse	AGCGGGGGGAAGTCGGTGAA		Tsang <i>et al.</i> (2014)
<i>H3</i>			55	
AF	Forward	ATGGCTCGTACCAAGCAGACVGC		Colgan <i>et al.</i> (1998)
AR	Reverse	ATATCCTTRGGCATRATRGTGAC		Colgan <i>et al.</i> (1998)
<i>RP</i>			57–60	
RP-for1	Forward	CACAAGATGAGTATGATGGG		Tsang <i>et al.</i> (2014)
RP-for4	Forward	GAYTTTGACGGCGAYGAGATGAA		Tsang <i>et al.</i> (2014)
RP-rev1	Reverse	CGTGCCGTCGTAGTTGACCAT		Tsang <i>et al.</i> (2014)
RP-rev4	Reverse	GAGACCCTCRCGRCCWCCCAT		Tsang <i>et al.</i> (2014)

Sequences were aligned with MUSCLE (Edgar, 2004), in which the default parameter settings were applied, and the results were checked manually. Poorly aligned regions were removed from the dataset. The sequences from the six molecular markers were first individually analysed using maximum likelihood (ML) analyses to determine any conflict amongst the gene trees. The sequences were subsequently concatenated and partitioned by gene in the final analyses. The best-fit models for nucleotide substitution were determined using jModelTest 2.1 (Darriba *et al.*, 2012) for individual genes (partitions). The ML analysis was implemented using RAxML 8.0.2 (Stamatakis, 2014). The GTRGAMMAI model was used for all six partitions. The gamma distribution with individual shape parameters, GTR rates and base frequencies were estimated and optimized for each partition during the analyses. We performed 1000 bootstrap (BP) runs and searched for the ML tree with the highest score. Bayesian inference (BI) analysis was conducted using MrBayes v.3.2.1 (Ronquist *et al.*, 2012) with two independent runs performed using four differentially heated Metropolis-coupled Markov chain Monte Carlo computations for 10 million generations that started from a random tree. Model parameters were estimated during the analysis, and chains were sampled every 1000 generations. Convergence of the analyses was validated by the standard deviation of split frequencies reaching  $< 0.01$  and by graphically monitoring the likelihood values over time using Tracer v1.5 (Rambaut & Drummond, 2009). The trees created before stable log likelihood values (5000 trees) were discarded as burn-in. A 50% majority-rule consensus tree was constructed from the remaining trees to estimate posterior probabilities (PP).

Alternative a priori phylogenetic hypotheses from previous morphological analyses were statistically tested using the likelihood-based approximately unbiased (AU) test (Shimodaira, 2002). We tested for the monophyly of the superfamilies and families proposed by Newman & Ross (1976), Buckeridge (1983), Jones (2000) and Buckeridge & Newman (2010). The null hypothesis for all topology testing was that no difference existed between trees in the AU test. Alternative tree topologies were constructed using RAxML by setting constraints on taxa monophyly according to the a priori hypotheses. The per-site log likelihood values of individual sites for the trees were estimated using the same programme and subsequently the confidence values of the tree topologies were calculated using CONSEL (Shimodaira & Hasegawa, 2001) with 1000 BP replicates to access the *P*-values of the testing topology.

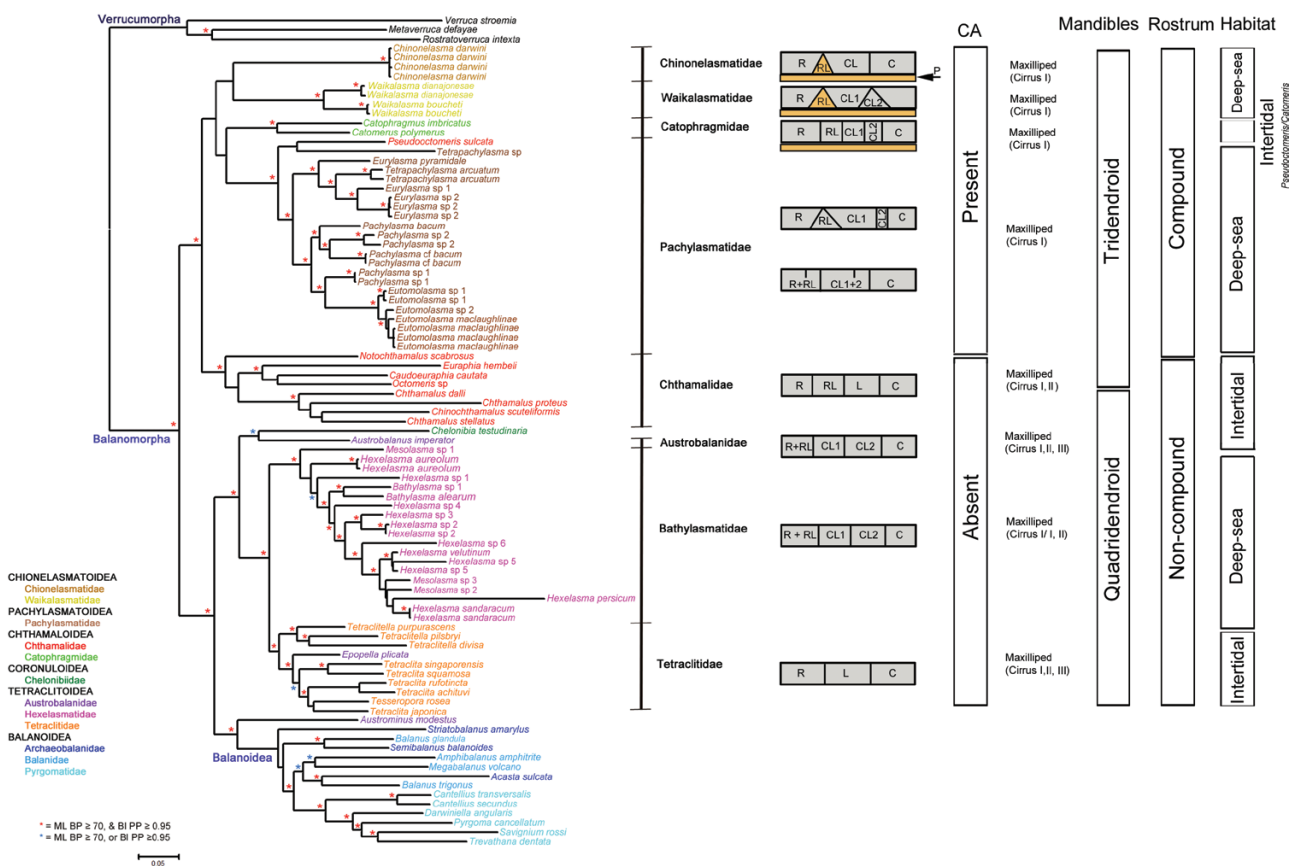
## RESULTS

In this study, 424 new sequences were generated and deposited in GenBank under the accession numbers KX230850–KX231271. The aligned sequences for *12S*, *16S*, *18S*, *EF1*, *H3* and *RPII* measured 411, 930, 1592, 894, 294 and 948 bp in length, respectively. None of the conflicting nodes were strongly supported in ML (BP  $\geq 70$ ) analyses of individual genes (Figs S1–S6). Therefore, we concatenated the sequences from the six genes and analysed the combined data set (5072 bp in total). The alignment is deposited as supplementary material. The ML and BI analyses resulted in largely consistent topologies, with the exception of the relationships amongst chionelasmatooid, chthamalooid and pachylasmatooid families (Figs 1, S 7). Both the ML and BI topologies strongly supported a Chionelasmatoidea, Chthamaloidea and Pachylasmatoidea clade (ML BP = 94; BI PP = 1.00). However, Chionelasmatidae, Pachylasmatidae and Wakailasmatidae formed a clade with Catophragmatidae and *Pseudoctomeris* in the ML topology, and this clade was the sister taxon of Chthamalidae (Fig. 1). Conversely, the BI topology suggested that Chionelasmatidae, Catophragmatidae and Waikalasmatidae were more closely related to Chthamalidae, whereas Pachylasmatidae + *Pseudoctomeris* Poltarukha, 1996, were the most early diverged taxa amongst them (Fig. S1). However, these conflicting nodes in the phylogenetic tree received low statistical support. Therefore, we presented the statistical evidence from the ML and BI analyses for the best ML topology (Fig. 1).

A monophyletic Balanomorpha was recovered in our molecular phylogeny. However, only Balanoidea Leach, 1817 was monophyletic according to the multiple exemplars analysed in the present study. Chthamaloidea, Pachylasmatoidea and Tetracitoidea were poly- or paraphyletic in our topology (Fig. 1). Members of Chionelasmatoidea, Chthamaloidea and Pachylasmatoidea intermingled and formed the earliest diverged lineage within Balanomorpha. The result rejected the monophyly of Chthamaloidea ( $P < 0.001$ ) and Pachylasmatoidea ( $P = 0.003$ ) on the basis of the AU test results. The Chionelasmatoidea, Chthamaloidea and Pachylasmatoidea clade comprised five well-supported lineages: Catophragmatidae, Chionelasmatidae, Chthamalidae (except for *Pseudoctomeris*), Pachylasmatidae + *Pseudoctomeris* and Waikalasmatidae. The phylogenetic relationships amongst these five lineages could not be resolved in the present gene tree. The chthamalid *Pseudoctomeris* nested within Pachylasmatidae and hence Chthamalidae and Pachylasmatidae were poly- and paraphyletic. The reciprocal monophyly of these two families was rejected by the AU test results ( $P < 0.001$  and  $P = 0.003$ , respectively).

The inferred phylogeny (Fig. 1) showed that Tetracitoidea or Coronuloidea was the sister group of





**Figure 1.** The phylogeny of combined mitochondrial *12S* and *16S*, and nuclear *EF1*, *H3*, *RPII* and *18S* gene sequences according to the maximum likelihood (ML) analysis. Nodal supports are denoted on the corresponding branches for a bootstrap value >70% for ML or posterior probability >0.95 for Bayesian analysis. The colour of the taxon names indicates that the classification is based on Buckeridge (1983) and Jones (2000). The family names to the right denote the revised familial classification proposed. The box to the right shows the morphological variations amongst families. Note the presence of primordial plates around the shells (p) of Catophragmidae, Waikalasmatidae and Chionelasmatidae. The Rostral lateral plates (RL) of Chionelasmatidae and Waikalasmatidae arise from the primordial plate and the summit of RL does not reach the sheath. In *Pachylasma*, the RL does not enter the sheath in the inner shell. In Catophragmidae and Chthamalidae, the RL enters the sheath in the inner shell. Caudal appendages are present in Chionelasmatidae, Waikalasmatidae, Catophragmidae and Pachylasmatidae, but absent in Chthamalidae, Austrobalanidae, Bathylasmatidae and Tetracitidae. In Chionelasmatidae, Waikalasmatidae, Catophragmidae and Pachylasmatidae, only the cirrus I is maxillipede.

Balanoidea + *Austrominius modestus*. Austrobalanidae Newman & Ross, 1976, was a polyphyletic assemblage. The austrobalanid *Austrobalanus* Pilsbry, 1916, was more closely related to the coronuloid *Chelonibia* Leach, 1817, than to other tetracitoids, although this arrangement of *Austrobalanus* was strongly supported only in the ML analysis. *Austrominius* Buckeridge, 1983, was strongly supported as sister to all Balanoidea taxa. *Epopella* Ross, 1970 was nested within Tetracitidae, thus making it a paraphyletic group. An alternative hypothesis of the monophyletic origin of Austrobalanidae ( $P < 0.001$ ) was rejected on the basis of the AU test; however, the monophyly of Tetracitoidea ( $P = 0.314$ ) or Tetracitidae ( $P = 0.182$ ) could not be rejected. Tetracitidae + *Epopella* and Bathylasmatidae formed reciprocally monophyletic assemblages.

At the generic level, monophyly of the majority of the Bathylasmatidae and Pachylasmatidae genera analysed was not supported. *Bathylasma* intermingled with *Hexelasma* species in Bathylasmatidae, whereas monophyly of only one out of the four genera (*Etomolasma* Jones, 2000, out of *Eurylasma* Jones, 2000, *Pachylasma* Darwin, 1854 and *Tetrapachylasma* Foster, 1988) from Pachylasmatidae was concordant.

## DISCUSSION

### PHYLOGENETIC RELATIONSHIPS AMONGST EARLY BALANOMORPHAN OFFSHOOTS

The evolutionary history of the lower balanomorphans has remained controversial because of the

complexities in the shell wall ontogeny and prevalence of plesiomorphic characteristics such that evolution polarity is difficult to determine. *Catophragmus*, *Eochionelasmus* Yamaguchi, 1990, *Pachylasma* and *Waikalasma* are considered the most primitive extant balanomorphans (Newman & Ross, 1976; Yamaguchi & Newman, 1990; Buckeridge & Newman, 1992; Buckeridge, 1995; Jones, 2000). However, no comprehensive cladistic or molecular analysis has been conducted to systematically address these hypotheses. In this study, we present the first molecular phylogenetic hypothesis on lower Balanomorpha evolution that is based on extensive taxon and gene sampling. Although the relationships amongst chionelasmatooids, chthamaloids and pachylasmatooids remain to be examined, our molecular phylogenetic analysis clearly suggests that the more derived balanomorphans have diverged from the extant chthamaloids or pachylasmatooids at the early stages of barnacle evolution. Subsequently, various morphological features have undergone complex evolutionary changes in different lineages (e.g. fusion of shell plates, loss of imbricating plate whorls, and modification of trophi). Accordingly, previous phylogenetic analyses based on morphological features have led to mixed conclusions because neither extant chthamaloids nor pachylasmatooids represent the stem group of balanomorphans.

The five major lineages recovered within the Chionelasmatoidea, Chthamaloidea and Pachylasmatoidea clade largely correspond to Catophragmatidae, Chionelasmatidae, Chthamalidae, Pachylasmatidae and Waikalasmatidae (Jones, 2000; Ross & Newman, 2001). However, on the basis of our molecular phylogenetic analysis, several taxa must be realigned to retain the natural family assemblages. We propose a revised familial classification on the basis of molecular evidence and morphological features. The phylogenetic relationships amongst the five clades could not be resolved in the current study; therefore, the superfamily status could not be thoroughly examined, and the families were tentatively retained in their original superfamilies until further examination. Families belonging to Chionelasmatoidea follow the classification of Jones (2000) (except for Bathylasmatidae) and Ross & Newman (2001), whereas those belonging to Chthamaloidea follow the classification of Buckeridge (1983) and Poltarukha (1996) (except for *Pseudoctomeris sulcata*).

#### PHYLOGENETIC RELATIONSHIPS OF PACHYLASMATIDAE, BATHYLASMATIDAE AND TETRACLITIDAE

Bathylasmatidae was recognized as Pachylasmatoidea by Buckeridge (1983) and Jones (2000). Buckeridge & Newman (2010) revised the classification of

Balanomorpha and grouped Bathylasmatidae and Tetraclitidae under the superfamily Tetraclitoidea. In our study, Bathylasmatidae was more closely associated with Tetraclitoidea than with Balanoidea or Pachylasmatoidea, which is consistent with the proposal by Newman & Ross (1971) and Buckeridge & Newman (2010) and previous findings of the molecular phylogenetic analysis of Tetraclitoidea (Tsang *et al.*, 2015). Bathylasmatid species differ markedly from pachylasmatid barnacles in morphology; for instance, bathylasmatids lack a compound rostrum or caudal appendages and possess quadridendorid instead of tridendroid mandibles compared with pachylasmatids. Furthermore, the bathylasmatid shell contains chitin-filled tubes (Jones, 2000) (characteristic of tetraclitoid barnacles), which are absent in pachylasmatids. Therefore, these morphological features and our molecular phylogenetic analysis provide strong evidence for placing Bathylasmatidae in Tetraclitoidea, which is in agreement with the proposal of Newman & Ross (1976) and Buckeridge & Newman (2010).

The sister relationship between Coronuloidea and Tetraclitoidea, proposed by Newman & Ross (1976) and Newman (1996) on the basis of morphology, has been consistently supported by molecular phylogenetic analyses (Hayashi *et al.*, 2013; Pérez-Losada *et al.*, 2014; Tsang *et al.*, 2015). Newman & Ross (1976) suggested that if Balanidae was derived from Coronuloidea Leach, 1817, then this occurred through Bathylasmatidae rather than Coronulidae or Tetraclitidae. However, this hypothesis is clearly rejected by the molecular evidence in the present study and the findings of previous studies (Pérez-Losada *et al.*, 2014; Tsang *et al.*, 2015). Extant Balanoidea is the sister lineage of Coronuloidea and Tetraclitoidea and is not derived from them.

Austrobalaninae Newman & Ross, 1971 was erected by Newman & Ross (1971) when they concluded that *Austrobalanus imperator* is a tetraclitoid that is closely allied with *Epopella*. Newman & Ross (1971) accommodated *Austrobalanus* and *Epopella* in Austrobalaninae to distinguish these two genera from other tetraclitoids. Buckeridge (1983) placed *Austrobalanus* with *Epopella* in the subfamily Austrobalaninae under Tetraclitidae. *Austrobalanus* is the only member of Tetraclitidae that exhibits six-plated shells (in contrast to the four-plated shells in other tetraclitids) and lacks interlaminar chitin. The presence of interlaminar chitin, regarded as a precursor for interlaminar longitudinal tube development in *Hexelasma* Hoek, 1913 and tetraclitoids wall plates, was proposed as strong evidence for a shared ancestry between the two taxa. However, whether the lack of interlaminar chitin in *Austrobalanus* was a secondary loss in the

primitive tetracitoids or a convergence in other tetracitoids and *Hexelasma* was unclear. Buckeridge & Newman (2010) proposed full family ranking for Austrobalaninae and Austrobalanidae. Two subfamilies, Elminiinae Foster, 1982 (including *Hexaminus* Foster, 1982, *Austrominus*, *Elminius* Leach, 1825 and the fossil genera *Matellonius* Buckeridge, 1983 and *Protelminius* Buckeridge & Newman, 2010) and Austrobalaninae (including *Austrobalanus* and *Epopella*) are recognized in this family (Buckeridge & Newman, 2010). Our molecular phylogenetic hypothesis suggested that Austrobalanidae proposed by Buckeridge & Newman (2010) is polyphyletic. *Austrobalanus* represents a lineage independent from other taxa, whereas *Epopella* is grouped with *Tetracitita* and *Tetracititella*. *Austrominus* is more closely related to Balanoidea and whether it shares a close affinity with *Austroiminus* and Tetracititoidea is not evident in the molecular data. *Austrobalanus* may be placed close to Coronuloidea (Hayashi *et al.*, 2013) or as a sister group to the Tetracititidae + Bathylasmatidae clade (Pérez-Losada *et al.*, 2014) in molecular phylogenetic studies. In either case, the taxon was firmly believed to have diverged before the emergence of the extant Tetracititidae and Bathylasmatidae and therefore likely represents an early offshoot within Tetracititoidea. Our results revealed that Austrobalanidae classification requires extensive revision. However, we did not propose a formal revision because numerous Austrobalanidae genera (particularly an exemplar of Eliminiinae) are missing and must be included in morphological and molecular evidence in future.

## TAXONOMIC REVISION OF PACHYLASMATOIDEA AND TETRACLITOIDEA

### SYSTEMATIC ACCOUNT

#### SUPERFAMILY PACHYLASMATOIDEA UTINOMI, 1968 EMEND.

Pachylasmatinae Utinomi, 1968: 37

Pachylasmatoidea Buckeridge, 1983: 60. – Jones, 2000: 156

*Diagnosis:* Shell wall comprises eight distinct compartmental plates, including rostrum, paired rostral lateral, carinal lateral 1 and carinal lateral 2, and carina. Shell with or without imbricating plates. Rostrum compound with RL but not entering sheath, solid parietes and radii absent. Base membranous, with solid calcareous not interdigitated with shell wall.

*Remarks:* Pachylasmatoidea contains the family Pachylasmatidae.

#### FAMILY PACHYLASMATIDAE UTINOMI, 1968 EMEND

Pachylasmatinae Utinomi, 1968: 37

Pachylasmatidae Foster, 1978: 76. – Buckeridge, 1983: 61. – Jones, 2000

*Diagnosis:* Shell wall comprises eight distinct calcareous plates (R-RL-CL1-CL2-C) with compound rostrum. Plates 8, 6 and 4 differentiated externally in adults. Caudal appendage present, cirrus III resembles cirrus II and not cirrus IV. Radii absent, and suture edges not complexly interlocked. Imbricating plates absent.

*Remarks:* *Pachylasma* was first identified by Darwin (1854), and these barnacles are considered exclusively deep-sea species. When Darwin examined the morphology of *Pachylasma*, he discovered that the *Pachylasma* shell is balanid-like; however, the somatic body is a feature of chthamalid species (Darwin, 1854). Darwin (1854) classified *Pachylasma* in Chthamalidae and concluded that *Pachylasma* was likely the point of contact in the chthamalid and balanid barnacle evolution (Darwin, 1854). The Jones (2000) classification includes five subfamilies in Pachylasmatidae, namely Eolasmatinae Jones, 2000, Pachylasmatinae, Metalasmatinae Jones, 2000, Bathylasmatinae and Hexelasmatinae. Our study indicated that Bathylasmatinae and Hexelasmatinae belonged to Tetracititoidea, supporting the proposal by Newman & Ross (1976) and Buckeridge & Newman (2010). Therefore, Pachylasmatidae currently comprises three subfamilies, Eolasmatinae, Pachylasmatinae and Metalasmatinae. The genera and species proposed in Metalasmatinae are based on the classification of Jones (2000). According to Jones (2000), Eolasmatinae comprises the fossil genera *Eolasma*, in which the specimen was described on the basis of a limited number of disarticulated plates, and *Waikalasma*. Ross & Newman (2001) erected a new family Waikalasmatidae in Chionelasmatoidea to accommodate *Waikalasma* because *Eolasma* lacks imbricating plates around the shells, unlike *Waikalasma*. In the present study, *Waikalasma* formed a distinct molecular clade with other familial groups, supporting the proposal of familial ranking for Waikalasmatidae Ross & Newman (2001).

In the present study, *Pseudoctomeris* was located in the same molecular clade as *Pachylasma*. The genera in the subfamily Pachylasmatinae was revised as follows:

## SUBFAMILY PACHYLASMATINAE UTINOMI, 1968 EMEND

Pachylasmatinae Utinomi, 1968: 37. – Newman & Ross, 1976: 36. – Jones, 2000: 163.

*Diagnosis (Emend):* Shell without imbricating plates and covered externally with eight, six, or four solid plates. Parietes without chitin. Opercular plates partially fused or separated. Membranous base. The subfamily includes intertidal and deep-sea species.

*Remarks:* The type genus is *Pachylasma*. Our study follows the classification of Jones (2000) that Pachylasmatinae contains *Eutomolasma*, *Microlasma* Jones, 2000, *Pachylasma*, *Eurylasma* and *Tetrapachylasma*, with the addition of *Pseudoctomeris*. *Pseudoctomeris sulcata* (Nilsson-Cantell, 1932) was grouped with *Pachylasma* in the present molecular phylogeny. *Pseudoctomeris sulcata* was first classified in the Chthamalidae (see Nilsson-Cantell, 1932) based on its solid shells and with a membranous base. However, Hiro (1939) re-examined *Pseudoctomeris* in detail and revealed that *P. sulcata* shared morphological similarities with *Octomeris* Sowerby, 1825 and *Pachylasma*. *Pseudoctomeris sulcata* was morphologically close to *Pachylasma* because it has *Pachylasma*-type opercular plates and a compound rostrum, in which the rostrum was joined with rostral laterals by a straight suture. Furthermore, *P. sulcata* has caudal appendages, whereas *Octomeris* lacked caudal appendages (Hiro, 1939). Hiro (1939) proposed that *P. sulcata* should be identified as *Pachylasma*. However, he believed that *Pachylasma* is an exclusive deep-sea taxon, whereas *Pseudoctomeris* is an intertidal inhabitant. He later recognized *P. sulcata* as a member of *Octomeris*. Poltarukha (1996), based on the distinct differences between *P. sulcata* and *Octomeris*, erected a new genus *Pseudoctomeris* to accommodate *P. sulcata*, under the subfamily Euraphinae in Chthamalidae. In the present study, molecular phylogenetic analysis showed *P. sulcata* was grouped in the clade of Pachylasmatidae. This result is also supported by the molecular analysis in Pérez-Losada *et al.* (2014) in which *P. sulcata* was positioned in the Pachylasmatoidae clade. On the basis of *Pseudoctomeris* possessing *Pachylasma*-type opercular plates, a compound rostrum and with the presences of caudal appendage, the present study grouped *Pseudoctomeris* in Pachylasmatidae. The genus *Pseudoctomeris* is monotypic, containing *P. sulcata* and this species represents the only intertidal species in Pachylasmatidae and has eight-plated shells (compound rostrum).

## TAXONOMIC REVISION OF

## TETRACLITOIDEA S.L. (BATHYLASMATIDAE)

## SUPERFAMILY TETRACLITOIDEA GRUVEL, 1903

Tetraclitidae Gruvel, 1903: 160.— Newman & Ross, 1976: 37

Tetraclitoidea Newman, 1993: 408.

*Diagnosis:* Six- or four-walled plates, solid parietes, permeated with chitin-filled tubes, with one or more rows of tubes containing living tissue or filled with calcareous materials. Plates with or without radii. Caudal appendages absent (diagnosis follows Newman & Ross, 1976).

*Remarks:* In the Tetraclitoidea superfamily, three families are proposed: Austrobalanidae, Bathylasmatidae and Tetraclitidae.

## BATHYLASMATIDAE NEWMAN &amp; ROSS, 1971

Bathylasmatidae Newman & Ross, 1971: 138. – Newman & Ross, 1976: 37; Buckeridge & Newman, 2010: 40; Table 1.

*Diagnosis:* Four- or six-plate shells, solid-plate shells or permeated with a single row of chitin-filled tubes. Radii absent. An inferior margin of mandible bearing a few small spines. All cirri without specialized setae. One or both rami of cirrus III, or sometimes cirrus II, may be antenniform.

*Remarks:* Hoek (1913) erected the genus *Hexelasma* in Balanidae to accommodate the deep-sea species discovered in the Challenger and Siboga expeditions, where the shells of these barnacles were balanid-like; however, the labrum lacked a notch, and cirrus III resembled cirrus IV instead of cirrus II. The morphology of such labrum and cirrus III of *Hexelasma* differed from those of the balanid barnacles. Pilsbry (1916), based on the morphology of the labrum and cirrus III, relocated *Hexelasma* from Balanidae to Chthamalidae. Bage (1938) argued that this position was the appropriate *Hexelasma* taxonomic position because this genus had features of both balanid and chthmalid barnacles. In Newman & Ross (1976), Bathylasmatidae was composed of two subfamilies: Bathylasmatinae and Hexelasmatinae. In the present study, we have samples only from *Hexelasma* and *Bathylasma* for molecular phylogenetic analysis. The taxonomic status of these two subfamilies should be evaluated following a phylogenetic reconstruction covering all five genera, including *Bathylasma*, *Mesolasma*, *Tessarelasma*, *Tetrachaelasma* (Jones, 2000) and *Hexelasma* (type genus). At present, we follow the classification of Newman & Ross (1976) that

Bathylasmatidae is composed of Bathylamatinae and Hexelasmatinae.

#### ACKNOWLEDGEMENTS

The authors thank the Principal Investigators of the Tropical Deep-Sea Benthos programme cruises; B. Richer de Forges, P. Bouchet S. Samadi and L. Corbari. Special thanks are also given to the crew of RV *ALIS*. This study has been supported by the ANR project France-Taiwan DeepEvo (Co PI S. Samadi, W.-J. Chen). The authors thank Marcos Perez-Losada for providing specimens of *Catophragmus* and Prof. Bill Newman for his teaching in *Bathylasma* and *Hexelasma* morphological features. This work was supported by grants from the Ministry of Science and Technology (MOST), Taiwan (NSC-102-2923-B-002-001 -MY3 to BKKC and NSC103-2621-B-019-004-MY2 to LMT).

#### REFERENCES

- Bage F. 1938.** Cirripedia. In: Australasian Antarctic expedition 1911–1914. *Science Reports Series C, Zoology and Botany* **2**(7): 5–13.
- Bouchet P, Héros V, Lozouet P, Maestrati P. 2008.** A quarter-century of deep-sea malacological exploration in the South and West Pacific: where do we stand? How far to go? In: Héros V, Cowie RH, Bouchet P, eds. *Tropical deep-sea Benthos 25. Mémoires du Muséum national d'Histoire naturelle* **196**. Paris: Muséum national d'Histoire naturelle, 9–40.
- Buckeridge JS. 1983.** Fossil barnacles (Cirripedia: Thoracica) of New Zealand and Australia. *New Zealand Geological Survey Paleontological Bulletin* **50**: 1–151.
- Buckeridge JS. 1995.** Phylogeny and biogeography of the primitive Sessilia and a consideration of a Tethyan origin for the group. In: Schram FR, Høeg JT, eds. *Crustacean issues 10, new frontiers in Barnacle evolution*. Rotterdam: A.A. Balkema, 255–268.
- Buckeridge JS. 1996.** A living fossil *Waikalasma boucheti* n. sp. (Cirripedia, Balanomorph) from Vanuatu (New Hebrides), Southwest Pacific. *Bulletin du Muséum National d'Histoire Naturelle (A)* **18**(3–4): 447–457.
- Buckeridge JS, Newman WA. 1992.** A reexamination of *Waikalasma* (Cirripedia, Thoracica) and its significance in balanomorph phylogeny. *Journal of Paleontology* **66**(2): 341–345.
- Buckeridge JS, Newman WA. 2010.** A review of the sub-family Elminiinae (Cirripedia: Thoracica: Austrobalanidae) including a new genus, *Protelminius* nov., from the Oligocene of New Zealand. *Zootaxa* **2349**: 39–54.
- Chan, BKK, Høeg JT. 2015.** Diversity of lifestyles, sexual systems, and larval development patterns in sessile crustaceans. In: Thiel, M. and Watling L. eds. *The natural history of the crustacea*, Vol. 2. Lifestyles and feeding biology. New York: Oxford University Press, 14–34.
- Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR. 1998.** Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* **46**: 419–437.
- Crandall KA, Fitzpatrick JF Jr. 1996.** Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology* **45**: 1–26.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012.** jModel-Test 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.
- Darwin C. 1854.** *A monograph of the sub-class Cirripedia, with figures of all the species. The Balanidae (or sessile cirripedes); the Verrucidae, etc.* London: Ray Society.
- Edgar RC. 2004.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.
- Foster BA. 1978.** The marine fauna of New Zealand: barnacles. *New Zealand Oceanographic Institute Memoir* **69**: 1–160.
- Gravel JA. 1903.** *Revision des cirripèdes ou Thecostraces*. Paris: Masson, 472p.
- Hayashi R, Chan BK, Simon-Blecher N, Watanabe H, Guy-Haim T, Yonezawa T, Levy Y, Shuto T, Achituv Y. 2013.** Phylogenetic position and evolutionary history of the turtle and whale barnacles (Cirripedia: Balanomorph: Coronuloidea). *Molecular Phylogenetics and Evolution* **67**: 9–14.
- Hiro F. 1939.** Studies on the Cirripedian fauna of Japan IV. Cirripedes of Formosa (Taiwan), with some geographical and ecological remarks on the littoral forms. *Memoirs of the College of Science, Kyoto Imperial University, Series B* **15**: 245–284.
- Hoek PPC. 1913.** *The Cirripedia of the Siboga Expedition. B. Cirripedia Sessilia. Siboga-Expeditie Monograph XXXIb i-xxv*. Leiden: E.J. Brill, 129–275.
- Jones DS. 2000.** Crustacea Cirripedia Thoracica: Chionelasmatoidea and Pachylasmatoidea (Balanomorpha) of New Caledonia, Vanuatu and Wallis and Futuna Islands, with a review of all currently assigned taxa. In: Crosnier A, ed. *Résultats des Campagnes MUSORSTOM, Volume 21. Mémoires du Muséum national d'Histoire naturelle* **184**: Paris: G. Dufour, 141–283.
- Jones DS. 2007.** The Cirripedia of New Caledonia. In: Payri CE, Richer de Forges B, eds. *Compendium of marine species from New Caledonia*. Noumea: Institut de Recherche pour le Développement, 289–294.
- Martin JW, Davis GE. 2001.** *An updated classification of the recent Crustacea*. Los Angeles, CA: Natural History Museum of Los Angeles County.
- Newman WA. 1993.** Darwin and cirripedology. In: Southward AJ, ed. *History of carcinology, crustacea issues, Vol. 8*. Rotterdam: A.A. Balkema, 349–434.
- Newman WA, Ross A. 1971.** Antarctic Cirripedia. *Antarctic Research Series* **14**: 1–257.
- Newman WA, Ross A. 1976.** *Revision of the balanomorph barnacles, including a catalog of the species, Vol. 9*. San Diego: San Diego Society of Natural History.
- Nilsson-Cantell CA. 1932.** Cirripedien aus Japan. *Arkiv For Zoologi* **24**: 1–30.

- Pérez-Losada M, Harp M, Høeg JT, Achituv Y, Jones D, Watanabe H, Crandall KA. 2008.** The tempo and mode of barnacle evolution. *Molecular Phylogenetics and Evolution* **46**: 328–346.
- Pérez-Losada M, Høeg JT, Simon-Blecher N, Achituv Y, Jones D, Crandall KA. 2014.** Molecular phylogeny, systematics and morphological evolution of the acorn barnacles (Thoracica: Sessilia: Balanomorpha). *Molecular Phylogenetics and Evolution* **81**: 147–158.
- Pilsbry HA. 1916.** The sessile barnacles (Cirripedia) contained in the collection of the US National Museum; including a monograph of the American species. *Bulletin of the United States National Museum* **93**: 1–366.
- Poltarukha OP. 1996.** Composition, phylogeny and position in system of the subfamily Notochthamalinae (Crustacea, Chthamalidae). *Zoologicheskii Zhurnal* **75**: 985–994 (in Russian).
- Rambaut A, Drummond AJ. 2009.** *Tracer v1.5*. Available at: <http://beast.bio.ed.ac.uk/Tracer>
- Richer de Forges B, Chan T-Y, Corbari L, Lemaitre E, Macpherson E, Ahyong ST, Ng PKL. 2013.** The MUSORSTOM-TDSB deep sea Benthos exploration programme (1976–2012): an overview of crustacean discoveries and new perspectives on deep-sea zoology and biogeography. In: Ahyong ST, Chan T-Y, Corbari L, Ng PKL, eds. *Tropical deep-sea Benthos*, Vol. 27, Paris: Muséum national d'Histoire naturelle, 13–66.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Ross A, Newman WA. 2001.** The Catophragmidae: members of the basal balanomorph radiation. *Sessile Organisms* **18**: 77–91.
- Shimodaira H. 2002.** An approximately unbiased test of phylogenetic tree selection. *Systematic Biology* **51**: 492–508.
- Shimodaira H, Hasegawa M. 2001.** CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* **17**(12): 1246–1247.
- Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
- Tsang LM, Chan BK, Shih FL, Chu KH, Allen Chen C. 2009.** Host-associated speciation in the coral barnacle *Wanella milleporae* (Cirripedia: Pyrgomatidae) inhabiting the *Millepora* coral. *Molecular Ecology* **18**: 1463–1475.
- Tsang LM, Chu KH, Nozawa Y, Chan BK. 2014.** Morphological and host specificity evolution in coral symbiont barnacles (Balanomorpha: Pyrgomatidae) inferred from a multi-locus phylogeny. *Molecular Phylogenetics and Evolution* **77**: 11–22.
- Tsang LM, Chu KH, Achituv Y, Chan BKK. 2015.** Molecular phylogeny of the acorn barnacle family Tetraclitidae (Cirripedia: Balanomorpha): validity of shell morphology and arthropodal characters in systematics of Tetraclitid barnacles. *Molecular Phylogenetic and Evolution* **82**: 324–329.
- Utinomi H. 1967.** Comments on some new and already known cirripedes with emended taxa, with special reference to the parietal structure. *Publications of the Seto Marine Laboratory* **15**(3): 199–237.
- Utinomi H. 1968.** A revision of the deep-sea barnacles *Pachylasma* and *Hexelasma* from Japan, with a proposal of new classification of the Chthamalidae (Cirripedia, Thoracica). *Publications of the Seto Marine Biological Laboratory* **16**: 21–39.
- Whiting MF. 2002.** Mecoptera is paraphyletic: multiple genes and phylogeny of *Mecoptera* and *Siphonaptera*. *Zoologica Scripta* **31**: 93–104.
- Yamaguchi T, Newman WA. 1990.** A new and primitive barnacle (Cirripedia: Balanomorpha) from the North Fiji Basin abyssal hydrothermal field, and its evolutionary implications. *Pacific Science* **44**: 135–155.

## SUPPORTING INFORMATION

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

**Figure S1.** Maximum likelihood topology of the mitochondrial *12S* gene sequences. Nodal supports are denoted on the corresponding branches for a bootstrap >50.

**Figure S2.** Maximum likelihood topology of the mitochondrial *16S* gene sequences. Nodal supports are denoted on the corresponding branches for a bootstrap >50.

**Figure S3.** Maximum likelihood topology of the nuclear *18S* gene sequences. Nodal supports are denoted on the corresponding branches for a bootstrap >50.

**Figure S4.** Maximum likelihood topology of the nuclear *EF1* gene sequences. Nodal supports are denoted on the corresponding branches for a bootstrap >50.

**Figure S5.** Maximum likelihood topology of the nuclear *H3* gene sequences. Nodal supports are denoted on the corresponding branches for a bootstrap >50.

**Figure S6.** Maximum likelihood topology of the nuclear *RP* gene sequences. Nodal supports are denoted on the corresponding branches for a bootstrap >50.

**Figure S7.** The phylogeny of combined mitochondrial *12S* and *16S*, and nuclear *EF1*, *H3*, *RPII* and *18S* gene sequences according to the Bayesian inference (BI) analysis. Nodal supports are denoted on the corresponding branches for a posterior probability > 0.90 for Bayesian analysis. The color of the taxon names indicates that the classification is based on [Buckeridge \(1983\)](#) and [Jones \(2000\)](#).