

The non-indigenous stolidobranch ascidian *Polyandrocarpa zorritensis* in the Mediterranean: description, larval morphology and pattern of vascular budding

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Abstract

The stolidobranch ascidian *Polyandrocarpa zorritensis* was detected, for the third time in the Mediterranean, in the harbour of Taranto (South Italy). Colonies develop vigorously on all hard substrata in shallow water and now represent one of the most important elements of the local fouling community. In this article specimens of the Mediterranean populations of the species are described. The morphology of the larva, which differs from that of other Polyzoinae, and a vascular budding mechanism of replication, similar to that known to occur in the Botryllinae, were both observed for the first time.

Key words: Non-indigenous ascidian, Mediterranean Sea, *Polyandrocarpa zorritensis*, vegetative replication, morphology, vascular budding, larvae

Introduction

The non-indigenous ascidian *Polyandrocarpa zorritensis* (Van Name 1931), originally described from Peru, was found in the northern Mediterranean in summer 1974 (Brunetti 1978–79). Subsequently, in 1986, the species was found in the eastern Mediterranean (Turon & Perera 1988). More recently (June 2001) during a research project undertaken to evaluate the conditions of the marine ecosystem (SPICAMAR, Italian Ministry of Scientific Research), the species was found in the southern Mediterranean (Taranto, Italy). The following description, including information on the mechanism of replication is based on the abundant Mediterranean population of this species.

Materials and Methods

Colonies were detected during scuba diving surveys carried out across the whole of the two basins ("little" and "large sea") of the harbour of Taranto, but periodic collections were made only in the two inlets of the "little sea" eleven and four metres deep respectively (Fig. 1). The salinity stays at about 37‰ in the 1st inlet and about 36–37‰ in the 2nd one and the temperature pattern is shown in Figure 1. There is no significant stratification. Animals were anesthetized with crystals of menthol and fixed with formalin (10% in sea water). Morphological observations were made on dissected material with a stereoscopic microscope and fragments of organs were stained with Mayer's haemalum, dehydrated with ethanol at crescent concentrations and after a final treatment with propanol, mounted in Euparal.

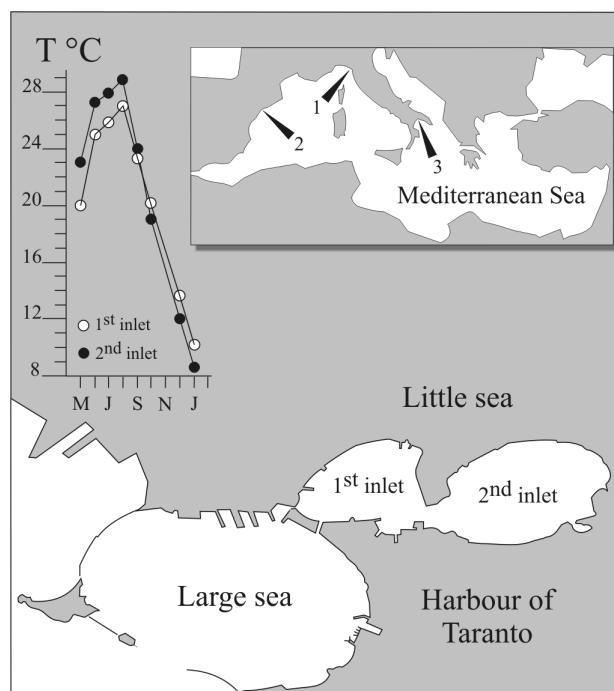


FIGURE 1 — The harbour of Taranto with mean water temperature (monthly average on the whole water column). Inset shows the locations where the species has been recorded in the Mediterranean: 1, harbour of La Spezia; 2, Ebro delta; 3, harbour of Taranto.

Results

Description of the species

Polyandrocarpa zorritensis (Van Name 1931) is a colonial Styelidae of the subfamily Polyzoinae. See: *Stolonica zorritensis* Van Name 1931, 218, *Polyandrocarpa zorritensis*: Van Name 1945, 245; Millar 1958, 505; Brunetti 1978–79, 647; Turon & Perera 1988, 84.

External morphology. The colony is a closely packed group of zooids of various sizes which are joined by basal stolons but otherwise remain independent of one another (Fig. 2). The basal portion of the colony is a tangle of stolons along which there are orange

globular bodies (buds) that will become new zooids. Adult zooids are sub cylindrical, yellow-green in color, with an apical oral siphon and a slightly eccentric atrial siphon. Each aperture is four-lobed with two dark, almost black, bands per lobe. The test is thin and leathery and is packed with branching vessels and spherical terminal ampullae. A number of large cells with granular cytoplasm accumulate on the external surface of the vessels and ampullae (Fig. 3, A).



FIGURE 2 — *Polyandrocarpa zorritensis*. Living zooids.

Internal morphology. Well-developed zooids, removed from the test, range in height from 9–10 to 12–13 mm. The body wall has delicate musculature with regularly arranged longitudinal and transverse fibres of the same size. Twenty to 30 simple tentacles of two lengths vary in number with zooid size. The dorsal tubercle has a wavy slit-shaped transverse opening. The dorsal lamina is flat with a smooth edge. The branchial sac has four narrow, low folds per side with up to 12 internal longitudinal vessels on the folds and up to two between. Muscle fibres are in the larger transverse vessels. A typical branchial formula is: E-0-(4)-0-(7)-1-(5)-1-(6)-2-DL-0-(6)-1-(6)-2-(7)-0-(4)-0-E and in more developed zooids E-1-(7)-1-(12)-2-(11)-3-(12)-2-DL-2-(8)-2-(11)-2-(7)-0-(12)-0-E. Some short lengths of parastigmatic vessel are present (Fig. 3, C). The gonads are oval polycarps in a row on each side of the endostyle, 9 to 10 on the right and 6 to 8 on the left (Fig. 3, B, D). All ovarian apertures are directed dorsally. The sexually mature zooids have several embryos in the peribranchial cavity, some adhering to the external wall of the branchial sac by a fragile mucous string derived from the external envelope of the embryo. Solitary brooding ascidians are known to retain their embryos in a sticky mucus (Lambert et al. 1995; Lambert 2004); this may be a similar adaptation. It is not known whether this mucous string is an artefact, or whether embryos are free as a consequence of fixation. The larval trunk, at the developmental stage in which the tail completely surrounds it, is about 530 µm in length (average from ten measurements = 524.5, min.= 458, max.= 600). Three tri-radially arranged adhesive organs are in a sessile frontal process from which three clusters of ampullae originate at metamorphosis (Fig. 3, E). The intestinal canal (gut), on the left of the branchial sac, consists of a short oesophagus, a trapezoid stomach with a folded wall (generally 10–15 folds in addition to the typhlosole). A finger-shaped gastric caecum with a spherical tip extends from the external postero-ventral corner of the stomach but is obscured by an endocarp located in the intestinal loop (Fig. 3, F). The diameter of the intestine gradually decreases from the pyloric end of the stomach to the plain edge anus near the atrial siphon.

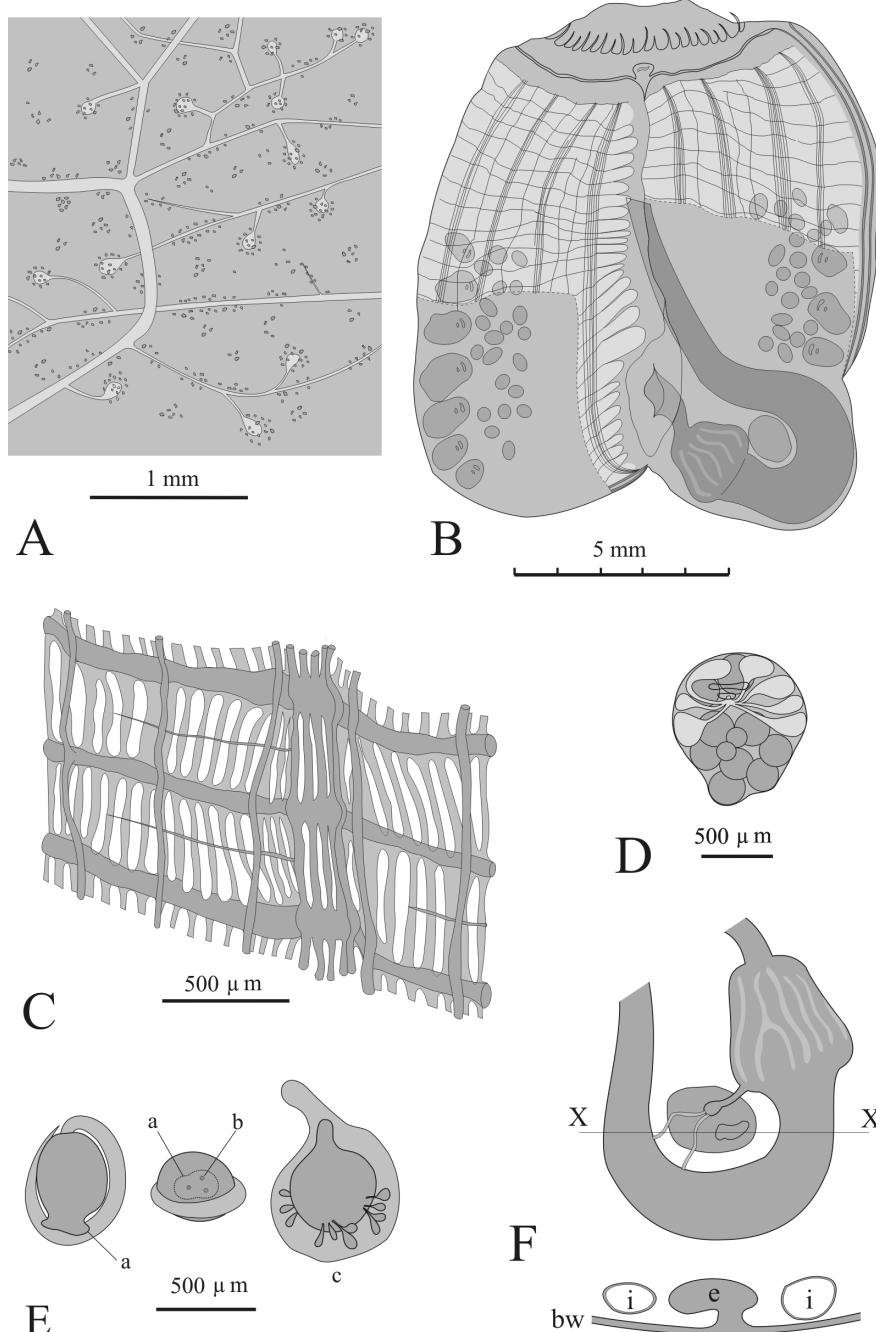


FIGURE 3—*Polyandrocarpa zorritensis*. **A**, Tunic vascular system. Note the large cells adjacent to the vessels and ampullae. **B**, dissected zooid opened ventrally, part of the branchial wall is removed, the stigmata are not drawn. **C**, part of branchial wall with fold and incomplete parastigmatic vessels. **D**, gonad. **E**, left: unhatched larva; middle: frontal process (a) in which the three adhesive organs are included (b); metamorphosed larva (c). **F**, intestinal loop drawn from the outer (left) side after removal of the body wall. Note the gastric caecum. Lower: cross section through X-X. bw, body wall; e, endocarp; i, intestine.

Vascular system and Replication. The basic circulatory plan is the same as in all ascidians: subendostylar vessel, perigastric lacunae, dorsal vessel, pericoronal vessel and again subendostyle vessel (for this terminology see Burighel & Brunetti 1971). The heart is large, extending along the posterior third of the branchial sac, surrounding it posteriorly then extending anteriorly as far as the stomach. At the level of the anterior end of the heart, a vessel branches off the subendostyle (or ventral) vessel, extends posteriorly ventral to the heart and enters the basal stolon (Fig. 4, A). The stolon branches and forms spherical swellings (buds) into which the stolonic vessel branches, each branch with a terminal ampulla filled with blood cells. New zooids develop inside these buds (Fig. 4, B), vascular connection with the parent closes and the ampullae around the buds empty themselves, are reduced in size and become the test vascular system of the new zooids. The latter are completely formed before reaching 2 mm in height and at 3 mm gonads often are present.

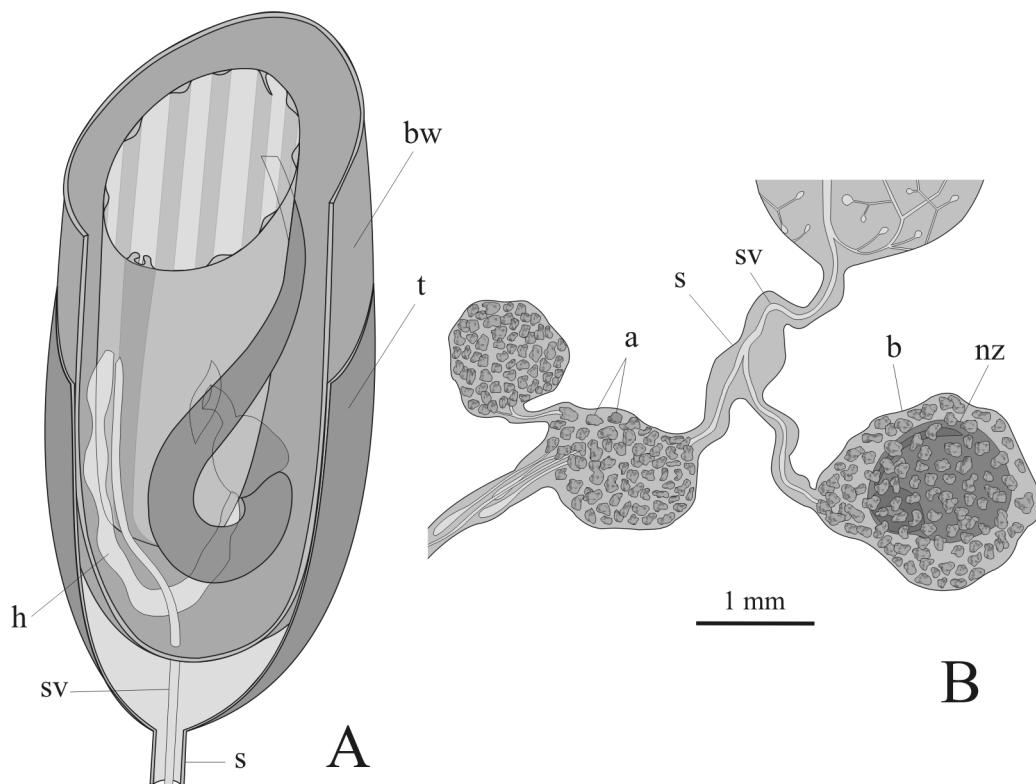


FIGURE 4 — *Polyandrocarpa zorritensis*. **A**, diagram showing the vascular connection between the zooid and the stolon. bw, body wall; h, heart; s, stolon; sv, stolonic vessel; t, test. **B**, stolon with buds. a, ampullae; b, bud; nz, developing new zooid; s, stolon; sv, stolonic vessel.

Ecology

In both basins the species colonized a significant proportion of the hard substrata present in shallow water down to a depth of 2 m. In particular, extended colonies were

found under iron buoys, on mussel breeding piles and on the steel wires connecting them. In November 2003 colonies collected in the two inlets were compared. The number of zooids present in three replicates of an area of 4 cm^2 was calculated and the percentage of small (3–6 mm), medium (6–10 mm) and large ($>10\text{ mm}$ in height) size were noted. As we can see in Figure 5, the percentage of large zooids is higher in the surface colonies of the 2nd inlet. This greater development may be due to the higher water temperatures registered during the previous months (Fig. 1) and probably also to a more eutrophic condition. At the deepest level colonies were present only in the 2nd inlet. However the percentage of medium and small zooids dramatically increases in comparison to the situation of the surface colonies. This was due to minor development of the bottom animals as confirmed by the observation that the gonads are low in number or absent.

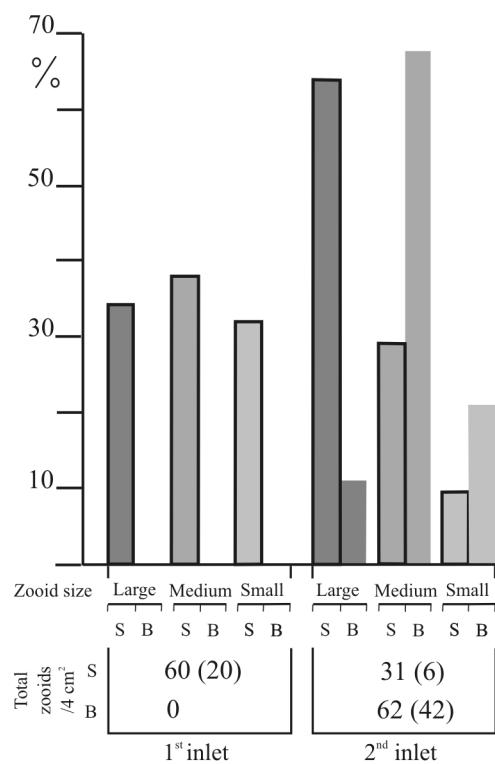


FIGURE 5 — Number of zooids per 4 cm^2 (average and, in brackets, SD; number of replications: 3) and percentages of large, median and small zooids (see text) in surface (S) and bottom (B) colonies.

Discussion

Polyandrocarpa zorritensis is a conspicuous shallow water species whose size and colonial habitus suggest that it would not have escaped the attention of the observers. Thus it is reasonable to assume that the Mediterranean records represent stages of a biological invasion. The species seems to prefer harbour environments, that is with eutrophic waters, where the colonies develop rapidly by prolific replication, while the zooids produce a larger number

of larvae, which show a positive phototaxis when ready to settle (Vázquez & Young 1996), a behaviour that enhances their restriction to hard substrata in shallow waters. The species is spreading in the Atlantic (Ruppert in Lambert & Lambert 1998) and the Pacific Ocean viz. in Japan (Nishikawa et al. 1993); and in South California where it has been recorded from 1994 (Lambert & Lambert 2003). Although there is no readily acceptable explanation for the phenomenon, Lambert & Lambert (2003, 159) present data suggesting that stolidobranch ascidians “both solitary and colonial survive long-range anthropogenic transport better than do other types of ascidians”. At this stage, it has been proposed that the warming of ocean waters could be the main cause of the apparent expansions for this and other species (Stachowicz et al. 2002; for Mediterranean see Francour et al. 1994). However these biological changes could be due to processes other than, or as well as, a simple increase in temperature (Bianchi 1997). The aggressive invasion of *P. zorritensis* could be due to its budding which makes possible the rebuilding of the colony even in the absence of adult zooids. The continued spread of this species around the Mediterranean may be due to short-range transport via hull fouling of small craft from its initial site of invasion (Wasson et al. 2001; Lambert & Lambert 2003). The basal stolons are extremely adherent, and could easily survive even if the zooids died in transit. Any brooded embryos in zooids that do survive would be released in the new harbour.

The species has some unusual morphological characters. The larvae, with three adhesive organs in a frontal process, differ from those of other Polyzoinae; and an equally wide test vessel system is present only in *Symplegma* and *Chorizocarpa*. However, it is the mechanism of replication that in our opinion is particularly interesting. In the few known cases, (for a review see Nakauchi 1982), the multipotent tissue involved in morphogenesis of buds in the Polyzoinae is the peribranchial epithelium. In *P. zorritensis* it is the blood vessel wall and the lymphocytes: and consequently it is a form of vascular budding. In her monumental work on the Australian Ascidiacea, Kott suggests the genus *Polyandrocarpa* might be polyphyletic (Kott 1985, 214). The results of our observations seem to be in agreement with the hypothesis of the great ascidiologist. This kind of budding is found also in Botryllinae, where in addition to the palleal budding, vascular budding is present both under natural (Oka & Watanabe 1957; Burighel et al. 1976) and experimentally-induced conditions (Oka & Watanabe 1959; Milkman 1967; Sabbadin et al. 1975). Finally, if the connection of the embryos to the outer wall of the branchial sac is confirmed to be more than just adhesion, this may be the first attempt at the brooding modality which is present, in a more evolved form, in *Botryllus delicatus* and *Botryllus sexiens* (Okuyama & Saito 2001).

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