Molecular data illuminate cryptic nudibranch species: the evolution of the Scyllaeidae (Nudibranchia: Dendronotina) with a revision of *Notobryon*

MARTA POLA[1,4]*, YOLANDA E. CAMACHO-GARCÍA[2,3] and TERRENCE M. GOSLINER[4]

[1]*Laboratorio de Biología Marina, Departamento de Biología, Edificio de Biología, C/ Darwin, 2, Universidad Autónoma, 28049 Madrid, Spain*
[2]*Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), Universidad de Costa Rica, San José, Costa Rica*
[3]*Museo de Zoología, Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica*
[4]*Department of Invertebrate Zoology and Geology, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA*

Scyllaeidae represents a small clade of dendronotoid nudibranchs. *Notobryon wardi* Odhner, 1936, has been reported to occur in tropical oceans from the Indo-Pacific and eastern Pacific to temperate South Africa. The systematics of *Notobryon* has not been reviewed using modern systematic tools. Here, specimens of *Notobryon* were examined from the eastern Pacific, the Indo-Pacific, and from temperate South Africa. Additionally, representatives of *Scyllaea* and *Crosslandia* were studied. Scyllaeidae was found to be monophyletic. *Notobryon* was also found to be monophyletic and is the sister group to *Crosslandia* plus *Scyllaea*. The molecular data also clearly indicate that within *Notobryon*, at least three distinct species are present, two of which are here described. Genetic distance data indicate that eastern Pacific and South African exemplars are 10–23% divergent from Indo-Pacific exemplars of *Notobryon wardi*. *Scyllaea pelagica* has been regarded as a single, circumtropical species. Our molecular studies clearly indicate that the Atlantic and Indo-Pacific populations are distinct and we resurrect *Scyllaea fulva* Quoy & Gaimard, 1824 for the Indo-Pacific species. Our morphological studies clearly corroborate our molecular findings and differences in morphology distinguish closely related species. Different species clearly have distinct penial morphology. These studies clearly reinforce the view that eastern Pacific, Indo-Pacific, and temperate biotas consist largely of distinct faunas, with only a minor degree of faunal overlap.

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INTRODUCTION

Notobryon Odhner, 1936 was described as a genus of Scyllaeidae within the Dendronotina (Odhner, 1936). The single species, *Notobryon wardi*, from Queensland, Australia was listed as the type species, by monotypy. The following year, Baba (1937) described two additional species, *Notobryon bijecurum* Baba,

1937 and *Notobryon clavigerum* Baba, 1937. Thompson & Brown (1981) described the anatomy of *N. wardi* and documented this species from temperate southern African waters. Since then, most specimens from other parts of the world have been documented as *N. wardi* (Gosliner, 1987). Recently, Camacho-García, Gosliner & Valdés (2005) and Gosliner, Behrens & Valdés (2008) listed several undescribed species from the tropical eastern Pacific and the Indo-Pacific, suggesting that there are maybe more undescribed taxa and that the group is in need of systematic revision.

*Corresponding author. E-mail: marta.pola@uam.es

In general, members of the Scyllaeidae are cryptic species in their natural environments where they often resemble algae. Owing to their resemblance to the substrate in which they are found, they are difficult to find in the field. In particular, the identification of members of *Notobryon* has been mainly based on external morphological features (including colour), anatomical traits, and radular morphology. The extreme external and anatomical similarity of these species has caused a great deal of confusion and specimens found in different biogeographical areas have been misidentified as the same species. Owing to this similarity, the presence of species complexes such as that found in *Notobryon* is also present in several groups amongst opisthobranchs (*Melanochlamys*: Krug *et al.*, 2008; sacoglossans: Carmona *et al.*, 2011; Arminidae: Gosliner & Fahey, 2011; *Navanax*: Camacho-García *et al.*, in press; Ornelas-Gatdula *et al.*, in press). In light of modern taxonomic techniques such as molecular systematics, taxonomists have a powerful tool that frequently resolves cases where traditional taxonomy has proven to be insufficient to delineate species boundaries.

Recently collected material from southern Africa, the Indo-Pacific tropics, and the tropical eastern Pacific, as well as a re-examination of the type material of *N. wardi*, permitted us to undertake a systematic revision of *Notobryon*. The new, freshly collected material also allowed us to include DNA sequence data, in addition to traditional morphological and anatomical data in the comparison of specimens from different geographical regions. We also studied the genetic distances of species with different geographical ranges in an attempt to determine whether these are in fact complexes of distinct, similarly coloured species. Finally, in the present paper, based on the addition of two new species in this genus, we propose a new diagnosis of the genus *Notobryon*.

MATERIAL AND METHODS

MORPHOLOGY

Specimens used for morphological studies included 40 individuals belonging to the genera *Scyllaea* (four individuals), *Notobryon* (35 individuals), and *Crosslandia* (one individual). Most of these specimens are *Notobryon* species because we hoped to clarify the systematics of this genus. Specimens studied anatomically are detailed in Table 1. Specimens were dissected by dorsal or ventral incision. The internal features were examined and drawn (for most of them) under a dissecting microscope with a camera lucida. Particularly informative and morphologically variable organs (jaws, stomach plates, and penis) were critical-point dried for scanning electron microscopy (SEM).

Special attention was paid to the morphology of the reproductive system. The buccal mass was removed and dissolved in 10% sodium hydroxide until the radula was isolated from the surrounding tissue. The radula was then rinsed in water, dried, and mounted for examination by SEM.

Voucher specimens are held either at the California Academy of Sciences, CASIZ (San Francisco, USA), Museo de Zoología de la Universidad de Costa Rica, MZUCR and MZUCR-INBIO (San José, Costa Rica), the Iziko South African Museum (SAM), or the Swedish Museum of Natural History, SMNH (Stockholm, Sweden).

MOLECULAR WORK

Taxon sampling
Sampling of Scyllaeidae specimens for the molecular study was limited because most of the specimens available were preserved in Bouin's fixative or formalin and thus useless for molecular work. Bornellidae, Dendronotidae, and other Dendronotina specimens as well as specimens of the genera *Armina*, *Bonisa*, and *Leminda* were also included to test the monophyly of Scyllaeidae. These outgroup taxa were chosen based on the results of Pola & Gosliner (2010). Sequences of these genera for 25 specimens already available in GenBank (from a previous study carried out by two of the authors of this paper, Pola & Gosliner, 2010) were included [25 for histone 3 (H3), 18 for cytochrome *c* oxidase subunit I (COI), and 24 for 16S]. All of the species and sequences (including those retrieved from GenBank) used in this study are listed in Table 2. The tissues came from specimens collected in recent field trips carried out by the authors as well as from specimens collected from different people around the world, specifically sent to us for this study. These specimens were preserved in 95% EtOH. In addition to the specimens collected specifically for molecular study, we were also able to use museum material that was either preserved in 70–75% EtOH or in other unknown preservatives.

DNA extraction, amplification, and sequencing
Genomic DNA was extracted from small pieces of foot tissue for most samples using Qiagen DNeasy Tissue Kits. In those cases where animals were small, pieces from the dorsal processes were used. Amplification of DNA was conducted on a Bio-Rads MyCycler Thermocycler (software v. 1.065, Bio-Rad Laboratories). Partial sequences of the mitochondrial genes COI (658 bp) and 16S rRNA (485 bp) and the nuclear gene H3 (328 bp) were amplified using the primer pairs LCO1490 and HCO2198 (Folmer *et al.*, 1994), 16Sar-L and 16Sbr-H (Palumbi *et al.*, 1991), and H3a F and H3a R (Colgan *et al.*, 1998), respectively. PCR

Table 1. Specimens used for morphology, collection sites, vouchers, size (preserved; al, alive), radular formula, collection date and collectors

Species	Locality	Voucher	Size (mm) preserved (alive)	Depth (m)	Radular formula	Date	Collector
Notobryon wardi (syntypes)	East Australia, Queensland, Port Curtis, off Gatcombe Head	SMNH 1346	50–55 (4 specs.)	16	12 × 21.0.21 (2 specs.)	vii.1929	N. Odhner
N. wardi	Papua New Guinea, near Madang, Matthew's Folly	CASIZ 075283	15	22	14 × 14.0.14	15.xi.1990	T. M. Gosliner
N. wardi	Papua New Guinea, near Madang, Matthew's Folly	CASIZ 086503	5 & 10	5	–	16.vi.1992	T. M. Gosliner
N. wardi	Indonesia, Banda Sea, Reong Island	CASIZ 117360	18	9	17 × 17.0.17	3.xi.1998	P. Fiene-Severns
N. wardi	Hawaii, Maui, Makena	CASIZ 104662	13 (al)	?	–	19.v.1995	P. Fiene
N. wardi	Hawaii, Maui, Black Rock	CASIZ 101129	5	10	–	20.x.1994	C. Pittman
N. wardi	Hawaii, Maui, Airport Beach	CASIZ 101109	2	9	–	1.ix.1994	C. Pittman
N. wardi	Hawaii, Oahu, Kepuhi Point	CASIZ 116801	20	35	–	3.xii.1985	S. Johnson
N. wardi	Marshall Islands: Kwajalein Atoll, South Loi Island	CASIZ 180378	25 (al)	8	–	18.xi.2007	S. Johnson
N. wardi	Philippines, Batangas, Anilao, Mainit Bubbles	CASIZ 177589	20	16 max.	16 × 20.0.20	16.iv.2008	T. M. Gosliner *et al.*
N. wardi	Philippines, Batangas, Anilao, Mainit Bubbles	CASIZ 177591	25	16 max.	16 × 20.0.20	16.iv.2008	T. M. Gosliner *et al.*
N. wardi	Philippines, Batangas, Anilao, Mainit Bubbles	CASIZ 177537	20	23 max.	–	21.iii.2008	T. M. Gosliner *et al.*
N. wardi	Philippines, Batangas, Anilao, Mainit Bubbles	CASIZ 177540	15	23 max.	–	21.iii.2008	T. M. Gosliner *et al.*
Notobryon sp. B	Philippines, Batangas, Anilao, Mainit Bubbles	CASIZ 177759	25	17 max.	16 × 28.0.28	22.iv.2008	T. M. Gosliner *et al.*
Notobryon panamica	Panama, Pacific coast, Isla Montuosa	CASIZ 088163	12	12 max.	9 × 13.0.13	15.iv.1993	T. M. Gosliner
N. panamica	Panama, Pacific coast, Isla Jicarita	CASIZ 088164	6	?	–	16.iv.1993	T. M. Gosliner
N. panamica	Panama, Pacific coast, Isla Jicarita	CASIZ 088177	20	Anchorage	12 × 15.0.15	16.iv.1993	T. M. Gosliner
N. panamica	Costa Rica, Guanacaste, West Cataline Island	MZUCRINB0003118069	15	Intertidal	13 × 20.0.20	23.i.2001	S. Avila
N. panamica	Costa Rica, Punta Ballena, Isla del Caño	MZUCR 6356	20	8	12 × 16.0.16	30.iv.2006	Y. Camacho
N. panamica	Mexico, Jalisco, Bahia de Banderas, Los Arcos	CASIZ 175778	15	?	18 × 20.0.20	12.vi.2003	A. Hermosillo
N. panamica	Mexico: Jalisco, Bahia de Banderas, Mismaloya	CASIZ 180376	–	?	–	3.iv.2009	A. Hermosillo
Notobryon bijecurum	Japan, Ryukyu Islands, Okinawa, Seragaki Beach	CASIZ 089003	57 (al)	40	16 × 24.0.24	29.xi.1992	R. Bolland
Notobryon thompsoni	South Africa, Cape Pr., W False Bay, Dale Brooks	CASIZ 074100	21	–	11 × 17.0.17	–	T. M. Gosliner

Table 1. *Continued*

Species	Locality	Voucher	Size (mm) preserved (alive)	Depth (m)	Radular formula	Date	Collector
N. thompsoni	South Africa, Cape Pr., Western False Bay, Dale Brooks	SAM 33981	3, 5, 8, 10 & 15	–	–	–	T. M. Gosliner
N. thompsoni	South Africa, Cape Pr., Atlantic coast, Oudekraal, Mushroom Rock	CASIZ 176277	18	14 max.	$19 \times 20.0.20$	5.i.2008	T. M. Gosliner *et al.*
N. thompsoni	South Africa, Cape Pr., West False Bay, Dale Brooks	CASIZ 176362	20	Intertidal	$12 \times 16.0.16$	7.i.2008	T. M. Gosliner *et al.*
N. thompsoni	South Africa, Cape Pr., West False Bay, Dale Brooks	CASIZ 176363	18	Intertidal	–	7.i.2008	T. M. Gosliner *et al.*
N. thompsoni	South Africa, Cape Pr., West False Bay, Dale Brooks	CASIZ 176364	10	Intertidal	–	7.i.2008	T. M. Gosliner *et al.*
N. thompsoni	South Africa, Cape Pr., Atl. coast, Oudekraal, Mushroom Rock	CASIZ 176925	12	12 max.	–	13.i.2008	T. M. Gosliner *et al.*
N. thompsoni	South Africa, Cape Province, Atl. coast, Oudekraal, Coral gardens	CASIZ 176956	12	17.5 max.	$14 \times 15.0.15$	14.i.2008	T. M. Gosliner *et al.*
N. thompsoni	South Africa, Cape Pr., Oudekraal, Coral gardens	CASIZ 073189	15	?	–	i.1981	T. M. Gosliner
N. thompsoni	South Africa, Cape Pr., Atl. Coast, off Bloubergstrand	CASIZ 073964	40	4.6	–	14.ii.1980	W.R. Liltved
N. thompsoni	South Africa, Cape Province, Ecological Survey	SAM CP 614B	20	?	$14 \times 19.0.19$	22.iii.1958	?
N. thompsoni	South Africa, Cape Province, Ecological Survey	SAM KNY214C	30	?	–	7.vii.1960	?
Scyllaea pelagica	Texas, Galveston County, Galveston	CASIZ 175651	–	?	–	28.vi.2007	G. Hightower
S. pelagica	Philippines, Batangas, Anilao, Luzon Is.,	CASIZ 182820	–	32	–	20.v.2010	T. M. Gosliner
S. pelagica	Philippines, Batangas, Anilao, Luzon Is.,	CASIZ 182823	–	32	–	20.v.2010	T. M. Gosliner
S. pelagica	Philippines, Batangas, Anilao, Luzon Is.,	CASIZ 184317	–	–	–	2.x.2010	T. M. Gosliner
Crosslandia daedali	Ecuador, Galapagos Islands, Las Marielas	CASIZ 172042	–	Shallow water	–	2.xii.2004	C. Hickman

Atl., Atlantic; Is., Island; Pr., Province.

Table 2. Specimens used for molecular analyses, collection sites, vouchers, GenBank accession numbers, and collectors

Species	Locality	Voucher	GenBank accession numbers			Collector
			H3	COI	16S	
Armina semperi	Philippines: Batangas, Anilao, Luzon Is., Mainit Bubble	CASIZ 177534	HM162512	HM162696	HM162606	T. M. Gosliner et al.
Bonisa nakaza	South Africa: Percy's Hole, Gordon's Bay, Eastern False Bay, Cape Pr.	CASIZ 176146	HM162579	HM162746	HM162670	T. M. Gosliner et al.
Leminda millecra	South Africa: Western Cape Pr.: False Bay, Smitswinkel Bay, wreck of HMSAS Good Hope	CASIZ 176348	HM162578	HM162745	HM162669	A. Taylor
Bornella anguilla	Marshall Is.: Kwajalein Atoll	CASIZ 175402	JN869424	–	–	S. Johnson
Bor. anguilla	Vanuatu: Espiritu Santo Island, south-east Urélapa Island	CASIZ 176811	JN869425	–	–	M. Pola, Y. Camacho
Bornella valdae	South Africa: Durban, Kwazulu-Natal	SAM A55952	JN869426	JN869449	JN869405	V. Fraser
Bor. valdae	South Africa: Durban, Kwazulu-Natal	CASIZ 176832	HM162532	HM162706	HM162626	V. Fraser
Bornella calcarata	Brazil: Bahía, Marau, Barra Grande de Camamu	MZSP 84448	HM162533	HM162707	HM162627	C. Sampaio
Bor. calcarata	Brazil: Espírito Santo, Guarapari	MZSP 39127	JN869427	–	–	C. M. Cunha
Bornella johnsonorum	Marshalls Is.: Kwajalein Atoll	CASIZ 175406	HM162530	HM162704	HM162624	S. Johnson
Bor. johnsonorum	Marshalls Is.: Kwajalein Atoll	CASIZ 175407	JN869419	JN869445	JN869401	S. Johnson
Bornella sarape	Mexico: Isla Montosa, Bahia Tangolunda	LACM 177780	JN869428	–	–	A. Hermosillo
Bornella stellifer	Hawaii: Lanai	CASIZ 167989	HM162529	HM162703	HM162623	Tom Powers
Bor. stellifer	Marshall Is.: Enewetak Atoll	CASIZ 175403	JN869417	–	–	S. Johnson
Bor. stellifer	Marshall Is.: Kwajalein Atoll	CASIZ 175725	JN869418	–	JN869400	S. Johnson
Bornella hermanni	Marshall Is.: Kwajalein Atoll	CASIZ 175404	JN869422	JN869448	JN869404	S. Johnson
Bor. hermanni	Australia: New South Wales	AMC 138000	JN869423	–	–	B. W. Rudman
Bor. hermanni	Malaysia: Pulau Tenggol North Point	CASIZ 175745	JN869421	JN869447	JN869403	T. M. Gosliner
Bor. hermanni	Malaysia: Tokong Kamundi	CASIZ 175743	HM162531	HM162705	HM162625	T. M. Gosliner
Bor. hermanni	Malaysia: Tokong Kamundi	CASIZ 175744	JN869420	JN869446	JN869402	T. M. Gosliner
Dendronotus orientalis	China: Daisong Bay	CASIZ 174989	HM162534	–	HM162628	S. Xikum
De. orientalis	China: Luoyuan Bay	CASIZ 174988	JN869432	–	–	S. Xikum
Dendronotus regius	Philippines: Batangas, Anilao, Tingloy Island, Kirby's Rock	CASIZ 179492	HM162535	HM162708	HM162629	T. M. Gosliner et al.
De. regius	Philippines: Batangas, Anilao, Tingloy Island, Kirby's Rock	CASIZ 179493	JN869430	JN869451	JN869407	T. M. Gosliner et al.
Dendronotus venustus	California: Santa Monica, Redondo Canyon LACM 174850	HM162536	HM162709	HM162630		K. Lee
Dendronotus iris	Washington: Gig Harbor	CASIZ 174471	HM162537	–	HM162631	T. M. Gosliner et al.
De. iris	California: San Diego, La Jolla Canyon	LACM 174858	JN869431	–	–	C. Stout & K. Lee
Dendronotus subramosus	Washington: Hudson's Point	LACM 174854	HM162539	–	HM162632	R. Zade
Dendronotus lacteus	Scotland: Garvellachs Islands	LACM 174877	HM162538	HM162710	–	J. Anderson
Dendronotus frondosus	Scotland: Garvellachs Islands	LACM 174860	JN869429	JN869450	JN869406	Anderson
Doto coronata	South Africa: Cape Province, Mushroom Rock, Oudekraal	CASIZ 176278	HM162566	HM162734	HM162657	T. M. Gosliner et al.
Hancockia cf. uncinata.	Italy: Calae cicale	CASIZ 175721	HM162528	–	HM162622	B. Moore, E. Trainito
Hancockia californica	Costa Rica: Guanacaste	CASIZ 175722	HM162527	HM162702	HM162621	Y. Camacho
H. californica	Mexico: Jalisco, Puerto Vallarta, Los Arcos	LACM 174934	JN869433	JN869452	JN869408	A. Hermosillo
Lomanotus sp. E	Mexico: Jalisco, Puerto Vallarta, Los Arcos	LACM 174962	HM162547	HM162715	HM162640	A. Hermosillo
Lomanotus sp.	Philippines, Batangas, Anilao, Luzon Is., Mainit Bubbles	CASIZ 177751	JN869434	JN869453	JN869409	T. M. Gosliner et al.
Lomanotus vermiformis	Mexico: Bahia Banderas, Los Arcos, Mismaloya	CASIZ 175963	JN869435	–	–	A. Hermosillo

COI, cytochrome c oxidase subunit I; H3, histone 3; Is, Island; Pr, Province.

amplifications were carried out in a 25 μL reaction volume including 1 μL 10 × PCR buffer, 0.2 μL dNTPs (10 mM stock), 1.5 μL MgCl (25 mM stock), 0.025 μL Taq (1.25 units/μL)-Apex, 0.2 μL of each primer (25 μM stock), and 1 μL genomic DNA. Standard PCRs for COI consisted of: an initial denaturing step at 94 °C for 3 min; 40 cycles of denaturing at 94 °C for 30 s, annealing at 48–50 °C for 30 s; and final extending at 72 °C for 5 min. The partial 16S amplifications followed the following parameters: an initial denaturing step at 94 °C for 3 min; 39 cycles of denaturing at 94 °C for 30 s, annealing at 50–52 °C for 30 s; and extension at 72 °C for 2 min and 25 °C for 2 min. Finally, the PCR conditions for the H3 amplification consisted of an initial denaturing step at 94 °C for 3 min; 35 amplification cycles (94 °C for 35 s, 50 °C for 1 min, and 72 °C for 1 min 15 s); and a final step at 72 °C for 2 min. Double-stranded amplified products were electrophoresed in a 0.5% TBE agarose gel stained with ethidium bromide. Amplified products were purified with ExoSAP-IT (USB Scientific). Cycle-sequencing reactions were performed using ABI Prism Big Dye Terminator (Applied Biosystems) (total volume 10 μL) and analysed using the automated sequencer ABI 3130 Genetic Analyzer (Applied Biosystems) in the Center for Comparative Genomics at the California Academy of Sciences (San Francisco, USA). All new DNA sequences have been deposited in GenBank (Table 2).

Sequence alignment and analysis
The authenticity of the sequences was verified by BLAST comparisons. H3 and COI sequences were edited and aligned using GENEIOUS 4.5.4 (Drummond *et al.*, 2009) and checked by eye. Protein-coding sequences were translated into amino acids using MacClade 4.08 (Maddison & Maddison, 2005) for confirmation of alignment. 16S sequences were aligned using MUSCLE (Edgar, 2004), using the default settings. GBLOCKS 0.91b (Castresana, 2000) was used to study poorly aligned positions and variable regions of the alignments. Alignments were further optimized by eye using GENEIOUS (Biomatters) and MacClade 4.08 (Maddison & Maddison, 2005). For the 16S rRNA locus, two types of tests were run in which we either took into account or did not consider the most variable regions. Results were not different from each other so these ambiguously aligned regions were finally included in the analysis. To test for the possible saturation types, we plotted the number of transitions (Ti) and transversions (Tv) against the uncorrected pairwise distances. Saturation plots were also examined separately for the first, second, and third positions of protein-coding genes. The partition-homogeneity test (Swofford, 2002) was performed to

test if the three genes could be combined in a single data set.

Model selection and phylogenetic analyses
Two different phylogenetic methods, maximum likelihood (ML) and Bayesian inference (MB) were used to infer evolutionary relationships. Separate analyses were conducted for the following data sets: (1) H3, (2) COI, (3) 16S, (4) combined mitochondrial DNA (mtDNA), and (5) combined mtDNA and nuclear DNA. Evolutionary models for each data set were selected using MrModelTest 2.3 (Nylander *et al.*, 2004) under the Akaike information criterion (Akaike, 1974). Support for nodes in the ML analysis was assessed with nonparametric bootstrapping (BP) using GARLI v.0.951 (Zwickl, 2006) with 300 pseudoreplicates, and RAxML v7.04 (Stamatakis, Hoover & Rougemont, 2008) random starting trees, using 5000 rapid bootstraps. The five data sets mentioned above were also analysed in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Models for each partition were chosen in MrModelTest as explained above. Analyses were initiated with random starting trees and run for 5×10^6 generations and four chains. Markov chains were sampled at intervals of 1000 generations. The program TRACER v.1.3 (Drummond & Rambaut, 2007) was used to determine when the log likelihood (lnL) of sampled trees reached a stationary distribution. Generations sampled before the chain reached stationarity were discarded as burn-in, and the remaining trees were used to create 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities.

Genetic distances
In order to compare the genetic distances amongst specimens of *Notobryon* and *Scyllaea* from different biogeographical areas, we calculated the mean genetic distances for the three molecular markers using PAUP* 4.0 b 10.0. All codon positions for COI and H3 genes were considered for the analysis.

RESULTS

SYSTEMATICS
NUDIBRANCHIA CUVIER, 1817
CLADOBRANCHIA WILLAN & MORTON, 1984
FAMILY SCYLLAEIDAE ALDER & HANCOCK, 1855

Synopsis of the family Scyllaeidae Odhner, 1936
Dorsal margin produced into large lobes (continuous or separated, with a trace on the rhinophoral sheaths) covered more or less with branchial tufts. Velum indistinct; no frontal papillae. Rhinophore club perfoliate. Anus lateral or laterodorsal. Eyes with very short optic nerves. Jaws covering pharynx. Radula

with several laterals; median tooth present or absent. Nephroproct adjacent to anus. Digestive gland divided into two to four compact, not branching, globular masses. Stomach plates present. Gonad separated from digestive gland, appearing in a few globular masses. Vas deferens emerges from the albumen gland.

GENUS NOTOBRYON

Synopsis of the genus Notobryon Odhner, 1936
Body sides smooth. Two pairs of dorsal lobes with long bases. Anus lateral, between the right lobes. Rhinophoral sheaths with an elevated posterior crest. Digestive gland divided into three compact masses (right, left anterior, and posterior). Radula without median tooth, rachis broad. Penis short, conical with flattened edge-shaped and bearing a small projecting lobe.

Type species: Notobryon wardi Odhner, 1936

NOTOBRYON WARDI ODHNER, 1936
(FIGS 1, 2, 3, 4, 5A–B)

Notobryon wardi Odhner, 1936: 1099, plate 1, figures 1–3, text figures 31–38; Baba, 1949: 90, pl. 36, figures 131–132, text figure 114. Nakano, 2004: 217, bottom photo.
Notobryon sp. 1 Gosliner, Behrens &Valdés, 2008: 327, second photo from bottom.
Notobryon sp. 2 Gosliner, Behrens & Valdés, 2008: 327, bottom photo.
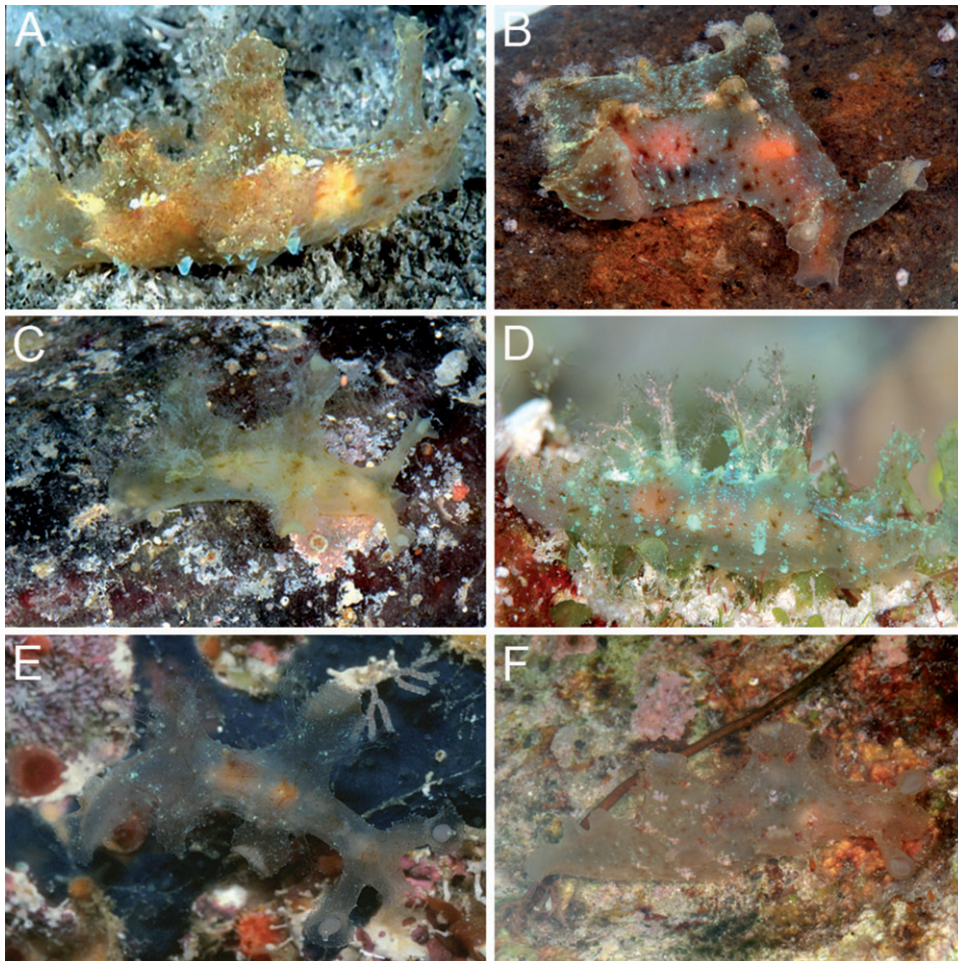
Figure 1. *Notobryon wardi* Odhner, 1936. Living animals. A, Australia, New South Wales, Port Stephens, photo by Ron Greer; B, Philippines, Luzon Island, Batangas Province, Anilao, Photo by T. M. Gosliner, CASIZ 177589; C, Philippines, Luzon Island, Batangas Province, Calumpan Peninsula, photo by T. M. Gosliner, CASIZ 177537; D, Marshall Islands, Kwajalein Atoll, South Loi Island, photo by J. Johnson, CASIZ 180378; E, Papua New Guinea, North coast, near Madang, photo by T. M. Gosliner, CASIZ 075283; F, *Notobryon* sp.B, Philippines, Luzon Island, Batangas Province, Anilao, Mainit Bubbles, photo by T. M. Gosliner, CASIZ 177759.
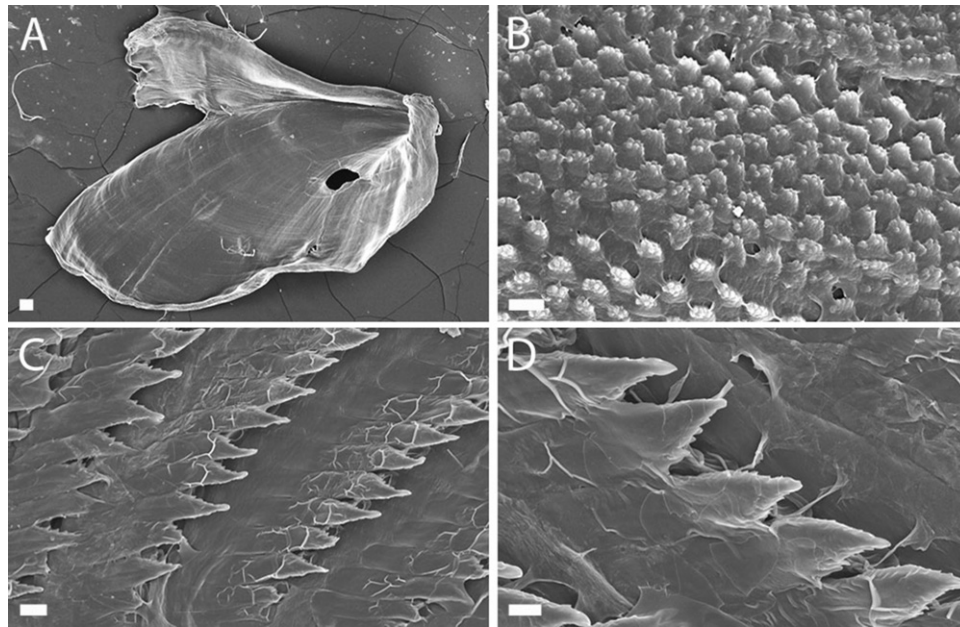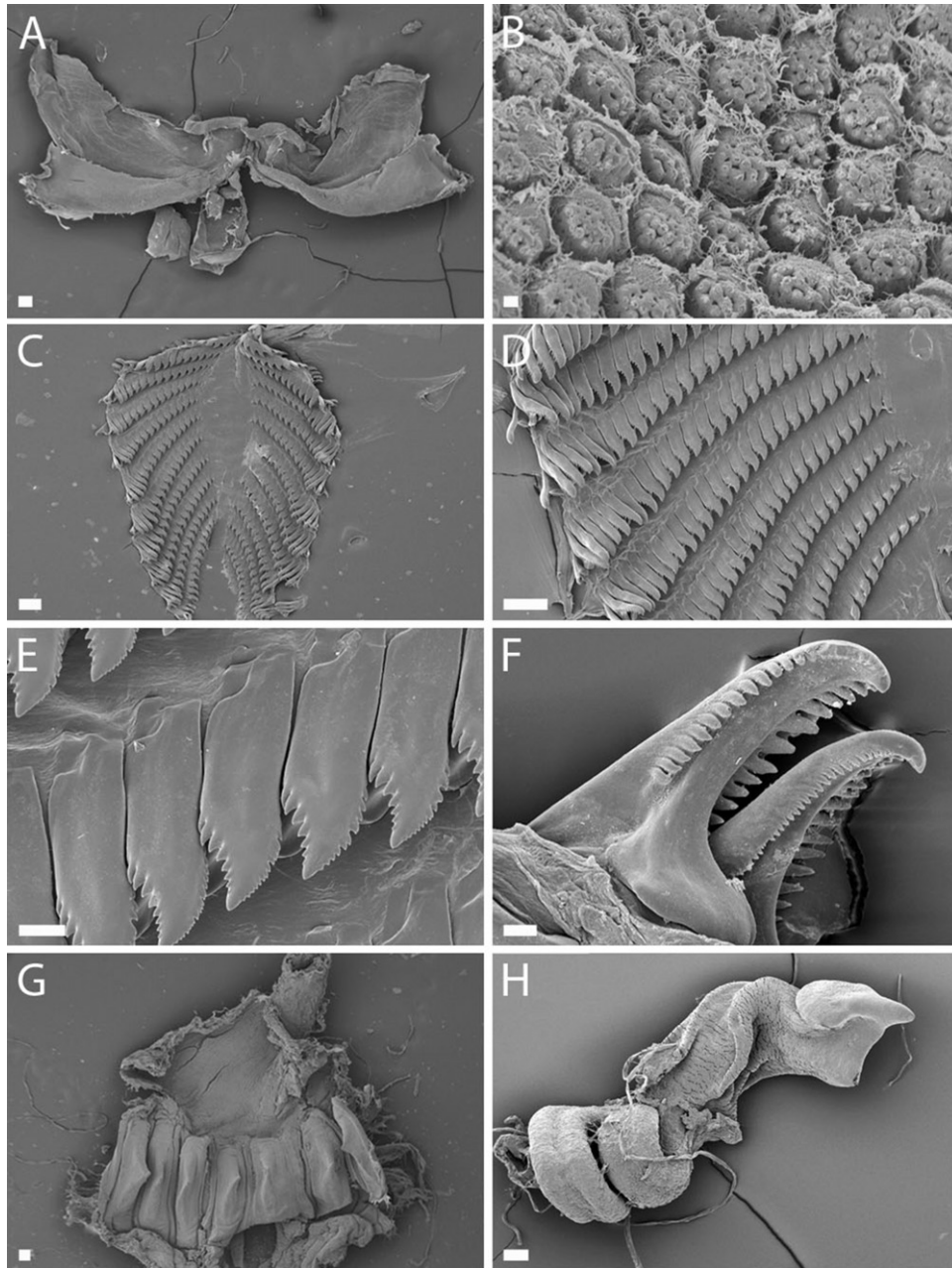
Figure 2. *Notobryon wardi* Odhner, 1936 (Paralectotype SMNH 1346), East Australia, Queensland. A, jaw, scale bar = 100 µm; B, jaw elements, scale bar = 10 µm; C, lateral teeth, scale bar = 30 µm; D, detail of lateral teeth, scale bar = 20 µm.

Type material: Lectotype (designated here): SMNH type no. 8092 *Notobryon wardi* Odhner, 1936, east Australia, Queensland, Port Curtis, off Gatcombe Head, one adult specimen 58 mm preserved length, 16 m depth, vii.1929, leg. M. Ward and W. Boardman. The lectotype is intact and was not dissected in this study. Externally, it is in good condition but it is difficult to visualize a few features because the specimen is swollen or some structures are contracted, probably because of the preservation method. *Paralectotypes (designated here)*: SMNH1346, east Australia, Queensland, Port Curtis, off Gatcombe Head, three adult specimens 37, 50, and 51 mm preserved length (two specimens dissected), 16 m depth, vii.1929, leg. M. Ward and W. Boardman. Specimens with four labels, three of them handwritten by Odhner: one records 'Notobryon wardi n. gen. n. sp. Type, Det. Odhner', the second records the number 4306, and the third one, 'Dredge off Gatcombe Head, Pt. Curtis about 9 f (illegible), July 1929. Coll. M. Ward & W. Boardman'. The fourth label corresponds to the printed label by the SMNH Museum with all the collecting information. Two of the three paralectotypes were dissected by Odhner. A vial inside the main container had one central nervous system, one complete jaw, two radulae, part of the buccal bulb, part of the stomach plates, and part of the digestive gland. One of the radulae was cleaned and mounted in a SEM stub by us. This is the radula that corresponds to the one that Odhner depicted in the original

description. All these structures with the exception of the second radula belong to the 50 mm paralectotype specimen. The second radula remained inside the tissue and was also cleaned and mounted for SEM. In the 51 mm paralectotype, the labial cuticle was still inside the buccal bulb. All the internal organs are present except for the heart and radula. The stomach was opened to remove the stomach plates, the same one that we found in the glass vial. We counted seven plates in the stomach and we believe that one is missing and should be the one that was inside the vial. This plate was also mounted for SEM.

Other specimens: Papua New Guinea: CASIZ 075283, Papua New Guinea, North coast, near Madang, inner side of barrier reef just south of Rasch Passage, Matthew's Folly, 22 m depth, one adult specimen 15 mm preserved, dissected, 15.xi.1990, collected by (coll.) T. M. Gosliner, photo slide. CASIZ 086503, Papua New Guinea, North coast, near Madang, inner side of Rasch Passage, 5 m depth, two immature specimens 5 & 10 mm preserved, dissected, 16.vi.1992, coll. T. M. Gosliner, photo slide. Indonesia: CASIZ 117360, Indonesia, Banda Sea, Reong Island, out at night, 9 m depth, one adult specimen 35 mm alive (18 mm preserved), dissected, 3.xi.1998, coll. P. Fiene-Severns, photo slide. Hawaii: CASIZ 104662, Hawaii, Maui, Makena, one immature specimen 13 mm alive, dissected, 19.v.1995, coll. P. Fiene-Severns, photo slide. CASIZ 101129, Hawaii, Maui,
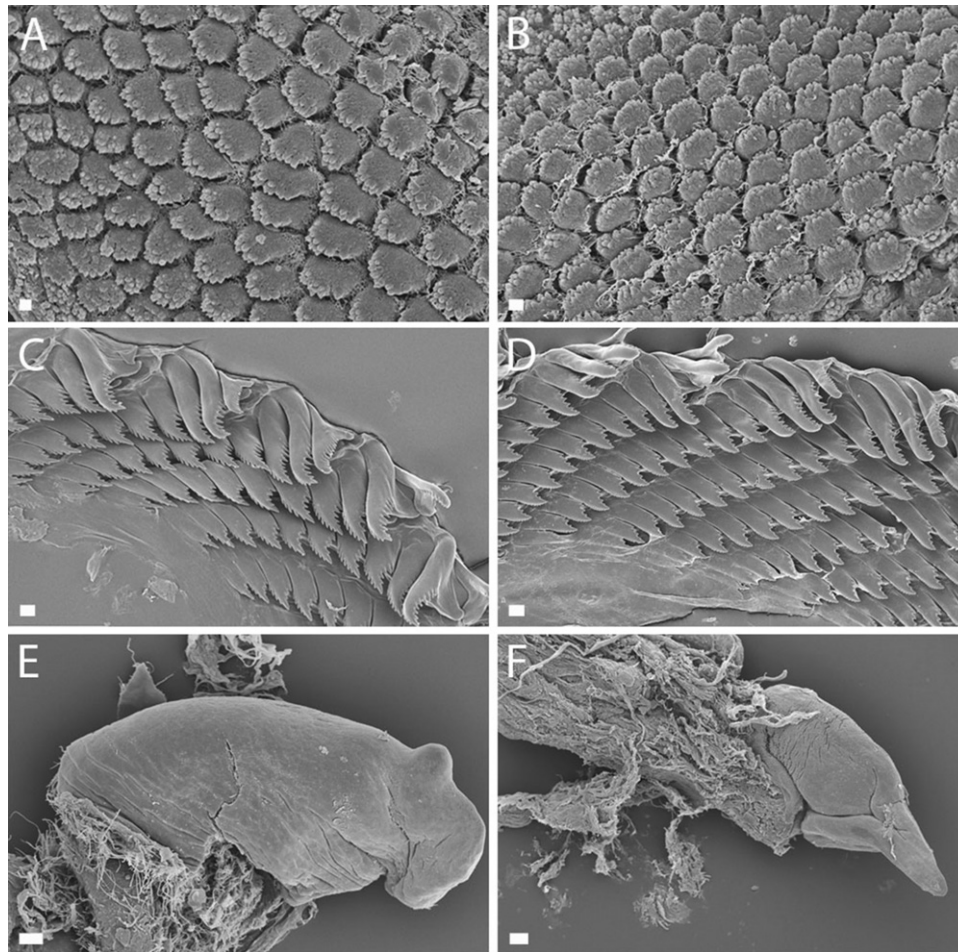
Figure 3. *Notobryon wardi* Odhner, 1936. Philippines specimens. A, CASIZ 177589, jaws, scale bar = 100 μm; B, CASIZ 177589, jaw elements, scale bar = 1 μm; C, CASIZ 177591, radula, scale bar = 100 μm; D, CASIZ 177589, left rows of teeth, scale bar = 100 μm; E, CASIZ 177589, central lateral teeth, scale bar = 20 μm; F, CASIZ 177589, detail of a tooth, scale bar = 10 μm; G, CASIZ 177589, stomach plates, scale bar = 100 μm; H, CASIZ 177589, penis, scale bar = 100 μm.

Black Rock, 10 m depth, two immature specimens 5 mm preserved, 20.x.1994, coll. C. Pittman, photo slide. CASIZ 101109, Hawaii, Maui, Airport Beach, on *Halimeda*, 9 m depth, one immature specimen 5 mm alive, 1.ix.1994, coll. C. Pittman, photo slide. CASIZ 105934, Hawaii, Oahu, off Haleiwa, 109 m, two specimens 20 mm preserved (one dissected), date and collector unknown. CASIZ 116801, Hawaii,

Oahu, Kepuhi Point, exposed on rubble at night, 35 m depth, one specimen 20 mm preserved, 3.xii.1985, coll. S. Johnson, photo slide. Philippines: CASIZ 177589, Philippines, Luzon Island, Batangas Province, Mabini, Mainit Bubbles, 16 m maximum depth, one adult specimen 20 mm preserved, dissected, 16.iv.2008, coll. T. M. Gosliner, digital pictures. CASIZ 177591, Philippines, Luzon Island,
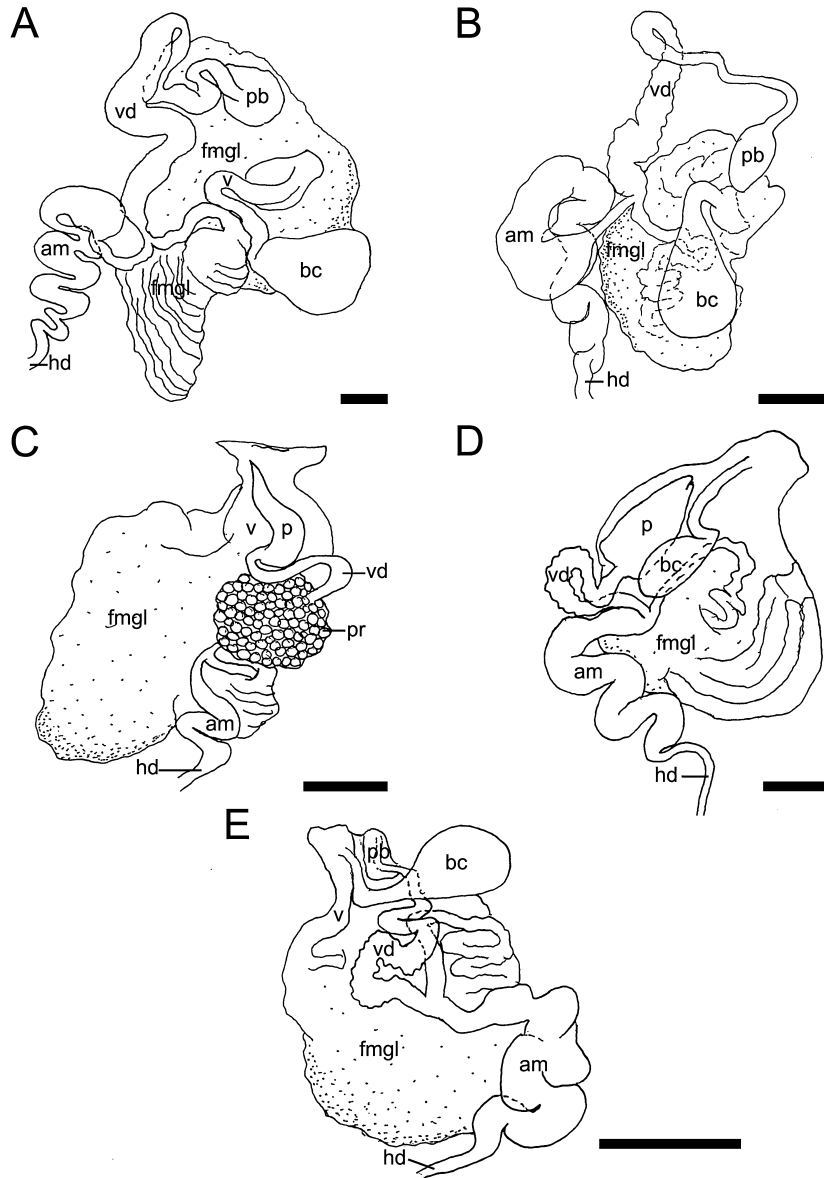
Figure 4. *Notobryon wardi* Odhner, 1936. Papua New Guinea (CASIZ 075283) and Indonesia (CASIZ 0117360) specimens. A, CASIZ 075283, jaw elements, scale bar = 1 µm; B, CASIZ 0117360, jaw elements, scale bar = 2 µm; C, CASIZ 075283, right rows of teeth, scale bar = 10 µm; D, CASIZ 0117360, right rows of teeth, scale bar = 20 µm; E, CASIZ 075283, penis, scale bar = 30 µm; F, CASIZ 0117360, penis, scale bar = 20 µm.

Batangas Province, Mabini, Mainit Bubbles, 16 m maximum depth, one adult specimen 25 mm preserved, dissected, 16.iv.2008, coll. T. M. Gosliner, digital pictures. CASIZ 177759, Philippines, Luzon Island, Batangas Province, Mabini, Mainit Bubbles, 17 m maximum depth, one adult specimen 25 mm preserved, dissected, 22.iv.2008, coll. T. M. Gosliner, digital pictures. CASIZ 177537, Philippines, Luzon Island, Batangas Province, Mabini, Calumpan Peninsula, Mainit Bubbles, 23 m maximum depth, one adult specimen 20 mm preserved, 21.iii.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & A. Alejandrino, digital pictures. CASIZ 177540, Philippines, Luzon Island, Batangas Province, Mabini, Calumpan Peninsula, Mainit Bubbles, 23 m max depth, one specimen 15 mm preserved, 21.iii.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & A. Alejandrino, digital pictures.

Pacific Ocean: CASIZ 180378, Marshall Islands: Kwajalein Atoll: South Loi Island: 'South Loi sand-spit', 8 m depth, one adult specimen 25 mm alive, 18.xi.2007, coll. S. Johnson, digital pictures.

Geographical distribution: Indo-West Pacific: originally described from Australia (Odhner, 1936; Greer, 2000) this species is also found in the Philippines (Köhler, 2001; Gosliner *et al.*, 2008 identified as *Notobryon* sp.2, present study), Papua New Guinea (Coleman, 2008; Gosliner *et al.*, 2008 identified as *Notobryon* sp.2, present study), Japan (Nakano, 2004), and Indonesia, Hawaii, and Marshall Islands (Debelius & Kuiter, 2007; present study).

Etymology: This species was named after one of the collectors, M. Ward who collected the first four specimens by dredging in July 1929.

Figure 5. Reproductive system. A, B, *Notobryon wardi* Odhner, 1936. A, Paralectotype SMNH 1346; B, CASIZ 177589; C, *Notobryon bijerecum* Baba, 1837, CASIZ 089003; D, ***Notobryon thompsoni* sp. nov.**, CASIZ 07400; E, ***Notobryon panamica* sp. nov.**, MZUCR-INB0003118069; am, ampulla; bc, bursa copulatrix; fmgl, female gland mass; hd, hermaphroditic duct; p, penis; pb, penial bulb; pr, prostate; v, vagina; vd, vas deferens. Scale bar = 1 cm.

*External morphology (*Fig. 1*):* The body is slender, soft, flaccid, and elevated but laterally compressed. There is a dorsal crest behind the posterior lobes that ends in a keel-like tail. The margin is continuous anteriorly to the narrowest point near the middle of the body. There are some additional more elevated parts, running towards and forming a marked crest on the elevated rhinophoral sheaths. The rhinophores are perfoliate with about ten to 20 lamellae. The front of the head is bilobed but without processes or tentacles. There are two pairs of dorsolateral lobes.

These lobes are well developed, the first pair slightly larger than the posterior pair and nearly continuous at their bases leaving U-shaped spaces between them. Each dorsolateral lobe bears four delicately branched secondary gills on their upper surface that look like small trees, two on the inner surface of each lobe and two usually smaller 'gills' on the outer margin of each lobe. There are also one or two extra secondary tripinnate 'gills' located just in front of the posterior end of the foot. The anal papilla is located on the right side, between the two lobes. The genital opening is on the

right side, beneath the anterior end of the first lobe. Minute holes or subcutaneous glands, whose function is indeterminate, cover the entire body.

The body surface is quite smooth and semitransparent or reddish-brownish but with fairly numerous small light blue spots, some coalescing into slightly larger blotches. There are also traces of light brown scattered all over the body surface. The secondary 'gill' stalks are mostly transparent but some have thin brown cores. Externally the 'gills' are sporadically scattered with shiny opaque white, usually near the bases.

Anatomy: The alimentary canal begins anteriorly with the buccal bulb that has a pair of elongate jaws that are somewhat brown and thickened along the masticatory process. The masticatory edge of the jaws is expanded into a wing-like flap (Figs 2A, 3A). Over the edge of this flap are a series of polygonal rodlets that have a series of denticles along their outer edge. These rodlets form a honey-comb pattern over the entire surface of the masticatory edge (Figs 2B, 3B, 4A–B). The radula is broad and lacks rachidian teeth (Fig. 3C). The radular formula of most of the dissected specimens is detailed in Table 1. The radula formula described by Odhner (1936) is 14×22–$24.0.22$–24. The formula of two of the syntypes is $12 \times 21.0.21$. Each lateral tooth bore denticles on both sides (Figs 2C–D, 3D–F, 4C–D). The denticulation is usually stronger on the outer face of each cusp (Fig. 3E–F). The teeth gradually increase in size towards the outer margin except for the two to three outermost teeth. The salivary glands are a flocculent mass of fine branching tubules, surrounding the foremost part of the very wide oesophagus. The stomach is well developed and has thick folds on the walls forming a girdle of eight thick plate-like elevations, broadly triangular, with broad base and a central apex (Fig. 3G). The digestive gland forms three distinct unbranched lobes.

The reproductive system of two specimens is shown in Figure 5A–B. The ovotestis consists of two large and globular gonads lying on the upper side of the posterior liver mass. From each gonad a thin-walled duct passes forwards to the hermaphroditic duct. The distal end of the hermaphroditic duct gets wider and expands into a long and convoluted ampulla that branch into the vas deferens and the oviduct. The oviduct enters the female gland mass. The vas deferens is long, and somewhat folded. It lacks a morphologically well-differentiated prostate gland mass but about half of its length has a different texture and appearance. The remaining deferent duct is narrower and smoother ending in a dilated penis. The penis is a short conical shape with its external margin compressed into an edge ending in a triangular lobe with

two short cups (Figs 3H, 4E–F). The vagina is short with an elongate and large bursa copulatrix.

NOTOBRYON BIJECURUM BABA, 1937
(FIGS 5C, 6A–D, 7)

Notobryon bijecurum Baba, 1937: 166 figures 1–3; Baba 1949: 91, plate 37, figure 133, text figure 115. Nakano, 2004: 217, middle photo.

Material examined: CASIZ 089003, Japan, Ryukyu Islands, Okinawa, 1.3 km east-north-east of Maeki-zaki, Seragaki Beach, 26°30.4′N, 127°52.6′E, mixed sand coral rubble, 40 m depth, one adult specimen 57 mm alive, dissected, 29.xi.1992, coll. R. Bolland, photo slide.

Geographical distribution: Currently known only from Japan (Baba, 1937, 1949; Nakano, 2004; present study).

*External morphology (*Fig. 6A–D*):* The body is slender, soft, flaccid, and elevated, but laterally compressed. The foot is narrow. The posterior crest is moderately large with an entire margin. The rhinophoral sheaths have a posterior longitudinal crest. The rhinophores are perfoliate with 16 lamellae. The front of the head is expanded in a semicircular veil with a wavy margin. There are two pairs of dorsolateral lobes, the posterior ones are much smaller that the anterior ones. These lobes are nearly continuous at their bases leaving a U-shaped space between them. The margins of the dorsolateral lobes are entire. Each dorsolateral lobe bears four large and dendritic 'gills' on their upper surface and one on the tail. The anal papilla is located on the right side, between the two lobes. The genital opening is on the right side, beneath the anterior end of the first lobe.

The body surface is quite smooth, varying from transparent to semitransparent light orange or light yellow with bold opaque marking on the back and the sides. Sometimes there are small light blue spots, some coalescing into usually two larger blotches. The 'gills' are mostly transparent. Minute holes or subcutaneous glands of indeterminate function cover the entire body.

Anatomy: The alimentary canal begins anteriorly with a buccal bulb that has a pair of elongate jaws (Fig. 7A). The masticatory edge of the jaw-plate is formed into a wide flange closely covered with numerous scale-like armatures (Fig. 7B). The radula is broad and lacks rachidian teeth (Fig. 7C). The radula formula described by Baba (1937) for a 30 mm long specimen is 12×14–$16.0.14$–16. The radula formula of the 57 mm specimen studied in this paper is

$16 \times 24.0.24$. Each lateral tooth bears denticles on both sides except for the first inner lateral tooth that lack any denticles (Fig. 7D, E). The teeth gradually increase in size towards the outer margin except for the last two outermost teeth that become smaller. The salivary glands are very elongate and flocculent surrounding the foremost part of the very long and narrow oesophagus. There are seven stomach plates that are thick and broadly triangular (Fig. 7F).

The reproductive system is shown in Figure 5C. The ovotestis is comprised of two large and globular gonad groups lying on the upper side of the posterior digestive gland mass. The anterior group is composed of four hermaphroditic glands whereas the posterior group consists of five hermaphroditic glands. The hermaphroditic duct expands into a wider and folded duct that functions as an ampulla. The ampulla branches into the vas deferens and the oviduct. The oviduct enters the female gland mass. The vas deferens has a mor-

phologically well-differentiated prostate gland mass. The prostate is large and globular consisting of many small rounded glands (Fig. 7G). From the prostate continues a relatively short and folded duct that ends in a conical and pointed penis (Fig. 7H). The vagina is short and lacks a bursa copulatrix.

NOTOBRYON CLAVIGERUM BABA, 1937 (FIG. 6E, F)

Notobryon clavigerum Baba, 1937: 168, figures 4–6; Baba, 1949: 92, plate 37, figure 134, text figure 116. Nakano, 2004: 218, top three photos.

No specimens of *N. clavigerum* Baba, 1937 have been studied in the present paper because types were not traceable. This species was originally described by Baba (1937) from a single specimen of 80 mm in length collected in Japan, Sagami Bay, Amadaiba, in July 1935. The general shape of the body and most of the external and internal features are very similar to the two previously described species but Baba
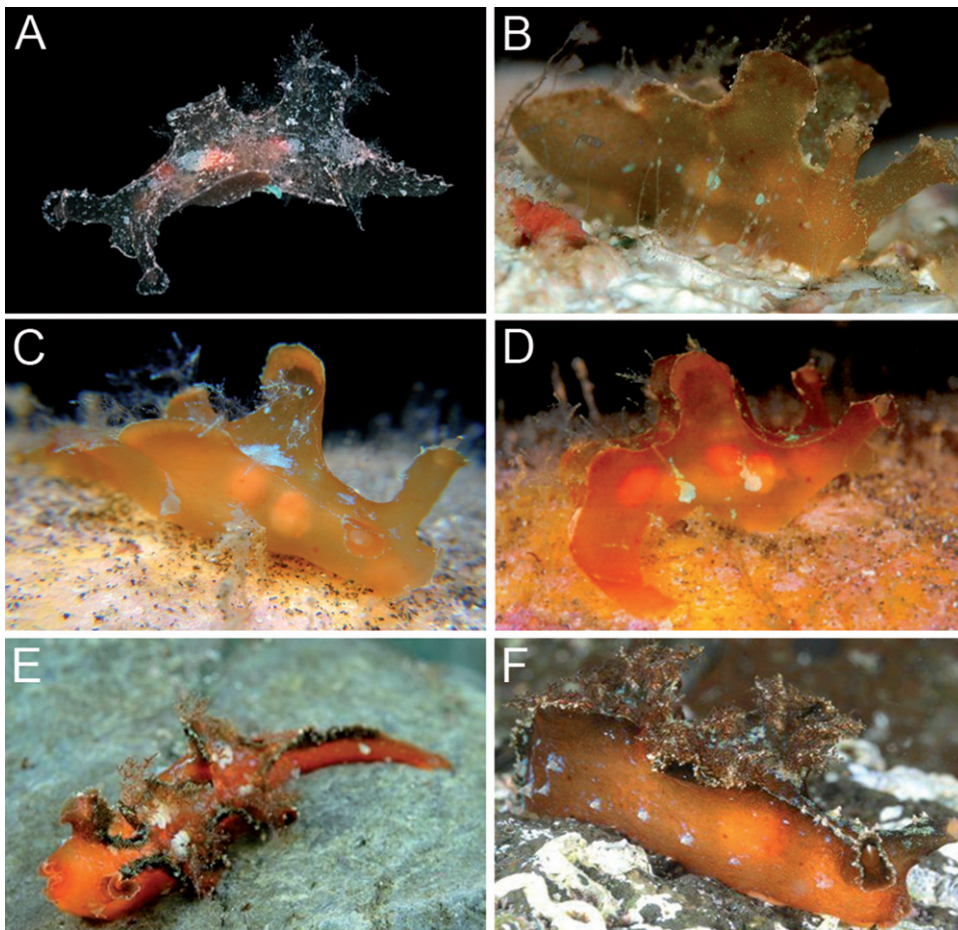

Figure 6. A–D, *Notobryon bijerecum* Baba, 1837, living animals. A, Japan, Ryukyu Islands, Okinawa, Seragaki Beach, photo by R. Bolland, CASIZ 089003; B, Japan, Shizuoka prefecture, photo by S. Yamamoto; C, Japan, Shizuoka prefecture, photo by T. Kurihara; D, Japan, Izu-Oshima Island, photo by H. Yamada. E-F, *Notobryon clavigerum* Baba, 1837, living animals. E, Japan, Fukui prefecture, photo by Y. Shamoto; F, Japan, Shizuoka prefecture, photo by A. Kawahara.
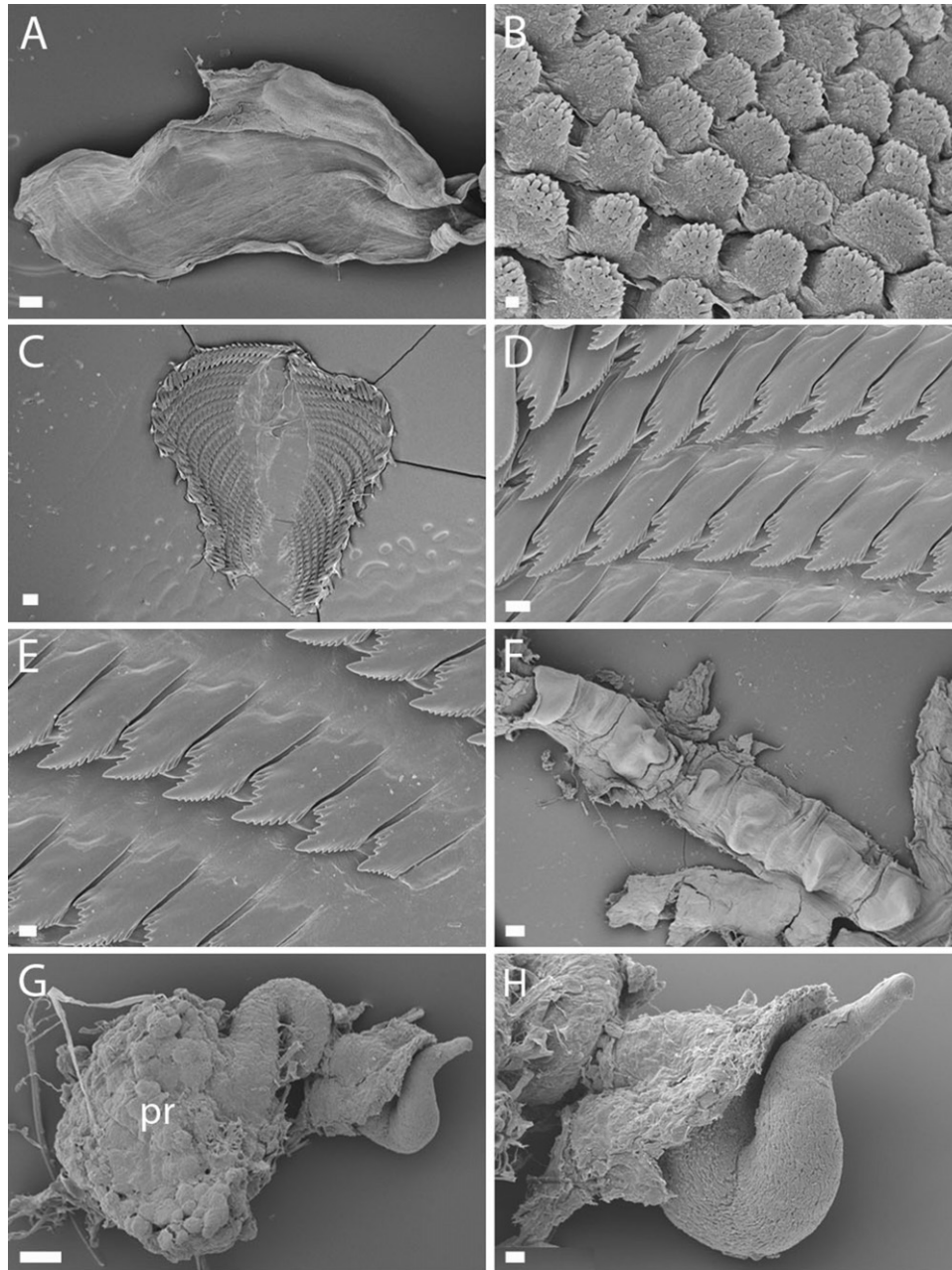
Figure 7. *Notobryon bijerecum* Baba, 1837, CASIZ 089003. A, jaws, scale bar = 100 μm; B, jaw elements, scale bar = 1 μm; C, radula, scale bar = 100 μm; D, Inner lateral teeth, scale bar = 10 μm; E, central lateral teeth, scale bar = 20 μm; F, stomach plates, scale bar = 200 μm; G, penis and prostate, scale bar = 100 μm; H, penis, scale bar = 20 μm.

claimed that there are enough differences to describe *N. clavigerum* as a different species. Thus, Baba stated that in *N. clavigerum* the rhinophoral sheaths have a longitudinal crest not only along the posterior margin but also along the anterior margin that ends distally in a wavy margin. Another interesting difference is that the dorsolateral lobes have smooth edges except at the tip, which stands recurved having a row of small claw-like papillae, five on the anterior and

three on the posterior lobes, respectively. Moreover, in *N. clavigerum*, the posterior end is long and has a lower crest on the middle line than in the previously described species. The remaining external and internal features described by Baba (1937) are very similar to *N. wardi* and *N. bijecurum* except for the description of hermaphroditic glands consisting of nine separates lobes and the presence of only six gastric plates. The reproductive system is not described.
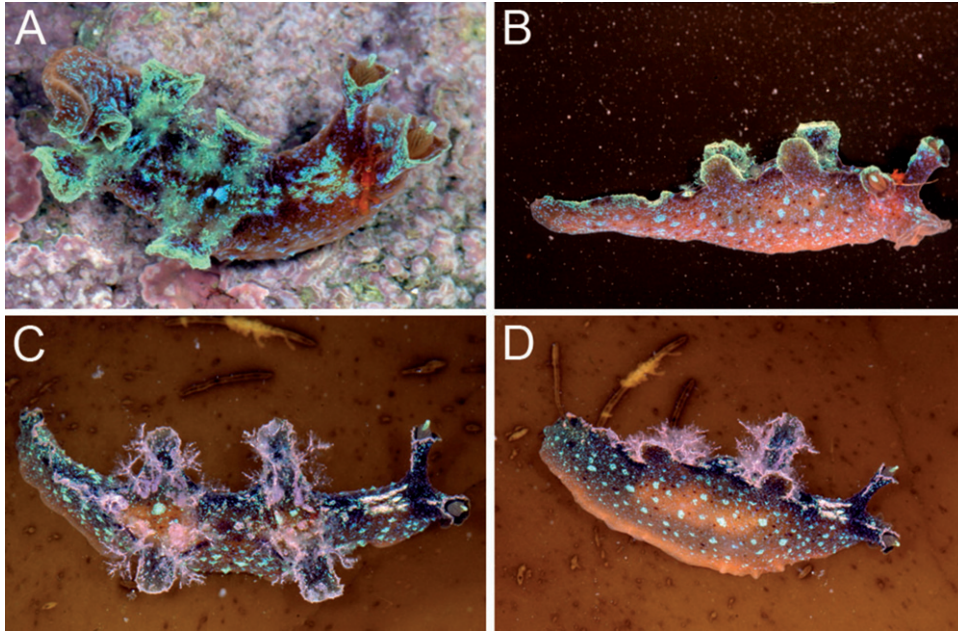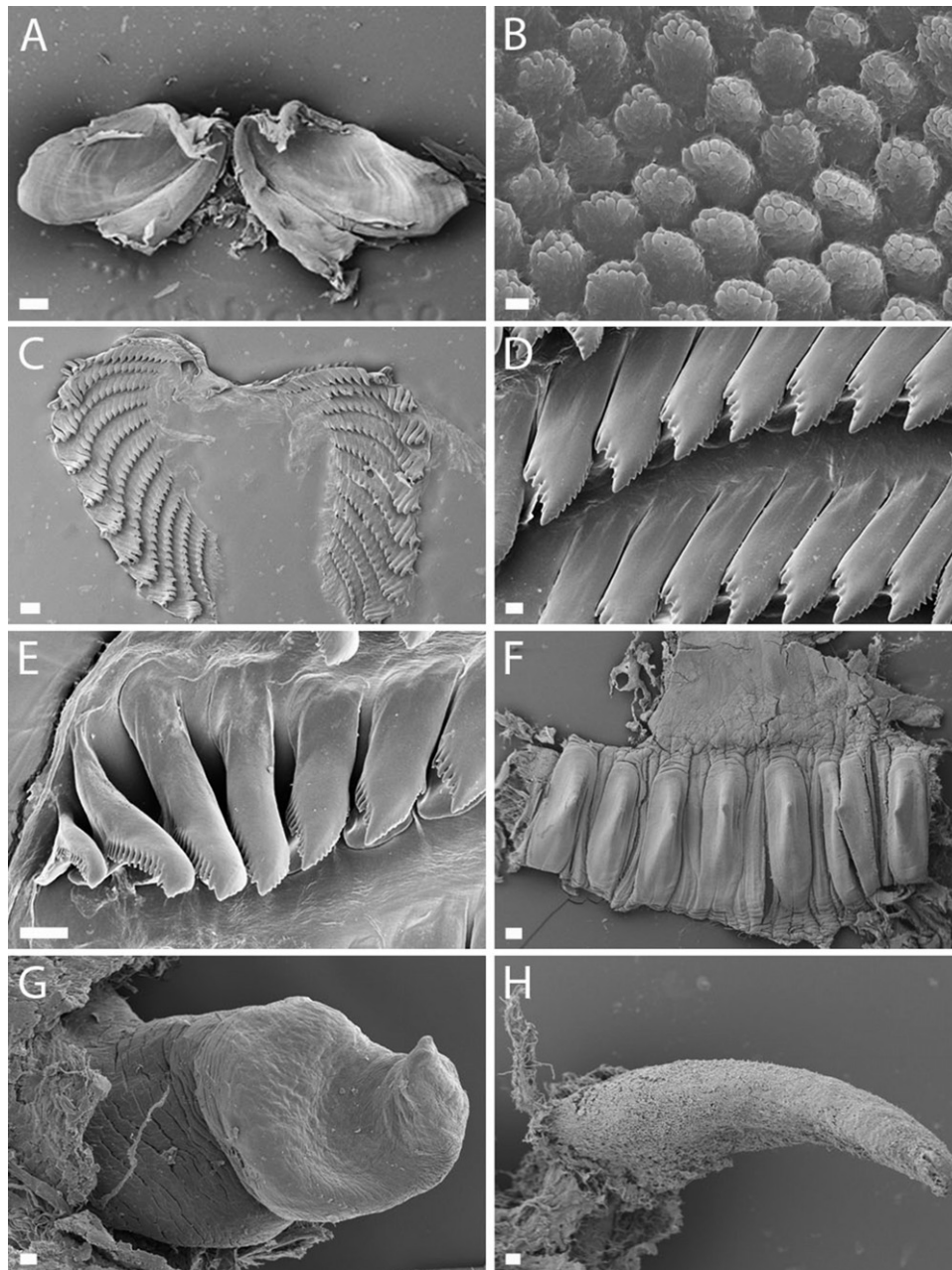
Figure 8. *Notobryon thompsoni* **sp. nov.** Living animals. A-B, South Africa, Cape Province, Oudekraal, photo by T. M. Gosliner, CASIZ 176277; C-D, South Africa, Cape Province, west False Bay, Dale Brooks, photo by T. M. Gosliner, CASIZ 176362.

NOTOBRYON THOMPSONI SP. NOV. (FIGS 5D, 8, 9)

Notobryon wardi Thompson & Brown, 1981: 437, figures 1–2, misidentification; Gosliner, 1987: 105, middle photo, misidentification; Zsilavecz, 2007: 67, 4 figs, misidentification.

Material examined: Type material: Holotype: CASIZ 176363, South Africa, western Cape Province, west False Bay, Dale Brook, 34°7.50′S, 18°27.12′E, intertidal zone, one adult specimen 18 mm preserved, 7.i.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & C. Stout, digital photograph. Paratypes: CASIZ 176362, South Africa, western Cape Province, west False Bay, Dale Brook, 34°7.50′S, 18°27.12′E, intertidal zone, one adult specimen 20 mm preserved, dissected, 7.i.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & C. Stout, digital photograph. CASIZ 176364, South Africa, western Cape Province, west False Bay, Dale Brook, 34°7.50′S, 18°27.12′E, intertidal zone, one immature specimen 10 mm preserved, 7.i.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & C. Stout, digital photograph. CASIZ 176277, South Africa, Cape Province, Atlantic coast, Oudekraal, Mushroom Rock, 33°58.91′S, 18°21.61′E, 14 m maximum depth, one adult specimen 18 mm preserved, dissected, 5.i.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & C. Stout, digital photograph. CASIZ 176925, South Africa, Cape Province, Atlantic coast, Oudekraal, Oudekraal, 33°58.91′S, 18°21.61′E, 12 m maximum depth, one specimen 12 mm preserved, dissected, 13.i.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & C. Stout, digital photograph. CASIZ 176956, South Africa, Cape Province, Atlantic coast, Oudekraal, Coral Gardens, Hottentot's Huisie, 33°59.29′S, 18°20.98′E, 17.5 m maximum depth, one immature specimen 12 mm preserved, dissected, 14.i.2008, coll. T. M. Gosliner, A. Valdés, M. Pola, L. Witzel, B. Moore & C. Stout, digital photograph. CASIZ 073189, South Africa, Cape Province, Atlantic coast, Oudekraal, Coral Gardens, Hottentot's Huisie, north of Llandudno, one specimen 15 mm preserved, i.1981, coll. T. M. Gosliner. CASIZ 074100, South Africa, Cape Province, False Bay, Dale Brook, 34°7.50′S, 18°27.12′E, one adult specimen 21 mm preserved, collection date unknown, coll. T. M. Gosliner, digital photograph. CASIZ 073964, South Africa, Cape Province, Atlantic coast, off Bloubergstrand, 4.6 m depth, one adult specimen 40 mm preserved, 14.ii.1980, coll. W. R. Liltved. SAM 33981, South Africa, Cape Province, False Bay, Dale Brooks, intertidal, five immature specimens 3, 5, 8, 10, and 15 mm preserved, collection date unknown, coll. T. M. Gosliner. SAM CP614B, South Africa, Cape Province, one adult specimen 20 mm preserved, dissected, 22.iii.1958, Ecological Survey, University of Cape Town. SAM KNY214C, South Africa, Cape Province, one adult specimen 30 mm preserved, 7.vii.1960, ecological survey, University of Cape Town.

Figure 9. *Notobryon thompsoni* **sp. nov.** A, SAM CP641B, jaws, scale bar = 200 μm; B, CASIZ 176956, jaw elements, scale bar = 2 μm; C, SAM CP641B, radula, scale bar = 100 μm; D, SAM CP641B, central lateral teeth, scale bar = 10 μm; E, CASIZ 176277, outer lateral teeth, scale bar = 30 μm; F, SAM CP641B, stomach plates, scale bar = 100 μm; G, SAM CP641B, penis, scale bar = 20 μm; H, CASIZ 176362, penis, scale bar = 20 μm.

Geographical distribution: Currently known from South Africa (Thompson & Brown, 1981; Gosliner, 1987; Debelius & Kuiter, 2007; Zsilavecz, 2007; present study), all specimens previously identified as *N. wardi.*

Etymology: Notobryon thompsoni is named after T. E. Thompson who was the first to describe this species based on several specimens that he collected in South

Africa (Thompson & Brown, 1981), but he mistakenly identified this species as *N. wardi.*

*External morphology (*Fig. 8*):* The body is slender and laterally compressed. The front of the head is bilobed forming a thin oral veil. There is a distinctive posterior dorsal crest. The rhinophores are perfoliate with about ten to 15 lamellae. The rhinophoral sheaths have the characteristic elevated crest

on its posterior face. There are two pairs of dorsolateral lobes. These lobes are markedly concave on the medial faces and the anterior pair is quite separate from the posterior pair leaving a large and straight space between the two of them. Both dorsolateral lobes are very similar in size, and more rounded rather than triangular, the first pair is sometimes slightly larger than the posterior pair. Each dorsolateral lobe bears four delicate secondary 'gills', although in one specimen (CASIZ 074100) we counted up to six on the right side of the second pair of lobes. All the 'gills' are about the same size and very branched, looking like small trees. There is an extra secondary tripinnate 'gill' located just in front of the posterior end of the body. The anal papilla is located on the right side, immediately in front of the posterior lobe. The genital opening is on the right side, at a level behind and ventral to the right rhinophore. Minute holes or subcutaneous glands of unknown function cover the entire body.

The ground colour is pale brown or brown reddish with specks and some large blotchy areas of superficial blue-green pigment, often forming glistening apices on the pointed papillae of the flanks. There are also scattered dark brown spots over the sides and notum and dark brown pigment edges the dorsolateral lobes and the metapodial ridges. The medial faces of the spoon-like lobes show iridescent pink or green areas. The posterior crest of the rhinophoral sheaths, as well as their upper wavy margin, has the same iridescent pigment as the lobes. The tips of the rhinophores are iridescent green or blue. The lamellae are pale brown. The 'gills' are transparent but exhibit the same iridescent pigment that is present in the lobes, rhinophoral sheaths, and the posterior end.

Anatomy: The alimentary canal begins anteriorly with a buccal bulb that has a pair of elongate jaws (Fig. 9A). The masticatory edge of the jaws is expanded into a wing-like flap. Over the edge of this flap is a series of polygonal rodlets that have a series of denticles along their outer edge. These rodlets form a honey-comb pattern over the entire surface of the masticatory edge (Fig. 9B). The radula is broad and lacks rachidian teeth (Fig. 9C). The radula formula is $12–19 \times 16–20.0.16–20$. The specific formula for some of the specimens studied in this paper is shown in Table 1. Although it is difficult to see it seems that except for the inner and outermost teeth, each cusp bears denticles on both sides, always stronger on the outer face of each cusp (Fig. 9D, E). The teeth gradually increase in size towards the outer margin except for the last two to three outer teeth that become smaller (Fig. 9E). The salivary glands are elongate and flocculent surrounding the foremost part of the long and narrow oesophagus. We found eight conical gastric plates in the stomach (Fig. 9F). The digestive gland forms three distinct unbranched lobes.

The reproductive system is shown in Figure 5D. The ovotestis consists of two globular gonads lying on the upper side of the posterior digestive gland mass. From each gonad a thin-walled duct passes forwards to the hermaphroditic duct. The distal end of the hermaphroditic duct gets wider and expands into a long and convoluted ampulla that branches into the vas deferens and the oviduct. The oviduct enters the female gland mass. The vas deferens is short, wide, and somewhat folded. It lacks a morphologically well-differentiated prostate gland mass but about half of its length has a different texture and appearance. The vas deferens ends in a digitiform, unarmed penis (Fig. 9G, H). The vagina is short with an elongate bursa copulatrix.

Natural history: Found from the intertidal zone down to at least 15 m, but most commonly found intertidally. Feeds on hydroids. Prefers densely overgrown reefs (Zsilavecz, 2007). This species, when disturbed, swims by lateral movements of the body.

NOTOBRYON PANAMICA SP. NOV. (FIGS 5E, 10, 11)

Notobryon wardi Behrens & Hermosillo, 2005: 99, upper photo, misidentification; Hermosillo *et al.*, 2006: 167, bottom photo, misidentification.
Notobryon sp. Camacho-García *et al.*, 2005: 95, upper photo.

Material examined: Type material: Holotype: Mexico: CASIZ 180376, Mexico, Jalisco, Bahía de Banderas, Mismaloya, one adult specimen, dissected, 3.iv.2009, coll: A. Hermosillo, digital photograph. Paratypes: CASIZ 175778, Mexico, Jalisco, Bahía de Banderas, Los Arcos, one adult specimen 15 mm preserved, dissected, 12.vi.2003, coll. A. Hermosillo, photo slide. Costa Rica: MZUCR-INB0003118069, Costa Rica, Guanacaste, West Isla Catalina, intertidal, 10°28′47″N, 85°52′17″W, one adult specimen 15 mm preserved, dissected, 23.i.2001, coll. S. Ávila. MZUCR 6356, Costa Rica, Punta Ballena, Isla del Caño, 8 m depth, one adult specimen 20 mm preserved, dissected, 30.iv.2006, coll. Y. Camacho, digital photograph. Panama: CASIZ 088163, Panama, Pacific coast, Isla Montuosa, 12 m maximum depth, one immature specimen 12 mm preserved, dissected, 15.iv.1993, coll. T. M. Gosliner, photo slide. CASIZ 088164, Panama, Pacific coast, Isla Jicarita, southeast side, 7°13′N, 81°48′30″W, 16.iv.1993, one immature specimen 6 mm preserved, 16.iv.1993, coll. T. M. Gosliner, photo slide. CASIZ 088177, Panama, Pacific coast, Isla Jicarita, anchorage, one adult specimen 20 mm preserved, dissected, 16.iv.1993, coll. T. M. Gosliner, photo slide.
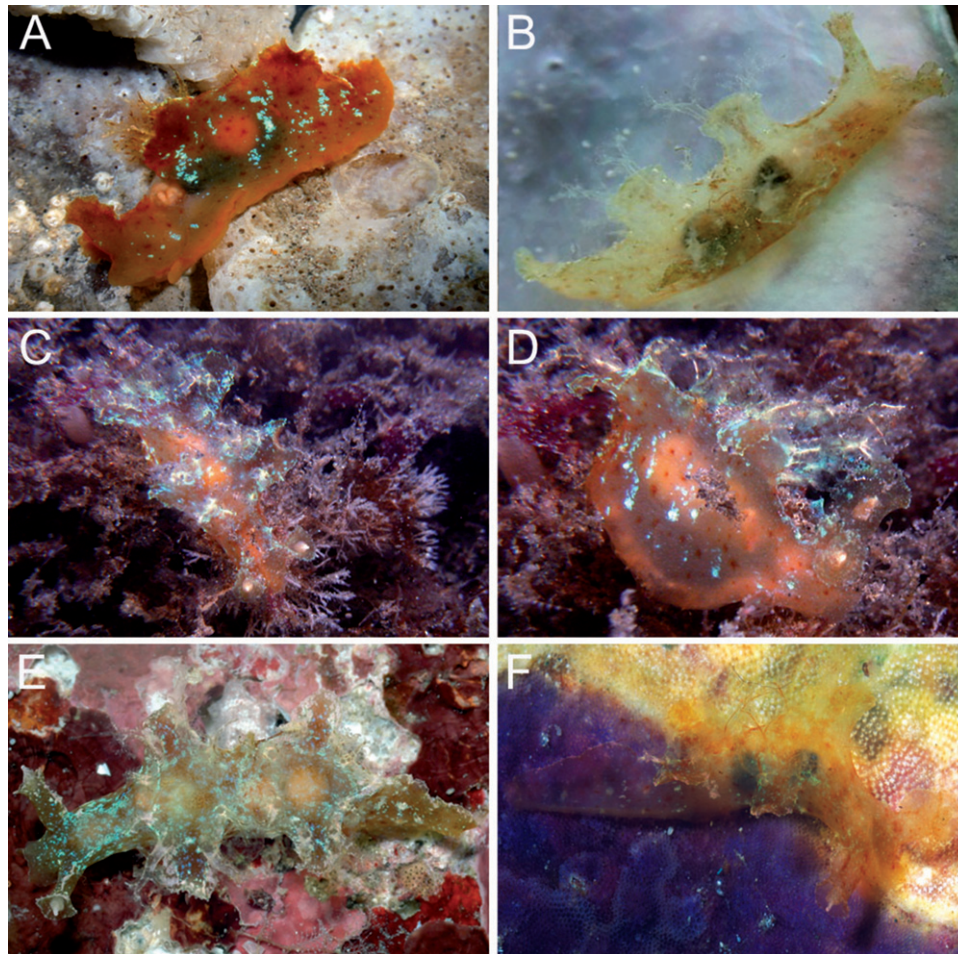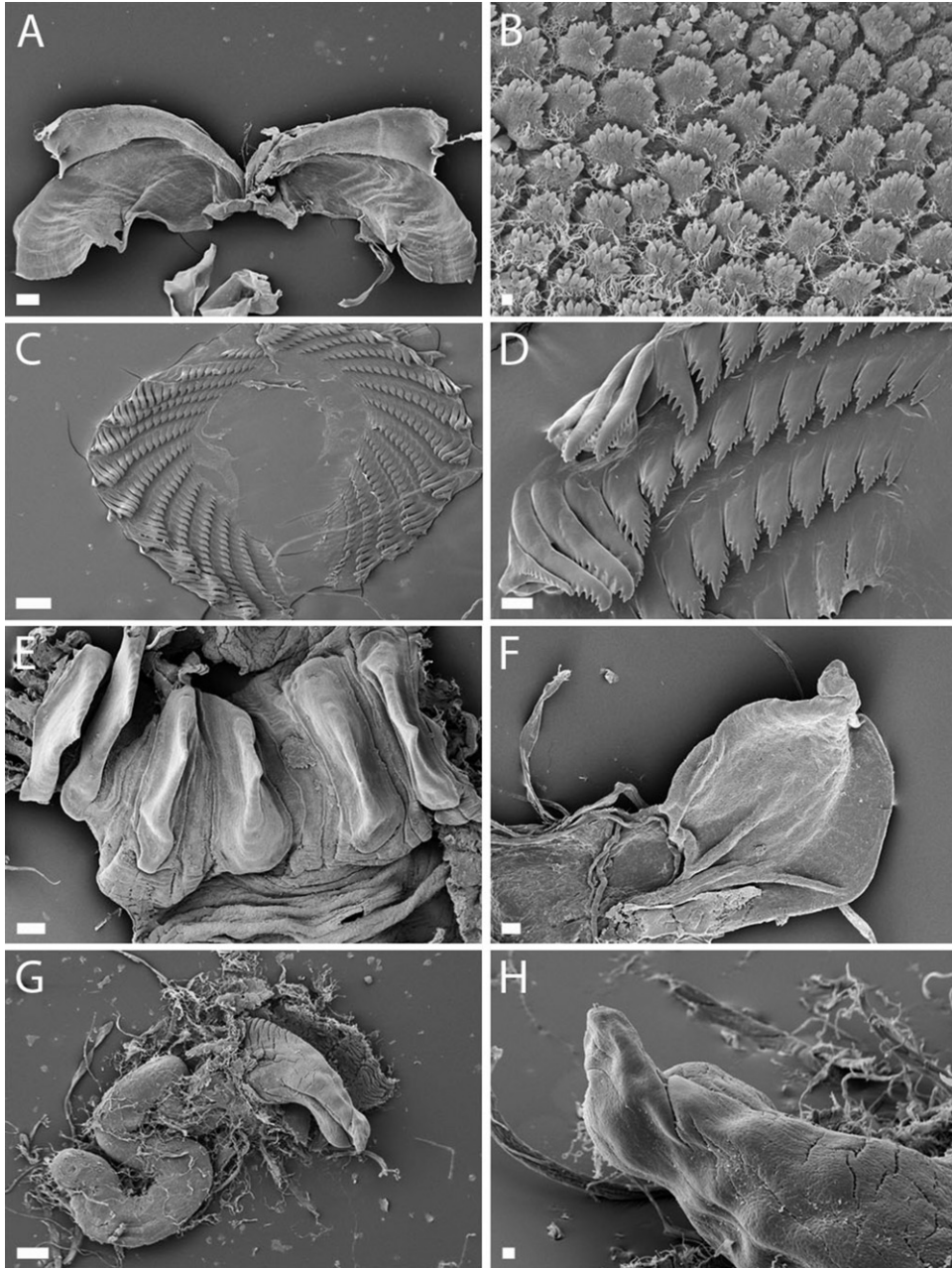
Figure 10. *Notobryon panamica* sp. nov. Living animals. A, Mexico, Jalisco, Bahia de Banderas, Los Arcos, photo by A. Hermosillo, CASIZ 175778; B, Pacific Coast of Mexico, Michoacan, Faro Buceiras, photo by A. Hermosillo; C-D, Mexico, Jalisco, Bahia de Banderas, Mismaloya, photo by A. Hermosillo, CASIZ 180376; E, Costa Rica, Punta Ballena, Isla del Caño, photo by Y. Camacho, MZUCR 6356; F, Panama, Pacific coast, Isla Jicarita, photo by T. M. Gosliner, CASIZ 088177.

Geographical distribution: This species has been recorded from the Pacific coast of southern Mexico to Costa Rica and Panama (Hermosillo, 2002a, b; Behrens & Hermosillo, 2005; Camacho-García *et al*., 2005 identified as *Notobryon* sp., Hermosillo, Behrens & Rios Jara, 2006 identified as *N. wardi*) and the Caribbean coast of Honduras, Santa Lucía, Virgin Islands, St. Vincent, and the Grenadines (Valdés *et al*., 2006 provisionally identified as *N. wardi* but probably an undescribed species).

Etymology: This species is named after the Panamic Biogeographical Province, to which this species appears to be restricted.

*External morphology (*Fig. 10*):* The body is limaciform, tapering to the acute posterior end of the foot. The anterior end of the head is indented. The foot is paddle shaped with a dorsal sail-shaped crest. The rhinophoral sheaths are wide and tall with a posteriorly directed crest. They expand into a wide opening through which the perfoliate rhinophores are visible. The crest is crenulated with a few tubercles at the base of its posterior side. The rhinophores are perfoliate with about 15 lamellae. The three lobes of the digestive gland are readily visible through the body. Small rounded tubercles are present on the body surface. The body has four lateral rounded lobes that are extended laterally when the animal is at rest or vertically when it is actively crawling. Each lobe has four secondary 'gills' of about the same length. These 'gills' are higher than the dorsal lobes. One pair is located below the base of each lobe, on the notum and the other pair just above them, but on the lobe. The secondary 'gills' are tripinnate. In one of the specimens examined (MZUCR6356), there is one extra secondary 'gill' located at the anterior part of the tail. Both pairs of dorsolateral lobes are very similar in

Figure 11. *Notobryon panamica* sp. nov. A. MZUCR-INB0003118069, jaws, scale bar = 100 μm; B, CASIZ 088177, jaw elements, scale bar = 1 μm; C, MZUCR-INB0003118069, radula, scale bar = 100 μm; D, CASIZ 088177, left rows of teeth, scale bar = 20 μm; E, MZUCR-INB0003118069, stomach plates, scale bar = 100 μm; F, MZUCR 6359, penis, scale bar = 30 μm; G, MZUCR-INB0003118069, penis, scale bar = 100 μm; H, MZUCR-INB0003118069, penis, scale bar = 10 μm.

size, the first pair sometimes slightly larger than the posterior pair. The upper margin of the lobes, the rhinophoral sheaths, and the tail are markedly crenulated. The anal papilla is elevated and lobated, located on the right side, immediately in front of the posterior lobe. The genital opening is located laterally at a level behind and ventral to the right rhinophore.

The body colour is translucent brown. The internal organs are orangish yellow. The notum is covered with opaque yellowish white, olive green, and blue traces of pigment. These spots are also spread over the oral veil, rhinophoral sheaths, dorsolateral lobes, and tail crest. Some pinkish spots also occur on the margins of the dorsolateral lobes and the branchial sheaths. Scattered brown spots are also found over

the notum, the laterals, and the posterior metapodium. The tubercles are opaque white to light brown in colour. The rhinophoral sheaths are the same colour as the notum with some blue, green, and pink specks. The rhinophoral apex is opaque white. The spotting on the body may be present and more notable in some animals than others. The branchial leaves are almost transparent, some of them speckled with pink. Ventrally, the foot is light grey.

Anatomy: The alimentary canal begins anteriorly with an oval, muscular buccal bulb that has a pair of strong and elongate jaws (Fig. 11A). The masticatory edge of the jaws is expanded into a wing-like flap. Over the edge of this flap is a series of polygonal rodlets that have a series of denticles along their outer edge. These rodlets form a honey-comb pattern over the entire surface of the masticatory edge (Fig. 11B). The radula is broad and lacks rachidian teeth (Fig. 11C). The radula formula is $9–18 \times 13–20.0.13–20$. The specific formula for some of the specimens studied in this paper is shown in Table 1. Most of the cusps bear denticles on both sides, stronger but less numerous on the outer face of each cusp (Fig. 11C, D). The inner and outermost teeth may lack any denticulation or have a very faint one. The teeth gradually increase in size towards the outer margin except for the last two to three outermost teeth, which are smaller (Fig. 11C, D). The salivary glands are elongate and flocculent surrounding the foremost part of the long and narrow oesophagus. We found six to seven thick plates-like elevations on the stomach, which are broadly triangular, with a broad base and a central apex (Fig. 11E). The digestive gland forms three distinct unbranched lobes.

The reproductive system is shown in Figure 5E. The ovotestis consists of two globular gonads lying on the upper side of the posterior digestive gland mass. From each gonad a thin-walled duct passes forwards to the hermaphroditic duct. The distal end of the hermaphroditic duct gets wider and expands into a long and convoluted ampulla that branches into the vas deferens and the oviduct. The oviduct enters the female gland mass. The vas deferens is short, wide, and somewhat folded. It lacks a morphologically well-differentiated prostate gland mass but about half of its length has a different texture and appearance. The penis is flattened and paddle-shaped with a symmetrical apex. It lacks any auxiliary projections and is unarmed (Fig. 11F–H). The vagina is short with an elongate bursa copulatrix.

Natural history: Rare, subtidal. This species, when disturbed, swims by lateral movements of the body. Feeds on *Lytocarpus* hydroids (Hermosillo, 2002a, b; Behrens & Hermosillo, 2005; Hermosillo *et al.*, 2006).

MOLECULAR RESULTS

Data set comparisons and parameters

In this molecular study, 53 specimens were included, representing 30 species and 129 sequences, for a total of 1451 characters (843 constant, 531 variable/parsimony informative, 77 variable/parsimony uninformative). A total of 62 sequences was generated in this study and 67 were extracted from GenBank (Table 2).

Test of saturation and conflict amongst partitions [incongruence length difference (ILD) test]

The saturation analysis showed no saturation for all three genes even when the third codon positions of COI and H3 were analysed independently. The ILD test showed no significant conflicting signals amongst the three genes combined ($P = 1$).

Phylogenetic analysis

The present molecular phylogenetic analysis clearly demonstrates that Scyllaeidae is a monophyletic group [posterior probability (Pp) = 1; bootstrap (bs) = 98] and that this clade is probably sister to Dendronotidae (Pp = 0.92) (Fig. 12). Within Scyllaeidae, *Crosslandia* is sister to *Scyllaea* (Pp = 1; bs = 100) and both are sister taxa to *Notobryon*. Atlantic and Pacific species of *Scyllaea* also appear to be distinct. Within *Notobryon* (Pp = 1; bs = 99), there are several distinct lineages that probably represent distinct species. These lineages correspond to the species included in the morphological investigations outlined in this paper. Thus, the clade containing the three specimens of *Notobryon* from South Africa (*N. thompsoni*) is highly supported but the relationship with the well-supported clade including most of the *Notobryon* specimens from the Philippines and Marshall Island is not clear. Specimens from Mexico also cluster together but with low support. However, this fact could be explained because only the COI sequence was available for the specimen CASIZ 175778. An interesting case is also represented for the Philippine specimen CASIZ 177759 [referred to as *Notobryon* sp. B here for consistency because it was used in a previous paper (Pola & Gosliner, 2010) and can be found in GenBank with that reference]. This specimen is found outside the clade including the remaining specimens from the Indo-Pacific. Initially we thought that this was because of the lack of a COI sequence but the previous situation was found in all single analyses (not shown) and the combined analysis. The systematic relationships of this second sympatric species in the Philippines require additional study. The molecular phylogenetic analysis clearly illuminated the presence of distinct cryptic species that are separated by only minor morphological differences.
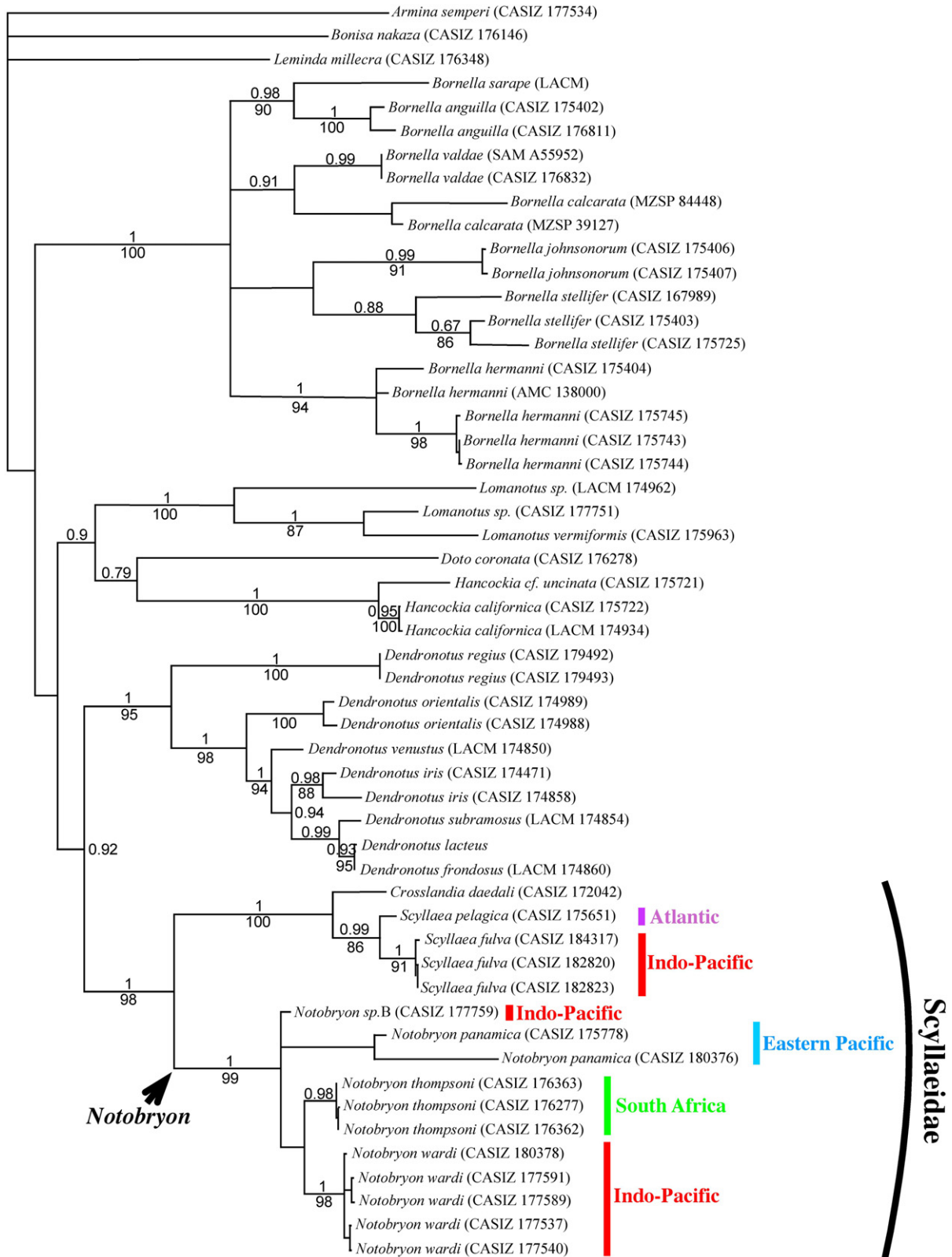
Figure 12. Phylogenetic hypothesis based on combined molecular data (H3+COI+16S) represented by Bayesian inference. Numbers above branches represent posterior probabilities from Bayesian inference (over 95%). Numbers below branches indicate bootstrap values for ML (over 85%).

Table 3. Genetic distances amongst *Notobryon* and *Scyllaea* species for COI, 16S, and H3 markers

	Genetic distances (%)		
Species	COI	16S	H3
Notobryon panamica (EP) vs. *Notobryon wardi* (IP)	23	1.9	0.6
Notobryon panamica (EP) vs. *Notobryon thompsoni* (SA)	22	2	0.0
Notobryon thompsoni (SA) vs. *Notobryon wardi* (IP)	10	2	0.6
Scyllaea pelagica (ATL) vs. *Scyllaea fulva* (IP)	7	2	0.0

ATL, Atlantic; COI, cytochrome *c* oxidase subunit I; EP, Eastern Pacific; H3, histone 3; IP, Indo-Pacific; SA, South Africa.

Genetic distances between specimens of Notobryon
The genetic distances were calculated and compared for the three molecular fragments. The results are shown in Table 3. The rates of genetic divergence for H3 ranged from 0.0 to 0.6%, from 10 to 23% for COI, and 2% for 16S from the mtDNA data set. The highest distance values (23%) for the COI fragment occurred between the specimens of *Notobryon* from the Indo-Pacific/South African versus the eastern Pacific specimens (Table 3). Likewise, the lowest distance values (10%) occurred between the Indo-Pacific and South African specimens.

DISCUSSION

The anatomical studies that we have undertaken clearly indicate that there are morphological differences amongst closely related species, especially in the structure of the male genital system. Moreover, as indicated in the Results section, molecular evidence based on the COI marker shows substantial genetic divergence amongst the Indo-Pacific, South African, and eastern Pacific populations of *Notobryon*. For this reason, we consider individuals from these three regions to belong to different species (Table 3). This conclusion is also supported by our morphological findings, which we discuss below. Similarly, specimens identified as *Scyllaea pelagica* Linnaeus, 1758, from the Atlantic and Pacific Oceans are found in two distinct clades. These taxa have been considered to represent a single circumtropical species by most recent authors. The evidence presented here suggests that these represent two distinct species.

Odhner (1936) described *N. wardi* from Queensland, South Australia. Since then, most *Notobryon* specimens recorded from anywhere around the world except Japan have been identified as *N. wardi*, Odhner, 1936. The specimens studied in the present paper from Australia, Papua New Guinea, Indonesia, Hawaii, the Philippines, and Marshall Islands perfectly match the original description and our re-examination of the type material. The one notable exception in the synopsis and the original description of *Notobryon* was that the author stated that the reproductive system had no ampulla. Nevertheless, he also said that the ampulla could only be indicated as a widening of the duct.

Thompson & Brown (1981) redescribed *N. wardi* based on several specimens collected at Dale Brook, False Bay, South Africa. The external and internal descriptions of the specimens studied in this paper perfectly match with the description given by the authors. The only difference between both descriptions is that Thompson & Brown numbered nine gastric plates, whereas we only counted eight plates in our specimens. The radular formulae given by Thompson & Brown for a 45 mm and a 60 mm specimen were $12 \times 18.0.18$ and $13 \times 20.0.20$, respectively, which fits our given range of $12–19 \times 16–20.0.16–20$. The reproductive system is here described for the first time.

However, our new molecular and morphological findings clearly demonstrate that the specimens found in South Africa correspond to a different species. Externally, the main differences between *N. thompsoni* from South Africa and *N. wardi* are the external coloration and the shape and size of the dorsolateral processes and tail. The dorsolateral processes of *N. thompsoni* from South Africa are smaller, more rounded rather than triangular than those of *N. wardi*, and the two pairs are mostly equally in size, whereas the first pair of *N. wardi* are larger than the second pair. Additionally, the two pairs of dorsolateral processes of *N. thompsoni* from South Africa are much more separated than those of *N. wardi*. In *N. wardi* the two pairs are nearly continuous at their bases leaving a U-shaped space between them. The dorsal crest behind the posterior lobes that ends in a keel-like tail is wider in *N. wardi*, whereas in *N. thompsoni* it is longer and more slender.

Anatomically, both species are very similar but there is an important and consistent difference. The shape of the penis in *N. thompsoni* is digitiform (which fits Thompson & Brown's description), whereas in *N. wardi* the penis is conical and ends in a triangular lobe with two short cups (which fits with the original description by Odhner, 1936 and our re-examination of the type material).

Found in Dale Brook by Thompson & Brown (1981) and by us at the same locality (under boulders in shallow pools), there is no doubt that the specimens

described by the previous authors and the specimens studied in this paper belong to the same species and that *N. thompsoni* is different from *N. wardi* from the Indo-Pacific. In addition, the molecular data obtained in this study (Table 3) support the description of these specimens as a new species of *Notobryon*. Based on the COI marker, the genetic distances between these two species is 10%, whereas the 16S genetic distance between them is 2%.

Baba (1937) described *N. bijecurum* from Japan. He stated that this species differed from *N. wardi* mainly in having unequal sized mantle lobes, the anterior being larger than the posterior pair. In addition to this feature the external and internal description provided by Baba for *N. bijecurum* matches the single specimen available for study in this paper. Moreover, the reproductive system is here described for the first time. It shows a number of important differences from *N. wardi*. The first difference, already noticed by Baba, is the number of hermaphroditic glands forming each group of gonads. In *N. bijecurum*, the anterior group is composed of four hermaphroditic glands whereas the posterior group consists of five hermaphroditic glands. This difference is not visible in *N. wardi*. However, even more important and characteristic of *N. bijecurum*, and what makes this species easily distinguishable from the remaining *Notobryon* species is the presence of a morphologically well-differentiated prostate, the shape of the penis, and the lack of a bursa copulatrix. Although we have only been able to study one specimen, its state of preservation was very good and the reproductive system was well developed and entirely preserved. We have no doubt that the bursa copulatrix does not exist and thus it is not a dissection artefact. Nevertheless, this character should be rechecked as soon as additional material becomes available.

We were not able to include *N. bijecurum* in our molecular phylogeny because the single available specimen was preserved in formalin and was thus useless for genetic analysis. Debelius & Kuiter (2007) recorded *N. bijecurum* for Australia, the Philippines, and Hawaii but based on the geographical distribution it is more likely that these specimens are *N. wardi*.

In the Remarks section of the description of *N. clavigerum*, Baba (1937) stated that this species is easily distinguishable from *N. wardi* and *N. bijecurum* based on the previously detailed features. Nevertheless, the internal features do not seem to show any particular differences from the other two species, with the possible exception of the number of gastric plates and hermaphroditic glands. In the present study, we were not able to locate the type material or any identified specimen of *N. clavigerum* Baba, 1937. However, based on the features of the incomplete original description, we decided to con-

sider *N. clavigerum* as a valid species until further examination of fresh material allows us to confirm the identity of this species. Figure 6E and F show specimens identified as *N. clavigerum*. Pictures identified as *N. clavigerum* can also be found in Suzuki (2000), Nakano (2004), Koh (2006), and Debelius & Kuiter (2007).

Specimens of *Notobryon panamica* from Costa Rica, Mexico, and Panama have previously been identified as *N. wardi* (Behrens & Hermosillo, 2005; Hermosillo *et al*., 2006). Valdés *et al*. (2006) provisionally identified specimens recorded from Honduras, Santa Lucía, Virgin Islands, St. Vincent, and the Grenadines as *N.* cf. *wardi*. However, the authors stated that those specimens could be undescribed and probably more than one species was illustrated in that field guide.

Notobryon panamica from the eastern Pacific is externally very similar to *N. wardi* and *N. thompsoni*, but several characteristics are distinguishing. The dorsal processes of the eastern Pacific and Indo-Pacific specimens are larger than the ones from South Africa. However, in *N. wardi*, both pairs of processes are nearly continuous at their bases leaving U-shaped spaces between them. In *N. thompsoni*, both pairs are quite separated whereas in *N. panamica* the distance between pairs is intermediate between the other two species. Although the coloration varies in different specimens, there are also some consistent differences. For instance, specimens of *N. thompsoni* are pale brown or reddish brown, whereas in *N. wardi* and *N. panamica* from the eastern Pacific, the coloration is mostly translucent yellow or brownish to orange. Moreover, the lamellae of the rhinophores are pale brown with iridescent green or blue in *N. thompsoni*, whereas the lamellae and their tips are translucent in *N. wardi* and *N. panamica*.

Internally, the three species are very similar, but again the most important and consistent difference is the shape of the penis. The penis of *N. panamica* is flattened and paddle-shaped with a symmetrical apex. It lacks any auxiliary projections on the penis that are found in *N. wardi*. The penis of *N. thompsoni* is symmetrical but is conical rather than flattened. The number of stomach plates found in *N. panamica* (seven plates) also distinguishes it from *N. wardi* and *N. thompsoni* (eight to nine).

The molecular data obtained in this study also support the description of these specimens as new species of *Notobryon*. Based on the COI marker, the genetic distances from *N. wardi* is 23%, whereas the 16S genetic distance between them is almost 2%. Furthermore, the genetic distances based on COI and 16S markers between South African and Eastern Pacific specimens are 22 and 2%, respectively (Table 3).

Genetic divergence amongst species has been evaluated by several authors. Johns & Avise (1998) stated that 2% was enough to separate vertebrate species using the molecular marker cytochrome *b* whereas Hebert *et al*. (2004) suggested a limit of 2.7% for birds. For invertebrates, Hebert *et al*. (2003) proposed a 3% interspecific limit for insects and Williams, Reid & Littlewood (2003) showed the existence of unequal evolutionary rates in Littorininae. Davison, Blackie & Scothern (2009) showed great variation for the COI sequence in terrestrial molluscs and thus, in that study, it was suggested that the limit of genetic divergence for molluscs species should be 4% (with an estimated identification error between 32 and 44%), whereas Malaquias & Reid (2009) established a cut-off value of 10% between sister species of the opisthobranch genus *Bulla*. Thus, it is clear that there is no universal agreement for using a single value for discriminating amongst species but the combined information based on morphological characters and genetic distances, as well as biogeographical information, can help us to elucidate unequivocally different species.

Therefore, the morphological and molecular data obtained in this study support the description of two new species of *Notobryon*. Based on the addition of these two new species in this genus (*N. thompsoni* and *N. panamica*), we propose a new diagnosis of the genus *Notobryon*. We add to the original diagnosis by Odhner, 1936 some other features that we consider important, such as the range in the number of stomach plates, the radular formula, the fact that the ampulla and prostate can be very differentiated or not, the presence of a well-defined bursa copulatrix or its absence, and the shape of the penis, bearing or lacking short cusps.

This study also demonstrates that the Atlantic and Indo-Pacific populations of *Scyllaea pelagica* form two distinct lineages in our phylogenetic analysis (Table 3). Traditionally, *S. pelagica* has been regarded as a single, circumtropical species (Gosliner *et al*., 2008). The present work supports the separation of the Indo-Pacific taxon from *S. pelagica*, based on Atlantic material. Whereas the Atlantic and Indo-Pacific specimens differ by 7% in the COI gene, there is little consensus as to how much difference constitutes enough variation to consider these taxa as distinct species (see above). If one looks at intraspecific differences of scyllaeid nudibranchs examined in the present study, their differences are only 0.4–0.5% different for COI, at least an order of magnitude lower than what is observed for the interspecific differences. Similarly, the differences between species of *Notobryon* for the 16S gene is around 2% and the same level of divergence is found for this gene between the Atlantic and Pacific

Scyllaea. Based on what has been found for other scyllaeids studied here, we are confident that the genetic distances observed here for the Atlantic and Pacific *Scyllaea* are consistent with considering them as distinct species. Amongst the many synonyms of *S. pelagica*, *Scyllaea fulva* Quoy& Gaimard, 1824, is the oldest name for specimens documented from the Indo-Pacific, having been described from New Guinea. Thus, *S. fulva* should be considered as the valid name for this species in the Indo-Pacific. Yokes (2002) and Petrusek (2002) recorded *S. pelagica* from Turkey and the Adriatic Sea, respectively. It is probable that these records actually represent the introduction of *S. fulva* into the Mediterranean via the Suez Canal. Certainly, it would be advisable to have future studies compare specimens of *Scyllaea* from a broader geographical range to test further if there are two distinct species of *Scyllaea* and to aid in understanding the origins of Mediterranean records of this genus.

Combining morphological and molecular phylogenetics is an excellent way to clarify the evolution of complex groups such as the one found in *Notobryon* and *Scyllaea*. The molecular data clearly demonstrated the presence of distinct lineages and prompted more careful morphological study that revealed differences in these cryptic species.

REVISED DIAGNOSIS OF *NOTOBRYON*

Body compressed, bearing a low keel at the tail and two pairs of triangular dorsolateral lobes, nearly contiguous at their bases. Each dorsolateral lobe bears several branched 'gills' on their surfaces. Rhinophoral sheaths elevated, margin running up as a continuous low crest. Rhinophore clubs laminated by several leaflets. Anus lateral between the two right lobes. Nephroproct next to the anus. Radula with broad naked rachis, ranging from $9–18 \times 14–24.0.14–24$, inner laterals with short cusp, denticulate on inner margin, outer laterals with gradually stronger denticulation in outer margin; each tooth with a process fitting into an excavation of the adjacent tooth. Salivary glands broadly triangular, flocculent. Stomach with a pyloric girdle of six to nine thick plates. Digestive gland with three distinct unbranched lobes. Ovotestis with two large and globular gonads, hermaphroditic duct with a well-differentiated ampulla or a poorly defined one, dividing into the vas deferens and oviduct, which expands into a globular mass; vagina normally with an elongate bursa copulatrix or without a bursa copulatrix (in *N. bijecurum*); with or without a well-developed prostate; penis short with conical or flattened edge ending in a single apex or with two short cusps.

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