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# A NEW LARGE AND COMMON SPECIES OF *DORIS* (GASTROPODA, NUDIBRANCHIA) FROM THE WESTERN INDIAN OCEAN

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*Abstract* A new species of cryptobranch dorid nudibranch is described from the Western Indian Ocean. *Doris ananas* sp. nov. has previously been recorded from South Africa to Tanzania. The studied specimens were from the sub-tropical waters of southern Mozambique, from 10 to 40m deep and are frequently associated with yellow sponges. The new species is characterized by having several conical simple small tubercles surrounding each large tubercle. The integument is yellow and the top of the tubercles are pigmented in brown or black. This species distinguishes from other described taxa on both external and internal characteristics explored herein, mainly in the reproductive system, with the presence of two bags with internal spines annexed to the vagina. This conformation is described for the first time for cryptobranchs.

*Key words* *Doris*, *Dorid*, *Nudibranchs*, *Mozambique*, *Western Indian Ocean*

## INTRODUCTION

The nudibranchs of the Western Indian Ocean are relatively poorly understood. Many species, even common and large ones, still remain undescribed or misidentified. The majority of nudibranch research in the region has been conducted in Tanzania (Eliot, 1902, 1903, 1904a–c, 1905), and South Africa (Barnard, 1927; Bergh, 1907; Fahey & Gosliner, 1999; Gosliner, 1987, 1994; MacNae, 1971), leaving a gap in Mozambique despite a few general marine invertebrate assessments (MacNae & Kalk, 1958, 1962; Martens, 1879).

The genus *Doris* is characterized by the dorsum covered by simple, rounded tubercles, stiffened by integumentary spicules, which do not protrude from the dorsal surface. A head with two lateral prolongations. An anterior border of the foot grooved but not notched. A labial cuticle lacking rodlets. A radula composed of simple, hamate teeth, and outermost teeth being simple or denticulate. A reproductive system with tubular, granular and simple prostate; penis and vagina devoid of hooks; and vestibular or accessory glands absent (Valdés, 2002).

The genus was described based on *Doris verrucosa* Linnaeus, 1758 from the Atlantic Ocean, the type species. Presently, it consists of 44 species of which 20 are from the Indo-Pacific region, six being unidentified species (Coleman, 2008;

Bouchet & Gofas, 2014). Samples of the here introduced species, *Doris ananas* sp. nov., had been found in the Western Indian Ocean, particularly South Africa, Tanzania (Gosliner, 1987; Gosliner *et al.*, 2011) and Mozambique (present study). Additional records in Madagascar, Mayotte and Seychelles Islands are found on-line ([www.seaslugs.free.fr](http://www.seaslugs.free.fr)). The description includes anatomical information, which has been used in an ongoing wider comparative, phylogenetic study on the doridaceans.

## MATERIAL AND METHODS

The examined material was hand-picked during SCUBA dives in Zavora and Ponta do Ouro, Southern Mozambique. The material was first stored at the A.C.C.M. – Zavora Marine Lab. and is currently deposited at the Museum of Kwazulu-Natal, Museu de Zoologia de São Paulo and Museu de História Natural de Maputo.

After collection, all specimens were individually photographed and notes were taken with all the data being entered in a database. The specimens were either relaxed in magnesium chloride 7% solution or by freezing. Shortly after this, they were transferred to formalin 4% (3 specimens), ethanol 70% (1 specimen) or ethanol 96% (5 specimens). Their dissections were performed under a stereomicroscope using standard techniques, with the specimens immersed in fixative.

Digital photos of each step of the dissection were obtained, as well as drawings aided by a camera lucida. The radula was removed and placed in 10% sodium hydroxide in order to isolate it from the soft tissue. A scanning electron microscope (SEM) was employed to view details of the radula in the Laboratório de Microscopia Eletrônica de Museu de Zoologia da Universidade de São Paulo.

The following abbreviations are used in the figures: **am**: ampulla; **au**: auricle; **at**: aortic trunk; **bc**: bursa copulatrix; **bg**: blood gland; **bm**: buccal mass; **bs**: buccal sphincter; **cb**: buccal commissure; **ce**: cerebral ganglia; **cg**: connective buccal ganglia; **cp**: pedal commissure; **cu**: caecum; **dd**: duct of digestive gland; **dg**: digestive gland; **es**: esophagus; **ey**: eye; **fg**: female gland; **ft**: foot; **gb**: buccal ganglia; **gc**: gill circle; **gf**: gill filament; **gg**: gastroesophageal ganglia; **gp**: pedal ganglia; **gr**: rhinophoral ganglia; **hd**: hermaphrodite duct; **in**: intestine; **mo**: mouth; **m2** – **m10**: odontophore muscles; **mt**: oral tube muscle; **oc**: odontophore cartilage; **od**: odontophore; **ot**: oral tube; **ov**: oviduct; **pa**: papilla; **pc**: pericardium; **pe**: penis; **pl**: pleural ganglia; **pr**: prostate; **ra**: radula; **rc**: renal chamber; **ri**: rhinophore; **rm**: retractor muscle gill; **rp**: reproductive system; **rs**: radular sac; **rv**: renal vesicle; **sg**: salivary gland. **st**: stomach; **sn**: nervous system; **sr**: seminal receptacle; **ud**: uterine duct; **va**: vagina; **vd**: vas deferent; **ve**: ventricle; **vp**: vaginal pouches; **vv**: auricoventricular valve.

#### *Institutional Abbreviations*

KZN – Museum of Kwazulu-Natal, South Africa.  
MHN – Museu de História Natural de Maputo, Mozambique.  
MZSP – Museu de Zoologia da Universidade de São Paulo, São Paulo.

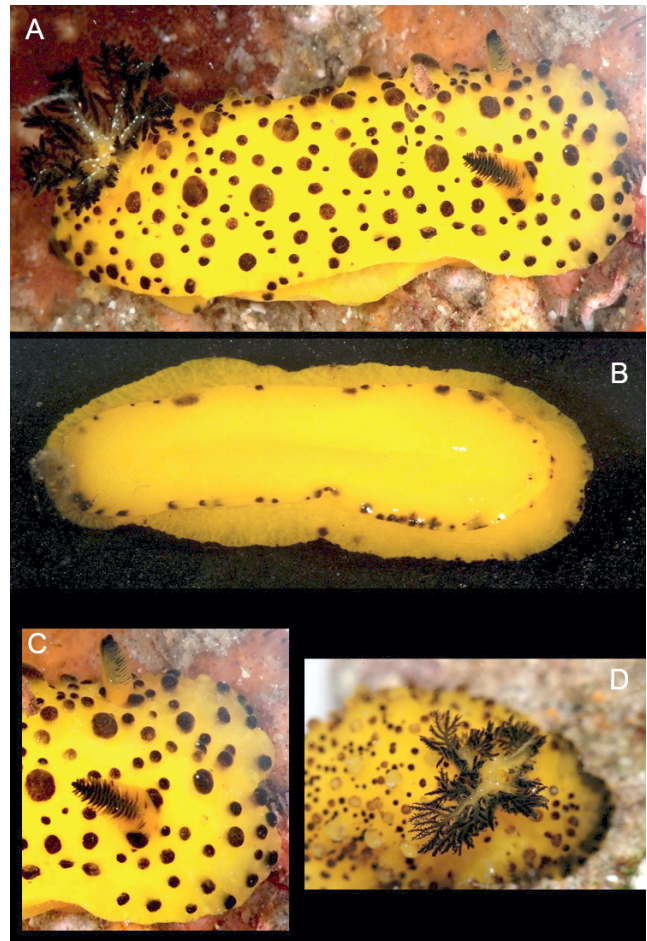
#### SYSTEMATICS

Family Doridae Rafinesque, 1815  
*Doris* Linnaeus, 1758

Type species *Doris verrucosa* Linnaeus, 1758

*Doris ananas* sp. nov.  
(Figs 1–7)

*Holotype* MOZAMBIQUE, Inhambane, Inharrime, Zavora Beach, 24°31'S 35°12'E (Y. Tibiriça col., 06/v/2010, 30m depth), MZSP 111010.

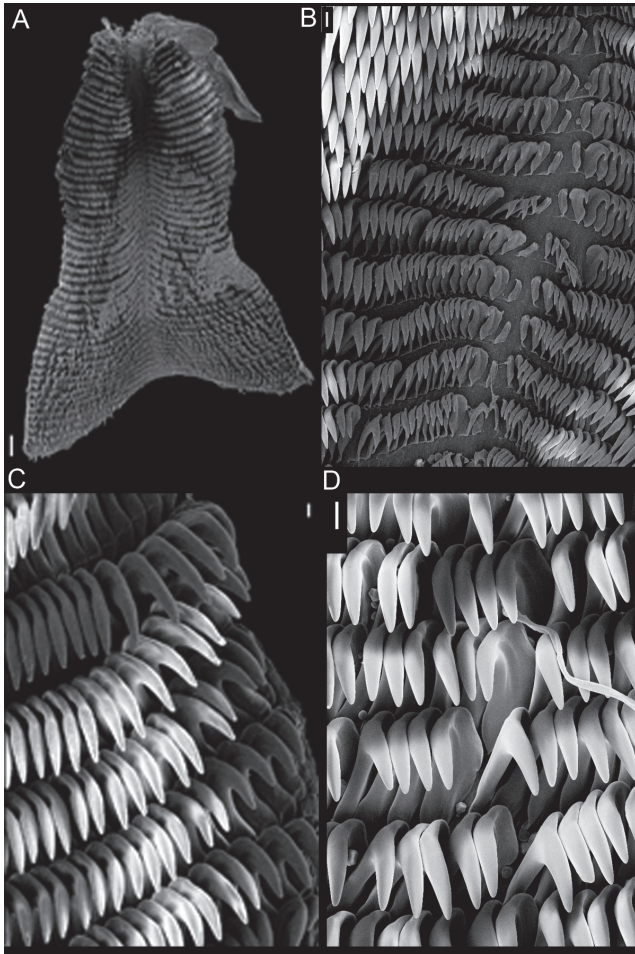


**Figure 1** *Doris ananas* live specimen. **A.** Dorsal-slightly right view. **B.** Ventral view. **C.** Detail anterior region, dorsal-slightly right view. **D.** Detail of extended gill circle, dorsal view.

*Paratypes* MOZAMBIQUE, Inhambane, Inharrime, Zavora Beach (Y. Tibiriça col.), MZSP 109879, 1 ex. (10/iii/2011, 12m depth); MZSP 109880, 1 ex. (10/iii/2011, 12m depth); MZSP 109882, 1 ex. (14/iii/2012, 18m depth); MZSP 109884, 1 ex. (10/viii/2012, 17m depth); MZSP 109885, 1 ex. (15/viii/2012, 17m depth); MZSP 109887, 1 ex. (06/iv/2010, 14m depth); NMSA L9730/T4025, 1 ex. (14/III/2011, 16m depth); NMSA L9731/T4026, 1 ex. (09/VIII/2011, 18m depth); NMSA L9732/T4027, 1 ex. (17/III/2013).

#### *Description*

*External morphology* (Figs 1A–D; 3A; D; F) Size of fixed animal 16–31–50mm. Color uniform yellow with black tubercles. Body oval, elongated, dorsum covered by rounded tubercles, located mainly in median dorsal region; tubercles decreasing in size towards edge. Rhinophores



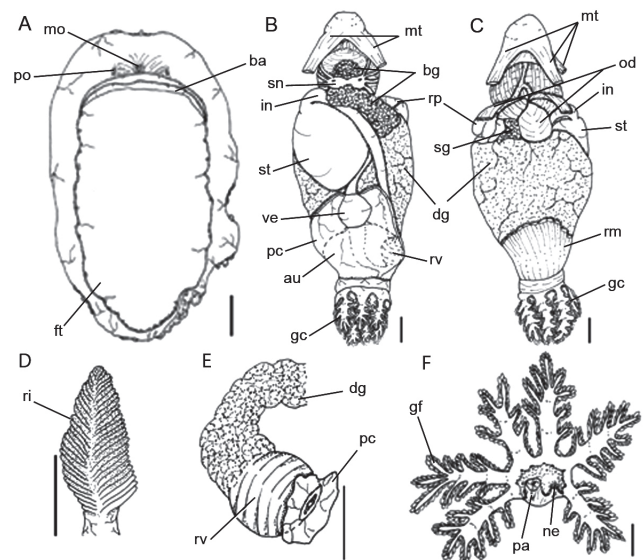
**Figure 2** *Doris ananas*, radula in SEM. **A.** Panoramic view (MZSP109884). Scale: 300 $\mu$ m. **B.** Higher magnification in central region (MZSP109880). Scale: 20 $\mu$ m. **C.** Outer lateral teeth (MZSP109884). Scale: 20 $\mu$ m. **D.** Detail of lateral teeth (MZSP109885). Scale: 30 $\mu$ m.

with 20–25 transverse lamellae, yellowish base, 2/3 black apex; rhinophoral sheaths with small tubercles, two larger tubercles on left and right sides. Gill composed of 6–7 tripinnate gill (Fig. 3F), occupying ~6% of haemocoel volume, yellowish base and black apex, arranged in circular fashion surrounding anus; branchial sheaths with small tubercles. Mouth opening in anterior ventral region between anterior region of notum and foot. Lateral extensions pore-like, on each side of mouth (Fig. 3A). Anterior border of foot grooved, but not notched. Foot yellow with some small black dots.

**Haemocoel organs** (Figs 3B–C) Pericardium and gill circle in posterior half of visceral mass occupying ~15% of haemocoel volume. Buccal mass located anteriorly, occupying ~25% of haemocoel

volume. Nervous system dorsal to buccal mass, covered by blood gland, occupying ~5% of haemocoel volume. Reproductive system on right side of animal, occupying ~10% of haemocoel volume. Stomach on left side, intestine with small curve at anterior portion, immersed in haemocoel, both occupying ~15% of haemocoel. Digestive gland/gonad occupying ~30% of haemocoel volume.

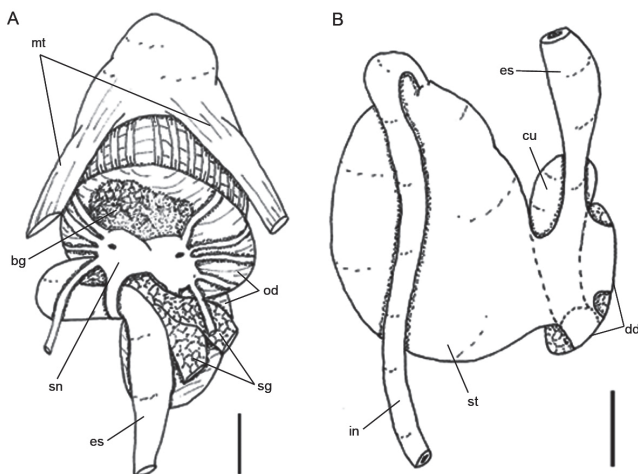
**Circulatory and excretory systems** (Fig. 3B; E–F) Pericardial cavity dorsal and posterior to digestive gland, anterior to gill circle. Gill retractor muscle originating from base of gill circle, running longitudinally up to 1/4 of foot level, inserting into dorsal surface of foot. Auricle funnel-like (wider anteriorly) with thin walls. Ventricle slightly taller than wide, with thick muscular walls. Aortic trunk branched into posterior artery irrigating stomach, caecum and digestive gland; anterior artery irrigating reproductive system, buccal mass, odontophore and nervous system inserting on blood gland. Blood gland (bg) with posterior portion three times larger than anterior portion (Fig. 3B). Medial sinus connected to afferent branchial ring, irrigating entire digestive gland. Renal vesicle located on right dorsal side of pericardium, near base of auricle, connected to inner surface of pericardium (Fig. 3B); renal



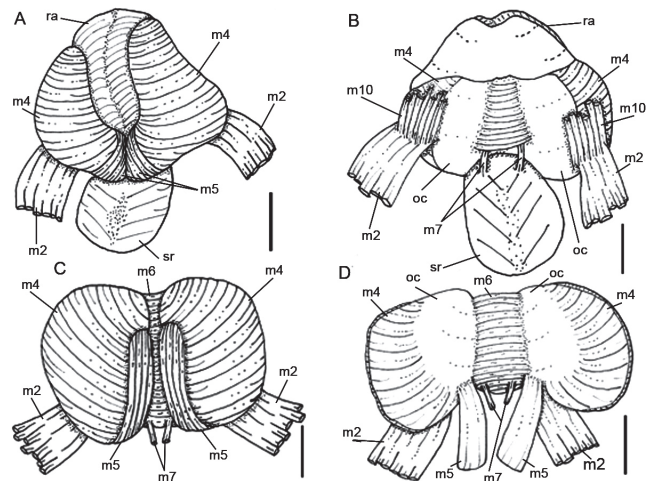
**Figure 3** Anatomical details of *Doris ananas*. **A.** Whole ventral view. Scale: 2mm. **B.** Dorsal view of extracted visceral mass. Scale: 1mm. **C.** Same, ventral view. **D.** Rhinophore, dorsal view. Scale: 1mm. **E.** Detail of renal vesicle with the connection with pericardium. Scale: 1mm. **F.** Gill circle, dorsal view. Scales: 1mm.

chamber elliptical, color light yellow, with longitudinal folds, same size as ventricle. Renal chamber extending from dorsal to medial sinus, previously connected to renal vesicle, extending posteriorly to center of gill circle and opening in nephrostome papilla, next to base of anal papilla.

**Digestive system** (Figs 4A-B; 5A-D) Oral tube composed of outer lip, with pleats lengthwise; inner lip with transverse fold. Main oral tube muscle, **mt**, three pairs of retractor muscles of buccal mass, originating on oral tube, running dorsally and ventrally to oral tube, inserting on body side, about six times as wide and twice as long as m10. Odontophore oval, connected to oral tube by pair of *ventral* protractor muscles (**m10**); thin longitudinal, dorsal and ventrolateral protractors of oral sphincter, originating in anterior region of odontophore, inserted in posterior region of integument, close to oral tube (Fig. 4A). Oral sphincter surrounding chitinous part of oral tube. Odontophore muscles: **m2**, pair of strong retractor muscles of buccal mass, twice as long as wide, origin on anterior dorsal odontophore, running laterally to m4 and inserted ventrally on dorsal portion of foot; **m4**, main pair of dorsal tensor muscles, strong and broad, 1/3 as long as wide, almost completely covering cartilage of odontophore, inserted on ventral portion of subradular membrane; **m5**, pair of dorsal auxiliary tensor muscles, twice as long as wide, originating mostly in posterior region of odontophore cartilages, covering ~1/3 of



**Figure 4** *Doris ananas* details of digestive system. **A.** Foregut, dorsal view, some adjacent structures also shown as in situ. **B.** Midgut as in situ, dorsal view. Scales: 1mm.

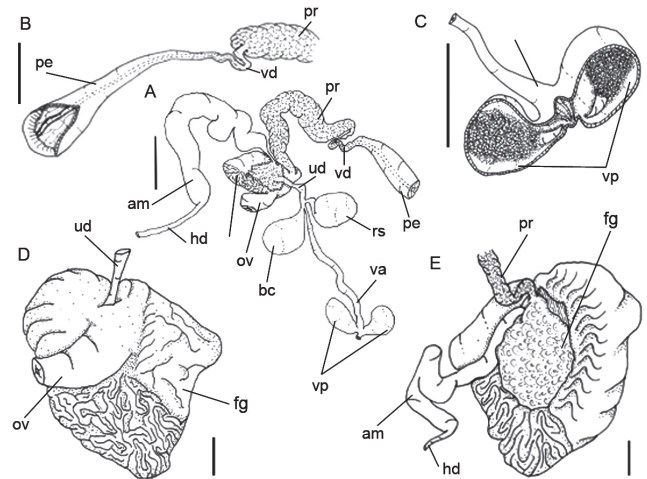


**Figure 5** *Doris ananas* odontophore anatomy. **A.** Whole dorsal view, esophagus removed. **B.** Whole ventral view, sphincter removed. **C.** Dorsal view, radula removed, each cartilage slightly deflected. **D.** Same, m4 and m5 deflected downwards to expose odontophore cartilage. Scales: 1mm.

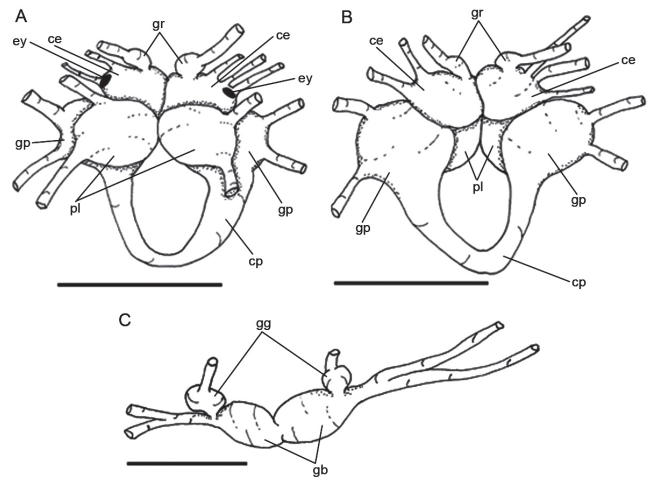
posterior cavity of odontophore, as long as, and with ~1/3 of m4 width, inserting on ventral side of subradular membrane, around radular sac; **m6**, unpaired horizontal muscle, with transverse fibers connecting to median surface of left and right odontophore cartilages, about same length and half as wide as m4, posterior and anterior portion about same width as m4 (Fig. 5D); **m7**, pair of thin and short muscles, running parallel to dorsal portion, originated in posterior part of m6 and inserting on radular sac (Fig. 5B). Pair of odontophore cartilages elliptical. Subradular membrane thin, strong, translucent. Radular sac ~1/3 of odontophore length (Fig. 5A). Radular teeth (Figs 2A-D): rachidian teeth absent; formula 58×50.0.50 (in 25mm long specimen). Each lateral tooth with broad base, tapering towards apex, hook-shaped, with single terminal cusp; outermost teeth thinner than internal teeth, inner base width about half of lateral teeth width, apex also hook-shaped, with single terminal cusp. Pair of salivary glands long, tubular (Fig. 4A); sponge-like, duct inserting in anterior region of esophagus, extending posteriorly to anterior region of digestive gland. Esophagus connected with odontophore, making fold up to nerve ring, running longitudinally until its connection with stomach (Fig. 4A). Stomach oval (Fig. 4B), with folds at entire inner surface; longitudinal pleats thicker posteriorly, close to anterior region of intestine. Intestine with longitudinal folds along

its entire length, diameter about half esophagus diameter, but more uniform. Caecum as elongated sac, located ventrally to stomach, opening in anterior portion of stomach, close to esophageal insertion,  $\sim 2/3$  the length and  $\sim 1/3$  the width of stomach; typically containing dark brown substance (Fig. 4B). Common opening for esophagus, stomach and caecum located on digestive gland. Digestive gland dark beige, cone-shaped, being largest organ of visceral mass, occupying  $\sim 30\%$  of its volume; anterior portion about twice wider than posterior portion, inner face of gland sponge-like, bearing distinct main duct and various secondary ducts. Anus opening into anal papilla at center of gill circle,  $\sim 1/4$  of gill filament length (Fig. 3F).

**Genital system** (Fig. 6A–E) Located between buccal mass and digestive gland, mainly on right-dorsal side. Gonad immersed into digestive gland, difficult to distinguish between them. Hermaphrodite duct thin, long. Ampulla located on female gland, elongated and tubular. Prostate tubular, granular,  $2/3$  of length of ampulla, narrowing in vas deferens, with about half of length of prostate, expanding up to penis (Fig. 6A). Penis' muscle absent. Penis muscular, cylindrical and elongated, about half of length of prostate. Female gland well-developed, rounded, occupying  $\sim 20\%$  of reproductive system volume, divided into mucus gland ( $\sim 2/3$  of female gland, color beige), and albumen gland ( $\sim 1/3$  of anterior most region, dilated, irregularly shaped, color dark brown) (Fig. 6E). Oviduct occupying  $\sim 1/4$  of female gland volume. Uterine duct thin, relatively short, length  $\sim 1/6$  of vagina length, located at base of vagina, inserted in female gland near oviduct. Seminal receptacle rounded, as large as bursa copulatrix, length  $\sim 1/3$  of vagina length, connected to vagina near uterine duct through short stalk. Bursa copulatrix rounded, length  $\sim 1/3$  of vagina length, connected to vagina after seminal receptacle, also through short stalk. Vagina cylindrical, elongated, approximately  $\sim$ twice wider than penis, followed ventrally by prostate and located parallel to penis in genital opening. Pair of pyriform vaginal pouches  $\sim 1/2$  of bursa copulatrix, one on each side of vaginal opening, internally with white mass and chitinous spine (Fig. 6C). Gonopore on right side, anterior quarter of length of animal from head, located between foot and notum.



**Figure 6** *Doris ananas* reproductive system. **A.** Dorsal whole view, most structures uncoiled. **B.** Detail of penis view. **C.** Detail of vaginal pouches, dorsal view, dorsal portion of walls artificially removed to show inner spines. **D.** Female gland, dorsal view, uterine duct and oviduct present. **E.** Same, ventral view, ampulla and prostate connections present. Scales: 1mm.



**Figure 7** *Doris ananas* central nervous system. **A.** Dorsal view. Scale: 1mm. **B.** Ventral view. Scale: 1mm. **C.** Detail of buccal and gastroesophageal ganglia, ventral view. Scales: 0.5mm.

**Central nervous system** (Fig. 3B; 4A; 7A–C) Located dorsally to odontophore, mostly covered by blood gland. Pair of cerebral and pleural ganglia fused with one another. Pedal ganglia fused with cerebral and pleural ventrally, but not fused among themselves. Pedal commissure simple, surrounding esophagus and salivary glands, same length as fused ganglia (cerebral, pleural and pedal) (Fig. 7A–B). Buccal ganglia short, located ventrally to odontophore, between

radular sac and anterior portion of esophagus, connected to cerebral ganglia through long and slender connective tissue, united to gastro-esophageal ganglia by short connective tissue. Gastro-esophageal ganglia length about  $\sim 1/3$  of buccal ganglia length, circular (Fig. 7C). Rhinophoral (olfactory) ganglia bulb-shaped, connected to anterior portion of cerebral ganglia. Eyes dorsal, located on cerebral ganglia (Fig. 7A). Statocysts small and iridescent, located ventrally to pedal ganglia.

*Etymology* The name refers to the external similarity with a pineapple, one of the most common fruits in Mozambique. *Ananas* is the pineapple genus and it comes from the Tupi word *nanas* for the fruit.

*Ecology* Subtropical rocky reef, from 15 to 33 meters deep; throughout the whole year, with higher abundance in June, when the water temperature starts to drop from an average of 26°C to 24°C. Specimens are usually seen crawling on the reef, once, a specimen has been observed embedded in an unidentified yellow sponge with its gills retracted. Despite its large size, when embedded in the sponge, the species became well camouflaged and could be easily missed by divers. They are relatively common but have never been seen in great numbers, usually only one or two individuals at a time.

*Distribution* Confirmed from Kenya to South Africa, with possible records in Madagascar, Mayotte, Tanzania, Indonesia, Queensland and Marshall Islands (internet sources: [http://www.nudipixel.net/species/doris\\_sp/](http://www.nudipixel.net/species/doris_sp/); <http://seaslugs.free.fr/>; Gosliner, 1987; Gosliner *et al.*, 2011; present study).

## DISCUSSION

The external and internal anatomy of the species described here is typical of the genus *Doris* (Valdés, 2002), with the exception of the two vaginal pouches, each one with a spine, located very close to the vaginal opening (Figs 6A, C). These vaginal pouches have never been recorded for the genus before.

Because of its originality, the terminology of vaginal pouches appears to be adequate. In the literature, there are two other denominations for genital accessory structures: accessory gland and

vestibular gland (Valdés *et al.*, 2010). *Doris ananas* has the vaginal pouches positioned in the vaginal opening, and may have distinct functions different from those in the literature. Other species from different families may also have genital accessory structures. For example, *Geitodoris pusae* (Marcus, 1955) has a single pouch around the vagina without a spine, considered a vestibular gland (Marcus, 1955; Alvim & Pimenta, 2014); and, *Platydorid dierythros* Fahey & Valdés, 2003 has an accessory gland connected to the penial side (Fahey & Valdés, 2003).

Two genera of Dorididae have similar structure in the atrium with the vagina: *Goslineria* and *Pharodoris*, both from the West-Pacific deep water. *Pharodoris* has two large glands containing a long, bifid, rigid spine, however it has a characteristic elevated branchial sheath (Valdés, 2001) not present in *Doris ananas*. The only species belong to the genus *Goslineria*, *Goslineria callosa* has several large sacs in the atrium, each containing a long, simple and flexible spine (Valdés, 2001). A notable difference between *Doris* and *Goslineria* is the prostate, which is tubular in *Doris* and flattened in *Goslineria*.

Despite having armed appendices in the vagina in common, *D. ananas* does not appear to belong to the genera *Goslineria* or *Pharodoris* because of the quantity of further similarities with *D. verrucosa* and several differences from species of those genera (summarized in Table 1).

We decided to compare the new species with the type species *Doris verrucosa*, because its recent review provides more complete information of the anatomy (Lima & Simone, 2015). Furthermore, we compare the new species with the common Indo-Pacific species: *Doris granulosa* (Pease, 1860) and *Doris immonda* Risbec, 1928, based on literature data (Valdés, 2002).

*D. ananas* differs from *D. granulosa* and *D. immonda* in lacking lateral prolongations, with only one pore on each side of the mouth (Fig. 3A). It is interesting to note that *D. verrucosa* has the lateral prolongations on the mouth, which are well developed and with triangle-shaped and lateral groove on each one, while *D. granulosa* and *D. immonda* present blunt prolongation.

The penis' muscles of *D. ananas* are absent, like in other species of *Doris*, such as *D. granulosa* and *D. immonda* (Marcus, 1955; Valdés, 2002; Camacho-García & Gosliner, 2008), further confirming the generic statement.

**Table 1** Some comparative anatomical data among the type species of the genus *Doris*, *Goslineria* and *Pharodoris* with *Doris ananas*.

	<i>Doris ananas</i>	<i>Doris verrucosa</i>	<i>Goslineria callosa</i>	<i>Pharodoris diaphora</i>
<b>Perfoliate rhinophores</b>	20–25 lamellae	13 lamellae	22 lamellae	30 lamellae
<b>Branchial leaves</b>	6–7 tripinate	15–17 unipinate	7 tripinate	5 bipinate
<b>Branchial sheath</b>	Bearing small tubercles	Eight elongated tubercles	Somewhat elevated bearing numerous tubercles	Very elevated
<b>Oral tentacles</b>	Absent	Two triangular prolongation on each side of the buccal area	Absent	Two triangular prolongation on each side of the buccal area
<b>Notum/foot</b>	Dorsum margin wider than foot	Dorsum margin wider than foot	Dorsum margin as wide as foot	Dorsum margin narrower than foot
<b>Radular formula (animal size)</b>	34×43.0.43 (21mm long)	32×40.0.40 (9mm long)	39×42.0.42 (21mm long)	34×39.0.39 (16mm long)
<b>Blood gland</b>	Divided (two)	undivided	undivided	Divided (two)
<b>Color of rhinophores and gill</b>	Different color (black) of the dorsum (yellow);	Same color as the dorsum	Same color as the dorsum	Same color as the dorsum
<b>Outermost lateral teeth</b>	Denticles absent	Denticles absent	Small denticles	Small denticles
<b>Prostate shape</b>	Tubular and glandular	Tubular and glandular	Flattened	Tubular
<b>Vaginal pouches</b>	Two vaginal pouches with spine	Absent	Several large sacs, each containing a long, simple, flexible spine	Two large glands, each containing a long, bifid, rigid spine
<b>Papilla of nephrostome</b>	Present	Absent	Absent	Absent
<b>Pedal commissure</b>	Simple	Simple	Simple	With a visible triple division in connection with the left pedal ganglia
<b>Optical ganglia</b>	Not pedunculated	Not pedunculated	Pedunculated	Pedunculated

The blood gland of *D. ananas* seems to be divided with the posterior portion three time greater than the anterior one (Fig. 3B), as with *D. immonda*, while with *D. verrucosa* the blood gland is undivided and covers the whole nervous system. Normally, the species of the family Discodorididae have a divided blood gland (Dayrat, 2010).

Another striking difference of *D. ananas* is the presence of a papilla in the nephrostome (Fig. 3F), which does not appear in *D. verrucosa*, *D. granulosa* and *D. immonda*. The renal vesicle of *D. ananas* is as large as ventricle (Fig. 3B), while the ratio of vesicle/ventricle is normally  $\frac{1}{4}$  the size of ventricle in *D. verrucosa*. The gill filaments of *D. ananas* are tripinnate (Fig. 3F), as well as *D. granulosa* and *D. immonda*, and not unipinnate as in *D. verrucosa*.

Despite of the geographic distance between *D. ananas* with the typo species *D. verrucosa*, as well as, anatomic differences with other known doridids, mainly in the genital organs, a conservative approach has been applied here in considering it in the genus *Doris*. However, a more complete taxonomic and molecular revision of the group might prove different.

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Científico e Tecnológico (CNPq) proc. 159446/2012-0.

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