European Journal of Taxonomy 196: 1–13
http://dx.doi.org/10.5852/ejt.2016.196

ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2016 · Garić R. & Batistić M.

Research article

urn:lsid:zoobank.org:pub:38CC4797-768A-44C5-8B85-EA3DCB6678CB

Description of *Brooksia lacromae* sp. nov. (Tunicata, Thaliacea) from the Adriatic Sea

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Abstract. *Brooksia lacromae* sp. nov. is described from zooplankton material collected at a marine monitoring station in the South Adriatic in the autumn of 2014. The description of both solitary and aggregate forms is given along with 18S rRNA and mitochondrial *cox1* sequence data that provides strong evidence that both forms belong to the same species. The described species is morphologically markedly different from *B. rostrata* (Traustedt, 1893) and *B. berneri* van Soest, 1975, previously the only two species in the genus *Brooksia*. Genetic analysis based on 18S rRNA gene confirmed distinctness of *B. lacromae* sp. nov. from *B. rostrata* (1.5% uncorrected pairwise distance). The appendicularian *Fritillaria helenae* Bückmann, 1924, so far known from the Atlantic only, was found in the same samples as *B. lacromae* sp. nov. Co-occurrence of *B. lacromae* sp. nov. with an Atlantic appendicularian suggests an Atlantic or Western Mediterranean origin for this new taxon.

Keywords. *Brooksia lacromae* sp. nov., new species, climate changes, gelatinous zooplankton, *Fritillaria helenae*.

Garić R. & Batistić M. 2016. Description of *Brooksia lacromae* sp. nov. (Tunicata, Thaliacea) from the Adriatic Sea. *European Journal of Taxonomy* 196: 1–13. http://dx.doi.org/10.5852/ejt.2016.196

Introduction

Thaliacea is a well investigated group, primarily because of their bloom potential and substantial grazing impact on phytoplankton communities (Madin & Deibel 1998; Andersen 1998). The last new species within the order Salpida were described 40 years ago (van Soest 1975), when, among other species, *Brooksia berneri* van Soest, 1975 was described. *Brooksia berneri* and *Brooksia rostrata* (Traustedt, 1893) are two morphologically very similar species that make up the genus *Brooksia* (van Soest 1975; Godeaux 1998). The blastozooid of *Brooksia berneri* is unknown or identical to that of *Brooksia rostrata* (van Soest 1975). Both species are known from tropical and temperate parts of the Atlantic, Indian and Pacific Oceans and their ranges seem to overlap (van Soest 1975). In the Mediterranean there have been only three records of *Brooksia rostrata* (Sigl 1912; Fedele 1926; Batistić *et al.* 2014), while *Brooksia*

berneri has not been recorded. The last record of *Brooksia rostrata* was of a single oozooid in 2008 at Lokrum station (Batistić *et al.* 2014; Fig. 1). After the discovery of *Brooksia lacromae* sp. nov. in 2014, the material from 2008 is re-examined in this paper and it proved in fact to be an individual of *Brooksia lacromae* sp. nov.

Brooksia lacromae sp. nov. was found in samples collected at the Lokrum station (South Adriatic, Fig. 1) in October 2014. The Lokrum station is located off the south-east Adriatic coast of Croatia where it is under the direct influence of incoming currents from the Ionian Sea. Such entering currents are known to modify Adriatic zooplankton community composition and to bring alien species of Atlantic/Western Mediterranean or Eastern Mediterranean/Lessepsian origin into the Adriatic on a pluriannual scale (Batistić *et al.* 2014; Gačić *et al.* 2010; Civitarese *et al.* 2010).

The life cycle of salps consists of two alternating generations: the solitary asexual generation (oozooids) and the aggregate sexual generation (blastozooids), which morphologically differ greatly. Here, we describe both forms of *Brooksia lacromae* sp. nov. Given the considerable morphological differences between these reproductive forms, and the limited differences between *Brooksia* species in aggregate morphology, we also use genetic data to corroborate our descriptions. DNA sequences from both the nuclear ribosomal small subunit RNA (18S) and mitochondrial cytochrome oxidase subunit I (*cox1*) genes were generated, as these have previously been successfully used for integrative taxonomic research on closely related salp species (Goodall-Copestake 2014). Finally, we also discuss the potential origin of *Brooksia lacromae* sp. nov.

Materials and methods

Collection of zooplankton samples

Zooplankton samples were collected on 3 Oct. 2014 at the Lokrum station (42°37'21" N, 18°06'05"E; Fig. 1) by vertical tows using a 53 µm mesh (55 cm diameter, 250 cm length) and a 200 µm mesh (56.5 cm diameter, 340 cm length) Nansen net. The samples were collected in two layers: 0–50 m and 50–90 m. After collection, the samples for morphological analysis and analysis of planktonic tunicate community composition and abundance were preserved in 2.5% $CaCO_3$ buffered formaldehyde solution. Temperature and salinity at the time of sample collection were measured using a SBE 19plus CTD

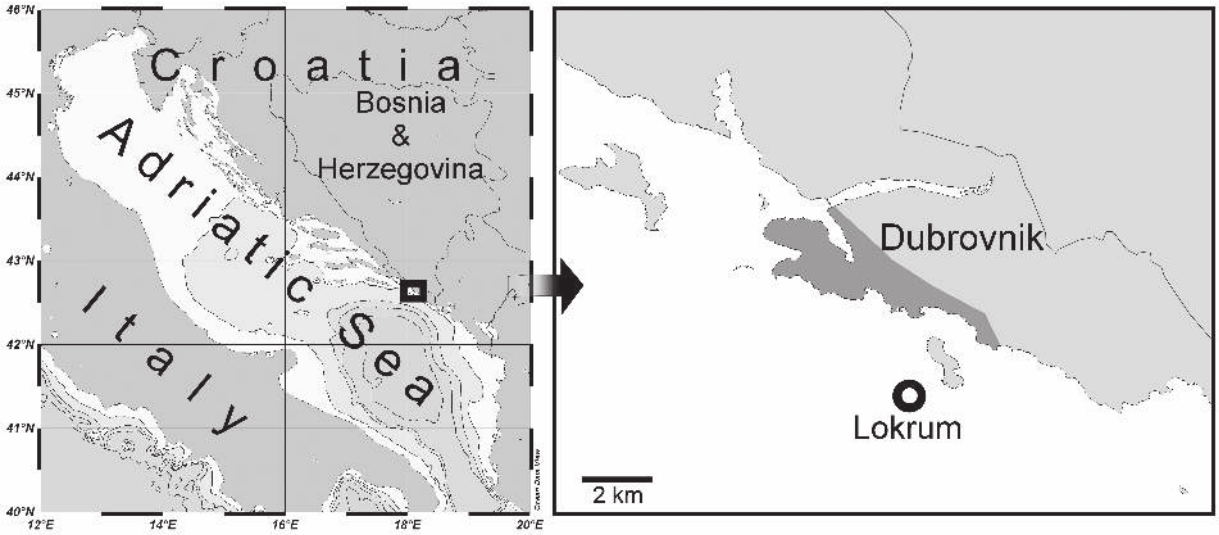

Fig. 1. Position of the Lokrum station in the South Adriatic, where specimens of *Brooksia lacromae* sp. nov. were collected.

Table 1. PCR primers used for amplification of *B. lacromae* 18S rRNA and *cox1* gene fragments.

Primer	5'-3' sequence	Reference
	18S	
app2f	ATCTGGTTGATCCTGCCAGT	modified from
n1800r	GATCCTTCCGCAGGTTCACCT	Medlin *et al.* 1988
500f	ATTGGAGGGCAAGTCTGGTG	
1300f	GGTGGTGCATGGCCGTTCTTAG	universal
1500r	CTAAGGGCATCACAGACCTG	
uni800r	AGGTCTGCTTTGAACACTCTA	
oiko800f	GAAAAAATTAGAGTGTTCAAAGC	this study
app1200r	CCGTGTTGAGTCAAATTAAGC	
	coxl	
coi-70f	ATGTCTACTAATCATAAAGATATT	this study
coi-930r	GCAGTAAAATAAGCACGAGAATC	

instrument (Seabird) from the surface to 90 m depth at every metre. The planktonic tunicate community composition was determined using an Olympus SZX-16 stereo microscope by analysis of entire zooplankton samples to the species level. The specimens of *B. lacromae* sp. nov. for morphological analysis were additionally stained with a few drops of 0.1% Janus Green dye, which made muscle bands clearly visible. Specimens were photographed using a DP25 (Olympus) digital camera mounted onto the stereo microscope and measured from the photographs using Cell^D software (Olympus). In addition to formalin-fixed samples, material for genetic analysis was taken with a 53 μm mesh net from 90 m depth to the surface. The plankton material was then filtered through a 53 μm mesh to remove sea water and subsequently preserved in 95% ethanol, for no more than 2 days. The ethanol sample was sorted for *Brooksia lacromae* sp. nov. oozooids and blastozooids and then cleaned of attached debris using dissection needles. Cleaned salps were placed in separate tubes containing pure acetone for long-term storage at -20°C.

DNA isolation, PCR and sequencing

DNA was isolated from one oozooid individual (branchial bar tissue) and one blastozooid individual (muscle and test tissue). About 1–2 mm^2 of the tissue was cut and used for DNA isolation. After removal of acetone and drying the tissue for 20 min at 55°C, tissue fragments were lysed for 2 hours at 55°C in 30 μl of a solution containing 10 mM Tris-HCl pH 8.0, 0.5% SDS, and 0.5 mM EDTA, with the addition of 1.5 μl of 20 mg ml^{-1} proteinase K (Fermentas). After lysis, the DNA was purified using the modified method described by Karatani *et al.* (2006) based on the anti-chaotropic effect of saturated $(NH_4)_2SO_4$ solution on DNA molecules. The lysate was mixed with five times the volume of saturated ammonium sulphate water solution and put in hand-made spin columns. The hand-made spin columns with glass filters were made by making two holes with a red hot needle in a 0.5 ml polypropylene tube, one in the cap and the other in the bottom of the tube. Two glass filters, 4 mm in diameter each, were then put in every column. The glass filters were cut out of GF/F (Whatmann) filter using a flame-sterilised hollow punch tool. After binding of DNA to the glass filters, the columns were washed with 300 μl of a buffer made by mixing 10 ml of 10 mM Tris (pH 7.5) with 40 ml of 96% ethanol. After drying the columns by centrifugation at maximum for 3 min, the DNA was eluted with 35 μl of TE buffer pH 8.0 (10 mM Tris, 1 mM EDTA).

Table 2. Planktonic tunicate community composition (ind. m^{-3}) on 3 Oct. 2014 at Lokrum station using 200 µm and 53 µm mesh nets in 0–50 m and 50–90 m layers. Abbreviations: bls = blastozooid; ooz = oozooid.

	200-µm		53-µm	
	0–50	50–90	0–50	50–90
Appendicularia				
Oikopleura longicauda (Vogt, 1854)	15.2	8.5	11.1	11.5
O. fusiformis Fol, 1872	21.5	6.7	7.4	9.5
O. cophocerca (Gegenbaur, 1855)	0.7	0.4	2.6	1.6
O. gracilis Lohmann, 1896	2.8	0.1	1.4	0.9
O. parva Lohmann, 1896	0.2	11.8	1.1	5.6
O. dioica Fol, 1872	0.2		0.2	0.4
Folia mediterranea (Lohmann, 1899)			0.1	
Stegosoma magnum (Langerhans, 1880)	0.5	0.2	0.2	
Mesoikopleura haranti (Vernières, 1934)	1.1	0.4	0.1	0.3
Fritillaria haplostoma Fol, 1872	5.9		6.2	8.9
F. borealis sargassi Lohmann, 1896	4.9	0.7	1.2	4
F. borealis intermedia Lohmann, 1905	0.3			
F. pellucida (Busch, 1851)	2.6	0.3	0.7	0.8
F. messanensis Lohmann *in* Bückmann, 1924	0.8		1	0.5
F. formica tuberculata Lohmann *in* Bückmann, 1926	1.2	0.4	0.4	0.9
F. formica digitata Lohmann *in* Bückmann, 1926	1.0		0.1	0.6
F. megachile Fol, 1872	0.2	0.2	0.2	
F. fraudax Lohmann, 1896	0.1	3.7		1.8
F. helenae Bückmann, 1924	0.3			0.4
Appendicularia sicula Fol, 1874		0.1	0.6	0.8
A. tregouboffi Fenaux, 1960				0.1
Kowalevskia tenuis Fol, 1872	0.5			
K. oceanica Lohmann, 1899	0.4	0.2	1.1	1.4
Thaliacea				
Doliolina muelleri (Krohn, 1852) (ooz)	2.6	0.7	1.4	0.2
D. muelleri (Krohn, 1852) nurse	0.2			
Doliolum nationalis Borgert, 1893 (bls)	1.3	0.1	0.1	
Dolioletta gegenbauri Uljanin, 1884 (bls)	0.9		0.1	
Dolioloides rarum (Grobben, 1882) (ooz)	0.3	0.2	0.2	
Doliolida larvae	0.4	0.1	0.2	
Thalia orientalis Tokioka, 1937 (ooz)	0.1	0.1	0.1	
T. orientalis (bls)		0.1	0.1	
Brooksia lacromae sp. nov. (bls)	0.2	0.9	0.5	0.4
Brooksia lacromae sp. nov. (ooz)			0.1	0.1

The 18S rRNA gene was PCR amplified and sequenced in four overlapping fragments using the primer pairs app2f-uni800r, 500f-app1200r, oiko800f-1500r, and 1300f-n1800r (Table 1); the overlap between any two fragments was 200–400 base pairs. The *cox1* fragment was amplified using primers coi-70f and coi-930r (Table 1), which were designed by aligning complete *cox1* sequences of multiple tunicate species available from GenBank.

The PCR was performed in a 100 µl PCR mix containing: 1 × PCR buffer, 0.2 mM of each dNTP, 3 mM $MgCl_2$, 0.2 µM of each primer, 1.2 U of recombinant Taq polymerase (Thermo Scientific) and 2 µl of template DNA. All amplifications of 18S DNA were performed using a PCR programme with a 2 min denaturation step at 94°C, with 40 subsequent cycles of 94°C for 20 s, 50°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 5 min. The *cox1* fragment was amplified using a PCR programme with a 2 min denaturation step at 94°C, with 40 subsequent cycles of 94°C for 20 s, 45°C for 2 min, 72°C for 3 min, and a final extension step at 72°C for 5 min.

PCR products were purified for sequencing using the same method as for DNA isolation and eluted in 17 µl of 10 mM Tris buffer (pH 8.0).

All PCR products were sequenced by the Macrogen company (South Korea) in both directions using the same primers as used for original amplification. Obtained 18S rRNA and *cox1* gene sequences from an oozooid and a blastozooid were deposited in GenBank under accession numbers: KR057222 (oozooid 18S), KR057223 (blastozooid 18S), KT818685 (oozooid *cox1*) and KT818686 (blastozooid *cox1*).

DNA data analysis

Sequence assemblies from 18S overlapping fragments were made using the BioEdit software (Ibis Biosciences). Uncorrected pairwise distances were calculated between *B. lacromae* sp. nov. 18S sequences and a *B. rostrata* 18S sequence obtained from GenBank, as well as between different *B. lacromae* sp. nov. sequences. *Cox1* sequences were translated using ascidian mitochondrial genetic code to check for nuclear pseudogenes and no stop codons were detected.

Results

Planktonic tunicate community composition

A total of 29 species of planktonic tunicates was recorded on 3 Oct. 2014 at the Lokrum station (Table 2). Out of 29 species, there were 23 species of Appendicularia, four of Doliolida and two of Salpida. The most abundant appendicularians were *Oikopleura fusiformis* Fol, 1872, *Oikopleura longicauda* (Vogt, 1854), *Oikopleura parva* Lohmann, 1896 and *Fritillaria haplostoma* Fol, 1872. The most abundant doliolid was *Doliolina muelleri* Krohn, 1852. The most abundant salp was *Brooksia lacromae* sp. nov. (0.2–0.9 ind. m^{-3}), while *Thalia orientalis* Tokioka, 1937 was found in low numbers (0–0.1 ind. m^{-3}).

Systematics

Phylum Chordata Haeckel, 1874
Subphylum Tunicata Lamarck, 1816
Class Thaliacea Van der Haeven, 1850
Order Salpida Forbes, 1853
Family Salpidae Lahille, 1888
Genus *Brooksia* Matcalf, 1918

Brooksia lacromae sp. nov.
urn:lsid:zoobank.org:act:2B9ACCBD-8B20-437D-B013-468F8E956864
Figs 1–4

Diagnosis

Oozooid (solitary form; Fig. 2)
Muscles II and III fuse dorsally before fusing with muscle I mid-dorsally. Muscles III and IV fuse briefly dorsolaterally. Muscles IV and V fuse before approaching muscles VI and VII mid-dorsally. There is one longitudinal ventral muscle which extends anteriorly into the proboscis. In addition, the proboscis

contains two lateral longitudinal muscles. The ventral longitudinal muscle branches posteriorly into two branches. There are two slit-like openings in the ventral longitudinal muscle, one below the anterior end of the endostyle, and the other slightly anteriorly from the first one. Muscle I fuses with branches of the

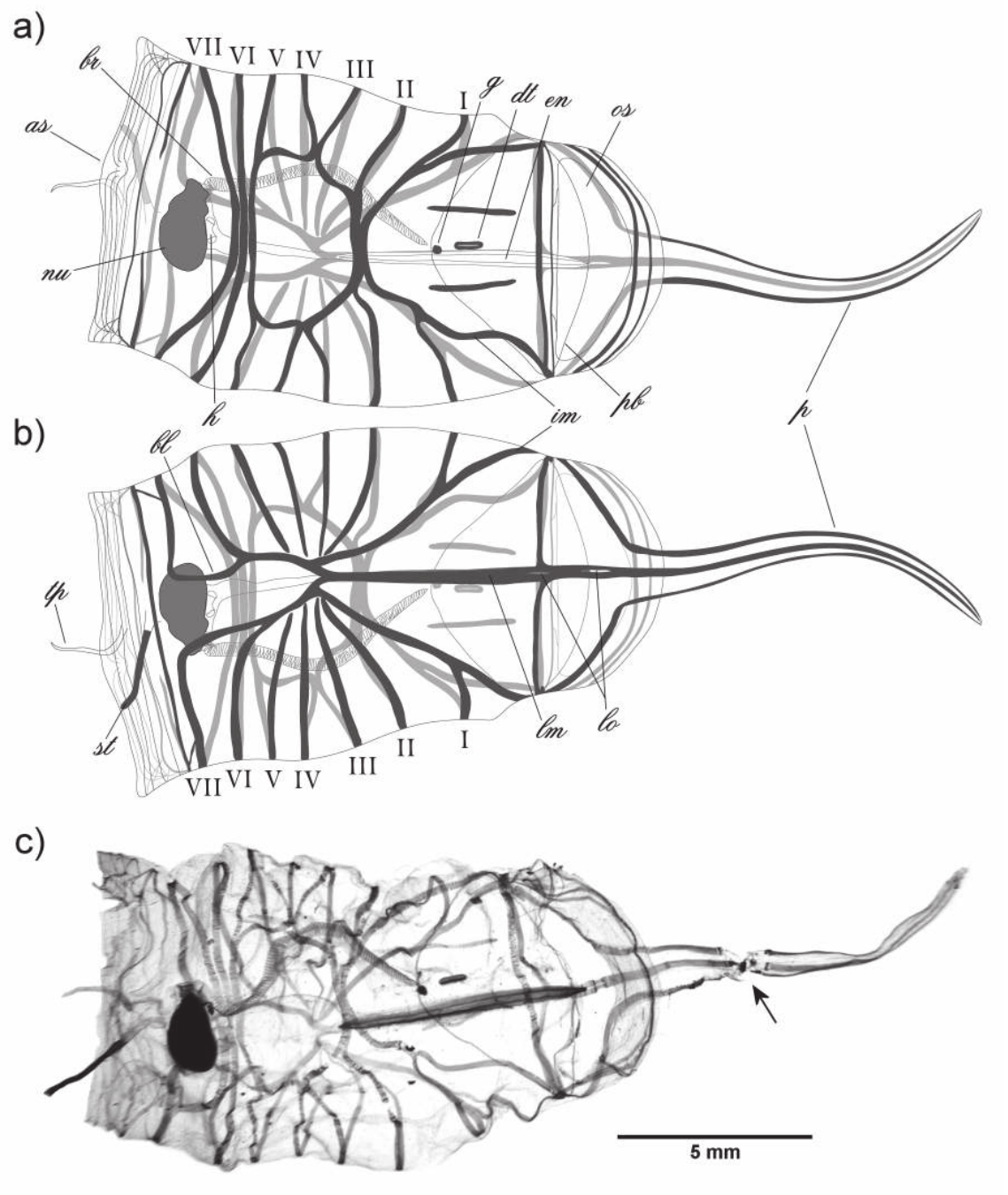

Fig. 2. Oozooid of *Brooksia lacromae* sp. nov. **A**. Dorsal view, drawing. **B**. Ventral view, drawing. **C**. Dorsal view, photograph of an individual dyed with Janus Green, with an arrow indicating breakage in the proboscis during collection. Abbreviations: as = atrial siphon; bl = branching of the ventral longitudinal muscle; br = branchial bar; dt = dorsal tubercle; en = endostyle; g = ganglion; im = intermediate muscle; lm = longitudinal muscle; lo = openings in the longitudinal muscle; nu = nucleus; os = oral siphon; p = proboscis; pb = peripharyngeal band; st = stolon; tp = test processus; I–VII = body muscles.

ventral longitudinal muscle at their anterior part, while muscles VI and VII fuse with them posteriorly. Muscles II, III, IV and V converge ventrally towards the junction between muscle I and the branches of the ventral longitudinal muscle. Muscle II can be slightly fused with muscle I just before its connection with branches of the ventral longitudinal muscle. Endostyle straight. Stolon emerges from the test ventrally, slightly posteriorly from the nucleus. There is one test processus just posteriorly from the stolon (Fig. 4D). Muscles I–VII with 52–58 muscle fibers in total, measured laterally in two examined individuals.

Blastozooid (aggregate form; Figs 3, 4A–C)

Sinistral individual – Four muscles on the left side, three muscles on the right side. Dorsal IM1 continues ventrally as the ventral muscle IM1, without extending to the anterior attachment processus (ap1). A branch of dorsal muscle IM1 (IM1-a) extends to the anterior attachment processus (ap1). Dorsal muscles IR, IIR and IIIR branch dichotomously. Dorsal muscle IR-a continues ventrally as ventral muscle IR. Dorsal muscle IR-b continues ventrally as muscle IIR-c. A small branch from muscle IR-b/IIR-c enters the lateral attachment processus (ap2). Dorsal muscle IIR-a continues ventrally as ventral muscle IIR-a. Muscle IIR-b ends blindly on the ventral side of the animal without entering the posterior attachment processus (ap3). Dorsal and ventral muscles IIIR enter the posterior attachment processus (ap3) without joining. Muscles IVL-a and IIIR-a extend from muscles IVL and IIIR, respectively, towards the nucleus. Endostyle curved anteriorly. Muscles IL-IIIL, IR-a,b and IIR-a,b,c with 3 muscle fibers each in all examined individuals. Muscle IVL with from 3 to 9 muscle fibers, muscle IIIR with from 5 to 7 muscle fibers.

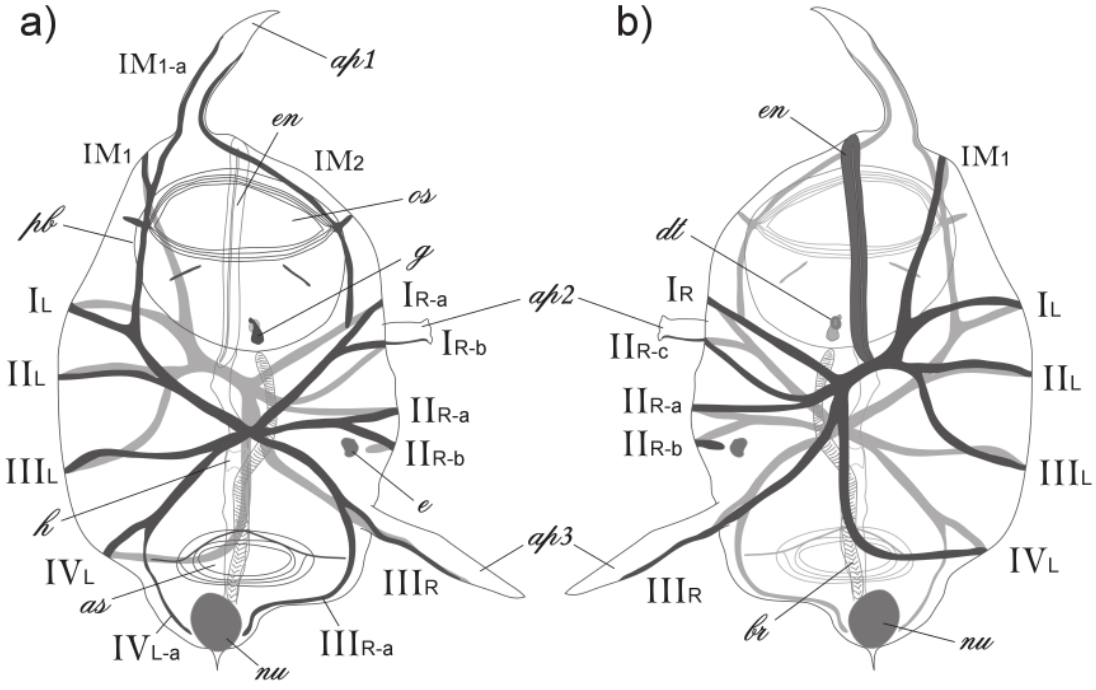

Fig. 3. Drawing of the blastozooid of *Brooksia lacromae* sp. nov. (sinistral individual). **A**. Dorsal view. **B**. Ventral view. Abbreviations: ap1–3 = attachment processes; as = atrial siphon; br = branchial bar; dt = dorsal tubercle; e = embryo; en = endostyle; g = ganglion; h = heart; nu = nucleus; os = oral siphon; pb = peripharyngeal band; I_L–IV_L = left side muscles; I_R–III_R = right side muscles; IM_1 and IM_2 = intermediate muscles.

Dextral individual

Mirror image of sinistral individual.

Etymology

Brooksia lacromae sp. nov. is named after the island of Lokrum near which it has been found. The Latin name of the island of Lokrum is Lacroma.

Material examined

Two oozooids and 19 blastozooids.

Holotype

One oozooid (14.8 mm body length, 23.9 mm total length including proboscis), collected from a 0–50 m depth layer on 3 Oct. 2014 in a 53 μm mesh plankton net. It is deposited in the Tunicata collection of the Croatian Natural History Museum under inventory number CNHM Inv. br. 44/1.

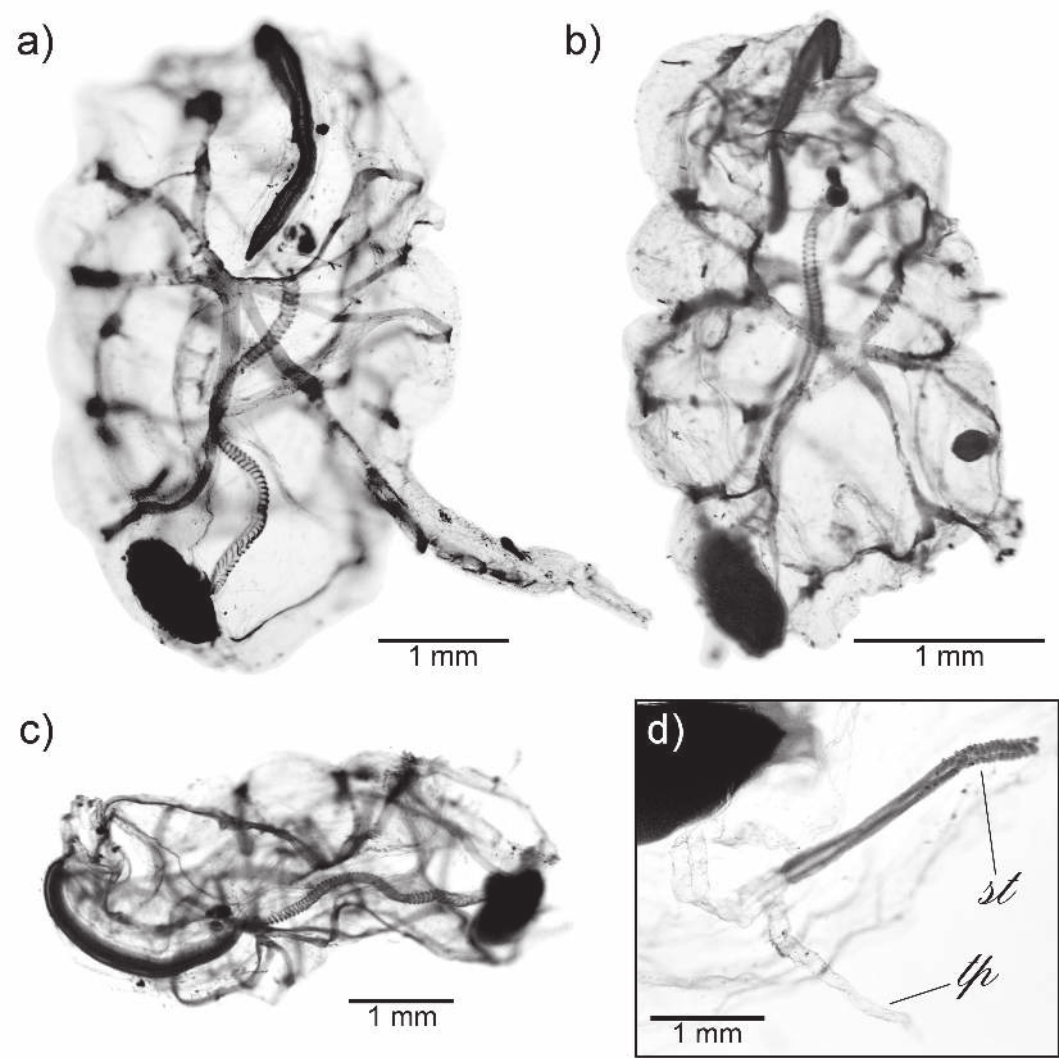

Fig. 4. *Brooksia lacromae* sp. nov. **A**. Photograph of the ventral side of the dextral blastozooid. **B**. Photograph of the dorsal side of the sinistral blastozooid. **C**. Photograph of the lateral side of a blastozooid. **D**. Detail of the ventral posterior end of an oozooid. All indivuduals were dyed with Janus Green dye. Abbreviations: st = stolon; tp = test processus.

Allotype

One sinistral blastozooid (4.2 mm body length, without attachment processes), collected from a 0–50 m depth layer on 3 Oct. 2014 in a 53 μm mesh plankton net. It is deposited in the Tunicata collection of the Croatian Natural History Museum under inventory number CNHM Inv. br. 44/2

Paratypes

One oozooid (11.5 mm body length, 18.2 mm total length including proboscis) and one dextral blastozooid (4.8 mm body length, without attachment processes), collected from a 50–100 m depth layer on 3 Oct. 2014 using a 53 μm mesh plankton net, deposited in the Institute for Coastal and Marine Research (University of Dubrovnik) under inventory number IMP-002; one dextral blastozooid (4.1 mm body length, without attachment processes) collected from a 0–50 m depth layer on 3 Oct. 2014 with a 200 μm mesh plankton net, deposited in the Dubrovnik Natural History Museum under inventory number PMD 2106.

Other material

16 blastozooids deposited in the Institute for Coastal and Marine research (University of Dubrovnik) under inventory number IMP-003.

Type locality

42°37'21"N, 018°06'05"E, off Dubrovnik, South Adriatic (Mediterranean Sea; Fig. 1).

The temperature average in the 0–50 m layer was 19.0°C, with a range between 16.2°C and 22.2°C, while the salinity average was 38.36, with a range between 37.79 and 38.68. In the 50–90 m layer the temperature average was 15.8°C, with a range between 15.5°C and 16.1°C, while the salinity average was 38.71, with a range between 38.67 and 38.77.

Remarks

Oozooid. *Brooksia rostrata* and *Brooksia berneri* oozooids are very similar, differing only in the fact that in *B. berneri* oozooid muscle I, joined with the intermediate muscle (im), is separated from muscle II and discontinuous mid-dorsally. Due to this fact we will only compare the *B. lacromae* sp. nov. oozooid to the *B. rostrata* oozooid. The *Brooksia lacromae* sp. nov. oozooid has one ventral longitudinal muscle which extends into the proboscis, while *B. rostrata* has two (Fig. 2). The proboscides in both collected oozooids broke off during collection, but were found in the sample (Fig. 2c). The proboscis of *Brooksia lacromae* sp. nov. seems to be thinner at the base than in *Brooksia rostrata* and in both species it contains two lateral longitudinal muscles. The ventral longitudinal muscle in *B. lacromae* sp. nov. has two small slit-like openings anteriorly, one below the endostyle and the other just anteriorly to it, partly overlapping with the anterior end of the endostyle. In *Brooksia lacromae* sp. nov., in contrast to *B. rostrata*, body muscles are not arranged in a barrel-like structure where muscles are perpendicular to the body axis dorsally, as well as ventrally, but they converge to the posterior third of the body. Only muscles I, VI and VII fuse with the branches of the ventral longitudinal muscle, while muscles II, III, IV and V converge to the connection between muscle I and branches of the ventral longitudinal muscle, without fusing with either. Only muscle II can sometimes be slightly connected with muscle I ventrally. The stolon in *Brooksia lacromae* sp. nov. seems to emerge from the test, slightly posteriorly from the nucleus (Fig. 4D), while in *B. rostrata* it emerges from the anterior part of the nucleus (Thompson 1948).

Blastozooid (sinistral individual). The *Brooksia lacromae* sp. nov. and *Brooksia rostrata* blastozooids are very similar. In *Brooksia lacromae* sp. nov., muscle IIR-b ends blindly on the ventral side of the animal without entering the posterior attachment processus (ap3), while in *Brooksia rostrata* it enters the posterior attachment processus (ap3). The left intermediate muscle (IM1) in *Brooksia lacromae* sp. nov. is continuous dorsally and ventrally. Its branch IM1-a enters the anterior attachment processus (ap1). In

Brooksia rostrata the left intermediate muscle is discontinuous. Its dorsal and ventral counterparts both enter the anterior attachment processus. Because of this, the anterior attachment processus in *B. lacromae* sp. nov. possesses two muscles (IM1-a and IM2), while in *B. rostrata* it possesses three muscles (dorsal and ventral left intermediate muscle and right intermediate muscle). According to Thompson (1948), the right intermediate muscle of the sinistral individual of *B. rostrata* ends blindly, while after Tokioka (1954) and Godeaux (1998) it connects to muscle I. In *B. lacromae* sp. nov. the right intermediate muscle (IM2) ends blindly.

Genetic analysis

There were no differences between blastozooid and oozooid 18S sequences. Between two *cox1* sequences there were 7 substitutions out of 837 nucleotides, which results in 0.84% uncorrected pairwise distance. *Cox1* sequences were translated using ascidian mitochondrial code and there were no differences in amino acid sequence between them. The uncorrected pairwise distance between the *Brooksia rostrata* 18S sequence (HQ015403) and the *Brooksia lacromae* sp. nov. 18S sequence (KR057223) was 1.5% (including gaps). Out of 26 differences in 1740 nucleotides between these two sequences, there were 21 transitions, 4 transversions and one single nucleotide gap.

Discussion

In the Mediterranean there are only two finds of *Brooksia rostrata* according to our knowledge. The first record was in 1911 on the eastern side of the Middle Adriatic (Sigl 1912) and the second from the Gulf of Naples (Fedele 1926). The find of *B. rostrata* in the South Adriatic in 2008 (Batistić *et al.* 2014) proved to be in fact an individual of *B. lacromae* sp. nov., after careful examination of one oozooid in poor condition. It thus seems that *Brooksia* species are rarely recorded in the Mediterranean.

Recent investigations have shown that entering currents, of either Atlantic/Western Mediterranean or Eastern Mediterranean origin, modify the composition of the gelatinous zooplankon community in the South Adriatic and each type of current brings different newcomers (Batistić *et al.* 2014). According to a recently postulated BiOS theory, the entrance of different water masses into the Adriatic depends on the direction of circulation of the North Ionian Gyre (Civitarese *et al.* 2010; Gačić *et al.* 2010). The finding of the appendicularian *Fritillaria helenae* Bückmann, 1924, so far only known from the Atlantic, in the same samples as *Brooksia lacromae* sp. nov., suggests that *B. lacromae* sp. nov. is of either Atlantic or Western Mediterranean origin. In addition to *F. helenae*, *Kowalevskia oceanica* Lohmann, 1899 and *Fritillaria formica digitata* Lohmann *in* Lohmann & Buckmann, 1926 were found in the same samples, which is their first peer-review published record from the Adriatic (Garić & Batistić 2013). *Fritillaria fraudax* Lohmann, 1896, a rare appendicularian in the Adriatic as well as in the Mediterranean (Skaramuca 1980; Fenaux 1967; Fenaux *et al.* 1998), was found in high numbers, which might also be related to the increased inflow of water of Atlantic or Western Mediterranean origin. Curiously, the oozooids of *Brooksia lacromae* sp. nov. were only found in a 53 μm mesh net, which suggests that the filtration speed in a 200 μm mesh net may be too high to preserve the delicate oozooid body.

The morphological differences between *Brooksia rostrata* and *Brooksia lacromae* sp. nov. are quite high for congeneric salp species (Godeaux 1998). The number of oozooid body muscles is the same in both species but their dorsal fusing pattern and overall arrangement is markedly different. In *B. rostrata* body muscles are parallel to each other and they fuse ventrally with the two longitudinal ventral muscles, forming a barrel-like pattern. In *B. lacromae* sp. nov. body muscles converge ventrally to the posterior third of the body and, except for muscles I, VI and VII, they don't fuse with the single longitudinal ventral muscle or its branches. The blastozooids are much more similar. There are only two differences between *B. rostrata* and *B. lacromae* sp. nov. blastozooids. In *B. rostrata* the muscle IM1 enters the anterior attachment processus and the muscle IIR-b enters the posterior attachment processus, while in

B. lacromae sp. nov. only a branch of the first intermediate muscle (IM1-a) enters the anterior attachment processus (ap1) and the muscle IIR-b does not enter the posterior attachment processus (ap3). Tokioka (1954) described a long testicular processus emerging from the posterior end of mature blastozooids of *B. rostrata*. No such structure could be seen in examined *B. lacromae* sp. nov. blastozooids, but that is perhaps due to their immaturity.

The 18S rRNA gene is very conservative and tends to underestimate the number of species when used in biodiversity studies (Tanga *et al.* 2012; Raupach *et al.* 2010). It is generally used for phylogenies of distant species or higher taxonomic groups. The pairwise distance of 1.5% between *B. rostrata* and *B. lacromae* sp. nov. 18S sequences was higher than most intrageneric species distances of Thaliacea (Govindarajan *et al.* 2011), supporting the designation of *B. rostrata* and *B. lacromae* sp. nov. as separate species. In addition to 18S sequence data, *cox1* sequences from *B. lacromae* sp. nov. are also provided. Although only one oozooid and one blastozooid sequence were generated, the pairwise difference between them was consistent with expectations for intra-specific divergences in salps (cf. Goodall-Copestake 2014). There are only a few published thaliacean *cox1* sequences (Yokobori *et al.* 2005; Leray *et al.* 2013; Goodall-Copestake 2014), mainly because of their high evolutionary rate, which makes common universal *cox1* primers unusable. The *cox1* sequences generated for the present study will aid in the future identification of *B. lacromae* sp. nov. as well as help with further refinements of Thaliacea-specific *cox1* PCR primers.

In the last couple of years two new species of gelatinous zooplankton were described from the Adriatic Sea (Garić & Batistić 2011; Piraino *et al.* 2014), while one species was re-established more than 100 years after its first description (Batistić & Garić 2016). This incidence of new species of gelatinous zooplankton in the Adriatic is likely partly due to climate change, with more and more alien species entering the Adriatic (Batistić *et al.* 2014), and partly as a consequence of poor quality investigations of marine zooplankton at the species level (Batistić & Garić 2016). In order to improve our ability to detect climate-induced changes in marine ecosystems in the future, we suggest more research effort be placed on taxonomically challenging groups such as the gelatinous zooplankton described here.

Acknowledgements

Thanks to Dr. Per Flood (Bergen, Norway) for providing us with Janus Green dye which was used for dying *Brooksia lacromae* sp. nov. muscles. Many thanks to Nikola Arkulin for sampling assistance during the rough sea. This research was supported by the Croatian Science Foundation (grant number: HRZZ IP-2014-09-2945).

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Manuscrip received: 25 September 2015
Manuscript accepted: 11 January 2016
Published on: 12 May 2016
Topic editor: Rudy Jocqué
Desk editor: Kristiaan Hoedemakers

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'Histoire naturelle, Paris, France; Botanic Garden Meise, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Natural History Museum, London, United Kingdom; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark.