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New contributions to the subfamily Polycerinae (Nudibranchia, Polyceridae): description of three new species and one new genus

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Abstract

The subfamily Polycerinae includes eight genera, from the monospecific *Lamellana* and *Lecithophorus* to the diverse *Polycera* and *Gymnodoris*, with 33 and 26 valid species, respectively. The monophyly of the subfamily has been tested by molecular data although not all genera were included. To date, relationships within the subfamily are not supported. In the present paper, three new species of polycerid nudibranchs are fully described based on specimens collected in Marshall Islands and Australia: one *Palio* species (*Palio gaeli* sp. nov.), one *Polycera* species (*Polycera nimbsi* sp. nov.) and a new genus (*Paliota galactica* gen. and sp. nov.). The new genus is described based on its peculiar radular teeth and genetic divergence.

The internal anatomy was studied by dissections and scanning electron microscope photographs. Partial sequences of mitochondrial cytochrome oxidase *c* subunit I (*COI*) and 16S ribosomal RNA (*16S*) as well as nuclear histone H3 (*H3*) were also obtained. A phylogenetic framework for two of these three species is proposed, also including for the first time the species *Paliolla templadoi* and *Polycera melanosticta*.

Introduction

Polycerinae Alder and Hancock, 1845 is one of the five subfamilies included in the family Polyceridae Alder and Hancock, 1845, together with Triophinae Odhner, 1941; Kalinginae Pruvot-Fol, 1956; Nembrothinae Burn, 1967, and Kankelibranchinae Ortea, Espinosa & Caballer, 2005. Polycerinae includes eight genera: *Polycera* Cuvier, 1816; *Thecacera* Fleming, 1828; *Gymnodoris* Stimpson, 1855; *Palio* Gray, 1857; *Polycerella* Verrill, 1880; *Lecithophorus* Macnae, 1958; *Paliolla* Burn, 1958, and *Lamellana* Lin, 1992 (MolluscaBase, 2023a). The morphological diagnosis of the subfamily is not clear since they are mostly characterised by having a limaciform body which may have simple tentacular processes on the margin of the oral veil and, on the sides, the mantle is reduced to a few tentacular papillae that can be connected by a ridge (Cuvier, 1816; Pola *et al.*, 2014). However, they may have no oral tentacular or mantle processes, as in *Lamellana*, *Lecithophorus*, or *Gymnodoris*. Rhinophores are lamellated (except in *Polycerella emertoni* Verrill, 1880) but gills are often simply pinnate. They lack buccal pump and usually have a large chitinous labial cuticle with a wing-like accessory process. The radula is usually present but may be absent as in the genera *Lamellana* and *Lecithophorus* (Macnae, 1958; Lin, 1992; Miller, 1996; Palomar *et al.*, 2014).

Some of the genera of this subfamily are difficult to assess. *Lamellana gymnota* Lin, 1992 was described from Hong Kong. The author described the external anatomy and stated that the buccal mass did not have a radula, but no other anatomical details were given (Lin, 1992) and nothing more is known beyond its original description. Another interesting monospecific genus was described for *Lecithophorus capensis* Macnae, 1958, also characterised by the lack of radular teeth. This species appears to be one of the most common subtidal nudibranchs on both sides of the Cape Peninsula, South Africa, and endemic to the Cape Province (Gosliner, 1987). *Paliolla* is another small genus that includes only two species: *Paliolla cooki* (Angas, 1864), distributed in Australia from central New South Wales to South Australia (Burn, 1958, 1975, 2015) and *Paliolla templadoi* (Ortea, 1989), so far found in Cape Verde Island and São Tomé (Ortea, 1989; Ortea *et al.*, 1992). *Paliolla* is characterised by an unusual radula with acicular teeth forming a tube (Burn, 1958; Ortea, 1989; Ortea *et al.*, 1992), which makes it unmistakable. To date, its phylogenetic affinities are unknown. The genus *Palio*, with six valid species (MolluscaBase, 2023b), includes species with a short bilobate veil bearing small tubercules along its edge, a ridge with a row of tubercles on each side of the back, 4 to 8 branched gills, and with more than one small tubercular exo-branchial appendage on each side (Gray, 1857; Miller, 1996). On the other hand, the genus *Polycera* is the most diverse within Polycerinae, with 33 species described worldwide to date (MolluscaBase, 2023c). Nimbs and Smith (2016) include five species in their inventory of New South Wales, with their Australian distribution; *Polycera capensis* Quoy and Gaimard, 1824, *P. hedgpethi* Er Marcus, 1964, *P. janjukia* Burn, 1962, *P. melanosticta* Miller, 1996 and, *P. risbeci* Odhner, 1941. Burn (1958, 2006) also cited *P. parvula* (Burn, 1958) for south-eastern Australia. Today, the diagnosis of the genus *Polycera* is not entirely clear, with a mixture of features: the notum, or mantle, can be smooth, or covered, partially or totally, with papillae or tubercles, and with or without extra-branchial processes. The anterior margin of the head expands to form a frontal veil, which may or may not have tentacular filaments or projections. It has short, lobed oral tentacles. The rhinophores have up to 26 lamellae and are not retractile. The gills, up to 11, can be simple and pinnate to tripinnate, and arranged in a semicircle around the anus. They have paired jaws, the radula has up to 20 rows of teeth; the inner lateral teeth are hooked, the second lateral teeth being larger than the first, the outer laterals being small. They have a large prostate gland, and a penis armed with spines (Gray, 1857; Pruvot-Fol, 1954; Thompson, 1975; Palomar *et al.*, 2014; Pola *et al.*, 2014; Sørensen *et al.*, 2020).

Although the monophyly of the subfamily Polycerinae was supported by molecular data (Palomar *et al.*, 2014) confirming its phylogenetic identity as revealed by morphological studies (Alder and Hancock, 1845; Odhner, 1941), unfortunately, the relationships within Polycerinae as well as the monophyly of *Polycera* were never recovered (Palomar *et al.*, 2014; Sørensen *et al.*, 2020; Knutson and Gosliner, 2022).

The objective of this study is to contribute to further increasing the knowledge about the diversity of this subfamily. A new genus of Polycerinae is described from Australian waters, based on another peculiar radula different from the one found in *Paliolla*, as well as on molecular data. One new species of *Palio* and one new *Polycera* are described from the Marshall Islands and Australia, respectively. An updated phylogenetic framework for Polyceridae including the first sequences for the species *P. templadoi* and *Polycera melanosticta* is presented.

Materials and methods

Taxon sampling

A total of 11 specimens belonging to three new species of the subfamily Polycerinae, as well as *P. melanosticta*, and *P. templadoi* were collected by SCUBA diving from 1992 to 2017 in Australia, the Marshall Islands, and the Cape Verde archipelago. Specimens from Australia were loaned by the Australian Museum (Sydney) and the Queensland Museum (Melbourne). Specimens from the Marshall Islands and Cape Verde were deposited in the Museo Nacional de Ciencias Naturales (Madrid, Spain). Molecular analyses included ten new specimens, in addition to 27 specimens belonging to 22 species of Polycerinae from GenBank. Seven further species of Nudibranchia were included as the outgroup (Table 1). Sequences from the Marshall Islands specimens were not obtained likely due to their originally storage conditions.

Molecular study

DNA extraction, amplification, and sequencing

DNA was extracted from a piece of foot tissue at the University of Cádiz, using Qiagen DNeasy Blood and Tissue Kit samples (Qiagen, Valencia, CA, USA). Partial sequences of Cytochrome Oxidase *c* subunit I (*COI*), 16S ribosomal RNA (*16S*) and histone H3 (*H3*) were amplified by polymerase chain reaction (PCR) using universal primers for invertebrates LCO1490 and HCO2198 for *COI* (Folmer *et al.*, 1994), 16S ar-L and 16S br-H for *16S* (Palumbi *et al.*, 1991) and H3AD5′3′ and H3BD5′3′ for *H3* (Colgan *et al.*, 1998). All PCRs were performed in 25 µl volume reactions. Each PCR was prepared using 2.5 µl Qiagen buffer (10x), 2.5 µl dNTPs (2 mM stock each of dATP, dGTP, dCTP, dTTP), 5 µl 'Q-solution' (5×), a gene-dependent amount of magnesium chloride (25 mM stock), 1 µl of each forward and reverse primer (10 µM stock), 0.025 µl Taq polymerase (1.25 units/µl)-Apex, and 2 µl DNA template. *COI* amplifications were performed with an initial denaturation for 3 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 46°C (annealing temperature), and 1 min at 72°C, with a final extension of 5 min at 72°C. *16S* amplification was performed with an initial denaturation of 5 min at 95.0°C, followed by 35 cycles of 30 s at 94.0°C, 30 s at 44.0°C (annealing temperature) and 1 min at 72.0°C with a final extension of 7 min at 72.0°C. *H3* amplification was performed with an initial denaturation of 3 min at 95.0°C, followed by 40 cycles of 45 s at 94.0°C, 45 s at 50.0°C (annealing temperature), 2 min at 72.0°C with a final extension of 10 min at 72.0°C. Successful PCR products obtained were sequenced by Macrogen, Inc. All new sequences obtained were deposited in GenBank (Table 1).

Phylogenetic analyses

Sequences were assembled and edited using Geneious 11.1.4 (http://www.geneious.com, Kearse *et al.*, 2012). All sequences were tested for similarity with BLAST algorithm and aligned using MEGA7 (Kumar *et al.*, 2016). Evolutionary models *16S* and each codon position of *COI* and *H3* were obtained using jModelTest2 on XEDE, CIPRES Science Gateway (Miller *et al.*, 2010), under Akaike information criteria (AIC) (Akaike, 1974). The models TrN + I, TPM1uf, HKY + G, were selected for first, second, and third codon position for *COI*. The evolutionary model for *16S* was TPM3uf + G. For gene *H3*, TIM2 + I was selected for first codon position, JC for second codon position, and GTR + I + G for third codon position. Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses were carried out using a matrix of individual gene and concatenated sequences, including *COI-16S*, *COI-H3* and, *16S-H3* including all available sequences, *COI* + *16S* + *H3* with at least two genes per specimen, and *COI* + *16S* + *H3* using all sequences. BI analyses were performed using MrBayes on XSEDE, CIPRES Science Gateway (Miller *et al.*, 2010), for ten million generations, four independent runs, and a sampling frequency of 1000. Nodes with posterior probabilities ⩾0.96 were considered supported (Alfaro *et al.*, 2003). ML analyses were performed using RAxML-NG (Kozlov *et al.*, 2019), using a Bootstopping cut-off of 0.03 implemented. ML nodes were considered statistically significant by bootstraps values ⩾75 (Hillis and Bull, 1993). All trees were rooted using the species *Crimora lutea*. Gene pairwise uncorrected *P*-distances for *COI* (Table 2), *16S* (Table 3), and *H3* (Table 4) were obtained using MEGA7 (Kumar *et al.*, 2016). The resulting tree was visualised using FigTree (Rambaut, 2009) and edited in Adobe Photoshop CC 2014.

Morphological study

External morphology was studied at the Universidad Autónoma de Madrid using photographs of living animals and observations of preserved specimens under a stereomicroscope Nikon SMZ-1500 equipped with a *camera lucida*. Internal anatomy was studied by dissections. The animals were opened by a dorsal incision, and the reproductive system and buccal mass were removed. The buccal mass was dissolved in a 10% sodium hydroxide solution until the labial cuticle and radula had been cleaned from their surrounding tissue. These structures were then rinsed with water and examined and photographed under a light microscope using the Life Sciences Imaging software cellSense (v. 1.18). The reproductive system was drawn using a *camera lucida*, and the penis was isolated and opened so that it could be examined and photographed using the light microscope. The penises were not prepared for scanning electron microscope (SEM) due to their tiny sizes. The labial cuticles were critical point dried using hexamethyldisilazane. The radulae and labial cuticles

Table 1. Specimens used for molecular analyses, including species, museum voucher, locality, and GenBank vouchers

Species	Voucher	Locality	COI	16S	H3
Crimora lutea	MNCN 15.05/46737	Australia, Western Australia, Abrolhos Island.	EF142903	EF142950	–
Gymnodoris alba	MNCN 15.05/55472	Australia, New South Wales, Nelson Bay.	JX274101	JX274063	
Gymnodoris citrina	CASIZ 192269	Saudi Arabia, Red Sea, North of Shuma Reef.	MZ382636	MZ409278	MZ399418
Gymnodoris impudica	CASIZ 179109	Vanuatu, Espíritu Santo Island, Perumamasa Island.	MZ382641	MZ409283	MZ399423
Gymnodoris okinawae	CASIZ 189442	Hawaii, Maui.	MZ382656	MZ409299	MZ399439
Gymnodoris pattani	CASIZ 190606	Thailand, Malay Peninsula, Thale Sap.	MZ382661	MZ409302	MZ399443
Kalinga ornata	ZMMU Op-83	Vietnam	MN224072	MN224103	–
Lecithophorus capensis	CASIZ 176174	South Africa, Western Cape Province, False Bay.	MZ382783	MZ409432	MZ399573
Limacia inesae	MNCN 15.05/46736	Spain, Cádiz.	EF142906	EF142952	–
Nembrotha purpureolineata	CASIZ 177006	Mozambique: Inhambane Province.	MZ382785	MZ409434	MZ399575
Palio dubia	CASIZ 182030	USA, Maine, Portland.	MZ382786	MZ409246	MZ399576
P. dubia	GB	Sweden, Bohuslän, Kristineberg.	AJ223272	AJ225197	–
Paliolla templadoi	MNCN 15.05/94863	Cape Verde Island.	**OQ676220**	**OQ685050**	**OQ686698**
***Paliota galactica* sp. nov.**	AM C.547850	Australia, New South Wales, Nelson Bay.	–	**OQ685049**	–
***P. galactica* sp. nov.**	AM C.547851	Australia, New South Wales, Nelson Bay.	**OQ676219**	–	–
***P. galactica* sp. nov.**	QM MO 86040	Australia, New South Wales, Nelson Bay.	–	–	**OQ686697**
Plocamopherus ceylonicus	CASIZ 185147	Hawaii, Maui, Maalaea Bay.	MZ382787	MZ409436	MZ399577
Polycera abei	CASIZ 180290	Hawaii, Maui, Maalaea Bay.	MZ382788	MZ409437	MZ399578
Polycera alabe	LACM 140737	Sonora, Mexico.	–	KF425272	KF425284
Polycera atra	CPIC 00806	California, San Francisco.	–	KF425277	KF425291
Polycera atra	CASIZ 170506a	California.	JX274084	JX274052	–
Polycera aurantiomarginata	MNCN 15.05/55492	Morocco, Aghroud.	JX274068	JX274038	–
Polycera capensis	CASIZ 176206	South Africa, Western Cape Province, False Bay.	MZ382789	MZ409439	MZ399580
Polycera faeroensis	ZMMU Op-774	United Kingdom.	MZ425340	MZ420427	–
Polycera hedgpethi	CPIC 00805	California, San Francisco.	–	KF425278	KF425292
Polycera cf. *hedgpethi*	MNCN 15.05/55493	Morocco, Aghroud.	JX274086	–	–
Polycera japonica	BMOO-09353	–	KC706901	–	–
Polycera kernowensis	FD012	The Netherlands.	MZ425376	MZ420466	–
Polycera melanostincta	QM MO 86039	Australia, New South Wales.	–	**OQ685051**	–
Polycera norvegica	ZMBN 126023	Norway, Uthaug, Orland, Trondelag.	MT477917	–	–
Polycera quadrilineata	MNCN 15.05/46738	United Kingdom, Oban.	EF142907	EF142953	–
P. quadrilineata	MNCN 15.05/55462	Sweden, Gothenborg, Tjärnö.	JX274070	JX274041	–
Polycera tricolor	CASIZ 176438a	California, San Francisco Bay, Marin County.	JX274087	JX274054	–
***Polycera nimbsi* sp. nov.**	AM C.547847	Australia, New South Wales, Nelson Bay.	–	–	**OQ686699**
***P. nimbsi* sp. nov.**	AM C.547846	Australia, New South Wales, Nelson Bay.	**OQ676221**	**OQ685052**	–
***P. nimbsi* sp. nov.**	AM C.547848	Australia, New South Wales, Nelson Bay.	**OQ676222**	–	–
***P. nimbsi* sp. nov.**	QM MO 86041	Australia, New South Wales, Nelson Bay.	**OQ676223**	–	**OQ686700**
***P. nimbsi* sp. nov.**	AM C. 547849	Australia, New South Wales, Low Reef.	**OQ676224**	–	–
Polycerella emertoni	MNCN15.05/55482a	Spain, Cádiz.	JX274098	JX274061	–
Polycerella emertoni	MNCN 15.05/55480	Spain, Cádiz, Santi Petri.	JX274095	JX274060	–
Roboastra gracilis	CASIZ 188582	Mauritius, Ilot Gabriel.	MZ382793	MZ409443	MZ399584
Tambja marbellensis	CASIZ 180379	Portugal, Atlantic Coast, Setubal, Outão.	HM162689	HM162599	HM162505
Thecacera picta	CASIZ 182281	–	KP871652	KP871701	KP871676
Thecacera pennigera	–	Spain, Cádiz.	AJ223277	AJ225202	–

Newly sequenced specimens are highlighted in bold. Institutional acronyms: Australian Museum (AM), California Academy of Sciences (CASIZ), Cal Poly Pomona research collections (CPIC), Bureau Waardenburg BV, Aquatic ecology, the Netherlands (FD), Natural History Museum of Los Angeles County (LACM), Museo Nacional de Ciencias Naturales (MNCN), Queensland Museum (QMMO), Zoological Museum, Moscow Lomonosov State University (ZMMU).

Table 2. COI gene pairwise uncorrected *P*-distances (%) within and between species for comparison

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Crimora lutea* MNCN 15.05/46737																		
2	*Gymnodoris alba* MNCN 15.05/55472	21,03																	
3	*G. citrina* CASIZ 192269	19,94	12,66																
4	*G. impudica* CASIZ 179109	20,97	14,19	12,79															
5	*G. okinawae* CASIZ 189442	20,24	14,20	13,29	12,28														
6	*G. pattani* CASIZ 190606	20,06	13,08	10,60	10,97	11,26													
7	*Kalinga ornata* ZMMU Op-83	20,67	21,18	20,41	21,94	22,07	19,91												
8	*Lecithophorus capensis* CASIZ176174	20,06	15,89	14,72	14,35	15,83	12,46	20,06											
9	*Limacia inesae* MNCN 15.05/46736	18,69	18,38	17,88	18,87	18,72	17,02	20,36	17,48										
10	*Nembrotha purpureolineata* CASIZ 177006	18,84	20,72	19,78	20,00	19,18	19,00	21,73	20,36	16,26									
11	*Palio dubia* CASIZ 182030	19,60	17,91	14,72	18,23	16,13	15,81	23,25	16,11	19,15	20,36								
12	*Palio dubia* GB	19,57	18,17	14,59	18,17	17,08	16,25	22,22	16,25	19,90	20,40	0,83							
13	***Paliolla templadoi* MNCN 15.05/94863**	20,52	17,13	14,40	16,13	14,76	12,61	21,43	13,37	18,39	19,30	17,48	17,58						
14	***Paliota galactica* sp. nov. AM C.547851**	22,92	20,07	18,91	20,65	19,87	18,91	24,04	20,19	22,92	22,92	**19,39**	**19,57**	**18,43**					
15	*Plocamopherus ceylonicus* CASIZ 185147	20,52	19,78	19,46	19,52	20,70	19,15	21,28	19,15	17,48	19,30	18,24	17,74	19,45	23,24				
16	*Polycera abei* CASIZ 180290	19,02	15,69	14,21	15,16	14,92	11,89	21,71	12,68	16,80	17,59	13,63	13,75	13,63	17,42	18,07			
17	*P. atra* CASIZ 170506a	17,86	18,57	20,58	18,41	17,86	18,88	20,92	18,37	19,39	19,56	18,20	18,37	18,03	18,03	22,11	16,67		
18	*P. aurantiomarginata* MNCN 15.05/55492	19,28	16,82	15,24	16,77	17,13	13,37	20,53	14,62	18,82	19,28	16,02	16,81	17,57	19,38	19,60	12,04	17,18	
19	*P. capensis* CASIZ 176206	19,30	16,04	14,87	16,61	16,44	12,61	21,43	14,13	17,48	19,30	15,81	16,25	16,11	18,91	18,84	11,09	16,84	4,98
20	*P. faeroensis* ZMMU Op774	20,06	18,38	17,41	17,26	17,66	15,35	21,43	17,33	20,21	20,36	17,78	18,41	17,93	18,27	20,52	14,26	17,69	10,11
21	*P. hedgpethi* MNCN 15.05/55493	18,38	16,20	15,26	16,13	15,44	13,24	21,96	13,40	15,73	18,22	13,86	15,00	16,82	17,43	19,63	12,04	15,84	14,49
22	*P. japonica* BMOO09353	20,77	19,17	19,51	17,25	16,99	15,65	24,60	16,29	18,21	19,81	15,34	15,87	17,25	20,43	19,49	11,50	18,99	15,65
23	*P. kernowensis* FD012	20,36	17,60	17,88	17,26	17,81	15,96	22,49	17,33	21,12	20,52	17,33	17,74	18,54	19,39	19,60	14,42	19,22	10,42
24	***Polycera nimbsi* sp. nov. AM C.547846**	19,17	18,37	18,21	18,21	17,67	16,56	23,16	15,95	19,79	21,17	16,56	16,22	18,40	**20,55**	18,71	13,58	17,84	15,52
25	***Polycera nimbsi* sp. nov. AM C.547848**	19,76	18,69	18,51	18,71	18,26	16,26	23,10	15,96	19,60	20,82	16,87	16,92	18,24	**20,03**	19,00	13,47	17,86	15,24
26	***Polycera nimbsi* sp. nov.AM C.547849**	19,60	19,16	18,99	18,87	18,11	16,41	23,25	16,11	19,76	20,97	17,02	17,08	18,54	**19,87**	18,69	13,63	18,03	15,09
27	***Polycera nimbsi* sp. nov. QM MO 86041**	19,30	18,85	18,83	18,87	18,42	16,41	23,56	16,11	19,76	20,97	16,72	16,92	18,24	**20,03**	19,00	13,63	17,86	15,09
28	*P. norvegica* ZMBN 126023	20,12	16,69	17,37	17,29	18,13	15,76	21,84	16,69	20,90	19,34	16,54	16,67	19,03	17,93	19,66	14,13	17,38	12,32
29	*P. quadrilineata* MNCN 15.05/46738	21,28	17,13	17,09	18,71	18,26	15,50	20,82	16,41	18,84	20,36	16,41	16,58	18,24	17,79	19,76	14,10	16,67	11,66
30	*P. quadrilineata* MNCN 15.05/55462	21,31	16,67	16,53	19,52	18,54	16,17	20,53	16,95	19,60	20,53	16,95	17,14	18,97	18,72	19,60	14,74	16,67	11,35
31	*P. tricolor* CASIZ 176438a	20,84	16,20	15,40	16,94	16,20	13,37	21,93	16,02	17,11	18,82	14,93	15,47	16,02	19,87	18,04	13,95	16,50	16,02
32	*Polycerella emertoni* MNCN 15.05/55480	18,97	18,22	15,56	18,39	17,13	15,86	21,77	16,33	18,20	18,51	18,35	19,13	16,64	18,23	19,91	14,90	17,35	14,46

(*Continued*)

Table 2. (*Continued.*)

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
33	*Polycerella emertoni* MNCN 15.05/55482	19,19	18,25	15,77	18,55	17,19	16,07	21,84	16,54	17,94	18,88	18,41	19,20	16,85	17,96	19,97	15,06	17,24	14,82
34	*Roboastra gracilis* CASIZ 188582	18,54	21,96	19,62	20,97	21,31	19,60	20,67	19,76	19,45	17,48	21,58	21,72	19,00	21,96	19,76	17,43	21,26	19,75
35	*Tambja marbellensis* CASIZ 180379	17,93	20,25	17,72	18,71	19,79	17,02	19,45	19,00	15,81	16,57	19,00	19,07	17,48	20,51	17,17	14,10	19,22	17,88
36	*Thecacera pennigera* GB	21,17	15,83	14,67	17,13	17,67	15,17	22,33	15,17	18,00	22,67	17,67	17,83	15,50	17,83	20,00	14,94	18,06	16,17
37	*Thecacera picta* CASIZ 182281	19,00	16,20	15,66	15,48	16,74	14,44	20,97	15,05	17,17	21,43	16,87	17,25	17,78	19,07	19,91	15,37	16,33	16,95

	Species	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
20	*Polycera faeroensis* ZMMU Op774	8,97																	
21	*P. hedgpethi* MNCN 15.05/55493	13,71	16,51																
22	*P. japonica* BMOO09353	17,57	16,61	15,65															
23	*P. kernowensis* FD012	9,57	5,78	16,36	17,25														
24	***Polycera nimbsi* sp. nov. AM C.547846**	14,88	17,33	14,60	17,48	18,10													
25	***Polycera nimbsi* sp. nov. AM C.547848**	13,83	16,57	14,49	19,17	17,63	**1,38**												
26	***Polycera nimbsi* sp. nov.AM C.547849**	13,98	16,72	14,95	18,85	17,48	**1,23**	**0,46**											
27	***Polycera nimbsi* sp. nov. QM MO 86041**	13,98	16,72	14,64	19,17	17,48	**1,23**	**0,46**	**0,30**										
28	*P. norvegica* ZMBN 126023	11,86	9,20	14,51	17,63	11,08	15,88	15,91	16,07	15,91									
29	*P. quadrilineata* MNCN 15.05/46738	10,64	**11,70**	16,20	20,13	**12,61**	16,41	16,26	16,41	16,41	11,39								
30	*P. quadrilineata* MNCN 15.05/55462	11,35	**12,75**	16,36	20,45	**13,37**	16,93	17,11	17,26	17,26	12,17	2,02							
31	*P. tricolor* CASIZ 176438a	15,71	17,73	12,77	18,85	18,20	16,93	16,95	17,11	17,11	18,41	16,33	15,86						
32	*Polycerella emertoni* MNCN 15.05/55480	15,24	15,71	15,11	18,85	15,71	17,40	17,42	17,26	17,26	17,78	18,20	17,42	16,33					
33	*Polycerella emertoni* MNCN 15.05/55482	15,44	15,76	15,29	18,85	16,07	17,14	17,00	16,85	16,85	17,97	18,25	17,47	16,38	0,62				
34	*Roboastra gracilis* CASIZ 188582	20,36	20,36	20,25	20,45	20,06	21,01	21,12	20,82	20,82	20,59	21,43	21,00	20,68	20,53	20,59			
35	*Tambja marbellensis* CASIZ 180379	17,48	18,39	19,00	18,53	19,15	17,94	18,24	18,09	18,09	19,03	19,15	19,44	18,20	16,64	16,85	16,87		
36	*Thecacera pennigera* GB	16,33	17,33	15,83	17,34	18,33	17,31	17,50	17,67	17,83	18,00	16,67	16,33	16,83	17,00	17,53	19,50	19,83	
37	*Thecacera picta* CASIZ 182281	15,81	16,41	15,26	19,17	17,78	18,71	18,54	19,00	18,69	16,38	15,50	16,80	15,09	16,80	17,16	21,43	17,63	13,50

Numbers in bold are mentioned in the text.

Table 3. 16S gene pairwise uncorrected *P*-distances (%) within and between species for comparison

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*Crimora lutea* MNCN 15.05/46737																				
2	*Gymnodoris alba* MNCN 15.05/55472	11,63																			
3	*G citrina* CASIZ 192269	11,39	8,19																		
4	*G.impudica* CASIZ 179109	10,80	7,73	8,79																	
5	*G.okinawae* CASIZ 189442	12,19	9,41	9,70	4,12																
6	*G.pattani* CASIZ 190606	11,36	8,27	8,81	5,43	5,43															
7	*Kalinga ornata* ZMMU Op83	11,39	17,37	17,15	16,84	17,11	15,83														
8	*Lecithophorus capensis* CASIZ 176174	11,36	14,32	15,67	12,50	14,06	13,54	16,36													
9	*Limacia inesae* MNCN 15.05/46736	10,25	12,19	12,50	11,36	12,47	9,70	8,61	12,74												
10	*Nembrotha purpureolineata* CASIZ 77006	10,80	12,79	13,61	13,84	13,84	12,57	13,76	12,57	10,80											
11	*Palio dubia* CASIZ 182030	8,61	11,60	13,66	11,92	12,37	11,43	12,93	11,75	9,44	10,73										
12	*Palio dubia* GB	8,83	11,11	12,96	11,44	11,64	10,93	13,48	11,26	9,12	10,22	0,53									
13	***Paliota galactica* sp. nov. AM C.547850**	9,70	13,92	14,73	11,89	12,63	11,66	14,21	13,02	11,63	14,10	8,79	9,28								
14	***Paliolla templadoi* MNCN 15.05/94863**	9,14	10,63	12,53	10,16	11,45	9,66	12,14	10,44	8,86	9,40	5,97	5,33	8,57							
15	*Plocamopherus ceylonicus* CASIZ 185147	10,86	16,19	17,54	15,93	16,97	15,45	10,32	15,71	8,08	13,12	11,52	11,02	14,10	11,26						
16	*Polycera abei* CASIZ180290	8,86	13,44	13,47	12,18	13,70	11,69	12,11	12,50	9,70	12,53	9,59	9,04	9,56	9,35	12,27					
17	*P. alabe* LACM 140737	9,95	16,81	16,03	15,61	17,23	15,25	14,41	14,16	9,95	13,30	10,59	10,62	10,97	9,40	15,09	11,02				
18	*P. atra* CPIC00806	9,43	17,67	15,98	15,48	18,70	14,71	14,72	14,47	9,91	12,82	10,88	10,92	11,30	10,66	15,38	11,34	2,94			
19	*P. atra* CASIZ 170506a	8,89	13,14	12,18	11,89	12,89	11,66	11,87	13,58	9,72	12,04	9,87	9,33	9,07	7,55	12,30	8,83	3,80	1,26		
20	*P. aurantiomarginata* MNCN 15.05/55492	7,52	12,11	12,89	11,69	12,66	11,98	14,29	11,26	10,86	11,81	8,05	7,73	8,85	7,29	13,39	8,88	9,83	10,08	8,33	
21	*P. capensis* CASIZ 176206	7,80	12,37	13,40	11,95	12,92	12,24	14,29	11,52	10,86	12,07	8,31	8,00	8,85	7,81	12,86	8,88	9,40	9,66	8,33	**1,55**
22	*P. hedgpethi* CPIC00805	7,22	11,20	11,23	11,23	12,76	10,99	13,00	11,61	9,72	11,05	7,33	6,99	8,09	6,82	11,38	8,12	5,53	5,11	6,01	6,05
23	*P. kernowensis* FD012	8,08	12,63	14,18	12,73	12,66	11,98	12,70	12,83	10,86	12,86	7,27	7,73	7,29	7,55	13,12	9,14	8,12	9,24	8,07	4,38
24	*P. melanosticta* QM MO 86039	9,97	13,14	13,44	10,34	11,34	10,10	14,47	13,02	10,53	13,58	9,56	8,75	9,28	9,61	13,84	9,82	10,97	12,13	10,10	10,16
25	*P. quadrilineata* MNCN 15.05/46738	7,87	9,42	9,97	9,47	9,70	9,22	12,85	12,01	10,11	11,14	6,67	6,27	6,13	5,82	11,20	8,08	7,18	7,08	6,42	3,60
26	*P. quadrilineata* MNCN 15.05/55462	8,64	12,11	13,14	11,17	11,63	10,94	13,23	12,83	10,31	12,34	7,53	7,20	7,03	7,03	12,34	8,62	8,12	8,82	7,55	4,64
27	*P. tricolor* CASIZ 76438a	10,28	13,05	15,71	14,66	14,36	12,60	14,32	15,75	11,94	14,92	9,16	8,60	9,92	9,40	15,00	11,49	9,40	8,97	9,42	10,26
28	***Polycera nimbsi* sp. nov. AM C.547846**	12,45	14,73	15,12	12,03	11,64	11,03	16,84	14,93	13,21	15,68	11,68	11,03	10,27	10,73	14,98	**12,37**	**15,60**	**16,78**	**11,72**	11,81
29	*Polycerella emertoni* MNCN 15.05/55482	7,78	11,98	11,98	10,99	11,98	10,76	12,20	12,60	10,00	12,04	6,51	5,88	9,40	7,29	11,05	9,14	8,15	7,66	7,09	7,33
30	*Polycerella emertoni* MNCN 15.05/55480	7,69	12,27	12,27	11,26	12,00	11,02	12,20	12,37	9,97	12,33	6,40	5,88	9,09	7,47	11,05	9,36	8,48	7,96	7,26	7,24
31	*Roboastra gracilis* CASIZ 188582	12,47	17,57	17,88	17,62	17,83	18,70	14,47	18,49	12,19	13,58	15,28	15,16	17,57	14,29	15,14	16,54	19,92	19,75	14,81	14,88
32	*Tambja marbellensis* CASIZ 180379	10,80	14,18	16,28	12,92	14,18	13,21	13,42	13,54	9,42	7,57	11,11	10,88	13,14	9,35	12,27	11,11	13,92	14,64	11,40	10,94

(*Continued*)

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Table 3. (*Continued.*)

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
33	*Thecacera picta* CASIZ 182281	8,89	14,99	14,03	12,99	13,99	12,24	13,46	12,79	9,44	11,78	9,40	9,12	10,16	9,64	13,09	8,85	8,09	9,28	8,55	9,38
34	*Thecacera pennigera* GB	8,52	14,03	13,39	12,14	13,09	11,38	12,63	13,07	9,09	12,03	10,05	9,28	10,32	9,74	13,10	9,28	11,40	11,49	8,44	10,55
35	*Polycera faeroensis* ZMMU Op774	8,59	14,18	15,08	12,89	13,53	12,14	13,68	12,76	11,36	13,05	7,75	7,96	8,01	8,38	13,32	9,33	8,86	11,34	8,76	3,87

	Species	21	22	23	24	25	26	27	28	29	30	31	32	33	34
22	*Polycera hedgpethi* CPIC00805	5,79													
23	*P. kernowensis* FD012	4,64	6,32												
24	*P. melanosticta* QM MO 86039	10,16	7,57	8,85											
25	*P. quadrilineata* MNCN 15.05/46738	3,88	5,31	1,94	7,24										
26	*P. quadrilineata* MNCN 15.05/55462	4,64	6,32	2,32	8,59	0,28									
27	*P. tricolor* CASIZ 76438a	10,00	7,09	8,16	11,49	9,22	9,47								
28	***Polycera nimbsi* sp. nov. AM C.547846**	11,81	9,76	10,07	**2,40**	**7,98**	**9,72**	12,89							
29	*Polycerella emertoni* MNCN 15.05/55482	6,28	4,74	7,59	8,36	6,39	7,07	10,47	9,76						
30	*Polycerella emertoni* MNCN 15.05/55480	6,17	4,58	7,51	7,75	6,27	6,97	9,12	9,76	0,00					
31	*Roboastra gracilis* CASIZ 188582	15,14	14,92	16,97	16,80	13,09	15,93	18,80	19,24	15,67	15,78				
32	*Tambja marbellensis* CASIZ 180379	11,20	9,92	11,46	12,63	9,75	10,42	13,05	16,10	10,44	10,43	12,14			
33	*Thecacera picta* CASIZ 182281	9,38	8,66	10,68	10,94	8,38	10,16	12,57	11,81	7,35	7,53	15,63	12,8		
34	*Thecacera pennigera* GB	10,29	8,82	10,82	10,85	7,67	9,76	10,72	12,37	8,29	8,29	14,59	11,6	6,6	
35	*Polycera faeroensis* ZMMU Op774	4,12	6,53	1,55	9,30	1,94	2,32	8,90	10,65	7,05	6,95	17,10	11,6	10,6	11,9

Numbers in bold are mentioned in the text.

Table 4. H3 gene pairwise uncorrected *P*-distances (%) within and between species for comparison

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*Gymnodoris citrina* CASIZ 192269																			
2	*Gymnodoris impudica* CASIZ 179109	3,96																		
3	*Gymnodoris okinawae* CASIZ 189442	3,35	1,83																	
4	*Gymnodoris pattani* CASIZ 190606	4,27	3,96	2,74																
5	*Lecithophorus capensis* CASIZ 176174	5,79	5,49	6,10	6,71															
6	*Nembrotha purpureolineata* CASIZ 177006	10,09	10,40	9,79	11,01	10,40														
7	*Palio dubia* CASIZ182030	8,84	10,37	9,15	10,67	10,06	11,93													
8	***Paliota galactica* sp. nov. QM MO 86040**	11,59	13,72	13,41	12,20	13,72	16,82	10,67												
9	***Paliolla templadoi* MNCN 15.05/94863**	6,10	5,79	5,79	7,62	4,88	9,48	8,54	13,11											
10	*Plocamopherus ceylonicus* CASIZ 185147	9,45	10,67	9,45	11,28	11,89	10,40	9,76	15,24	9,76										
11	*Polycera abei* CASIZ 180290	7,93	9,45	8,84	8,54	10,37	11,62	9,76	10,37	10,37	11,59									
12	*Polycera alabe* LACM140737	13,11	14,02	14,94	13,41	12,80	13,76	12,20	9,45	11,59	15,24	12,20								
13	*Polycera atra* CPIC00806	13,41	14,33	14,63	13,72	13,72	14,37	13,11	10,98	13,41	14,63	12,50	4,57							
14	*Polycera capensis* CASIZ 176206	11,59	13,41	13,41	11,28	13,72	15,90	10,98	7,62	14,33	14,63	10,06	9,45	11,28						
15	*Polycera hedgpethi* CPIC00805	8,54	10,67	10,06	10,06	10,98	12,84	10,37	9,45	10,98	12,20	9,45	8,84	10,98	7,93					
16	***Polycera nimbsi* sp. nov. AM C.547847**	10,67	13,41	12,50	11,28	12,50	15,29	9,45	**8,54**	11,28	13,41	**10,67**	**9,76**	**10,67**	8,54	7,62				
17	***Polycera nimbsi* sp. nov. QM MO 86041**	10,67	13,41	12,50	11,28	12,50	15,29	9,45	**8,54**	11,28	13,41	**10,67**	**9,76**	**10,67**	8,54	7,62	**0,00**			
18	*Roboastra gracilis* CASIZ 188582	7,01	9,15	7,93	8,84	9,15	7,65	9,15	10,98	8,54	8,84	9,15	11,28	14,02	10,37	7,32	11,28	11,28		
19	*Tambja marbellensis* CASIZ 180379	6,40	8,54	7,32	8,23	7,32	5,81	9,15	12,20	6,71	7,93	9,76	11,89	14,63	11,59	9,15	11,89	11,89	1,83	
20	*Thecacera picta* CASIZ 182281	8,23	7,62	8,23	8,84	7,01	11,93	10,37	14,33	8,54	10,67	12,20	13,72	14,33	14,02	11,59	14,02	14,02	10,98	9,76

Numbers in bold are mentioned in the text.

were mounted on metallic stubs for SEM, and sputter coated with gold-palladium. Observations were made with a Hitachi S-3000 SEM-machine. This work has been registered under ZooBank Accession: urn:lsid:zoobank.org:pub:977248D2-B20F-414D-A4C4-44B5D0BA2D50.

Results

Phylogenetic analysis and species delimitation analysis

The molecular phylogenetic analysis included a total of 37 sequences for *COI*, 34 for *16S*, and 19 for *H3* (Table 1). The concatenated *COI + H3 + 16S* with all sequences included 44 taxa, while the same concatenated but with a minimum of two genes per specimen included 34 taxa (not shown because it does not include *Paliota galactica*). Since most of the newly sequenced specimens were only successful for a single gene, we provide the individual phylogenetic tree obtained with *COI* (Figure 1), and the single *16S*, *H3*, concatenated *COI-16S*, and complete concatenated *COI + H3 + 16S* phylogenetic trees are shown as supplementary material (Figures S1, S2, S3, S4). Figure 1 and concatenated trees (Figures S3, S4) indicate high support of the monophyly of the family Polycerinae but the

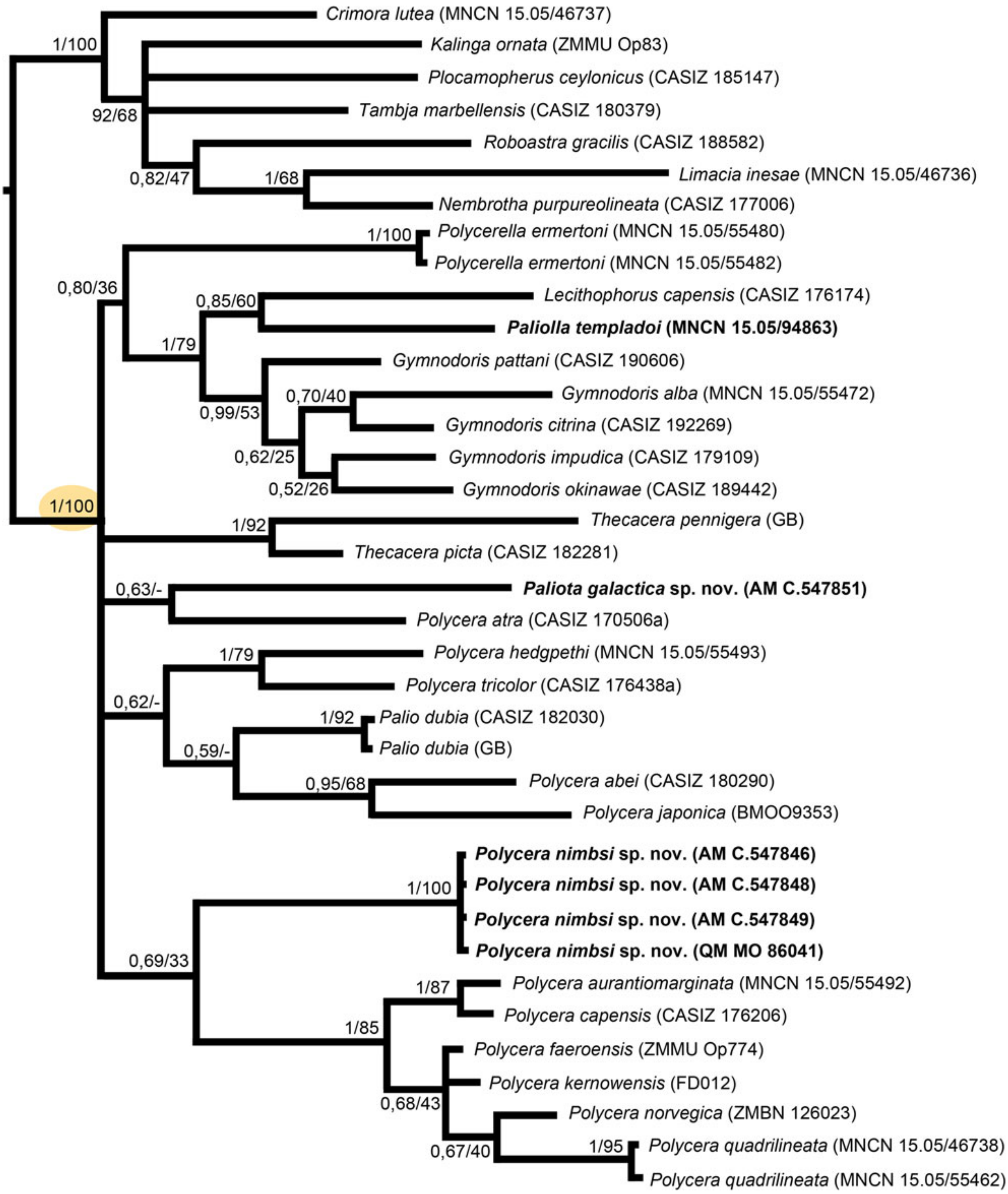

Figure 1. Phylogenetic relationships (BI/ML) based on the mitochondrial COI gene marker. The family Polycerinae is highlighted with an orange spot. The newly sequenced specimens are in bold.

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relationships between the genera remain unresolved. The genus *Polycera* was never rendered monophyletic; however, all the specimens of *Polycera nimbsi* sp. nov. clustered together with maximum support (PP = 1/BS = 100) (Figures 1, S3, S4), with a range of intraspecific variability for *COI* uncorrected pairwise genetic distance between 0.3 and 1.2% (Table 2). *Paliolla templadoi* is included in a clade containing *L. capensis* and *Gymnodoris* spp. (Figures 1, S3, S4). *COI* uncorrected pairwise genetic distances showed 18.43% difference between *P. templadoi* and *P. galactica* sp. nov., and 19.39–19.47% between *Palio dubia* and *Paliota* gen. nov. (Table 2). A small clade containing *Polycera quadrilineata*, the type species of the genus, gathers *P. norvegica* Sørensen, Rauch, Pola & Malaquias, 2021, *P. kernowensis* Korshunova, Driessen, Picton & Martynov, 2021, and *P. faeroensis* Lemche, 1929 as sister to *P. aurantiomarginata* García-Gómez & Bobo, 1984 and *P. capensis* (Figures 1, S3, S4). *COI* uncorrected pairwise genetic distances among these species varies between 12.61–13.37% (*P. quadrilineata* vs *P. kernowensis*) and 11.70–12.75% (*P. quadrilineata* vs *P. faeroensis*) (Table 2).

Systematics

Nudibranchia Cuvier, 1817
Family POLYCERIDAE Alder and Hancock, 1845
Genus *Palio* Gray, 1857
***Palio gaeli* sp. nov.** (Figures 2A–C, 3A, B, 4)
LSID urn:lsid:zoobank.org:
act:99C9BE0D-E682-4E5C-9837-2929479A0B6D
Polycera sp. 8 in Gosliner *et al.* (2018): 29.

Diagnosis

Body ground translucent with numerous tubercles on the notum and the margins of the body. Numerous brown and black spots. Dense white pigmentation. Four small tuberculate velar processes. Three tripinnate trident-shape gill branches. Three small extra-branchial processes. 5–7 rhinophoral lamellae. Many spicules embedded in the mantle. Radular formula 9 × 2(3).2.0.2.2(3). Very large prostate. Armed penis.

Material examined

Holotype. MNCN 15.05/200208H. Marshall Islands, Kwajalein Atoll, Eller U pinnacle, under dead coral with eggs, 9 mm alive, 16 September 2013, col. by S. Johnson. Originally preserved in 70 isopropyl. Dissected (SEM: radula, labial cuticle). *Paratype.* MNCN 15.05/200208P. Marshall Islands, Kwajalein Atoll, Eller U pinnacle, under dead coral with eggs, 9 mm alive, 16 September 2013, col. by S. Johnson. Originally preserved in 70% isopropyl alcohol. Dissected (SEM: radula, labial cuticle).

Etymology

The species is named after Gael Pola, nephew of the first author of this paper. Also, in the Celtic language, 'gaelico' means 'from the islands'.

External morphology (Figure 2A–C)

Body elongated, limaciform. Body surface smooth but apparently rough in texture due to presence of differently sized tubercles located on dorsal and lateral parts of body. Medium-sized tubercles mark mantle edge, from anterior part of head to behind gill, where they join and run as a ridge to end of tail. Large tubercles also form a longitudinal line on either side of body. Four frontal veil processes short, rounded; two larger in central area and two smaller on each side. Elongated and cross-shaped spicules evenly distributed throughout body, within mantle. Rhinophores perfoliate with 5–7 lamellae, lacking sheaths. Oral tentacles not clearly observed. Gill non-retractile into pocket, with three long tripinnate gill branches that surround anterior part of anus. Gill semicircular and large compared to body, characterised by trident shape. Three small lobed extra-branchial process on each side of gill, the closer to gill larger than the others. Foot long, narrow, slightly widened anteriorly, with two propodial tentacles in its anterior end. Genital opening on right side, closer to rhinophores than to gills.

Colour pattern (Figure 2A–C)

Translucent body with small, pale brown and black spots dispersed evenly throughout. Black spots mostly arranged on tubercles, while gill branches and rhinophores bare mostly pale brown spots. Dense white pigmentation observed in most tubercles and outer rachises of gill branches. This colouration delimits edge of notum accompanying tubercles from cephalic part to end of tail. Dense white pigmentation also forms diamond-shaped patch behind rhinophores on middorsal line.

Internal anatomy (Figures 3A, 4A–E)

Oral tube slightly larger than buccal bulb. Pair of elongate, granular salivary glands attached at each side of oesophagus where it enters buccal bulb (Figure 3A). Labial cuticle large, robust, with two large elongated lateral wings; inner part of wings and central area with delicate rods and striations (Figure 4A, B). Radular formula 9 × 2(3).2.0.2. 2(3). Rachidian tooth absent (Figure 4C). All laterals elongated, hamate, with strong prominent distal cusp; inner lateral smaller, with narrow base and triangular, spatulate distal cusp. This distal cusp has a small protrusion on its outer margin (Figure 4C, D), outer laterals larger, more than twice size of inner laterals; both laterals with hook-shaped wing-like expansion which is sharper on inner laterals. Marginal teeth smaller, flat, plate-like, pseudo-rectangular, decreasing in size outward (Figure 4E). Last marginal very small, only visible in some rows (Figure 4E).

Reproductive system (Figures 3B, 4F)

Triaulic; hermaphroditic duct elongate, thin. Ampulla small, sausage-shaped; post-ampullary duct not seen. Prostate gland very large, narrowing towards distal vas deferens. Penis armed with numerous hooked-shaped spines (Figure 4F). Vaginal duct elongated, bent, same length as vas deferens but wider, ending in large oval bursa copulatrix. Bursa copulatrix connects to small, rounded receptaculum seminis by a very long and conspicuous duct, emerging from approximately the halfway point of the length of the vagina. Short uterine duct emerging close to vagina, entering female gland.

Distribution

Marshall Islands (present study) and the Philippines (Gosliner *et al.*, 2018).

Remarks

This species is very easy to distinguish from any other described *Palio* by its external morphology and colour pattern (Sars, 1829; Pease, 1871; O´Donoghue, 1924; Baba, 1960; Behrens, 1991; Picton and Morrow, 1994; Martynov *et al.*, 2015). The background colour of *Palio amakusana* Baba, 1960 and *P. dubia* (Sars, 1829) is yellowish brown, while it is pale cream ornamented with small orange yellow papillae in *P. gracilis* (Pease, 1871), greenish black varied with chestnut and green and covered with irregular spots of a pale -yellow or fawn-colour in *P. nothus* (G. Johnson, 1838), translucent with microscopic brown specks sometime sparse but usually quite dense in *P. zosterae*

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Figure 2. Photographs of living animals. A–C *Palio gaeli* sp. nov. A, C. MNCN 15.05/200208H; B. MNCN 15.05/200208P; photographs by S. Johnson. D–E *Polycera nimbsi* sp. nov. D, C.547846; photograph by M. Nimbs. E, C.547849; photograph by S.D.A. Smith.

(O´Donoghue, 1924), and olive green or pale brown in *P. ionica* Korshunova, Sanamyan & Martynov, 2015. In addition, *P. amakusana*, *P. dubia*, *P. zosterae*, and *P. nothus* have five tripinnate gill branches, *P. ionica* has four or five, and *P. gracilis* has eight very small arborescent branches. However, our specimens only have three large gill branches, and with a very characteristic trident shape. The number and shape of the frontal papillae are also different among species, being small lobes in *P. amakusana* (12–14), *P. dubia* (8), *P. nothus* (several), *P. zosterae* (several), and *P. gaeli* sp. nov. (4), while they are quite long in *P. gracilis* (8). They are unknown in *P. ionica* since the description is very incomplete and there is no photograph or drawing of the specimen (Martynov *et al.*, 2015). Finally, the number of extra-branchial appendages on each side of the gill is also important. There are four to six in *P. amakusana*, five in *P. dubia*, two or three in *P. nothus* and five or six in *P. zosterae.*, The extra-branchial processes are not described or drawn in the original work of *P. gracilis* although there is a large number of tentacular processes in the margin and dorsal areas. This feature is also unknown for *P. ionica*. Our specimens have three lobed appendages, the one closest to the gill larger than the others. These appendages are difficult to distinguish due to the colouration and the rest of the tubercles that are present on the notum and margins. Regarding internal anatomy, it is very difficult to compare species since most lack this information (Sars, 1829; Pease, 1871; O´Donoghue, 1924; Baba, 1960; Martynov *et al.*, 2015). Unfortunately, we were not able to obtain sequences for these specimens from Marshall Islands, likely because of its original fixative. However, all these features described above make *Palio gaeli* sp. nov. be very easy to differentiate from the other species of *Palio* and the remaining genera of the subfamiliy Polycerinae.

Genus *Polycera* Cuvier, 1816

***Polycera nimbsi* sp. nov.** (Figures 2D, E, 3C, D, 5)

LSID urn:lsid:zoobank.org:act:39988A8E-BF43-4D7B-BC2A-038A37FDBA19

Diagnosis

Different colour morphs. Notal margin with medium-sized pointed tubercles. 4–6 velar processes elongated. No extra-branchial appendages. Large, robust labial cuticle with lateral wings and stretch marks. Typical *Polycera* radula. Cup-shaped structure indicating end of prostatic section. Penis armed with two types of spines.

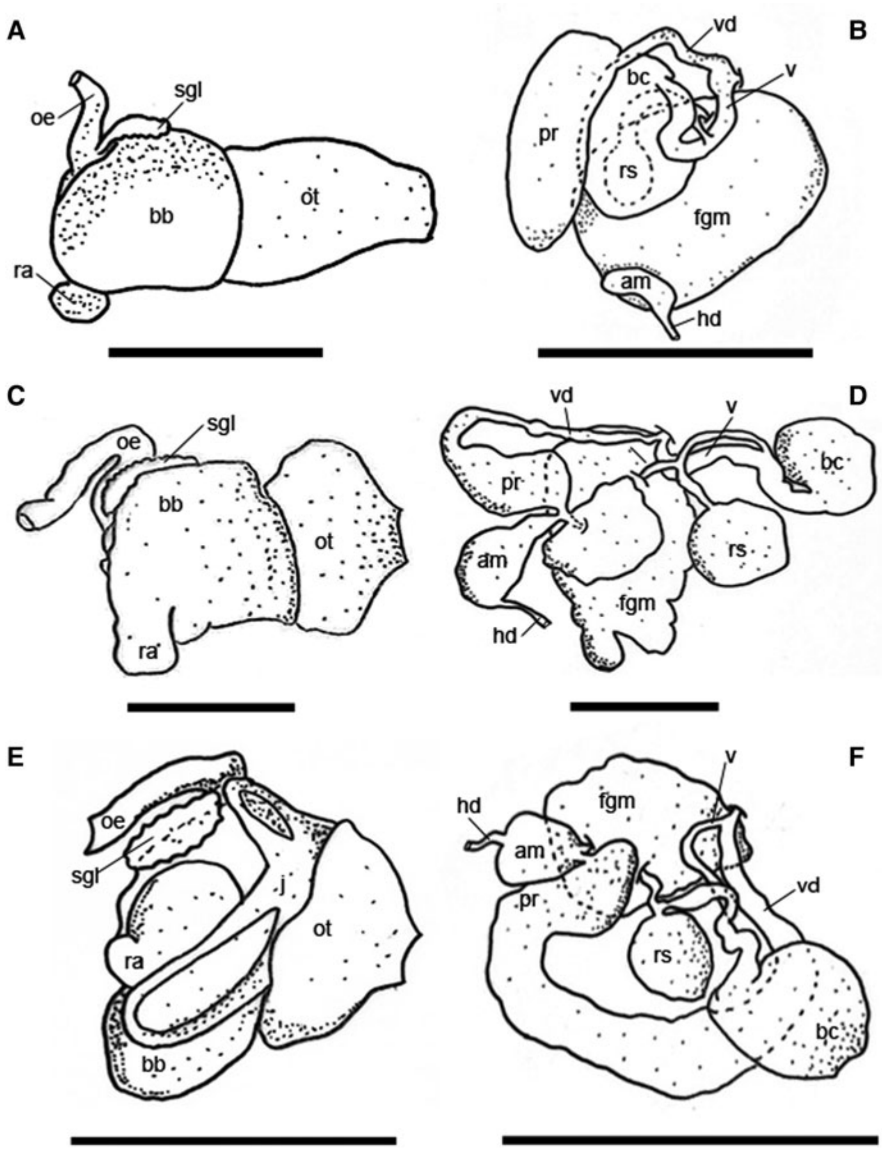

Figure 3. Drawings of internal anatomy. A,B, buccal bulb and reproductive system of *Palio gaeli* sp. nov. C, D, buccal bulb, and reproductive system of *Polycera nimbsi* sp. nov. E, F, buccal bulb, and reproductive system of *Paliota galactica* sp. nov. Abbreviations: am, ampulla; bb, buccal bulb; bc, bursa copulatrix; fgm, female gland mass; hd, hermaphroditic duct; oe, oesophagus; pr, prostate; ra, radular sac; rs, receptaculum seminis; sgl, salivary gland; va, vagina; vd, vas deferens. Scale bars = 1 mm.

Material examined

Holotype. AM C.547846, Australia, New South Wales, Nelson Bay, Port Stephens, 6 mm preserved, 12 September 2015, col. by M. Nimbs. Dissected (SEM: radula, labial cuticle) and sequenced (*COI, 16S*). *Paratypes.* AM C.547847, Australia, New South Wales, Nelson Bay, Port Stephens, 6 mm preserved, 12 September 2015, col. by M. Nimbs. Dissected and sequenced (*H3*). AM C.547848, Australia, New South Wales, Nelson Bay, Port Stephens, 5 mm preserved, 12 September 2015, col. by M. Nimbs. Dissected (SEM: radula, labial cuticle) and sequenced (*COI*). QM MO 86041. Australia, New South Wales, Fly Point, 4 mm preserved, 11 March 2017, col. by M. Nimbs. Dissected (SEM: radula, labial cuticle) and sequenced (*COI, H3*). AM C.547849, Australia, New South Wales, Low Reef, 4 mm preserved, 04 December 2014, col. by S.D.A. Smith. Dissected (SEM: radula, labial cuticle) and sequenced (*COI*). All the specimens preserved in 96% EtOH.

Etymology

The species is named after Matt Nimbs, who collected and photographed this species, in gratitude for always being willing to help.

External morphology (Figure 2D, E)

Body elongated, limaciform, wrinkled due to presence of numerous small conical tubercles all over body. Notal margin with low crest that enlarges anteriorly into oral veil. Notal margin with medium-sized pointed tubercles. Anterior margin of head expanded with frontal veil bearing 4–6 elongated velar processes. Small oral tentacles. Rhinophores perfoliate with nine lamellae, lacking sheaths, tips smooth. Gill non-retractile into pocket, surrounding anus in semi-circle. Five or six bipinnated gill branches, central branch longer than posteriors. Genital opening on right side of body.

Colour pattern (Figure 2D, E)

Two different colour morphs may be present. (1) Beige with brownish marbled pattern, including velar processes, oral tentacles, and gill branches. Dark lamellae, yellowish on the upper part. Notum with dense bright white spots (Figure 2D). (2) Orangish to pale brownish with dense minute bright white spots scattered on notum and notal margin, also on velar processes and gill branches. Velar processes, oral tentacles, outer side of gill branches, most of lamellae and tip of rhinophores black, and all these structures with yellowish in some upper areas (Figure 2E). There is probably also a third colour morph, as in the photograph taken by M. Nimbs at Serenity Bay (Solitary Islands Marine Park), NSW, Australia, in 2016, (https://www.flickr.com/photos/128938954@N03/31440861523/in/photostream/) that shows a specimen very similar to ours but with olive colouration and much more white pigment dorsally.

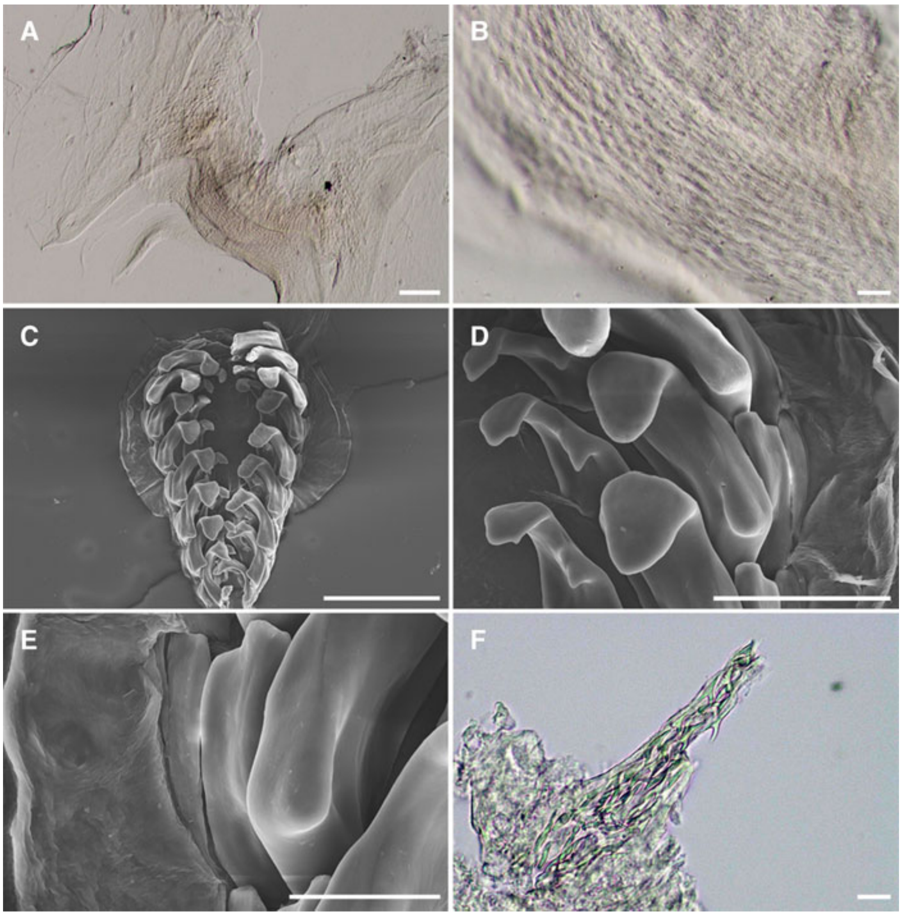

Figure 4. Scanning electron micrographs (SEM) and light microscope photographs (LMP) of *Palio gaeli* sp. nov. A, labial cuticle (MNCN 15.05/200208H) (LMP); B, detail of labial cuticle (MNCN 15.05/200208H) (LMP); C, complete radula (MNCN 15.05/200208H) (SEM); D, detail of inner and outer lateral teeth (MNCN 15.05/200208P) (SEM); E, detail of marginal teeth (MNCN 15.05/200208P) (SEM); F, detail of penial spines (MNCN 15.05/200208H) (LMP). Scale bars: A, 50 µm; B, 10 µm; C, 300 µm; D, 100 µm; E, 50 µm; F, 10 µm.

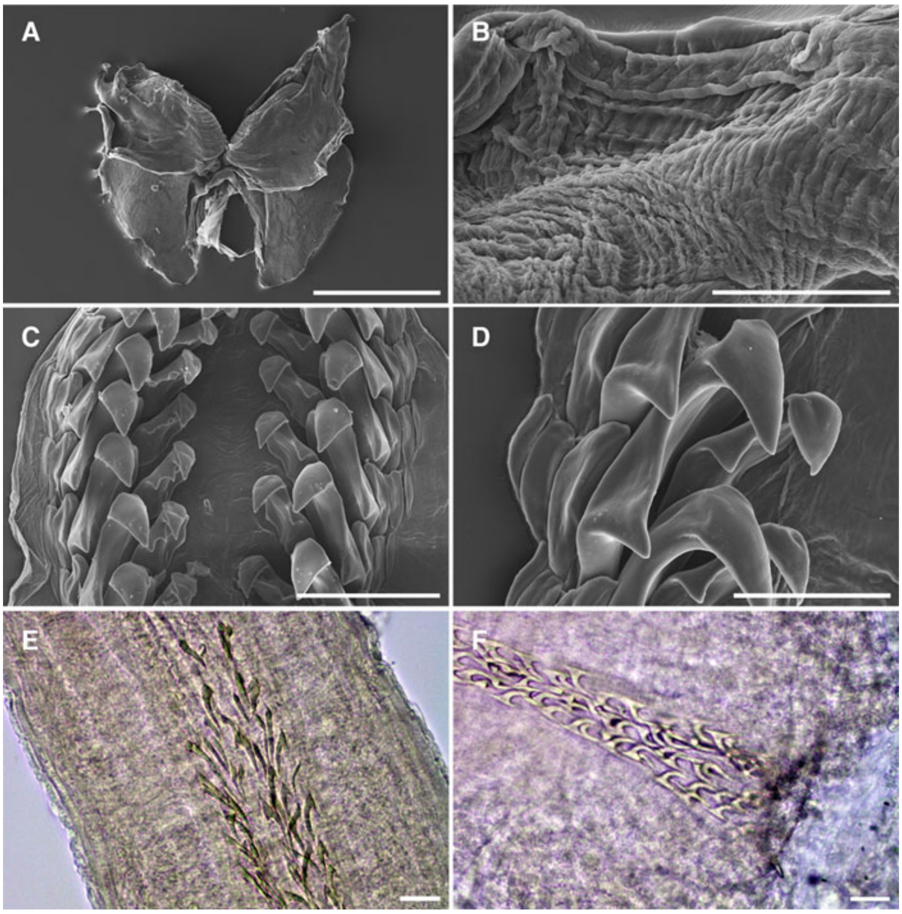

Figure 5. Scanning electron micrographs (SEM) and light microscope photographs (LMP) of *Polycera nimbsi* sp. nov. A, labial cuticle (MNCN 15.05/200210); B, detail of labial cuticle (AM C.547846) (SEM); C, section of radula at rows 3–7 (MNCN 15.05/200210) (SEM); D, detail of a row of teeth (AM C.547846) (SEM); E, penial spines located more proximal to base (AM C.547848) (LMP); F, penial spines located distally (AM C.547848) (LMP); Scale bars: A, 500 µm; B, 50 µm; C, 200 µm; D, 100 µm; E, 50 µm; F, 50 µm.

Internal anatomy (Figures 3C, 5A–D)

Oral tube smaller than buccal bulb. Pair of elongate, granular salivary glands attached at each side of oesophagus where it enters buccal bulb (Figure 3C). Labial cuticle large, robust with two large and elongated lateral wings, with stretch marks (Figure 5A, B). Radular formula 12 × 3.2.0.2.3, rachidian tooth absent (Figure 5C). Outer lateral larger, thicker than inner; all laterals with hook-shaped wing-like expansion; wing-like expansion less prominent on inner laterals. Outer lateral elongated, hamate, with strong prominent distal cusp; inner lateral with narrow base and triangular, spatulate distal cusp (Figure 5C, D). Marginal teeth smaller, flat, plate-like, pseudo-rectangular, decreasing in size outward (Figure 5C, D).

Reproductive system (Figures 3D, 5E, F)

Triaulic; hermaphroditic duct elongate, thin. Ampulla small, kidney-shaped; post-ampullary duct bifurcating into short oviduct leading to large female gland mass and vas deferens through prostrate portion. Prostate small, kidney-shaped narrowing towards distal vas deferens. In its original position, prostate surrounds bursa copulatrix. Inside vas deferens a cup-shaped structure indicates end of prostatic section. Vas deferens short, narrow. Penial bulb reduced. Penis armed with two types of chitinous spines; spines closest to prostate more elongate; spines closest to genital opening hook-shaped (Figure 5E, F). Vaginal duct elongated, slightly bent, longer and wider than vas deferens. Vagina ends in large oval bursa copulatrix. Bursa copulatrix connects to large, oval receptaculum seminis by a very long, and thin duct, emerging from about half length of vagina. Long and thin uterine duct emerging past length of duct, entering female gland.

Distribution

New South Wales, Australia (present study).

Remarks

In our phylogenetic analysis, the genus *Polycera* is not supported as monophyletic, as was the case in analyses previously carried out by other authors (Palomar *et al.*, 2014; Sorensen *et al.*, 2020; Knutson and Gosliner, 2022). However, although *Polycera* is not supported, *P. nimbsi* sp. nov. always appears in clades containing other *Polycera* species (Figures 1, S1–S4), and its morphological features agree with the mix of characters known for other species described to date (Miller, 1996). In Figure S2 (*H3* single gene) the new genus described below (*Paliota* gen. nov.) also falls within a cluster with other *Polycera* species. This could be explained because it is an artefact of having too few sequences included in this analysis. But looking at Table 4, the *H3* uncorrected pairwise genetic distance between *P. nimbsi* sp. nov. vs *P. galactica* sp. nov. is 8.54%, which is less than the percentages found between *P. nimbsi* sp. nov. and *P. alabe* (9.76), and *P. abei* and *P. atra* (10.67). However, these three *Polycera* spp. never cluster with the clade containing the type species *P. quadrilineata*, which in fact could mean that they need to be transferred to another genus. At this point, we need a more robust and complete phylogeny to make this taxonomic decision. *COI* uncorrected pairwise genetic distance between *P. nimbsi* sp. nov. and *P. galactica* sp. nov. is 19.89–20.55 (Table 2), higher than with any other *Polycera* spp. A comparison of the uncorrected pairwise genetic distances for each gene is shown in Tables 2–4 (*COI, 16S*, and *H3*, respectively).

Thus, until the phylogeny of the Polycerinae is better resolved, including the maximum number of species and the largest number of genes possible, we will always be left wondering if we are not making mistakes when assigning this species to the genus *Polycera*. In this study, we have decided to make that decision based on the presence of long velar appendages, a diagnostic character at this time for the genus. That, together with the radula, its union with other *Polycera* species in the phylogenetic trees, and the comparison of the uncorrected pairwise genetic distances of all three genes, makes us think that we are making the right decision. Regarding the two-colour forms described here, the range of intraspecific variability for *COI* uncorrected pairwise genetic distance is between 0.3 and 1.38, allowing us to be confident in our description (Table 2).

Of all the *Polycera* species recorded throughout the Indo-Pacific and Australia in particular, none are similar to *P. nimbsi* sp. nov. *P. capensis*, *P. hedgpethi, P. janjukia, P. melanosticta, P. risbeci*, and *P. parvula* all differ in colouration from the two described colour morphs of *P. nimbsi* sp. nov. Additionally, the new species lacks extra-branchial processes present in *P. capensis* and *P. hedgpethi*. Regarding *P. melanosticta*, Miller (1996: 445) described its colouration as 'white, translucent, grey or greyish brown all over subepidermally, sometimes very dense, opaque white spherules within bases of papillae; black pigment, evenly and widely spaced over surface of body, on terminal knob, lamellae of rhinophores, outer face of gills at tip and base, lightly on inner face, tip of tail and middle section of foot angles; orange pigment in between base of velar processes and subterminally on tail, i.e. anterior of black patch, on lower rhinophore lamellae and stalk, middle section of outer face of gills, upper surface of base of foot angles, anterior foot groove; sole of foot pigmentless; viscera showing through yellowish brown.'. Clearly, although with a few similarities, this description differs from *P. nimbsi* sp. nov. Internally, the radulae are very similar, but there are substantial differences in the reproductive system; the bursa copulatrix and, above all, the seminal receptacle of *P. melanosticta* are very small compared to those of *P. nimbsi* sp. nov. Also, the vagina and oviduct are very short in *P. melanosticta* (Miller, 1996), while in the species described here those ducts are much longer. Moreover, *P. melanosticta* lacks the spherical thickening of the vas deferens characteristic of *P. nimbsi* sp. nov. Figure S1 shows that these two species are closely related, but with an uncorrected pairwise genetic distance of 2.4 between them for *16S* (Table 3). As shown in Table 3 for *16S*, the differentiation between species in this gene can be considered from 1.55 (*P. capensis* vs *P. aurantiomarginata*).

***Paliota* gen. nov.**

Diagnosis

Body elongated, limaciform, with distinct marginal ridge and tubercles. Medium size digitate velar processes, rhinophores perfoliate lacking rhinophoral sheaths; gill branches bipinnate and not retractile; extra-branchial processes absent. Prominent jaws. Labial cuticle with wing-like processes. Radula tubiform in its original position, teeth forming an enclosed structure. Inner lateral teeth scythe-shaped, smooth, elongated, and wide, tapering to tip. Prostate very well developed. Penis armed.

Etymology

The generic name *Paliota* is a play on words to highlight the resemblance to related genera such as *Palio*, *Paliolla*, and *Polycera*, within the Polycerinae.

Paliota galactica* sp. nov. *(Figures 3E, F, 6A–C, 7)

LSID urn:lsid:zoobank.org:act:5EF65DF5-AEC9-4C4B-BF27-5739532CDFB1

Polycera *sp. in Coleman (2008): 360*

Material examined

Holotype. AM C.547850, Australia, New South Wales, Nelson Bay, 7 m depth, 4 mm preserved, November 2014, col. by D. Harasti.

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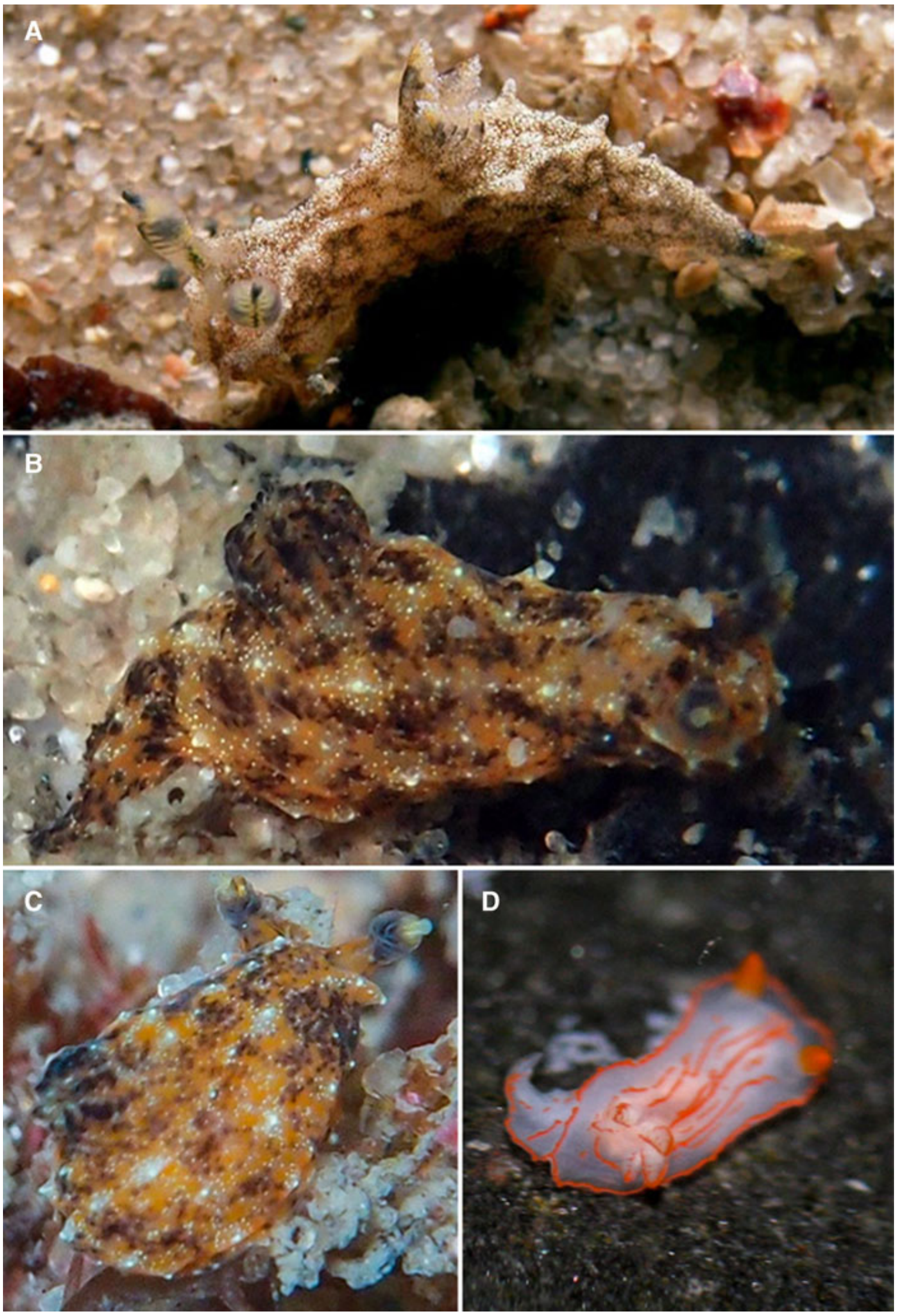

Figure 6. Photographs of living animals. A-C. *Paliota galactica* sp. nov. A, B, AM C.547850; C, AM C.547851; photographs by D. Harasti. D. *Paliolla templadoi* (MNCN 15.05/94863); photograph by M. Pola.

Dissected (SEM: radula, labial cuticle) and sequenced (*16S*). *Paratypes.* AM C.547850, Australia, New South Wales, Nelson Bay, 7 m depth, 4 mm preserved, 05 October 2014, col. by D. Harasti. Dissected (SEM: radula, labial cuticle) and sequenced (*COI*). QM MO 86040, Australia, New South Wales, Nelson Bay, 7 m depth, 3 mm preserved, 05 October 2014, col. by D. Harasti. Dissected (SEM: radula, labial cuticle) and sequenced (*H3*). All specimens preserved in 96% EtOH.

Etymology

The specific epithet refers to a galaxy due to the colour pattern of the species.

External morphology (Figure 6A–C)

Body elongated, limaciform, with distinct marginal ridge; highest at middle length: slightly constricted laterally between head and mid-region; notum with uneven appearance due to presence of tubercles. Ten tubercles aligned along mantle rim, larger than those dispersed all over remaining body. Anterior margin of head expanded with frontal veil bearing six digitate medium-size velar processes. Rhinophores perfoliate with eight lamellae, lacking sheaths. Gill non-retractile into pocket, with eight bipinnate branches surrounding anus in semi-circle. Foot forms two propodial tentacles at its anterior end. Inner side of notum covered by long spicules. Numerous small white or orangish granules arranged irregularly in this area, constituting in some parts circle-shaped concentrations. Genital pore darkly pigmented and located on the right side, very close under rhinophore.

Colour pattern (Figure 6A–C)

Notum translucent to pale cream or orange; dark brown stains all over body creating an irregular pattern. Tubercles translucent white; many tiny bright white dots scattered all over the mantle. Rhinophores stalks same colour pattern as notum with banded yellow, black, and yellow lamellae with a translucent tip. Outer rachises of the gill branches dark brown with yellow tips, inner plumes whitish. Oral tentacles and end of tail coloured dark and yellow as rhinophores and gill.

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Internal anatomy (Figures 3E, 7A–D)

Oral tube very short, not muscular. Buccal bulb much larger than oral tube, with two very prominent brownish jaws that project back to sides of buccal bulb (Figure 3E). Granular droplet-shaped salivary glands at each side of oesophagus where it enters buccal bulb (Figure 3E). Labial cuticle with two elongated downwards wing-like processes; fine striations present at edges (Figure 7A, B). Peculiar radula, tubiform in its original position. At first view, number and shape of rows make it impossible to formulate a normal radula. Teeth form a closed structure, all facing inward, overlapping each other (Figure 7C, D). Radular formula approximately 19 × (2) 1.2.0.2.1(2). Rachidian teeth absent (Figure 7C, D). Two lateral teeth with scythe-shape, being smooth, elongated, and wide, tapering to tip (Figure 7C, D). Both laterals very similar in size; second inner tooth appears to have a small cusp near its base. Marginal teeth are one or two small plates. First one easy to locate (Figure 7C, D), but second is not appreciated in all rows and specimens due to its small size (Figure 7E).

Reproductive system (Figures 3F, 7F)

Triaulic. Long, thin, hermaphroditic duct connects with hazelnut-shaped ampulla. Short post-ampullary duct emerges from ampulla, which branches on one side into prostate, and on other side into small duct that enters female gland. Prostate very well developed, long and wide narrowing to vas deferens (Figure 3F). End of vas deferens with small penis, armed with four longitudinal rows of small hook-shaped spines (Figure 7F). Vagina long, narrow, ending in bursa copulatrix large and rounded. From vagina, before reaching bursa copulatrix, a large and conspicuous duct leads into pyriform seminal receptacle, smaller than bursa copulatrix. Finally, uterine duct leaves seminal receptacle to enter female gland.

Distribution

Australia: New South Wales (present study) and Victoria (Coleman, 2008).

Remarks

The new genus described here has a unique and different anterior part of the digestive system from the other Polyceridae genera. Since *Paliolla* is the only other Polycerinae genus with an unusual pharynx (Burn, 1975; Ortea, 1989; Ortea *et al*., 1992), we first thought that we had found a third species belonging to that genus. However, there are several significant differences. In our specimens, the oral tube is much smaller than the buccal bulb and the latter is surrounded by two very prominent brown jaws (Figure 3E). These jaws are large, elongate, and very difficult to miss if present, as they surround the bulb on both sides, and are very robust and dark brown in colour. For the two species of *Paliolla* described, the presence of jaws is not mentioned (Angas, 1864; Burn, 1958; Ortea, 1989; Ortea *et al*., 1992), while for *Palio*, Baba (1960) and Miller (1996) describe them as triangular. However, we think, based on the drawing by Baba (1960: Pl. VI, Figure 2B) that the author was referring to the labial cuticle. This nomenclatural confusion also occurs in *Polycerella*, where jaws are described as oval for *P. glandulosa* by Behrens and Gosliner (1988) but, in fact, the authors refer to the labial cuticle. *Polycera* species also lack distinct jaws (Sørensen *et al*., 2020). Additionally, the salivary glands are much smaller than those described to *P. cooki* by Burn (1975). Regarding teeth, Burn (1958, 1975: 109, Figures 2, 3) described them as 'needle-like in shape with slender compressed base. Each tooth rising from a separate socket and not connected by or joined to a chitinous odontophoral ribbon'. This description and the one by Ortea (1989) do not match with our specimens since although at first

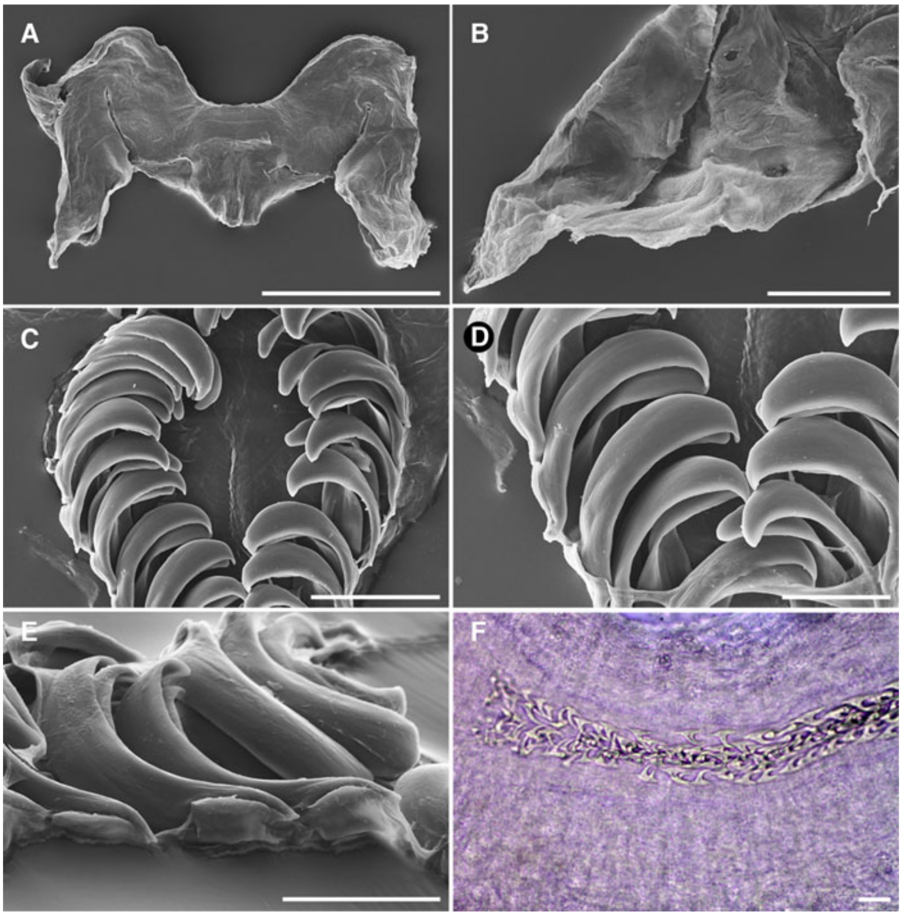

Figure 7. Scanning electron micrographs (SEM) and light microscope photographs (LMP) of *Paliota galactica* sp. nov. A, labial cuticle (AM C.547850); B, detail of labial cuticle (MNCM 15.05/200209) (SEM); C, view of radula (AM C.547851) (SEM); D, detail of lateral teeth (AM C.547851) (SEM); E, detail of marginal teeth (MNCM 15.05/200209); F, penial spines (AM C.547851) (LMP). Scale bars: A, 500 µm; B, 100 µm; C, 100 µm; D, 50 µm; E, 50; F, 10 µm.

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sight the teeth appear as a closed structure, after careful observation rows can be seen (Figure 7C, D). Inner laterals are elongate but far from being needle-like, and instead they are very wide and blade-like. Moreover, one or two small outer plates can be distinguished, which are absent in *P. cooki* (Burn, 1975) and they are very different from the ones described by Ortea *et al.* (1992) for *P. templadoi.* There are also some differences in the reproductive systems as described by Burn (1975) and Ortea *et al.* (1992). Ortea *et al.* (1992) described a vestibular gland that is not present in the new genus, and in Burn´s description, the position of the receptaculum seminis appears much closer to the prostate than to the vagina. Finally, *COI* pairwise distances between the new genus and *P. templadoi* is 18.43 (Table 2). For all the reasons mentioned above, we are confident that this species belongs to a new genus herein described, for which the major synapomorphies are the large brown jaws and the peculiar tubiform radula with scythe-shaped lateral teeth.

Discussion

The family Polycerinae clearly needs to be studied in more depth. Although its monophyly was confirmed by Pola *et al.* (2014) and Sørensen *et al.* (2020), it is true that not all the genera or species that constitute it were included. In fact, when Knutson and Gosliner (2022) included an undescribed species of *Vayssierea* Risbec, 1928 (currently included in Okadaiidae Baba, 1930) in their phylogenetic study of *Gymnodoris*, it appeared within Polycerinae, albeit with a very long branch. We also included it in the preliminary analysis, but since it is not described (not confirmed morphologically) and with such a distant branch, we decided not to include it in our analysis. Once again, in Knutson and Gosliner (2022), some other genera and most of the species in the subfamily were not studied. For example, *Lamellana* has never been included in a phylogenetic framework, since it has never been found since its original description, and just recently, *Lecithophorus* Macnae, 1958 was sequenced for their study (Knutson and Gosliner, 2022). In our study, for the first time we include a species of *Paliolla* in the phylogeny (Figure 1), as well as the first colour photograph of an individual of *P. templadoi* alive (Figure 6D). In all previous studies, the relationships between the genera were not defined and, it should be repeated that *Polycera* was never recovered as monophyletic.

There are some morphological characteristics that can help us to separate the genera: starting with the easiest, *Lamellana* and *Lecithophorus* both lack a radula and have a smooth body, the mantle is reduced, and there are no processes. However, *L. gymnota* was described from Hong Kong with 'yellowish, back flecked black and yellow around the branchiae' (Lin, 1992: 182), while *L. capensis* is 'translucent white and the internal organs may be seen shining through as brownish areas. Pale lemon-yellow patches indicate the position of the buccal mass and gonad' (Macnae, 1958: 362). Whereas *Lecithophorus* has many spicules embedded in its skin, there is no mention of spicules in *Lamellana* (Macnae, 1958; Lin, 1992). *Gymnodoris* is easy to distinguish from species of other Polycerinae genera by its predatory trophic behaviour. Its species prey upon other nudibranchs and thus, its radula has many more outer lateral teeth than in the other genera (Knutson *et al.*, 2011). Regarding '*Polycera*', *Palio*, *Polycerella*, and *Thecacera,* they all have quite similar radular teeth and formulae, but there are some features that allow us to differentiate between some of them. Thus, *Thecacera* species are very easy to distinguish since they have well-developed rhinophoral sheaths (Baba, 1960; Fischer, 2006). Species of *Palio* are small and do not have elongated appendages either on the oral veil or as extra-branchial processes. Instead, their velar processes are small and tuberculate, and the extra-branchial processes are small and lobed, these being the main differences with *Polycera* (Gray, 1857; Baba, 1960; Rudman, 2003). Additionally, Rivest (1984) argued that the differences in reproductive anatomy support the case for considering *Palio* to be a genus distinct from *Polycera. Polycerella* species have more rows of teeth than *Polycera,* despite its radula being smaller. Both radulae also differ in the shape of their teeth (Verrill, 1880; Behrens and Gosliner, 1988; Miller, 1996). Finally, *Paliolla*, as stated in the introduction, is unmistakable by its unusual radula with acicular teeth forming a tube (Burn, 1958; Ortea, 1989; Ortea *et al.*, 1992) but, as discussed under Remarks, clearly different from *Paliota* gen. nov. The main problem continues to be the genus *Polycera*, as the combination of traits within the genus and the lack of support for its monophyly make the species difficult to diagnose. However, one interesting fact is that the species clustering in Figure 1 (that includes the type species) are those having large and elongated oral veil and extra-branchial processes.

Conclusions

The biodiversity of the subfamily Polycerinae, as well as the phylogenetic relationships within the family Polyceridae, need further studies. One new species of *Palio*, one new species of *Polycera*, and a new genus are described in this work based on external and internal features, supported by molecular analysis. More fresh specimens and taxonomical studies are necessary to fully understand the richness and evolution of this diverse and poorly studied family of nudibranchs.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315423000607

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Authors' contributions. **M. P.** designed and carried out the study, analysed data, interpreted the results, and wrote the manuscript. **M. M. G.** analysed data and helped write and format the manuscript. **S. P. S.** analysed data, helped to carried out the study, interpreted the results, and helped write the manuscript.

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