New data on *Aplidium tabarquense* (Tunicata: Ascidiacea) distribution in the Adriatic Sea

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**Abstract:** The colonial ascidian *Aplidium tabarquense* Ramos-Esplá 1991 was recorded in Montenegro during a monitory survey carried out by scuba divers on an underwater outfall of the wastewater discharge system in Trašte Bay. Several colonies were present on artificial substrata at a depth of 27 m. This is the first record of the species in the South Adriatic Sea. Further observations are recommended both to delineate the effective distribution and population density of *A. tabarquense* in the Mediterranean Sea and to widen the knowledge on ascidian diversity along Montenegro’s coasts.

**Key words:** *Aplidium tabarquense*, Tunicata, ascidian, new record, Montenegro, Adriatic Sea, Mediterranean

*Aplidium tabarquense* is a Mediterranean endemic species. Ramos-Esplá (1991) documented this species from the littoral zone of the Iberian Peninsula as new for science and as the first record for the Mediterranean, respectively. *Aplidium tabarquense* was described as *A. tabarquensis* by Ramos-Esplá but, considering the neuter gender of the genus *Aplidium*, the species is correctly named as *tabarquense* (Brunetti and Mastrototaro, 2017). Available literature data indicate the presence of this species both in the Ionian Sea and North Adriatic Sea (Mastrototaro and Tursi, 2001; Ponti and Mastrototaro, 2006). Further data about the distribution of *A. tabarquense* are very poor. Moreover, the knowledge of ascidian diversity in Montenegro is still limited and available only from gray literature (VV AA, 2017). This contribution aims to report a new occurrence of *A. tabarquense* for the Mediterranean Sea and a first record in the southern Adriatic waters.

Colonies of *A. tabarquense* were first observed and then manually collected by scuba divers during a monitoring survey of an underwater outfall of the wastewater discharge system in Trašte Bay-Montenegro (42.36765°N, 18.66910°E), on 1 November 2016 (Figure 1A). More specifically, specimens collected were attached to the discharge pipe at a depth of 27 m (Figures 1B and 2A–2C). At the moment of sampling, the temperature of the water was of 20 °C, with a salinity of 36.6 psu and pH 8.22.

The diversity of the study area was mainly characterized by sciophilous populations of hard substrata, such as sponges belonging to the species *Phorbas tenacior* (Topsent, 1925), *Ircinia variabilis* (Schmidt, 1862), *Ircinia oros* (Schmidt, 1864), and *Aplysina aerophoba* (Nardo, 1833); bryozoans such as *Schizobrachiella sanguinea* (Norman, 1868); and the ascidians *Halocynthia papillosa* (Linnaeus, 1767) and *Microcosmus* spp. (Figure 1B).

Upon sampling, ascidians were narcotized with menthol crystals and then preserved in a 4% formaldehyde solution in seawater. The identification was carried out at the Biology Department of the University of Bari, Italy. Zooids and larvae were extracted from the test and colored with Mayer’s hemalum solution for identification, using the most updated taxonomic identification keys (Ramos-Esplá, 1991; Brunetti and Mastrototaro, 2017).

The collected specimens were identified as *Aplidium tabarquense* Ramos-Esplá, 1991 based on numerous morphological characters of colonies, zooids, and mature larvae (Figures 2–4) (Ramos-Esplá, 1991; Mastrototaro and Tursi, 2001; Brunetti and Mastrototaro, 2017).

Phylum: Chordata
Subphylum: Tunicata
Class: Ascidiacea
Order: Enterogona
Suborder: Aplousobranchia
Family: Polyclinidae
Genus: *Aplidium*
Species: *Aplidium tabarquense* Ramos-Esplá, 1991

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Figure 1. A) Map of Trašte Bay (Montenegro) showing the sampling site of *Aplidium tabarquense* (November 2016). In the lower left corner, a view of Mediterranean Sea. B) Colony of *A. tabarquense* sampled on hard substrata in association with the ascidian *Microcosmus* sp. and the bryozoan *Schizobrachiella sanguinea*.
Sampled material showed contracted ovoid colonies (despite the anesthetic treatment with menthol crystals in sea water) about 30 mm high. The test was transparent and jelly-like, scarcely impregnated with sand (Figure 2C). Zooids were arranged in systems of two parallel series (Figure 2B), which opened at the top of the colony; they gave an orange coloring to the fresh colony, while formalin-conserved colonies became whitish-gray (Figures 2A and 2C).

Extracted zooids were orange in color and about 8–12 mm long, with the thorax about as long as the abdomen (Figure 3A). The thorax and the abdomen measured about 2 mm each, while the posterior abdomen reached up to 8 mm in length, with a relation between thorax, abdomen, and postabdomen of 1/1/4. The branchial siphon was terminal at the anterior end of the zooid, while the atrial siphon opened on the dorsal side of the thorax. The oral siphon had six triangular lobes, which appeared to be rounded in contracted zooids. A short atrial siphon, smooth-edged, opened between the third and fourth row of stigmata; anteriorly to it, there was a simple languet (Figure 3B). The longitudinal musculature was composed of 8–10 fine fibers on each side of the thorax, which extended to the entire length of the abdomen and postabdomen.

The branchial sac had about 15–16 rows of stigmata, with 9–10 stigmata per row on each side (Figure 3C). Several zooids had 2–3 mature larvae in their peribranchial cavity.

The abdomen region contained the alimentary canal; in fully extended zooids it was possible to identify the characteristic stomach shape with 5 well-marked vertical folds (Figures 3D and 3E). The anus was smooth and opened at the level between the thirteenth and the fourteenth rows of stigmata (Figure 3C).

There was no constriction between the abdomen and the posterior abdomen regions. The gonads were situated in the whole posterior abdomen of the zooids,
with the ovary situated anteriorly to testis (Figure 4A). The ovary, with 3–6 oocytes, was in the anterior portion of the postabdomen (Figure 4B). The testis, distributed throughout the postabdomen length, consisted of up to 40 rounded follicles, aligned in a single row or sometimes arranged in pairs (Figure 4C). A vas deferens connected the testicular follicles.

Several mature larvae were recorded from both the test matrix and the peribranchial cavity of numerous zooids, in numbers of two or three in each zooid (Figure
Larval trunk measured approximately 0.5 mm long. The tail was already formed and wrapped around the trunk. In the dorsal part of the trunk, posteriorly, there was a well-developed ocellus. At the anterior end of the trunk there were three adhesive organs in a single median vertical plane and two pointed ampullae. Several oval ectodermal vesicles, arranged around the visceral mass, were distributed on both sides of it (Figure 4D).

The family Polyclinidae was defined by Kott (1992) as colonial species with oral siphon lobed, zooids arranged in systems with a common cloaca, and gonads and heart in the posterior abdomen. Larvae with otolith and ocellus, three median adhesive organs, ectodermal ampullae and vesicles (Kott, 1992). This family includes genera characterized by a branchial sac with or without stigmata and stomach longitudinally folded or not (Brunetti and

![Figure 4. A) Posterior region of a zooid showing the gonads in the postabdomen. ab, abdomen; pa, postabdomen; ov, ovary; ts, testis. B) Postabdomen region with ovary, composed of three oocytes and an embryo. oo, oocytes; em, embryo. C) Postabdomen with testicular follicles. tf, testicular follicles. D) Larva with ectodermal vesicles, white arrows pointing out 3 adhesive organs. ev, ectodermal vesicles; am, pointed ampullae; ao, adhesive organs.](image_url)
Mastrototaro, 2017). Based on the presence of stigmata and a stomach with vertical folds, it was possible to assign the genus *Aplidium* to our colonies.

The genus *Aplidium* is characterized by branchial siphons with six or sometimes eight lobes, no constriction between abdomen and posterior abdomen, stomach usually with vertical folds, larvae with three median adhesive organs and several ectodermal ampullae (Brunetti and Mastrototaro, 2017). In the recent monography of European ascidians, Brunetti and Mastrototaro (2017) report 36 species of *Aplidium* and, among these, 27 species with a 6-lobed siphon. Furthermore, 14 species with 6-lobed siphon have a simple languet. Among them, 10 are characterized by a plicated stomach with well-marked folds. Among these 10 species, based on the number of rows of the branchial sac stigmata, there are only three species with approximately 15–16 rows: *A. tabarquense*, *Aplidium densum* (Giard, 1872), and *Aplidium bermudae* (Van Name, 1902). Concerning *A. densum*, its colony has a characteristic investing or cushion shape up to 10 mm thick, coated with sand. Moreover, its zooids have thorax about twice as long as abdomen and 18–20 stigmata per half row. The stomach has 10–16 folds and the anus, opening halfway through the thorax, is bilobed. The testicular follicles are arranged in a double row (Ramos and Ros, 2004; Brunetti and Mastrototaro, 2017). *Aplidium bermudae* colonies are never impregnated by sand. The zooids of this species have thorax twice as long as abdomen. Four characteristic red spots are visible at the level of the peribranchial groove. The stomach has usually eight folds and the anus opens halfway through the thorax. The larvae have ectodermal vesicles arranged dorsally and ventrally to the adhesive organs (Brunetti and Mastrototaro, 2017).

Our colony, as described by Brunetti and Mastrototaro (2017), has a 6-lobed oral siphon, a simple languet, a plicated stomach with five folds, 15–16 rows of stigmata on the branchial sac (9–10 stigmata per half row), and an ovoid-shaped colony impregnated with sand. Moreover, the simple languet of the atrial aperture is characteristically situated anteriorly and separated from it. The thorax is as long as the abdomen and the anus, smooth-edged, opens at the level of the posterior part of the thorax (Ramos-Esplá, 1991). All these mentioned characters, as well as features of the larvae, such as the arrangement of the ectodermal vesicles all around the visceral mass, confirm that the colony collected in Trašte Bay belonged to the species *A. tabarquense*.

This finding represents the first record of *A. tabarquense* in the South Adriatic Sea. Previous data on an *A. tabarquense* population were reported by Ponti and Mastrototaro (2006) for the North Adriatic Sea (Chioggia-Venice), describing a medium density of 33.9 ±7.9 colony/ m². During the survey carried out in Trašte Bay (November 2016), it was possible to observe many different colonies along the discharge pipe; it could thus be interesting to carry out further studies in order to better understand the distribution of this species and make a comparison with the above-mentioned study.

References


