The Occurrence of *Amphiglena mediterranea* (Leydig, 1851) (Annelida: Sabellidae) at the Romanian Coast of the Black Sea: A Case on an Unsuccessful Invasion?

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Abstract—During a study of fauna associated with a shallow-water Zostera (Zosterella) noltei meadow from the southern part of the Romanian Black Sea coast, in August 2015, a sabellid polychaete identified as Amphiglena mediterranea was found. This represents a new record for the Romanian coast of the Black Sea. Three individuals were collected from muddy fine sand at 1.5 m depth at Mangalia, at salinity of 17.4 PSU and temperature of 23.2°C. Subsequent repeated sampling in the same area (in 2018 and 2019) did not revealed any additional individuals of this species. Therefore, the occurrence of A. mediterranea at the Romanian Black Sea coast might be regarded as an unsuccessful invasion.

Keywords: Polychaetes, new record, faunistics, Romania, unsuccessful invasion, alien species **DOI:** 10.1134/S207511172003011X

INTRODUCTION

Seven species in seven genera of Sabellidae have been reported in the Black Sea: *Amphicorina armandi* (Claparède, 1864) (Vinogradov, 1931, 1949; Dumitrescu, 1960), *Amphiglena mediterranea* (Leydig, 1851) (Makkaveeva, 1983; Kisseleva, 2004), *Paradialychone filicaudata* (Southern, 1914) (Sezgin et al., 2010; Kurt Şahin et al., 2017), *Pseudopotamilla reniformis* (Bruguière, 1789) (Boltachova and Lisitskaya, 2016), *Jasmineira caudata* Langerhans, 1880 (Marinov, 1959), *Acromegalomma vesiculosum* (Montagu, 1813) (Rullier, 1963), and *Potamilla torelli* (Malmgren, 1866) (Rullier, 1963), the last three in the Prebosphoric waters only. Of these species, only *Amphicorina armandi* is known from the Romanian Black Sea coast (Dumitrescu, 1957; Băcescu, 1963; Băcescu et al., 1965).

Recently, another two species of sabellids—*Aracia* sp. and *Laonome xeprovala* Bick & Bastrop, in Bick et al., 2018 (as *Laonome calida* Capa, 2007)—were reported in the adjacent Sea of Azov by Syomin et al. (2015) and Boltachova et al. (2017), respectively. Thus, it is expected that those two species might occur as well in the Black Sea, especially given that *Laonome xeprovala* is considered as an introduced species in European waters (Capa et al., 2014; Bick et al., 2018).

During a study that was carried out with the aim to reveal the fauna associated with a dwarf eelgrass (*Zostera (Zosterella) noltei*) canopy from the southern part

of the Romanian Black Sea coast in 2015, among the material collected, a previously unreported sabellid polychaete was observed. The aim of this paper is to report and to describe the sabellid species that is new for the Romanian coast of the Black Sea.

MATERIAL AND METHODS

Specimens identified as A. mediterranea were collected on August 6, 2015 at Mangalia (43.8042° N, 28.5917° E. Romanian coast. Black Sea) from fine muddy sands with Zostera (Zosterella) noltei at about 1.5 m deep. Sediment samples were taken with a handheld corer, 10 cm in diameter, volume ca. 0.8–1, penetration ~20 cm. Samples of sediment were sieved in situ with seawater through a 0.5 mm mesh screen and the remaining residue was preserved in 10% buffered solution of formalin in seawater and subsequently transferred to 80% ethanol. Romanian specimens were compared with other collected in the Western Mediterranean, from port environments in Palma, Mallorca at about 0.5-1.5 m deep, on vertical concrete walls of piers and preserved in 100% ethanol. All specimens were examined with light (LM) and phase contrast (PCM) microscopy after mounting them in polyvinyl lactophenol for one week. Micrographs were taken with a Leica DFG500 digital camera attached to a Leica DM750 compound microscope. For scanning



Fig. 1. *Amphiglena mediterranea* (LM micrographs of the Black Sea specimen). (a) Whole specimen, right lateral view; (b) branchial crown, lateral view; (c) axial cells; (d) collar chaeta; (e) superior thoracic broadly-hooded notochaeta (in the left) and inferior thoracic paleate notochaeta (in the right), chaetiger 3; (f) thoracic uncinus, side view; (g) companion chaeta, chaetiger 7; (h) mid-abdominal uncinus, side view; (i) mid-abdominal uncini, apical view; (j) elongate broadly-hooded abdominal neuro-chaetae; (k) pygidium, dorsal view. Scale bars: (a) 1 mm; (b) 0.5 mm; (c, k) 0.1 mm; (d–j) 0.05 mm.



Fig. 2. Amphiglena mediterranea (SEM micrographs of the Black Sea specimen). (a) Whole specimen, left lateral view; (b) anterior end and base of branchial crown, dorso-lateral view; (c) anterior end and base of branchial crown, ventro-lateral view; (d) thoracic notochaeta; (e) thoracic uncinus; (f) thoracic uncini (above) and companion chaetae (below); (g) abdominal elongate broadly-hooded neurochaeta; (h) abdominal uncini; (i) abdominal elongate broadly-hooded neurochaetae (in the left) and notopodial uncini (in the right). Scale bars: (a-c) 0.1 mm; (d) 5 µm; (e) 1 µm; (f-i) 0.5 µm.

electron microscopy (SEM), specimens were dehydrated in a graded ethanol series, and subsequently in a series of mixtures of absolute ethanol and Hexamethyl disilazane (HMDS) with the following ratios 2:1, 1:1, 1:2, and then into pure HMDS. The prepared samples were mounted on holders, sputtercoated with gold (10 nm thickness) and examined with a HITACHI S-3400N scanning electron microscope at the University of the Balearic Islands. Measurements of body width refer to the distance between the distal-most structures on the widest chaetiger seen on the anterior end (without chaetae). The specimens from the Black Sea were deposited in the collections of the Grigore Antipa National Museum of Natural His-

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tory, Bucharest, Romania (MGAB PLY157) and at the University of the Balearic Islands (SEM stubs).

RESULTS AND DISCUSSION

All three Romanian specimens identified as A. mediterranea are complete, with eight thoracic and 4-16 abdominal chaetigers. Total length, including crown, 0.9-3.1 mm, branchial crown 0.29-0.85 mm long, body width 0.146–0.366 mm. Body cylindrical, tapering posteriorly (Figs. 1a, 2a). Preserved specimens whitish or yellowish. Branchial crown is up to half body length, with four pairs of radioles, each with about 5-6 pairs of pinnules arranged in two alternat-



Fig. 3. *Amphiglena mediterranea* (LM micrographs of the Palma de Mallorca specimens). (a) anterior end and base of branchial crown, ventro-lateral view; (b) thoracic uncini and companion chaetae; (c) superior thoracic broadly-hooded notochaeta and inferior thoracic paleate notochaetae; (d) abdominal uncini; (e) pygidium, dorsal view. Scale bars: (a–e) 10 μm.

Fig. 4. *Amphiglena mediterranea* (SEM micrographs of the Palma de Mallorca specimens). (a) Whole specimen, ventral view; (b) anterior end and base of branchial crown, ventro-lateral view; (c) anterior end and base of branchial crown, ventro-lateral view; (c) anterior end and base of branchial crown, dorsal view; (e) thoracic uncini (above) and companion chaetae (below), chaetiger 6; (f) apical view of thoracic uncinus, anterior chaetiger; (g) lateral view of thoracic uncinus, chaetiger 6; (h) thoracic notochaetae, chaetiger 3; (i) thoracic notochaeta, chaetiger 3; (j) detail of thoracic paleate notochaetae, chaetiger 8; (k) mid-abdominal elongate broadly-hooded neurochaetae; (m) mid-abdominal uncini. Scale bars: $(a-d) 0.1 \text{ mm}; (e, h, i, k, l) 10 \,\mu\text{m}; (f, g, j, m) 5 \,\mu\text{m}.$



ing rows, increasing in size distally (Figs. 1b, 2b); tips of radioles without pinnules, slightly longer than length of basal pinnules. Radiolar skeleton with two rows of axis cells (Fig. 1c). Palmate membrane and radiolar flanges absent. Dorsal radiolar appendages not discerned. Anterior peristomial ring shorter than posterior ring, only visible from lateral and ventral sides (Fig. 2c). Posterior peristomial ring with ventral incision reaching half of peristomium and with ventral basal flanges attached. Thorax longer than wide. First thoracic chaetiger with only 1-2 elongate, narrowlyhooded collar chaetae in notopodial lobe (Fig. 1d). Superior thoracic notochaetae of chaetigers 2-8 broadlyhooded, one (very rarely 2) per fascicle (Figs. 1e, 2d). Inferior thoracic notochaetae of chaetigers 2-8 paleate chaetae, in a single transverse row, 2 (very rarely 1 or 3) per fascicle (Fig. 1e). Thoracic uncini in one row, 2-6 per torus, progressively decreasing in number from anterior to posterior chaetigers; avicular uncini with several rows of small teeth of similar size above main fang (Fig. 2e), breast well developed, handles of medium length (Fig. 1f). Companion chaetae, 1-5 per row, with straight shaft and long denticulate mucro (Figs. 1g, 2f), also decreasing in number from anterior to posterior chaetigers, alternating with uncini. Abdominal uncini, 2-3 (rarely four) per torus, with several rows of small teeth of similar size above main fang (Fig. 2h), breast well developed, handles short (Fig. 1h, 1i). Abdominal neurochaetae in a single transverse row, elongate, broadlyhooded, two (very rarely 3) per fascicle (Figs. 1j, 2g, 2i). Peristomial eyes, statocysts and pygidial eyes not seen (Fig. 1k). Tube unknown.

There are currently 14 accepted species of Amphiglena (Tilic et al., 2019). Specimens from the Romanian Black Sea match the description of A. mediterranea (Rouse and Gambi, 1997; Capa and Rouse, 2007) and the Mallor can specimens used for comparison (Figs. 3, 4) in having a unique combination of characters with: dorsal radiolar appendages about one-quarter the length of the crown, anterior margin of posterior peristomial ring projected forward ventrally (not perpendicular to body axis), ventral basal flanges stopping at the base of the crown length of thoracic uncini handles between one and two times the distance from the main fang to the base of the uncini breast (that is medium length handles) (Rouse and Gambi, 1997; Capa and Rouse, 2007). The three Romanian specimens differ from A. mediterranea described in the literature in having four pairs of radioles rather than five pairs, but this is also the case in some of the smaller specimens collected in Mallorcan waters.

Amphiglena mediterranea was originally described from Nice, France, and it is considered as one of the most common polychaetes in shallow hard bottom communities in the Mediterranean Sea (Leydig, 1851; Claparède, 1864; Sarda, 1991; Rouse and Gambi, 1997). It has also been reported from the Atlantic coast of Europe (Saint-Joseph, 1894; Rioja, 1923; Fauvel, 1927; Hayward and Ryland, 1990), South Africa (Day, 1967), and Antarctica (Rouse and Gambi, 1997). Previously, A. mediterranea was mentioned in the Black Sea only once from Zostera meadows of the area of Sevastopol Bay (Kazach'ya Bay) at 1-3 m depth (Makkaveeva, 1983). Because there are no other subsequent records of this species in the Black Sea since then and our repeated sampling in 2018 and 2019 in the same area at the Romanian coast where the Amphiglena specimens were found did not revealed any additional individuals, its occurrence is regarded here as an unsuccessful invasion. It is important to mention that both areas (Sevastopol Bay and Mangalia) are important seaports of the Black Sea which are usually well surveyed (Surugiu et al., 2013; Surugiu, unpublished data; Boltacheva and Lisitskava, pers. comm., 10-09-2019). Now, the records outside of the Mediterranean should be revised in order to discriminate potential misinterpretation of the diagnostic features (wrong identification) and when possible, the assessment of potential unintentional translocations. A recent study indicates that A. mediterranea, with a great genetic diversity but homogenous morphology, could in fact be a complex of cryptic species (Tilic et al., 2019). Unfortunately, the three specimens collected for the present study were preserved in formaldehyde and therefore not suitable for DNA sequencing, so genetic comparison between populations was not feasible.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All research pertaining to this article did not require any research permits.

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