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ORIGINAL ARTICLE

A new species of *Tambja* (Mollusca, Gastropoda, Nudibranchia) from the Mediterranean Sea: description of the first species of the genus from the Balearic Islands and Malta

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Abstract A new species of polycerid nudibranchs of the genus *Tambja* is described from Mallorca Island (Spain) and Malta. So far, only two species of *Tambja* had been recorded in the Mediterranean Sea with a distribution limited to southern Spain. With *Tambja mediterranea* sp. nov., the distribution of the genus in the Mediterranean Sea is extended, and the new species represents the first occurrence of *Tambja* at the Balearic Islands and Malta. Externally, the new species is mainly characterized by having ground orange-red colour, dorsum covered with rounded whitish tubercles, rhinophores red with whitish tips and three gill branches with orange-reddish rachis and whitish branches. In the present paper, external and internal features of *T. mediterranea* are described and compared with other species of the genus, especially with its most similar species, *T. limaciformis*. Additionally, phylogenetic analyses (Bayesian and maximum likelihood) based on mitochondrial sequences (COI) show that *T. mediterranea* sp. nov. is sister to *T. divae* and that both species cluster together with *T. limaciformis* and *T. amakusana* with the maximum support.

Keywords Polyceridae · Nembrothinae · Mediterranean Sea · New species · Balearic Island · Malta

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Introduction

The genus *Tambja* Burn, 1962 is one of the three genera of the subfamily Nembrothinae Burn (1967), the others being *Nembrotha* Bergh, 1877 and *Roboastra* Bergh, 1877 (Burn 1967). In the last decade, morphological and molecular analyses have shown that the phylogenetic relationships of Nembrothinae are still unresolved and that *Nembrotha* is the only genus so far verified to be monophyletic (Pola et al. 2006a, b, 2007, 2008). However, the taxonomical changes proposed by Pola et al. (2007) have not been implemented yet, and it is clear that further analyses comprising all described and still undescribed species of the subfamily are crucial to fully understand the phylogenetic relationship within the subfamily Nembrothinae (Pola et al. 2007, 2008).

Traditionally, the genus *Tambja* s.l. Burn, 1962 has been characterized by the presence of a thick and smooth labial cuticle, rectangular rachidian radular tooth with notched or smooth upper margin, lateral tooth bifid or with a simple cusp, and three to seven marginal plates. In addition, the prostate gland is small and confined to a glandular section of the vas deferens, and the penis is armed (Burn 1967; Pola et al. 2006a). However, Pola et al. (2006a, b, c, 2007, 2008) have shown that some "*Tambja*" species are quite different (Table 1). For instance, *Tambja limaciformis* (Eliot 1908), *T. amakusana* (Baba 1987) and *T. divae* (Marcus, 1958) have lateral pouches located between the

Table 1 "Unusual" features found in some species of "*Tambja*" s.l. Burn, 1962

	Lateral pouches	Vestigial teeth	Vaginal gland	Prostate surrounds bursa copulatrix	Wide vagina
Tambja capensis (Bergh, 1907)	No	Yes	Yes	No	No
T. limaciformis (Eliot 1908)	Yes	Yes	No	Yes	Yes
T. divae (Er. Marcus, 1958)	Yes	No	No	Yes	Yes
T. abdere Farmer, 1978	No	No	No	Yes	No
T. amakusana (Baba 1987)	Yes	Yes	No	Yes	Yes
T. blacki Pola et al. (2006a)	No	No	No	Yes	Yes
T. mediterranea sp. nov.	Yes	No	No	Yes	Yes

oral tube and the buccal bulb; *T. limaciformis*, *T. amakusana* and *T. capensis* (Bergh, 1907) have vestigial teeth between the rachidian and the inner lateral teeth. In addition, *T. limaciformis*, *T. amakusana*, *T. divae*, *T. blacki* Pola et al. 2006a and *T. abdere* Farmer, 1978 have a quite different arrangement of the reproductive system (see "Discussion").

To date, 27 valid species of the genus *Tambja* have been described worldwide, but many others are still undescribed (e.g. Nakano 2004; Cobb and Willan 2006; Debelius and Kuiter 2007; Coleman 2008; Gosliner et al. 2008), or in the process of being described Pola et al. (2014). Most of them have been recorded in the Indo-Pacific, but the geographical distribution of *Tambja* includes the Atlantic Ocean, the eastern Pacific and the Galápagos Islands (Pola et al. 2006a). Two species, *T. ceutae* García-Gómez and Ortea 1988 and *T. marbellensis* Schick and Cervera (1998), have been recorded in the Alborán Sea (Cervera et al. 2004). To date, the distribution of the genus in the Mediterranean Sea is limited to this area of southern Spain next to the Strait of Gibraltar.

In the present paper, we describe a new species of "*Tambja*" s.l. Burn, 1962 from Mallorca Island and Malta, *Tambja mediterranea* sp. nov. This is the first record of the genus for the Balearic Islands and Malta and the only known species of the genus in this part of the Mediterranean Sea. We compare and discuss the new species with other species of the genus "*Tambja*" s.l. Burn, 1962.

Materials and methods

In July 2012, six specimens of *Tambja* sp. nov. were found in southern Mallorca (Balearic Islands, Spain) and two specimens in July 2014 in Fomm Ir-Rih Bay (Malta). The sea slugs from Mallorca were found in one out of 41 samples collected on board of the RV Ramón Margalef, as part of a study focused on mapping the benthic habitats of the continental shelf between Sa Dragonera and Cabrera (DRAGONSAL Project). The sample was collected at 53 m depth, in a medium-grained sandy bottom, in a zone located in front of the municipality of Llucmajor, eastern Bay of Palma (39°18′18.5″N, 02°47′50.8″E). Sampling was performed using a beam trawl of 2 m width provided with a cod end of 20 mm mesh size, which was trawled for 3 min at 2 knots. The sample was predominantly composed by the red algae *Phyllophora crispa.* Once on board, the sample was washed to eliminate the sediment and the taxonomic groups were separated for the identification of algae and fauna species. Specimens from Malta (35°54′26.30″N, 14°20′22.42″E) were found in bycatch from fishermen's nets at 50–60 m depth, along with *Posidonia* leaves and maerl.

Type series are deposited in the National Museum of Natural Sciences (MNCN) in Madrid, and one specimen is kept in the Biological Reference Collections (CRB) of the Institute of Marine Science in Barcelona.

Photographs of the new species of *Tambja* were taken from live specimens. The specimens were first examined alive and then preserved in 96 % ethanol. In the laboratory, four individuals were dissected by dorsal longitudinal incision under a dissecting microscope. Thereafter, the internal features were observed, and both the buccal mass and the reproductive system were extracted for further detailed studies. The buccal mass was put into sodium peroxide to eliminate the soft tissue. Then, the labial cuticle and radulae were examined and photographed under a dissecting microscope, and line drawings of the radulae were done with the aid of a microscope. Radulae, lateral pouches and penises were rinsed in water, dried and mounted for examination by scanning electron microscope (SEM) at the Scientific and Technical Services of the Balearic Islands University and the Autonomous University of Madrid.

Partial sequences of the mitochondrial gene cytochrome c oxidase subunit I (658 bp) were obtained for three specimens of *T. mediterranea* sp. nov. following the protocols of Pola et al. (2014a). The new sequences were checked for contamination with BLAST (Altschul et al. 1990) implemented in the GenBank database and added to a previous molecular dataset (Pola et al. 2014a). The best-fit models of evolution were determined using the Akaike information criterion

(Akaike 1974) implemented in MrModeltest 2.3 (Nylander 2004). The selected models by positions were as follows: GTR+I+G for COI-1st and COI-3rd, F81 for COI-2nd, and GTR. Bayesian inference (BI) analysis was conducted using MrBayes 3.1.2b (Ronquist and Huelsenbeck 2003) for ten million generations and four chains, with two independent runs and a sampling frequency of 1000. In order to compare the genetic distances amongst *T. mediterranea* sp. nov. and *T. limaciformis*, we calculated the pairwise uncorrected p-distances for COI using PAUP* 4.0b 10.0. All codon positions were considered for the analysis.

Nomenclatural acts

This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is urn:lsid:zoobank.org:pub:2BFE313C-B8B5-4742-B7FB-7797C6627F8C.

Results

Family Polyceridae Alder & Hancock 1845
Subfamily Nembrothinae Burn (1967)
Genus *Tambja* Burn, 1962
Tambja mediterranea sp. nov. (Figs. 1, 2, 3)
LSID. urn:lsid:zoobank.org:act:0B9B5A8A-551B-48D5-93C6-8D92D815C236.

Type material: Holotype: MNCN 15.05/60151H. Southern Mallorca, Balearic Islands, Spain (39°18′N, 02°47′E). 53 m depth, one adult specimen 10 mm long. Dissected and sequenced (GenBank accession numbers: KP793056 and KP793059 for COI and H3, respectively).

Paratypes: MNCN 15.05/60151P1, MNCN 15.05/60151P2 (dissected), MNCN 15.05/60151P3 (dissected), MNCN 15.05/60151P4 (dissected). 10 mm long. Same data as holotype.

MNCN 15.05/60152, Fomm Ir-Rih Bay, Malta (35°54′26.30″N, 14°20′22.42″E). 50–60 m depth, one adult specimen 10 mm long. Sequenced (GenBank accession numbers: KP793057 and KP793060 for COI and H3, respectively).

MNCN 15.05/60153. Fomm Ir-Rih Bay, Malta (35°54′26.30″N, 14°20′22.42″E). 50–60 m depth, one adult specimen 10 mm long. Sequenced (GenBank accession numbers: KP793058 and KP793061 for COI and H3, respectively).

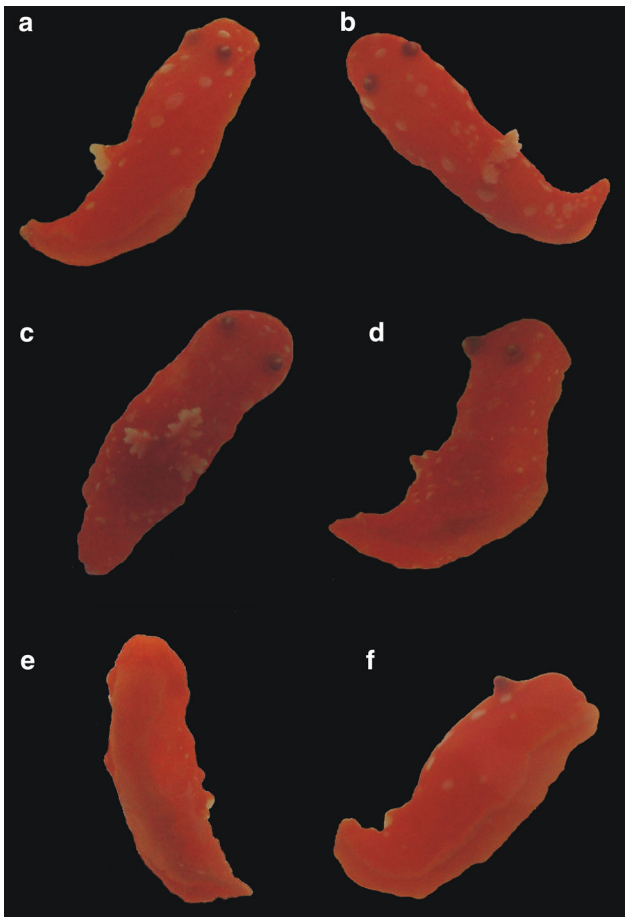

Fig. 1 *Tambja mediterranea* sp. nov. Photographs of living animals. **a–d** Dorsal view. **e–f** Ventral view

Other material: CRB ICMB000001. One adult specimen 10 mm long. Same data and locality as type series from Mallorca.

Distribution: To date, only found in the southern continental shelf of Mallorca (Balearic Islands, Spain) and Fomm Ir-Rih Bay (Malta).

Etymology: The specific name refers to Mediterranean Sea, where the specimens were found.

Diagnosis: Body elongate and limaciform, ending in a pointed tail. Living animal up to 10 mm long. Ground colour orange-red (Fig. 1). Dorsum covered with rounded whitish tubercles. In some specimens, the tubercles are large and most of them arranged in three rows along the body (Fig. 1a, b). In others, the tubercles are smaller and irregularly scattered over the dorsum (Fig. 1c, d). Head rounded anteriorly, with a pair of perfoliate rhinophores with about 15–20 lamellae, retractile in elevated sheaths. Rhinophores dark red coloured with whitish tip. Oral tentacles short and grooved. Three tripinnate gill branches, non-retractile, with orange-reddish rachis and whitish

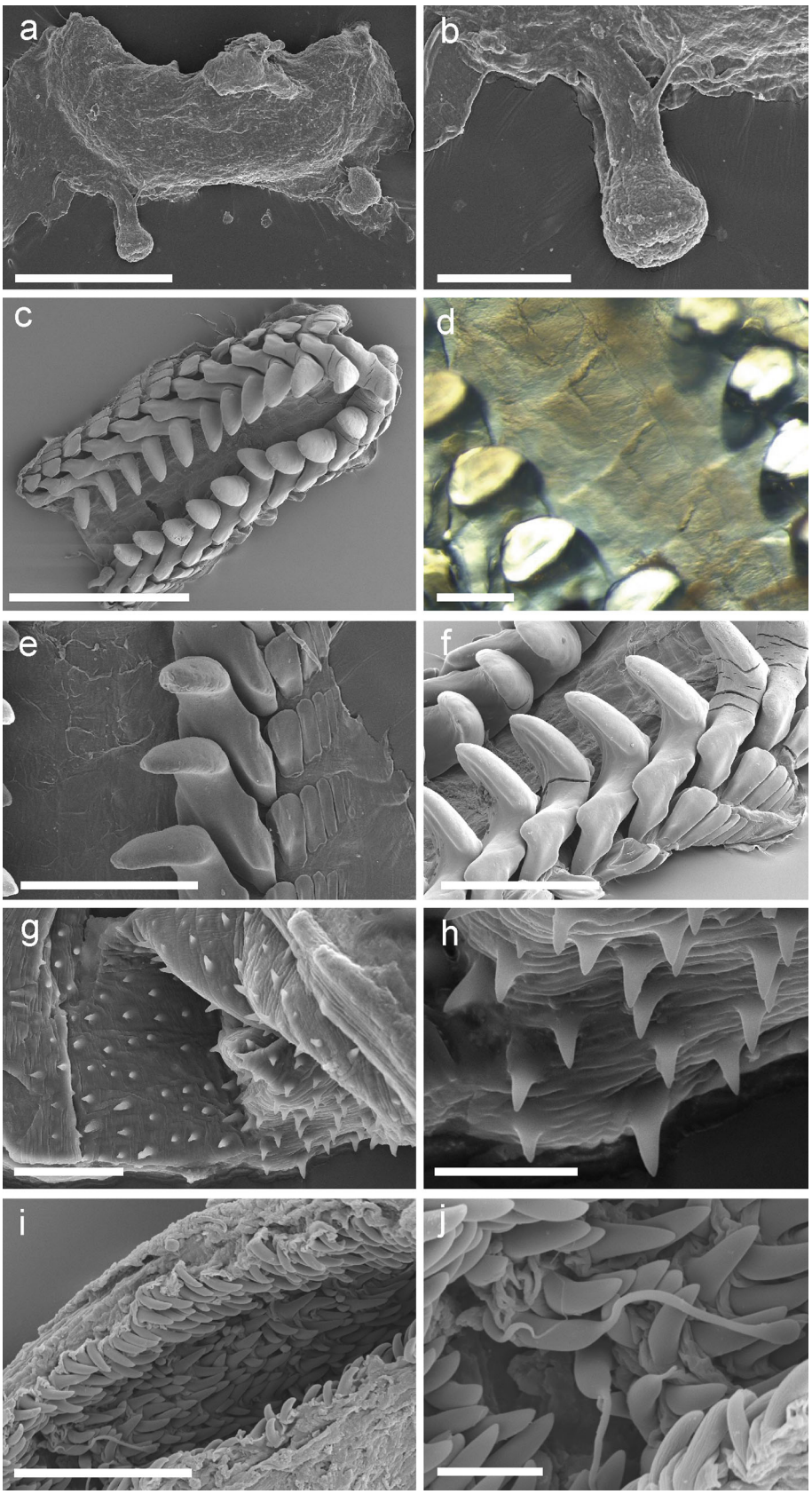

Fig. 2 *Tambja mediterranea* sp. nov. SEM (**a**–**c**, **e**–**j**) and binocular dissecting scope micrographs (**d**). **a** Oral tube with lateral pouches (MNCN 15.05/60151H). **b** Lateral pouch (MNCN 15.05/60151H). **c** Radula (MNCN 15.05/60151P3). **d** Central part of the radula showing the weak rachidian tooth (MNCN 15.05/60151P3). **e** Right half of the radula (MNCN 15.05/60151H). **f** Detail of innermost lateral teeth (MNCN 15.05/60151P3). **g**–**j** Penial spines. **g** Distal penial spines (MNCN 15.05/60151H). **h** Detail of the distal penial spines (MNCN 15.05/60151H). **i** Basal penial spines (MNCN 15.05/60151P3). **j** Detail of the rare spines (MNCN 15.05/60151P3). *Scale bar* **a** 1 mm, **b** 300 μm, **c** 1 mm, **d** 200 μm, **e** 500 μm, **f** 500 μm, **g** 50 μm, **h** 20 μm, **i** 500 μm, **j** 10 μm

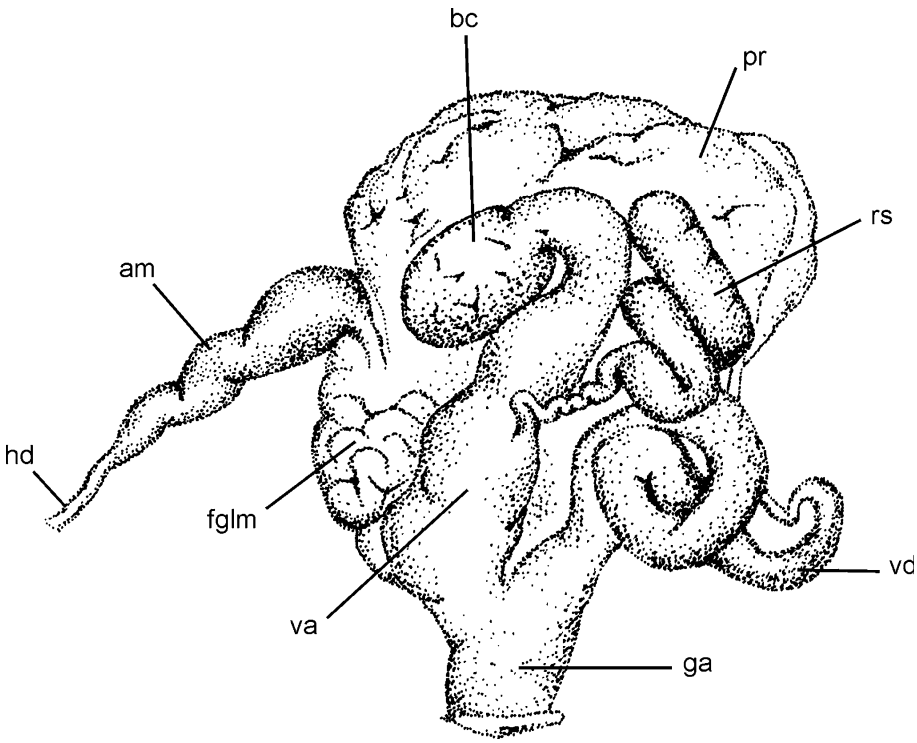

Fig. 3 Reproductive system of *T. mediterranea* sp. nov. *am* ampulla, *bc* bursa copulatrix, *fglm* female gland mass, *ga* genital atrium, *hd* hermaphrodite duct, *pr* prostate, *rs* receptaculum seminis, *va* vagina, *vd* vas deferens. *Scale bar* 1 mm

branches. Lateral slots below the rhinophores. Foot and oral tentacles orange-red (Fig. 1e, f).

Internally, a short muscular oral tube continues into the buccal mass. There is a pair of small, elongate lateral pouches at the junction of the oral tube and the buccal mass (Fig. 2a, b). Labial cuticle chitinous, smooth and very thin. Radular formula of the dissected specimens 9–13 × 6–5.1.1.1.5–6 (Fig. 2c). Rachidian teeth subquadrate without denticles and smooth, slightly curved upper margin, very thin and weak (Fig. 2d, e). Rachidian teeth are very difficult to see when the radula is dried (Fig. 2c, e, f). Innermost lateral tooth elongate and large with two cusps: upper cusp hamate, well developed, with a rounded prominence on the outer side of the tooth; and outer cusp small. A wide body joins both cusps (Fig. 2e, f). The remaining lateral teeth are represented by 5–6 rectangular plates with rounded edges (Fig. 2e, f). The innermost plate has a rounded prominence on the inner side, as if it were a vestigial cusp axe-shaped. The next plates are oval-shaped and become progressively smaller towards the outer margin.

The reproductive system is triaulic (Fig. 3). The elongate and narrow hermaphrodite duct continues into a large, wide and folded ampulla, which is divided into a short oviduct and the vas deferens. The oviduct enters the female gland. The vas deferens widens into a very large, coiled massive prostate. Next to the prostate is the female gland mass. The prostatic section of the vas deferens narrows again to a thin duct, which descends through the centre of the large, wider and highly convoluted final portion of the vas deferens. In situ, the bursa copulatrix is almost entirely wrapped by the prostate. The penis is located on the larger muscular portion of the vas deferens, covered with two different types of spines. Scattered distal spines are short and broad (Fig. 2g, h), some of them very small (Fig. 2g); the remaining spines are numerous and closely packed; they are elongate, thick and slightly curved (Fig. 2h, i). One specimen has some unusual spines, which have an elongate narrow lengthening structure and rounded distal part (Fig. 2i, j). These latter spines are located on the basal part of the penis. The bursa copulatrix is large and round. The seminal receptacle is elongate, wide and longer than the bursa copulatrix. The seminal receptacle joins the vagina by a long and highly convoluted duct. The vagina is large and wide and leads to the genital atrium next to the vas deferens. There is no vaginal gland.

Discussion

The genus *Tambja* includes numerous species distributed worldwide, but only two species known from European waters: *Tambja ceutae* García-Gómez and Ortea 1988 and *Tambja marbellensis* Schick and Cervera 1998. *Tambja ceutae* has been recorded from the Strait of Gibraltar (Ceuta), southern Andalusia, Madeira, Azores and the Canary Islands (Pola et al. 2006a), while *T. marbellensis* is found in southern Spain and Portugal (Pola et al. 2006a).

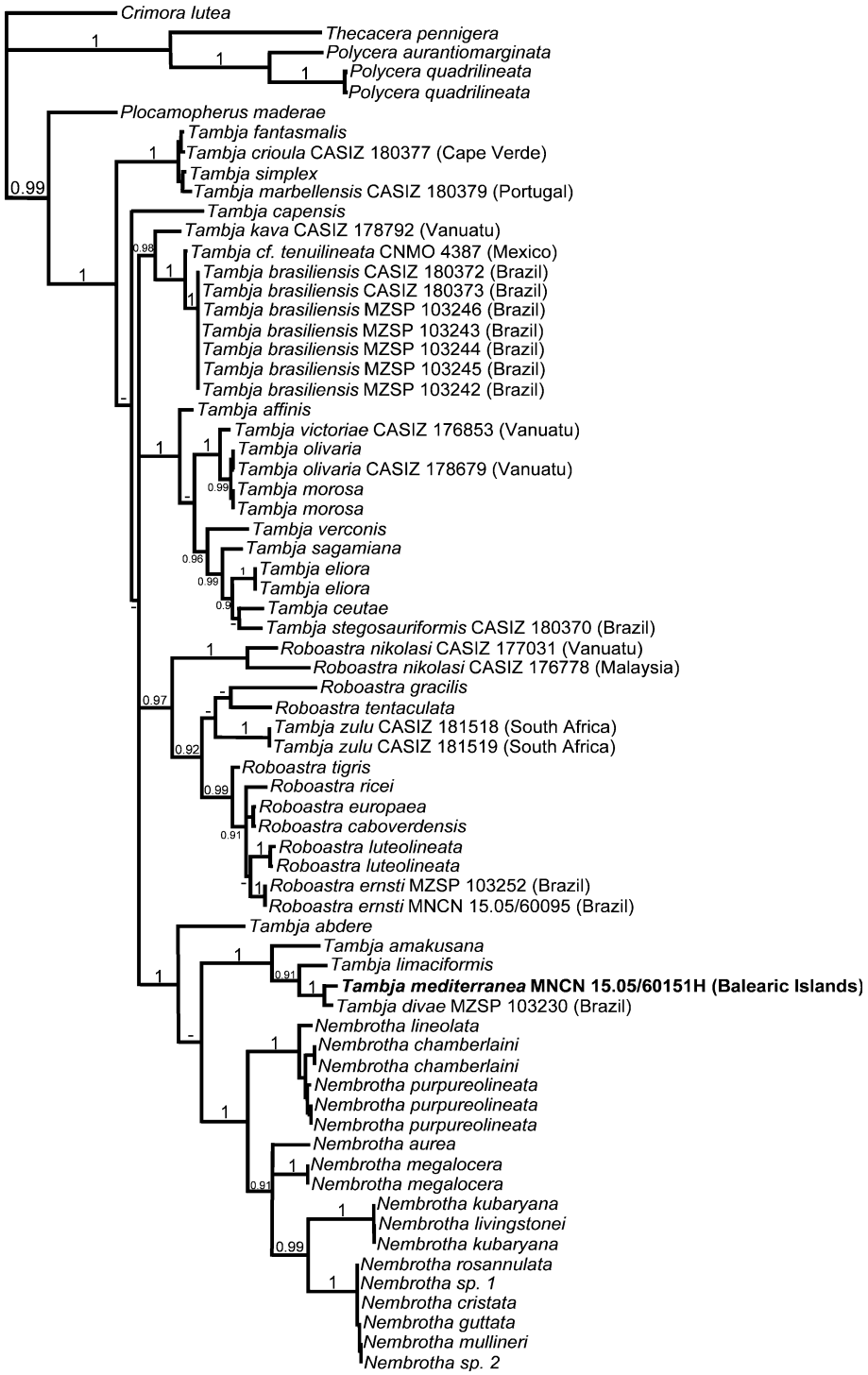

Fig. 4 Phylogenetic hypothesis based on COI sequences represented by Bayesian inference. *Numbers* represent posterior probabilities from Bayesian inference

The presence of this genus in the Mediterranean Sea is restricted to the Alboran Sea, and to date, no species of *Tambja* has been recorded further east in the Mediterranean. Both Mediterranean species clearly differ in their external features from the new species. *Tambja mediterranea* sp. nov. has an orange-red notum with rounded whitish tubercles, while *T. ceutae* has a green-blue ground colour with longitudinal yellow bands and small conic papillae around the notum (García-Gómez and Ortea 1988) and *T. marbellensis* is dark blue or dark green-blue with several yellow stripes and marks shaded with brown (Schick and Cervera 1998).

The species most similar to *T. mediterranea* sp. nov. is *T. limaciformis*, described by Eliot (1908) from the Red

Sea. Both species share several morphological and anatomical features. Externally, *T. limaciformis* resembles the new species as it has a deep saffron-red ground colour with small yellowish to white dots scattered on the back and the sides of the body. However, the presence of purple on the tips of the rhinophores and the tips of the gill is distinctive in *T. limaciformis* (Baba 1960; Pola et al. 2006a). The radular teeth are quite similar, the central tooth is subquadrate without denticles, first lateral tooth large and elongate with upper cusp hamate and well developed, but all specimens of *T. mediterranea* sp. nov. studied have a very thin rachidian tooth. These teeth are difficult to see and at a cursory glance seem to be lacking. Also, Pola et al. (2006a) described the presence of a vestigial tooth between the rachidian and the inner lateral for *T. limaciformis*. In *T. mediterranea* sp. nov., the vestigial teeth are lacking or at least much less well developed than in *T. limaciformis*.

Regarding the reproductive system, both species are very similar, but *T. limaciformis* has three different kinds of penial spines (Pola et al. 2006a), whereas *T. mediterranea* sp. nov. has only two of them. Moreover, their shape and number differ from those of *T. limaciformis*, which has smaller and sharper spines, which are less closely packed than in *T. mediterranea* sp. nov. The number of spines in *T. mediterranea* sp. nov. is significantly greater.

In summary, there is no doubt that the only species quite similar to *T. mediterranea* sp. nov. is *T. limaciformis*. However, *T. limaciformis* has been reported from the Red Sea, Japan, Papua New Guinea, Philippines, Mariana Islands, Hawaii, Western Australia and Heron Island, but never from the Atlantic or Mediterranean waters (Pola et al. 2006a). Finally, in order to discard the possibility that *T. mediterranea* sp. nov. is actually *T. limaciformis* introduced through the Suez Canal, we obtained COI sequences for three specimens of *T. mediterranea* sp. nov. and compared them with *T. limaciformis* from Pola et al. (2007) (GenBank accession number: EF142878.1). In this case, the minimum uncorrected p-distance for COI between *T. mediterranea* sp. nov. and *T. limaciformis* was 11.0 %. Since there are no available sequences of H3 for *T. limaciformis*, this gene has not been used here.

The distribution together with some external and internal differences and the minimum uncorrected p-distance for COI allow us to describe the first species of the genus for the Balearic Islands and Malta.

In addition, it is clear that *T. mediterranea* sp. nov. shares several important features with only some "*Tambja*" s.l. Burn, 1962 (Table 1). Only five other species of the genus described to date lack a vaginal gland, i.e. *T. limaciformis*, *T. amakusana*, *T. abdere*, *T. divae* and *T. blacki*. These same five species are the only ones with the bursa copulatrix surrounded in situ by the prostate, and all but *T. abdere* have a large and wide vagina. In the case of *T. abdere*, the vagina is much narrower, long and coiled (Pola et al. 2006b). Some other interesting features are present only in *T. mediterranea* sp. nov. and a few of the latter species. In fact, only *T. limaciformis*, *T. amakusana* and *T. divae* have small, elongate pouches opening at the junction of the oral tube and the buccal mass. The function of these structures is still unknown. Finally, only *T. limaciformis*, *T. amakusana* and *T. capensis* have vestigial teeth between the rachidians and the inner laterals. In the case of *T. mediterranea* sp. nov., it is not clear whether these vestigial teeth are really present. Since the rachidian teeth are so thin and weak, we might have failed to notice them, but in the other mentioned species these teeth are always clearly visible.

All these peculiar morphological features especially shared by *T. limaciformis* and *T. amakusana* probably make that these two species clustered together in a very well-supported clade (Pola et al. 2006a, 2007, 2008). In fact, *T. divae* was not included in Pola et al. (2007, 2008) because the specimens were missing, but it is included in Pola et al. (2014a) and it clusters together with *T. limaciformis* and *T. amakusana*. Molecular sequences for several genes are still missing for some species such as *T. blacki*, but the morphological and molecular analyses so far clearly show that *T. limaciformis*, *T. amakusana* and *T. divae* are grouped together and well as are *T. abdere* and *T. blacki*. When including COI sequence for *T. mediterranea* sp. nov. in the molecular dataset of Pola et al. (2014a), *T. mediterranea* sp. nov. clusters together with maximum support (PP = 1.00) with *T. limaciformis*, *T. amakusana* and *T. divae* (Fig. 4).

Material from further oceanographic surveys will help us to continue unravelling the intricate phylogenetic relationships of this group of sea slugs.

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