

UPON AN UN-IDENTIFIED *SCHMIDTEA* SPECIES FROM SW
ROMANIA (PLATYHELMINTHES, TRICLADIDA, DUGESIIDAE)

ASUPRA UNEI SPECII NEIDENTIFICATE A GENULUI
SCHMIDTEA DIN SV ROMÂNIEI (PLATYHELMINTHES,
TRICLADIDA, DUGESIIDAE)

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Abstract: This paper presents the general organization of a DugesIIDae population from Dăbuleni locality (SW Romania) with emphasis on the anatomy of the copulatory apparatus. The copulatory apparatus is characterized by intermingled bursal canal musculature and two distinct penis bulbs with two large seminal vesicles. The copulatory apparatus is also characterised by the presence of an atrial fold, feature typical of *Schmidtea mediterranea* (Benazzi, Baguñà, Ballester, Puccinelli, Del Papa 1975). The characteristics of the copulatory apparatus of the investigated population are comparatively discussed with all the other *Schmidtea* species. Data on the reproductive biology are also given. The presence of *Schmidtea mediterranea* in SW Romania is discussed in relation with the paleogeographic evolution of land and aquatic basins of Europe. The possibility that the *Schmidtea* population from Dăbuleni (SW Romania) be a candidate for a new species is also considered.

Key-words: *Schmidtea* sp., SW Romania, copulatory apparatus, sexual reproduction, asexual reproduction by fission, biogeographic analysis.

Cuvinte cheie: *Schmidtea* sp., SV România, aparat copulator, reproducere sexuală, reproducere asexuală prin fisiune, analiză biogeografică.

1. INTRODUCTION

Schmidtea Ball, 1974 is a genus of freshwater flatworms belonging to Order Tricladida. Tricladida is classified into 3 suborders: Maricola Hallez, Cavernicola and Continenticola (Sluys et al., 2009), out of which only Continenticola is present in Romania. An important Continenticola subtaxon is the Family DugesIIDae with 11 genera including the genus *Schmidtea*.

The genus *Schmidtea* consists of only four species with west-palearctic distribution: *S. lugubris* (Schmidt, 1861), *S. polychroa* (Schmidt, 1861), *S. mediterranea* (Benazzi, Baguñà, Ballester, Puccinelli, Del Papa, 1975) and *S. nova* (Benazzi, 1982) (Leria et al., 2018).

In Romania, the genus *Schmidtea* is represented only by the species *S. lugubris* and *S. nova*. *S. nova* was found in the north-western part of Romania at

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Huedin (Leria et al., 2018). The literature registers the presence of *S. lugubris* in the south and east regions - South Plain (the Romanian Plain), Dobrogea, the Danube Delta, Siret Plain (Năstăsescu, 1973) and in the north-western part at Aiud (Leria et al., 2018). On *S. lugubris* from Oltenia Plain (SW Romania), data on the geographical distribution, habitat and biocenoses are brought by Rogoz (1979), without giving any description of the copulatory apparatus.

2. DATA AND METHODS

Worms were collected by author from Dăbuleni – ANIF Sadova (National Agency for Land Improvement, 43° 47' N, 24° 07' E) (Fig. 1), with a paintbrush, from the underside of immersed pebbles. The sampling sites (Fig. 2) were a concrete drainage and a small basin of stagnant water polluted with hydrocarbons (pollution indicated by the oily appearance of the water surface). Temperatures: July – water 12 °C, air 37 °C; November – water 10 °C, air 10 °C.



Fig. 1 The sampling location in SW Romania at Dăbuleni locality

(Source: <https://www.google.ro/maps/place/Rom%C3%A2nia/@45.9533581,22.9436635,530830m/data=!3m1!1e3!4m5!3m4!1s0x40b1f26958976c3:0x84ef4f92a804b194!8m2!3d45.943161!4d24.96676>)

The specimens were fixed in Beauchamp's fluid (ethanol 96°: 6 volumes, formaldehyde 37%: 3 volumes, glacial acetic acid: 1 volume, dr. D. Codreanu-Bălcescu personal communication) for 24 hours, and thereafter stored in 75% ethanol. Histological sections were made at intervals of 5µm and were stained in Haematoxylin-Eosin.

The study of the copulatory apparatus is based on serial frontal and sagittal histological sections on 7 specimens deposited in author private collection at the University of Craiova:

- AFB-TRI-no.14 – Dăbuleni, 11 July 2017, immature specimen, frontal sections on 8 slides;
 - AFB-TRI-Dg1 – Dăbuleni, 24 November 2017, sagittal sections on 32 slides;
 - AFB-TRI-Dg2 – Dăbuleni, 24 November 2017, frontal sections on 8 slides;
 - AFB-TRI-Sc1 – Dăbuleni, 24 November 2017, sagittal sections on 91 slides;
 - AFB-TRI-Sc2 – Dăbuleni, 24 November 2017, sagittal sections on 54 slides;
 - AFB-TRI-Sc3 – Dăbuleni, 24 November 2017, frontal sections on 14 slides;
 - AFB-TRI-Sc5 – Dăbuleni, 14 October 2021, frontal sections on 18 slides
- (where Dg1, 2, Sc1 to Sc5 represent the symbols for labelling each investigated worm).

Abbreviations used in the figures: af: atrial fold, b1: the first penis bulb, b2: the second penis bulb, bc: bursal canal, cb: copulatory bursa, ej: ejaculatory duct, go: gonopore, ma: male atrium, od: oviduct, pp: penis papilla, sd-v: spermiducal vesicles, sv1: the first seminal vesicle, sv2: the second seminal vesicle, vd: vas deferens.

All photos were taken by the author.



Fig. 2 The sampling sites at Dăbuleni (a – concrete drainage, b – small basin with polluted water; white arrow indicates the worms)

3. RESULTS AND DISCUSSIONS

3.1 Results

A) Systematic

Order Tricladida Lang, 1884

Suborder Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguña & Riutort, 1998

Family DugesIIDae Ball, 1974

Genus *Schmidtea* Ball, 1974

Schmidtea sp.

B) General organization

The size of the sexuate living specimens ranged between 7 mm long (specimen AFB-TRI-no. 14, July) to 12 mm long (specimen AFB-TRI-Dg1, November). The head has a rounded-subtriangular shape with usually two close eyes set in pigment-free patches; few specimens possess supernumerary eyes (Fig. 3a). The head narrows very slightly in an indistinctly marked neck region. Two auricular grooves are well visible as depigmented, yellow lines, parallel with the neck margins (Fig. 3a). The dorsal surface of the living worm is dark brown; the ventral surface is paler (Fig. 3b). The fixed worms have a dark brown body with a sandy, granular appearance.

The pharynx is located at the middle of the body, with the mouth opening in the posterior third part of the body. The inner pharyngeal muscular coat is typical for DugesIIDae, with the circular and longitudinal layers separated, the longitudinal layer most developed. One specimen presents a supplementary pharynx.

The testes (Fig. 4) have a dorsal position in the mesenchyme. They extend throughout the body length on the left and right sides, while in the medial (central) part of the body they are more numerous in the pre-pharyngeal region.

The vasa deferentia (spermiducts) are not clearly visible; they form large spermiducal vesicles on either side of the pharynx. The spermiducal vesicles pass beyond the level of both penis bulbs (Figs. 5, 11) and judging by their position, the spermiducts probably take a bent course before opening separately in the posterior wall of the first bulb (Fig. 6b).

The penis (Figs. 5, 6, 7, 8, 11) has two distinct bulbs nearly of the same size: the first bulb (b1) has an antero-dorsal position, the second bulb (b2) is placed postero-ventral. The first bulb has a developed muscular wall with layers of intermingled and circular musculature. It contains the first seminal vesicle, large and rounded, lined with a wave-shaped epithelium, with cells of different heights. The wall of the second bulb consists of un-oriented musculature. The shape of the second seminal vesicle is rather an individual character, large and elongated in some specimens (Fig. 7) or branched/dichotomic branched in others (Figs. 5, 8). The separation of the penis bulb in two distinct parts is almost imperceptible in the immature specimen AFB-TRI-no. 14. The two bulbs communicate through a narrow duct (Figs. 7b, c; 11). The penis papilla is housed by a single genital atrium, not separated into a male and a female atrium. This genital atrium can be named the male atrium because it contains the penis papilla, as well the hermaphrodite

atrium, being the place where the bursal canal opens. The penis papilla is elongate, with a median compression produced by the atrial fold and with the ejaculatory duct running slightly ventrally (Figs. 7a, 8, 11). No nipple was observed at the opening of the ejaculatory duct. The ejaculatory duct is lined with a low epithelium sitting on a thin muscular layer.

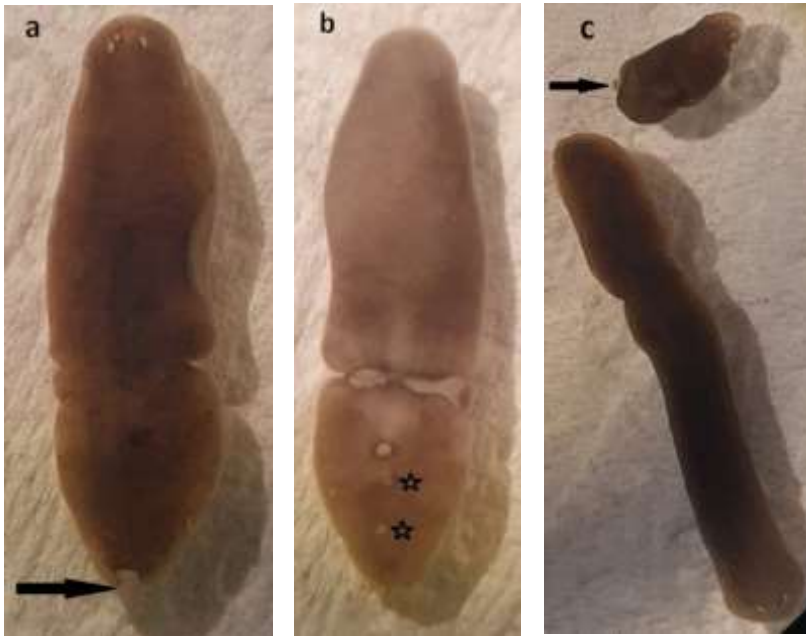


Fig. 3 External morphology in fixed and living worms collected in March 2022

(a, b – a fixed worm in dorsal and ventral view, showing fission, c – the same worm in living and a small worm, possibly resulted from the larger one; the stars indicate the two subpharyngeal orifices, the arrows indicate the place of fission)

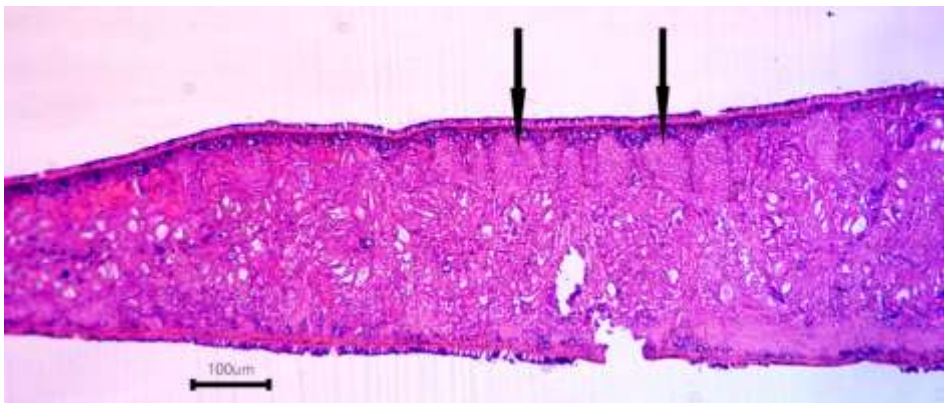


Fig. 4 Microphotograph of a sagittal section (specimen Dg1) indicating the dorsal testes



Fig. 5 Microphotograph of the copulatory apparatus in frontal section (specimen Dg2)

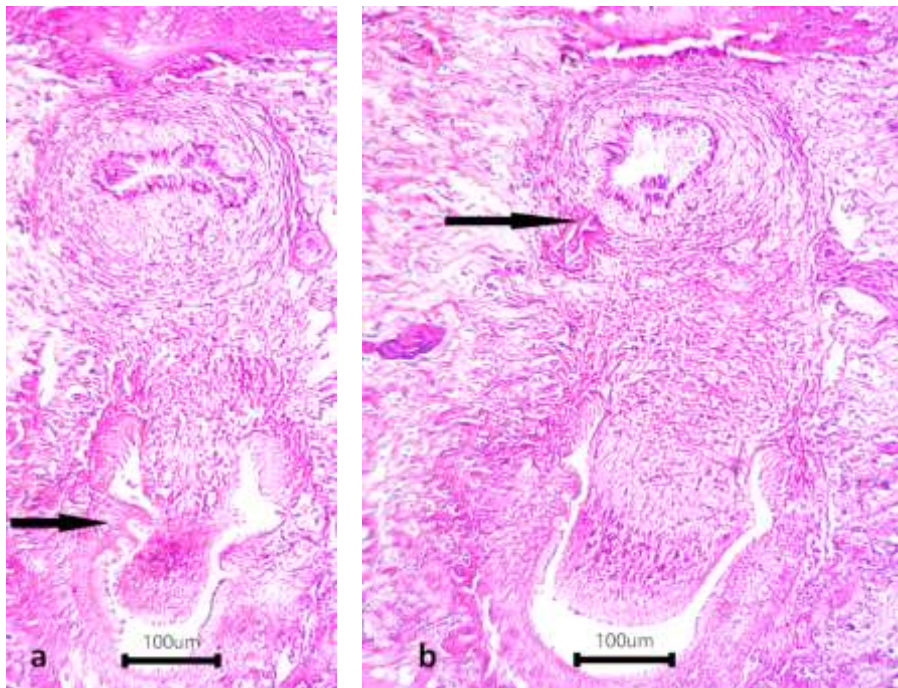


Fig. 6 Microphotographs of the copulatory apparatus in frontal sections (specimen Sc3): a – the atrial fold, b – the opening of the spermiducts in the posterior part of the first penis bulb



Fig. 7 Microphotographs of the copulatory apparatus in sagittal sections (specimen Dg1)

The epithelium of the genital atrium produces a large fold which compresses the penis papilla at its middle (Figs. 5; 6a, 7a, 8, 11). The atrial fold is present in all mature analysed specimens. The atrial epithelium consists of low cylindrical, pillar-shaped cells, sitting on a thin muscular layer.

The copulatory bursa (Figs. 5, 7, 8, 9, 11) is a large sac lined with a tall, pseudostratified nucleated and vacuolated epithelium sitting on a very thin muscular layer. The bursal canal appears to have intermingled musculature and it is lined with a low epithelium, discharging small vacuoles into the lumen (Fig. 9).

The ovaries consist of large oocytes (Fig. 10). The two oviducts open separately into the bursal canal, close to where it opens into the male atrium (Fig. 11).



Fig. 8 Microphotograph of the copulatory apparatus in sagittal section (specimen Sc1)



Fig. 9 Microphotograph of a sagittal section (specimen Sc1) showing the copulatory bursa and the bursal canal

C) Reproductive biology

In July the worms are immature and undergo fission. They become mature in October and November. In March, sexuate worms undergo fission (Fig. 3). The small worm in Fig. 3c seems to be resulted by fission from the larger worm in Fig. 3a, b. This larger worm has two subpharyngeal orifices

out of which at least one is the genital orifice. The presence of two subpharyngeal orifices in a worm undergoing fission is unusual and their significance is unknown.

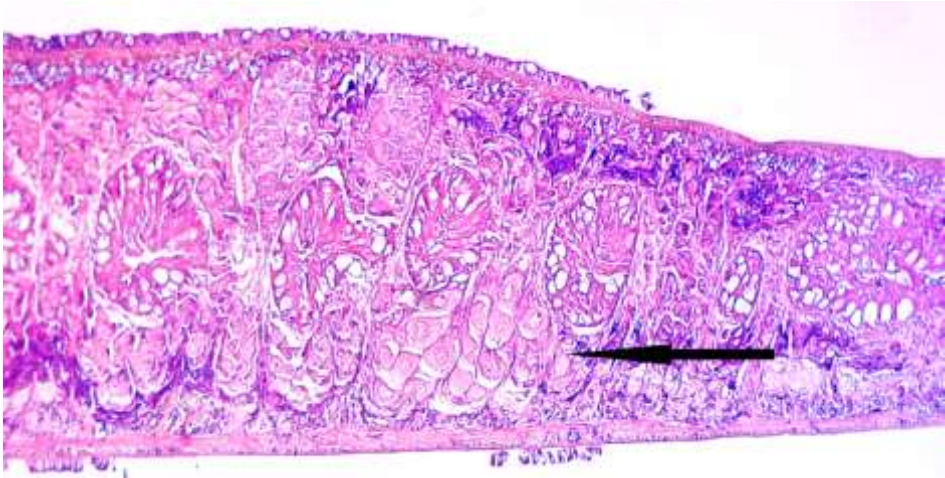


Fig. 10 Microphotograph of a sagittal section (specimen Sc1) indicating the ovary

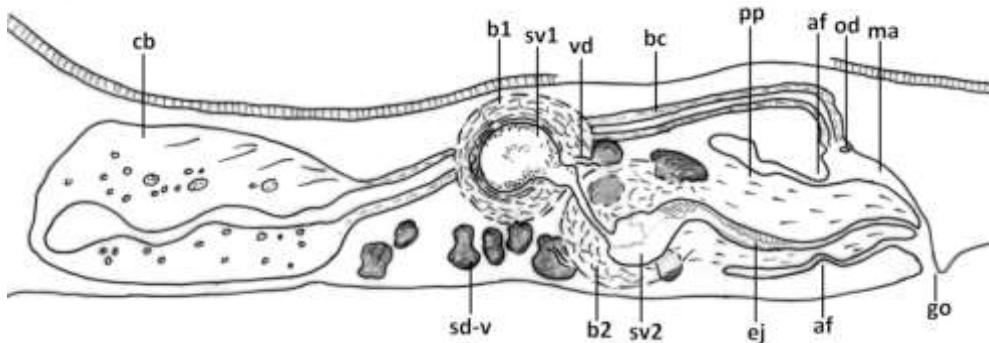


Fig. 11 Reconstructed copulatory apparatus (anterior to the left)

3.2 Discussions

A) The copulatory apparatus

The literature presents distinctive morphological characters for each of the four species of the genus *Schmidtea*. For *S. lugubris*, a permanent nipple on the tip of the penis is considered distinctive (Reynoldson & Belamy, 1970; Ball & Reynoldson, 1981; Leria et al., 2018). The particularities of *S. polychroa* are a dorsal hump on the penis papilla, (Ball & Reynoldson, 1981; Leria et al., 2018), a ventral ejaculatory duct and the lack of a permanent nipple (Reynoldson & Belamy, 1970). *S. mediterranea* presents a developed

atrial fold (Benazzi et al., 1975; Leria et al., 2018). *S. nova* stands apart by several unique features (Leria et al., 2018).

However, a morphological discrimination between *S. lugubris*, *S. polychroa* and *S. mediterranea* is not very sharp (precise). For instance, an atrial fold is also recorded on a small percentage in *S. polychroa* and *S. lugubris* (Benazzi et al., 1975). Consequently, the morphology of the penis bulb does not seem to be a sharp discriminant character. A penis bulb consisting of two distinct, separated bulbs with two large seminal vesicles nearly of the same size is present in *S. mediterranea* from Italy and Spain (Benazzi et al., 1975, Figs. 1, 2, 3) and *S. lugubris* from Romania (Năstăsescu, 1973, Figs. 3, 4, 5, 7, 8). Instead, in *S. lugubris* of the British fauna, the penis bulb is not separated in two distinct bulbs, not even by an external, superficial constriction, while “two vesicular structures within the bulb” are given: a true anterior, larger seminal vesicle and a smaller, posterior, secondary seminal vesicle (Ball & Reynoldson, 1981). In *S. mediterranea* from Tunisia the penis bulb is also figured as two distinct bulbs (Harrath et al. 2004, fig. 1), but not separated. A bulbar constriction is reported by Harrath et al. (2012) in *S. polychroa* from the Palearctic section of the African continent. The penis bulb in *S. nova* consists of two parts separated by a constriction. Only Benazzi et al. (1975), citing Böhmig, present a clear distinction between the species *S. lugubris* and *S. polychroa*. Thus, although both species have the penis bulb “divided into two sections”, an anterior dorsal part with a rounded seminal vesicle and a posterior ventral part with an elongated posterior vesicle, the distinction between species appears only at the external level: “in *lugubris* the two parts are separated, while in *polychroa*, owing to the strong development of the muscular mass, they are not clearly distinct”.

Considering the above characters, the worms from the SW Romania at Dăbuleni differ from *S. lugubris* by lacking a nipple on the tip of the penis and are different from *S. polychroa* in lacking a dorsal hump on the penis papilla. They also differ from *S. lugubris* studied by Năstăsescu (1973) by having an atrial fold, thus pointing to *S. mediterranea*. However, the atrial fold of *S. mediterranea* (as described in 1975) is located at the level of the penis papilla tip and it separates the male and female genital atria, while in Dăbuleni worms the atrial fold has a different position and only one genital atrium is present in all analysed specimens. With respect to the genital atrium, it is noteworthy that the internal organization of the genital atrium space (only one atrium or two atria) might be an individual or populational feature, variable within or among populations, as it was presented for *S. lugubris* from Romania (Năstăsescu, 1973). The bent course of the vasa deferentia before opening into the penis bulb is more characteristic of *S. mediterranea* (Harrath et al., 2004, Fig. 1) than of *S. lugubris* presented in the descriptive literature.

Finally, it is important to mention the communication between the 2 penis bulbs through an external duct (located in the mesenchyme) in the

specimens of *S. lugubris* from Sf. Gheorghe and Mangalia (Năstăsescu, 1973, Figs. 4, 5).

B) Comments upon individual and anomalous features

Individual features might be considered the genital atrium space (in general) and the openings of the spermiducts into the first or into the second penis bulb in our population. The latter situation is suggested by the dichotomic branches of the second seminal vesicle in some specimens, branches which might correspond to the spermiducts entrance.

The presence of a supplementary pharynx is an anomalous feature. Anatomical anomalies have been reported for instance in *S. mediterranea* (Benazzi et al., 1975).

C) Biogeography

The presence of *Schmidtea mediterranea* in Romania is to be questioned.

S. mediterranea is a species with a limited geographical range. It covers the western-Mediterranean region (the Catalan coast, Menorca, Mallorca, Corsica, Sardinia, Sicily, Tunisia) where the species is represented by local populations with two types of reproductive biology. Most populations are sexual amphimictic diploids. Few asexual fissiparous populations occur in Catalonia and Balearic Island where sexual populations were not found to date (Lázaro et al., 2011).

A phylogeographic study based on molecular markers (Lázaro et al., 2011) suggests that *S. mediterranea* is an old, single species of pre-Oligocene-Miocene age and its current west-Mediterranean distribution “is a relic of the split from a former larger geographical distribution”.

In the absence of molecular analysis, the species identity of the population from Dăbuleni might be considered:

a) *S. mediterranea*, implying that this species is a very old one but not surviving only a few Mediterranean islands as questioned by Lázaro et al. (2011). The presence of the species in SW-Romania should be analysed in relation with the paleogeographic evolution of land and aquatic basins in Europe. This paleogeographic evolution was marked by serial events that shaped and re-shaped the borders of water and land, also the salinity of the water from salty to brackish and back. The paleogeographic events and their influence on the freshwater fauna is presented by Oncescu (1965), Müller (1985), Bănărescu (1992). During the Oligocene and Eocene, Europe was covered by the Tethys Sea. During the middle Miocene, the Paratethys Sea separated from the Tethys in the north. During the Upper Miocene, the Paratethys separates into the Mediterranean, Sarmatian Seas and the Pontian Lake (the Pontian Basin). During the Pliocene and the Quaternary, the surface of the Paratethys Sea reduces and there appear distinct basins, such as Black, Caspian and Aral Seas, due to the uplift of the Carpathian and the Caucasus Mountains (Popov et al., 2006). During the Messinian salinity crisis (the end of the Miocene) a continuous hydrographic network was established

(Bănărescu, 1992). During the Pleistocene, the hydrographic network probably takes shape in a form very similar to the present one. At the end of Pleistocene, the Mediterranean overflows the Pontian Lake. It is the so called “biological mediterraneanisation and faunistic infusions” (Müller, 1995) which must have had a deep influence on the hydrographic network too. During the glacial period there are described more temporary connections through which the Mediterranean overflowed the Pontian Lake.

The spread of *S. mediterranea* in SW Romania might have been possible through both sea and fluvial basins at different moments, hypothesis that implies a great environment plasticity of the species, namely a great tolerance to brackish water. A similar dispersal pattern was analysed for the genus *Dugesia* (Solà et al., 2013).

b) Dăbuleni population belongs to *S. lugubris* with an atrial fold and the atrial fold is not a morphological distinctive character for *S. mediterranea*.

c) Dăbuleni population belongs to a new species. The reproductive biology of Dăbuleni population, with asexual and sexual individuals differs from that of *S. mediterranea* and *S. lugubris*. So far, no case of asexual reproduction was reported in the Romanian populations of *S. lugubris*.

4. CONCLUSIONS

1. The taxonomic diversity of the genus Schmidtea is not fully known.
2. From a morphological point of view, the specimens analysed in this paper, show a more pronounced morphological similarity with *S. mediterranea* than with *S. lugubris*, however, they are not identical with *S. mediterranea*.
3. The cryptical biodiversity in triclads is known to be high and thus, a cryptical diversity for the triclads on the Romanian territory is also expected.
4. More and more, the description of new Dugesiid species (and not only), species delineation, inferring phylogenetic relationships rely on integrative methods (e. g. Carranza et al., 1996; Baguña et al., 1999; Lázaro et al., 2009; Lázaro et al., 2011; Sluys et al., 2013; Stocchino et al., 2013; Harrath et al., 2019; Leria et al., 2020; Song et al., 2020; Wang et al., 2021). The assignment of the morphotype described in this paper to a species is a future task of integrative studies. Additional molecular and morphological analysis could also establish whether the atrial fold is a character specific to *S. mediterranea* or is present in other species.

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