PRESENCE OF *BRANCHIOSTOMA LANCEOLATUM* (PALLAS, 1774) LARVAE IN THE BLACK SEA ROMANIAN WATERS

MIHAELA MUREȘAN[*], ADRIAN TEACĂ[**], MARIUS SKOLKA[**]

The paper reports the first occurrence of *Branchiostoma lanceolatum* (Pallas, 1774) larvae in the Romanian waters in samples collected in July and August 2018 in four stations located in coastal and offshore waters of the Romanian shelf.

Keywords: *Branchiostama lanceolatum*, Black Sea, Romania.

INTRODUCTION

After almost fifty years since the first observation of an adult of *Branchiostoma lanceolatum* (Pallas, 1774) in the benthic samples collected in shallow waters of the Romanian littoral (Gomoiu, *pers. comm.*), here we report the first occurrence of pelagic larvae of the species in the national waters.

Branchiostoma lanceolatum is known as a relatively rare species in the Black Sea. Grigore Antipa, during the expedition performed in 1893 all around the Black Sea, documented the presence of amphioxus from Sinope, on the northern Anatolian coast, and from Sevastopol, where it had been found by Ostroumov on coarse sands in association with *Ophelia taurica*, *Polygordius ponticus*, *Glycera* sp., and two species of *Synapta* (Antipa, 1941).

Only few recent records in literature were found reporting, for example, abundances of 1,109 indv. m^{-2} (Luth, 2004) in 2004. In the period 1999–2014, *B. lanceolatum* was found in 38% of the samples collected on the Crimean soft bottoms (Shalovenkov, 2017). Shiganova *et al*. (2010) signals the appearance of specimens of the lancelet larvae (1.5–5 mm in length) in the layer of 0–160 m above 500 m (sampling point: 44°513' N, 37°933' E) at a temperature of 26°C, inferring its potential intrusion in the Black Sea from the Mediterranean Sea. At the Bulgarian coast, it forms in shallow waters (10–30 m) on shelly, medium and coarse sand an association with *Protodorvillea kefersteini* and *Gouldia minima* (Todorova *et al.*, 2015; Todorova, 2017). Lately (2011–2017), *B. lanceolatum* larvae have been constantly observed in the summer-autumn season mainly at depths of

15–40 m in coastal and inner shelf area and rarely, on the outer shelf and open sea. Its average abundance varies insignificantly around 2 ind.m^{-3} (SD± 5), an exception being recorded in front of Ropotamo river where a peak density of 32 ind.m^{-3} was found in 2016 (Stefanova K. & Stefanova E., *pers. data*). To our knowledge, no data is available for the Black Sea, from the Turkish and Georgian coast.

This species has been included in the Black Sea Red Data Book and in the Vulnerable category according to the IUCN criteria, in 1996, due to its continuous decreasing population since the 60's as result of anthropogenic pressure (Konsulova, in Dumont, 1999). Most data in the literature suggest that its demise is generally related to the change in texture and eutrophication of the coastal sediments.

MATERIAL AND METHOD

The larvae were found in the zooplankton samples collected by a Juday net with 150 µ mesh size, deployed at certain depths in the water column in July and August 2018. Out of the eight collected larvae, five specimens were found in the samples collected in front of Mamaia Bay at depths between 23–25 m, and the other three in the samples taken along Sf. Gheorghe and Constanţa transects in offshore waters (Fig. 1).

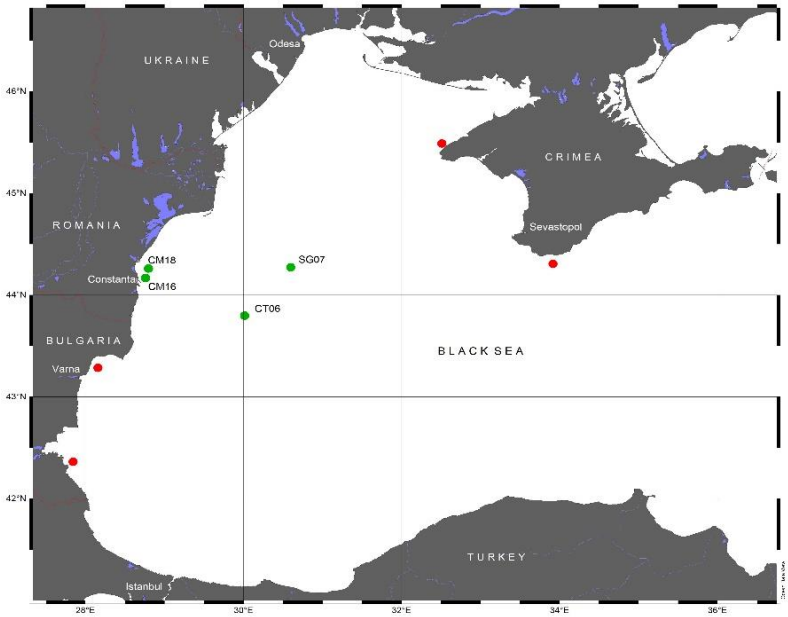

Fig. 1. The sampling stations (green points) where *B. lanceolatum* larvae occurred in July and August 2018 at the Romanian coast and its occurrence (red points) (according to Konsulova, 1999).

RESULTS

Description of larvae

Along the body three regions are seen: the head with the pharyngeal region, the midgut, the hindgut with the anus opening and the spear like tail. The notochord and the nerve chord with pigment spots span along entire body length. In the anterior head region the main observable morphological features were: the preoral organ, the club shaped gland and the anterior and posterior endostyle ridges (Fig. 2b). Within the pharyngeal part, 10–11 primary gill slits were accounted, while a canal-like atrial chamber formed from the level of the third posterior gill slit to the anterior midgut region (atriopore) was noticed (Fig. 2c). In the hindgut, the cells forming the wall corresponding to the future ileocolonic ring (Urata *et al*., 2007) of brownish colour and the anal opening could be seen (Fig. 2d). The larvae were 5–5.5 mm long, flattened in transverse plan. After all appearances, the larvae were in their late stage of development (premetamorphic larvae). *B. lanceolatum* larvae metamorphose with at least 14 gill slits, 45 to 50 days after fertilization at 23°C (Fuentes *et al*., 2007).

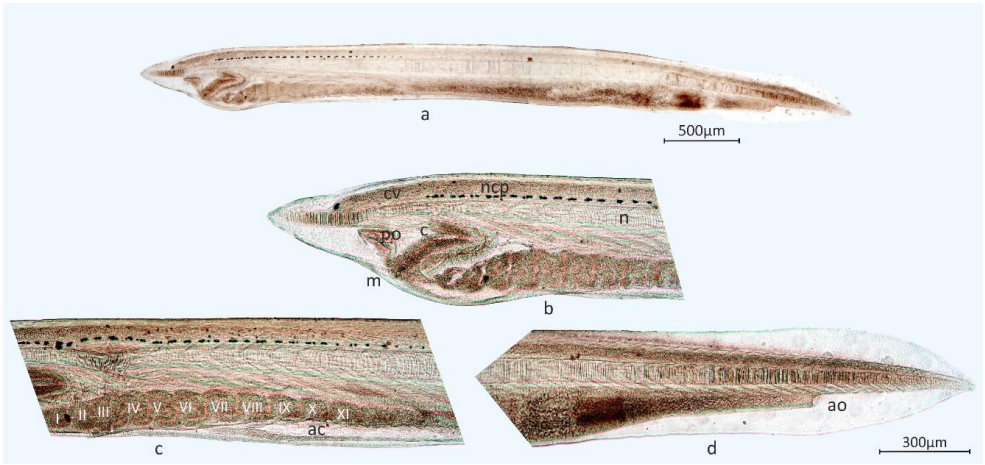

Fig. 2. a. Larva before metamorphosis into juvenile; b. Left sided head region of larva, cv. – cerebral vesicle, ncp.– nerve cord with pigment spots; c. – club-shaped gland; m. – mouth margin; n. – notochord; po. – preoral organ; c. Branchial region of larva with 11 primary gill slits (I–XI); ac – the canal-like atrial chamber formed from the level of the third gill slit to the anterior midgut region; d. Hindgut with brownish wall cells and the ao – anal opening on the left body side, and the spear shaped tail region (Photo: Teacă A.).

Distribution of specimens found at the Romanian littoral

The 4 specimens collected in July in the station CM 16 were found in the upper surface layer of 0–5 m depth as well as within the thermocline layer at 5–15 m, respectively. Likewise, in the station CM18 the fifth specimens was collected also within the thermocline layer at 10–18 m depth. The distribution of all three specimens

found in August was confined to the under thermocline layers. The larvae is believed to execute diurnal vertical migration due to their light-guided behaviour (Pergner & Kozmik, 2017). Their diet include algae such as diatoms, dinoflagellates, e.g., *Ceratium* (Kehayias, 2015), the latter being present in great abundances during sampling period. However, the larvae ingest also calanoid copepods or other organic material and small particles of a size similar to that of the larval mouth (Webb, 1969).

The salinity and temperature at the sampling site varied between 17.19 (surface layer) and 18.91 PSU (under thermocline layer) and 23.97°C (surface) and 8.41°C (bottom layer) (Table 1). In the Black Sea, the species has a reproductive peak during the summer season (Konsulova, 1992).

Table 1

Physico-chemical parameters of seawater at depths where larvae have been collected in July and August 2018

Station	**CM16**	**CM16**	**CM18**	**SG07**	**CT06**
Date	23.07.2018	23.07.2018	24.07.2018	20.08.2018	21.08.2018
Longitude [deg. E]	28.75747	28.75747	28.79524	30.59493	30.01361
Latitude [deg. N]	44.16838	44.16838	44.26112	44.27365	43.79941
Bot Depth [m]	27.4	27.4	27	107	89.7
Depth [m]	0–5	5–15	10–18	25–60	35–85
Chla [ug/L]	0.41	9.89	9.67		
T°C	23.97	12.73	13.65	10.11	8.41
PSU	17.19	18.49	18.57	18.46	18.91
O_2 mg.L^{-1}	7.24	7.06	7.95		6.99
Substrate type	coarse (shell debris)	coarse (shell debris)	sand	shelly mud	shelly mud
No. Ind./sample	2	2	1	2	1

DISCUSSION

At this moment there is no evidence for the species presence in the adult stage on the Romanian shelf. In our opinion, there are three hypothetic explanations that could be drawn concerning the larvae appearance.

Firstly, it is likely that adult populations to be actually present in the Romanian waters, but with distribution and abundances limited to few habitats, such as the sandy bottoms from the coast and/or circalittoral. For example, the habitats investigated in July correspond to the circalittoral sand and circalittoral coarse (shell debris) sediments (Teacă *et al*., 2018) that are likely suitable for adults dwelling and spawning.

Secondly, the Romanian sector is on the north-south cyclonic current trajectory, thus the larvae could have been conveyed in our research area simultaneously with the water masses from the Crimean coast.

Third, the favourable hydrometeorological conditions in the collecting period and especially in the previous days, characterised by predominantly W-NW winds that favoured upwelling and increased surface and subsurface cyclonic and anticyclonic eddies in the region of the Romanian shelf.

CONCLUSIONS

After more than fifty years since first observation of an adult of *Branchiostoma lanceolatum* at the Romanian littoral (Gomoiu, *pers. comm.*), here we report the first occurrence of pelagic larvae of the species, eight specimens being found in the coastal and offshore Romanian waters within the surface and under thermocline layers.

Three hypothesis regarding their occurrence were assumed: one, the species actually lives within the Romanian habitats but its presence in adult stage has not been observed due to its limited distribution and abundance, the second, the larvae were drifted from the Crimean coast to the Romanian waters with the north-south cyclonic current, and the third, as result of favourable hydromorphological conditions prior and during the sampling period.

Acknowledging that the species has already been declared in the Vulnerable category at the Black Sea level, according to the IUCN criteria and has been enlisted in the 1999 Black Sea Red Data Book, we also propose re-evaluation of its status at the Romanian littoral if its presence will be further confirmed.

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REFERENCES

ANTIPA GR., 1941, *Marea Neagră, Vol. I. Oceanografia, Bionomia și Biologia generală a Mării Negre*. Imprimeria Națională, București, 313 pp..

FUENTES M., BENITO E., BERTRAND S., PARIS M., MIGNARDOT A., GODOY L., JIMENEZ-DELGADO S., OLIVERI D., CANDIANI S., HIRSINGER E., D'ANIELLO S., PASCUAL-ANAYA J., MAESO I., PESTARINO M., VERNIER P., NICOLAS J. F., SCHUBERT M., LAUDET V., GENEVIERE A. M., ALBALAT R., GARCIA FERNANDEZ J., HOLLAND N. D., ESCRIVA H., 2007, *Insights into spawning behavior and development of the European amphioxus* (*Branchiostoma lanceolatum*). Journal of Experimental Zoology, Part B, Molecular and Developmental Evolution, **308** (4): 484–93.

KEHAYIAS G., 2015, *Spatial and temporal variation of Branchiostoma lanceolatum larvae (Cephalochordata) in a hypoxic bay*. Biologia, **70** (9): 1234–1244.

KONSULOVA T., 1992, *Seasonal structure and ecological status of Varna Bay (Black Sea) sandy and muddy macrozoobenthic coenoses*. Rapp. Comm. Int Mer Medit., **33**: 42.

KONSULOVA T., 1999, *Amphioxus lanceolatum* Pallas, 1774. *In*: DUMONT H., 1999. *Black Sea Red Data Book*, UNOPS, New York, 413 pp..

LUTH U., 2004, *The benthos of the oxic/anoxic interface in the western Black Sea: comparative macro-and meiofauna investigations on transects from the Ukrainian, Romanian and Turkish shelf*: 2–70. *In:* ÖZTÜRK B., MOKIEVSKY V. O., TOPALOĞLU B. (Eds.), International Workshop on Black Sea Benthos. Published by Turkish Marine Research Foundation Turkey, 245 pp..

PERGNER J., KOZMIK Z.., 2017, *Amphioxus photoreceptors - insights into the evolution of vertebrate opsins, vision and circadian rhythmicity*. International Journal of Developmental Biology, **61**: 665–681.

SHALOVENKOV N., 2017, *Non-native zoobenthic species of the Crimean Black Sea Coast. Mediterranean Marine Science*. DOI: http://dx.doi.org/10.12681/mms.1925

SHIGANOVA T. A., MUSAEVA E. I., LUKASHEVA T. A., STUPNIKOVA A. N., ZAS'KO D. N., ANOKHINA L. L., SIVKOVICH A. E., GAGARIN V. I., BULGAKOVA YU. V., 2012, *Increase in Findings of Mediterranean Nonnative Species in the Black Sea*. Russian Journal of Biological Invasions, **3** (4): 255–280.

TEACĂ A., MUREȘAN M., BEGUN T., POPA A., 2018, *Marine benthic habitats within a physical disturbed site from the Romanian coast of the Black Sea*. Pp.: 231-236. *In*: ÖZCAN G., TARKAN A. S., ÖZCAN T. (Eds.), Proceeding Book, International Marine & Freshwater Sciences Symposium, 18–21 October 2018, Kemer-Antalya, Turkey, 402 pp..

TODOROVA V., 2017, *Macrozoobenthos*. *In*: Monitoring report "*Analysis of marine environmental status – 2016" Agreement -33-5/28.01.2016 between MEW and IO-BAS for implementation of obligations under the monitoring of the Black Sea, on the grounds of Article 17, Paragraph 2, item 3 of the Water Act*, pp.: 207–232 (in Bulgarian)

TODOROVA V., DIMITROV L., DONCHEVA V., TRIFONOVA E., PRODANOV B., 2015, *Benthic Habitat Mapping in the Bulgarian Black Sea*. Proceedings of the Twelfth International Conference on the Mediterranean Coastal Environment 15, 06-10 October 2015, Varna, Bulgaria, Mediterranean Coastal Foundation, Dalyan, Mugla, Turkey, **1**: 251– 262.

URATA M., YAMAGUCHI N., HENMI Y., YASUI K., 2007, *Larval Development of the Oriental Lancelet, Branchiostoma belcheri, in Laboratory Mass Culture*. Zoological Science, **24**: 787–797.

WEBB J. E., 1969, *On the feeding and behaviour of the larva of Branchiostoma lanceolatum*. Marine Biology, **3**: 58.

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[*] *National Institute for Research and Development on Marine Geology and Geo-ecology – GeoEcoMar, Romania*
e-mail: mmuresan@geoecomar.ro

[**] *The Faculty of Natural and Agricultural Sciences, Ovidius University, Constanţa, Romania*
e-mail: mskolka@gmail.com