



# A new phylogeny of the Cephalaspidea (Gastropoda: Heterobranchia) based on expanded taxon sampling and gene markers <sup>☆</sup>



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## ABSTRACT

The Cephalaspidea is a diverse marine clade of euthyneuran gastropods with many groups still known largely from shells or scant anatomical data. The definition of the group and the relationships between members has been hampered by the difficulty of establishing sound synapomorphies, but the advent of molecular phylogenetics is helping to change significantly this situation. Yet, because of limited taxon sampling and few genetic markers employed in previous studies, many questions about the sister relationships and monophyletic status of several families remained open.

In this study 109 species of Cephalaspidea were included covering 100% of traditional family-level diversity (12 families) and 50% of all genera (33 genera). Bayesian and maximum likelihood phylogenetics analyses based on two mitochondrial (COI, 16S rRNA) and two nuclear gene markers (28S rRNA and Histone-3) were used to infer the relationships of Cephalaspidea. The monophyly of the Cephalaspidea was confirmed. The families Cylichnidae, Diaphanidae, Haminoeidae, Philinidae, and Retusidae were found non-monophyletic. This result suggests that the family level taxonomy of the Cephalaspidea warrants a profound revision and several new family and genus names are required to reflect the new phylogenetic hypothesis presented here. We propose a new classification of the Cephalaspidea including five new families (Alacuppidae, Colinatydidae, Colpodaspididae, Mnestiidae, Philinorbidae) and one new genus (*Alacuppa*). Two family names (Acteocinidae, Laonidae) and two genera (*Laona*, *Philinorbis*) are reinstated as valid. An additional lineage with family rank (Philinidae “Clade 4”) was unravelled, but no genus and species names are available to reflect the phylogeny and formal description will take place elsewhere.

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## 1. Introduction

The Cephalaspidea (bubble snails) is a major lineage of marine heterobranch gastropods with about 634 species (Jörger et al., 2010; WoRMS, 2015) distributed worldwide across all latitudes, reaching highest diversity in tropical and subtropical waters of the Indo-West Pacific (Lin and Qi, 1985; Bouchet et al., 2002; Gosliner et al., 2008; Too et al., 2014). Cephalaspids occur from the intertidal zone (e.g. *Haminoea* Turton & Kingston, 1830) to the deep-sea (e.g. *Inopinodon* Bouchet, 1975, *Meloscaphander* Schepman, 1913, *Scaphander* Montfort, 1810; Bouchet, 1975; Valdés, 2008; Eilertsen and Malaquias, 2013a), but seem to be more abundant in shallow areas to 40 m deep (Gosliner et al.,

2008). Most species inhabit soft bottoms of sand or mud, but several occur in close association with seagrass and algae and others in coral rubble and sponges (Burn and Thompson, 1998; Gosliner et al., 2008). A single genus is known to inhabit exposed rocky shores (*Smaragdinella* A. Adams, 1848; Gosliner et al., 2008).

Malaquias et al. (2009) suggested that diet specialization played a chief role in the evolution and diversification of cephalaspids. Within the group there are herbivorous and carnivorous lineages, which feed preferentially upon diatoms, filamentous algae, foraminiferans, small bivalves, gastropods and polychaetes (see Malaquias et al., 2009; Göbbeler and Klussmann-Kolb, 2011; Eilertsen and Malaquias, 2013b, for reviews).

Mikkelsen (1993) highlighted the difficulties on establishing synapomorphies and the sharp discrepancies between proposed classifications. Consequently, the first attempts to study the relationships of the group within a cladistic framework were hampered not only by incomplete taxon sampling, but mostly by difficulties on the interpretation of morphological characters and

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resulted in poorly resolved hypotheses (Mikkelsen, 1996, 2002; Dayrat and Tillier, 2001; Wägele and Klussmann-Kolb, 2005). Nevertheless, these studies backed the view that some traditional cephalaspidean groups such as Acteonoidea, Aplustridae, Bullinidae Gray, 1850, and Ringiculidae Philippi, 1853 were likely not closely related to the remaining lineages, a hypothesis originally suggested by Haszprunar (1985).

Since the late 1990s the advent of molecular phylogenetics yielded significant new insights about the relationships of euthyneuran gastropods and the Cephalaspidea was no exception (reviewed in Malaquias et al., 2009). Molecular studies contributed to an objective redefinition of the concept of Cephalaspidea and become well established that traditional taxa such as the Acteonoidea (*Acteon* Montfort, 1810, *Hydatina* Schumacher, 1817, *Micromelo* Pilsbry, 1895, *Pupa* Röding, 1798, *Rictaxis* Dall, 1871) and Runcinacea (*Runcina* Forbes, 1851, *Ilbia* Burn, 1963) are not members of the clade Cephalaspidea. Also, the Diaphanoidea Odhner, 1914 (*Diaphana* Brown, 1827, *Toledonia* Dall, 1902) represents the basal lineages of the Cephalaspidea *sensu stricto* (*sensu* Malaquias et al., 2009; Jörger et al., 2010; Göbbeler and Klussmann-Kolb, 2011). The “Cephalaspidea” went from being considered as basal “primitive” opisthobranchs (e.g. Gosliner, 1981) to derived and specialized members of the Euthyneura Spengel, 1881 within the monophyletic Euopisthobranchia (*sensu* Jörger et al., 2010; Schrödl et al., 2011; Brenzinger et al., 2013; Wägele et al., 2014).

Yet, the systematics and evolutionary relationships of the Cephalaspidea remain far from completely understood. The study by Malaquias et al. (2009) is still the only one to specifically focus on the internal relationships of the Cephalaspidea *sensu stricto* within a molecular phylogenetic framework. The authors used three gene markers (mitochondrial COI and nuclear 18S rRNA and 28S rRNA) and included representatives of nine families, 22 genera, and 41 species. They found the families Cylichnidae, Diaphanidae, Haminoeidae, and Retusidae to be polyphyletic and in need of revision and reinstated the family names Rhizoridae (for *Volvulella* Newton, 1891) and Scaphandridae (for *Scaphander*). The family relationships were generally poorly resolved, but sister relationships were found between Philinidae and Aglajidae and between Philinoglossidae and Gastropteridae. A possible sister relationship between Bullidae and Retusidae was hinted, albeit support was marginal (PP = 0.94, BS < 75; Malaquias et al., 2009: Fig. 4A–C) and the traditional superfamilies Philinoidea Gray, 1850 and Bulloidea Gray, 1827 (*sensu* Mikkelsen, 1996) were not supported. Based on their results the authors proposed a new classification for the Cephalaspidea including 13 families (Malaquias et al., 2009: Tab. 4).

The dataset available to Malaquias et al. (2009), still barely comprised a partial representation of the generic diversity of the group (ca. 30%), and several families with extensive morphological diversity were represented by only one or two species (e.g. Cylichnidae, Diaphanidae, Gastropteridae, Philinidae, Philinoglossidae, Scaphandridae). Later studies of euthyneuran gastropods did not change the state of the art because they used essentially the same datasets that were included in previous works (Jörger et al., 2010; Göbbeler and Klussmann-Kolb, 2011).

In this study we present a complete family level phylogeny of the Cephalaspidea including 109 species covering 50% (33 of 66 genera, not counting fossil genera) of the traditionally accepted generic diversity of the group (*sensu* Burn and Thompson, 1998; Bouchet and Rocroi, 2005; WoRMS, 2015; see Table 1). This work aims to (1) produce a new hypothesis of relationships for the Cephalaspidea gastropods by means of multi-locus phylogenetic analyses, (2) to infer relationships between families, (3) to test the monophyletic status of traditional families and genera, and

(4) to test and revise the classification proposed by Malaquias et al. (2009) for the Cephalaspidea.

## 2. Methods

### 2.1. Sampling

Novel material used in the present study was self-collected in a series of shore based expeditions and deep water cruises conducted by the Museum National d'Histoire Naturelle (MNHN) in the tropical Indo-Pacific; and from the University Museum of Bergen (Natural History), Norway (ZMBN). Whenever possible at least four species per genus were included in the analyses. In some situations we have added additional species, namely for genera/families of uncertain systematic status (e.g. Retusidae; Malaquias et al., 2009) and when previous unpublished preliminary work undertaken by us have hinted possible cases of unexpected non-monophyly (e.g. *Chelidonura* A. Adams, 1850, *Haminoea*, Philinidae). Furthermore, when specimens did not have full gene coverage (the four genes used in this study), we have used additional representatives to complement the gene diversity (see Table 1).

DNA extracts of *Philinoglossa praelongata* and *Pluscula cuica* were provided by colleagues from The Bavarian State Collection of Zoology, Germany (ZSM). Additionally 153 sequences from 60 specimens were mined from GenBank (Table 1).

Outgroup taxa consisting of 30 species from 24 genera representing nine higher euthyneuran clades of ranking similar to that of Cephalaspidea (Jörger et al., 2010) were included in the analyses, namely Acochlidia, Acteonoidea, Anaspidea, Nudipleura, Pteropoda (Gymnosomata and Thecosomata), Pyramidelloidea, Runcinacea, Sacoglossa, and Umbraculida. The trees were rooted with the caenogastropod species *Littorina littorea* a sister lineage to the Heterobranchia molluscs (Zapata et al., 2014). In total this study includes 177 specimens (145 Cephalaspidea, 31 Euthyneura outgroups, and 1 Caenogastropoda) and a total of 596 sequences (Table 1).

### 2.2. DNA extraction, amplification, and sequencing

DNA was extracted from tissue obtained from the foot or parapodial lobes using the Qiagen DNeasy® Blood and Tissue Kit following the protocol recommended by the manufacturer. Partial sequences of the mitochondrial genes cytochrome *c* oxidase subunit I (COI; ca. 660 bp, primers: LCO1490 (F) GGTCAACAAATCATAAAGATATTGG, HCO2198 (R) TAAACTTCAGGGTGACCAAAA TCA; Folmer et al., 1994) and 16S rRNA (ca. 506 bp, primers: 16S ar-L CGCCTGTTTATCAAAAACAT, 16S br-H CCGGTCTGAACCTCAGATCACGT; Palumbi et al., 1991) and the nuclear genes Histone-3 (H3; ca. 345 bp, primers: H3AD5'3' (F) ATGGCTCGTACCAAGCAGACVGC, H3BD5'3' (R) ATATCTTRGGCATRATRGTGAC; Colgan et al., 1998), and 28S rRNA (ca. 1178 bp, primers: LSU5-F TAGGTCCGACCCGCTGAAYTTAAGCA (Littlewood et al., 2000); 900-F CCGTCTTGAACACGGACCAAG (Olson et al., 2003); LSU1600-R (Williams et al., 2003); ECD2S-R CTTGGTCCGTGTTTCAAGACGG (modified from primers by Littlewood et al., 2000)) were amplified and sequenced.

For the COI and 28S genes, amplification follows the protocols described by Malaquias et al. (2009). Polymerase chain reactions (PCR) for the 16S and H3 genes were equally performed in 50 µL volume, including 17.5 µL Sigma water, 5 µL CoraLoad buffer, 5 µL dNTP, 10 µL Q-solution, 7 µL MgCl<sub>2</sub>, 2 µL of each of the primers, 0.5 µL Taq, and 1 µL DNA. For the H3 gene only 4 µL of MgCl<sub>2</sub> were used and the volume of Sigma water was adjusted to 20.5 µL.



Table 1 (continued)

Higher taxa	Family	Species	New taxonomic assignment	Code	Locality	Voucher no.	COI	16S	28S	H3		
Diaphanidae Odhner, 1914		<i>Colinatys</i> sp. A	Colinatydidae Oskars, Bouchet & Malaquias n. fam	c15	Panglao, the Philippines	MNHN 42244	DQ974665	KJ022776*	DQ927223	KJ022946*		
		<i>Colinatys</i> sp. A	Colinatydidae Oskars, Bouchet & Malaquias n. fam	c39	Panglao, the Philippines	MNHN 42254	DQ974666	KJ022783*	DQ927224	KJ022939*		
		<b><i>Colobocephalus costellatus</i> M. Sars, 1870</b>	Colpodaspididae Oskars, Bouchet & Malaquias n. fam	to38	Aurlandfjorden, Norway	ZMBN 91084	KF992207*	KJ02286*	KJ023013*	KJ022873*		
		<i>Colpodaspis thompsoni</i> G. H. Brown, 1979	Colpodaspididae Oskars, Bouchet & Malaquias n. fam	c11	Panglao, the Philippines	MNHN 42241	KF992158*	KJ022774*	DQ927222	KJ022947*		
		<i>Diaphana globosa</i> (Lovén, 1846)	Cylichnidae H. Adams & A. Adams, 1854	cn27	Hauglandsosen, Norway	ZMBN 88018	KF992162*	KJ022791*	KJ023056*	KJ022930*		
		<i>Toledonia globosa</i> Hedley, 1916		gb26	Scotia Arc, Antarctica	EED-Phy-475	EF489395	EF489327	EF489375	-		
		Gastropteridae Swainson, 1840		<i>Gastropteridae</i> sp.		to36	Surprise Island, New Caledonia	MNHN IM-2009-4370	KF992206*	KJ022864*	KJ023014*	KJ022874*
				<b><i>Sagaminopteron ornatum</i> Tokioka &amp; Baba, 1964</b>		gb10	GenBank	Sag. iso. 240	AM421857	AM421814	AM421937	-
				<i>Sagaminopteron psychedelicum</i> Carlson & Hoff, 1974		c44	Kalakajoro, Madagascar	Cas-Cephas3	DQ974667	KJ022787*	DQ927225	KJ022934*
				<b><i>Siphopteron tigrinum</i> Gosliner, 1989</b>		c45	Kalakajoro, Madagascar	Cas-Cephas4	DQ974668	KJ022788*	DQ927226	KJ022933*
<i>Siphopteron brunneomarginatum</i> (Carlson & Hoff, 1974)				gb12	GenBank	Siph.b. iso. e4	AM421864	AM421816	AM421939	-		
<i>Siphopteron quadrispinosum</i> Gosliner, 1989				gb11	GenBank	Sip. iso.179	AM421860	AM421819	-	-		
<i>Siphopteron quadrispinosum</i> Gosliner, 1989				gb67	GenBank	Siph. Iso. 189	-	-	AM421941	-		
<i>Aliculastrum debilis</i> (Pease, 1871)				348	Maui, Hawaii	ZMBN 81658	KF992193*	KJ022823*	KJ023026*	KJ022895*		
<i>Aliculastrum paralella</i> (Gould, 1847)				219	Bile Bay, Marianas islands, Guam	UF 374138	KF992171*	KJ022802*	KJ023047*	KJ022902*		
<i>Aliculastrum paralella</i> (Gould, 1847)				340	Guam	ZMBN 81670	KF992188*	KJ022818*	KJ023031*	KJ022901*		
Haminoeidae Pilsbry, 1895		<i>Aliculastrum</i> sp.		238	Bile Bay, Marianas islands, Guam	UF 374152	KF992177*	KJ022808*	KJ023041*	KJ022896*		
		<i>Atys kuhnsi</i> Pilsbry, 1917		350	Maui, Hawaii	ZMBN 81660	KF992194*	KJ022824*	KJ023025*	KJ022894*		
		<b><i>Atys naucum</i> (Linnaeus, 1758)</b>		236	Palau, Hawaii	UF 301586	KF992176*	KJ022807*	KJ023042*	KJ022913*		
		<i>Atys semistriata</i> Pease, 1860		347	Maui, Hawaii	ZMBN 81656	KF992192*	KJ022822*	KJ023027*	KJ022897*		
		<i>Atys semistriata</i> Pease, 1860		222	Tepung channel, Marianas islands, Guam.	UF 374125	KF992174*	KJ022805*	KJ023044*	KJ022915*		
		<b><i>Bullacta exarata</i> (Philippi, 1849)</b>		213	South Korea	NHMUK 20070444	GQ332576	KJ022800*	HM100714	KJ022920*		
		<b><i>Bullacta exarata</i> (Philippi, 1849)</b>		gb44	Wenzhou, China	LSGB 25302	HQ834118	HQ833986	-	HQ834193		
		<i>Diniatys costulosa</i> (Pease, 1869)		344	Maui, Hawaii	ZMBN 81802	KF992191*	KJ022821*	KJ023028*	KJ022898*		
		<b><i>Diniatys dentifer</i> (A. Adams, 1850)</b>		343	Maui, Hawaii	ZMBN 81706	KF992190*	KJ022820*	KJ023029*	KJ022899*		
		<i>Diniatys dubia</i> (Schepman, 1913)		220	Bile Bay, Marianas islands, Guam	UF 374148	KF992173*	KJ022804*	KJ023045*	KJ022916*		
<i>Diniatys monodonta</i> (A. Adams, 1850)		239	Cocos, Marianas, Guam	UF 376788	KF992178*	KJ022809*	KJ023040*	KJ022912*				
<i>Haminoea cymbalum</i> (Quoy & Gaimard, 1832)		323	Magliao, Guam	ZMBN 81711	KF992182*	KJ022812*	KJ023037*	KJ022908*				
<b><i>Haminoea hydatis</i> (Linnaeus, 1758)</b>		166	Port Barcarès, Salses-Leucaté Lake, France, Mediterranean	NHMUK 20060326	KF615841	KJ022796*	KF615802	KJ022925*				

Table 1 (continued)

Higher taxa	Family	Species	New taxonomic assignment	Code	Locality	Voucher no.	COI	16S	28S	H3
		<i>Haminoea orbignyana</i> (Férussac, 1822)		1	Faro, Portugal	NHMUK 20030296	KF615813	KJ022794*	KF615776	KJ022927*
		<i>Haminoea ovalis</i> Pease, 1868		333	Maui, Hawaii	ZMBN 81689	KF992184*	KJ022814*	KJ023035*	KJ022906*
		<i>Liloea curta</i> (A. Adams, 1850)		248	Tepung channel, Marianas islands, Guam.	UF 374131	KF992180*	–	KJ023039*	KJ022910*
		<i>Liloea porcellana</i> (Gould, 1859)		451	Maui, Hawaii	ZMBN 89712	KF992202*	KJ022836*	KJ023018*	KJ022887*
		<i>Liloea</i> sp.		338	Guam	ZMBN 81663	KF992187*	KJ022817*	KJ023032*	KJ022903*
		<i>Mnestia villica</i> (Gould, 1859)	Mnestiidae Oskars, Bouchet & Malaquias n. fam	c8	Panglao, the Philippines	MNHN 42239	KF992161*	KJ022789*	DQ927236	KJ022931*
		<i>Mnestia vilica</i> (Gould, 1859)	Mnestiidae Oskars, Bouchet & Malaquias n. fam	337	Maui, Hawaii	ZMBN 81716	KF992186*	KJ022816*	KJ023033*	KJ022904*
		<i>Phanerophthalmus cylindricus</i> (Pease, 1861)		341	Maui, Hawaii	ZMBN 81693	KF992189*	KJ022819*	KJ023030*	KJ022900*
		<i>Phanerophthalmus smaragdinus</i> (Rüppell & Leuckart, 1830)		218	Mangrove Bay, Egypt, Red Sea	NHMUK 20070584	KF992170*	–	KJ023048*	KJ022918*
		<i>Phanerophthalmus</i> sp.		c4	Sulu, Indonesia	NHMUK 20050661	KF992160*	KJ022784*	DQ927241	KJ022938*
		<i>Smaragdinella</i> cf. <i>calyculata</i> (Broderip & G. B. Sowerby I, 1829)		c42	Panglao, the Philippines	MNHN 42257	DQ974682	KJ022786*	DQ927242	KJ022936*
		<b><i>Smaragdinella calyculata</i> (Broderip &amp; G. B. Sowerby I, 1829)</b>		336	Maui, Hawaii	ZMBN 81646	KF992185*	KJ022815*	KJ023034*	KJ022905*
		<i>Smaragdinella</i> sp. A		184	Pulau Jung, Singapore Strait, Singapore	NHMUK 20070586	KF992166*	–	KJ023052*	KJ022923*
		<i>Smaragdinella</i> sp. A		186	Pulau Jung, Singapore Strait, Singapore	NHMUK 20070586	KF992167*	KJ022797*	KJ023051*	KJ022922*
	Philinidae Gray, 1850	<b><i>Philine aperta</i> (Linnaeus, 1767)</b>		gb59	South Africa	CASIZ 176332	–	JQ691679	–	–
		<i>Philine angasi</i> (Crosse, 1865)		gb61	New Zealand	CASIZ 188571	–	JQ691675	–	–
		<i>Philine angasi</i> (Crosse, 1865)		gb62	Australia	NHMUK 2388	–	JQ691677	–	–
		<i>Philine auriformis</i> Suter, 1909		gb63	New Zealand	CASIZ 188573	–	JQ691680	–	–
		<i>Philine auriformis</i> Suter, 1909		gb64	California, USA	CASIZ 188580	–	JQ691681	–	–
		<i>Philine babai</i> Valdés, 2008		to25	Bohol Sea, the Philippines	MNHN IM-2009-4352	KF877702*	KJ022854*	KJ022989*	KJ022968*
		<i>Philine babai</i> Valdés, 2008		to13/384	Panglao, the Philippines	MNHN IM-2009-4344	KF877703*	KJ022843*	KJ023061*	KJ022982*
		<i>Philine confusa</i> Ohnheiser and Malaquias, 2013	Laona Adams, 1865; Laonidae Pruvot-Fol, 1954	446	Hauglandsosen, Norway	ZMBN 87081	JX944804	KJ022835*	KJ023012*	KJ022949*
		<i>Philine exigua</i> Challis, 1969		gb60	Guadalcanal, Solomon I., West Pacific	ZSM Mol-20080752	HQ168450	HQ168412	HQ168438	–
		<i>Philine grandioculi</i> Ohnheiser and Malaquias, 2013	Laona Adams, 1865; Laonidae Pruvot-Fol, 1954	cn12	Off Lofoten, Norway	ZMBN 88009	JX944805	KJ022790*	KJ023058*	–
		<i>Philine indistincta</i> Ohnheiser and Malaquias, 2013		413	Silavågen, Norway	ZMBN 82108	JX944798	KJ022832*	–	KJ022950*
		<i>Philine orientalis</i> A. Adams, 1854		gb65	Japan	NHMUK 1996409	–	JQ691684	–	–
		<i>Philine orientalis</i> A. Adams, 1854		gb66	California, USA	CASIZ 188556	–	JQ691686	–	–
		<i>Philine paucipapillata</i> Price, Gosliner & Valdés, 2011		gb56	Taiwan	BMHN 20070190	–	JQ691691	–	–
		<i>Philine pruinosa</i> (Clark, 1827)	Laona Adams, 1865; Laonidae Pruvot-Fol, 1954	444	Tjärnö, Sweden	ZMBN 87076	JX944808	KJ022834*	KJ023011*	KJ022951*
		<i>Philine quadrata</i> (S. Wood, 1839)	Laona Adams, 1865; Laonidae Pruvot-Fol, 1954	cn06	West of Lofoten, Norway	ZMBN88012.1	JX944809	KJ022793*	KJ023010*	KJ022952*

Table 1 (continued)

Higher taxa	Family	Species	New taxonomic assignment	Code	Locality	Voucher no.	COI	16S	28S	H3
		<i>Philine quadripartita</i> Ascanius, 1772		gb55	Spain	?	AY345016	–	–	–
		<i>Philine quadripartita</i> Ascanius, 1772		gb57	Tjärnö, Sweden	MCZ DNA101778	GQ160767	–	–	–
		<i>Philine quadripartita</i> Ascanius, 1772		gb58	Tjärnö, Sweden	MCZ DNA101268	–	DQ093482	DQ279988	DQ093508
		<i>Philine scabra</i> (O. F. Müller, 1784)		439	Hauglandsosen, Norway	ZMBN 81821	JX944796	KJ022833*	KJ023009*	KJ022953*
		<i>Philine ventricosa</i> (Jeffreys, 1865)	<i>Laona</i> Adams, 1865; Laonidae Pruvot-Fol, 1954	402	Bergen, Norway	ZMBN 87080.1	JX944803	KJ022831*	KJ023008*	KJ022978*
		<i>Philine</i> sp. A		to18	Sulu Sea, the Philippines	MNHN IM-2009-4380	–	–	KJ022983*	KJ022963*
		<i>Philine</i> sp. B		to26	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4360	KF877697*	KJ022855*	KJ022984*	KJ022969*
		<i>Philine</i> sp. B		to19	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4359	–	KJ022847*	KJ022995*	KJ022975*
		<i>Philine</i> sp. C		to8	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4358	KF877698*	KJ022870*	KJ022998*	KJ022961*
		<i>Philine</i> sp. C		to20	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4357	KF877699*	KJ022849*	KJ022994*	KJ022964*
		<i>Philine</i> sp. C		to21	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4356	KF992157*	KJ022850*	KJ022993*	KJ022965*
		<i>Philine</i> sp. C		to22	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4355	KF877700*	KJ022851*	KJ022992*	KJ022966*
		<i>Philine</i> sp. C		to23	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4354	KF877701*	KJ022852*	KJ022991*	KJ022967*
		<i>Philine</i> sp. C		to17	Baler Bay, the Philippines	MNHN IM-2009-4368	KF877691*	KJ022846*	KJ022996*	KJ022955*
		<i>Philine</i> sp. C		to16	Pavuvu, Solomon Islands	MNHN IM-2009-4335	KF877707*	KJ022845*	KJ022997*	KJ022962*
		<i>Philine</i> sp. C		to32	Pavuvu, Solomon Islands	MNHN IM-2009-4334	KF877690*	KJ022862*	KJ022986*	KJ022972*
		<i>Philine</i> sp. C		to1	Pavuvu, Solomon Islands	MNHN IM-2009-4333	KF877708*	KJ022839*	KJ023004*	KJ022957*
		<i>Philine</i> sp. D		to15	Taiwan	MNHN IM-2009-4328	KF877689*	–	–	KJ022956*
		<i>Philine</i> sp. D		to31	Panglao, the Philippines	MNHN IM-2009-4338	KF877704*	KJ022861*	KJ022987*	KJ022971*
		<i>Philine</i> sp. D		to11	Panglao, the Philippines	MNHN IM-2009-4337	KF877705*	KJ022841*	KJ023060*	KJ022980*
		<i>Philine</i> sp. D		to12	Panglao, the Philippines	MNHN IM-2009-4336	KF877706*	KJ022842*	KJ023059*	KJ022981*
		<i>Philine</i> sp. E		to28	Surprise Island, New Caledonia	MNHN IM-2009-4375	KF877693*	KJ022857*	KJ023006*	KJ022970*
		<i>Philine</i> sp. E		to29	Surprise Island, New Caledonia	MNHN IM-2009-4374	KF877695*	KJ022858*	KJ023005*	KJ022976*
		<i>Philine</i> sp. E		to30	Between Surprise and Pott Is. New Caledonia	MNHN IM-2009-4373	KF877694*	KJ022860*	KJ023007*	KJ022977*
		<i>Philine</i> sp. F		to6	East of Lamon Bay, the Philippines	MNHN IM-2009-4367	KF877692*	KJ022868*	–	KJ022954*
		<i>Philine</i> sp. F		to27	East of Lamon Bay, the Philippines	MNHN IM-2009-4366	KF877709*	KJ022856*	KJ022988*	KJ022878*
		<i>Philine</i> sp. F		to14	East of Lamon Bay, the Philippines	MNHN IM-2009-4365	KF877696*	KJ022844*	–	–
		<i>Philine</i> sp. G		to5	Bohol Sea, the Philippines	MNHN IM-2009-4341	KF877696*	KJ022867*	KJ023000*	KJ022872*
		" <i>Philine</i> " sp. A	Philinidae clade 4	to10/382	North of Lamon Bay, the Philippines	MNHN IM-2009-4361	KF877714*	KJ022840*	KJ023062*	KJ022880*

(continued on next page)

Table 1 (continued)

Higher taxa	Family	Species	New taxonomic assignment	Code	Locality	Voucher no.	COI	16S	28S	H3
		" <i>Philine</i> " sp. B	Philinidae clade 4	to9/ 381	East of Lamon Bay, the Philippines	MNHN IM-2009-4362	KF877713*	KJ022871*	KJ023063*	KJ022979*
		" <i>Philine</i> " sp. C	Philinidae clade 4	to4	Surprise Island, New Caledonia	MNHN IM-2009-4372	KF877712*	KJ022866*	KJ023001*	KJ022974*
		" <i>Philine</i> " sp. D	Philinidae clade 4	to37	East of Lamon Bay, Philippines	MNHN IM-2009-4363	KF877711*	KJ022838*	KJ022985*	KJ022973*
		<i>Philinorbis</i> sp. A	Philinorbidae Oskars, Bouchet & Malaquias n. fam.	to7	East of Lamon Bay, Philippines	MNHN IM-2009-4369	KF877715*	KJ022869*	KJ022999*	KJ022960*
		<i>Philinorbis</i> sp. B	Philinorbidae Oskars, Bouchet & Malaquias n. fam.	to24	Bohol Sea, Philippines	MNHN IM-2009-4353	KF877716*	KJ022853*	KJ022990*	KJ022879*
		<i>Philinorbis</i> sp. C	Philinorbidae Oskars, Bouchet & Malaquias n. fam.	to3	Savu, Indonesia	MNHN IM-2009-4340	–	KJ022859*	KJ023002*	KJ022959*
		<i>Philinorbis</i> sp. D	Philinorbidae Oskars, Bouchet & Malaquias n. fam.	to2	Surprise Island, New Caledonia	MNHN IM-2009-4316	–	KJ022848*	KJ023003*	KJ022958*
Philinoglossidae Hertling, 1932		<i>Philinoglossa praelongata</i> Salvini-Plawen, 1973		ph1	Rovinj, Istria, Croatia, Mediterranean Sea	ZSM Mol-20080917	–	HQ168411	KJ023017*	–
		<i>Philinoglossa praelongata</i> Salvini-Plawen, 1973		ph2	Rovinj, Istria, Croatia, Mediterranean Sea	ZSM Mol-20080918	–	–	–	KJ022882*
		<b><i>Pluscula cuica</i> Er. Marcus, 1972</b>		pl1	Sino da Pedra, Ilha Bela, São Paulo, Brazil	ZSM Mol-20100325	KF992203*	KJ022837*	KJ023016*	KJ022881*
Retusidae Thiele, 1925		<i>Pyrunculus</i> sp. A		377	Maui, Hawaii	MNHN IM-2009-4322	KF992198*	–	KJ023021*	KJ022890*
		<i>Pyrunculus</i> sp. B		c10	Panglao, the Philippines	MNHN 42240	DQ974678	KJ022773*	DQ927237	KJ022948*
		<i>Retusa umbilicata</i> (Montagu, 1803)		cn40	Hauglandsosen, Norway	ZMBN 90143	KF992163*	KJ022792*	KJ023055*	KJ022929*
		<i>Retusa</i> sp. A		378	North of Lamon Bay, the Philippines	MNHN IM-2009-4364	KF992199*	KJ022828*	KJ023020*	KJ022889*
		<i>Retusa</i> sp. B		383	Panglao, the Philippines	MNHN IM-2009-4346	KF992201*	KJ022830*	KJ023019*	KJ022888*
		<i>Retusa</i> sp. C		c14	Panglao, the Philippines	MNHN 42243	DQ974679	KJ022775*	DQ927238	KJ022884*
		<i>Retusa</i> sp. D		329	Hong Kong, China	ZMBN 81712	KF992183*	KJ022813*	KJ023036*	KJ022907*
Rhizoridae Dell, 1952		<i>Volvulella</i> sp.		c41	Panglao, the Philippines	MNHN 42256	DQ974684	KJ022785*	DQ927244	KJ022937*
Scaphandridae Sars, 1878		<i>Sabatia</i> sp. A	<i>Alacuppa</i> n. gen.; Alacuppidae Oskars, Bouchet & Malaquias n. fam.	to33	Baler Bay, the Philippines	MNHN IM-2009-4572	KF992204*	KJ022863*	KJ023015*	KJ022876*
		<i>Sabatia</i> sp. A	<i>Alacuppa</i> n. gen.; Alacuppidae Oskars, Bouchet & Malaquias n. fam.	379	Baler Bay, the Philippines	MNHN IM-2009-4323	KF992200*	KJ022829*	–	KJ022877*
		<i>Sabatia</i> sp. B	<i>Alacuppa</i> n. gen.; Alacuppidae Oskars, Bouchet & Malaquias n. fam.	to34	Panglao, the Philippines	MNHN IM-2009-4382	KF992205*	–	–	KJ022875*
		<b><i>Scaphander lignarius</i> (Linnaeus, 1758)</b>		s37	Bergen, Norway	ZMBN 88000	KC351563	KC351526	KC351545	KJ094553*
		<i>Scaphander mundus</i> Watson, 1883		s29	East of Lamon Bay, the Philippines	MNHN IM-2009-4319	KC351565	KC351529	KC351547	KJ094556*
		<i>Scaphander punctostriatus</i> (Mighels & Adams, 1842)		s34	Norway	ZMBN 88006	KC351571	KC351536	KC351553	KJ094554*
		<i>Scaphander</i> sp.		s30	Between Surprise and Pott Island, New Caledonia	MNHN IM-2009-4317	KC351572	KC351537	KC351554	KJ094555*

Table 1 (continued)

Higher taxa	Family	Species	New taxonomic assignment	Code	Locality	Voucher no.	COI	16S	28S	H3
Outgroups										
Acteonoidea	Acteonidae d'Orbigny, 1843	<i>Acteon</i> sp.		c35	Panglao, the Philippines	MNHN 42253	DQ974648	KJ022782*	DQ927213	KJ022940*
	Aplustridae Gray, 1847	<i>Pupa solidula</i> (Linnaeus, 1758)		gb5	Dingo Beach, Australia		DQ238006	EF489319	AY427481	EF133483
		<i>Hydatina physis</i> (Linnaeus, 1758)		27	Madagascar	NHMUK 20060098	DQ986572	DQ986637	DQ986699	–
		<i>Hydatina physis</i> (Linnaeus, 1758)		c17	Madagascar	NHMUK 20060098	DQ974651	–	–	KJ022945*
		<b><i>Micromelo undatus</i> (Bruguère, 1792)</b>		c19	Tenerife, Canary Islands	NHMUK 20030800	DQ974653	KJ022778*	DQ927214	KJ022944*
Acochlidia	Acochliidae Kütze, 1935	<i>Acochlidium fijiense</i> Haynes & Kenchington, 1991		gb46	GenBank		HQ168458	HQ168420	HQ168446	–
		<b><i>Strubellia paradoxa</i> (Strubell, 1892)</b>		gb51	GenBank		HQ168457	HQ168419	HQ168445	–
Anaspidea	Akeridae Mazzarelli, 1891	<b><i>Akera bullata</i> O. F. Müller, 1776</b>		127	Algarve Portugal	NHMUK 20020723	KF992164*	KJ022795*	KJ023054*	KJ022926*
	Aplysiidae Lamarck, 1809	<i>Aplysia californica</i> J. G. Cooper, 1863		gb1	California		AF077759	AF192295	AY026366	–
		<i>Aplysia dactylomela</i> Rang, 1829		21	Cape Verde	NHMUK 20030795/ 20030796	KF992168*	KJ022798*	KJ023050*	KJ022921*
Caenogastropoda	Littorinidae Children, 1834	<b><i>Littorina littorea</i> (Linnaeus, 1758)</b>		gb43	GenBank	MCZ DNA101389	DQ093525	DQ093481	FJ977692	DQ093507
Lower Heterobranchia	Pyramidellidae Gray, 1840	<i>Eulimella ventricosa</i> (Forbes, 1844)		gb45	Gnejna Bay, Malta.	EED-Phy-5	FJ917274	FJ917255	FJ917235	–
Nudipleura	Cadlinidae Bergh, 1891	<i>Turbonilla</i> sp. <i>Aldisa smaragdina</i> Ortea, Pérez & Llera, 1982		gb32	New Zealand	EED-Phy-572	FJ917276	FJ917257	FJ917236	EF133489
	Chromodorididae Bergh, 1891	<i>Felimare picta</i> (Schultz in Philippi, 1836)		24	Senegal	NHMUK 20030798	KF992175*	KJ022806*	KJ023043*	KJ022914*
	Pleurobrachiidae Chun, 1880	<i>Berthella martensi</i> (Pilsbry, 1896)		gb48	Panama, Las Secas, Islas sin nombre	MZUCR 6982	HM162683	HM162592	–	HM162498
		<i>Berthella medietas</i> Burn, 1962		gb30	Victoria, Australia		FJ917491	FJ917433	FJ917473	–
		<i>Berthellina edwardsi</i> (Vayssière, 1896)		17	Cape Verde	NHMUK 20030794	KF992165*	–	KJ023053*	KJ022924*
		<i>Pleurobranchus membranaceus</i> (Montagu, 1815)		gb31	Mediterranean Sea, France		FJ917496	FJ917437	FJ917478	–
Pteropoda (Thecosomata)	Cliidae Jeffreys, 1869	<b><i>Clio pyramidata</i> Linnaeus, 1767</b>		gb2	Canary Islands		DQ238000	–	DQ237986	EF133477
Pteropoda	Creseidae Rampal, 1973 (Gymnosomata)	<i>Creseis</i> sp.		gb49	GenBank	<i>Creseis</i> sp. GG-2005	DQ280021	–	DQ279989	DQ280012
		Pneumodermatidae Latreille, 1825	<b><i>Spongiobranchaea australis</i> d'Orbigny, 1834</b>	gb3		Scotia Arc, Antarctica	DQ238002	–	–	DQ237988
–		<b><i>Pneumoderma violaceum</i> d'Orbigny, 1834</b> (in GenBank as <i>P. atlanticum</i> )		gb4	Atlantic Ocean off USA		DQ238003	–	DQ237989	EF133478
Runcinacea	Runcinidae H. Adams & A. Adams, 1854	<i>Runcina africana</i> Pruvot-Fol, 1953		c20	Tenerife, Canary Islands	NHMUK 20030791/1	DQ974680	KJ022780*	DQ927240	KJ022942*
		<i>Runcina divae</i> (Marcus & Marcus, 1963)		359	Ferry Reach, Bermuda	ZMBN 82997	KF992195*	KJ022825*	KJ023024*	KJ022893*
Sacoglossa	Plakobranchidae Gray, 1840	<i>Elysia papillosa</i> Verrill, 1909		gb34	Cuba	MNCN 24.922	HQ616844	HQ616815	–	HQ616869
		<i>Elysia pusilla</i> (Bergh, 1879)		gb38	Pago Bay, Guam	Isolate 09	JQ914601	JQ914630	–	JQ914646
		<b><i>Elysia timida</i> (Risso, 1818)</b>		gb50	Cuba	MNCN 15.05/53680	HQ616847	HQ616818	–	HQ616872

(continued on next page)



Table 1 (continued)

Higher taxa	Family	Species	New taxonomic assignment	Code	Locality	Voucher no.	COI	16S	28S	H3
	Volvatrellidae 1895	<i>Ascobuilla</i> sp. A		c32	Panglao, the Philippines	MNHN 42250	DQ974683	KJ022781*	DQ927243	KJ022883*
		<i>Ascobuilla</i> sp. B		215	Sugarloaf Canal, Sugarloaf Key, Florida, USA	NHMLUK 20070601	KF992169*	KJ022801*	KJ023049*	KJ022919*
Umbraculida	Tyloidiidae Gray, 1847	<i>Tyloдина perversa</i> (Gmelin, 1791)		22	Tenerife, Canary Islands	NHMLUK 20031483	KF992172*	KJ022803*	KJ023046*	KJ022917*
	Umbraculidae Dall, 1889	<i>Umbraculum</i> sp.		gb33	GenBank	EED-Phy-661	GU213058	GU213044	GU213053	-

Annealing temperatures for 16S and for H3 were 51.5 °C and 50 °C, respectively.

For samples that did not amplify with Qiagen Taq, additional 25 µL reactions were set with TaKaRa Ex Taq Polymerase HS (250 U) (Cat. number: RR006A), with 17.35 µL of Sigma water, 2.5 µL of buffer, 2 µL dNTP, 1 µL of each primer, 0.15 µL of TaKaRa Taq, and 1 µL of DNA. A hot start step was included at 94 °C for 5 min followed by five cycles with initial denaturation at 94 °C for 45 s, annealing phase at 45 °C for 30 s, and extension at 72 °C for 1 min, followed by 30 cycles including denaturation at 94 °C for 45 s, gene specific annealing temperature for 30 s (temperatures as for amplification with Qiagen Taq), extension at 72 °C for 1 min, and one final extension at 72 °C for 10 min.

The quality and quantity of PCR products were assessed by gel-electrophoresis following standards methods (see Eilertsen and Malaquias, 2013a). Successful PCR products were purified according to the EXO-SAP method described by Eilertsen and Malaquias (2013a). Sequence reactions were run on an ABI 3730XL DNA Analyser (Applied Biosystems).

### 2.3. Phylogenetic analyses

Sequencher (v. 4.10.1, Gene Codes Corp.) and Geneious (v. 6.1.4 Biomatters Ltd.) were used to inspect, edit, and assemble the chromatograms of the forward and reverse DNA strands. All sequences were blasted in GenBank to check for contamination. Single gene sequences were aligned with Muscle (Edgar, 2004a, 2004b) implemented in Geneious. Alignments were trimmed to a position at which at least 50% of the sequences had nucleotides and missing positions at the ends were coded as missing data (?). All new sequences were deposited in GenBank (Table 1), and the concatenated alignment and consensus tree is listed in TreeBASE (<http://purl.org/phylo/treebase/phylo/works/study/TB2:S17443>).

Blocks of ambiguous data in the single gene alignments were identified and excluded using Gblocks with stringent and relaxed settings (Talavera and Castresana, 2007; Kück et al., 2010; Table 2). Saturation was tested for the first, second, and third codon positions of the protein coding genes COI and H3 using MEGA5 (Tamura et al., 2011) by plotting general time-reversible (GTR) pairwise distances against total substitutions (transitions + transversions). The JModeltest software (Darrriba et al., 2012) was used to find the best-fit model of evolution for each single gene dataset under the Akaike information criterion (Akaike, 1974) (Table 3).

Eight individual gene analyses were initially performed: COI, COI (3rd codon excluded), 16S Gblocks-stringent, 16S Gblocks-relaxed, 28S Gblocks-stringent, 28S Gblocks-relaxed, H3, and H3 (3rd codon excluded).

To determine if the individual gene sets selected for final concatenation had conflicting phylogenetic signals the incongruence length difference test (ILD) (Farris et al., 1995a, 1995b), implemented in PAUP\*4.0 (Swofford, 2003) as the partition homogeneity test was conducted. Prior to running the ILD test all uninformative characters were removed (Lee, 2001). The analysis was run with heuristic search and 500 replicates with ten stepwise random additions, holding one tree at each step, followed by TBR swapping with the multrees function in effect (Farrell, 2014).

Concatenations were based in sequences from the same specimen with the single exception of the samples of *Philine quadripartita* (GenBank Accession Nos: COI: GQ160767, 16S: DQ093482, 28S: DQ279988, H3: DQ093508), in which the COI sequence belongs to a different specimen from the one which yielded the other three sequences. This was done because both specimens were collected at the same time from the same population in Tjärnö, Sweden (Aktipis and Giribet, 2012).

The Bayesian analyses were performed in MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) using three parallel runs of 15 million generations for the single gene analyses and 25 million generations for concatenated datasets, with sampling every 100 generations. Concatenated datasets were partitioned by gene and each partition was run under the best-fit model of evolution. The majority of Bayesian analyses were run through the Lifeportal data cluster of the University of Oslo (Kumar et al., 2009; Bioportal, 2013). Convergence of runs was inspected in Tracer v1.5 (Rambaut and Drummond, 2007) with a burn-in set to 25%. Additionally a maximum likelihood analysis was performed on the combined dataset of all-genes using RAxML v.7.2.6 (Stamatakis, 2006). The analysis was run for 20 replicates with a random seed using the GTR+G+I model with the dataset partitioned by gene and 1000 bootstrap replicates were generated. Consensus phylogenies were annotated and converted to graphics in FigTree v1.3.1 (Rambaut and Drummond, 2009), and final adjustments were made in Inkscape 0.48.4 (Inkscape Team, 2013) and Gimp 2.8.10 (Mattis et al., 1995; Natterer and Neumann, 2013).

### 3. Results

#### 3.1. Sequence analysis

The COI and 16S rRNA genes better resolved relationships at family and genus levels, whereas the H3 gene proved to be better for recovering generic diversity, as found previously in other studies of Heterobranchia gastropods (e.g. Dinapoli et al., 2006); the 28S rRNA gene yielded better resolution at family level (see Figs. S1–S4). The result of the ILD test may imply potential conflicting signals between the four genes ( $P = 0.002$ ), but according to Cunningham (1997) values of  $P < 0.001$  can be a more realistic criterion for rejection of combinability than the originally proposed level of 0.05 (Farris et al., 1995a, 1995b). Phylogenetic incongruence between genes is widespread and expected as unlinked genes can have different evolutionary histories (Huelsenbeck et al., 1996; Maddison, 1997; Rokas et al., 2003), and several studies with gastropods have already showed that even when the ILD test shows incongruence between data sets the combined analysis can provide better resolved trees (e.g. Collin, 2003; Vonnemann et al., 2005; Williams and Ozawa, 2006; Malaquias et al., 2008; Camacho-García et al., 2014). This is clear the case of the current study where single genes (Figs. S1–S4), mitochondrial genes combined (Fig. S5), and nuclear genes combined (Fig. S6) led to trees poorly resolved when compared with the all-genes combined analysis (Figs. 1 and 2).

Both COI and H3 genes showed saturation in third codon positions. Because third codon positions may contain phylogenetic information (Williams and Ozawa, 2006; Malaquias et al., 2009), gene analyses with and without third codons were performed. In both cases the tree topology was better resolved when third codon

**Table 2**  
Gblocks masking parameters.

	16S relaxed	16S stringent	28S relaxed	28S stringent
Min. nr seq. for conserved pos.	80	80	75	75
Min. nr seq. for flank pos.	134	134	125	125
Max. nr contig. nonconserved pos.	12	12	12	12
Min. length of block	5	5	5	2
Allowed gap pos.	All	Half (none)	All	Half (none)
Gblocks alignment	469	346	1116	857

**Table 3**  
Best-fit models and parameters calculated in JModeltest.

Parameters	COI	16S rRNA	28S rRNA	Histone 3
Dataset	3rd codon included	Relaxed masking	Relaxed masking	3rd codon included
No. of specimens used in this study	160	158	145	133
No. of included characters	644	469	1116	330
Best-fit model	TVM+I+G	GTR+I+G	GTR+I+G	TVM+I+G
Frequency A	0.4976	0.3724	0.1465	0.2116
Frequency C	0.1137	0.0973	0.3225	0.3892
Frequency G	0.1122	0.1532	0.3541	0.1789
Frequency T	0.2765	0.3771	0.1769	0.2203
$\Gamma$ shape (G)	0.3540	0.4770	0.5960	0.9840
Proportion of invariant sites (I)	0.3210	0.2260	0.2550	0.5330
R-matrix [A–C]	6.2048	1.0960	0.6708	1.8747
R-matrix [A–G]	74.7204	5.2805	1.7750	5.3739
R-matrix [A–T]	4.5018	2.2867	1.7551	2.2576
R-matrix [C–G]	13.8178	0.4358	0.3803	1.4908
R-matrix [C–T]	74.7204	7.6525	4.9105	5.3739
R-matrix [G–T]	1.0000	1.0000	1.0000	1.0000

positions were included (Figs. S1 and S4). The results of the Gblocks analyses under relaxed settings yielded better resolved trees (Figs. S2 and S3).

Therefore, final analyses were made based on the COI with third codon (644 bp; 160 sequences), H3 with third codon (330 bp; 133 sequences), 16S-relaxed (469 bp; 158 sequences), and 28S-relaxed (1116 bp; 145 sequences) datasets. In addition to the individual gene analyses, three concatenated datasets were tested: combined mitochondrial genes (COI + 16S rRNA; 174 sequences), combined nuclear genes (28S rRNA + H3; 164 sequences), and all-gene markers combined (175 sequences).

#### 3.2. Monophyly of Cephalaspidea

Bayesian and maximum likelihood analyses were largely congruent particularly concerning terminal nodes (i.e. family level), with the former often retrieving high support values (Fig. 1). The monophyly of Cephalaspidea was supported in the all-genes combined analyses (PP = 1, BS = 88, Fig. 1) with *Diaphana globosa* in a basal position. The monophyly of Cephalaspidea was also supported in the 28S analysis (PP = 0.96, Fig. S3).

In the COI analysis all taxa, but three basal lineages (*Cylichna cylindracea*, *Toledonia globosa*, *Diaphana globosa*) formed a clade with maximum support (PP = 1, Fig. S1), whereas in the 16S analysis the majority of the taxa clustered together, but with no support, with Aglajidae branching separately in the tree (PP = 0.77, Fig. S2). The H3 analysis rendered the Cephalaspidea non-monophyletic with family groups clustering separately in various parts of the tree (Fig. S4). Analysis of the mitochondrial dataset (COI + 16S) equally rendered the Cephalaspidea non-monophyletic with the majority of taxa clustering together with marginal support (PP = 0.93, Fig. S5), but with the basal species *Diaphana globosa*, *Toledonia globosa*, *Cylichna gelida* and *C. cylindracea* branching outside the main Cephalaspidea assemblage (Fig. S5). The analysis of the nuclear dataset (28S + H3) grouped all taxa of the Cephalaspidea together albeit with no support (PP = 0.52, Fig. S6).

#### 3.3. Family level phylogeny of Cephalaspidea

Diaphanidae was found to be polyphyletic in all analyses with the genera *Diaphana*, *Toledonia*, *Colpodaspis* M. Sars, 1870 + *Colobocephalus* M. Sars, 1870 branching off in different parts

of the trees. The species *Diaphana globosa* branched alone and was basal to the remaining Cephalaspidea (PP = 1, BS = 88, Fig. 1).

In the all-genes analysis *Toledonia globosa* was retrieved within a basal clade containing two species of *Cylichna* Lovén, 1846 (Cylichnidae) (PP = 1, Fig. 2; BS = 70, Fig. S7). A clade containing *Colpodaspis* + *Colobocephalus* (Colpodaspididae, new family) was monophyletic in nearly all analyses (16S, 28S, COI + 16S, 28S + H3, all-genes combined) with moderate to maximum support (PP = 1, Figs. 1, S2, S3 and S5; PP = 0.91, Fig. S4; PP = 0.98, Fig. S6; BS = 100, Figs. 1 and S7). Colpodaspididae was sister to Gastropteridae in the combined analysis with marginal support (PP = 0.94, Fig. 2) and with high support in the COI + 16S analysis (PP = 0.99, Fig. S5), but this sister relationship was not supported in the maximum likelihood analysis (BS = 69, Figs. 1 and S7). The Gastropteridae received high support (PP ≥ 0.99, BS = 100, Figs. 1 and 2) in all analyses except for COI (PP = 0.93, Fig. S1) and 16S analysis (Fig. S2).

Cylichnidae was rendered polyphyletic with representatives of the genus *Cylichna* and the “diaphanid” genus *Toledonia* forming a basal clade in the all-genes analysis (Cylichnidae Clade 1; PP = 1, BS = 70, Fig. 1) and *Acteocina* Gray, 1847, a genus traditionally classified in Cylichnidae, branching off elsewhere in the tree (Fig. 1). A clade with *Cylichna* and “diaphanids” was also obtained in the 16S single gene analysis albeit with no support (PP = 0.76).

Haminoeidae without *Mnestia* H. Adams & A. Adams, 1854 (= *Ventomnestia* Iredale, 1936) was found to be monophyletic in the all-genes analysis (PP = 1, BS = 87, Fig. 1). Members of this family also clustered together in the 28S analysis however with no support for the inclusion of *Bullacta* Bergh, 1901 (28S; PP = 0.87, Fig. S3), but a clade with the remaining taxa received maximum support (PP = 1, Fig. S3; BS = 100, Fig. S7). In the 28S + H3 analysis Haminoeidae was not monophyletic because *Bullacta* clustered elsewhere in the tree; however, the remaining Haminoeidae taxa were rendered monophyletic (PP = 1, Fig. S6).

Philinidae was found to be polyphyletic with species clustering in four different clades. The Philinidae *sensu stricto* (with worldwide representatives including the type species *Philine aperta*; Fig. 2), received high support in the all-genes, 28S, 28S + H3 analyses (PP = 1, BS = 89, Figs. 1, S4 and S6), and was not supported in the 16S (PP = 0.82, Fig. S2). This clade was retrieved as sister to the Aglajidae in the combined analysis of all-genes (PP = 1, BS = 79, Figs. 1 and 2) and 28S analysis (PP = 1, Fig. S3) and received marginal support in the 28S + H3 analysis (PP = 0.94, Fig. S6).

The Philinorbidae (new family) represented by West Pacific deep-sea species received maximum support (PP = 1, BS = 100) in all analyses. A sister relationship between the latter clade and *Alacuppa* (new genus) was recovered with maximum support (PP = 1) in the all-genes, COI + 16S, and COI analyses (Figs. 1, 2, S1 and S5). This relationship received marginal support in the 28S analysis (PP = 0.94, Fig. S3), and was not supported in the maximum likelihood analysis of all-genes combined (BS = 59, Fig. 1).

Philinidae Clade 4 with deep-sea West Pacific species received high support in the all-genes, 16S, 28S, COI + 16S and 28S + H3 analyses (Figs. 1, 2, S2, S3, S5 and S6), but was not supported in the COI (PP = 0.82, Fig. S1) and H3 gene (PP = 0.89, Fig. S4) analyses.

The Laonidae, with species from the Atlantic Ocean, received high support in the Bayesian analysis of all-genes combined (PP = 0.98; Figs. 1 and 2) and was nearly supported in the maximum likelihood analysis (BS = 73, Fig. 1). However in the remaining analyses the species *Laona quadrata* showed an unstable position either branching off in a basal position within Laonidae or elsewhere in the tree (16S, PP = 0.82, Fig. S2; COI + 16S, PP = 0.64, Fig. S5; 28S + H3, PP = 0.68, Fig. S6), but the remaining assemblage of laonid taxa was always monophyletic with high support (PP = 0.99–1, Figs. S1–S6). This was also the case for the

maximum likelihood analysis of all genes combined (BS = 100, Fig. S7).

The family Aglajidae was monophyletic with marginal support in the all-genes (PP = 0.93, Figs. 1 and 2) and 28S analyses (PP = 0.94, Fig. S3) and was not supported in the 28S + H3 analysis (PP = 0.88, Fig. S6). In the 16S and COI + 16S analysis the Aglajidae was not monophyletic as *Odontoglaja guamensis* did not cluster within Aglajidae, however the remaining members were supported (16S, PP = 0.98, Fig. S2; COI + 16S, PP = 0.95, Fig. S5). Surprisingly, the Aglajidae was not supported in the maximum likelihood analysis of all-genes combined (BS = 51, Figs. 1 and S7).

The Philinoglossidae was rendered monophyletic with maximum support in the all-genes combined analysis (PP = 1, BS = 99, Figs. 1 and 2) and received no support in the COI + 16S (PP = 0.71, Fig. S5), 28S (PP = 0.85, Fig. S3), and H3 (PP = 0.68, Fig. S4) analyses. The Philinoglossidae was sister to a clade including the Gastropteridae and the new taxon Colpodaspididae (*Colpodaspis* + *Colobocephalus*; PP = 1, BS = 81, Figs. 1 and 2).

The Scaphandridae, with *Scaphander*, and the new family Alacuppidae, with *Alacuppa*, were monophyletic in all analyses with the exception of the H3 and 28S + H3 analyses where *Alacuppa* was rendered polyphyletic (Figs. S4 and S6).

The Retusidae was not monophyletic with species clustering in four non-resolved branches; two branches containing species of *Retusa* Brown, 1827 plus two independent lineages of *Pyrunculus* Pilsbry, 1895 (Figs. 2 and S7). A putative close relationship of Retusidae Bullidae, Rhizoridae (*Volvulella*), and Acteocinidae (*Acteocina*) was hinted by the COI (PP = 1, Fig. S1) and COI + 16S (PP = 0.98, Fig. S5) analyses, but this relationship was not supported in the all-genes analysis (PP = 0.58, BS = 25, Figs. 1, 2 and S7).

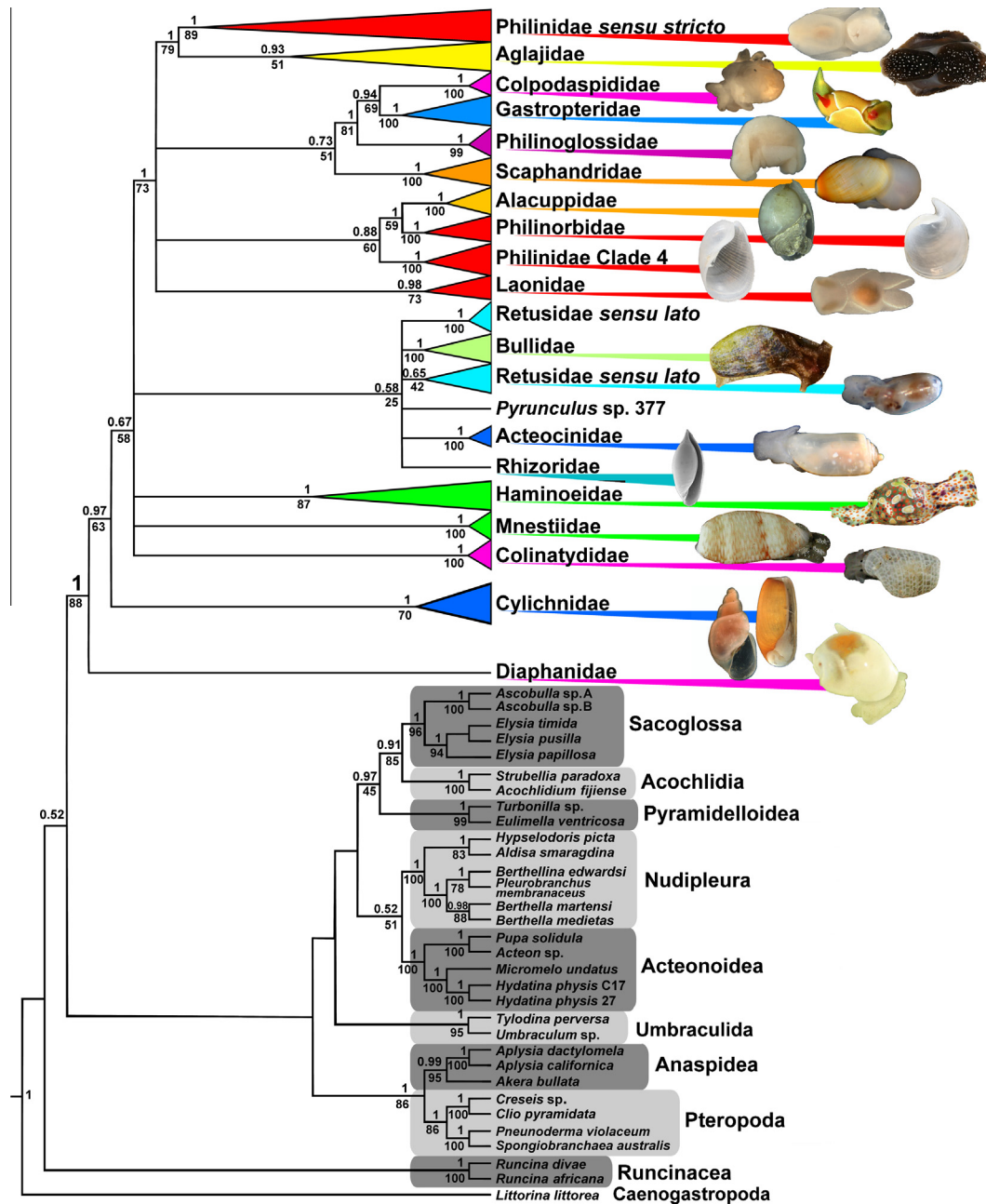
Bullidae was rendered monophyletic with high support in all analyses and received maximum support in the concatenated analysis of all-genes (PP = 1, BS = 100, Figs. 1 and 2). The Rhizoridae was represented by a single species of *Volvulella*, and therefore its monophyly could not be tested.

## 4. Discussion

### 4.1. The monophyly of Cephalaspidea and its main lineages

The expanded taxon sampling used in the current research confirmed the monophyletic status of Cephalaspidea without the Runcinacea and the basal position of the genus *Diaphana* (Diaphanidae). This evolutionary scenario was first suggested based in molecular phylogenetics by Malaquias et al. (2009) and later supported by Jörger et al. (2010).

If the limits of the Cephalaspidea can now be considered well established, the same cannot be said about its internal relationships and composition of families and genera; and the traditional division of the Cephalaspidea in two main evolutionary lineages – Philinoidea and Bulloidea (*sensu* Mikkelsen, 1996) – has been challenged by several phylogenetic studies (e.g. Malaquias et al., 2009; Jörger et al., 2010; Göbbeler and Klusmann-Kolb, 2011). Our results are not conclusive regarding the deep relationships of the Cephalaspidea, but they showed support for a clade Philinoidea including all its traditional members except for Cylichnidae and Retusidae (PP = 1, Fig. 1) (*sensu* Bouchet and Rocroi, 2005) as well as for a clade Haminoeidea (*sensu* Bouchet and Rocroi, 2005; PP = 1, Fig. 1). The relationships of the remaining taxa are obscured by lack of phylogenetic resolution and discovery of high levels of paraphyly in same traditional families (e.g. Cylichnidae) with strong implications for our understanding of the systematics of the group.



**Fig. 1.** Bayesian phylogeny of the Cephalaspeida based on the combined analysis of the mitochondrial COI and 16S rRNA and nuclear 28S rRNA and Histone-3 genes. Figures above branches are posterior probabilities and below branches bootstrap values derived by maximum likelihood analysis. Collapsed clades refer to Cephalaspeida family/clades; gray boxes refer to outgroup taxa used in the analysis. The tree was rooted with the Caenogastropod species *Littorina littorea*.

The current and previous studies on the phylogeny of heterobranch gastropods showed that single gene analyses either based on mitochondrial or nuclear markers are largely insufficient to resolve the relationships of these molluscs. Even the concatenation of few mitochondrial or nuclear genes produce poor results, while the best supported hypotheses are rendered from the combination of mitochondrial and nuclear gene markers (e.g. Wägele et al., 2003; Vonnemann et al., 2005; Klussmann-Kolb et al., 2008; Malaquias et al., 2009; Dinapoli and Klussmann-Kolb, 2010; Jörger et al., 2010). Phylogenetic inference of heterobranch molluscs has been grossly limited to multi-locus approaches typically including three to four genes (COI, 16S rRNA, 18S rRNA, 28S rRNA, Histone-3; see references above), but recently, Kocot et al. (2013) and Zapata et al. (2014) published the first phylogenomic results for heterobranch gastropods, the former including 14 lineages

(no cephalaspid) and the previous 21 lineages (two cephalaspid). Both studies produced well resolved hypotheses and yielded high support for basal nodes and internal relationships anticipating the power of phylogenomics to address relationships in heterobranch molluscs.

#### 4.2. The family level phylogeny

The most recent family classification of the Cephalaspeida was proposed by Malaquias et al. (2009; Tab. 4) and included 13 families, of which two were considered of uncertain taxonomic validity (Bullactidae Thiele, 1926 and Plusculidae Franc, 1968). The authors found the families Cylichnidae, Diaphanidae, and Retusidae to be paraphyletic and reinstated the family names Scaphandridae (for *Scaphander*, previously in Cylichnidae) and Rhizoridae (for

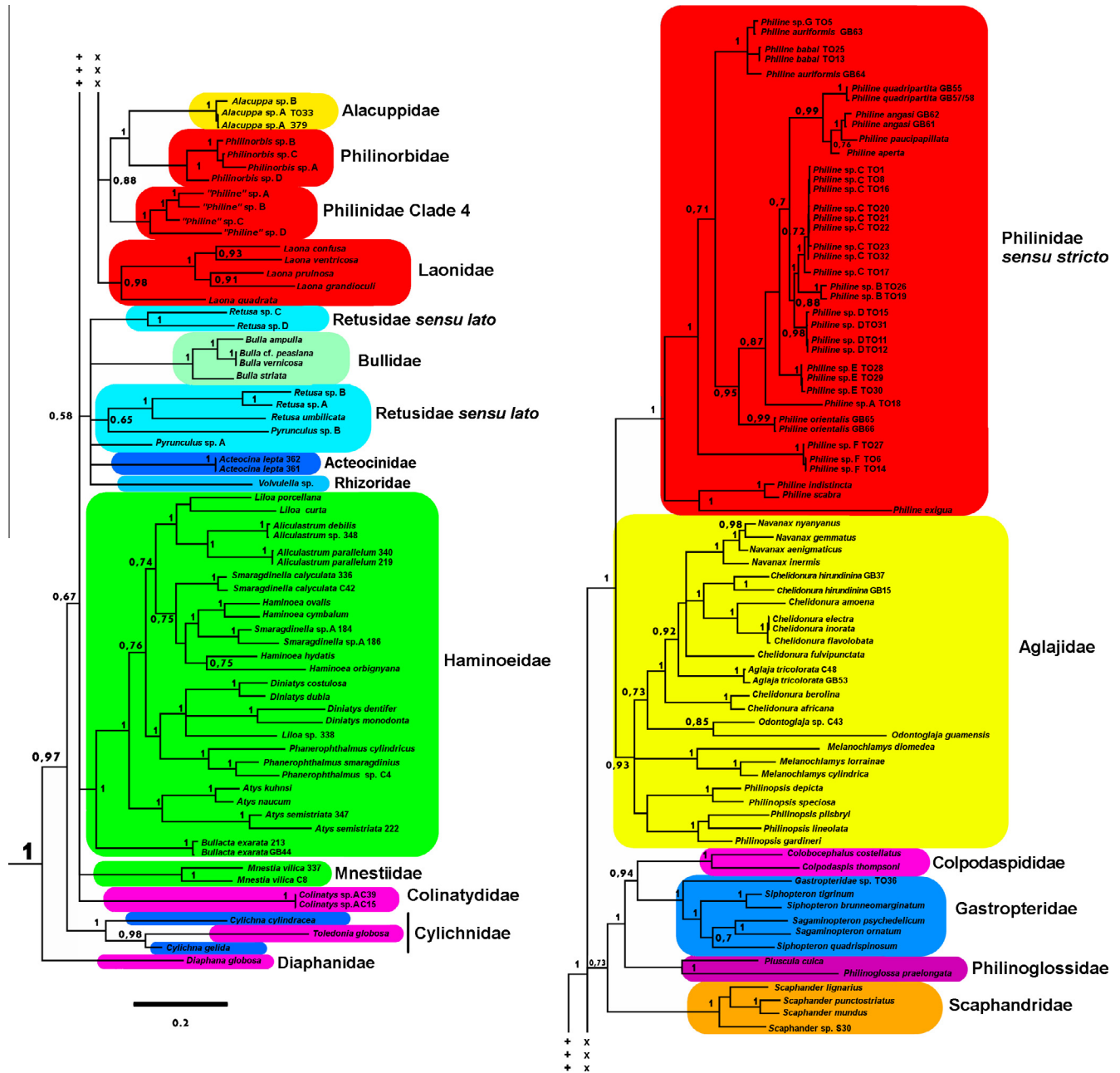


Fig. 2. Same tree as in Fig. 1 depicting the phylogenetic relationships of Cephalaspidea taxa. Outgroups taxa removed for clarity. Figures on nodes are posterior probabilities.

*Volvulella*, previously in Retusidae) to accommodate the new findings. Two families were represented by single species (Philinidae, Philinoglossidae) and therefore their monophyly could not be tested (Malaquias et al., 2009).

The new hypothesis presented here is based on an expanded taxon sampling with better coverage of traditional family and generic diversity (Fig. 2), and yielded a new scenario of the evolution of cephalaspidean gastropods which, in some cases, is radically different from the previously accepted hypothesis (for example in the cases of the families “Diaphanidae” and “Philinidae”), but also confirmed several of its aspects such as the monophyly of the families Aglajidae, Bullidae, and Gastropteridae among others (further discussed below).

#### 4.2.1. Family Acteocinidae

The genus *Acteocina* has been accepted as part of the family Cylichnidae (see Burn and Thompson, 1998; Hori, 2000a; Valdés,

2008; Rosenberg and Gofas, 2014). Nevertheless, Mikkelsen (1996) has highlighted the possibility that *Acteocina* could belong to a different clade from *Cylichna*. Our results are not conclusive about the phylogenetic placement of *Acteocina*, but they strongly suggest exclusion of the genus from the Cylichnidae; none of the phylogenetic analyses supported a close relationship between *Acteocina* and Cylichnidae (*Cylichna*) (Figs. 1, 2 and S1–S7). Species of *Acteocina* have heavily calcified gizzard plates whereas in *Cylichna* they are corneous (Mikkelsen and Mikkelsen, 1987; Burn and Thompson, 1998; Valdés, 2008). The shells of *Acteocina* (and also *Tornatina* Adams, 1850 another genus of “Cylichnidae” not tested here) are characterized by the presence of a moderate spire with a projecting conspicuous heterostrophic protoconch in contrast with the cylindrical shells with sunken spire of the cylichnid genera *Adamnestia* Iredale, 1936, *Austrocylichna* Burn, 1972, *Cylichna*, and *Eocylichna* Kuroda & Habe, 1952 (Burn and Thompson, 1998; Hori, 2000a). These features are unique among

cephalaspidean gastropods and could be regarded as autopomorphies of the group. Therefore, we reinstate the family Acteocinidae (so far considered a junior synonym of Cylichnidae) as valid to reflect these differences and the current phylogenetic hypothesis (see Section 5).

#### 4.2.2. Bullidae, Retusidae sensu lato and Rhizoridae

Taxa of the Retusidae (genera *Retusa* and *Pyrrunculus*) were rendered in three/four different groups and their relationships were not resolved (Figs. 1, 2 and S7), which suggests that Retusidae may be an artificial group in need of systematic revision. The family Rhizoridae was represented in this study by a single species (*Volvulella* sp.) and therefore its monophyly could not be tested.

Taxa of the Retusidae and Rhizoridae clustered together with *Acteocina* (Acteocinidae) and Bullidae, but without support (PP = 0.58, BS = 25, Figs. 1, 2 and S7); although mitochondrial gene analyses yielded strong support for this relationship (COI; COI + 16S; PP = 0.98, Figs. S1 and S5). Mikkelsen (1996) retrieved a similar clade including *Cylichna*, *Acteocina* and *Retusa*, but mentioned that these genera are only superficially similar, while she previously suggested *Retusa* to be closely related to Bullidae/Bulloidea (Mikkelsen, 1993, 1994). Ghiselin (1966) has also pointed out a possible close relationship between Retusidae and Bulloidea based on the presence of a “spermatid bulb” (=blind caecum sensu Malaquias and Reid, 2008). Moreover, based on molecular phylogenetics, Malaquias et al. (2009: Fig. 4A, PP = 0.94) and Göbbeler and Klussmann-Kolb (2011: Fig. 1, PP = 1) have hinted a putative relationship between these two families. Few morphological similarities exist between Retusidae and Bullidae and further molecular and morphological analyses including additional taxa are needed to clarify the systematics of Retusidae and the affinities of these two families.

#### 4.2.3. Scaphandridae and the new family Alacuppidae

Samples from the West Pacific identified originally by us as *Sabatia* Bellardi, 1877 a genus in the family Scaphandridae (Bouchet, 2014), did not cluster together with *Scaphander* (the type genus of Scaphandridae), but as sister to a clade of philinid-like slugs (Figs. 1, 2 and S7). Our provisional identification of these samples followed Valdés (2008: 690, Fig. 52), who ascribed these specimens to *Sabatia* because of similarities of the shell (sculpture and presence of columellar callus). Nevertheless, the author pointed out several differences such as the absence of gizzard plates and the presence of a conspicuous wing expansion in the posterior lip of the shell, which led him to stress that such differences might warrant a new generic assignment that only a phylogenetic study could confirm.

Our results clearly demonstrate that these “*Sabatia*” snails are not part of the family Scaphandridae, but are possibly related to the Philinorbidae, a group of philinids with plate-like internal shells and reduced or vestigial gizzard plates (Philinorbidae; Figs. 1, 2 and S7, PP = 1, BS = 59; discussed below). The first description of a specimen belonging to this morphotype of “*Sabatia*” was ascribed to the genus *Atys* Montfort, 1810 (*Atys supracancellata* Schepman, 1913: pl. 32, Fig. 4), a genus in the family Haminoeidae to which these snails clearly do not belong (Figs. 1 and 2). No names are available to reflect the present phylogenetic classification and we here introduce the family and genus names Alacuppidae and *Alacuppa*, respectively (see Section 5).

#### 4.2.4. Haminoeidae and the new family Mnestiidae

Malaquias et al. (2009) discussed the systematics of the family Haminoeidae and showed that *Smaragdinellidae* (with *Smaragdinella* and *Phanerophthalmus* A. Adams, 1850; sensu Burn and Thompson, 1998) was not a valid family, but a junior synonym of Haminoeidae. Later Malaquias (2010) using a reduced dataset

studied the phylogenetic affinities of the enigmatic monotypic genus *Bullacta* (family Bullactidae; Burn and Thompson, 1998; Bouchet and Rocroi, 2005) and suggested the genus to be the basal lineage of the Haminoeidae, a result corroborated by the present study. Even if the limits of the family Haminoeidae are presently well established, the relationships of its members and generic classification are still in need of a considerable amount of systematic and targeted phylogenetic work, as illustrated by the paraphyletic status of the genera *Haminoea* and *Smaragdinella* rendered in this study (Fig. 2) or by the difficulties to define the genus *Atys* as recently discussed by Too et al. (2014).

*Mnestia* (= *Ventomnestia*) was not recovered together with the other haminoeid genera (Figs. 1 and 2), a result obtained previously by Malaquias et al. (2009) who had regarded the genus to be of uncertain taxonomic position within the Cephalaspeida. The present study confirms the exclusion of *Mnestia* from the Haminoeidae, but the phylogenetic affinity of the genus remains unresolved (Figs. 1 and 2). Early studies have assigned *Mnestia* (as *Ventomnestia*) to the Cylichnidae (e.g. Gould, 1859; Pease, 1860), or to the Retusidae (Mikkelsen, 1996) due to similarities in the shell, but more often to the Haminoeidae (Burn and Thompson, 1998; Carlson and Hoff, 2000). While the ridged gizzard plates of *Mnestia villica* suggest affinity to the Haminoeidae, the radular formula with a central tooth and two lateral teeth on each side does not match the typical haminoeids (Carlson and Hoff, 2000) and also excludes these snails from the Retusidae, which is characterized by lack of radula (Burn and Thompson, 1998).

*Mnestia* has unique features among cephalaspids such as the presence of small, thick, cylindrical and colored shells (shells are white in all other genera with similar shell shapes). The combination of these features together with the presence of spiral striae throughout the shell, presence of radula, ridged gizzard plates, and absorption of the inner whorls of the shell (Burn and Thompson, 1998; Carlson and Hoff, 2000) make this group unique among cephalaspids which is corroborated by molecular phylogenetics (Malaquias et al., 2009; present study). Since no family name is available to reflect the phylogenetic classification we here introduce the new family name Mnestiidae (see Section 5).

#### 4.2.5. Diaphanidae sensu lato

The monophyletic status of the family Diaphanidae has been disputed because of lack of synapomorphies uniting the entire group (reviewed by Ohnheiser and Malaquias, 2014). Malaquias et al. (2009) provided the first molecular evidence supporting the putative non-monophyly of Diaphanidae (with “*Diaphana*” and *Colpodaspis*) and later Jörger et al. (2010) and Göbbeler and Klussmann-Kolb (2011), both including representatives of genera *Diaphana* and *Toledonia*, have retrieved similar results.

The present study, which includes a larger taxon sampling of “Diaphanidae” with representatives of four genera, confirmed the polyphyly of the group and the basal position among cephalaspids of the genus *Diaphana* (with *D. globosa* – a species phylogenetically closely allied to the type species *D. minuta* T. Brown, 1827; see Schiøtte, 1998) (Figs. 1 and 2). *Toledonia globosa* clustered together with *Cylichna* (family Cylichnidae) (PP = 1, BS = 70) and *Colpodaspis* + *Colobocephalus* formed a clade (PP = 1, BS = 100) sister to Gastropteridae (PP = 0.94, BS = 69), while specimens of an undescribed species of “*Diaphana*” from the Philippines (previously used by Malaquias et al. (2009) as true *Diaphana*) branched off alone (*Colinatys*, discussed below) (see Figs. 1 and 2). The affinities of three genera (i.e. *Bogasonia* Warén, 1989, *Woodbridgea* Berry, 1953 and *Newnesia* Smith, 1902) attributed to the family remain to be tested. The Antarctic genus *Newnesia* has a shell and radular inner lateral teeth similar to *Diaphana*, however the unilobed, denticulated rachidian tooth resembles *Bogasonia* and *Toledonia*, which

have shells with raised spires (Smith, 1902; Marcus, 1976; Schiøtte, 1998; Warén, 1989; Ohnheiser and Malaquias, 2014).

**4.2.5.1. *Diaphanidae sensu stricto*.** The *Diaphanidae sensu stricto* are represented in our dataset by the single species *Diaphana globosa*. Thus, the composition of the family/genus remains to be thoroughly tested, but a morphological cladistics analysis by Schiøtte (1998) showed this species to have phylogenetic affinities for example with the type species *D. minuta*, and *D. hiemalis* Couthouy, 1839; furthermore, work in progress based on DNA from northern European cephalaspids confirm that *D. globosa* is closely related to *D. hiemalis* (Ohnheiser and Malaquias, work in progress). The *Diaphanidae sensu stricto* seems to encompass species with a globose external, thin, fragile, and umbilicate shell with a radula formed by bilobed rachidian teeth and arched lateral teeth with fine denticulation along the inner edge (Warén, 1989; Schiøtte, 1998; Ohnheiser and Malaquias, 2014).

**4.2.5.2. *The new family Colpodaspididae*.** Our phylogenetic results rendered a new group formed by the sister lineages *Colobocephalus* and *Colpodaspis*, both traditionally considered to belong in the *Diaphanidae*. The phylogeny suggested affinity of these genera to the families *Gastropteridae* and *Philinoglossidae* (discussed below). The uniqueness of *Colobocephalus* and *Colpodaspis* among diaphanids was recently stressed by Ohnheiser and Malaquias (2014) who claimed these snails to be united by unique features not present in any other diaphanids such as the presence of an internal, sculptured, and globose shell with short, but protruding spires, and radulae lacking rachidian teeth, with smooth hook-shaped lateral teeth (Brown, 1979; Ohnheiser and Malaquias, 2014). No family name is available to reflect this new systematic arrangement, and therefore we here introduce the new family name *Colpodaspididae* (see Section 5).

**4.2.5.3. *The phylogenetic position of Toledonia and the family Cylichnidae*.** An unexpected result of this study was the position in the Cephalaspidea tree of the “diaphanid” genus *Toledonia* (represented by *T. globosa*), which branched within *Cylichnidae sensu stricto* (=genus *Cylichna*) with *Cylichna gelida* and *Cylichna cylindracea* (PP = 1, BS = 70; Fig. 2). Shells of *Toledonia* with their raised spire are very different from those of *Cylichna*, which despite some variability are grossly cylindrical and made up of the last whorl with a flat or slightly involute spire and an aperture running lengthwise to the axis of the shell (Burn and Thompson, 1998). The radulae of these two genera are also quite distinct; whereas *Toledonia* has an unilobed, multi-cusped rachidian tooth and plate-like lateral teeth (Ohnheiser and Malaquias, 2014), the *cylichnid* radula is formed by a broad bilobed denticulate rachidian with several curved laterals depicting denticulation along the inner edge (Marcus, 1976; Gosliner, 1994). Despite these morphological differences the male reproductive system of *Cylichna* and *Toledonia* show some resemblances with an undivided tubular structure.

Based on the phylogenetic results we suggest the inclusion of the genus *Toledonia* in the family *Cylichnidae* (with *Cylichna*). We however stress that inclusion of a larger representation of the diversity of *Cylichnidae* may imply the need to revise the systematics of the family; for example the genus *Cylichna* itself was rendered paraphyletic in our tree, with *Toledonia globosa* and *Cylichna gelida* – two Antarctic species – showing closer affinities than the two species recognized in the genus *Cylichna* (Fig. 2). Jensen (1996) and Schiøtte (1998) suggested that *Toledonia* could be closely related to *Newnesia* and *Bogasonia* forming their own family (for which the name *Toledoniidae* would be available), but the latter two genera have not been tested in the present study. Moreover, the results of both criteria of analyses (Bayesian inference and maximum likelihood) rendered conflicting results.

Whereas, in the Bayesian analysis *Cylichnidae* with *Toledonia* received maximum support (PP = 1, Figs. 1 and 2), the maximum likelihood analysis did not render support for this clade (BS = 70, Figs. 1 and S7).

Burn and Thompson (1998) and Malaquias et al. (2009) stressed the fact that *Cylichnidae* as traditionally defined (*sensu* Burn and Thompson, 1998) is one of the most diverse families of the Cephalaspidea, but nevertheless no synapomorphies exist to unite the family as highlighted by Mikkelsen (1996). Thus, it is not surprising that DNA-based phylogenetic studies are separating the family in distinct evolutionary lineages. First, Klussmann-Kolb et al. (2008) showed that *Scaphander* was not closely allied to *Cylichna*, a result corroborated by Malaquias et al. (2009), Jörger et al. (2010), and Göbbeler and Klussmann-Kolb (2011). In the present study we re-assign the genus *Acteocina* to its own family (*Acteocinidae*; discussed above) and show that a morphotype previously recognized as *Sabatia* in the family *Cylichnidae* (Valdés, 2008) corresponds to a new evolutionary lineage to which we introduce a new family and generic names (*Alacuppidae* and *Alacuppa*; discussed above). Further inclusion of representatives from other genera (e.g. *Roxania* Leach, 1847) will certainly contribute to the redefinition of the family *Cylichnidae*.

#### 4.2.6. *The new family Colinatydidae*

Our results provide clear evidence that specimens from the Philippines cited by Malaquias et al. (2009) as *Diaphana* sp. (Voucher MNHN 42244 and MNHN 42254) belong to a lineage of cephalaspids different from the true *Diaphana* (represented here by *Diaphana globosa*; discussed above).

A reassessment of the morphology of the Philippine specimens shows that they are different from the animals with globose and smooth shells characterizing *Diaphana*. The Philippine species appears to be conspecific with material from Hawaii illustrated (as *Diaphana* sp.1) by Pittman and Fiene (2013). This group is characterized by the presence of quadrangular shells wider anteriorly, with slightly convex sides, covered by a conspicuous reticulated pattern of whitish squares, unique among cephalaspidean shells (see Espinosa and Ortea, 2004; Valdés et al., 2006; Pittman and Fiene, 2013). A species of this group is also known in the Caribbean Sea, western Atlantic. First described as *Alys alayoi* by Espinosa and Ortea (2004), it was later assigned to *Retusa* (Valdés et al., 2006; Redfern, 2013), and recently made the type of a new genus *Colinatys*, classified in the family *Haminoeidae* based on the presence of radula with rachidian tooth and two lateral teeth (Ortea et al., 2013).

Our phylogeny shows that these snails do not belong in the family *Haminoeidae* and no family name is available. Therefore, we introduce the name *Colinatydidae* (see Section 5).

#### 4.2.7. *The enigmatic Notodiaphanidae*

An overview of the taxonomic confusion surrounding the controversial family *Notodiaphanidae* can be found in the studies of Burn and Thompson (1998) and Ortea et al. (2013). In brief, Thiele (1917, 1931) introduced the genus *Notodiaphana* Thiele, 1917 and family *Notodiaphanidae* Thiele, 1931 for a shell described by Vélain (1877) as *Bulla fragilis* Vélain, 1877 and later transferred by Pilsbry (1895–1896) to *Diaphana*. Thiele (1912) allegedly redescribed Vélain’s species based on a new specimen from the same locality (St Paul I.) in the Indian Ocean, but nevertheless with a shell considerably different from that of Vélain (see Thiele, 1912: 277, pl. 19, Fig. 17; Thiele, 1931; Burn and Thompson, 1998: 959, Fig. 16.39; Ortea et al., 2013: 16, Figs. 1 and 2). Thiele found his specimen to have a radula with a unique morphology, and this was the base that led him to establish the new genus and family (Thiele, 1912, 1917, 1931). However, the differences in shell shape between Thiele’s and Vélain’s specimens

strongly suggest that Thiele had misidentified his material. The uniqueness of the radula depicted by Thiele (1912) even led Burn and Thompson (1998) to speculate that the radula could have been damaged with the inner laterals representing parts of a broken central tooth. Because of this confusing situation Notodiaphanidae has been considered by several authors (e.g. Burn and Thompson, 1998; Malaquias et al., 2009) a family of *incertae sedis* position in the Cephalaspidia.

Ortea et al. (2013) recently described the new species *Notodiaphana atlantica* Ortea, Moro & Espinosa, 2013 from the (sub)tropical Atlantic, based on claimed similarities of the shell, radula, and absence of gizzard plates. We have some difficulties to accept this taxonomic assignment, first because of the challenge to ascertain what is *Notodiaphana*, and second because of the mismatch between the description by Ortea et al. (2013) and those of Vélain (1877) and Thiele (1917, 1931). For example, the radula of *N. atlantica* is described as asymmetrical with a different teeth count (three lateral teeth in *N. fragilis* vs. two in *N. atlantica*); the shape of the radular teeth is different with the second lateral tooth hook-shaped in *N. atlantica* and plate-like in *N. fragilis* (see for comparison Thiele, 1912: 277, pl. 19, Fig. 22; Thiele, 1931 [1992]: 637–638, Fig. 481; Ortea et al., 2013: 18, Fig. 4A–E); the shells are different with dense spiral striae in *N. fragilis* and a reticulate pattern in *N. atlantica* (see Valdés et al., 2006: 22 as *Retusa* sp.1; Ortea et al., 2013: 24, L1; Redfern, 2013: 256–257, Fig. 722A–D as *Retusa* sp.).

Our phylogenetic results have highlighted the complexity of the taxonomy of diaphanid–cyllichnid–retusid-like taxa, which is far from understood and in need of much work including broader taxon sampling across oceans. Hence, we suggest that the names *Notodiaphana* and Notodiaphanidae should be treated as *nomina dubia*, until freshly collected material from St Paul Island allows anatomical and molecular investigations.

#### 4.2.8. A common origin for the Philinoglossidae, Colpodaspididae, and Gastropteridae?

Malaquias et al. (2009) have considered the family Plusculidae of uncertain taxonomic validity and Brenzinger et al. (2013) after a thorough anatomical investigation of its type species – *Pluscula cuica* – have regarded Plusculidae a synonym of Philinoglossidae, a conclusion supported by our phylogenetic results (Fig. 1).

Previous studies suggested a close relationship between the Gastropteridae and Philinoglossidae (Malaquias et al., 2009; Göbbeler and Klussmann-Kolb, 2011), but the phylogenetic affiliation of the Colpodaspididae (*Colobocephalus* and *Colpodaspis*) was never adequately tested because of limitations in taxon sampling. Malaquias et al. (2009) had included a representative of the Colpodaspididae (*Colpodaspis thompsoni*), but their results were weakened by the fact that a single gene (28S rRNA) was sequenced, which nevertheless supported a sister relationship between Colpodaspididae and the Gastropteridae + Philinoglossidae. The current study rendered strong support for a close relationship between the three lineages (PP = 1, BS = 81, Fig. 1) and Bayesian analysis suggested a sister relation between Colpodaspididae and Gastropteridae (PP = 0.94, Fig. 1), which however was not corroborated by the maximum likelihood analysis (BS = 69, Fig. 1).

The Colpodaspididae and Gastropteridae have several morpho-anatomical resemblances, but the true extent of homology remains to be tested. For example, Gosliner (1989) recognized the presence of an anterior cleft foot as an apomorphy of the Gastropteridae, a character also present in *Colobocephalus* and *Colpodaspis*. This character may constitute instead a synapomorphy of the clade Colpodaspididae + Gastropteridae that was lost in several lineages of the Gastropteridae. Another possible synapomorphy is the presence of an elaborate cephalic shield with chemoreceptors. *Colpodaspis* and *Colobocephalus* have developed, enrolled tentacles (Ohnheiser and Malaquias, 2014) and the Gastropteridae have a

siphon in the posterior part of the cephalic shield (Gosliner, 1989); all these structures possibly act as chemosensors.

The Philinoglossidae retain a radula that has an arrangement and shape largely similar to that of Gastropteridae (Gosliner, 1989, 1994; Cadien, 1998; Behrens, 2004) and interestingly the male genital opening in Philinoglossidae is located inside the mouth (Marcus, 1953, 1959; Marcus and Marcus, 1958; Salvini-Plawen, 1973; Brenzinger et al., 2013), a configuration closer to that found in the Gastropteridae, where the male genital opening is located under the anterior part of the cephalic shield (Anthes and Michiels, 2007a, 2007b). Yet, this may result from an adaptation to a meiofaunal lifestyle, as other interstitial groups of gastropods also have an anteriorly orientated male reproductive system (Swedmark, 1964; Challis, 1969; Jörger et al., 2008, 2009; Brenzinger et al., 2013).

#### 4.2.9. The Philinidae sensu lato

Gonzales and Gosliner (2014) were the first to question the monophyly of the family Philinidae. Based on a 16S rRNA phylogenetic analysis including representatives of Philinidae and Aglajidae, the authors found that philinids with pigmented bodies and plate-less muscular gizzards had stronger affinities with the Aglajidae. Nevertheless, the use of a single gene marker, reduced representation of outgroups, and general lack of node support, hampered any sound conclusion and led the authors to wish for more studies including larger taxon and character sampling (Gonzales and Gosliner, 2014).

Our study included a broader taxon sampling and geographical coverage of philinids and rendered strong support for the polyphyly of the family, with at least four independent lineages (Fig. 1). In addition, it has unravelled a large diversity of unknown West Pacific deep-sea philinids, which will be the focus of a dedicated paper (Oskars and Malaquias, in preparation).

4.2.9.1. *Philinidae sensu stricto*. A clade with philinids including *Philine aperta*, the type species of the type genus of the family, was rendered with maximum support and was sister to the family Aglajidae (PP = 1, BS = 79). This close relationship was suggested earlier based on morphological evidence (Guiart, 1901; Boettger, 1954; Rudman, 1978; Gosliner, 1980) and previous molecular phylogenies (e.g. Malaquias et al., 2009; Göbbeler and Klussmann-Kolb, 2011).

Taxa in the *Philinidae sensu stricto* can be differentiated from other philinid lineages by the shared presence of a muscular gizzard with calcified plates, developed penial papilla, and a long convoluted prostate (Price et al., 2011; Gonzales and Gosliner, 2014; Oskars and Malaquias, in preparation).

4.2.9.2. *The new family Philinorbidae*. Another lineage of philinids branched off separately from the *Philinidae sensu stricto* and received maximum support (Philinorbidae; PP = 1, Fig. 1). Species in this clade exhibit a long and rhomboid cephalic shield; an internal plate-like shell that can be flat or concave, smooth or exhibiting linear spiral striae (Mattox, 1958; Marcus, 1974; Habe, 1950, 1976; Hori, 2000b; Chaban, 2011; Price et al., 2011; Oskars and Malaquias, in preparation).

The anatomy of these species (Oskars and Malaquias, in preparation) hints of a close relationship with the well-described species *Philine alba* Mattox, 1958 (from the Eastern Pacific) and *Philine alboides* Price, Gosliner & Valdés, 2011 (from the Caribbean) (Mattox, 1958; Marcus, 1974; Price et al., 2011), and to species ascribed to the elusive West Pacific genera *Pseudophiline* (*P. hayashii* Habe, 1950) and *Philinorbis* (*P. teramachii* Habe, 1976) (Habe, 1950, 1976; Hori, 2000b; Chaban, 2011). They share the unique presence of a distinctly rounded shell with a shoulder extending beyond the apex, a non-muscular gizzard with



reduced (or even absent) brown chitinous gizzard plates, a radula that has short, broad and hook-shaped inner lateral teeth, which are often smooth, but sometimes finely denticulated, and a penial atrium embedded in the tissue anteriorly to the body cavity, while the prostate is free within the body cavity (Mattox, 1958; Kitao and Habe, 1982; Price et al., 2011; Chaban, 2011; Gonzales and Gosliner, 2014; Oskars and Malaquias, in preparation).

Two generic names *Philinorbis* and *Pseudophilinae* are available for species in this clade. These genera only differ by the presence of spiral striae on the shell and Chaban (2011) considered *Pseudophilinae* Habe, 1976 to be a synonym of *Philinorbis* Habe, 1950. Therefore, we ascribe species in this clade to the genus *Philinorbis* and introduce the family name *Philinorbidae* to reflect the present phylogeny (see Section 5) and suggest future usage of these names for species characterized by the aforementioned synapomorphies.

**4.2.9.3. The family Laonidae.** An additional clade of philinid snails was rendered monophyletic (Laonidae; PP = 0.98, BS = 73, Figs. 1, 2 and S7). Species in this clade are characterized by the unique presence of a parietal wall extending into the posterior half of the shell aperture and a non-muscularized gizzard lacking plates (Ohnheiser and Malaquias, 2013: see descriptions of *P. confusa*, *P. grandioculi*, *P. quadrata*, *P. pruinosa*, *P. ventricosa*; Oskars and Malaquias, in preparation). All species except one also have umbilicated shells with smooth or net-like surface, but *P. quadrata* has a non-umbilicated quadrangular shell sculptured with chain-like spiral lines (Ohnheiser and Malaquias, 2013). These differences and the fact that this latter species branched in a basal position sister to all others, which together received maximum support in both Bayesian and maximum likelihood analyses (Figs. 2 and S7), might eventually reflect a different phylogenetic affiliation that only future studies using additional taxa can confirm.

A. Adams (1865) introduced the name *Laona* (type species *Philine* (*Laona*) *zonata* from O-Sima, Yohuko, Japan) for philinids with a net-like sculpture covering the shell and suggested that the European species *Philine pruinosa* should be transferred to this group. Nothing is known about the anatomy of *Laona zonata*, but its shell shares the net-like sculpture with *P. pruinosa* and it possesses a parietal wall extending into the aperture (Habe, 1976: 157, pl. 1, Figs. 1–3) that characterizes the species in this clade. On the other hand “*Philine*” (*Laona*) *pruinosa* was included in this study and branched together with the other species of this clade. Pruvot-Fol (1954) had erected the subfamily Laoninae to encompass species of the *Philinidae* that lacked gizzard plates and had a colored periostracum, and she included *Laona pruinosa* in this subfamily.

Therefore, it is here suggested to reinstate as valid the genus *Laona* (considered by Ohnheiser and Malaquias (2013) a synonym of *Philine*) and to raise Laoninae to family level to include all philinid species with the aforementioned characters (see Section 5).

**4.2.9.4. *Philinidae* Clade 4.** *Philinidae* Clade 4 (PP = 1, BS = 100, Figs. 1, 2 and S7) includes an assemblage of species characterized by unique traits such as the presence of a thick, well-calcified shell with a developed columellar callus, and a non-muscular gizzard with brown chitinous plates lightly calcified on the active surface. The radula can be either absent or present with a reduced rachidian tooth (Oskars and Malaquias, in preparation).

Two species not included in this study, which based on morphological similarities could be closely allied, are *Philine gibba* Strebel, 1908 and *Philine falklandica* Powell, 1951 (Rudman, 1972: 173, 174, Figs. 1a–b, 2a), but this remains to be confirmed. At present there is no genus or family names available for this clade and none of the described species can be attributed with certainty to it. Formal

species descriptions and generic and familial assignments will take place elsewhere (Oskars and Malaquias, in preparation).

#### 4.3. Conclusions and a revised classification for the Cephalaspidea

The results obtained with this study stressed the importance of taxon sampling to infer phylogenetic relationships in the Cephalaspidea. Preconceived ideas of monophyly can lead to a misleading choice of the taxa to be included in analyses and thus to biased results. Future work should account for this bias and include taxa covering the generic and/or morphological disparities of each traditional family. Perhaps the most striking example that emerged from this study was the polyphyly of *Philinidae* with its four distinct clades. Most likely this is not even the “end of the story” since the generic diversity of some of these clades is most likely under-represented (e.g. *Philinorbidae*, *Laonidae*, and *Philinidae* Clade 4). For example the level of inter-specific genetic and morphological variability recognized in Clade 4 is very high (discussed above), and it would not be surprising if the inclusion of additional taxa allied to these clades unravels additional lineages requiring further taxonomic breakdown.

Based on a 16S rRNA phylogeny including 11 species, Gonzales and Gosliner (2014) were the first to suggest the possible non monophyletic status of *Philinidae*, but their results were not statistically supported. They hypothesized that philinid species with pigmented bodies and muscular gizzards without plates could be more closely related to Aglajidae slugs (the latter family characterized by pigmented bodies and muscular gizzards lacking plates) than to other philinids. They speculated that the European species *Philine quadrata* – characterized by a pigmented-body and a plate-less gizzard – could also be part of a clade made up of pigmented-body philinids. Unfortunately we did not have the opportunity to include in this analysis Indo-West Pacific colored philinid species, but we did include *P. quadrata*, which branched off alone within *Laonidae* (Fig. 2), suggesting a possible relationship to species not represented in our analysis. The relationships and phylogenetic affiliation of the “colored and plate-less” philinids remains to be thoroughly tested and should constitute a topic of future studies in cephalaspidean systematics.

Another group that should be prioritised in future Cephalaspidea research is the family Retusidae, here rendered paraphyletic. The systematics of Retusidae is complicated by the small size of most species, with whitish, nearly smooth, shells that are sometimes difficult to distinguish from those of other families (e.g. *Cylichnidae*). Our results do not reject a possible monophyly of Retusidae, but the phylogenetic status, affinities, and composition of the group need to be a topic of future analysis including a better representation of its diversity, geography (Atlantic + Indo-Pacific), and ecology (shallow + deep-sea species).

The monophyletic status of the family *Cylichnidae* as traditionally defined has been questioned (e.g. Mikkelsen, 1996; Malaquias et al., 2009) and we here further demonstrate that this group is an artificial assemblage of taxa. Yet, additional work is still necessary to clarify the composition of the family; for example the phylogenetic position of the “cylichnid” genus *Roxania* remains untested and the affinity of *Cylichna* to the genus *Toledonia* must be further investigated.

The much debated monophyletic status of *Diaphanidae* was, at least in part, clarified, but several taxa remain untested like the elusive Antarctic monospecific genus *Newnesia*.

Sound evidence for the exclusion of *Mnestia* from *Haminoeidae* was produced, but the relationships of this lineage are not yet resolved. Also the phylogenetic affinities of the rare “haminoeid” genus *Cylichnium* Dall, 1908 remain to be tested.

For the first time four gene markers were used to infer relationships at family level in Cephalaspidea gastropods. Even so, it is

evident that this is not sufficient to resolve the basal relationships within the group. Most sister relationships between families of cephalaspids remain unknown. This can be the result of inadequacy of these genes to recover phylogenetic signal in deep time – Jörger et al. (2010) and Zapata et al. (2014) estimated that the origin of Cephalaspidea lineages can be as old as 170–160 My (medium age estimate ca. 120–105 My) – and/or an effect of incomplete taxon sampling. Despite our efforts to include a broad representation of the cephalaspidean diversity we acknowledge that several representatives of important groups were still not included or have been misrepresented (e.g. *Cylichnium*, *Hamineobulla* Habe, 1950, *Newnesia*, *Noalda* Iredale, 1936, *Roxania*, “colored philinids”, philinoglossids, cylichnid-like genera – *Acteocina*, *Tornatina*, *Paracteocina* Minichev, 1966).

As an outcome of our results we attempt a new operational classification for the Cephalaspidea contemplating all resolved and unresolved nodes of our phylogeny (Table 4). We consider four main groups of superfamily status; two received maximum support in our Bayesian phylogenetic hypothesis, namely Haminoeidea and Philinoidea (and moderate to marginal support in the maximum likelihood analysis; BS = 87 and 73, respectively, see Fig. 1), one was not supported – the Bulloidea, and one is polyphyletic – Diaphanoidea. These four groups correspond to the traditional superfamily division of the Cephalaspidea (see Burn and Thompson, 1998; Bouchet and Rocroi, 2005), but the composition of each of them was drastically rearranged (Table 4). Undoubtedly, subsequent work will lead to new modifications of this classification, but this is the fate of any classification.

## 5. Taxonomic section

### Acteocinidae Dall, 1913

**Diagnosis:** Adult shells 2–4 mm high, white, smooth, fine axial lines can be present, thick, cylindrical, apex slightly projecting as a moderate spire; protoconch conspicuously protruding on top of spire. Radula formula 1.1.1; bilobed denticulate rachidian teeth, large curved denticulated lateral teeth. Gizzard plates calcified (Mikkelsen and Mikkelsen, 1984, 1987; Burn and Thompson, 1998).

**Type genus:** *Acteocina* Gray, 1847; **Type species:** *Acteon wetherellii* Lea, 1833; by original designation; New Jersey, USA, Miocene.

### Alacuppidae Oskars, Bouchet, and Malaquias n. fam.

**Combined family and genus diagnosis (ICZN Art. 13.5):** Adult shells 7–10 mm high, with only one whorl visible, solid, oval, with convex sides. Apex not umbilicated. Lip expanding posteriorly beyond shell. Columella thick. Sculpture of several punctuated spiral grooves more densely concentrated towards posterior end. Gizzard plates absent. Radula 1.0.1, with smooth hook-shape lateral teeth (see Valdés, 2008: 690, Fig. 52).

**Type genus:** *Alacuppa* Oskars, Bouchet, and Malaquias, new genus; **Type species:** *Atys supracancellata* Schepman, 1913; 535 m depth, Sulu Archipelago, the Philippines (6°4.1'N–120°44'E), tropical west Pacific.

**Etymology:** The name of this genus derives from the presence of a wing-like projection in the posterior part of the lip (Lat. *ala*) and the barrel shape of the shell (Lat. *cuppa*).

### Mnestiidae Oskars, Bouchet, and Malaquias n. fam.

**Diagnosis:** Adult shells about 5 mm high, thick, cylindrical, and colored with brown–reddish flames or bands in a whitish background; spiral striae present throughout shell. Radular formula 2.1.2. Gizzard plates with 6–7 ridges (Burn and Thompson, 1998; Carlson and Hoff, 2000).

**Type genus:** *Mnestia* H. Adams and A. Adams, 1854; **Type species:** *Bulla marmorata* A. Adams, 1850; by subsequent designation

**Table 4**

New taxonomic classification for the Cephalaspidea.

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Order Cephalaspidea Fischer, 1887
Superfamily “Bulloidea” Gray 1827 [not monophyletic]
Family Bullidae Gray, 1827
<i>Bulla</i>
Family “Retusidae” Thiele, 1925 [not monophyletic]
<i>Pyrunculus</i>
<i>Retusa</i>
Family Rhizoridae Dell, 1952
<i>Volvulella</i>
Family Acteocinidae Dall, 1913
<i>Acteocina</i>
Superfamily Haminoeidea Pilsbry, 1895
Family Haminoeidae Pilsbry 1895
<i>Aliculastrum</i>
<i>Atys</i>
<i>Bullacta</i>
<i>Diniatys</i>
<i>Haminoea</i>
<i>Liloa</i>
<i>Phanerophthalmus</i>
<i>Smaragdinella</i>
Superfamily Philinoidea Gray (1850) 1815
Family Philinidae Gray, 1850 (1815)
<i>Philine</i>
Family Aglajidae Pilsbry 1895
<i>Aglaja</i>
<i>Chelidonura</i>
<i>Melanochlamys</i>
<i>Navanax</i>
<i>Odontogljaja</i>
<i>Philinopsis</i>
Family Colpodaspididae Oskars, Bouchet, and Malaquias n. fam.
<i>Colobocephalus</i>
<i>Colpodaspis</i>
Family Gastropteridae Swainson, 1840
<i>Gastropteron</i>
<i>Siphopteron</i>
<i>Sagaminopteron</i>
Family Philinoglossidae Hertling, 1932
<i>Philinoglossa</i>
<i>Pluscula</i>
Family Scaphandridae G. O Sars, 1878
<i>Scaphander</i>
Family Alacuppidae Oskars, Bouchet, and Malaquias n. fam.
<i>Alacuppa</i> n. gen.
Family Philinorbidae Oskars, Bouchet, and Malaquias n. fam.
<i>Philinorbis</i>
Family Laonidae Pruvot-Fol, 1954
<i>Laona</i>
Family Philinidae Clade 4
“ <i>Philine</i> ”
Superfamily “Diaphanoidea” Odhner, 1914 (1857) [not monophyletic]
Family Mnestiidae Oskars, Bouchet, and Malaquias n. fam.
<i>Mnestia</i>
Family Colinatydidae Oskars, Bouchet, and Malaquias n. fam.
<i>Colinatys</i>
Family Cylichnidae H. Adams & A. Adams, 1854
<i>Cylichna</i>
<i>Toledonia</i>
Family Diaphanidae Odhner, 1914 (1857)
<i>Diaphana</i>

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of Kobelt (1879); Island of Capul, the Philippines, tropical West Pacific.

### Colpodaspididae Oskars, Bouchet, and Malaquias n. fam.

**Diagnosis:** Adult shells about 2 mm; shell internal, whitish transparent, sculptured, globose with short, but protruding spire. Foot anteriorly lobed, cephalic shield with rolled lobes. Radula 1.0.1, with smooth hook-shaped lateral teeth (Brown, 1979; Ohnheiser and Malaquias, 2014).

**Type genus:** *Colpodaspis* M. Sars, 1870; **type species:** *Colpodaspis pusilla* M. Sars, 1870; by monotypy; Norway

### Colinatydidae Oskars, Bouchet, and Malaquias n. fam.

**Diagnosis:** Adult shells about 2 mm; shell quadrangular, wider anteriorly, with slightly convex sides above the mid-point, spire involute. Lip protruding slightly over spire. Color pattern made of conspicuous reticulated whitish squares. Cephalic shield anteriorly bilobed, with two preeminent eye spots and short, widely separated cephalic tentacles (see *Espinosa and Ortea, 2004; Valdés et al., 2006; Pittman and Fiene, 2013*).

**Type genus:** *Colinatys* Ortea, Moro and Espinosa, 2013; **Type species:** *Atys alayoi* *Espinosa and Ortea, 2004*; by original designation; Habana, Cuba, Caribbean Sea.

#### **Philinorbidae Oskars, Bouchet, and Malaquias n. fam.**

**Diagnosis:** Cephalic shield broad and rhomboid. Pallial lobe elongate, lacking posterior notch. Shell internal, rounded with shoulder extending beyond apex; sculpture smooth or with linear spiral striae. Non-muscular gizzard with reduced brown chitinous gizzard plates; plates sometimes absent. Radula formula 2.1.0/1.1.2, with short, broad, and hook-shaped inner laterals; inner laterals smooth or with fine denticulation along inner edge. Penial atrium embedded in tissue anteriorly to body cavity (*Mattox, 1958; Marcus, 1974; Habe, 1950, 1976; Kitao and Habe, 1982; Hori, 2000b; Chaban, 2011; Price et al., 2011*).

**Type genus:** *Philinorbis* *Habe, 1950*; **Type species:** *Philinorbis teramachii* *Habe, 1950*; by monotypy; Japan.

#### **Laonidae Pruvot-Fol, 1954 [new rank; ex Laoninae]**

**Diagnosis:** Shell rounded to quadrangular; smooth or with chain-like or net-like sculpture. Parietal wall protruding into posterior half of aperture. Gizzard plates absent (*Ohnheiser and Malaquias, 2013*).

**Type genus:** *Laona* A. Adams, 1865; **Type species:** *Laona zonata* A. Adams, 1865; by monotypy; Japan.

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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.jmpev.2015.04.011>.

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