A new large and colourful species of the genus *Doto* (Nudibranchia: Dotidae) from South Africa

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A new species of the genus *Doto* is described from the Cape Peninsula of the Western Cape Province, South Africa. To date, the genus *Doto* is probably one of the more complex and poorly defined genera within nudibranchs. The very small body size and very similar external and internal features make this genus problematic and, therefore, poorly studied. Despite the large number of described species around the world, only three species are known to be present in South Africa: *Doto coronata* (Gmelin, 1791), *Doto pinnatifida* (Montagu, 1804) and *Doto rosea* Trinchese, 1881. Morphologically, *Doto splendida* sp. nov. is easily distinguished from all its South African congeneric species by its conspicuous colouration. In addition, mitochondrial and nuclear genes clearly separate the new species from other species from southern Africa. A molecular phylogeny based on two mitochondrial (COI and 16S) and one nuclear (H3) gene is herein presented. This phylogeny includes all available species of *Doto* (valid and unidentified) as well as several other traditionally closed related species retrieved from GenBank.

<http://zoobank.org/urn:lsid:zoobank.org:pub:3764C38DF6BB-415F-958C-E3132A1A9524>

Keywords: Cape Peninsula; Heterobranchia; molecular phylogeny; Mollusca; new species; taxonomy

Introduction

The genus *Doto* Oken, 1815 includes 93 species, of which 75 are considered valid (WORMS: <http://www.marinespecies.org/aphia.php?p=taxdetails&id=137916>). Most of these species have not been completely described; most lack any detailed description of the reproductive system and often there has been no description of the radula. Most species appear to have relatively narrow distributions, but several species are more widely distributed. For example, *Doto ussi* Ortea, 1982 is known from the western Indian Ocean to the western Pacific (Gosliner et al. 2008). While many species have been recorded from the temperate north-eastern Atlantic (e.g. Lemche 1976; Ortea and Bouchet 1989; Morrow et al. 1992), relatively few species have been documented from the temperate south-eastern Atlantic (Gosliner 1987). The three species that have been recorded from the coast of southern Africa are all species that are also known from the north Atlantic: *Doto coronata* (Gmelin, 1791), *Doto pinnatifida* (Montagu, 1804) and *Doto rosea* Trinchese, 1881. While it is assumed that these are conspecific with European taxa (Gosliner 1987), no detailed anatomical or

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molecular studies have confirmed this. Most of the descriptions are based solely on external morphology, and mainly the details of coloration, and it is unclear how definitive external features are in Dotidae. Additionally, the lack of detailed descriptions of anatomical structures, such as the radula and the reproductive system, at present does not permit a determination of how definitive these internal structures are in determining species boundaries and delimitation.

This paper provides precise and detailed description of a new species of *Doto*, recently collected along the False Bay coast of Cape Peninsula, South Africa. Scanning electron micrograph (SEM) pictures of the radula and penis in addition to a drawing of the reproductive system are included. This taxon, although clearly assignable to the genus *Doto*, is different from the other species of the genus. Its coloration is strongly divergent from the rest of the species of the genus and its external morphology is also unlike any other taxon. These external features, together with some internal features and molecular data, characterize it as a very interesting and remarkable new species of this genus. Since the goal of this study is not to conduct a review of the genus *Doto*, the new species is compared only with species of the genus reported for the same geographic area and others that have similar anatomical features, where the anatomy is known. Molecular data for the new species obtained by Pola and Gosliner (2010) are also included in a small phylogeny based on molecular data available in GenBank.

Material and methods

Specimens of the new species were obtained from museum samples deposited in the California Academy of Sciences Department of Invertebrate Zoology and Geology in San Francisco (CASIZ). Features of living animals were recorded from photographs. Specimens were dissected under a microscope and external and internal features were recorded. The buccal mass was extracted and soaked in a 10% sodium hydroxide solution to dissolve the connective and muscle tissue, leaving only the radula and mandibles. The penis was critical point dried. The coated radula, jaws and penis of each species were examined and images were obtained using SEM (Leo 1450 VP, Carl Zeiss Inc., Oberkochen, Germany).

Phylogenetic trees were obtained using all available species of *Doto* (valid and unidentified) in GenBank. Six new specimens were sequenced following the same protocol as in Pola and Gosliner (2010). All species used in this study, localities, vouchers and GenBank accession numbers for partial sequences of the mitochondrial genes cytochrome *c* oxidase subunit I and 16S rRNA, and the nuclear gene histone 3 are found in Table 1.

DNA sequences were assembled and edited using Geneious Pro 4.7.6 (Drummond et al. 2009). The alignments were checked by eye using MacClade 4.06 (Maddison and Maddison 2005). Protein-coding sequences were translated into amino acids for confirmation of alignment. Pairwise uncorrected p-distance values between each taxon were calculated for the COI gene using PAUP* 4.0b 10.0 (Swofford 2002). All codon positions were considered for the analysis. The most variable regions from 16S rRNA alignments were removed using both the default settings and the standard options for stringent and less stringent selection in GBLOCKS (Castresana 2000) and MAFFT (Katoh et al. 2009). After primer removal, sequences of COI, 16S, and H3 were trimmed to 658, 486 (including

Table 1. Species, localities, vouchers and GenBank accession numbers of the specimens used in this study.

Species	Locality	Voucher	GenBank Accession nos.		
			H3	COI	16 S
<i>Bathydoris clavigera</i>	Antarctica	CASIZ 167553	KP940463	JX274106	JX274067
<i>Berthella martensi</i>	Panama	MZUCR6982	HM162498	HM162683	HM162592
<i>Aeolidia papillosa</i>	Chile	ZSM20020700	KF317859	KF317849	KF317837
<i>Facelina annulicornis</i>	Portugal	CASIZ186793	JQ996986	JQ997076	JQ99688
<i>Phidiana lynceus</i>	Cuba	MNCN/ADN51995	JX087633	JX087562	JX087497
<i>Favorinus elenalexianum</i>	Costa Rica	CASIZ 178875	HM162588	HM162755	HM162679
<i>Sakuraeaolis enosimensis</i>	California	CASIZ 178876	HM162591	HM162758	HM162682
<i>Babakina indopacifica</i>	Philippines	CASIZ 177458	HM162587	HM162754	HM162678
<i>Godiva quadricolor</i>	South Africa	CASIZ 176385	HM162589	HM162756	HM162680
<i>Phyllodesmium horridum</i>	South Africa	CASIZ 176127	HM162590	HM162757	HM162681
<i>Armina semperi</i>	Philippines	CASIZ 177534	HM162512	HM162696	HM162606
<i>Dermatobranchius</i> sp.A	South Africa	CASIZ 176273	HM162515	HM162697	HM162609
<i>Hancockia californica</i>	Costa Rica	CASIZ 175722	HM162527	HM162702	HM162621
<i>Bonisa nakaza</i>	South Africa	CASIZ 176146	HM162579	HM162746	HM162670
<i>Janolus capensis</i>	South Africa	CASIZ 176974	KP940458	KP940453	KP940448
<i>Bornella valdæ</i>	South Africa	CASIZ 176832	HM162532	HM162706	HM162626
<i>Dendronotus regius</i>	Philippines	CASIZ 179493	JN869430	JN869451	JN869407
<i>Dendronotus venustus</i>	California	LACM 174850	HM162536	HM162709	HM162630
<i>Notobryon thompsoni</i>	South Africa	CASIZ 176363	HM162543	HM162713	HM162636
<i>Tritonia nilsdhneri</i>	South Africa	CASIZ 176220	KP940459	KP940454	KP940449
<i>Marionia arborescens</i>	Philippines	CASIZ 177394	KP940460	KP940455	KP940450
<i>Doto antarctica</i>	Antarctica	—	—	GQ292025	—
<i>Doto coronata</i>	South Africa	CASIZ 176278	HM162566	HM162734	HM162657
<i>Doto coronata</i>	Kattegat	—	—	AF249794	—
<i>Doto columbiana</i>	USA	—	—	GQ292026	—

(Continued)

Table 1. (Continued).

Species	Locality	Voucher	GenBank Accession nos.		
			H3	COI	16 S
<i>Doto eireana</i>	Spain	—	—	—	AF249248
<i>Doto floridicola</i>	Spain	—	—	AF249820	—
<i>Doto splendida</i> sp. nov	South Africa	CASIZ 176123	HM162573	HM162741	HM162664
<i>Doto koenneckeri</i>	Portugal	CASIZ 178247	HM162567	HM162735	HM162658
<i>Doto koenneckeri</i>	Portugal	CASIZ 178248	KP940461	KP940456	KP940451
<i>Doto koenneckeri</i>	Spain	—	—	AF249797	AF249249
<i>Doto pinnatifida</i>	Spain	—	—	AF249793	AF249250
<i>Doto ussi</i>	Philippines	CASIZ 177438	HM162568	HM162736	HM162659
<i>Doto ussi</i>	Philippines	CASIZ 177514	KP940462	KP940457	KP940452
<i>Doto sp. 2</i>	Philippines	CASIZ 177543	HM162569	HM162737	HM162660
<i>Doto sp. 7</i>	Philippines	CASIZ 177542	HM162570	HM162738	HM162661
<i>Doto sp. H</i>	Mexico (Pacific coast)	LACM 174964	HM162572	HM162740	HM162663
<i>Doto sp. J</i>	Italy	CASIZ 175711	HM162574	HM162742	HM162665
<i>Doto sp. K</i>	Philippines	CASIZ 177460	HM162575	HM162743	HM162666

*New sequences.

gaps), and 328 base pairs, respectively. The tree resulting from the trimmed 16S sequences was poorly resolved and had lower node support.

Individual gene analyses and a concatenated analysis were performed. The best-fit models for evolution for each gene were determined using the Akaike information criterion (Akaike 1974) implemented in MrModeltest 2.3 (Nylander 2004). The GTR +G+I model was selected for COI and 16S genes. GTR+G was selected for H3.

Maximum likelihood (ML) analyses were performed using the software RAxML 7.04 (Stamatakis 2006). Node support was assessed with non-parametric bootstrapping (BS) with 50,000 replicates, random starting trees, and parameters estimated from each data set under the model selected for the original data set. Bayesian inference (BI) analyses were conducted using MrBayes 3.1.2b (Ronquist and Huelsenbeck 2003) for 10 million generations with two independent runs and a sampling frequency of 1000. The models implemented were those estimated with MrModeltest 2.3. The combined data set was partitioned among genes, and the 'unlink' command was used to allow all parameters to vary independently within each partition. Node support was assessed with posterior probabilities (PPs). Only nodes supported by $BS \geq 75$ and $PP \geq 0.95$ are considered robust and discussed.

Systematics

Class **GASTROPODA** Cuvier, 1795
 Order **NUDIBRANCHIA** Cuvier, 1817
 Suborder **DEXIARCHIA** Schrödl et al. 2001
 Family **DOTIDAE** Gray, 1853
 Genus *Doto* Oken, 1815

Type species: *Doris coronata* Gmelin, 1791. ICZN opinion no. 697

Doto splendida sp. nov.
 (Figures 1–3)

Doto sp. I Pola and Gosliner, 2010: 935

Type material

Holotype: adult specimen, 5 mm preserved (South Africa, western Cape Province, western False Bay, Miller's Point, 34°13.84'S, 18°28.47'E; intertidal, found feeding on *Eudendrium* [California Academy of Sciences (CASIZ176124)]. Collected by T.M. Gosliner, 3 January 2008.

Paratype: one adult specimen completely dissected and sequenced for molecular studies, 20 mm alive (10 mm preserved), water depth: 3 m (same locality, date and collector as holotype) [CASIZ176123].

Figure 1. *Doto splendida* nov. sp. Paratype (CASIZ176123), South Africa, western Cape Province, western False Bay, Miller's Point. 20 mm in length. Photo by T.M. Gosliner. (A) Living animal; (B) detail of a cera.

Figure 2. *Doto splendida* nov. sp. Paratype (CASIZ176123), SEM pictures of the radula and penis. (A) Radula, scale bar: 10 μ m; (B) detail of the teeth, scale bar: 3 μ m; (C) penis, scale bar: 10 μ m; (D) detail of the penis, scale bar: 10 μ m.

Figure 3. Reproductive system of *Doto splendida* nov. sp. Paratype (CASIZ176123). Abbreviations: am, ampulla; fglm, female gland mass; hd, hermaphrodite duct; ov, oviduct; pb, penial bulb; pr, prostate; sr, seminal receptacle; v, vagina; vd, vas deferens. Scale bars, 1 mm.

Etymology

The specific name refers to the beautiful and conspicuous colour of the animal. From the Latin word *splendidus*, meaning brilliant, magnificent appearance.

Distribution

Thus far this species is only known from Western Cape Province, South Africa.

External morphology (Figure 1)

The body is slender and elongate (Figure 1A). Its length reaches 20 mm alive. The head bears a pair of smooth rhinophores with blunt apices, surrounded by

rhinophoral sheaths that reach half of the total length of the rhinophores in living specimens (Figure 1A). The sheaths are rather slender, the diameter being double that of the rhinophore itself. Above, the sheaths are undulate, trumpet-shaped with their anterior side ending with a rather long, tongue-like projection at the upper end (Figure 1A). A wide oral veil is present in front of the rhinophores. It is entire, rounded and expanded laterally, without projections. On each side of the body, up to 7 cerata occur, the hindmost usually being smaller than the anterior and middle cerata. The cerata are large, elongate, with rounded tubercles that decrease in size basally towards the body. The base of the cerata is not pedunculate. Each ceras bears six or seven rings of six–seven simple or more complex semiglobular tubercles and one larger tubercle at the top (Figure 1A, B). At the inner side of a ceras a pseudobranch is situated with 4–5 ramifications of varying lengths. The genital opening is located slightly below and in front of the first ceras on the right side, and protrudes as a cylindrical genital papilla. On this side, between the first and second cerata, the anus and nephroproct together form a prominent papilla. The pericardium starts just behind the first ceras. The posterior end of the foot is long and limaciform (Figure 1A). The posterior end of the foot is narrow and extends slightly from under the mantle. The pericardium forms a swelling on the back, between the first and second cerata.

Colouration (Figure 1). The body is transparent, with a faint orange hue covered with occasional small opaque white dots dispersed over the frontal veil, the upper half of the rhinophores, the borders of their sheaths, the posterior end of the foot and the tubercles of the cerata. The yellow digestive gland is clearly visible through the transparent faint orange body wall. The branches of the digestive gland in the cerata are intense pink. The tubercles of the cerata are faint orange, similar to the ground colour with small white dots more or less abundant (Figure 1A, B). The foot is transparent.

Internal anatomy (Figures 2, 3)

The jaws are very thin with a smooth masticatory process. The uniseriate radula is composed of 85 horseshoe-shaped teeth with three strong lateral denticles on either side of the median cusp (Figure 2A, B). The salivary glands are located just in front of the buccal bulb. They have a globular appearance opening into the oesophagus via a thin duct. Furthermore, above and at the posterior end of the salivary glands, there are three rounded giant cells. The pedal gland lies at the anterior part of the foot and around the oral opening. It is composed of numerous semispherical follicles.

The reproductive system is androdiaulic (Figure 3). The hermaphroditic gland is granulate, appearing as a compact mass clearly visible under the mantle. It is connected with the ampulla by a narrow hermaphroditic duct. The ampulla is large, thick and elongate. The rather long, postampullary duct splits into the vas deferens and the oviduct. The vas deferens begins as a large pyriform prostate gland and then tapers into a long and thin vas deferens with several loops and entering into the muscular penial bulb. Within the penial bulb is the elongate, conical and unarmed penis (Figure 2B, C). The oviduct is rather wide and elongated, forming a U-shaped loop before it dilates into the seminal receptacle, a spacious sac similar in length and width

to the prostate gland. It continues with the vagina, which is short and slender. The female glands are very well developed and open together with the vagina.

Molecular data (Figure 4)

As indicated in Table 1, some gene fragments were not available for the analysis; in particular some nuclear fragments of the gene H3 and some 16S fragment for some isolated species of *Doto* are missing. After alignment 1472 bp were used. We obtained 18 new sequences; 6 for H3, 6 for COI and 6 for 16S. The saturation plots of uncorrected differences against corrected sequence divergence divided by codon indicated no saturation for any gene (not shown). Figure 4 shows the phylogenetic hypothesis based on the combined data set represented by BI.

Figure 4 clearly indicates that *D. splendida* sp. nov. represents a distinct lineage and that it clusters with two eastern Pacific (from the Pacific coast of Mexico and California, respectively) species rather than with any other Atlantic taxa. The available species of *Doto* constitute a clade with the maximum support (PP = 1; BS = 100). *Doto pinnatifida*, *D. coronata*, *D. eireana*, *D. floridicola* and *D. koenneckeri* form a well-supported clade (PP = 0.98; BS = 78) while *D. splendida* sp. nov. clusters with *D. columbiana* and *Doto* sp. H (PP = 0.99; BS = 72). The phylogenetic relationship of this clade with the one including *D. antarctica*, *Doto* sp. 2, *Doto* sp. 7, *Doto* sp. K and *D. ussi* (PP = 0.98; BS = 87) is not resolved. DNA sequences for *Doto rosea* are not available, but the morphological differences highlighted below clearly separate *D. rosea* of the new species. The sister taxon to the genus *Doto* is not yet fully resolved. Figure 4 shows that *Hancockia californica* is closer than *Notobryon* or *Dendronotus*, but this relationship is not supported either by the analysis of Bayesian inference nor by maximum-likelihood analysis.

Discussion

Three species of the genus *Doto* have been reported from South Africa: *Doto coronata* (Gmelin, 1791), *Doto pinnatifida* (Montagu, 1804) and *Doto rosea* Trinchese, 1881 (Gosliner 1987; Zsilavecz 2007). However, none of the species previously mentioned were originally described from South Africa or nearby. *Doto coronata* was originally described from the Dutch coast (Gmelin 1791), *Doto pinnatifida* from the British Isles (Montagu 1804) and *Doto rosea* from the Mediterranean (Trinchese 1881). Gosliner (1987) and Zsilavecz (2007) recorded these three species for South African waters, specifically for the Cape Peninsula and False Bay. Nevertheless, these authors did not undertake any morphological or molecular study to confirm the correct identification of these taxa. Thompson et al. (1990) stated that the identity of the '*Doto rosea*' recorded from Southern Africa by Barnard (1927) and illustrated by Gosliner (1987), is enigmatic. Barnard (1927) reported it as *Doto cinerea*, which is actually accepted as *Doto rosea* after Pruvot-Fol (1954) synonymized both species. However, Odhner (1936), Just and Edmunds (1985), Ballesteros et al. (1986) and Cervera et al. (1988, 2004) consider *D. cinerea* as a valid species, distinct from *D. rosea*. It should be noted that *D. rosea* (illustrated by Schmekel and Portmann 1982) has dark brown spots on the notum, while the South African specimens have opaque white spots (Gosliner 1987). Therefore, taking into account the scarcity of morphological and molecular studies that exist within this genus, in this paper we simply assume that those

Figure 4. Phylogenetic hypothesis based on the combined data set (COI+16S+H3) inferred by Bayesian analysis (BI). Numbers above branches represent posterior probabilities from BI. Numbers below branches indicate bootstrap values for ML.

identifications by Gosliner (1987) and Zsilavecz (2007) are correct to compare them with *Doto splendida* sp. nov. However, it is obvious that detailed descriptions, with good pictures and internal structures as well as molecular data, are sorely needed to improve our knowledge within this baffling group of nudibranchs.

Both the molecular phylogenetic tree based upon mitochondrial and nuclear markers shown in Pola and Gosliner (2010), as well as our new results (Figure 4), clearly indicate that *D. splendida* sp. nov. is a distinctly different species from *D. pinnatifida* and *D. coronata*, based on the fact that it is nested in a clade together with two eastern Pacific species rather than with these other two Atlantic species. Regarding *D. coronata*, our analysis demonstrates that whether specimens from South Africa are well identified as *D. coronata* or not, both South African and North Sea specimens are different from our new species. DNA sequences for *Doto rosea* are not available at this time, but the morphological differences highlighted below also clearly distinguish *D. rosea* from the new species.

Comparison among *D. splendida* sp. nov., *Doto coronata*, *D. pinnatifida* and *D. rosea* based on morphological characters (Figure 5)

Doto coronata is a small nudibranch that attains 15 mm in extended length but usually does not exceed 10–12 mm (Thompson and Brown 1976, 1984) (Figure 5A, B). *Doto*

Figure 5. Living specimens. (A) *Doto coronata* (Gmelin, 1791). Specimen from Northern Ireland. Photo by Bernard Picton; (B) *Doto* cf. *coronata*. Specimen from South Africa, Cape Province, Oudekraal. Photo by T.M. Gosliner; (C) *Doto rosea* Trinchese, 1881. Specimen from Catalonia, Spain. Photo by Antoni López-Arenas; (D) *Doto pinnatifida* (Montagu, 1804). Specimen from Northern Ireland. Photo by Bernard Picton.

rosea (Figure 5C) was originally described by Trinchese (1881) with a maximum length of 8 mm. Thompson et al. (1990) studied specimens of 5 and 6 mm but Schmekel and Portmann (1982) studied specimens of 12 mm. It should be noted that the South African specimens of *D. coronata* lack the dark spots that are present in European specimens. *Doto pinnatifida* is larger than the previous species, reaching 29–30 mm (Thompson and Brown 1976, 1984; Zsilavecz 2007) (Figure 5D). Therefore, with respect to body size, *D. pinnatifida* is the only species that may be compared to the new species. Nonetheless, the colour of the body, cerata and digestive gland branches is completely different in all of them (Figure 5). The original description of *D. pinnatifida* says ‘colour gray, spotted with olive green; blue gray papillae marked with a black spot at their tip: the stem or center part of the peduncle is a mixture of olive green and rufous brown’ (Montagu 1804, table VII, figures 2, 3). Surprisingly, this is not the colour that appears in other descriptions of the species (Thompson and Brown 1976, 1984; Schmekel and Kress 1977; Picton and Morrow 1994) and is not shown in the photographs of the specimens from South Africa (Gosliner 1987; Zsilavecz 2007). No trace of olive green is found in those descriptions. After Montagu (1804) all pictures and colour description are similar to those found in Thompson and Brown (1976, p. 76): ‘pale fawn with dark brown to black mottling above: conspicuous dark brown spots around the rim of each rhinophore sheath and on the tips of small tubercles down each side of the body; cerata with tubercles each of which terminates in a dark brown or black spot’. Specimens illustrated from South Africa (Gosliner 1987; Zsilavecz 2007) are white with some occasional red spots and streaks along the mid-line of the body. The tip of each ceratal tubercle is generally black. *Doto coronata* and *D. rosea* also differ in colour from *D. splendida* sp. nov. *Doto coronata* is pale yellow or white with red or purplish pigment above, with streaks along the mid-line of the dorsum or covering much of it and also often parts of the sides. The cerata are pale and bear tubercles ending with a dark red spot (Gmelin 1791; Alder and Hancock 1845–1855; Lemche 1976; Thompson and Brown 1976, 1984; Gosliner 1987; Zsilavecz 2007). Figure 5A shows a specimen from Northern Ireland and Figure 5B shows the specimen collected in South Africa and sequenced in this study. In the original description of *Doto rosea* (Figure 5D), Trinchese (1881) stated that its body colour is yellow, dotted with small black dots or very dark ochre. Besides, the colour of the digestive gland branches in these three species is very different from *D. splendida* sp. nov., which is intense pink. In short, whether the identifications of the specimens observed in different parts of the world are correct or not, the colour of these three species differs widely from the new species.

The number of pairs of cerata and the number of tubercles per ceras are not very different among species. However, pseudobranchs are indistinct in *D. coronata* and *D. pinnatifida* (Thompson and Brown 1984) whereas they are present in *D. rosea* and *D. splendida* sp. nov. Thompson et al. (1990) described a simple flap-like pseudobranch but Schmekel and Portmann (1982) mentioned that the pseudobranch had branches. In our new species, each pseudobranch has at least 4–5 ramifications of varying lengths.

Regarding the radula, no SEM pictures of the teeth exist for these species. A few drawings and short descriptions are found in several older papers (Pruvot-Fol 1954; Kress 1968; Schmekel and Kress 1977; Schmekel and Portmann 1982; Thompson and Brown 1984; Thompson et al. 1990) but, in general, no pictures or drawings of the specimens are linked to those descriptions. Therefore, it is almost impossible to make

good comparisons between the radula of these three species. It seems that the number of rows of teeth is quite variable ($n \times 0.1.0$), since n goes from 51 to 68 in *D. coronata* (Schmekel and Kress 1977; Thompson and Brown 1984; Thompson et al. 1990), from 55 to 100 in *D. pinnatifida* (Miller 1958; Schmekel and Kress 1977; Thompson and Brown 1984) and from 75 to 101 in *D. rosea* (Schmekel and Portmann 1982; Thompson et al. 1990). Our paratype specimen of *D. splendida* sp. nov. has 85 teeth. The real problem with radular descriptions is that each of these descriptions is different regarding the number and size of the subsidiary denticles of the median cusp. For example, in *D. coronata* the descriptions vary from ‘one (sometimes two) robust subsidiary denticle present on each side of the median cusp’ (Thompson et al. 1990, p. 394), ‘3–5 subsidiary denticles present on each side of the median cusp’ (Kress 1968, p. 472) to ‘3 subsidiary denticles present on each side of the median cusp’ (Thompson and Brown 1984, p. 27). For *D. pinnatifida*, Thompson and Brown (1984) described each tooth as having ‘a slender erect cusp, flanked on either side by up to 5 irregular, tiny, subsidiary denticles’ (p. 35). The same apparent variation occurs in *D. rosea*, for which descriptions by the different authors are as follows: ‘One subsidiary denticle present on each side of the stout median cusp’ (Thompson et al. 1990, p. 399, for specimens from Greece); ‘two prominent sharp denticles on each side of the median cusp’ (Thompson et al. 1990, p. 399, for specimens from Italy); and ‘2 or 3 denticles on one side and 3 or 4 on the other’ (Schmekel and Portmann 1982, p. 168; specimens from Naples). All these different descriptions are considered today as variation within the same species but easily could reflect different cases of cryptic species (Morrow et al. 1992; Pola et al. 2012, 2014; Carmona et al. 2014). For future comparisons, our species clearly has 85 horseshoe-shaped teeth with three strong lateral denticles on either side of the median cusp (Figure 2A, B).

Comparisons among reproductive system are not feasible since very few of them have been fully or even partially described. For example, the reproductive systems of *D. pinnatifida* is unknown. Trinchese (1881) described and figured the genitalia of *D. costae* but the drawing is impossible to interpret (table LX, figure 2). Odhner (1922) described and figured a long-stalked receptaculum seminis opening into the vestibulum in *D. coronata*. Schmekel and Portmann (1982, Abb. 7.41a-f) depicted schematic arrangements of the reproductive systems of *D. acuta*, *D. coronata*, *D. doerga*, *D. floridicola*, *D. paulinae* and *D. rosea*. Differences can be found among these schemes. In all species they depicted, except *D. doerga*, they illustrated a separate duct for the receptaculum seminis rather than the receptaculum seminis being a swelling of the vaginal duct, as in *D. splendida*. No trace of distinct duct with a terminal receptaculum is present in *D. splendida* (Figure 3). Other differences are shown in these schemes as the length and curvature of the vas deferens, the size of the penial bulb and also the length and form of the duct to the receptaculum seminis, when present. These differences could be used to separate species but were not used by subsequent authors. MacFarland (1966) described and figured in detailed the reproductive system of *Doto varians* MacFarland, 1966. This reproductive system is very similar to *D. splendida* sp. nov. However, the oviduct forms a U-shaped loop instead of two loops as in our species. Also, both the vas deferens and the vagina appear shorter than in *D. splendida* sp. nov. Marcus (1961) also depicted the reproductive systems of *D. columbiana* (O’Donoghue, 1921, *D. amyra* Marcus, 1961, *Doto ganda* Marcus, 1961, *Doto kya* Marcus, 1961 and *Doto wara* Marcus, 1961. All of these have a similar configuration of reproductive organs to *D. doerga* and *D. splendida* and lack a

distinct duct with a terminal receptaculum seminis. However, in these five species from the Pacific coast of North America, the receptaculum is situated at the distal end of the vagina while in *D. doerga* and *D. splendida* the receptaculum is more basal near the gonopore. It is also important to note that the only described species with this reproductive anatomy, other than *D. splendida*, for which molecular data are also available, is *D. columbiana*. Interestingly, these species are found in the same clade, suggesting that reproductive anatomy found in these species may also be an indicator of phylogenetic relatedness.

These few examples of comparisons among radulae and reproductive systems demonstrate that these features should always be entirely described in every single species of *Doto*. Small differences could represent intra-specific variability but more likely they could represent different species and thus, they could be very useful to discriminate among species. Therefore, detailed morphological studies, when combined with molecular studies, provide different lines of evidence that greatly aid species delimitation.

Recent molecular studies on heterobranch gastropods (Carmona et al. 2011, 2014; Cooke et al. 2014; Pola et al. 2014; Padula et al. 2014), especially when combined with detailed morphological studies, have revealed the presence of numerous cryptic species. These molecular markers provide compelling new tools to facilitate rapid species delimitation and recognition of previously undetected biodiversity.

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