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A new species of deep-sea *Dendronotus* Alder & Hancock (Mollusca : Nudibranchia) from California, with an expanded phylogeny of the genus

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Abstract. *Dendronotus patricki*, sp. nov. is a new species collected from a whalefall in the Monterey Canyon, California. This new species is characterised by having a small number of dorsal appendages compared with similarly sized species of *Dendronotus* Alder & Hancock, 1845. Anatomically, *D. patricki*, sp. nov. has a small prostate with just a few alveoli, a very small seminal receptacle situated near the distal end of the vagina, and a relatively short and small ampulla. The rachidian radular teeth of *D. patricki*, sp. nov. are unique among *Dendronotus* as they have a well differentiated, conical cusp with very small denticles on either side, but most denticles are located on the sides of the teeth, rather than on the sides of the cusp. *Dendronotus patricki*, sp. nov., is genetically distinct from other species of *Dendronotus* for which sequence data are available. A phylogenetic analysis of *Dendronotus* based on COI, 16S, and H3 sequence data reveals that *D. patricki*, sp. nov. forms a polytomy with *Dendronotus orientalis* (Baba, 1932) and a clade of the shallow temperate and cold water species. The tropical Indo-Pacific species *D. regius* Pola & Stout, 2008 is the sister group to all other *Dendronotus* species.

Additional keywords: COI, Dendronotina, Dendronotoidea, 16S, H3, taxonomy, whalefalls.

Introduction

Although there is a relatively diverse deep-sea fauna of opisthobranch molluscs, with some species reaching 4435 m depth (Bergh 1884), these molluscs are poorly represented in chemosynthetic environments. Only one species is known from hydrothermal vents, the nudibranch *Dendronotus comteti* Valdés & Bouchet, 1998, described from the Lucky Strike area in the Mid-Atlantic Ridge at 1685 m depth. Valdés and Bouchet (1998) suggested that this species most likely occurs at some distance from the core of the vent, in areas where lower temperatures and reduced water toxicity allow their hydroid prey to become abundant. The unique, less toxic chemical composition of the Lucky Strike site (Langmuir *et al*. 1997) may explain the presence of a shell-less organism in otherwise highly toxic environments.

The second species of opisthobranch found in chemosynthetic environments is *Marionia tedi* Ev. Marcus, 1983, originally described based on several specimens collected from the southern Gulf of Mexico, the Straits of Florida, and the south-eastern Caribbean Sea at depths between 60 and 348 m (Marcus 1983). This species was subsequently reported from hydrocarbon cold seeps in the Mississippi Canyon and the Vioska Knoll, Gulf of Mexico, at 540–624 m depths (Valdés 2006). Whereas *D. comteti* appears to be endemic to hydrothermal vents, and has never been reported from any other deep-sea environment, *M. tedi* is most likely an occasional visitor to cold seeps. The latter was previously recorded from five other localities (Marcus 1983), none of which appear to correspond with known cold seep environments. Hydrocarbon cold seeps in the Gulf of Mexico often produce carbonate substrates by authigenic precipitation that generates hard bottoms (Roberts *et al*. 2007) in which the gorgonian prey of *M. tedi* can settle, favouring colonisation by the nudibranchs (Valdés 2006).

This paper describes a third nudibranch species from chemosynthetic environments, a new *Dendronotus* species collected at a whalefall in the Monterey Canyon, California.

Materials and methods

Collection and deposition of specimens

Two specimens were collected during whalefall cruises led by Bob Vrijenhoek at the Monterey Bay Research Institute (MBARI). Both specimens were collected from the area housing an experimentally implanted whale carcass known as 'Patrick' in the Monterey Canyon, at ~1820 m depth (description in Braby *et al*. 2007) (Fig. 1). The specimens

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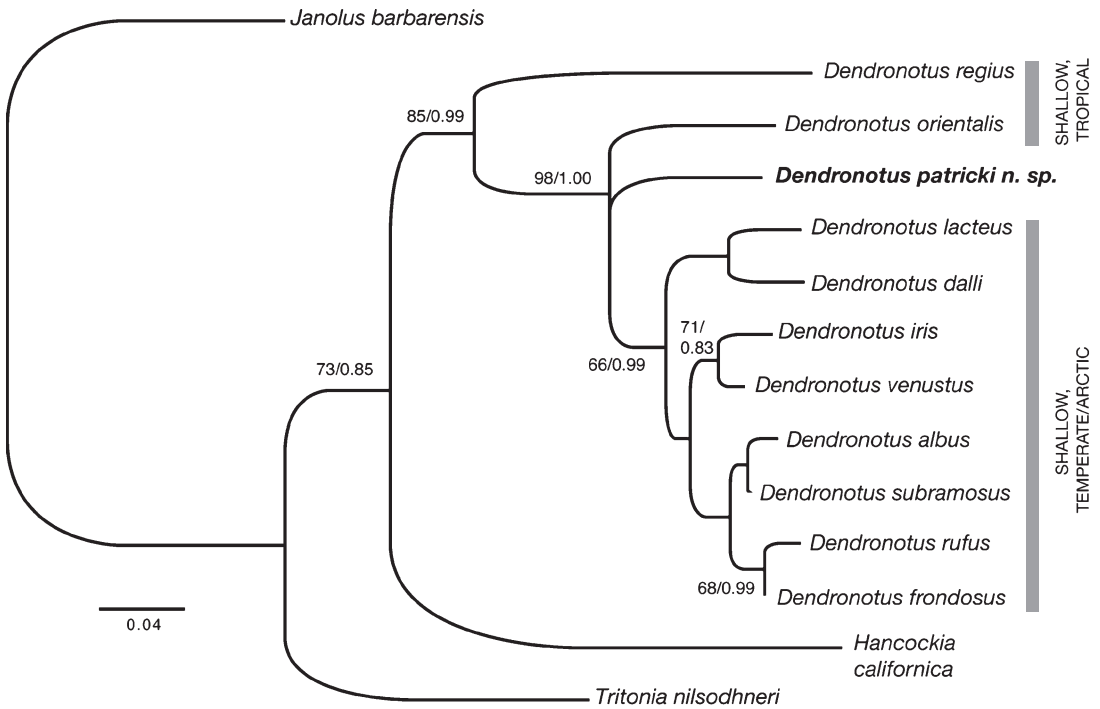

Fig. 1. Maximum likelihood tree of concatenated dataset (COI, 16S, H3). Support values for likelihood and Bayesian approaches are shown only for nodes with bootstrap values over 70 or posterior probability values greater than 0.97.

examined are deposited in the Benthic Invertebrate Collections of the Scripps Institution of Oceanography (SIO-BIC). Other specimens sequenced for the phylogenetic analyses are deposited in the Natural History Museum of Los Angeles County (LACM) and the California Academy of Sciences Invertebrate Zoology and Geology collections (CASIZ). Additional video records that expand the distribution are archived at MBARI, Monterey, California.

Morphological examination

The paratype was dissected and reproductive system features were examined and drawn using a dissecting microscope, Nikon SMZ-100 (www.nikon.com), with a camera lucida attachment. The buccal mass was removed and dissolved in 10% sodium hydroxide until the radula and jaw were isolated from the surrounding tissue. The radula and jaw were then rinsed in water, dried, mounted and sputter coated for examination by scanning electron microscopy (SEM), Hitachi S-3000N (www.hitachi.com), at the Natural History Museum of Los Angeles County. A sample of the intestinal contents of the paratype was removed and examined under an Olympus CX 31 microscope (www.olympus.com).

Molecular data

Sequences from the paratype of *D. patricki*, sp. nov. were generated for partial 18S rDNA, 16S and COI sequences and deposited in GenBank. To place the new species in a phylogenetic framework, we used previously published sequences of Cytochrome Oxidase I (COI), 16S rRNA (16S) and Histone H3 (H3) from GenBank (Table 1). Sequences of 18S rDNA were not used in the analysis due to the lack of data for most *Dendronotus* species. A set of new H3 sequences were also generated for several species to supplement H3 sequence data available in GenBank.

DNA extraction and sequencing

Approximately 3 mm of tissue from the foot of each individual was chopped into fine pieces using a sterile razor and forceps. DNA was extracted using either a DNEasy Extraction Kit (Qiagen, Valencia, CA, USA) or Chelex-100 Resin (Bio-Rad, Hercules, CA). With DNEasy, protocols provided by the manufacturer were followed, with the exception of increasing the 56°C incubation period (tissue lysis) from 1–3 h to 12–18 h (overnight incubation). For Chelex extractions, tissue was placed in 200 µl of 10% Chelex in 1X TE solution, incubated at 56°C in a water bath for 20 min, and vortexed for 10 s. Samples were incubated in a dry heat block at 100°C for 8 min and again vortexed for 10 s. After centrifugation for 3 min at 15 000×*g*, the supernatant was ready for polymerase chain reaction (PCR). Primers used to amplify and sequence the H3 nuclear gene (H3AF 5′-ATGGCTCGTACCAAGCAGACGGC-3′, H3 AR 5′-ATATCCTTGGGCATGATGGTGAC-3′) were developed by Colgan *et al*. (1998). The 16S primers (16S ar-L 5′-CGCC TGTTTATCAAAAACAT-3′, 16S br-H 5′-CCGGTCTGAACT CAGATCACGT-3′) were developed by Palumbi *et al*. (1991). Partial 18S and COI sequences were also derived for the new species using primers (18S 1F 5′-TACCTGGTTGATCCTGC CAGTAG-3′, 18S 5R 5′-CTTGGCAAATGCTTTCGC-3′) from Giribet *et al.* (1996) and (LCO1490 5′-GGTCAACAAATCA

Table 1. List of species included in phylogenetic analyses, including locality, museum voucher numbers and GenBank accession numbers

Accession numbers of new sequences generated for this study are highlighted in bold

Species	Locality	Voucher	16S	COI	H3
Dendronotus albus	California	LACM174845	GU339185	–	**HQ267088**
D. frondosus	Scotland	LACM174860	GU339187	AJ223261	**HQ267089**
D. dalli	USA, Atlantic[A]	–	AF249252	AF249800	–
D. iris	Washington	LACM174471	GU339189	–	**HQ267090**
D. lacteus	Scotland	LACM174877	HM162538	HM162710	–
D. orientalis	China	CASIZ174989	HM162628	–	HM162534
D. regius	Philippines	CASIZ179492	HM162629	HM162708	HM162535
D. rufus	Alaska	LACM174861	GU339191	–	**HQ267091**
D. patricki	California	SIO-BIC M12134	**HQ225829**	**HQ225828**	–
D. subramosus	Washington	LACM174855	GU339196	–	**HQ267092**
D. venustus	California	LACM174852	GU339200	HM162709	**HQ267093**
Hancockia californica	Costa Rica	CASIZ175722	HM162621	HM162702	HM162527
Tritonia nilsodhneri	South Africa	CASIZ176219	HM162641	HM162716	HM162548
Janolus barbarensis	California	CASIZ176833	HM162671	HM162747	HM162580

[A]Given as locality in published paper, but does not correspond with known distribution of species.

TAAAGATATTGG-3′, HCO2198, 5′-TAAACTTCAGGGTG ACCAAAAAATCA-3′) from Folmer *et al.* (1994).

Each 50 µl PCR reaction consisted of 50 mM KCl, 10 mM Tris, 2 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 mM of each primer, 0.25 µl Taq polymerase, and 2 µl DNA template. Conditions for PCR for both genes included a 2 min initial denaturation at 94°C, followed by 30 cycles of 94°C for 30 s (denaturation), 50°C for 30 s (annealing), 1 min at 72°C (extension), and ended with one final extension at 72°C for 7 min. Polymerase chain reaction products were purified using Montáge life science kit from Millipore (www.millipore.com) and sequenced at the City of Hope DNA sequencing laboratory in Duarte, CA. Geneious Pro 4.8.3 (Drummond *et al.* 2009) was used to obtain consensus sequences from assembled forward and reverse sequences and then to align them.

Analyses

Sequences for each gene were assembled and edited using Geneious Pro 4.8.3. Coding genes were aligned by eye and checked for reading frame shifts. The 16S data were aligned using MAAFT (Multiple Alignment using Fast Fourier Transform) implementing the Q-INS-I strategy that considers secondary structure. Alignments resulted in 657 bp of COI, 459 bp of 16S and 327 bp of H3 data and are available through TreeBASE. The levels of saturation for coding genes were assessed using the substitution saturation test developed by Xia *et al.* (2003) and Xia and Lemey (2009) implemented in the software package DAMBE (Xia and Xie 2001).

Outgroup selection for phylogenetic analyses considered Stout *et al.* (2010) and Pola and Gosliner (2010), and representatives of *Hancockia*, *Tritonia* and *Janolus* were chosen, rooting trees with the latter. Analyses were conducted for individual gene partitions, as well as the concatenated dataset. Maximum likelihood analyses were carried out with RAxML ver. 7.2.6 (Stamatakis 2006) under a GTR+Γ model (see Yang 2006), with joint branch length parameter estimation and assessment using 1000 rapid bootstrap replicates as above (Stamatakis *et al.* 2008). Bayesian analyses were executed in MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001), partitioned by gene (unlinked), with two runs of six chains for 5 million generations, sampling every 1000 generations. Effective sample sizes and convergence of runs were assessed using Tracer 1.4.1 (Rambaut and Drummond 2007) and the default 25% burn-in (500 000) was applied before constructing majority-rule consensus trees.

To estimate maximum likelihood corrected distances, the Akaike information criterion (Akaike 1974) was applied in jModelTest (Posada 2008) to determine the best-fit model of evolution. Both uncorrected and corrected ‘p’ distance matrices of pairwise distances between 16S and COI sequences (Tables 2 and 3) were generated using PAUP* (Swofford 2002).

Results

The concatenated maximum likelihood tree (Fig. 1) was relatively well resolved, although most branches were poorly supported in the bootstrap analyses. The Bayesian analysis resulted in an identical topology and is not shown; posterior probability values have been transcribed onto the likelihood tree for comparison. The tropical Indo-Pacific species *D. regius* is the sister to the rest of *Dendronotus*. The relationship between the tropical Indo-Pacific *D. orientalis*, the new deep-water *D. patricki* from California, and a clade including all other temperate and cold water species is unresolved. Either *D. orientalis* or *D. patricki* is sister to the temperate and cold water clade. Within the temperate and cold water clade only two groups of sister species pairs received any support: the north-eastern Pacific *D. iris* Cooper, 1863 + *D. venustus* MacFarland, 1966 and *D. rufus* O’Donoghue, 1921 + *D. frondosus* (Ascanius, 1774). From these phylogenetic analyses, it is clear that the new species examined here is genetically distinct from other species studied, and is formally described below. Tables 2 and 3 show the genetic distances between taxa for COI and 16S respectively. The saturation

Table 2. Uncorrected (lower diagonal) and maximum likelihood corrected distances (upper diagonal) under the TPM3uf+I+G model among species of *Dendronotus* for which cytochrome oxidase I data are available (as well as the three outgroup taxa)

	1	2	3	4	5	6	7	8	9
Dendronotus frondosus	–	0.15025	0.15581	0.08741	0.21466	0.16038	0.24161	0.20739	0.28843
D. dalli	0.13379	–	0.09850	0.14350	0.19101	0.19621	0.22534	0.21431	0.28186
D. lacteus	0.13812	0.09105	–	0.13707	0.19521	0.19690	0.23724	0.19878	0.29655
D. venustus	0.08144	0.12841	0.12310	–	0.19536	0.15509	0.21447	0.19152	0.27841
D. regius	0.18413	0.16734	0.17021	0.17021	–	0.19796	0.21750	0.22101	0.24393
D. patricki	0.14256	0.16837	0.16869	0.13830	0.17173	–	0.22963	0.19464	0.26727
Hancockia californica	0.20277	0.19232	0.20061	0.18389	0.18541	0.19453	–	0.19892	0.26770
Tritonia nilsodhneri	0.17898	0.18504	0.17325	0.16717	0.18845	0.17021	0.17325	–	0.23888
Janolus barbarensis	0.23818	0.23377	0.24319	0.23172	0.20730	0.22333	0.22365	0.20378	–

Table 3. Uncorrected (lower diagonal) and maximum likelihood corrected distances (upper diagonal) under the GTR+I+G model among species of *Dendronotus* for which 16S rDNA data are available (as well as the three outgroup taxa)

	1	2	3	4	5	6	7	8	9	10	11	12	13
Dendronotus frondosus	–	0.01676	0.01155	0.02090	0.01151	0.03280	0.02575	0.03537	0.06507	0.13154	0.15528	0.15758	0.15738
D. dalli	0.01655	–	0.01674	0.02429	0.01449	0.04180	0.02443	0.03432	0.05235	0.12299	0.14231	0.14157	0.14905
D. rufus	0.01144	0.01654	–	0.02797	0.01853	0.04483	0.03773	0.03770	0.06746	0.12823	0.16352	0.16303	0.16309
D. albus	0.02055	0.02387	0.02736	–	0.00917	0.03984	0.02795	0.03997	0.07238	0.11965	0.14870	0.15429	0.15921
D. subramosus	0.01140	0.01435	0.01826	0.00911	–	0.03733	0.02800	0.03996	0.06963	0.12788	0.15967	0.15963	0.15860
D. patricki	0.03201	0.04057	0.04333	0.03872	0.03636	–	0.03503	0.04214	0.06443	0.12475	0.14234	0.15988	0.16151
D. venustus	0.02515	0.02390	0.03649	0.02733	0.02734	0.03416	–	0.02083	0.05232	0.11704	0.14874	0.15981	0.15630
D. iris	0.03429	0.03342	0.03653	0.03870	0.03864	0.04091	0.02050	–	0.06699	0.12770	0.14545	0.16246	0.16808
D. orientalis	0.06172	0.05027	0.06394	0.06840	0.06596	0.06141	0.05016	0.06369	–	0.13052	0.16249	0.17126	0.17363
D. regius	0.11891	0.11215	0.11642	0.10932	0.11613	0.11388	0.10705	0.11614	0.11825	–	0.15998	0.20810	0.16471
Tritonia nilsodhneri	0.13932	0.12896	0.14584	0.13413	0.14297	0.12895	0.13428	0.13142	0.14502	0.14280	–	0.18831	0.14787
Hancockia californica	0.14168	0.12873	0.14604	0.13888	0.14328	0.14358	0.14351	0.14559	0.15230	0.18026	0.16541	–	0.18640
Janolus barbarensis	0.14133	0.13486	0.14591	0.14297	0.14248	0.14477	0.14060	0.15000	0.15415	0.14751	0.13390	0.16389	–

analysis showed insignificant levels of saturation for all three genes (COI: Iss < Iss.c, $P = 0.008$; 16S: Iss < Iss.c, $P = 0.000$; H3: Iss < Iss.c, $P = 0.000$) even when the third codon positions of COI and H3 were analysed independently.

Taxonomy

Dendronotus patricki, sp. nov.

(Figs 2–4)

Material examined

Holotype. Dive ROV Doc Ricketts 12, Whalefall 'Patrick' area (36°42′30.3120″N, 122°06′18.7200″W), Monterey Canyon, 1819 m depth, 13.iii.2009, 18 mm preserved length, leg. N. Wilson (SIO-BIC M12134).

Paratype. Dive ROV Tiburon 1163, Whalefall 'Patrick' area (36°42′29.4480″N, 122°06′19.0800″W), Monterey Canyon, 1822 m depth, 20.xii.2007, 25 mm preserved length, leg. N. Wilson, dissected (SIO-BIC M12133).

Additional material examined. Video records: dive ROV Tiburon 652, grey whalefall, Santa Cruz Basin (33°29′40.0200″N, 119°22′04.0800″W), 1676 m depth, 1.iii.2009, leg. L. Lundsten; dive ROV Ventana 359, canyon head (36°44′15.1800″N, 122°01′53.0400″W), Monterey Canyon, 898 m depth, 17.i.1992, leg. L. Lundsten; dive ROV Doc Ricketts 90, canyon floor (36°38′31.1280″N, 122°07′27.8400″W), Monterey Canyon, 2161 m depth, 23.x.2009, leg. L. Lundsten.

Etymology

This species is named after Commander Patrick Rouse, who spent much of his life patrolling the seas in the service of his country. The whalefall where the specimens were collected was also named after Pat.

External morphology

Live animals uniformly reddish brown, with digestive gland visible through semi-translucent body as a purple mass (Fig. 2). Buccal mass and hermaphroditic gland also visible as pale yellowish masses on anterior and posterior ends of the body, respectively. Dorsal appendages, velar appendages, and rhinophoral sheath papillae generally reddish brown with distal areas semi-translucent or devoid of pigment. Apices of dorsal appendage branches, velar appendages, and rhinophoral sheath papillae opaque white.

Dorsal appendages with fan-shaped branching pattern (Robilliard 1970) arranged in three (holotype) or four (paratype) pairs. Anterior pair located posterior to pericardium and much larger and ramified than the rest. Last two pairs small, with just a few branches. Velum with six highly branched appendages. Rhinophoral sheaths with long stalk and four tentacular, generally long, distal appendages. Distal

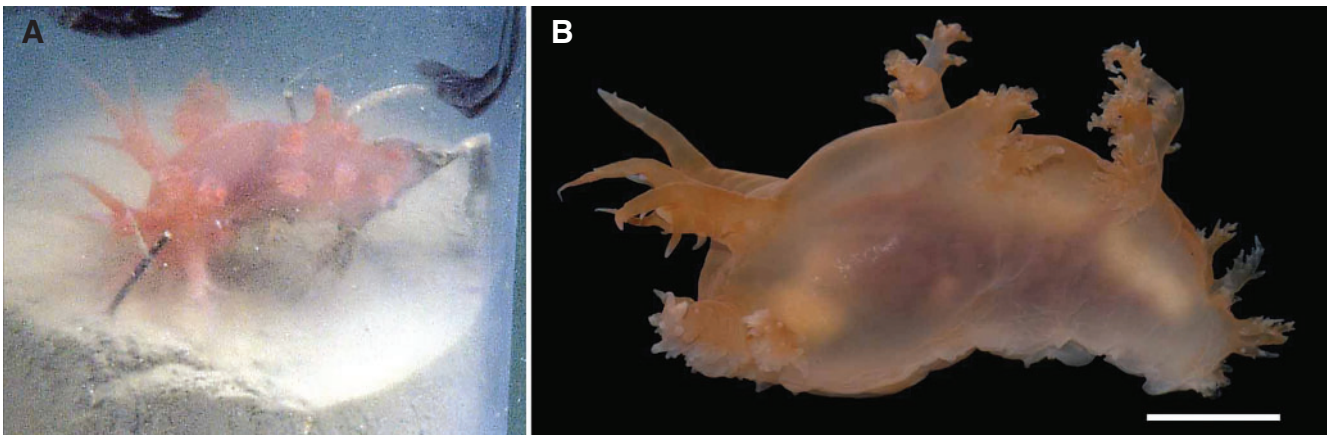

Fig. 2. *Dendronotus patricki*, sp. nov. (*A*) Living holotype in push core at time of collection (SIO-BIC M12134); (*B*) living paratype (SIO-BIC M12133).

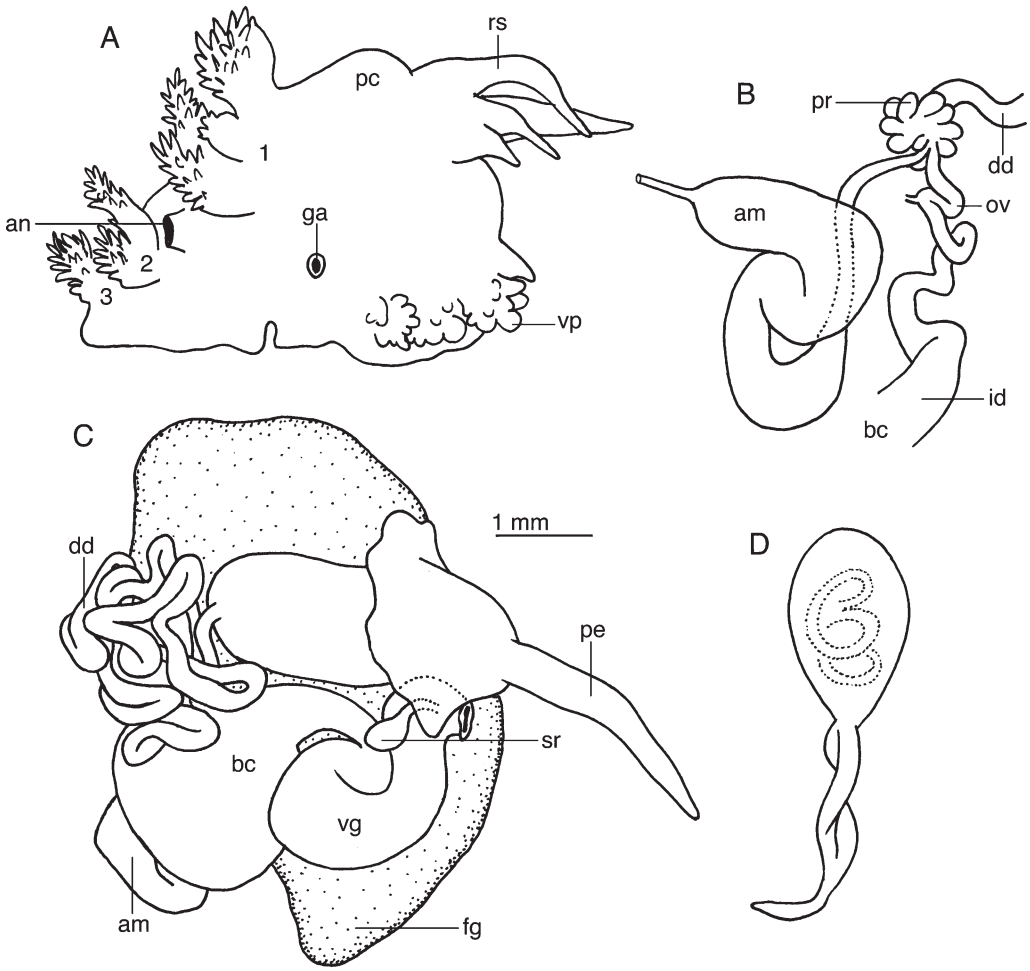

Fig. 3. *Dendronotus patricki*, sp. nov., drawings of the external morphology, anatomy and some intestine contents. (*A*) Lateral view of the preserved holotype (SIO-BIC M12134), numbers represent each pair of dorsal appendages; (*B*) detail of some reproductive organs visible after removal of the deferent duct (SIO-BIC M12133); (*C*) general view of the reproductive system of the paratype (SIO-BIC M12133); (*D*) possible nematocyst found among intestine contents. Abbreviations: am, ampula; an, anus; bc, bursa copulatrix; dd, deferent duct; fg, female gland complex; ga, genital aperture; id, insemination duct; ov, oviduct; pe, penis; pc, pericardium; pr, prostate; rs, rhinophoral sheath; sr, seminal receptacle; vg, vagina; vp, velar papilla.

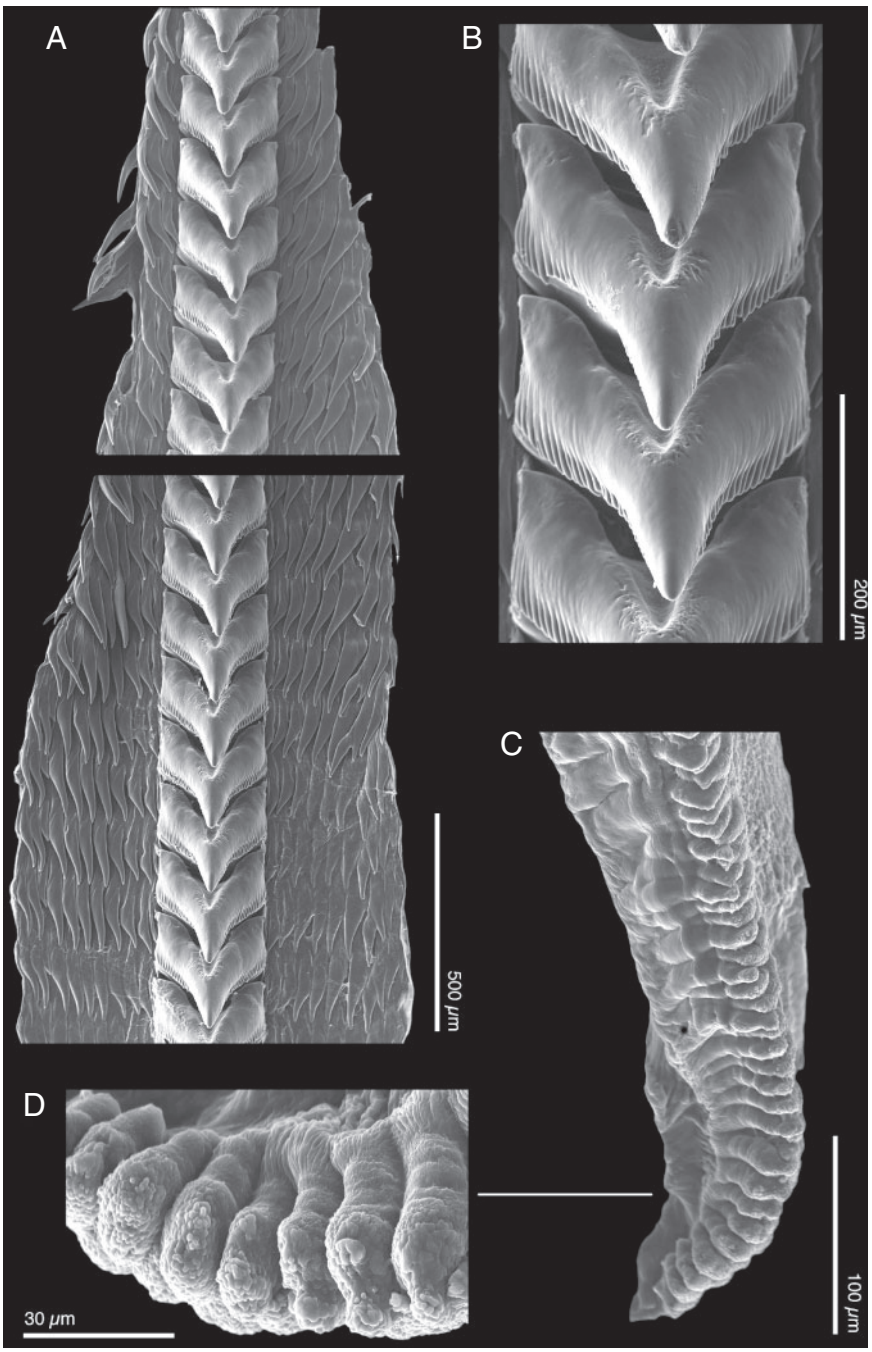

Fig. 4. *Dendronotus patricki*, sp. nov., scanning electron micrographs of the radula and jaws of the paratype (SIO-BIC M12133). (*A*) Several rows of radular teeth; (*B*) detail of the rachidian teeth; (*C*) jaw masticatory border; (*D*) detail of jaw rodlets.

appendages generally unbranched, but longest appendages with a few short, simple branches.

Reproductive opening on right side of body, below pericardium, anterior to first pair of dorsal appendages (Fig. 3*A*). Anus dorso-lateral, located between first and second pairs of dorsal appendages.

Internal anatomy

Reproductive system with ampulla merging with oviduct and connecting into the prostate (Fig. 3*B*). Oviduct continues distally, connecting into female gland complex through a short duct and insemination duct. Prostate small, composed of several glandular

alveoli and connected to very long and convoluted deferent duct. Deferent duct expands distally into a wide, muscular portion (Fig. 3*C*). Penis long, tentacular, lacking armature. Vagina opening posterior to penis, with a small seminal receptacle connecting near its proximal end. Vagina very long, folded, opening into an irregular bursa copulatrix. From the bursa copulatrix a relatively long insemination duct emerges, connecting into female gland complex and oviduct (Fig. 3*B*).

Radular formula 31 × 3–8.1.8–3 (paratype). Rachidian teeth large, broad, with strong central cusp and ~20 small denticles on each side (Fig. 4*A*, *B*). Lateral teeth elongate, with a sharp cusp lacking denticles (Fig. 4*A*); some with two cusps. The number of lateral teeth varies from 3 in anterior radular rows to ~8 on the most posterior. Jaws large, with masticatory border having a single row of rodlets (Fig. 4*C*). Rodlets are broad and irregular (Fig. 4*D*).

Biology

Intestine materials recovered from the paratype show an amorphous mixture of brown to black materials. Among the debris there are several structures that appear to be nematocysts (Fig. 3*D*). Collected specimens were crawling on the sediment surface close to the whalefall. Specimen records were collected from a range of oxygen concentrations (0.26–1.656 mL L^{-1} O_2) spanning the oxygen minimum zone, and with temperatures ranging from 1.962 to 4.223°C.

Distribution

Known from specimens and video records from Monterey Canyon, and from video records from the Santa Cruz Basin off southern California (Fig. 5), spanning a depth range of 898–2161 m.

Remarks

Dendronotus patricki, sp. nov. is consistently different from other species of *Dendronotus*. The rachidian teeth of *D. patricki*, sp. nov. have a well differentiated, conical cusp with very small denticles on either side, but most denticles (and the largest) are arranged on the sides of the teeth, rather on the sides of the cusp. This rachidian tooth morphology is very different from that of other species of *Dendronotus*. Most species, including *D. frondosus*, *D. dalli* Bergh, 1879, *D. rufus*, *D. gracilis* Baba, 1949, *D. subramosus* MacFarland, 1966, *D. albus* MacFarland, 1966, *D. albopunctatus* Robilliard, 1972, *D. regius* Pola & Stout, 2008, *D. noahi* Pola & Stout, 2008, *D. lacteus* (Thompson, 1840), and *D. comteti* do not have a well differentiated cusp, but the teeth look triangular (Robilliard 1970, 1972; Valdés and Bouchet 1998; Pola and Stout 2008). Only *D. iris*, *D. robustus* Verrill, 1870 and *D. albopunctatus* have well differentiated conical cusps. However, the rachidian radular teeth of *D. iris* are very different from those of *D. patricki*, sp. nov.; they are much narrower, have fewer and larger teeth, and the base of the teeth are narrower than the apical region (see Robilliard 1970; Pola and Stout 2008). The rachidian teeth of *D. robustus* are more similar to those of *D. patricki*, sp. nov., however, they are easily differentiated because they are narrower, the cusp is less robust, and the denticles are comparatively larger, with some space between them (see Pola and Stout 2008), whereas in *D. patricki*, sp. nov. the denticles are more closely arranged, with no gaps. Finally, the rachidian teeth of *D. albopunctatus* are also different to those of *D. patricki*, sp. nov.

Fig. 5. Known distribution of *Dendronotus patricki*, sp. nov.

as the denticles are much longer in the former and the cusp is not as clearly differentiated as in the latter (Robilliard 1972; Pola and Stout 2008).

The lateral radular teeth of *D. patricki*, sp. nov. have no denticles. Although this condition seems to be variable, it has only been found in *D. iris*, *D. robustus*, and *D. lacteus* (Pola and Stout 2008). As mentioned above, *D. iris* and *D. robustus* differ from *D. patricki*, sp. nov. in the morphology of the rachidian teeth. This is also the case for *D. lacteus*, which has rachidian teeth that are short and broad, wider than long, and lacking a differentiated cusp (Pola and Stout 2008).

The reproductive system of *D. patricki*, sp. nov. is characterised by having a very small prostate with just a few alveoli, a very small seminal receptacle situated near the distal end of the vagina, and a relatively short and small ampulla. Of the species for which anatomical information is available, only *D. subramosus*, *D. dalli*, and *D. diversicolor* Robilliard, 1970 have a similar configuration (Robilliard 1970, 1972; Pola and Stout 2008). Both *D. dalli*, and *D. diversicolor* have a short, wide penis (Robilliard 1970), very different from the very long, tentacular penis of *D. patricki*, sp. nov. *Dendronotus subramosus* also has a long, tentacular penis, but the bursa copulatrix is comparatively much smaller than that of *D. patricki*, sp. nov., and the prostate much larger (Robilliard 1970). For comparison, the ampulla is about four times as large as the bursa copulatrix in *D. subramosus* and about the same size in *D. patricki*, sp. nov.

Externally, *D. patricki*, sp. nov. is characterised by having a comparatively small number of dorsal appendages. The paratype, which is ~25 mm long preserved, has only four pairs of dorsal appendages.

Discussion

The systematics of species of *Dendronotus* was for the first time comprehensively reviewed by Robilliard (1970, 1972), who recognised 11 valid species, 7 of them present in the Pacific north-west. Later, Thollesson (1998) recognised *D. lacteus* as a distinct species from *D. frondosus* based on allozyme electrophoresis studies, Valdés and Bouchet (1998) described *D. comteti* from a hydrothermal vent in the Mid-Atlantic Ridge, and Pola and Stout (2008) described *D. regius* and *D. noahi*, two new species from the tropical Indo-Pacific. Recent morphological studies suggest *Pseudobornella* is more closely related to Dendronotidae (Pola *et al*. 2009). After confirmation with molecular data, Pola and Gosliner (2010) formally transferred *Pseudobornella orientalis* to *Dendronotus orientalis*.

Stout *et al*. (2010) published the first phylogenies of *Dendronotus* based on morphological and molecular characters. The morphological phylogeny shows *D. robustus* to be the most basal species, with the tropical Indo-Pacific taxa as derived. The hydrothermal vent species *D. comteti* is also basal. The molecular phylogeny did not contain sequences from the tropical Indo-Pacific taxa, the hydrothermal vent species, nor *D. robustus*, and the base of the tree remained largely unresolved. A more general phylogeny of the Cladobranchia, recently published by Pola and Gosliner (2010), and including a few species of *Dendronotus*, shows the tropical Indo-Pacific species to be the most basal.

The new phylogeny shown here expands on the data published by Stout *et al*. (2010) and Pola and Gosliner (2010) by including data for the new species described here, and by adding a new dataset for H3. In this newly generated phylogenetic hypothesis, previous results are corroborated, with the tropical Indo-Pacific species being the most basal (Pola and Gosliner 2010), and *D. venustus* maintaining its distinctness from *D. frondosus* (Stout *et al*. 2010). Because the morphological phylogeny proposed by Stout *et al*. (2010) shows the tropical Indo-Pacific species as the most derived members of *Dendronotus*, in light of the new molecular phylogeny, morphological data likely need re-evaluation. *Dendronotus patricki*, sp. nov. is confirmed to be genetically distinct from other species of *Dendronotus* (see Tables 2 and 3).

Although a well resolved phylogeny of *Dendronotus* remains elusive, all species in this group are well characterised based on morphological characters. The radular morphology and the reproductive anatomy have been proven to allow reliable identification of species, as they vary substantially between different taxa and are conserved within species (Robilliard 1970, 1972; Pola and Stout 2008). Some external characters, such as the branching pattern of the dorsal appendages, the velar appendages, and the rhinophoral sheath appendages have also been found to be reliable in species characterisation (Robilliard 1970, 1972; Pola and Stout 2008), but the external colouration is too variable to provide useful taxonomic information.

The molecular phylogeny presented here shows that *D. patricki*, sp. nov. does not appear in the same well supported clade that contains most of the shallow temperate and cold water species. One of the most interesting questions that remains is the relationships between *D. patricki*, sp. nov. and *D. comteti* (hydrothermal vent species). It has been suggested that organic falls, such as whale or wood, can act as a transition stage in the colonisation of hydrothermal vents and cold seeps (Smith *et al*. 1989; Smith and Baco 2003). This has been upheld for the Mytilidae mussels (Distel *et al*. 2000; Samadi *et al*. 2007; Lorion *et al*. 2009, 2010), where some organisms could occur broadly across these habitats, and where vent and seep organisms occupied derived positions in the phylogeny. Unfortunately, there is no suitable material of *D. comteti* available for molecular work at the present time, and other undescribed deep-sea *Dendronotus* are known only from photographs (pers. obs.). Available morphological phylogenies (Stout *et al*. 2010) indicate that *D. comteti* is part of a basal grade in *Dendronotus*, but again there are clear contradictions between morphological and molecular phylogenies.

Given the presumed cnidarian diet of *D. patricki*, sp. nov., it is likely that the whalefall simply provides organic nutrients and perhaps a substrate that attracts settlement of their prey. Therefore, it would be difficult to describe this species as a whalefall specialist, as it is likely that other substrates may provide similar prey availability (note video records showing specimens from presumed non-whalefall areas). According to Braby *et al*. (2007), 'Patrick' rests on the benthos only 500 m from a cold seep, which may also contribute to organismal diversity found at the whalefall. In any case, species of *Dendronotus* occur from tropical to temperate seas in multiple ocean basins, and occur from intertidal waters to the deep sea, including whalefalls and hydrothermal vents. A more comprehensive

phylogeny of this genus would offer intriguing evolutionary insights to both biogeographical patterns and habitat shifts.

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