



***Botrylloides pizoni*, a new species of Botryllinae (Asciacea) from the Mediterranean Sea**

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Abstract

A new ascidian species belonging to the Botryllinae was discovered in the Gulf of Taranto (South Italy). This new species was collected in different seasons over a period of several years, which allowed information about its biology to be obtained. The new species presents large zooids arranged in ladder systems. The zooids have several rows of stigmata, the second one complete, ovary posterior to testis, one larva per side developing in an incubatory pouch, and a peculiar arrangement of the gut loop. The new species is named *Botrylloides pizoni* after the great French zoologist Antoine Pizon (1860–1942).

Key words: Tunicata, Asciacea, Botryllinae, *Botrylloides pizoni* n. sp., Mediterranean Sea, South Italy

Introduction

The taxonomy of the Botryllinae is far from being satisfactory: the reduced size of zooids, ignorance of the degree of variability in most characters and the possibility of morphological variability in many structures, as a consequence of the particular stage of the colonial life history at the moment of collection. All these mean that specific determination is often uncertain and, unfortunately, many previous descriptions are not really discriminant. In this situation Saito & Okuyama (2003) emphasised that underlined details of the life history may be indispensable for a precise classification of these animals. Although this opinion is indubitably valid in many cases, unfortunately, practically all ascidian taxonomists are obliged to examine dead specimens. However if sufficient material collected over a period of several seasons is available, useful indications about the life history of the species can be obtained. In any case, until the true taxonomic values of the different morphological characters have been ascertained, descriptions of these species should be very accurate and involve the highest number of characters possible (Brunetti, 2009).

In this paper, several samples of a Botryllinae species, collected from the Gulf of Taranto over various seasons during 2003 to 2011, were analysed and recognised as belonging to a new species of the genus *Botrylloides*.

Material and methods

Collection site. The species was found in mussels farms of the *Mar Piccolo* of the Gulf of Taranto (South Italy). This area is composed of two basins: an external one, called *Mar Grande*, covering an area of 35.5 km² with a maximum depth of 42 m, is in ample communication with the open sea; and a more internal one called *Mar Piccolo*, covering 20.72 km², which is made up of two inlets called *I* and *II seno*, with maximum depths of 13 and 9 m respectively (Fig. 1). The three basins present a gradient in hydrological conditions with progressive reduction of water renewal and an increase in silting and suspended matter (Umgiesser et al. 2007). All the *Botrylloides* colonies were collected by scuba divers in the *I seno* of the *Mar Piccolo* at 2–3 m depth on the submerged chains of iron buoys.

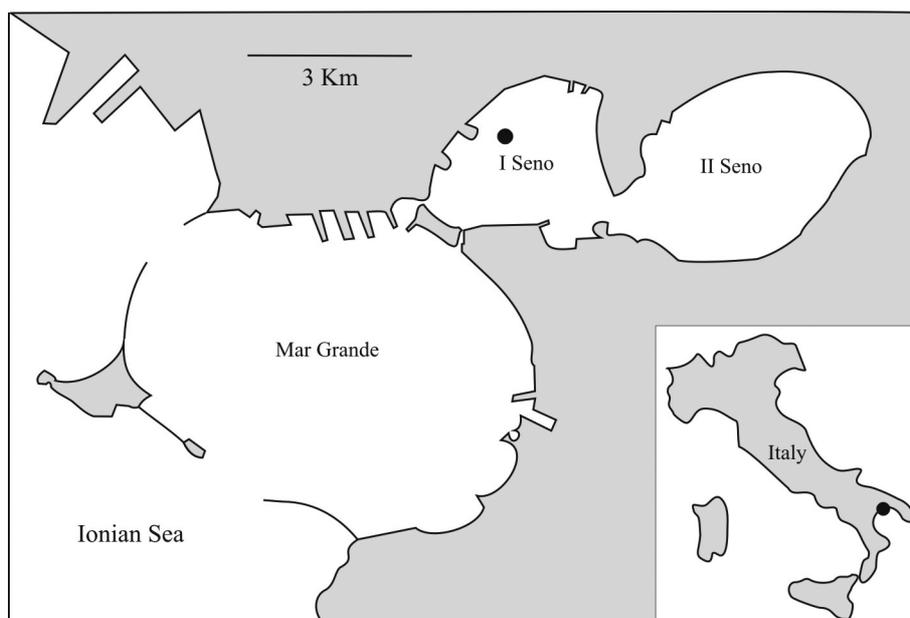


FIGURE 1. Map of the Gulf of Taranto with collection site.

Material examined. For each sample the time of collection and physiological condition of the colony are given, with the following symbols:

bcg = colony immediately before change of generation, that is, when the first order buds have the size almost equal to that of the filtering zooids, and on the second order buds new buds (difficult to see) are developing.

acg = colony immediately after change of generation, that is, when the old filtering zooids are regressing or regressed and the system is formed by new filtering zooids with first order buds on which new buds (difficult to see) are at their initial developmental stages.

ils = intermediate life stage

Z = filtering zooid

1B = first order bud

2B = second order bud

prim = gonadic primordia

tp = testis primordia

t = ripening testis

T = ripe testis

o = oocytes or ripening eggs

L = larva (or developing embryo)

ng = gonads absent

mam = *mammillated* colony (see below)

Example: “[acg]: Z, ng; 1B, prim” means, “colony immediately after the change of generation, with filtering zooid without gonads and first order buds with gonadic primordia”. The type series was deposited in the Natural History Museum of Venice.

Sample 1. Taranto, 20 Mar. 2003: [acg]: Z, ng; 1B, prim.

Sample 2. Taranto, 26 Aug. 2003: [ils]: Z, ng; 1B, ng; 2B, ng. [mam].

Sample 3. Taranto, 22 Sep. 2003: [acg]: ZT – 1Bt, o; [mam].

Sample 4. Taranto, 18 Nov. 2003: [bcg]: Z, ng; 1B, t; 2B, prim. Paratype MSNVE-22078

Sample 5. Taranto, 19 Dec. 2003: [acg]: Z, ng; 1B, ng.

Sample 6. Taranto, 22 Jun. 2004: [acg]: Z, T; 1B, tp, o. [mam].

Sample 7. Taranto, 1 Jul. 2004: [bcg]: Z, T, L; 1B, t, o; 2B, t, o.

Sample 8. Taranto, 29 Jul. 2004: [bcg]: Z, L; 1B, t, o; 2B, t, o. Holotype MSNVE-22077

Sample 9. Taranto, 4 Oct. 2004: [acg]: Z, T; 1B, t. Paratype MSNVE-22079

Sample 10. Taranto, 25 Oct. 2004: [acg]: Z, T; 1B, t. Paratype MSNVE-22080

Sample 11. Taranto, 6 Dec. 2004: [acg]: Z, ng; 1B, prim. [mam].

Sample 12. Taranto, 10 Jan. 2005: [ils]: Z, ng; 1B; ng; 2B, ng. Paratype MSNVE-22081

Sample 13. Taranto, Sep. 2007: [acg]: Z, T, O; 1B, prim. [mam]. Paratype MSNVE-22082

Sample 14. Taranto, Sep. 2011: [acg]: Z, T; 1B, prim.

Description

Botrylloides pizoni n. sp.

Etymology. The species is named after Antoine Pizon—the great French zoologist author of the fundamental work on the blastogenesis of the botryllids (1893).

Colonies. Living specimens are mainly violet or from lilac to violet in colour, but red and orange colonies were also found (Figs 2a–e). The investing colonies are often lobed, and in the eutrophic waters of the collection site; they may reach large dimensions (several square decimetres) and thicknesses of up to 2–3 cm. Zooids are arranged in “*leachii* type” systems (Brunetti, 2009). Zooid systems are usually in close proximity (Figs 2a–d) but in some colonies (here called *mammillated*) there are areas, between them without zooids, where the colonial test forms prominences (Figs 2 f–g) (see below).

Zooids. Zooids are cylindrical in shape and up to 4 mm long (Fig. 3a). The oral siphons are smooth edged; the atrial aperture is wide, exposing much of the branchial sac, as usual in animals with this type of system; however in this species, the posterior third or more of the branchial sac is not exposed, and all abdominal organs including the rectum lie inside a body wall bag which is embedded in the colonial matrix. The anterior rim of the atrial aperture extends in a more or less developed dorsal languet, according, as usual, the position of the zooid in the system.

In living specimens, 4 or 5 tentacles appear to be dominant (Fig. 2e). However, dissection shows that they number about 30, in 3 order of size regularly arranged according to the following scheme L,S,M,S,L (large, small, medium) (Fig. 3d). They rise from a single ring and are finger-like, not differing greatly in diameter from the base to the top. The anterior third of the oral siphons, from the tentacles ring to the edge, is run through by numerous thin circular muscle fibers, the remaining posterior two-thirds have longitudinal fibers (Fig. 3d). Thin longitudinal muscles fibers also descend along the whole body wall, approaching the edge of the atrial aperture and forming a band around it.

Branchial sac. The prepharyngeal ring does not form a V below the dorsal tubercle, which presents a simple, oval, vertically arranged aperture (Fig. 3d). The neural gland complex is about 200 μ m further down; it is oval in shape with the major longitudinal axis shorter than a stigmata. The prebranchial area is about half the height of a stigmata. There are usually 17 rows of stigmata, all complete, plus 3–4 irregular rows at the posterior end. There is a difference in length between the dorsal and endostylar sides of the branchia, because the latter 3–4 irregular rows of stigmata do not reach the dorsal side (Fig. 3e). However, on the whole, the branchial sac is cylindrical in shape, the same number of stigmata appearing in every row. The longitudinal vessels are not very large and not very protuberant, with a diameter not greater than the space between two stigmata (Fig. 3d). The branchial dorsal (D), lateral (L) and ventral (V) sectors are $D > V > L$ and the stigmata are distributed in a remarkably constant manner, according to the branchial formula: L 4,2,2,5 DL 6,2,2,5 R. The transversal vessels are large, externally protuberant, and filled with pigmented blood cells. Two large muscle bands run on both sides of the dorsal vessel and the endostyle. Other muscle fibers also run along the transversal vessel partly connecting with the longitudinal fibers at the side of the dorsal vessels, and partly passing over the latter and connecting with the fibers at the sides of the endostyle. Muscle fibers also run along the internal longitudinal branchial vessels (Figs 3d–5d).

Gut loop. The oesophagus presents four very evident longitudinal, grooves. The stomach is bell-shaped with 11 folds, excluding the typhlosolis (Fig. 4a₁–a₂). Anteriorly, at the cardiac end, the folds extend in ampullae around the oesophagus. On the right side of the stomach, the folds are as long as the stomach itself, posteriorly thinned, not closed, and with reduced glandular epithelium (Figs 4a₂–5b); on the left side (Nos 1, 2, 10, 11) they are much shorter (Fig. 4a₁). The typhlosolis runs along the first fold and does not extend over the posterior end of the stomach. The pyloric caecum rises about half-way along its length; it is finger-like, about one-quarter the length of the stomach, straight, or only slightly bent, and with an only slightly swollen tip. Between the typhlosolis and the 11th fold, there is a smooth polygonal area (Fig. 4a₁). The major axis of the stomach forms an angle of 45° with the

longitudinal axis of the branchial sac, so that the stomach lies almost completely behind the latter and the intestine forms an extensive S-shape (Figs 3a,b– 4a₁). After a short pyloric tract, the intestine is larger in diameter and has a pavement epithelium with cells regularly arranged in transversal rows. The pyloric gland forms a band around the intestine, and its duct opens into the caecum half-way along its length (Fig. 4a₁). The rectum is long, ascending along the dorsal side of the branchial sac as far as the rim of the atrial aperture (Figs. 3a, 5a). The anus is smooth-edged but when closed, appears to be bilobed (Fig. 4a₁).

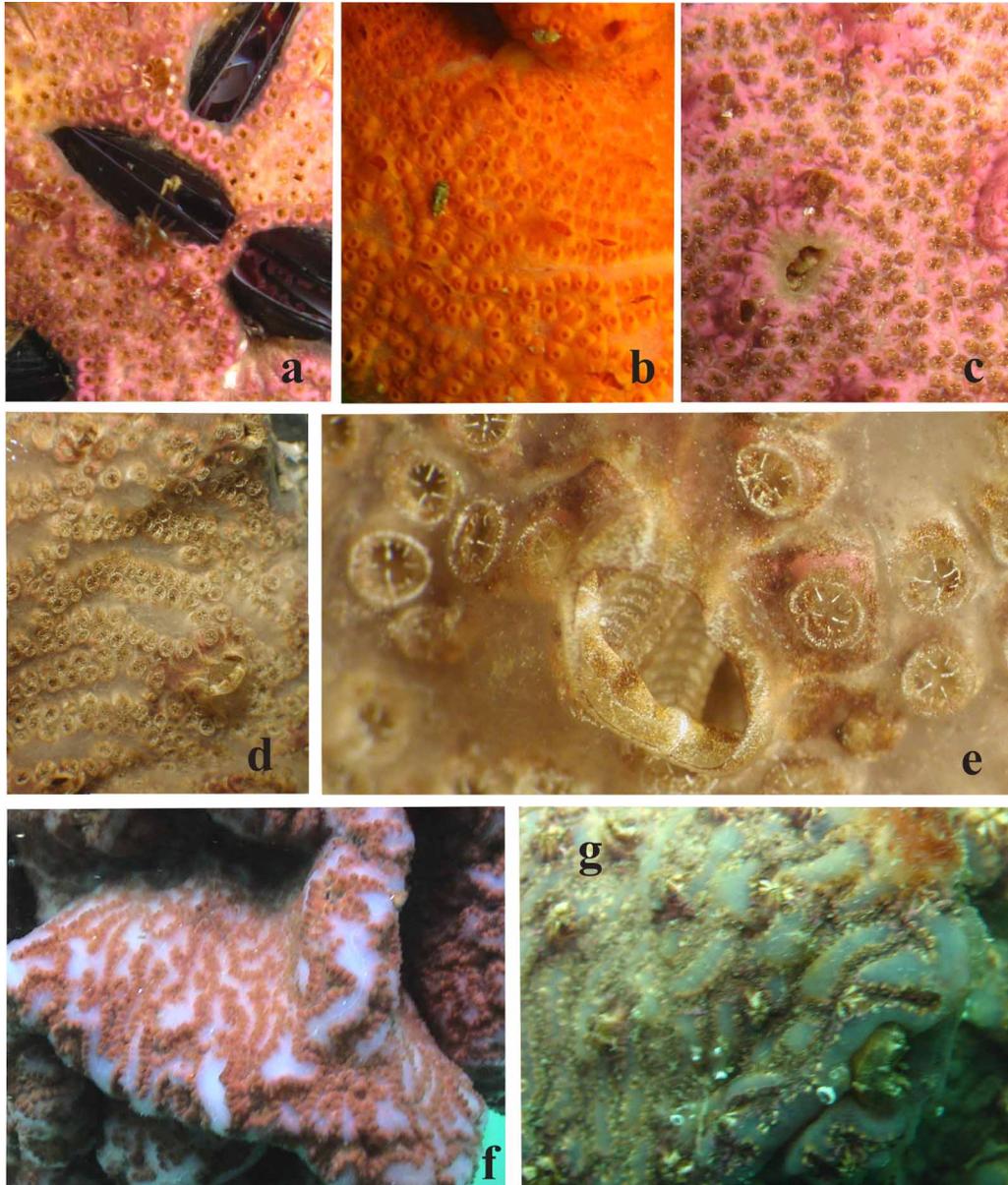


FIGURE 2. Colour morph of *Botrylloides pizoni* n. sp. a–e, normal colonies; e, enlarged detail of fig. d showing a common cloacal aperture and dilated oral aperture with tentacles; f–g, “mammillated” colonies.

Gonads. The testis and ovary lie immediately below the buds, the testis anterior to the ovary. The testis is usually formed of about 10 elongated follicles meeting in a single central deferent (Fig. 3f). When the ovary is present the testis is arranged as an arch on the ovary, however when only ripe testes are present the swollen follicles give them a mulberry appearance. In filtering zooids there is only one egg per side, projecting from the body wall. The embryo develops in the same position, enclosed in a brood pouch (Fig. 3a) as happens in some *Botrylloides* species. Sometimes only one embryo develops.

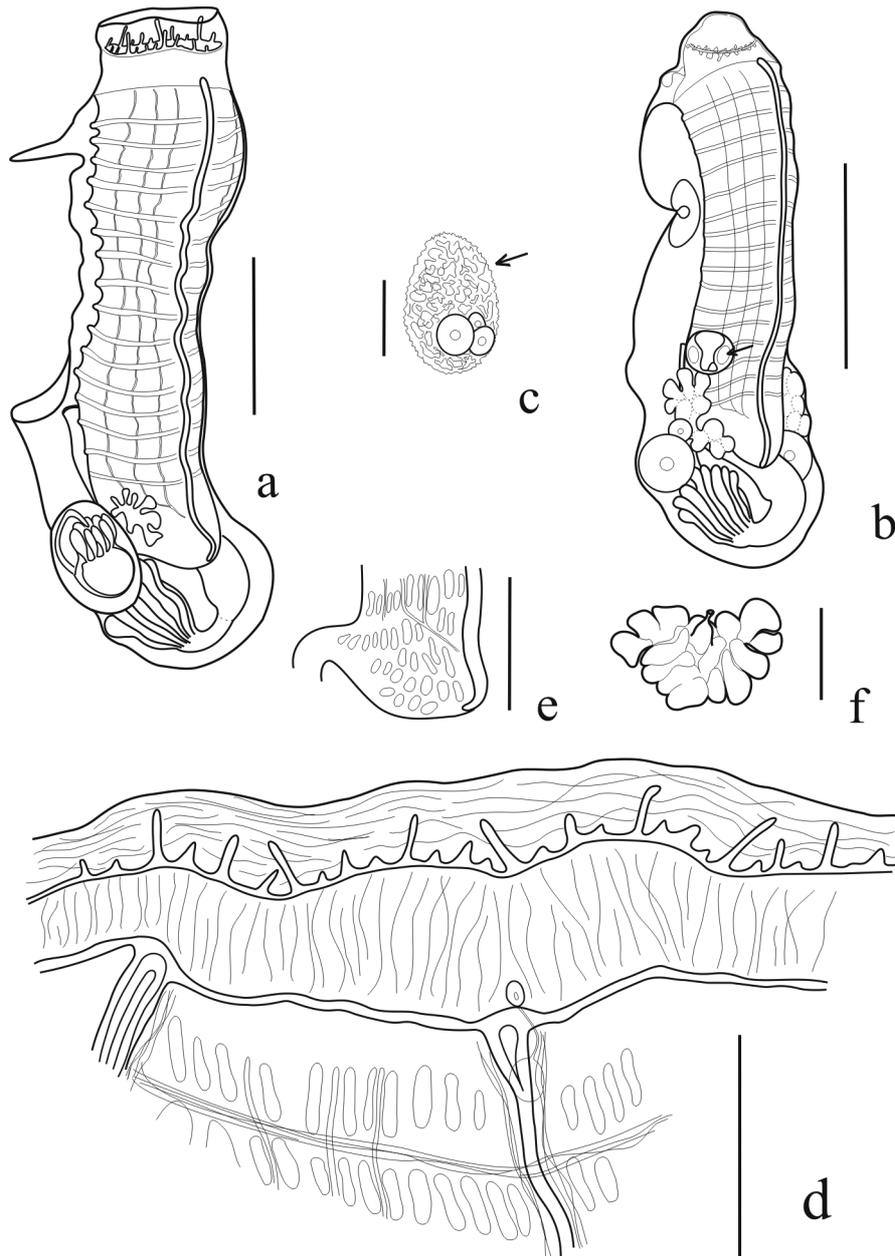


FIGURE 3. *Botrylloides pizoni* n. sp. a, filtering zooid with testes (empty or regressing) and larvae in the brood pouch; b, first order bud with testes and oocytes: primordia of gonads are present in the second order bud (arrow); c, gonadic primordia of fig. b enlarged; d, opened zooid showing tentacles and muscles; e, posterior part of the branchial sac; f, test. Scale bars: a: 1 mm; b: 200 µm; c: 20 µm; d: 500 µm; e: 600 µm; f: 80 µm.

Reproduction. Reproduction takes place from spring to early winter. Like all species of Botryllinae for which the reproductive physiology is known, the present species is protandric (Satoh, 1994). The first testis primordia and subsequently oocytes develop in buds (Figs 3b–c, 5c) but both regress during the change of generation (samples 1, 4, 11); then, if the season is in favour of sexual reproduction, the gonadic primordia of the buds ripen when the latter become filtering zooids: at first only the testis (samples 3, 6, 9, 10, 13), then also the ovary (samples 7), finally only the ovary (sample 8) whereas the testis are atrophic or absent in filtering zooids (Fig. 3a). Thus during the reproductive season colonies with “male”, “hermaphroditic” or “feminine” zooids can be found. Some colonies (probably young ones) may of course have no gonads in the reproductive season (samples 2, 14). Lastly, reproduction stops in winter (samples 5, 12). The situation of samples 4 and 11 represents the transition from the reproductive to resting season.

The larvae are liberated from cloacal apertures. However, the dissection of one colony, fixed during the spawning period (sample 8), showed that larvae are discharged from the brood pouch when its external wall breaks into the colonial matrix where many larvae were found. In addition, this colony, which was fixed during the *bcg* physiological stage (colony immediately preceding regression of filtering zooids or change of generation), the most external layer of the matrix, which is immediately below the cuticle and surrounds the filtering zooids is mucous. Although this may be an artefact due to a fixation error, we presume it may also be a natural, although temporary, condition which makes it easier for the larvae, which leave the maternal body in a such an unusual way, to reach the cloacal channels and thence leave it.

Swimming larvae are about 1.8 mm long (without fins), of which the trunk is about 600 μm . It has 8 ampullae and 3 adhesive papillae, like almost all species of the subfamily. When near to settling in the trunk, the structures of the future oozoids are visible (Fig. 4b).

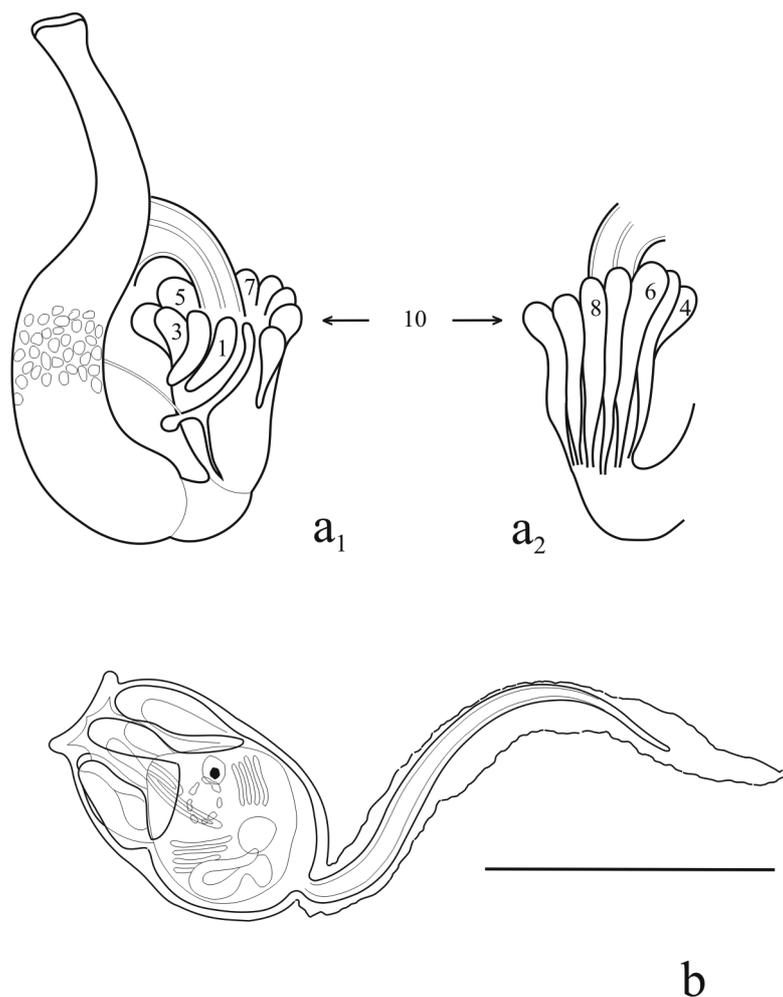


FIGURE 4. *Botrylloides pizoni* n. sp. *a*₁, and *a*₂, parietal and mesial sides of intestine; *b*, swimming larva. Scale bars: *a*₁ and *a*₂, 300 μm ; *b*: 600 μm .

“*Mammillated*” colonies. The curious appearance of the colonies shown in Figs 2 f–g is not common, since only 4 out of 14 samples present this aspect. As noted above, these colonies have areas of test without zooids between systems. A similar condition is not peculiar, and may be observed in many (or all) species of the Botryllinae. However, in this case, these areas are prominent, and almost enclose the systems in a furrow. In cross-section, these structures appear to be due to an expansion of the matrix, produced by a large number of vascular ampullae, branching out from vessels which rise vertically between the systems from the basal layer of the colony to the surface (Fig. 6a) They are not linked to any season or particular physiological stage (e.g. sexual reproduction) of the colony, and the reason for the phenomenon is still unclear. Although the zooids do not appear to be in poor conditions, they are clearly suffocated by the surrounding matrix, which may indicate a pathological condition (Figs 2g–6b).

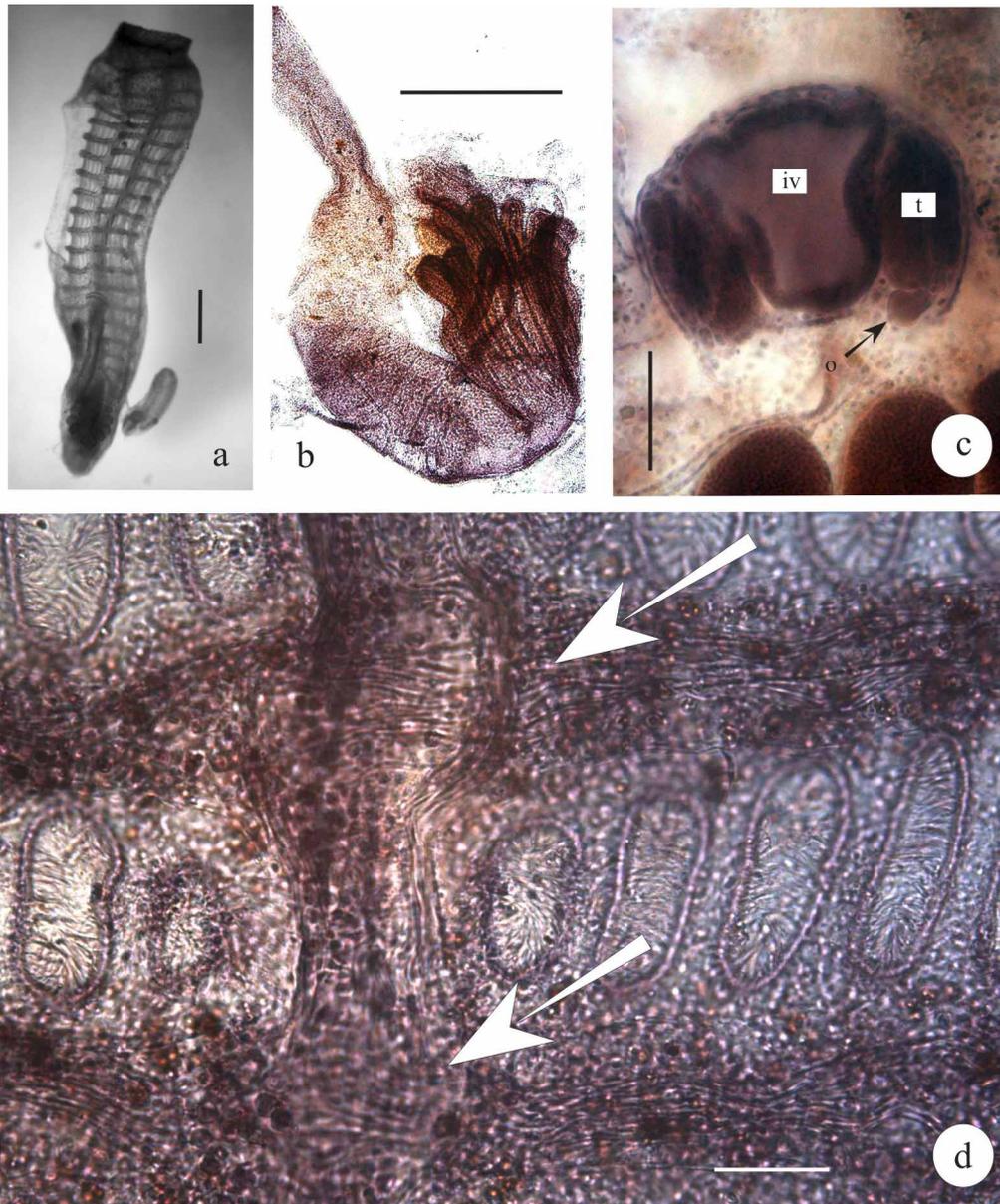


FIGURE 5. *Botrylloides pizoni* n. sp. a, filtering zooid with buds; b, stomach and intestine; c, second order bud: iv = internal vesicle in which the two peribranchial chambers are developing, t = testes primordia and o = oocytes; d, branchial wall, observe the presence of fibers muscle (arrow) in transversal vessels and along the dorsal vessel. Scale bars: a, 500 µm; b, 300 µm; c, 50 µm ; d, 40µm.

Remarks. Among Botryllinae with “*leachii* type” systems only one species may be related to the present one: *Botryllus perspicuus*: Monniot, F. & C., 2001 (originally described as *Botryllus firmus* (Monniot, F. & C., 1996, where the description is given). Both species are characterised by large zooids, with branchial sacs with numerous rows of stigmata, of which the second is complete, and furnished with a stomach larger at the cardiac than the pyloric end, but devoid of the large ovoid swelling at their cardiac end, which are present in many species of the group (i.e., in the type species *Botrylloides leachii*). However, the two species differs in the shape of gut loop and the atrial aperture. In *B. firmus* the stomach is horizontal, the intestinal loop describes a close loop, the anus opens one row of stigmata forward of the anterior edge of the intestinal loop and the atrial aperture is very large, reaching the level of the oesophageal opening. In addition, the stomach differs in the number and shape of its folds.

Another species with large cylindrical zooid with many rows of stigmata, *Botryllus eilatensis* (Shenkar & Monniot, 2006), must be compared, because the closeness of its type locality, the Gulf of Eilat, and the theoretical possibility of its introduction in the Mediterranean. However the latter species differs in some taxonomically

important characters, such as the shape of the stomach and pyloric caecum, the second row of stigmata incomplete, and other minor ones.

The new species described here was first recorded in 2001, but it is difficult to establish whether it is native or an invader. However, although in non-taxonomic studies Botryllinae are usually all assigned to the most famous local species (in this case, *Botryllus schlosseri* and *Botrylloides leachii*), it was probably really absent in the area previously, because its large zooids and the peculiar shape of the gut loop would have been noticed earlier.

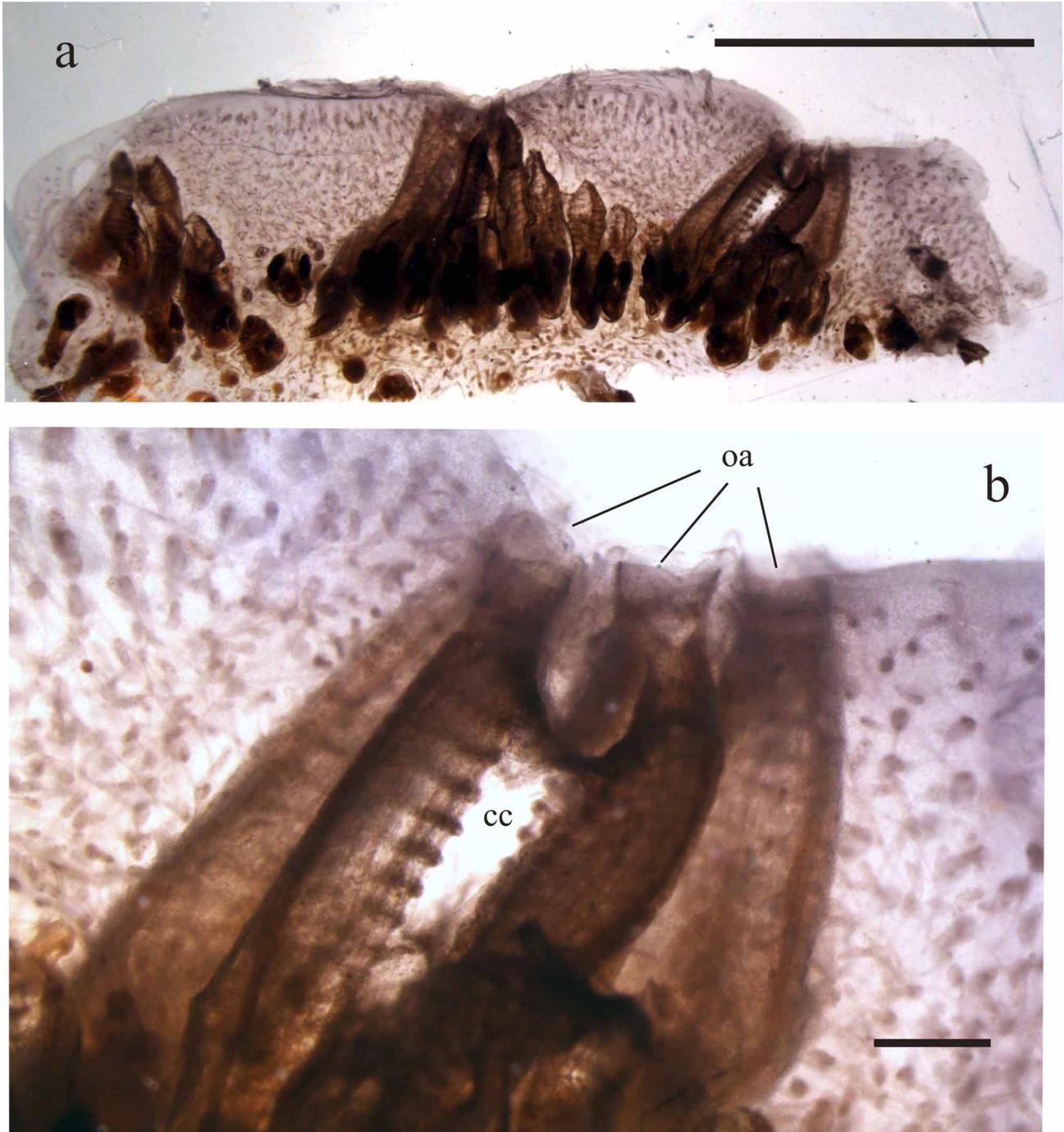


FIGURE 6. *Botrylloides pizoni* n. sp. a, cross section of a “mammillated” colony; b, enlarged detail from fig a: oa = oral apertures of zooids, cc = cloacal canal. Scale bars: a, 6 mm; b, 500 μ m.

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