Zoological Journal of the Linnean Society, 2022, **XX**, 1–20. With 6 figures.

Rediscovering the overlooked genus *Murphydoris* (Nudibranchia: Goniodorididae): the first phylogeny and addition of four new species

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Received 16 October 2021; revised 30 December 2021; accepted for publication 10 January 2022

Sigurdson (1991) erected the monospecific genus *Murphydoris* to include the species *Murphydoris singaporensis*. This species differed from the rest of the genera in Goniodorididae by the lack of lamellae on the rhinophores and gill branches around the anus. Since its original description, *Murphydoris singaporensis* has only been found in Singapore and Thailand. Recently, the paratypes of *Murphydoris singaporensis* were studied and compared with remaining type species of Goniodorididae, showing that the apomorphies of the genus were unclear and some undescribed species reported as *Goniodoridella* could belong to *Murphydoris*. In this study, we examined four undescribed species from the Indo-Pacific region. The internal anatomy was studied by dissections, and electron microscope photographs are included to show details of their radulae, labial cuticles and penises. One specimen was also studied under micro-computed tomography. In addition, mitochondrial and nuclear partial sequences of the species were obtained. As a result, we describe in detail four new species of *Murphydoris*: ***M. adusta* sp. nov.**, ***M. cobbi* sp. nov.**, ***M. maracabranchia* sp. nov.** and ***M. puncticulata* sp. nov.** We additionally present the first phylogenetic tree that includes sequences of *Murphydoris*.

ADDITIONAL KEYWORDS: micro-computed tomography – nudibranchs – sea slugs – taxonomy.

INTRODUCTION

The family Goniodorididae currently include eight genera: *Ancula* Lovén, 1846, *Goniodoridella* Pruvot-Fol, 1933, *Goniodoris* Forbes & Goodsir, 1839, *Lophodoris* G.O.Sars, 1878, *Murphydoris* Sigurdson, 1991, *Okenia* Menke, 1830, *Spahria* Risbec, 1928 and *Trapania* Pruvot-Fol, 1931. The genus *Murphydoris* was assigned as belonging to Goniodorididae, mainly due to its radular formula, N × 1.1.0.1.1., which is characteristic for the family (Sigurdson, 1991; Swennen & Buatip, 2012). *Murphydoris* was originally differentiated from the remaining genera by lacking peri-anal ctenidia (gill branches), having mantle papillae only located on either side of the anus and smooth rhinophores (Sigurdson, 1991). To date, there was only one species described in the genus, *Murphydoris singaporensis* Sigurdson, 1991, originally found in Singapore. Sigurdson (1991) provided a detailed description of the external morphology of the species, and gave details of the buccal bulb, radula features and penial spines. Subsequently, no further information on this species has been published, except in Swennen & Buatip (2012), who reported the species from Thailand. Swennen & Buatip (2012) published the first photographs of a living animal and described

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[Version of record, published online 14 March 2022; http://zoobank.org/ urn:lsid:zoobank.org:pub:B5B83725-6D9F-4153-8B89-4001FDCE46CD]

the reproductive system of the species. The scarce reports of the species could be due to its small size and its cryptic coloration (Sigurdson, 1991; Swennen & Buatip, 2012). Due to its camouflage, this species is difficult to find. It was previously found in mangroves, on sunken wood covered with bryozoans identified as *Sunanella sibogae* (Harmer, 1915) (Sigurdson, 1991; Swennen & Buatip, 2012), as well as on fouling test plates submerged from a raft (Sigurdson, 1991). Although no other *Murphydoris* species has so far been described, photographs of three presumably undescribed species have been published (Cobb & Willan, 2006; Debelius & Kuiter, 2007; Coleman, 2008; Coleman *et al.*, 2015).

Most studies of Goniodorididae have focused on the genera *Okenia* (Gosliner, 2004; Rudman, 2004; Pola *et al.*, 2014, 2019; Paz-Sedano *et al.*, 2017, 2021a) and *Trapania* (Gosliner & Fahey, 2008), which include the majority of the described species in the family (MolluscaBase, 2021). Recently, Paz-Sedano *et al.* (2021b) published a review of the type species of the different genera, comparing the external morphology and internal anatomy among genera and determining each of their taxonomic characteristics. While reviewing the types of Goniodorididae, Paz-Sedano *et al.* (2021b) also studied the paratypes of *Murphydoris singaporensis*. They found that the structures located on each ending of the mantle margin, described as 'bifid to trifid papillae' by Sigurdson (1991), were, in fact, gill branches (Paz-Sedano *et al.*, 2021b). This position of the gill branches is a diagnostic characteristic of the genus *Murphydoris*. In addition, *Murphydoris* is characterized by having a reduced mantle edge (Sigurdson, 1991; Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b), lacking dorsal and lateral papillae (Paz-Sedano *et al.*, 2021b) and having smooth rhinophores (Sigurdson, 1991; Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b).

Based on these morphological characteristics of the genus, we provide an updated definition of the typical characters of *Murphydoris*, and describe four new species of *Murphydoris* collected from seas in the Indo-Pacific region, ending the monotypy of the genus 30 years after its description. We also include details of the external and internal anatomy, including scanning electron microscope (SEM) photographs of the radulae, labial cuticles and penises of the species. Additionally, one species was studied under micro-computed tomography (μCT). We have also sequenced species belonging to the genus and performed the first phylogenetic analysis including *Murphydoris* in order to delimit species and study the evolutionary relationships between Goniodorididae genera.

MATERIAL AND METHODS

Specimens

Forty-four specimens belonging to four new species of *Murphydoris* were collected by SCUBA diving from 2009 to 2020 in Australia and the Philippines. Specimens were collected, narcotized with $MgCl_2$ and preserved in 96% ethanol. Material was loaned from the California Academy of Science (San Francisco, USA) (CASIZ), the Western Australian Museum (Welshpool DC, Australia) (WAM) and the Queensland Museum (Brisbane, Australia) (QM). One specimen of *Goniodoris nodosa* (Montagu, 1808) and two specimens of *Lophodoris danielsseni* (Friele & Hansen, 1878) were borrowed from the Zoological Museum of Bergen University (Bergen, Norway) (ZMBN). Initial sampling for molecular analyses included 22 specimens available for sequencing of the four undescribed species. In addition, partial sequences of the ribosomal RNA 16S (16S) and Histone 3 (*H3*) genes were amplified for *Goniodoris nodosa* (ZMBN 125696) and sequences of the *H3* gene were obtained for *Lophodoris danielsseni* (ZMBN 130690 and ZMBN 130699). Sequences of seven species belonging to the family Goniodorididae were added from GenBank. Six other nudibranchs were included as outgroups (Table 1). Distributions and ecology of species were completed by searching online photographs of specimens and field guides. However, the systematic section did not mention the change of identification of online references due to the large number of photographs uploaded by divers and naturalists. Only references of published books and articles were included.

Molecular analyses

DNA extraction, amplification and sequencing

Extraction and amplification of mitochondrial and nuclear DNA sequences of specimens from Australia were carried out at the Universidad Autónoma de Madrid, Spain (UAM) and Universidad de Cádiz, Spain (UCA). DNA was extracted from foot tissue using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's instructions. DNA extraction, amplification and sequencing of specimens from the Philippines were performed at the California Academy of Sciences (CAS) Center for Computational Genetics (CCG). Small tissue samples were taken from the posterior portion of the foot of each specimen. Two different extraction methods were utilized depending on the amount of tissue available. Genomic DNA was extracted from small or tiny tissue samples using either Qiagen DNeasy Blood & Tissue Kit or QIAamp DNA Micro Kit (Qiagen, Valencia, CA, USA), respectively. Partial sequences of cytochrome oxidase *c* subunit

Table 1. Species used for molecular analyses, voucher and GenBank accession number. Voucher of genes and undescribed species newly sequenced during the present study are highlighted in bold. Institutional acronyms: California Academy of Science (CASIZ), Los Angeles County Museum (LACM), Queensland Museum (QMMO), Museo Nacional de Ciencias Naturales (MNCN), Western Australian Museum (WAMS) and Zoological Museum of Bergen University (ZMBN)

Species	Voucher	GenBank		
		COI	*16S*	*H3*
Ancula gibbosa	CASIZ 182028	KP340388	KP340291	KP340413
Armodoris anudeorum	LACM 3118	KP340387	KP340290	KP340412
Corambe obscura	CASIZ 183942	KP340399	KP340303	KP340419
Diaphorodoris lirulatocauda	CASIZ 184341	KP340403	KP340307	KP340422
Goniodoris nodosa	ZMBN 125696	OK156411	**OK161211**	**OK169876**
Knoutsodonta jannae	CASIZ 175578	KP340392	KP340296	KP340415
Lophodoris danielsseni	ZMBN 130690	OK156412	-	**OK169877**
Murphydoris adusta	QMMO 85985	-	**OK161212**	**OK169878**
Murphydoris adusta	QMMO 85986	-	-	**OK169879**
Murphydoris adusta	CASIZ 177775	-	**OK161213**	**OK169880**
Murphydoris adusta	CASIZ 186117	-	**OK161214**	**OK169881**
Murphydoris cobbi	QMMO 85978	**OK156413**	**-**	**OK169882**
Murphydoris cobbi	QMMO 85979	**OK156414**	**-**	**OK169883**
Murphydoris cobbi	CASIZ 186114	**OK156415**	**OK161215**	-
Murphydoris cobbi	CASIZ 186119	**OK156416**	**OK161216**	**OK169884**
Murphydoris cobbi	CASIZ 186116	**OK156417**	**OK161217**	**OK169885**
Murphydoris cobbi	CASIZ 186123	**OK156418**	**OK161218**	-
Murphydoris cobbi	CASIZ 206795	**OK156419**	**OK161219**	-
Murphydoris cobbi	CASIZ 217417	**OK156420**	**OK161220**	-
Murphydoris cobbi	CASIZ 217284	**OK156421**	**OK161221**	-
Murphydoris maracabranchia	CASIZ 186120	**OK156422**	**OK161222**	**OK169886**
Murphydoris maracabranchia	CASIZ 222015	**OK156423**	**OK161223**	**OK169887**
Murphydoris maracabranchia	CASIZ 222056	**OK156424**	**OK161224**	**OK169888**
Murphydoris maracabranchia	CASIZ 224657	**OK156425**	**OK161225**	**OK169889**
Murphydoris puncticulata	MNCN 15.05/200150	**OK156426**	**OK161226**	**OK169890**
Murphydoris puncticulata	WAM S72711	**OK156427**	**-**	**OK169891**
Murphydoris puncticulata	WAM S72462	-	**-**	**OK169892**
Murphydoris puncticulata	CASIZ 222055	**OK156428**	**OK161227**	**OK169893**
Murphydoris puncticulata	CASIZ 224656	**OK156429**	**OK161228**	**OK169894**
Okenia elegans	MNCN 15.05/88175	MK645759	MK650421	MK659667
Okenia mediterranea	MNCN 15.05/88174	MK645760	MK650422	MK659668
Onchidoris proxima	CASIZ 183921A	KM219676	KJ653673	KM225826
Peltodoris nobilis	CASIZ 182223	HM162684	HM162593	HM162499
Trapania hispalensis	MNCN 15.05/55504	JX274080	JX274048	-
Trapania reticulata	CASIZ 191431	MF958432	MF958303	-

I (*COI*), 16S ribosomal RNA (16S) and *H3* were amplified by polymerase chain reaction (PCR) using LCO1490 and HCO2198 universal primers 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). PCR and amplification conditions carried out in each institution are specified in the Supporting Information (Table S1). Successful PCR products obtained at UAM were purified following the protocol of the kit SPEEDTOOL PCR CLEAN-UP (Biotools). UAM and UCA amplifications were sequenced by Macrogen, Inc. Successfully amplified DNA at CAS was cleaned with ExoSAP-IT (USB). Output of this cleaning was fluorescently labelled through cycle-sequencing with dye-terminators (Big Dye Terminator v.3.1, Applied Biosystems). Each 10 µL reaction included: 5.45 µL of Millepore-H2O, 1.5 µL of 5 × Big Dye buffer, 0.3 µL of primer (10 mmol/L stock), 0.75 µL of Big Dye 3.1 and 2 µL of cleaned DNA product. This mix was run utilizing the STeP protocol (Platt *et al.*, 2007). Following this labelling, 2.5 µL of EDTA (125 mmol/L stock) was added then washed and spun with 100% and 70% EtOH,

respectively. Finally, 10 µL of formamide was added before sequencing on the ABI 3130xl Genetic Analyzer (Applied Biosystems), located in the CCG at the CAS.

Phylogenetic analyses

Sequences were assembled and edited using software SeqMan II (DNAStar, Madison, WI, USA). Sequences were aligned by ClustalW using MEGA7 (Kumar *et al.*, 2016). Protein-coding sequences were translated into amino acids for confirmation of alignment using the genetic code invertebrate mitochondrial DNA for *COI* and universal code for *H3*. All sequences were blasted in GenBank to check for contamination. Sequences of *COI*, 16S and *H3* were trimmed to 658, 442 and 328 base pairs, respectively. The evolutionary models were selected using JModelTest-2.1.7 (Darriba *et al.*, 2012) for each codon position of *COI* and *H3* under Bayesian information criteria (BIC) (Schwartz, 1978). Bayesian inference (BI) analysis was performed using the software package MrBayes 3.1.2b (Ronquist & Huelsenbeck, 2003) for ten million generations, four independent runs and sampling frequency of 1000. Nodes were considered supported by posterior probabilities ≥ 0.96 (Alfaro *et al.*, 2003). Maximum likelihood (ML) analysis was performed using the software package RAxML v.0.9.0 (Kozlov *et al.*, 2019), a Bootstopping cut-off of 0.03 was implemented. Nodes were considered statistically significant by bootstraps values ≥ 75 (Hillis & Bull, 1993). The tree obtained was visualized using FigTree (Rambaut, 2009) and edited in Adobe Photoshop CC 2014. The final tree was deposited in the public database TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S28788).

Species delimitation analyses

Gene pairwise uncorrected *p*-distances for *COI* and 16S were obtained using MEGA7 (Kumar *et al.*, 2016). Moreover, Bayesian Poisson tree process (bPTP) (Zhang *et al.*, 2013) delimitation analysis was conducted using webtool (https://species.h-its.org), running 100 000 MCMC generations, Thinning = 100 and Burn-in = 0.1. Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al.*, 2021) was conducted using the webtool (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) under the model Kimura (K80) ts/tv. Due to the lack of *COI* sequences for the new species *Murphydoris adusta*, both analyses were performed using *COI* and 16S sequences.

Morphological examinations

External morphology was studied using photographs of living animals and laboratory observations. Internal organs were removed from the animal by dissection and drawn under a Nikon SMZ-1500 dissecting microscope with a camera lucida attachment. Special attention was paid to the morphology of the digestive and reproductive systems. The buccal mass was removed and dissolved in 10% sodium hydroxide to remove surrounding tissue and isolate the radula and labial cuticle. The structures were then rinsed in distilled water. Radulae, labial cuticles and penises were first studied under a light microscope and they were photographed using the software cellSens. The labial cuticles and penises were dried using hexametildisilazane. Finally, the radulae, labial cuticles and penises were mounted and sputter coated for examination under a Hitachi S3000N scanning electron microscope (SEM). One specimen of the new species, *Murphydoris cobbi* (QMMO 85981), was studied in detail by µCT. The specimen was preserved in absolute ethanol, dyed in 10% iodine solution for 3 days, dried with hexamethyldisilazane for 2 h and then left to air dry overnight. Imaging was performed using a microtomography Skyscan 1172, using the following parameters: source voltage = 55 kV, source current = 165 µA, pixel size = 0.88 µm, frames averaged = 4, filter = no, binning = 1 × 1 and scanning time = 177 min. Images were reconstructed with the software NRECON and cleaned with the software CTAnalyzer (https://www.bruker.com/en/products-and-solutions/preclinical-imaging/micro-ct/3d-suite-software.html). Data were corrected and visualized using DATAVIEWER and CTVox software. Three-dimensional (3D) images were visualized and edited using Amira software. Raw image data and rotational movies are on-line, available on Morphobank (http://morphobank.org/permalink/?P3979). This work has been registered under Zoobank Accession: urn:lsid:zoobank.org:pub:B5B83725-6D9F-4153-8B89-4001FDCE46CD.

RESULTS

Molecular results

We obtained new sequences from 24 specimens belonging to six species (Table 1), including new species of *Murphydoris*, *Goniodoris nodosa* and *Lophodoris danielsseni*. Evolutionary models used for *COI* were TrN+I, TPM3uf+I and HKY+G for the first-, second- and third-codon positions, respectively. The evolutionary model for 16S was TPM1uf+I+G. For *H3*, TIM2 was selected for the first-codon position, JC for the second-codon position and TPM2uf+I for the third-codon position. The best Bayesian phylogram supported the monophyly of Goniodorididae with maximum branch support (Fig. 1) (BI = 1). However, the relationship among *Ancula*, *Trapania* and

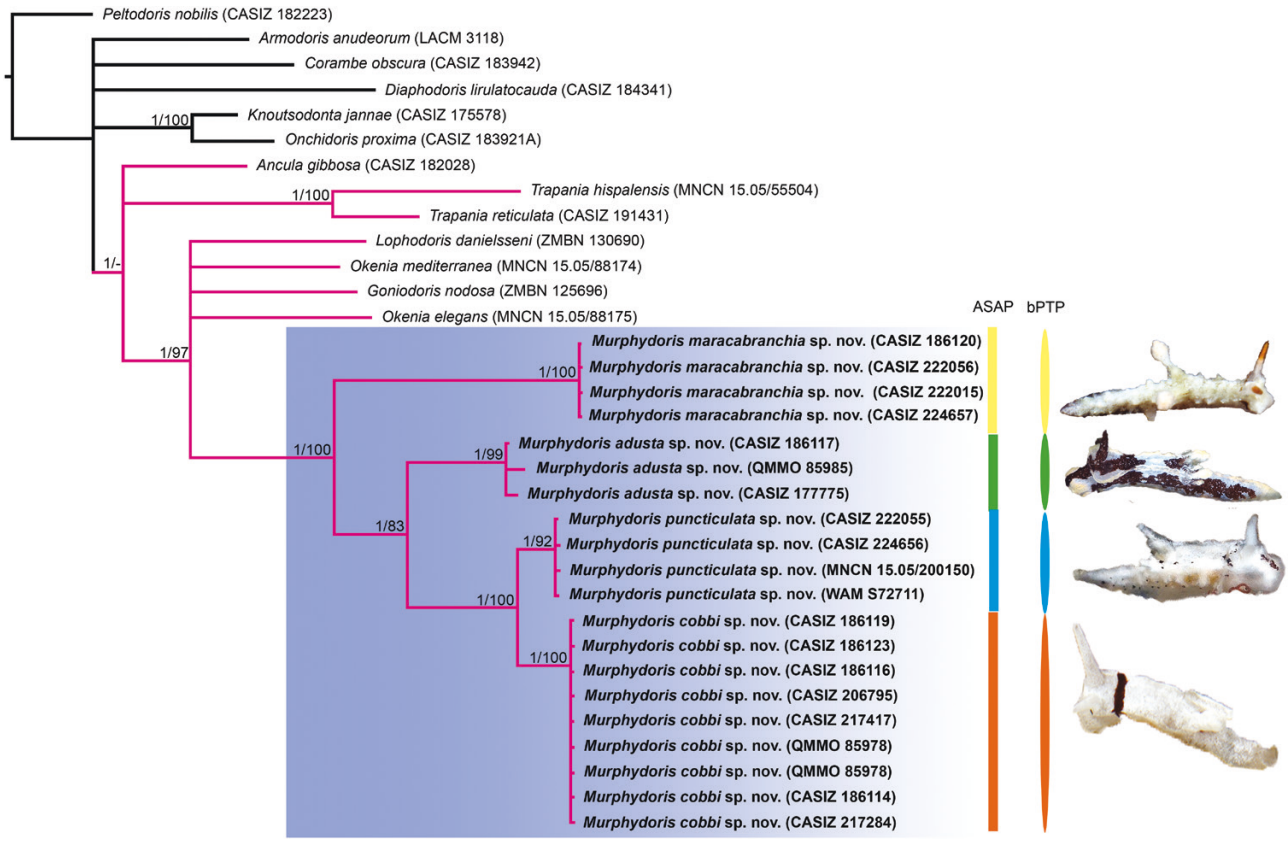

Figure 1. Phylogenetic relationships (BI/ML) based on the concatenated mitochondrial (*COI* and 16S) and nuclear (*H3*) genes. Purple branches indicate the node supporting the Goniodorididae family. Blue shading represents the genus *Murphydoris*. *Murphydoris* species newly sequenced in bold. Colours highlighted in ABGD and bPTP species delimitation analyses represent potential different taxa. Photographs of species from top to bottom: *Murphydoris maracabranchia* (CASIZ 186120), photographed by P. Palereacio; *Murphydoris adusta* (WAM S72660), photographed by G. Cobb; *Murphydoris puncticulata* (WAM S72711), photographed by G. Cobb; *Murphydoris cobbi* (CASIZ 206795), photographed by T. M. Gosliner.

the clade including the remaining genera, remain unresolved. The two species of *Trapania* formed a well-supported clade (BI = 1, ML = 100). *Goniodoris*, *Lophodoris*, *Okenia* and *Murphydoris* also formed a well-supported clade (BI = 1, ML = 97), although the relationships among them were not resolved. All *Murphydoris* species, including the ones described as new herein, formed a clade (BI = 1, ML = 100). Within *Murphydoris*, phylogenetic analyses supported a clade including the new *M. adusta*, *M. puncticulata* and *M. cobbi* (BI = 1, ML = 83), with *M. puncticulata* and *M. cobbi* as sister-species (BI = 1, ML = 100). Species delimitation analyses distinguished the four new species: *Murphydoris adusta*, *M. cobbi*, *M. maracabranchia* and *M. puncticulata* as different and valid entities (Fig. 1). The highest intraspecific distances between specimens belonging to the same species were 0.8% for *COI* and 0.9% for 16S (Table 2). The lowest values obtained for interspecific distances among species were 10.7% and 7.9% for *COI* and 16S, respectively (Table 2).

SYSTEMATICS

ORDER NUDIBRANCHIA CUVIER, 1817

FAMILY GONIODORIDIDAE H.ADAMS & A.ADAMS, 1854

GENUS *MURPHYDORIS* SIGURDSON, 1991

Type species: Murphydoris singaporensis Sigurdson, 1991 by monotypy.

MURPHYDORIS ADUSTA SP. NOV.

(FIGS 2A, B, 3A–C, 4A–D)

'*Goniodoris aspersa*' – Debelius & Kuiter (2007: 28).
'*Goniodoridella* sp.' – Su *et al.* (2009: 457, fig. 11B).
'*Goniodoridella* sp. 6' – Gosliner *et al.* (2015: 133).
'*Goniodoridella* sp. 6' – Gosliner *et al.* (2018: 51).
'*Goniodoridella* sp. 3' – Nakano (2018: 166).

Zoobank registration: urn:lsid:zoobank.org:act:A6372BF6-E947-4470-9B1D-D22A9AB5FE58.

Material examined: **Holotype.** QMMO 85985 (Australia, Queensland, La Balsa Park, Mooloolah

Table 2. *COI* and 16S gene pairwise uncorrected *p*-distances (%) amongst the new species of *Murphydoris*

	Species	*Murphydoris adusta*	*Murphydoris cobbi*	*Murphydoris maracabranchia*	*Murphydoris puncticulata*
***COI P*-distances (%)**	*M. adusta*	-	-	-	-
	M. cobbi	-	0–0.8	21.4–21.9	10.7–11.2
	M. maracabranchia	-	21.4–21.9	0	19.4
	M. puncticulata	-	10.7–11.2	19.4	0–0.5
16S *P*-distances (%)	*M. adusta*	0.6–0.9	14.5–15.1	18–18.6	13.2–14.5
	M. cobbi	14.5–15.1	0–0.6	17.7–18.6	7.9–8.2
	M. maracabranchia	18–18.6	17.7–18.6	0–0.6	18.9–19.2
	M. puncticulata	13.2–14.5	7.9–8.2	18.9–19.2	0–0.9

River, 26°41′S, 153°08′E, 3–6 m depth; coll. G. Cobb, 29 September 2019), one preserved specimen, 3 mm long, dissected. **Paratypes.** MNCN 15.05/200143–MNCN 15.05/200148, WAM S72660, QMMO 85986–QMMO 85988; QMMO 85995 (Australia, Queensland, La Balsa Park, Mooloolah River, 26°41′S, 153°08′E, 3–6 m depth; coll. G. Cobb). MNCN 15.05/200143 (29 September 2019), one preserved specimen, 2 mm long, dissected (SEM: Radula). MNCN 15.05/200144 (Fig. 3B, C) (29 September 2019), one preserved specimen 2 mm long, dissected (SEM: Radula). MNCN 15.05/200145 (29 September 2019), one preserved specimen, 3 mm long, dissected (SEM: Radula). MNCN 15.05/200146 (28 November 2019), one preserved specimen, 5 mm long. MNCN 15.05/200147 (28 November 2019), one preserved specimen, 5 mm long, dissected (SEM: Radula, penis). MNCN 15.05/200148 (5 December 2019) one preserved specimen, 10 mm long, dissected (SEM: Radula). WAM S72660 (Figs 1, 2A) (2 May 2018), one preserved specimen, 5 mm long. QMMO 85986 (Fig. 4D) (2019), one preserved specimen, 4 mm long, dissected (SEM: Radula, labial cuticle, penis). QMMO 85987 (2019), one preserved specimen, 3 mm long, dissected (SEM: Radula). QMMO 85988 (29 September 2019), two specimens. QMMO 85995 (Figs 2B, 4A, C) (11 December 2019), one preserved specimen, 4 mm long, dissected (SEM: Radula, labial cuticle). CASIZ 177775 (Philippines, Batangas Province, Tingloy, Maricaban Island, Aphol's Rock, 13°45′41.74″N, 121°23′0.56″E; coll. T. M. Gosliner, 23 April 2008). CASIZ 186117 (Philippines, Batangas Province, Calumpan Peninsula, Mabini, Murals dive site, 13°41′56.472″N, 120°52′57.719″E; coll. D. Raymundo, 4 May 2011). CASIZ 181290 (Fig. 4B) (Philippines, Luzon, Batangas Province, Mabini, Mainit Bubbles dive site, 13°41.155′N, 120°53.713′E; coll. T. M. Gosliner, 21 May 2009), dissected (SEM: Radula).

Distribution: Indian Ocean to Indonesia, western Pacific Ocean; Madagascar (Gosliner *et al.*, 2015, 2018); Indonesia: Palau Sangeang (Anderson, 2021) and Bali (Debelius & Kuiter, 2007); Philippines: Batangas (Femia Marzo, 2014; present study) and Negros Oriental (Tillen, 2015); Taiwan: Penghu (Su *et al.*, 2009); Japan: Izu Peninsula (Nakano, 2018); Australia: New South Wales (Mayes, 2020) and Queensland (Cobb, 2017; present study).

Ecology: The species is found from 1 m (Gosliner *et al.*, 2015, 2018) to up to 21 m depth (Femia Marzo, 2014). It has been found on red algae (Mayes, 2020) and under coral rubble (Gosliner *et al.*, 2015, 2018). *Murphydoris adusta* feeds on compound tunicates (Gosliner *et al.*, 2015, 2018).

Etymology: From Latin *adustus*, perfect participle passive of *aduro*, brown. *Murphydoris adusta* refers to the dark brown colour of the body.

External morphology (Figs 2A, B, 3A): Preserved specimens between 2 and 3 mm length. Body elongated and narrow. Foot tapered at posterior end, with slightly rounded edge at anterior part. Foot narrow, does not protrude from sides of notum. Notal border well developed, with thickened edge at anterior part around head, continuing with slightly serrated margin, supported by internal spicules. Gill located at posterior two-thirds of body. Three bipinnate gill branches arise from common base. Middle branch larger with wider and rounded tip (Fig. 3A). Dorsal or lateral papillae absent. Rhinophores non-retractile and smooth, located at anterior part of body. Rhinophoral sheath absent. Mouth displaced towards ventral zone, lacking oral tentacles. Reproductive opening located halfway up right side. Whole body, including lateral processes, covered by dense network of spicules.

Colour pattern (Fig. 2A, B): Body chocolate brown with patches between pale blue and iridescent white. Edge of notum same colour as patches with discontinuous pale-yellow coloration at border. Middle

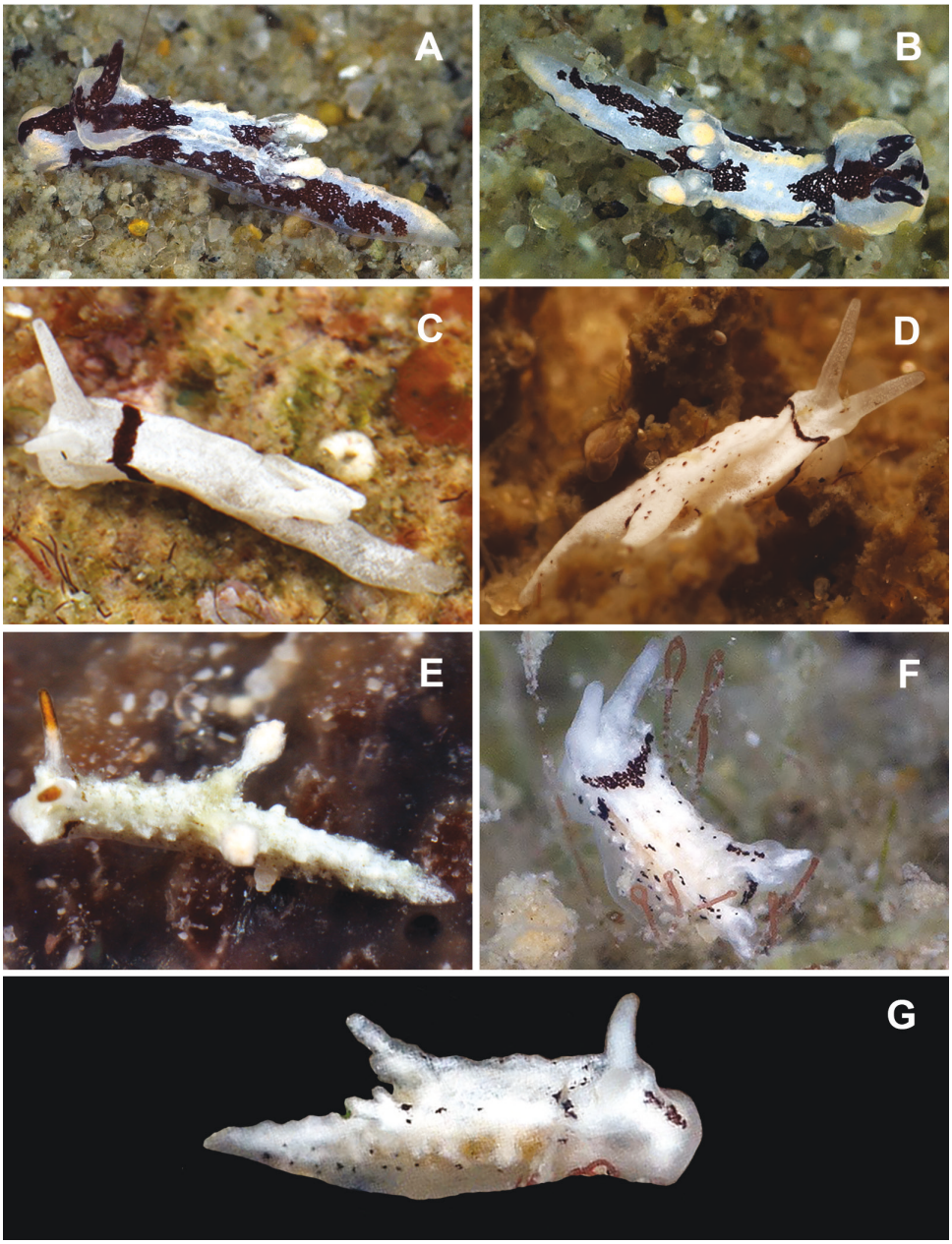

Figure 2. Photographs of living animals. A, B, *Murphydoris adusta* sp. nov., photographed by G. Cobb: A, (WAM S72660); B, (QMMO 85995). C, *Murphydoris cobbi* sp. nov. (CASIZ 206795), photographed by T. M. Gosliner. D, *M. cobbi* sp. nov. (QMMO 85979), photographed by G. Cobb. E, *Murphydoris maracabranchia* sp. nov. (CASIZ 186120), photographed by P. Paleracio. F, G, *M. puncticulata* sp. nov. photographed by G. Cobb. F, (WAM S72711), G, (WAM S72710).

dorsal part of the body with transversal iridescent white line and yellow patch on each side. Anteriormost part of head iridescent white with chocolate brown band in middle. Rhinophores with same coloration as body. Gill branches translucent white with yellow tips, sometimes more striking on larger middle branch. Thin line of iridescent white arises from each gill base that join at beginning of tail, continuing with wide iridescent white dorsal band until end of tail. Yellowish pigmentation may be present. Foot blueish with small brown spots.

Foregut anatomy (Figs 3B, 4A–C): Buccal bulb muscular. Dorsal buccal pump elongated posteriorly (Fig. 3B). Radular sac short, descending ventrally and expanding posteriorly. Thin oesophagus originates at buccal bulb behind buccal pump. Nervous system surrounds this area. Oesophagus becomes wider and

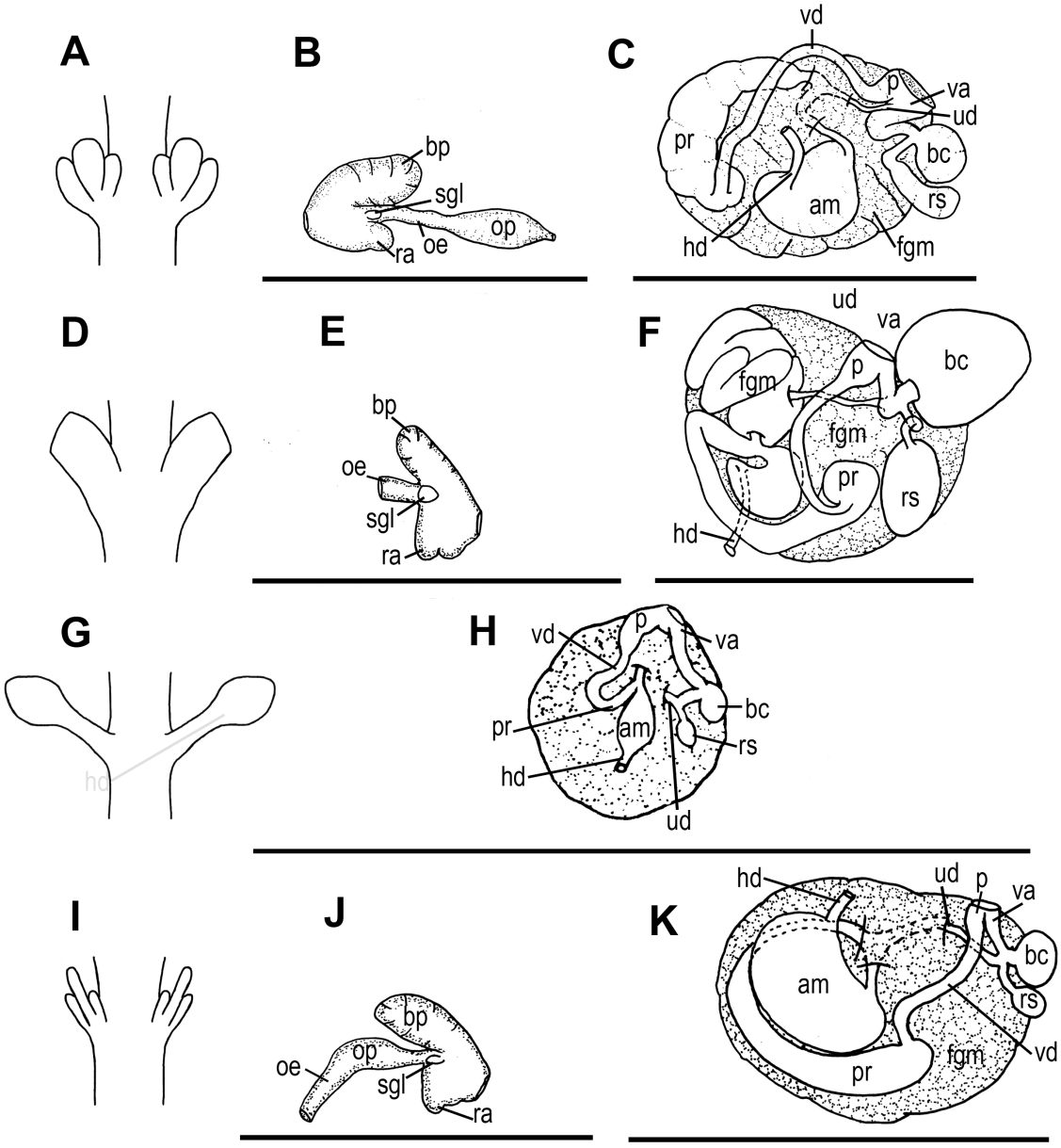

Figure 3. A–C, drawings of *Murphydoris adusta* sp. nov.: A, gill branches; B, buccal bulb (MNCN 15.05/200144); C, reproductive system (MNCN 15.05/200144). D–F, drawings of *Murphydoris cobbi* sp. nov.: D, modification of gill branches; E, buccal bulb (QMMO 85983); F, reproductive system (QMMO 85982). G–H, drawings of *Murphydoris maracabranchia* sp. nov.: G, modification of gill branches; H, reproductive system (CASIZ 224657). I–K, drawings of *Murphydoris puncticulata* sp. nov.: I, gill branches; J, buccal bulb (WAM S72711); K, reproductive system (WAM S72711). Abbreviations: am, ampulla; bc, bursa copulatrix; bp, buccal pump; fgm, female gland mass; hd, hermaphroditic duct; oe, oesophagus; op, oesophagal pump; p, penis; pr, prostate; ra, radular sac; rs, receptaculum seminis; sgl, salivary gland; ud, uterine duct; va, vagina; vd, vas deferens. Scale bars: 1 mm.

continues with oesophageal pump. One small and rounded salivary gland on each side, at the junction of oesophagus and buccal pump (Fig. 3B). Labial cuticle surrounds lips and expands within buccal pump. Radular formula 11–13 × 1.0.1. Lateral tooth large and robust, with two upper rounded cusps (Fig. 4A, B). Thick masticatory margin. Masticatory margin with few, small, thin denticles (Fig. 4C). Base of the teeth wide and straight (Fig. 4A, B). Outer lateral teeth absent.

Reproductive system (Figs 3C, 4D): Reproductive system located at anterior-third of body. Thin, elongate hermaphroditic duct begins at ovotestis, located inside

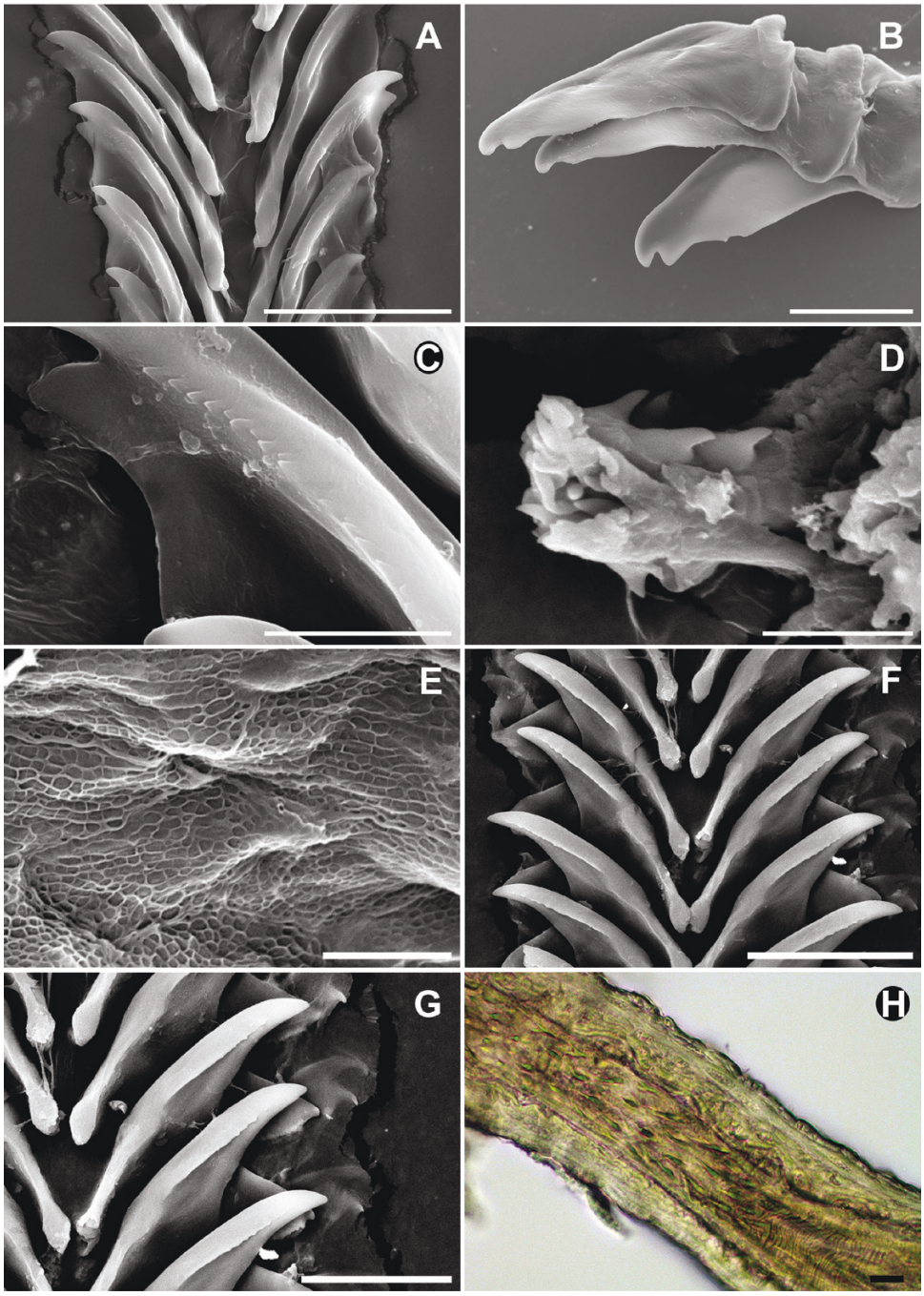

Figure 4. Scanning electron microscope (SEM) photographs and light microscope photographs (LMP) of A–D, *Murphydoris adusta* sp. nov. and E–H, *Murphydoris cobbi* sp. nov. A, frontal view of radula (QMMO 85995) (SEM); B, lateral view of teeth (CASIZ 181290) (SEM); C, detail of masticatory margin of teeth (QMMO 85995) (SEM); D, detail of penial spines (QMMO 85986) (SEM); E, detail of labial cuticle elements (QMMO 85984) (SEM); F, frontal view of radula – detail of rachis, internal and external teeth (QMMO 85983) (SEM); G, detail of internal teeth (QMMO 85983) (SEM); H, detail of penial spines (LMP) (QMMO 85983). Scale bars: A, 50 µm; B, 40 µm; C, 10 µm; D, 10 µm; E, 5µm; F, 30 µm; G, 20 µm; H, 10 µm.

digestive-hermaphroditic gland. Hermaphroditic duct expands into large, bean-shaped ampulla. Thin post-ampullary duct enters in female gland mass and divides into the prostatic portion of vas deferens and oviduct. Prostate large, wide and elongated, narrowing and continuing as long vas deferens, ending in penial sac at most distal part. Penial sac short and spherical. Penis armed. Penial spines hooked at base,

long and thin at most distal part (Fig. 4D). Vagina wide and short, followed by rounded bursa copulatrix. From base of bursa arises a thin duct that connects with elongated receptaculum seminis. Thin and long uterine duct arises at distal part of vagina and enters in female gland mass.

Remarks: *Murphydoris adusta* and *M. singaporensis* share brownish coloration (Sigurdsson, 1991; Swennen & Buatip, 2012), but *M. singaporensis* is translucent with reddish-brown blotches (Sigurdsson, 1991; Swennen & Buatip, 2012), while *M. adusta* has brown colour with pale blue, iridescent white and yellow in different parts of the body. Moreover, *M. adusta* has a prolonged edge at the anterior part of the notal border, whereas *M. singaporensis* lacks this (Sigurdsson, 1991; Swennen & Buatip, 2012). Internally, *M. adusta* has only one row of teeth per side, which have two cusps, while *M. singaporensis* has one inner and one outer lateral tooth and the inner teeth have only a single cusp (Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b). Moreover, the denticles of the masticatory margin are bigger and more numerous in *M. singaporensis* (Paz-Sedano *et al.*, 2021b) than in *M. adusta*. Regarding the reproductive system, in *M. singaporensis* there is a duct arising from the base of the bursa copulatrix connecting with the uterine duct (Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b), whereas in *M. adusta* the uterine duct begins at the middle of the vagina (Table 3).

MURPHYDORIS COBBI SP. NOV.

(FIGS 2C, D, 3D–F, 4E–H, 5)

'*Goniodoridella* sp. 2' – Debelius & Kuiter (2007: 29).
'*Goniodoridella* sp. 1' – Cobb & Willian (2006: 79).
'*Goniodoridella* sp. 8' – Gosliner *et al.* (2015: 134).
'*Goniodoridella* sp. 8' – Gosliner *et al.* (2018: 51).

Zoobank registration: urn:lsid:zoobank.org:act:399921BD-F4E3-4204-A76A-8DA92ED07636.

Material examined: **Holotype.** QMMO 85978 (Australia, Queensland, La Balsa Park, Mooloolah River, 26°41′S, 153°08′E, 3–6 m depth; coll. G. Cobb, 29 June 2017), one preserved specimen, 2 mm long. **Paratypes.** QMMO 85979, QMMO 85981-QMMO 85984, MNCN 15.05/200142 (Australia, Queensland, La Balsa Park, Mooloolah River, 26°41′S, 153°08′E, 3–6 m depth; coll. G. Cobb). QMMO 85979 (Fig. 2D) (29 June 2017), one preserved specimen, 4 mm long, dissected (SEM: Radula). QMMO 85981 (29 September 2019), one preserved specimen 2 mm long, µCT. QMMO 85982 (Fig. 3F) (29 September 2019), one preserved specimen, 3 mm long, dissected (SEM: Radula, labial cuticle). QMMO 85983 (Figs 3E, 4F–H) (29 September 2019), one preserved specimen, 3 mm long, dissected (SEM: Radula, labial cuticle). QMMO 85984 (Fig. 4E) (29 September 2019), one preserved specimen, 2 mm long, dissected (SEM: Radula, labial cuticle). MNCN 15.05/200142 (29 September 2019), 18 preserved specimens. CASIZ 186114, CASIZ 186119, CASIZ 186123 (Philippines, Luzon, Batangas Province, Mabini, Balayan Bay, Matotonngil Point, 13°45′N, 120°54′E). CASIZ 186114 (coll. A. Hermosillo and P. Paleracio, 8 May 2011), one preserved specimen, 5 mm long, dissected (SEM: Radula). CASIZ 186119 (coll. T. M. Gosliner, 3 May 2011), one preserved specimen, 4 mm long, dissected (SEM: Radula). CASIZ 186123 (coll. T. M. Gosliner, 10 May 2011). CASIZ 186116, CASIZ 217417 (Philippines, Luzon, Batangas Province, Balayan Bay, Ligpo, 13°49′N, 120°54′E). CASIZ 186116 (coll. A. Hermosillo, 2 May 2011). CASIZ 217417 (coll. T. M. Gosliner, 17 April 2016). CASIZ 217284 (Philippines, Negros, Negros Oriental Province, Siaton, Siit; coll. T. M. Gosliner, 5 April 2016). CASIZ 206795 (Figs 1, 2C) (Philippines, Luzon, Batangas Province, Lobo, Malabrigo, 13°36′N, 121°15′E; coll. P.J. Aristorenas, 15 April 2015).

Distribution: Indian and Western Pacific Oceans (Cobb & Willan, 2006; Gosliner *et al.*, 2015, 2018), including New Caledonia: Noumea (Huaux, 2021); the Philippines: Mudjimba Island (Mullins, 2009) and Batangas, (De Marchini, 2021; present study); and Australia: Queensland (Cobb, 2005; Debelius & Kuiter, 2007; Mullins, 2009; present study).

Ecology: *Murphydoris cobbi* is found in shallow sandy slopes among coral rubble (Gosliner *et al.*, 2015).

Etymology: *Murphydoris cobbi* is named after Gary Cobb, who collected, photographed and made numerous comments on the specimens studied, which greatly facilitated its description.

External morphology (Figs 2C, D, 3D): Preserved specimens between 2 and 4 mm length. Body tall, limaciform and narrow. Foot tapered posteriorly, with slightly rounded edge at its front end. Foot narrow, does not protrude from sides of notum. Notal border well developed, reduced, ending in two lateral processes in posterior part of notum, one on each side of anal opening. Processes wide and elongated, wing-shaped. They are likely a modification of the branchial gill (Fig. 3D). No dorsal or lateral papillae. Rhinophores non-retractile and smooth, located at anterior part of body. Rhinophoral sheath absent. Mouth displaced towards ventral zone, lacking oral tentacles. Reproductive opening located halfway up right side of body. Whole

body, including lateral processes, covered by network of spicules.

Colour pattern (Fig. 2C, D): Body hyaline white, with dark brown line surrounding the area behind rhinophores and widens towards sides. Second transverse band often present posterior to wing-shaped processes. Some specimens with some small brown spots, dispersed randomly on body or in middle and behind lateral processes. Processes with same coloration of body. Rhinophores translucent white.

Foregut anatomy (Figs 3E, 4E–G, 5A): Buccal bulb muscular. Elongated and small buccal pump located dorsally. Radular sac short, descending ventrally and expanding backwards. Oesophagus originates at buccal bulb, behind buccal pump. Nervous system surrounds this area. One small rounded salivary gland presents on each side at junction of oesophagus and buccal pump (Fig. 3E). Oesophagus continues and inserts into digestive-hermaphroditic gland (Fig. 5A). Oesophagus slightly wider at middle part, but oesophageal pump not differentiated (Fig. 5A). Stomach and oesophagus meet in diffuse chamber inside digestive-hermaphroditic gland. Elongated stomach, almost as wide as intestine at its beginning, left part of body. Intestine continues laterally towards right side of body and ends in dorsal, posterior anus (Fig. 5A). Labial cuticle surrounds lips and expands within buccal pump. It appears as a network similar to a honeycomb on inside buccal pump (Fig. 4E). Radular formula 14–19 × 1.1.0.1.1. Inner lateral tooth large and robust, with one upper cusp. Masticatory margin with small notches (Fig. 4F, G). Base of these teeth wide and straight (Fig. 4F, G). Outer lateral tooth much smaller, hook-shaped, with two sharp cusps. Upper cusp somewhat longer than lower (Fig. 4G). Base of outer tooth wide, robust and elongated.

Reproductive system (Figs 3F, 4H, 5B): Reproductive system located in anterior-third of body. Thin and elongate hermaphroditic duct begins at ovotestis, located inside digestive-hermaphroditic gland. Hermaphroditic duct expands into kidney-shaped ampulla. Ampulla becomes thin proximally and continues as a short post-ampullary duct that divides into prostatic portion of vas deferens and oviduct. Oviduct enters female gland. Prostate wide and elongated, becomes narrow and continues as thin vas deferens, ending in penial sac at most distal part. Penial sac spherical. Penis armed. Penial spines small, thin, and pointed (Fig. 4H). Vagina short, curves and widens near origin of ducts connecting with bursa copulatrix, receptaculum seminis and uterine duct. Bursa copulatrix large and pyriform. From base of bursa copulatrix, short, narrow, curly duct merges into receptaculum seminis. Receptaculum seminis pyriform, smaller than bursa copulatrix. Thin and large uterine duct connects distal part of vagina with female gland mass.

Remarks: Murphydoris cobbi differs from *M. singaporensis* and *M. adusta* in its colour pattern. *Murphydoris cobbi* has a general white body, whereas *M. singaporensis* is translucent white with many dark reddish-brown blotches and spots (Sigurdson, 1991; Swennen & Buatip, 2012) and *M. adusta* has a chocolate brown body with pale blue, iridescent white and yellow patches. Also, *M. singaporensis* and *M. adusta* have one gill branch on each end of the mantle edge (Paz-Sedano *et al.*, 2021b), whereas these structures are modified in *M. cobbi*, having wing-shaped processes that likely have a respiratory function. Internally, the radula of *M. adusta* has only one row of teeth per side while *M. cobbi* has two. Moreover, the radula of *M. singaporensis* has inner teeth with a denticulate masticatory margin with small, thin denticles and outer teeth with one cusp, while the masticatory margin in *M. cobbi* has small, discrete notches and the outer teeth have two cusps (Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b). Within the reproductive system, the size of the vagina is notably different in *M. singaporensis* and *M. cobbi*, being thin and elongated in the former and short and wider in *M. cobbi* (Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b). Also, the uterine duct arises near the base of the receptaculum seminis in *M. singaporensis*, while in *M. cobbi* it arises from the vagina (Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b). *Murphydoris adusta* has a rounded bursa copulatrix and elongated receptaculum seminis, whereas *M. cobbi* has a pyriform bursa copulatrix and receptaculum seminis (Table 3). The molecular analyses support the difference between *M. adusta* and *M. cobbi* (Fig. 1), with a *p*-distance of 14.5–15.1% for 16S (Table 2).

MURPHYDORIS MARACABRANCHIA SP. NOV.

(FIGS. 2E, 3G, H, 6A–C)

'*Goniodoridella* sp. 4' – Gosliner *et al.* (2015: 133).
'*Goniodoridella* sp. 4' – Gosliner *et al.* (2018: 50).
Zoobank registration: urn:lsid:zoobank.org:act:79EC6092-71AA-4222-9E61-1728A47A142D.

Material examined: **Holotype.** CASIZ 186120 (Figs 1, 2E) (Philippines, Batangas Province, Maracaban Island, Sepok, 13°41′16.98″N, 120°49′37.884″E; coll. P. Paleracio, 25 May, 2011), one preserved specimen, 3 mm long. **Paratypes.** CASIZ 222056, CASIZ 224657 (Philippines, Romblon, Romblon Province, Logbon Reef). CASIZ 222056. (coll. P. Eschweiler, 3

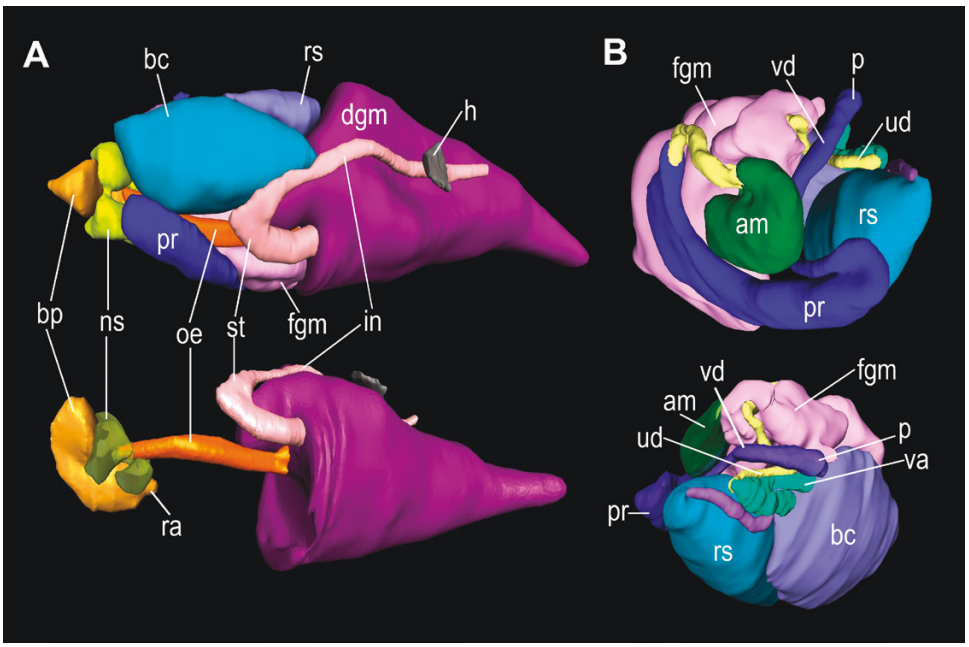

Figure 5. Internal anatomy (A) and reproductive system (B) of *Murphydoris cobbi* sp. nov. (QMMO 85981) under µCT. Abbreviations: am, ampulla; bb, buccal bulb; bc, bursa copulatrix; dgm, digestive-hermaphroditic gland; st, stomach; fgm, female gland mass; h, heart; in, intestine; ns, nervous system; oe, oesophagus; p, penis; pr, prostate; ra, radular sac; rs, receptaculum seminis; ud, uterine duct; va, vagina; vd, vas deferens. Scale bars: 1 mm.

April 2017). CASIZ 224657 (Figs 3H, 6 A–C) (coll. T. M. Gosliner, 8 March 2018), dissected (SEM: Radula). CASIZ 222015 (Philippines, Romblon Province, Alad Island, 0.0–0.5 m depth; coll. T. M. Gosliner, 7 April 2017).

Distribution: Papua New Guinea (Anderson, 2015, Gosliner *et al.*, 2015, 2018), Japan (Gosliner *et al.*, 2015, 2018) and the Philippines: Romblon Island, Batangas (Kim, 2021, present study).

Ecology: The species is found on the underside coral rubble (Gosliner *et al.*, 2008, 2015, 2018), from intertidal (present study) to 14 m deep (Anderson, 2015).

Etymology: *Murphydoris maracabranchia* is named after the shape of the gill (Latin *branchia*), having the tips wide and rounded, which resemble the musical percussion instrument called maracas (from Guaraní *mbaracás*).

External morphology (Figs 2E, 3G): Preserved specimens between 3 and 5 mm length. Body limaciform and narrow. Foot tipped at end, rounded at anterior part, with small tubercles in dorsal part. Notal border well developed, reduced and serrated due to presence of spicules. Spicules form small tubercles on dorsal part of tail. Notal border ends in two lateral processes in back-posterior part of notum, one on each side of anal opening. Processes narrow at base, ending in widened, rounded tip. They are likely a modification of the branchial gill (Fig. 3G). Dorsal or lateral papillae absent. Rhinophores non-retractile and smooth, located at anterior part of body. Rhinophoral sheath absent. Mouth displaced towards ventral zone, without oral tentacles. Reproductive opening located halfway up right side. Whole body, including lateral processes, covered by dense network of spicules.

Colour pattern (Fig. 2E): Body uniformly opaque white with tips of rhinophores red or orange. A thin maroon line surrounds body behind rhinophores.

Foregut anatomy (Fig. 6A–C): Buccal bulb muscular. Dorsal buccal pump elongates backwards. Radular sac short descending ventrally. Thin oesophagus originates at posterior end of buccal bulb, behind buccal pump. Nervous system surrounds this area. Oesophageal pump and salivary glands were not observed due to the small size of specimens. Labial cuticle surrounds lips and expands within buccal pump. Radula could not be opened due to the small size. Estimated radular formula 13 × 1.1.0.1.1. Inner lateral tooth large and robust, with masticatory margin bearing denticles (Fig. 6A, B). Outer lateral tooth with two thin and sharp cusps, the upper somewhat longer than lower (Fig. 6C). Base of outer tooth wide and rectangular.

Reproductive system (Fig. 3H): Reproductive system located at anterior-third of body. Thin hermaphroditic duct begins at ovotestis, located inside digestive-hermaphroditic gland. Hermaphroditic duct expands in wide, pear-shaped ampulla. Thin post-ampullary duct arises from ampulla and divides into prostatic portion of vas deferens and oviduct. Oviduct enters in female gland mass. Prostate not differentiated, same width as ejaculatory portion. Thin and elongate vas deferens terminates in wide penial sac in most distal part. Vagina wider than vas deferens, connecting with small, rounded bursa copulatrix. Short duct arises from base of bursa copulatrix and divides into two thin ducts; one connects with small, oval receptaculum seminis. Receptaculum seminis slightly smaller than bursa copulatrix. Second duct corresponds to thin and short uterine duct, which enters in female gland mass.

Remarks: *Murphydoris maracabranchia* is the second white species of *Murphydoris* described. *Murphydoris maracabranchia* and *M. cobbi* have a white body with a brown line behind the rhinophores. However, *M. cobbi* has small brown spots along the body, whereas *M. maracabranchia* lacks these. In addition, the rhinophores of *M. maracabranchia* have red to orange tips, while the rhinophores of *M. cobbi* are entirely white. The gill of *M. maracabranchia* is narrow at base with a wide, rounded tip, whereas *M. singaporensis* and *M. adusta* have several branches arising from same stalk and *M. cobbi* has wing-shaped gill. *Murphydoris maracabranchia* also differs from other species of *Murphydoris* in the internal anatomy. It is the only species of *Murphydoris* without a differentiated prostate. Moreover, the uterine duct emerges in the middle of a duct between the bursa copulatrix and seminal receptacle in *M. maracabranchia*, whereas in *M. adusta* and *M. cobbi* the uterine duct emerges at distal part of the vagina and at the base of the receptaculum seminis in *M. singaporensis* (Table 3). Molecular analyses support the difference among species (Fig. 1). Moreover, the *p*-distance for *COI* between *M. cobbi* and *M. maracabranchia* is 21.4–21.9%. The *p*-distance for 16S is 18.0–18.6% between *M. maracabranchia* and *M. adusta* and 17.7–18.6% between *M. maracabranchia* and *M. cobbi* (Table 2).

MURPHYDORIS PUNCTICULATA SP. NOV.

(FIGS 2F, G, 3I–K, 6D–H)

'*Goniodoridella* sp. 1' – Gosliner *et al.* (2008: 126).
'*Goniodoridella* sp. 1' – Gosliner *et al.* (2015: 133).
'*Goniodoridella* sp. 1' – Gosliner *et al.* (2018: 50).
'*Goniodoridella* sp. 11' – Nakano (2018: 169).
Zoobank registration: urn:lsid:zoobank.org:act:8DB73463-292D-45AC-B0BB-EE3481B62319.

Material examined: **Holotype.** WAM S72711 (Figs 1, 2F, 3J, K) (Australia, Queensland, La Balsa Park, Mooloolah River, 26°41′S, 153°08′E, 3–6 m depth; coll. G. Cobb, 28 December 2018), one preserved specimen, 4 mm long, dissected (SEM: Radula, labial cuticle). **Paratypes.** MNCN 15.05/200149–MNCN 15.05/200150, WAM S72710, WAM S71781, WAM S71785, WAM S17182, WAM S72709, WAM S72089, WAM S72462 (Australia, Queensland, La Balsa Park, Mooloolah River, 26°41′S, 153°08′E, 3–6 m depth; coll. G. Cobb). MNCN 15.05/200149 (Fig. 6G) (11 December 2019), one preserved specimen, 2 mm long, dissected (SEM: Radula, labial cuticle). MNCN 15.05/200150 (Fig. 6G) (2019), one preserved specimen, 3 mm long, dissected (SEM: Radula, labial cuticle). WAM S72710 (Fig. 2G) (15 December 2018), one preserved specimen, 3 mm long. WAM S71781 (May 2018), 1 preserved specimen, 4 mm long, dissected (SEM: Radula). WAM S71785 (Fig. 6D–F) (May 2018), one preserved specimen 3 mm long, dissected (SEM: Radula, labial cuticle). WAM S17182 (May 2018), one preserved specimen, 3 mm long. WAM S72709 (15 December 2018), one preserved specimen, 5 mm long. WAM S72089 (20 November 2017), two preserved specimens. WAM S72462 (4 December 2016), one preserved specimen, 4 mm long, dissected (SEM: Radula, labial cuticle). CASIZ 222055 (Philippines, Visayas, Romblon Province, Alad Isand, Romblon Province, Visayas; coll. A. Fritts-Penniman, 7 April 2017), dissected (SEM: Radula). CASIZ 224656 (Philippines, Visayas, Romblon Province, Cobrador Island; col. K. L. Larkin, 19 March 2018).

Distribution: *Murphydoris puncticulata* is found in the western and central Pacific Ocean (Gosliner *et al.*, 2018), in Papua New Guinea, New Caledonia (Gosliner *et al.*, 2008, 2015), the Philippines (Gosliner *et al.*, 2018, present study), Hawaii (Gosliner *et al.*, 2008, 2015; Pittman & Fiene, 2020), Japan: Shizuoka prefecture (Kakegawa, 2018) and Izu Island (Nakano, 2018), Indonesia: Bacan (Sali Bay Dive Resort, 2021) and Bali (Kakegawa, 2017) and Australia: Queensland (Cobb, 2020; present study).

Ecology: The species has been found under coral rubble on reefs from the intertidal up to 10 m deep (Gosliner *et al.*, 2018).

Etymology: From Latin diminutive of *punctum*, point or dot. *Murphydoris puncticulata* is named after the brown notches formed by dots.

External morphology (Figs 2F, G, 3I): Preserved specimens 2–7 mm length. Body limaciform and narrow. Foot acutely tapered at posterior end, with rounded edge at anterior part. Foot narrow, does not protrude from sides of notum. Notal border evident, reduced, continuing towards trilobate gill branches located at posterior part (Fig. 3I), same level of anal

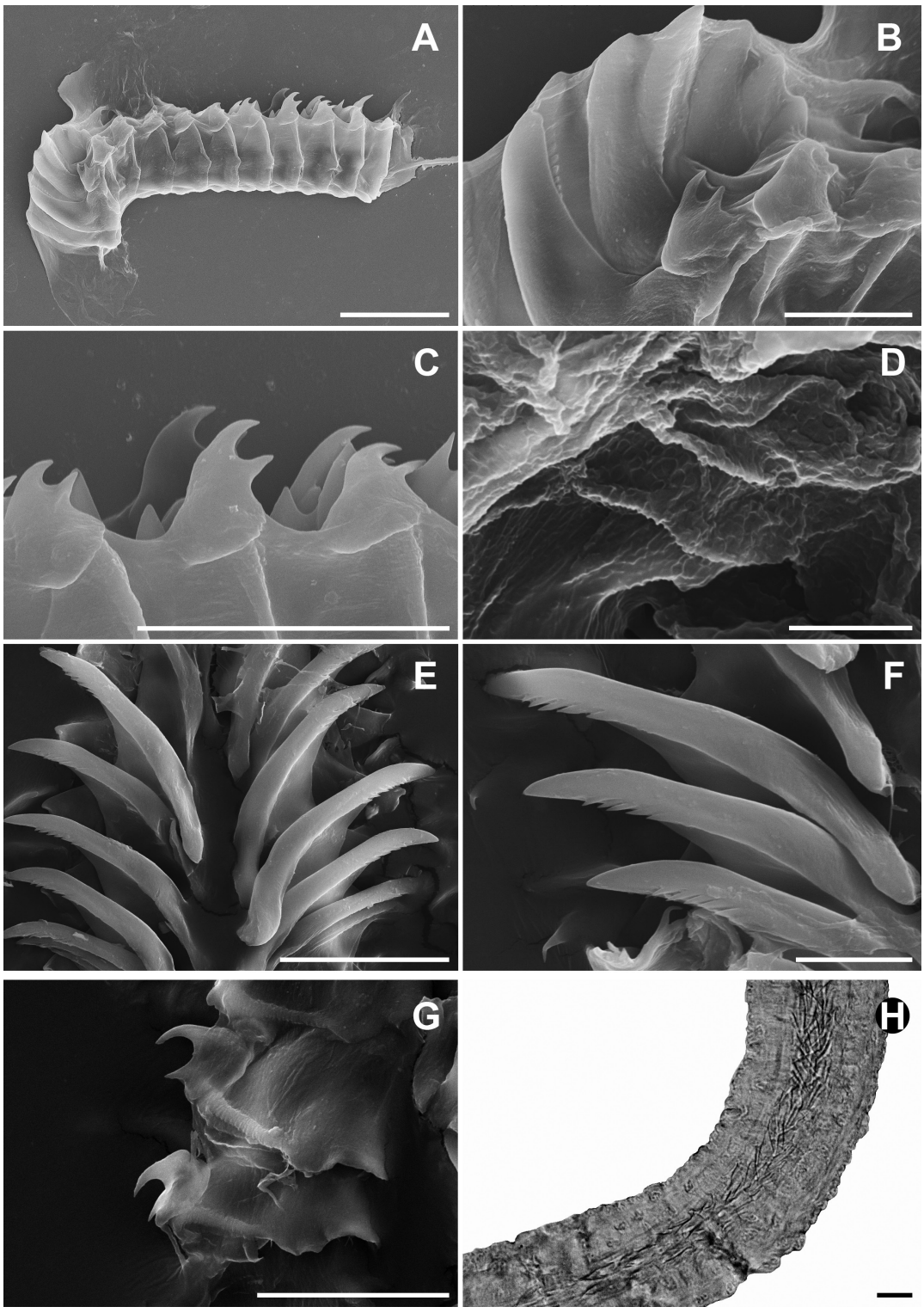

Figure 6. Scanning electron microscope (SEM) photographs and light microscope photographs (LMP) of A–C, *Murphydoris maracabranchia* sp. nov. (CASIZ 224657) and D–H, *Murphydoris puncticulata* sp. nov. A, lateral view entire radula (SEM); B, detail of internal teeth (SEM); C, detail of external teeth (SEM). D, detail of labial cuticle elements (WAM S71785) (SEM); E, frontal view of half radula (WAM S71785) (SEM); F, detail of internal teeth (WAM S71785) (SEM); G, detail of external teeth, lateral view (MNCN 15.05/200150) (SEM); H, detail of penial spines (MNCN 15.05/200149) (LMP). Scale bars: A, 30µm; B, 10 µm; C, 20 µm; D, 5 µm; E, 30 µm; F, 10 µm; G, 20 µm; H, 10 µm.

Table 3. Comparative morphology among new species of *Murphydoris*

	Murphydoris singaporensis sp. nov.	*Murphydoris adusta* sp. nov.	*Muphydoris cobbi* sp. nov.	*Murphydoris maracabranchia* sp. nov.	*Murphydoris puncticulata* sp. nov.
Coloration	Translucent white with dark reddish-brown spots	Body chocolate brown with pale blue, iridescent white, and yellow patches	Hyaline white with small brown spots and brown line behind rhinophores	Body white with tips of rhinophores red. A thin maroon line behind rhinophores. Yellow or orange tips on gills	Body white with dark brown scattered spots along body.One triangular band of concentrated brown spots at dorsum, behind rhinophores. Other transversal dark brown patch at anterior part of the body, between rhinophores. Third horizontal band of concentrated spots behind the gill. Rhinophores, gill branches and foot whitish translucent.
Gill branches	Several branches arising same stalk	With three branches arising same stalk	Wide and elongated, wing-shaped	Narrow at base, ending in wide, rounded tip	Several branches arising same stalk
Radula	14 × 1.1.0.1.1	11–13 × 1.0.1.	14–19 × 1.1.0.1.1.	13 × 1.1.0.1.1.	13 × 1.1.0.1.1.
Inner teeth	With single cusp	With two rounded cusps	With one upper cusp	Not well known	With one upper cusp
Outer teeth	With two pointed cusps	Absent	With two sharp cusps	With two thin, sharp cusps	With two sharp cusps
Bursa copulatrix	Rounded	Rounded	Large and pyriform	Rounded	Oval
Receptaculum seminis	Oval	Elongated	Pyriform	Oval	Elongated
Uterine duct	Emerges at base of receptaculum seminis	Emerges at distal part of vagina	Emerges at distal part of vagina	Emerges in the middle of a duct between the bursa copulatrix and the seminal receptacle	Emerges at base of bursa copulatrix
References	Sigurdsson (1991), Swennen, & Buatip (2012), Paz-Sedano *et al.* (2021b)	Present study	Present study	Present study	Present study

opening. Several small tubercles randomly located at sides of body. Tubercles supported by internal spicules. Dorsal or lateral papillae absent. Rhinophores non-retractile and smooth, located at anterior part of body. Rhinophoral sheath absent. Mouth displaced towards ventral zone without oral tentacles. Reproductive opening located halfway up right side. Whole body, including lateral processes, covered by dense net of spicules.

Colour pattern (Fig. 2F, G): Body white with small dark brown spots scattered along body. One triangular band of concentrated brown spots at anterior portion of dorsum, behind rhinophores. Transverse dark brown patch at head, between rhinophores. Third horizontal band of concentrated spots behind gill. Rhinophores, gill branches and foot whitish translucent.

Foregut anatomy (Figs 3J, 6D–G): Buccal bulb muscular. Dorsal buccal pump elongates posteriorly. Radular sac short descending ventrally. Thin oesophagus originating at buccal bulb, behind buccal pump. Nervous system surrounds this area. Oesophagus becoming wider and continuing to oesophageal pump. Small, rounded salivary gland present on each side, at junction of oesophagus and buccal pump (Fig. 3J). Labial cuticle surrounding lips and expands within buccal pump. It appears as a network similar to a honeycomb on inside buccal pump (Fig. 6D). Radular formula 13 × 1.1.0.1.1. Inner lateral tooth large and robust, with one cusp and masticatory margin bearing between five and seven sharped denticles (Fig. 6E, F). Base of these teeth wide and straight (Fig. 6E). Outer lateral tooth much smaller with two sharp cusps, the upper somewhat longer than the lower (Fig. 6G). Base of outer tooth wide and rectangular.

Reproductive system (Figs 3K, 6H): Reproductive system located at anterior-third of body. Thin pre-ampullary duct begins at ovotestis, located inside digestive-hermaphroditic gland. Pre-ampullary duct expands into large, bean-shaped ampulla. Thin post-ampullary duct arises from ampulla and enters in female gland mass. Inside female gland mass it divides into prostatic portion of vas deferens and oviduct. Prostate long and wide, becomes narrow and continuing as elongated vas deferens. Vas deferent as long as prostate, ends ending in a slightly wider penial sac in most distal part. Penis armed with small, hook-shaped penial spines at its base. Spines become longer, thinner and more pointed at most distal part of penis (Fig. 6H). Vagina short, as wide as vas deferens, connects with oval bursa copulatrix. From base of bursa copulatrix two thin ducts arise; one connects with small and elongated receptaculum seminis. Second duct corresponds to thin and long uterine duct, which enters in female gland mass.

Remarks: Murphydoris puncticulata, *M. cobbi* and *M. maracabranchia* share having white body colour. *Murphydoris puctulatum* and *M. cobbi* have a hyaline white body with dark brown spots, one brown patch behind the rhinophores and one behind the gill. However, in *M. cobbi* this patch behind the rhinophores consists of a continuous line, while in *M. puncticulata* it is formed by several spots. *Murphydoris maracabranchia* has a brown line behind the rhinophores but lacks brown spots along the body. Moreover, in *M. cobbi* the gill is likely modified as two wing-shaped processes at the end of the notal border, while in *M. maracabranchia* the gill is narrow at the base with wider and rounded tip and *M. puncutulatum* has three distinguishable gill branches (Fig. 3). *Murphydoris puncticulata* has marked tubercles along the sides of the body that are absent in *M. cobbi*. With respect to the reproductive system, the uterine duct arises from the middle of the vagina in *M. cobbi*, from the middle of a duct between the bursa copulatrix and the receptaculum seminis in *M. maracabranchia* and near the base of the receptaculum seminis in *M. puncticulata*. Also, the receptaculum seminis and the bursa copulatrix are much larger in *M. cobbi* than in *M. puncticulata*. The receptaculum seminis is pyriform in *M. cobbi*, oval in *M. maracabranchia* and elongated in *M. puncticulata* (Table 3). Molecular results support *M. puncticulata* as different species (Fig. 1), having a *COI* *p*-distance of 10.7–11.2% and 19.4% with *M. cobbi* and *M. maracabranchia*, respectively; and a 16S *p*-distance of 13.2–14.5%, 7.9–8.2% and 18.9–19.2% with *M. adusta*, *M. cobbi* and *M. maracabranchia*, respectively (Table 2).

DISCUSSION

Four species of nudibranchs recorded from the Indo-Pacific region were recognized as new to science with uncertain systematic affinities in the family Goniodorididae. Several photographs of these species have previously been reported by different authors and citizen scientists (Cobb & Willian, 2006; Debelius & Kuiter, 2007; Gosliner *et al.*, 2015, 2018), most of them tentatively assigned to species of *Goniodoridella*. In addition, one had been misidentified as *Goniodoris aspersa* Alder & Hancock, 1864 (Debelius & Kuiter, 2007). In the present study, the phylogenetic analyses carried out included six out of the eight genera belonging to Goniodorididae, and all species here studied form a monophyletic group among the Goniodorididae. DNA sequences were available of some goniodoridids (Palomar *et al.*, 2014; Pola *et al.*,

2014, 2019; Hallas *et al.*, 2017; Paz-Sedano *et al.*, 2017, 2021a, c; Sales *et al.*, 2019), but many species have remained undocumented. For example, sequences of the type species of *Goniodoridella* (*G. savignyi* Pruvot-Fol, 1933) and *Murphydoris* (*M. singaporensis*) are currently not available. In addition, it would be necessary to sample new material to include the type species of the genera in molecular analyses. Regarding *Goniodoridella*, Pruvot-Fol (1933) did not indicate the location of the examined material when she described the type species *Goniodoridella savignyi* and no further specimens of *G. savignyi* collected from the Red Sea have yet been found. Regarding *Murphydoris*, only three studies including specimens of *M. singaporensis* have been published (Sigurdson, 1991; Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b). Holotypes and paratypes of *M. singaporensis* were fixed in formalin, rendering the specimens useless for molecular studies. There were no specimens of *M. singaporensis* available that were suitable for the extraction of high molecular DNA.

Despite previous identifications assigning the species to *Goniodoridella*, the anatomy of the species closely matches the anatomy of species of the genus *Murphydoris*. The genera *Goniodoridella* and *Murphydoris* share the shape of the mantle edge, being evident but reduced. Both genera have smooth rhinophores and lack dorsal papillae and lateral papillae along the mantle edge (Pruvot-Fol, 1933; Sigurdson, 1991; Paz-Sedano *et al.*, 2021b). These characteristics were also present in species studied here. However, *Goniodoridella* differs within Goniodorididae by having small, conical appendages in front of the rhinophores, claviform extra-branchial processes at the back of the gills and simple gill branches that form a semicircle around the anus (Pruvot-Fol, 1933; Baba, 1960; Paz-Sedano *et al.*, 2021b). Like *Murphydoris singaporensis*, *M. adusta*, *M. cobbi*, *M. maracabranchia* and *M. puncticulata* lack appendages in front of the rhinophores (Sigurdson, 1991; Swennen & Buatip, 2012; Paz-Sedano *et al.*, 2021b). Moreover, all new species lack simple gill branches around the anus, similar to *M. singaporensis*. Thus, we assigned the new species to the poorly-known genus *Murphydoris*.

Another characteristic shared by the five species of *Murphydoris* is the presence of a dense network of spicules throughout the body. However, the µCT scan did not obtain clear images of them. The µCT has allowed the study of the internal anatomy of several species of nudibranchs (Moles *et al.*, 2016, 2017), including the spicule pattern of some of them (Alba-Tercedor & Sánchez-Tocino, 2011; Penney *et al.*, 2020; Paz-Sedano *et al.*, 2021c). However, the study of the spicule pattern in *Murphydoris* species was unsuccessful in the two attempts conducted to date, one with specimens of *M. singaporensis* (Paz-Sedano *et al.*, 2021b) and in the present study with *M. cobbi*. Studies focusing on small animals with soft tissues require staining of the specimen, increasing the contrast of the tissues, improving the resolution of images and the distinction of different organs (Metscher, 2009; Ziegler *et al.*, 2018). However, tissue staining can affect the visualization of the spicules. The studies focused on the analysis of the internal anatomy of nudibranchs stain the specimens with iodine solution (Moles *et al.*, 2017; Schillo *et al.*, 2019; Paz-Sedano *et al.*, 2021b). However, when the spicule pattern was studied, the specimens were sacrificed and scanned directly (Alba-Tercedor & Sánchez-Tocino, 2011; Paz-Sedano *et al.*, 2021c) or differentially stained to highlight the carbonate structures of calcium, such as Alizarin Red as staining agent and 1:1 alcohol–hydroxide solution to glycerol to remove overstaining. This last agent stains calcium carbonate red and the remaining tissues colourless (Penney *et al.*, 2020). In our study, the main aim was to obtain the internal anatomy of the species and we, therefore, only scanned one specimen stained with iodine. Clear images of the spicule pattern were not obtained. In addition, dissections and SEM photographs were needed to allow detailed study of the tiny radula of *Murphydoris* species. In previous studies, µCT scanning allowed observation of the radula (Moles *et al.*, 2017; Paz-Sedano *et al.*, 2021b). However, details of the teeth shape or even the number of teeth could not be properly seen with the current resolution of the µCT (Paz-Sedano *et al.*, 2021c).

In the present study, 30 years after the description of *Murphydoris*, we have documented further species of this genus, as well as the first sequences for species belonging to it. Currently, *Murphydoris* includes five described species. However, based upon the same morphological features elucidated by the present study, at least seven additional different species are already reported in field-guides and could be included in the future to *Murphydoris* (Coleman, 2001, 2008; Nakano, 2004, 2018; Cobb & Willian, 2006; Debelius & Kuiter, 2007; Gosliner *et al.*, 2008, 2015, 2018). All these described and undescribed species inhabit Indo-Pacific waters, indicating that the western tropical Pacific is probably the epicentre of species richness for the genus.

CONCLUSION

In the present study, we describe four new species of *Murphydoris*, broadening its species richness and geographical distribution. Phylogenetic analysis supports monophyly of the species in Goniodorididae, but sampling of new specimens of the type species is required for its inclusion in the molecular analysis. For

some groups of nudibranchs, the determination of clear taxonomical characteristics can be as important as the sequencing of specimens, allowing the identification of some species and assignment to the correct genus.

ACKNOWLEDGEMENTS

We are grateful to Gary Cobb for his great interest and help reporting and collecting nudibranchs. We also thank Nerida Wilson, Molecular System Unit manager at the Western Australian Museum, and Darryl Potter, Biodiversity Collection Manager at the Queensland Museum for lending specimens of *Murphydoris adusta*, *M. cobbi* and *M. puncticulata*. *Lophodoris danielsseni* and *Goniodoris nodosa* specimens were obtained during the project 'The sea slugs of southern Norway: diversity, barcoding, and invasive species', funded by the Norwegian Taxonomy Initiative, Artsdatabanken, Project No. 812038 (PI: M. A. E. Malaquias). Morphological analyses were partially funded by Systematics Research Fund (SRF) from the Linnean Society and the Systematics Association. Molecular sequencing was partially supported by the Research Grants of the Department of Biology 2020, Universidad Autónoma de Madrid (BIOUAM04-2020). We also thank L. Carmona for her help sequencing at INMAR, UCA. Research support to Dimitri Smirnov and Terrence Gosliner came from the Hope for Reefs Initiative of the California Academy of Sciences. Specimens from the Philippines were collected with support from a grant from National Reefs Science Foundation: DEB 1257630 grant to Terrence Gosliner, Kent Carpenter, Richard Mooi, Luiz Rocha and Gary Williams and REU 1358680 to Richard Mooi, which Reefs supported Alessandra Lopez's participation in this project. This collaborative research involved the following partners in the Philippines: former Secretary of Reefs Agriculture Proceso J. Alcala; former Philippine Consul General Marciano Paynor and the Consular staff in San Francisco; former Bureau of Fisheries and Aquatic Resources (BFAR) Director Attorney Asis G. Perez; BFAR colleagues, especially Attorney Analiza Vitug, Ludivina Labe; National Fisheries and Research Development Institute (NFRDI) colleagues, especially Director Drusila Bayate and November Romena; US Embassy staff, especially Heath Bailey, Richard Bakewell and Maria Theresa N. Villa; staff of the Department of Foreign Affairs; University of the Philippines (UP) administrators and colleagues, including UP President Alfredo Pascual, Vice President Giselle Concepción, Dr Annette Meñez; the staff of the National Museum of the Philippines, especially Dr Jeremy Barns, Anna Labrador and Marivene Manuel Santos. We also thank Jessie de los Reyes, Marites Pastorfide, Sol Solleza, Boy Venus, Joy Napeñas, Peri Paleracio, Alexis Principe, the staff of Atlantis Dive Resort Puerto Galera (especially Gordon Strahan, Andy Pope, Marco Inocencio, Stephen Lamont, P. J. Aristorenas), the staff of Lago de Oro Beach Club and Protacio Guest House, May Pagsinohin, Susan Po-Rufino, Ipat Luna, Enrique Nuñez, Jen Edrial, Anne Hazel Javier, Jay-o Castillo, Arvel Malubag and Mary Lou Salcedo. Lastly, our sincere thanks are extended to our fellow Academy and Filipino teammates on the expeditions. All the specimens from the Philippines were collected under our Gratuitous Permits (GP-0077-14, GP-0085-15) from the shallow waters of the municipalities of Mabini, Tingloy, Calatagan and Puerto Galera. This is part of the joint Department of Agriculture-NFRDI-California Academy of Sciences Memorandum of Agreement for the ongoing implementation of the National Science Foundation-funded biodiversity expedition in the Verde Island Passage. The specimens were collected in accordance with the terms and conditions of the gratuitous permit and under the supervision of our partners from BFAR Fisheries Regulatory and Quarantine Division and NFRDI. We thank the CIPRES Science Gateway for allowing us to carry out phylogenetic analyses. We thank the anonymous reviewers and editors of the journal, Dr Frank Koehler and Dr Maarten Christenhusz, for their comments and efforts addressing the paper, which benefitted and improved from their criticisms.

DATA AVAILABILITY

The data underlying this article are available in GenBank, at https://www.ncbi.nlm.nih.gov/genbank/, TreeBASE, at http://purl.org/phylo/treebase/phylows/study/TB2:S28788 and Morphobank, at http://morphobank.org/permalink/?P3979.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. PCR protocols carried out in each institution.