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Nudibranchia) from southern Mozambique*

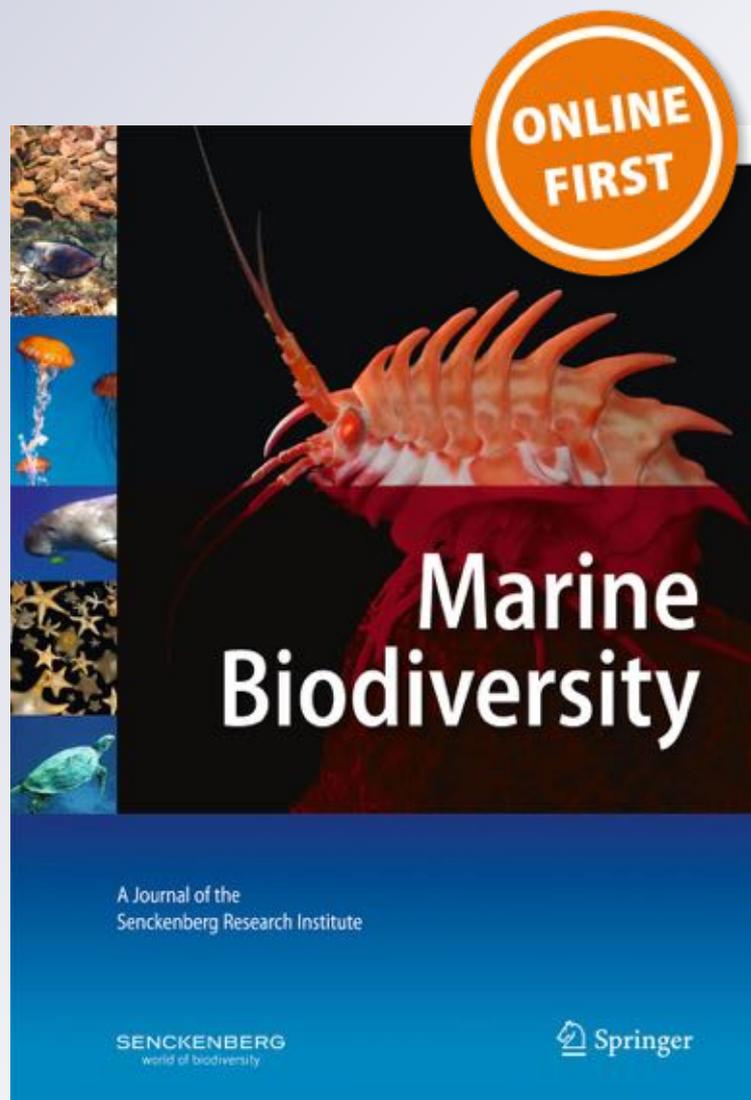
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Marine Biodiversity

ISSN 1867-1616

Mar Biodiv

DOI 10.1007/s12526-017-0752-x



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Two new species of the genus *Aldisa* Bergh, 1878 (Gastropoda, Heterobranchia, Nudibranchia) from southern Mozambique

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Abstract The genus *Aldisa* Bergh, 1878 is relatively poorly studied. Up to now, no species have been described from the Western Indian Ocean. Two new species of the genus *Aldisa* are described from Zavora (Mozambique), *Aldisa fragaria* sp. nov. and *Aldisa zavorensis* sp. nov. Both species are characterized by having two oval depressions on the dorsum, a red mantle with yellowish-white patches and red rhinophores. Moreover, *Aldisa fragaria* sp. nov. has large round tubercles on the dorsum tipped in black and a large flattened oral glandular mass, while *A. zavorensis* sp. nov. has tan gills, rounded red tubercles, branchial and rhinophores sheaths distinctively serrated, and a large oral gland mass with a semi-spherical shape. Partial sequences of mitochondrial (COI and 16S) and nuclear (H3) markers of both species are provided. Both anatomical and molecular data confirm that these species are different from other known species of the genus.

Keywords *Aldisa* · Cadlinidae · Nudibranchia · Heterobranchia · New species · Mozambique

Introduction

The taxonomic placement of the genus *Aldisa* Bergh, 1878 has a long and complex history. Bergh (1878) described *Aldisa* as a genus of the family Dorididae Rafinesque, 1815 characterized by a typical radula composed by extremely elongate teeth with a serrated margin and spatulate apex. Later, because of its aberrant radula teeth, the genus *Aldisa* was placed in its own family, Aldisidae (Odhner 1939). Since then, authors have included and excluded other genera in this family (Marcus and Marcus 1967; Marcus 1976; Franc 1968), but finally decided that the family Aldisidae was monotypic (see Millen and Gosliner 1985 for details). In 2002, Valdés provided a phylogenetic analysis of the cryptobranch dorids based on anatomical characters (Valdés 2002). According to his analysis, the genus *Cadlina* Bergh, 1878 was a sister clade of the genus *Chromodoris* Alder and Hancock, 1855, while the genus *Aldisa* was more closely related to other dorids such as the genera *Doris* Linnaeus, 1758, *Pharodoris* Valdés, 2001 and *Aphelodoris* Bergh, 1879. Contradictorily, recent phylogenetic analyses based on molecular data have supported the hypothesis of a sister relationship of *Aldisa* spp. and *Cadlina* spp. (Johnson 2011; Johnson and Gosliner 2012). As a result, the genus *Cadlina* was removed from the family Chromodorididae Bergh, 1891 and placed together with the genus *Aldisa* in the resurrected family Cadlinidae Bergh, 1891 (Johnson 2011).

The genus *Cadlina* is characterized by having spicules and small tubercles on the mantle, large glands forming a submarginal row or multiple rows, simple pinnate gills tending to be secondary bi- or tripinnate, buccal armature often with bifid rodlets, oral tube and oral bulb of similar size, radular shape similar to the

Communicated by V. Urgorri

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genus *Chromodoris* but with rachidean teeth, most species having an armed penis and the seminal receptacle opening off the exogenous sperm duct instead of the vagina (Rudman 1984). The genus *Aldisa* typically has tubercles on the dorsum, often presents dorsal oval pits, mantle with spicules, multipinnate gills, smooth labial armature, atypically long and thin teeth with denticulation on the top and a lateral and armed penis (Valdés 2002). While the genera *Cadlina* and *Aldisa* share some similarities, such as the mantle covered with spicules and tubercles as well as armed penis in most of the species, the reproductive system and the radula are strikingly different. The anatomical details that make these two genera closely related are not yet understood.

The new placement of the genus *Aldisa* within the family Cadlinidae was based on molecular analysis focused on the family Chromodorididae (Johnson 2011). Thus, that study had a clear bias in the number of *Aldisa* spp. versus *Cadlina* spp. The analysis included 11 species of the genus *Cadlina* (22 specimens), and only 2 species of the genus *Aldisa* (1 specimen of *Aldisa banyulensis* Pruvot-Fol, 1951 and 1 specimen of *Aldisa* sp.). Johnson (2011) mentioned that the genera *Aldisa* and *Cadlina* share some common characteristics such as tubercles on the mantle and a differentiated stomach; however, details of the anatomical features that supported this clade were not cited. Moreover, as shown by Johnson (2011), taxon-sampling might have a great influence in the phylogenetic results; therefore, adding more specimens of the genus *Aldisa* is important to confirm the taxonomical status of the family.

One of the main difficulties with studying *Aldisa* spp. is that most of them are relatively rare or nocturnal (Millen and Gosliner 1985). Hence, several species remain undescribed (Debelius and Kuitert 2007; Coleman 2008; Gosliner et al. 2008, 2015). Gosliner et al. (2015) suggested that there are at least nine undescribed species in the Indo-Pacific. Moreover, the number of specimens of the genus *Aldisa* sequenced to date is very low (only four species have been included in GenBank). In this paper, we describe two new species of the genus *Aldisa* from southern Mozambique and provide partial sequences of mitochondrial (COI and 16S) and nuclear (H3) markers for both species, contributing to the increase in baseline knowledge necessary to understand the phylogenetic relationships and characteristics of the family.

Material and methods

Several specimens of two putative undescribed species of the genus *Aldisa* were examined. The first author collected all of them by snorkelling or SCUBA diving in the same area, a rock pool located in Zavora Bay, Inhambane Province, Mozambique (24°31'09"S, 35°12'27"E). Individual information on date of collection and size are given under the descriptions. After collection, individuals were photographed and relaxed in a solution of MgCl₂ 7% and preserved in 96% ethanol or 4% formalin.

Anatomy

Specimens were dissected under a stereomicroscope by dorsal incision. Special attention was giving to the buccal bulb and reproductive system. Drawings of the dissected specimens were made with the assistance of a camera lucida and improved in Photoshop CS5. Scanning electronic microscope (SEM) photographs were taken of radulae and penis. For the radulae, the buccal mass was immersed in a solution of 10% sodium hydroxide to dissolve soft tissues, washed in water and mounted for SEM. For the penis, the penial bulb was separated and the penis was critical point-dried before being mounted for SEM.

Type specimens were deposited at the Museu Nacional de História e da Ciência de Lisboa (MB). Duplicates, when available, were deposited at the Museu de História Natural de Maputo (MHN, catalogue number not available), Mozambique.

Molecular markers

DNA extraction, amplification and sequencing

Two specimens of *Aldisa* sp. (1) and one specimen of *Aldisa* sp. (2) were sequenced (Table 1). Partial sequences of three molecular markers were obtained: the mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA and the nuclear Histone 3 (H3). All three markers were successfully amplified for all specimens except the 16S for one specimen (MB28–004392).

DNA samples were extracted from a small piece of the foot with DNeasy Blood and Tissue Kit (Qiagen) using universal primers from Folmer et al. (1994) for COI, Palumbi (1991) for 16S and Colgan et al. (2000) for H3. PCRs were performed in 25- μ l reactions with 2 μ l of DNA template. COI amplifications were performed with an initial denaturation for 3 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 46 °C and 1 min at 72 °C with a final extension of 5 min at 72 °C. 16S amplifications were performed with an initial denaturation for 3 min at 94–95 °C, followed by 40 cycles of 30 s at 94 °C, 30–45 s at 48–51 °C (annealing temperature), 1–2 min at 72 °C, with a final extension of 5–7 min at 72 °C. H3 amplifications were performed with an initial denaturation for 3 min at 95 °C, followed by 25 cycles of 45 s at 94 °C, 45 s at 50 °C (annealing temperature) and 2 min at 72 °C, with a final extension of 10 min at 72 °C. Successful PCR products were purified and sequenced by Macrogen.

Sequences were edited, aligned and concatenated using Geneious R6 (v.6.1.8) (<http://www.geneious.com>, Kearse et al. 2012). All sequences were checked for contamination using Blast in GenBank (Altschul et al. 1990). Alignments were generated by MUSCLE using default sets (Edgar 2004). For protein-coding, sequences alignment translations

Table 1 Specimens used in this study with respective voucher number and accession number

Specimen	Voucher no.	16S	COI	H3
<i>Aldisa fragaria</i> sp. nov.*	MB28-004392	–	MF288004	MF327390
<i>Aldisa fragaria</i> sp. nov.*	MB28-004393	MF288006	MF288003	MF327389
<i>Aldisa zavorensis</i> sp. nov.*	MB28-004397	MF288007	MF288005	MF327391
<i>Aldisa banyulensis</i>	–	–	AY345039.1	–
<i>Aldisa albotrossae</i>	–	KP871679.1	KP871632.1	KP871655.1
<i>Aldisa smaragdina</i>	–	KJ022806.1	KF992175.1	KJ022914.1
<i>Aldisa</i> sp.	CASIZ175733	EU982818.1	–	–
<i>Aldisa zetlandica</i>	–	–	KU695603.1	–
<i>Ardeadoris egretta</i>	CASIZ157481	EU982762.1	EU982713.1	–
<i>Cadlina sparsa</i>	CASIZ182932	EU982776.1	EU982726.1	–
<i>Cadlina rumina</i>	CASIZ175456	EU982775.1	EU982725.1	–
<i>Chromodoris magnifica</i>	CASIZ144119	JQ727731.1	JQ727852.1	–
<i>Discodoris cebuensis</i>	CASIZ185141	KP871687.1	KP871639.1	KP871663.1
<i>Hypselodoris zephyra</i>	CASIZ175555	JQ727797.1	JQ727905.1	–
<i>Berthella sideralis</i>	–	AJ225181.1	AJ223257.1	–

*Specimens sequenced for this study, all others sequences were obtained from GenBank

into amido acids were carried out in Genious using the translation tool (Genetic Code: Invertebrate Mitochondrial) to confirm accuracy. We tested the 16S alignment with all gap regions and excluded most of them. In the final analyses, we used the one excluding the gaps because, despite both alignments showing similar results for the BL analyses, the ML tree was poorly resolved when the gaps were included.

Saturation testing was carried out by plotting the absolute number of transitions and transversions at each condon position against p distance in an Excel plot graph. In order to compare the genetic distances between species, the pairwise uncorrected p distance for COI was calculated using MEGA (v.6.06-mac). Finally, all sequences were deposited in Genbank. Accession codes and museum vouchers are shown in Table 1.

Phylogenetic analyses

A phylogenetic tree was carried out using Bayesian inference and Maximum likelihood (ML) to verify the relationship between the new species and others *Aldisa* spp. available in GenBank. We tested all the marks separately and combined. The best-fit evolutionary model was chosen in JModeltest (v.2.1.7) using AIC selection (Akaike 1974). The evolutionary model obtained for the concatenated analysis (COI+16S) was TVM+I+G. Bayesian Inference (BI) analysis was accessed using the software MrBayes v.3.2.6 and 6 substitutions (nst = 6) (Ronquist and Huelsenbeck 2003). The analysis ran for 5,000,000 generations MCMC; the first and last 1250 generations were discarded. The node support for the ML analysis was assessed with non-parametric bootstrap (BS) with 5000 replicates, random starting trees and parameters estimated according to the model selected in J Modeltest. Mr. Bayes and

ML tree were visualized, collapsed (PP \geq 0.90, BS \geq 75) and edited in TreeGraph (v.2.7.1; Müller and Müller 2004). Final editions were carried out in Photoshop CS5.

Results

SYSTEMATIC DESCRIPTIONS

Class GASTROPODA Cuvier, 1797
 Subclass HETEROBRANCHIA Burmeister, 1837
 Order Nudibranchia Cuvier, 1817
 Suborder Euctenidiacea Tardy, 1970
 Infraorder Doridacea Thiele, 1931
 Superfamily Doridoidea Rafinesque, 1815
 Family Cadlinidae Bergh, 1891
 Genus *Aldisa* Bergh, 1878
Aldisa fragaria sp. nov.
 (Figs. 1a, b, c,d, e, f, 2a, b, c, 3a, b, c, d)

Diagnosis

Mantle red with whitish-yellow patches. Rhinophores and gills red. Large round red tubercles on dorsum. Two oval depressions (pits) on dorsum ringed by tubercles. Numerous elongate teeth, which are very thin, serrated on sides and folded on tips. Armed penis.

Differential diagnosis

Tubercles tipped in black. Large flattened oral glandular mass.

Derivatio nominis. The specific name refers to the external similarity with strawberries (*Fragaria* spp.).

Material examined

Holotype, depth 1 m, 37 mm in length alive (MB28–004392), 22 Apr. 2015, preserved in ethanol 96%, GenBank (pending number), dissected and sequenced, penis and radula mounted for SEM; Paratype 1, depth 0.3 m, 34 mm in length alive (MB28–004393), 05 Jan. 2014, preserved in alcohol 96%, GenBank (pending number), dissected and sequenced, penis and radula mounted for SEM; Paratype 2, depth 1 m, 25 mm in length alive (MB28–004394), 05 Nov. 2010 preserved in 4% formalin, dissected; Paratype 3, depth 1 m, 24 mm in length alive (MB28–004395), 26 May 2013, preserved in alcohol 96%, dissected; Paratype 4, depth 1 m, 33 mm in length alive (MB28–004396), 26 May 2014, preserved in 96% alcohol, dissected; Paratype 5, one exemplar deposited in the Natural History Museum of Maputo (no voucher number available); depth 2 m, 10 Dec. 2011, 14 mm in length preserved (immature), preserved in 4% formalin, not dissected..

Type locality: Zavora Bay, Inhambane Province, Mozambique (24°31'09"S, 35°12'27"E).

This species has been registered in ZooBank under the name *Aldisa fragraria* sp. nov. urn:lsid:zoobank.org:act:B9D75718-1DF0-4903-A849-D9795C0C4B4F

Description

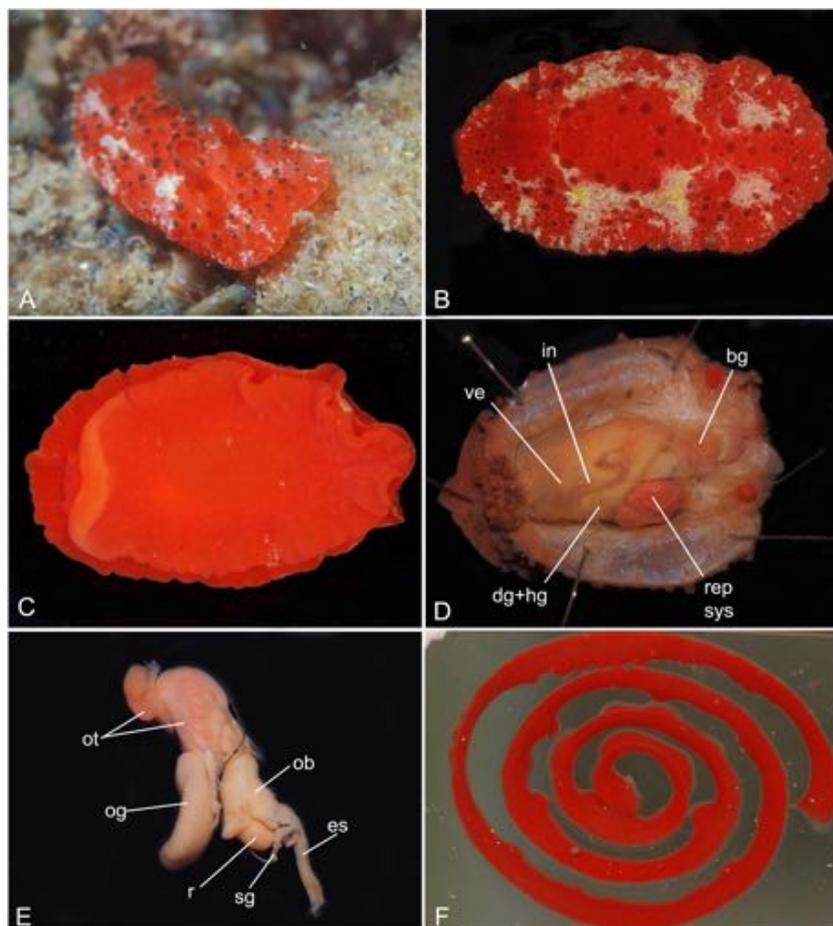
External anatomy (Fig. 1a–c). The body shape is oval, rigid, with two depressions on the dorsum. Each depression is ringed by tubercles. The tubercles are rounded, larger on the dorsum but smaller closer to the margin of the individuals (Fig. 1a, b). Under a dissecting microscope ($\times 250$), minute spicules can be seen covering the mantle. The oral tentacles are short and oval, near the mouth (Fig. 1c). The perfoliate rhinophores bear from 13 to 19 lamellae with slightly elevated sheaths. There are five bipinnate and retractile branchial leaves located dorsally in the posterior region of the dorsum. The anus is mid-dorsal, located in the centre of the branchial tuft. The foot is narrower than the mantle.

Coloration. The mantle is red with creamy-yellow patches on the sides. The tubercles are red with the top dark brown to black. A thin creamy-yellow ring often surrounds the tubercles. The rhinophores sheaths are red with creamy edges. The rhinophores are completely red. The branchial leaves are red. The underside of the mantle and the foot are also red (Fig. 1a–c).

Internal organs coloration. One specimen (MB28–004392) was dissected on the same day of collection

Fig. 1 *Aldisa fragraria* sp. nov.:

a living animal in the field (MB28–004396, Paratype 4); **b** dorsal view (MB28–004393, Paratype 1); **c** ventral view (MB28–004393, Paratype 1); **d** dorsal view showing internal coloration (MB28–004392, Holotype); **e** buccal mass showing internal coloration (MB28–004392, Holotype); **e** egg mass (MB28–004394, Paratype 2). *bg* blood gland; *dg + hg* digestive gland complex + hermaphrodite gland; *es* oesophagus; *in* intestine; *ob* oral bulb; *og* oral gland mass; *ot* oral tube; *r* radula; *rep sys* reproductive system; *sg* salivary gland; *ve* ventricle



allowing us to observe the internal coloration (Fig. 1d, e). The blood gland is salmon in colour (Fig. 1d). The oral tube is salmon. The oral bulb, oral glandular mass and radular sac have a creamy coloration. The salivary glands are light cream (Fig. 1e). The female gland is dark salmon, the prostate yellowish, the deferent duct and penis are white (not shown). The vagina and receptacle seminal are creamy and the bursa copulatrix salmon with grey (not shown). The digestive gland, the ventricle and intestine are yellowish.

General internal anatomy (Figs. 1d, e, 2). A large blood gland is located in front of the central nervous ring (Fig. 2a). The eyespots are connected to the cerebral ring by a short optic nerve, visible when the blood gland is removed. There is a distinctive large flattened oral glandular mass connected to the distal part of the oral tube. Such glandular mass covers most of the ventral surface of the oral bulb. The radular sac is round. There are two salivary glands connected at each side of the oesophagus. They are triangular at the base and thin at the end (Figs. 1e, 2b). The stomach is slightly dilated, but not clearly differentiated (Fig. 2a). The muscular oral tube is larger and wider than the oral bulb (Figs. 1e, 2b).

Radula (Fig. 3a–d). The radula is typical of *Aldisa* spp. with extremely narrow teeth. As in other species of the genus (e.g. *A. binotata* Pruvot-Fol, 1953; *A. smaragdina* Ortea, Perez and Llera, 1982; *A. pikokai* Bertsch and S. Johnson, 1982; *Aldisa andersoni* Gosliner and Behrens,

2004), the radular formula was impossible to determine, as the teeth are too numerous, thin and overlapping (Fig. 3a–c). In the 24-mm specimen (MB28–004395), the teeth measure around 420 μm in length. Each tooth bears from 10 to 15 long sharp lateral serrations (Fig. 3c). The teeth are triangular in the base and the apex is curved and has from 4 to 10 folded long denticles (Fig. 3d).

Reproductive system (Fig. 2c). The reproductive system is triaulic. The hermaphrodite duct leads to a bent ampulla. The ampulla is located ventrally, close to the prostate. The ampulla narrows in a short postampullatory duct, which forks into the prostate and oviduct. The oviduct enters into the female gland. The prostate has a U-shape with two different parts; the portion of the prostate connected to the postampullatory duct is narrower and darker than the second part of the prostate. The prostate narrows into the muscular part of the deferent duct. The distal part of the deferent duct leads to a small penial bulb, which opens in a common atrium with the vagina. The vagina is thin and long, forking into three: a very short uterine duct, a short duct that leads to the bursa copulatrix and a duct that ends in the receptaculum seminis. The bursa copulatrix is oval and bigger than the bean-shaped receptaculum seminis. The penis is armed with 11 rows of at least 18–24 spines each (Fig. 4). Each spine has a triangular shape; the base is wide, narrowing off to a thin tip (Fig. 4a, b). Some of the basal spines are fused on the base and bifurcate in two hooks (Fig. 4d).

Fig. 2 *Aldisa fragraria* sp. nov.: general internal anatomy of the Holotype (MB28–004392). **a**, dorsal view; **b**, buccal mass; **c**, reproductive system. *am* ampulla; *at* aortic trunk; *bc* bursa copulatrix; *bg* blood gland; *dd* deferent duct; *dg + hg* digestive gland complex + hermaphrodite gland; *es* oesophagus; *fg* female gland; *in* intestine; *mo* mouth; *ob* oral bulb; *og* oral gland mass; *ot* oral tube; *p* penis; *pr* prostate; *r* radular sac; *rep sys* reproductive system; *rv* renal vesicle; *sg* salivary gland; *st* stomach; *rs* receptaculum seminis; *ud* uterine duct; *v* vagina; *ve* ventricle

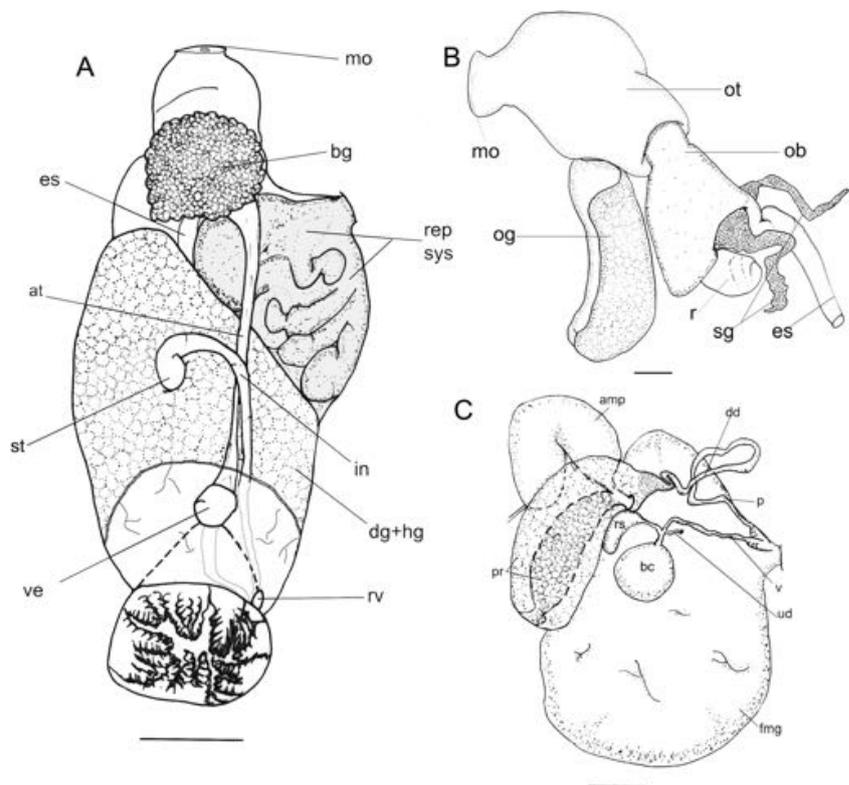
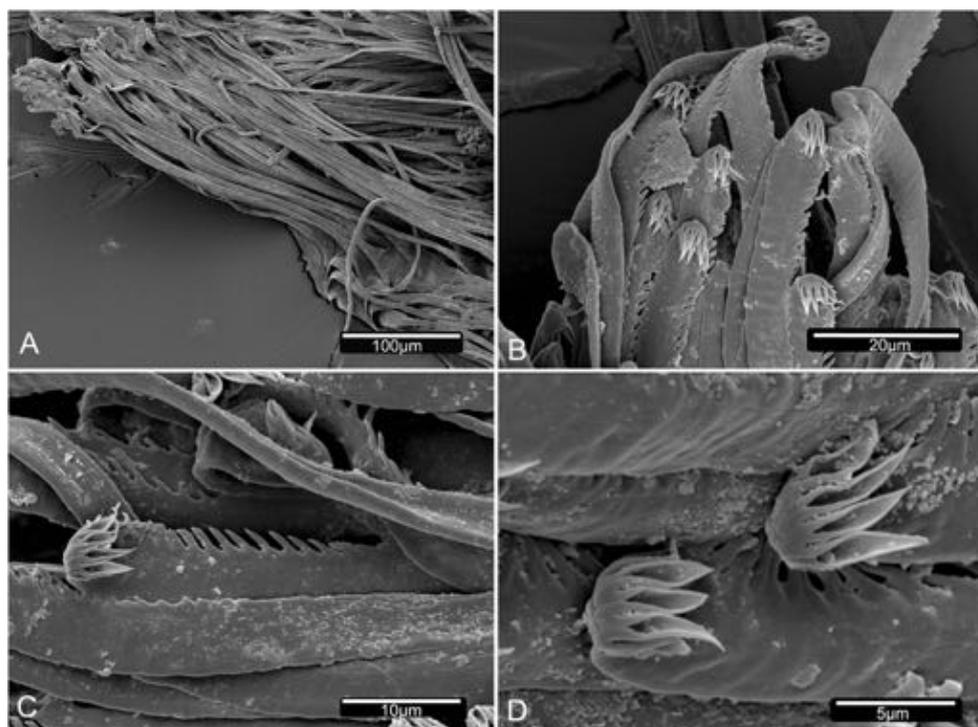


Fig. 3 *Aldisa fragaria* sp. nov., Zavora, Mozambique (MB28–004395, Paratype 3) SEMs of radula: **a** outer side showing overlapping of the teeth; **b** radular teeth; **c** teeth showing lateral serrations; **d** detail of the apex of the teeth



Natural history

This uncommon species was found in a large rock pool from 0.3 to 2 m deep. The egg mass colour is red (Fig. 1f).

Distribution

Mozambique (Gosliner et al. 2015, as *Aldisa* sp. 3; present study).

Aldisa zavorensis sp. nov.

Material examined

Holotype, depth 0.5 m, 16 Apr. 2014, 19 mm in length alive (MB28–004397), preserved in ethanol 96%, GenBank (pending number), dissected and sequenced, penis mounted on slide and radula mounted for SEM; paratype: depth 1 m, 08 Feb 2012, 22 mm in length alive (MB28–004398), preserved in 4% formalin, dissected and radula mounted for SEM.

Type locality: Zavora Bay, Inhambane Province, Mozambique (24°31'09"S, 35°12'27"E).

Diagnosis

Red mantle and rhinophores. Yellowish specks. Tan gills. Rounded tubercles. Smaller tubercles close to the margin of the specimens. Two pits on dorsum. Branchial and rhinophore sheaths with deeply serrated borders. Elongated teeth.

Differential diagnosis

Uniformly tan branchial leaves. Large oral gland mass with semi-spherical shape.

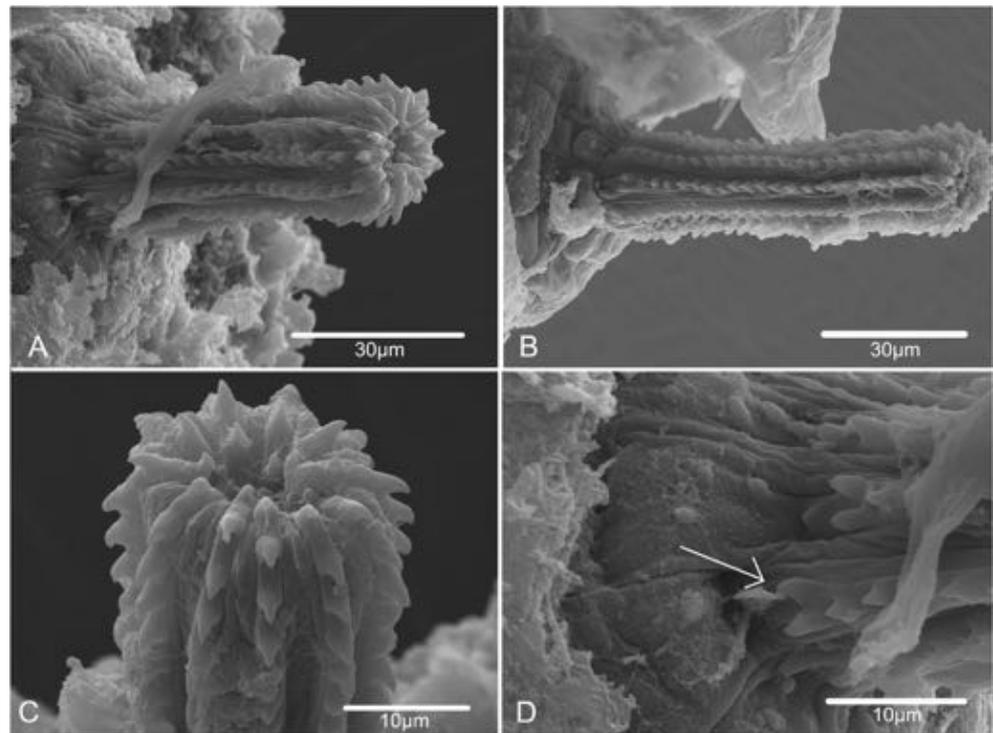
Derivatio nominis. The specific name “zavorensis” refers to the tidal rock pool of Zavora, the location where the holotype was found. The rock pool is a natural tidal pool approximately 110 m in diameter where more than 80 species of nudibranchs have been found (personal observation). Unfortunately, the high biodiversity of this area is currently under threat due to the illegal use of gill nets inside the pool. We named this species after the rock pool with the hope that it will bring more attention to this area and to the importance of conserving this fragile, easily accessible and rich environment.

This species has been registered in ZooBank under the name *Aldisa zavorensis* sp. nov. urn:lsid:zoobank.org:act:2B442691-7796-4879-8E65-FEC3F70C5403

Description

External anatomy (Fig. 5a–e). The body shape is elongated oval, the profile is low and it is rigid to the ouch, with two depressions on the dorsum ringed by small tubercles. The dorsum is covered by round tubercles. The tubercles are small closer to the edge of the mantle, larger on the sides and slightly smaller on the top of the dorsum (Fig. 5a). The oral tentacles are very short and rounded. The perfoliate rhinophores bear from 15 to 18 lamellae with slightly elevated sheaths. The

Fig. 4 *Aldisa fragaria* sp. nov. SEM pictures of the penial spines: **a** general view of *A. fragaria* sp. nov. (MB28-004393, Paratype 1) penial spines; **b** general view of *A. fragaria* sp. nov. (MB28-004392, Holotype) penial spines; **c** top view of the penial spines; **d** detail of a basal bifurcated spine (MB28-004392, Holotype)



rhinophores and branchial sheaths have a deeply scalloped edge, forming small triangles (Fig. 5c–e). There are five bipinnate and retractile branchial leaves located in the posterior portion of the dorsum, posterior to the second pit. The anus is

mid-dorsal located in the centre of the branchial circle in the mantle. The foot is narrower than the mantle (Fig. 5b).

Coloration. The mantle is red with yellowish speckles on the dorsum, which concentrate in a few areas forming creamy-

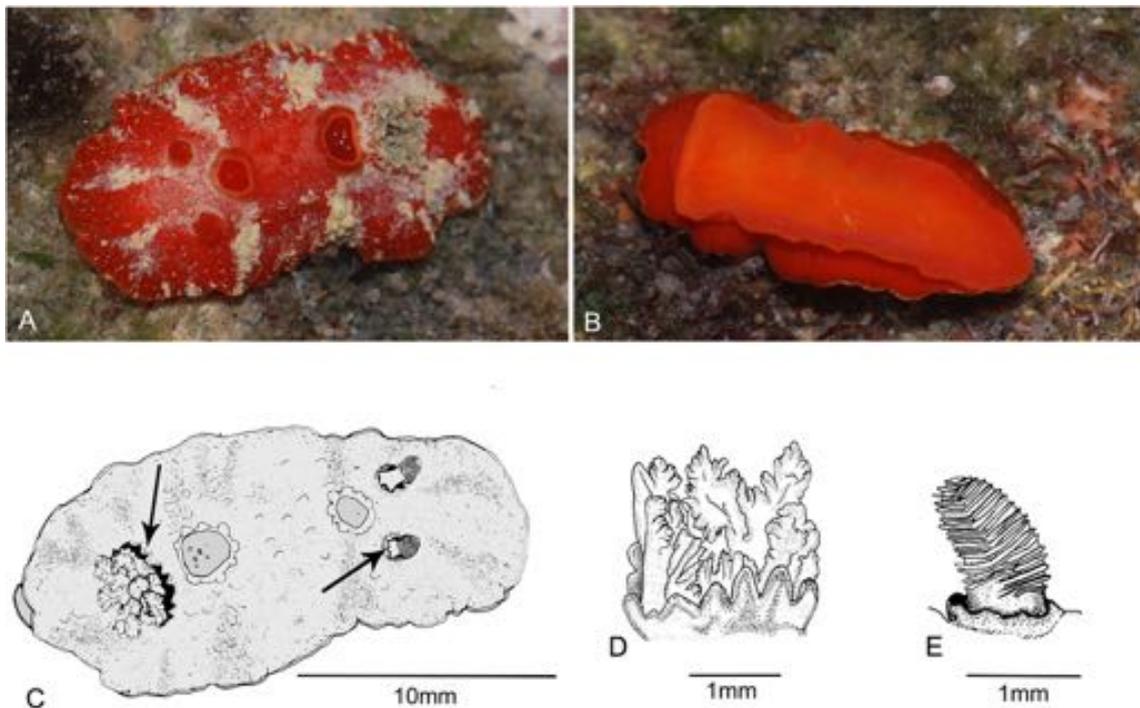
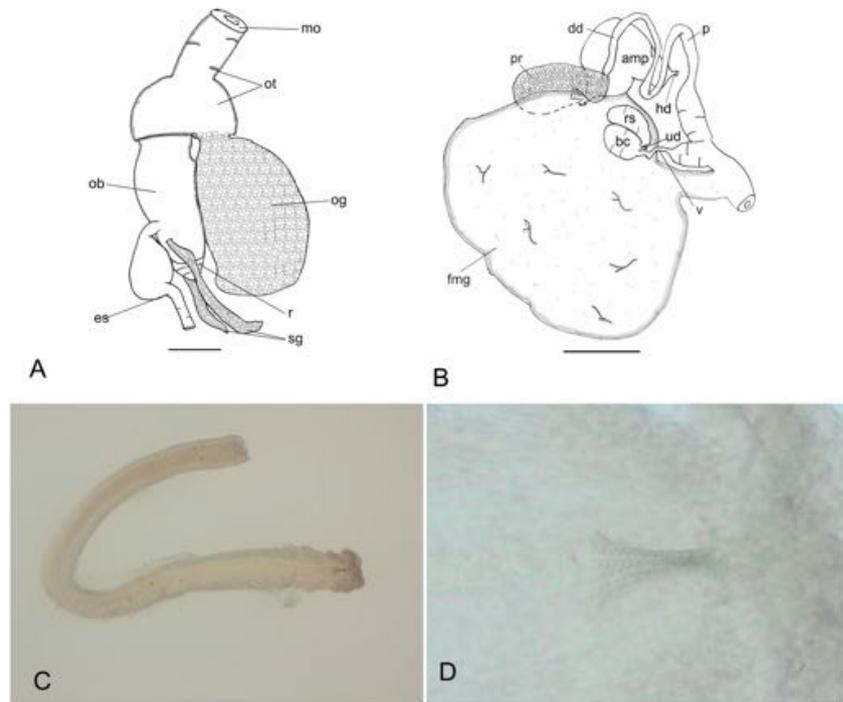


Fig. 5 *Aldisa zavorensis* sp. nov. (MB28-004398): dorsal (a) and ventral (b) view of the Paratype; c dorsal view showing details of the rhinophores and gill sheaths; d gill leaves and gill sheath details; e rhinophore and rhinophore sheath details

Fig. 6 *Aldisa zavorensis* sp. nov. internal anatomy of the Holotype (MB28-004397): **a** buccal mass; **b** reproductive system; **c** photography of the penial bulb; **d** detail of the armed penis. *am* ampulla; *bc* bursa copulatrix; *dd* deferent duct; *es* oesophagus; *fg* female gland; *hd* hemaphrodite duct; *mo* mouth; *ob* oral bulb; *og* oral gland mass; *ot* oral tube; *p* penis; *pr* prostate; *r* radular sac; *sg* salivary gland; *st* stomach; *rs* receptaculum seminis; *ud* uterine duct; *v* vagina



yellow patches. The edge of the mantle is orange-red. The tubercles are red. The rhinophores sheaths are red with creamy patches. The rhinophores are red. The red pits on the dorsum have orange edges (Fig. 5a). The branchial leaves are uniformly tan. The underside of the mantle is red with orange-red edges. The foot is orange-red (Fig. 5b).

General internal anatomy (Fig. 6a–d). The blood gland covers the nerve ring but not the eyes, which are connected by optical nerves. The oral tube is muscular and half-globular, with a large oral gland mass connected directly at its posterior side. The oral gland mass is quite thick and lies alongside the flat side of the oral bulb. The oral bulb has a semi-hemispheric shape, flat on the dorsum side and oval on the ventral part with a small round radular sac. There are two flat salivary glands connected at each side of the oesophagus (Fig. 6a). The stomach forms a small caecum clearly visible on the surface of the digestive gland.

Radula (Fig. 7a–d). The radular formula was impossible to determine. Around 62 rows could be seen through the dorsal view of the radula during dissection. The teeth are extremely long and overlapping (Fig. 7a, b). In the 19-mm individual, the teeth measured approximately 1.2 mm in length (Fig. 7b). The teeth are triangular at the base and very thin, tending to fold throughout their length (Fig. 7b, c). Therefore, it was impossible to observe lateral serrations in the two radulae examined. The distal end is wider and folds at the tip. This end is curved and has from 4 to 6 folded long denticles (Fig. 7d).

Reproductive system (Fig. 6b). The reproductive system is triaulic. The hermaphrodite duct leads to a thick, bent ampulla.

The ampulla narrows into a very short postampullatory duct, which forks into the prostate and the oviduct. The oviduct enters into the female gland. The curved thick prostate narrows abruptly into the muscular part of the deferent duct. The distal part of the deferent duct leads to the penial bulb that opens in a common atrium with the vagina. The short vagina leads to the bursa copulatrix. The receptaculum seminis is connected to the bursa copulatrix by a very short duct. The uterine duct, very short, leads the bursa copulatrix between the receptaculum seminis and the vagina to the female gland. The bursa copulatrix and the receptaculum seminis are both oval and of similar size, the receptaculum seminis is slightly more curved than the bursa copulatrix (Fig. 6b). The penis is armed with diminutive hooks. Unfortunately, the penis was inverted and because of the fragility of the diminutive hooks, we decided to not risk losing the material by trying to carry out the technique of the critical point for the SEM. Instead, we took photographs under an optical microscope ($\times 1000$). The penis contains at least six rows of spines with 15–17 spines each (Fig. 6c, d).

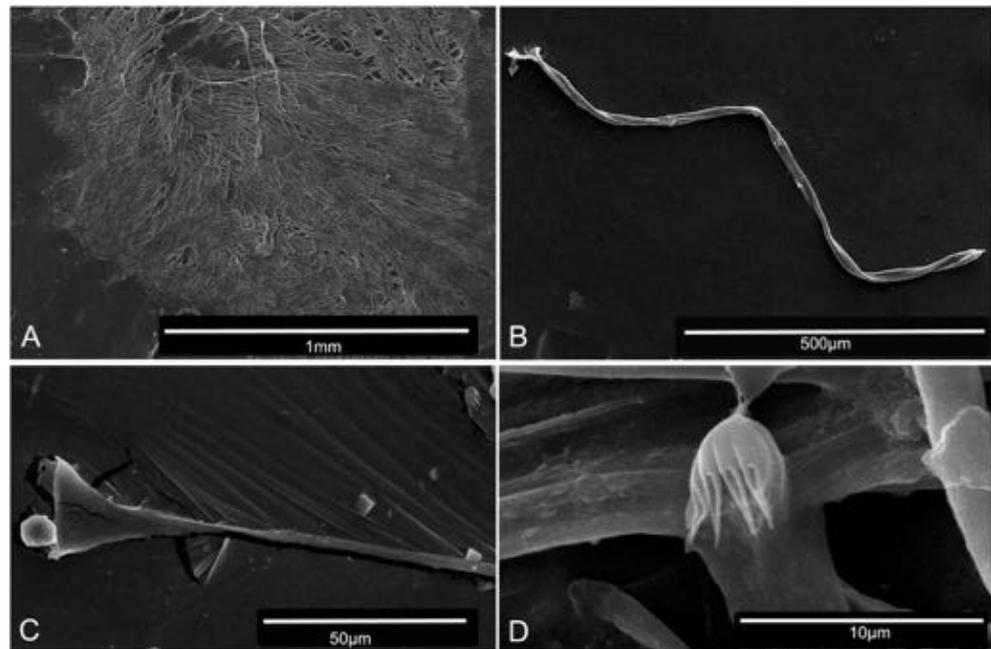
Natural history

This species is very rare and has only been seen twice during 5 years of sampling in Mozambique, both times in a larger rock pool around 0.5 m depth.

Distribution

Mozambique (present study).

Fig. 7 *Aldisa zavorensis* sp. nov., Zavora, Mozambique (MB28-004397, Holotype), SEMs of radula: **a** general view of the radula; **b** one isolated tooth; **c** triangular base of the teeth; **d** detail of the apex of the teeth



Molecular results

All codons were considered but no signal of saturation was observed for any of the marks (not shown). The tree topology with best resolution was obtained with the combined 16S and COI alignment (1055 pb) (Fig. 8). In general, the BI topology was similar to the ML topology; however, ML showed lower resolution. The monophyly of the genus *Aldisa* was not retrieved using only COI (not shown), but it was obtained using 16S (not shown), as well as the combined 16S and COI dataset (Fig. 8). The concatenate tree using the three marks did not show good resolution probably due the low number of H3 sequences (not shown).

The phylogenetic analyses confirms that the clade comprised by *Cadlina* spp. is sister to the clade constituted by *Aldisa* spp. Figure 8 shows that *A. zavorensis* sp. nov. is

closely related to an undescribed species of the genus *Aldisa* from GenBank (PP = 1, BL = 100) and these two species are sister to *A. fragaria* sp. nov. from Mozambique (PP = 1, BL = 96). Moreover, *A. zetlandica* (Alder and Hancock, 1854) and *A. albatrossae* Elwood, Valdés and Gosliner, 2000 from GenBank are closely related (PP = 0.96; BL = 79), and *A. smaragdina* Ortea, Pérez and Llera, 1982 and *A. banyulensis* from GenBank (PP = 1; BL = 100) seem to be the same species.

The *p*-uncorrected distance for COI between species of the genus *Aldisa* reached values from 10.05% (*A. smaragdina*/*A. banyulensis* vs. *A. albatrossae*) up to 16.58% (*A. fragaria* sp. nov. vs. *A. zetlandica*), except in the case of *A. smaragdina* vs. *A. banyulensis* (1.01%). The COI genetic divergence between *A. fragaria* sp. nov. and *A. zavorensis* sp. nov. is 15.08% (Table 2).

Fig. 8 Molecular phylogeny of available sequenced *Aldisa* spp. based on the combined dataset (COI + 16S) inferred by Bayesian analysis. Support values shown represent posterior probabilities from Bayesian interference and maximum likelihood (PP/ML). GB GenBank

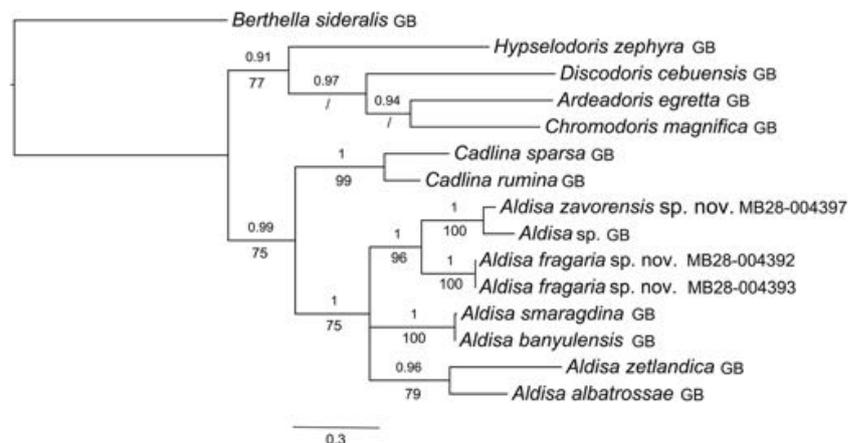


Table 2 Minimum pairwise uncorrected *p*-distances for COI between *Aldisa* spp.

	1	2	3	4	5
1. <i>A. smaragdina</i> Ortea, Perez and Llera, 1982	-	-	-	-	-
2. <i>A. banyulensis</i> Pruvot-Fol, 1951	1.01%	-	-	-	-
3. <i>A. albatrossae</i> Elwood, Valdés and Gosliner, 2000	10.05%	10.05%	-	-	-
4. <i>A. zetlandica</i> (Alder and Hancock, 1854)	12.06%	12.06%	11.06%	-	-
5. <i>A. fragaria</i> sp. nov.	12.31%	11.81%	14.32%	16.58%	-
6. <i>A. zavorensis</i> sp. nov.	13.57%	14.07%	14.57%	16.08%	15.08%

Discussion

So far, 18 species of the genus *Aldisa* have been described, but many are still waiting to be described and discovered (Debelius and Kuitert 2007; Coleman 2008; Yonow 2008; Gosliner et al. 2008, 2015). The Western Indian Ocean (WIO) is recognized as a hotspot for marine biodiversity with the second highest diversity of corals in the world. However, the diversity of marine invertebrates of the WIO is far less studied than in the Pacific Ocean (Obura 2012; Wilson and Kirkendale 2016). The two nudibranch species regarded here are the two first species of the genus *Aldisa* to be described with material collected from the Western Indian Ocean.

Anatomy

Several colours are found in the genus *Aldisa*, but two are dominant: whitish-blue and orange-red. Over eight species of red *Aldisa* spp. have been recorded for the Indo-Pacific (e.g. Gosliner et al. 2015), but only *Aldisa pikokai* has been described. Table 3 compares the different species of red *Aldisa* with those described here. *A. pikokai* was described from specimens from Hawaii Islands and clearly differs from all others described *Aldisa* spp. by having elevated reticulate ridges instead of the typical tubercles or papillae (Bertsch and Johnson 1982). *Aldisa fragaria* sp. nov. and *A. zavorensis* sp. nov. have rounded tubercles and two depressions on the dorsum, while *A. pikokai* has three craters and ridges, which are slightly elevated on the junctions (Bertsch and Johnson 1982).

Another species that resembles the species studied here is *Aldisa sanguinea* (Cooper 1873) described from specimens from San Diego Bay (eastern Pacific). This species has oval dark spots, which are similar to a depression (MacFarland 1905; Millen and Gosliner 1985). Millen and Gosliner (1985) reviewed *A. sanguinea* and state that the preserved animals sometimes had flattened tubercles. Both species described here are geographically far apart and have their tubercles prominent alive or preserved, and therefore could not be *A. sanguinea*.

The remaining red species with oval depressions on the dorsum are from the Eastern Atlantic and Mediterranean: *Aldisa binotata*, *Aldisa smaragdina* and *Aldisa banyulensis*.

A. banyulensis was described based on external anatomy of a single specimen from Banyuls-sur-Mer (France, Mediterranean) (Pruvot-Fol 1951). Millen and Gosliner (1985) refer to this species as having uniform notum without depression; however, both the illustration from Pruvot-Fol (1951; pl. 2, fig. 20) and the review by García et al. (1986) show a species with two depressions on the dorsum. This species differ externally from the one described here by having unipinnate gills and smaller tubercles.

A. banyulensis and *A. binotata* are very similar species. They are accepted as two separate species in the World Register of Marine Species (WoRMS) and by some authors (Calado and Urgorri 1999; Ávila et al. 2000), but as synonyms by others (Millen and Gosliner 1985; Cervera et al. 2006). Further anatomical and molecular studies are needed to clarify this issue. Independently, *A. fragaria* sp. nov. differs from all the above *Aldisa* spp. by the large black-tipped tubercles and from *A. zavorensis* sp. nov. by the tan colour of the gills.

A striking internal characteristic of *A. fragaria* sp. nov. and *A. zavorensis* sp. nov. is the unusual massive granular oral gland mass connected to the posterior end of the oral tube. This gland has not been mentioned for any of the other red *Aldisa* spp., except for *A. pikokai*. In both Mozambican species, the gland is located ventrally and extends beyond the oral bulb covering part of the radular sac. In *A. fragaria* sp. nov., the granular mass is ventrally flattened, while in *A. zavorensis* sp. nov., it is much thicker with a semi-hemispherical shape. This gland seems to be characteristic of many species of the genus (Gosliner and Behrens 2004). However, not much attention has been given to it, particularly in earlier descriptions. Millen and Gosliner (1985) briefly mention a glandular projection in the oral tube of *A. albomarginata* Millen in Millen and Gosliner, 1985, *A. tara* Millen in Millen and Gosliner, 1985 and *A. pikokai*, but no drawing or picture was provided. Later, Gosliner and Behrens (2004) described *A. andersoni* Gosliner and Behrens, 2004, highlighting and illustrating a large gland behind the oral tube. They then reviewed *A. williamsi* Elwood, Valdés and Gosliner, 2000 and *A. albatrossae* and found the same kind of gland but much smaller (Gosliner and Behrens 2004). The gland illustrated by Gosliner and Behrens (2004) for *A. andersoni* is considerably smaller than that observed in *A. fragaria* sp. nov. and *A. zavorensis* sp. nov. This aberrant gland is not mentioned in

Table 3 Comparative table of red-colored *Aldisa* spp. *Aldisa sanguinea*: Cooper (1863), Millen and Gosliner (1985); *Aldisa binotata*: Pruvot-Fol (1953), Millen and Gosliner (1985); *Aldisa banyulensis*: Pruvot-Fol (1951), Garcia et al. (1986); *Aldisa pikokai*: Bertsh and Johnson (1982); *Aldisa smaragdina* and *A. expleta*: Ortea, Pérez and Llera (1982); *Aldisa benguelae*: Millen and Gosliner (1985)

Species	Distribution	Coloration	Tubercles	Buccal Oral Mass	Rhinophores	Radula	Stomach with caecum	Penis hooks	Gills	Ecology
<i>Aldisa sanguinea</i> (f. G. Cooper, 1863)	Pacific side of North America	Red-orange with densely dotted black spots (original desc.) In Millen & Gosliner (1985), 2 mid-dorsal black spots	From conical to flattened	Not mentioned	Red-orange, 12–15 lamellae	70 × 100.0; 70–100	Yes	5–6 regular rows with approx. 14 spines per row	8–10 orange-red	Intertidal, peach--orange spawn
<i>Aldisa binotata</i> Pruvot-Fol, 1953	Morocco, North Atlantic Ocean	Red-orange with a pair of creamy diagonal stripes	Scattered rounded; two darker red spots in slight depression	Not mentioned	8–12 lamellae yellow, 4–5 deeply scalloped lobes around the margin of the rhinophores sheath	70 × 60.060 30–35 denticles along the top, tip is not folded	No	7–8 regular rows of recurved hooks 5 µm long	8–9 tripinnate, red--orange (5 to 9 in Millen and Gosliner 1985)	Intertidal. Red spawn
<i>Aldisa banyulensis</i> Pruvot-Fol, 1951	Eastern Atlantic and Mediterranean	Red-orange with light brown and white dots	No pit; around gills granulated, the rest of mantle fine granulated	Not mentioned	8–10 lamellae	60 × 40.0.40 large radular sac	Yes	7–13 µm; 6 regular row	5–8 unipinnate, red--orange	Subtidal 9–24 m
<i>Aldisa pikokai</i> Bertsch & S. Johnson, 1982	Hawaii	Red	Ridges instead of tubercles; 3 depressions	Yes	Red-orange	Teeth from 60 to 260 µm	Present inside the digestive gland	10 µm; 6 irregular row	Cream-white	Subtidal, Bright red, upright coil
<i>Aldisa smaragdina</i> Ortea, Pérez & Llera, 1982	Mediterranean to Atlantic Ocean (Canary Islands)	Red with tan dots and few white patches	Tubercles of different sizes, 2 darker round areas (pits)	Not mentioned	Red, 12–13 lamellae	50 rows impossible to estimate; 30 lateral serration and 4–5 at the tip 350 µm	Yes	12 rows; 17 curved hooks per row distributed irregular	5 tripinnate orange with white tip	Red spawn, intertidal
<i>Aldisa expleta</i> Ortea, Pérez & Llera, 1982	Mediterranean to Atlantic Ocean (Canary Islands)	Red with several small white patches	Conical with no pits	Not mentioned	Red, 16 lamellae	60 × 80.0.80; teeth 190–250 µm	Stomach no dilated	8 rows of curved hooks with 15 per row	5 tripinnate red with white tip	?
<i>Aldisa benguelae</i> Gosliner in Millen & Gosliner, 1985	Atlantic side of South Africa	Red-orange	Rounded, no pit	Not mentioned	9–10 lamellae, 4–11 tubercles around the margin of the rhinophores sheath	35 × 75.0.75; teeth from 80 to 750 µm; 28–40 serration on folded tip and side	Yes	12 rows of curved spines, 12 µm long and 8 µm wide at the base	6 tripinnate, red	Subtidal
<i>Aldisa fragaria</i> sp. nov.	So far only known from	Red often with whitish-creamy specks		Yes	13–19 lamellae	Indeterminate		11 rows of 18–24 hooks	5 tripinnate, red	Intertidal

Table 3 (continued)

Species	Distribution	Coloration	Tubercles	Buccal Oral Mass	Rhinophores	Radula	Stomach with caecum	Penis hooks	Gills	Ecology
<i>Aldisa zavorensis</i> sp. nov.	Mozambique So far only known from Mozambique	Red with whitish specks	Round, red with black top and two pits Round, red, two pits	Yes	15–18 lamellae	Indeterminate	Stomach slightly dilated Yes	Diminutive hooks, min. 6 rows with 14–17 hooks each	5 bipinnate, tan	Intertidal

early descriptions for many other species, such as *A. sanguinea* (MacFarland 1905, 1906), *A. cooperi* Robilliard and Baba, 1972 (Robilliard and Baba 1972), *A. expleta* Ortea, Pérez and Llera, 1982 (Ortea et al. 1982) and *A. puntallanensis* Moro and Ortea, 2011 (Moro and Ortea 2011). It is unlikely that a large gland such as the one found in *A. fragaria* sp. nov. and *A. zavorensis* sp. nov. went unnoticed by other authors, but as they can be quite small (Gosliner and Behrens 2004), it might need further review to confirm its absence in other species of the genus.

Phylogenetic analyses

To date, the number of specimens sequenced of the genus *Aldisa* is relatively low. The use of small taxon sampling can lead to a misinterpretation of the results, particularly with regards to complex questions (Tholeson, 2000; Johnson 2011). Therefore, the analyses performed here only give preliminary information about the relationship of the two new species and previously sequenced *Aldisa* spp., and in order to confirm the specific identity of both new species. Our study also reveals that the sequences of *A. smaragdina* and *A. banyulensis* deposited in Genbank appear to be from the same species. It is true that *A. smaragdina* and *A. banyulensis* share several common features (Ortea et al. 1982; Millen and Gosliner 1985); however, the type locality of *A. banyulensis* is the Banyuls sur mer (France, Mediterraneo), thus, without additional material from the Mediterranean and anatomical examination, which is beyond the scope of this study, it is not possible to synonymize them, but only to call attention to this issue.

Our results agree with Johnson (2011) supporting the relationship between the genera *Cadlina* and *Aldisa*. Genetically, the closest species to *A. zavorensis* sp. nov. appears to be an undescribed species from Malaysia (PP = 1, BS = 100).

Distribution

Specimens from other part of the world could not be examined. Thus, based only on external appearance, it is possible that both *A. fragaria* sp. nov. and *A. zavorensis* sp. nov. have an Indo-Pacific distribution. For *A. fragaria* sp. nov., photograph records of similar specimens exist for Reunion Island (http://seaslugs.free.fr/nudibranche/a_aldisa_sp2.htm), Australia (Marshall 1999; Debelius and Kuitert 2007; Coleman 2008; Gosliner et al. 2008) and the Philippines (Coleman 2008; Gosliner et al. 2008, 2015). Diagnostic features of the external anatomy such as the whitish-yellow patch, two dorsal depressions ringed by tubercles and large round red tubercles tipped in black can be seen in these photographs. Controversially, Gosliner et al. (2015) considered a specimen from the Pacific (*Aldisa* sp. 4, pg. 169, bottom left photo) as a different species from *A. fragaria* sp. nov.

(specimen examined by us and illustrated on p. 168, bottom right photo as *Aldisa* sp. 3), but no clear external difference could be seen between them, except that, in the Mozambican specimen illustrated in Gosliner (2015), the lighter ring surrounding the tubercles (which is often present in *A. fragaria* sp. nov.) are not so visible as in the Pacific specimen.

For *A. zavorensis* sp. nov., photograph records of specimens of similar coloration exist for Reunion Island (http://seaslugs.free.fr/nudibranche/a_aldisa_sp4.htm), Guam (<http://www.nudipixel.net/photo/00022022/>), Taiwan (<http://www.nudipixel.net/photo/00007497/>), and Malaysia (Gosliner et al. 2008). Despite the colour similarity, key diagnostic features such as the deeply serrated borders of the branchial and rhinophores sheaths could not be seen in any of the photographs. Nevertheless, our molecular study shows that the specimen from Malaysia, illustrated in Gosliner et al. 2008 (p. 160, second bottom), appears to be the closest species to *A. zavorensis* sp. nov. Unfortunately, the COI sequence of this material is not available to verify the genetic divergence between these two specimens (Johnson 2011). Therefore, with such limited information, it is not possible to confirm whether or not the Malaysian specimen is *A. zavorensis* sp. nov.

Despite the external similarities between the photograph records with the species examined here, the broader distribution of *A. fragaria* sp. nov. and *A. zavorensis* sp. nov. can only be confirmed after molecular and/or anatomical examination of additional material from other regions.

Conclusion

Our phylogenetic and anatomical studies both indicate that *Aldisa fragaria* sp. nov. and *Aldisa zavorensis* sp. nov. are two new different species. They differ from all other described species both externally and internally. Externally, *A. fragaria* sp. nov. has distinctive round tubercles tipped in black, while *A. zavorensis* sp. nov. has distinctive rhinophores and gill sheaths, as well as tan gill branches. Internally, the most striking feature is the massive granular oral gland mass, which no other red *Aldisa* spp. with tubercles has, at least of such size. Moreover, the minimum genetic distance of the species described here, compared with all other sequenced *Aldisa* spp., was as high as 11.81%, which clearly distinguishes them from the other sequenced species of the genus. Nevertheless, some anatomical details such as the lateral border of the teeth of *A. zavorensis* sp. nov. could not be analysed. However, due to the rarity of this species and the need to improve our knowledge about this group, we considered the anatomical and molecular information based on the two specimens examined here good enough to describe *A. zavorensis* sp. nov.

Acknowledgements The authors are grateful to the Rufford Small Grant Foundation for their support to conduct field trips for the collection

of material. The critical point and scanning electron micrographs of the penis were carried out at the Universidad Autónoma de Madrid with funding from the Department of Biology. The SEM of the radulae and the molecular studies were completed at the Universidad de Cádiz and supported by funds of the research group “Marine Biology and Fisheries” PAIDI RNM-213.

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