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ON TWO POLYCELIS SPECIES FROM ROMANIA – *POLYCELIS PANTHERINA* SP. NOV. AND *POLYCELIS FELINA LOMENSIS* BABALEAN, 2019 WITH EMENDATION AND COMPLETION OF THE 2019 NOMENCLATURAL ACT UNDER ARTICLE 10.1 (PLATYHELMINTHES, TRICLADIDA, PLANARIIDAE)

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ABSTRACT

This paper presents a new Polycelis species – Polycelis pantherina from S-W Romania, which can be separated from all the other Polycelis species by the general habitus and especially by the reproductive biology – asexual population with reproduction by fission/fragmentation. The description of the subspecies Polycelis felina lomensis Babalean, 2019 is emended and completed according to ICZN art. 10.1 The subspecies Polycelis felina lomensis is elevated at the species rank by reassigning it from the genus Polycelis to the genus Ijimia: Ijimia lomensis.

INTRODUCTION

Polycelis Ehrenberg, 1831 is a genus of free-living flatworm belonging to the family Planariidae Stimpson, 1857. The distinctive morphological character of the genus Polycelis is the presence of numerous eyes (oceli) “arranged in a row at the anterior end. “(Kenk, 1973).

During time, the systematic of the genus based on morphology suffered several changes, with the creation of the subgenera Polycelis Ehrenberg, 1831, Ijimia Bergendal, 1890 and Seidlia Zabusov, 1911. They can be separated by the characteristics of the copulatory apparatus: Polycelis – thin muscular coat of the male part of the genital atrium, lack of adenodactyls; Ijimia - thin muscular coat of the male part of the genital atrium, adenodactyls present; Seidlia – thick muscular coat of the male part of the genital atrium, adenodactyls absent. (Kenk 1953, Kenk 1973, Kawakatsu & Mitchell 1998). Sluys et al. (2009) recognize *Polycelis*, *Ijimia* and *Seidlia* as distinct genera within Planariidae.

In Romania, the genus Polycelis is represented by three species: *Polycelis nigra* (O.F. Müller, 1773), *Polycelis tenuis* Ijima 1884 and *Polycelis felina* (Dalyell, 1814) (Năstăsescu 1976). Babalean (2019) described the subspecies *Polycelis felina lomensis*.

MATERIAL AND METHODS

The specimens described as *Polycelis pantherina* were collected by author during October 2021 – late July 2022, with a paint brush, under immersed pebbles

and brick debris, from a small collector of a large limnocrene spring (Fig. 1), outskirts of locality Dăbuleni (Izvoarelor Street), SW Romania, as follows:
- 14 October 2021 – 16 specimens fixed in absolute ethanol
- 08 March 2022 – 78 specimens fixed in Beauchamp solution
- 24 May 2022 – 2 specimens fixed in absolute ethanol
- 28 July 2022 – 1 specimen fixed in absolute ethanol for histology

The fixed specimens are deposited in author private collection at University of Craiova and will be shared (donated) between Grigore Antipa Museum Bucharest and Naturalis Biodiversity Centre Leiden.

Figure 1. The sampling sites at Dăbuleni

RESULTS AND DISCUSSIONS

Results

Systematic according to Sluys et al. 2009

Ord. Tricladida Lang, 1884

Subord. Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguñà, Riutort, 1989

Fam. Planariidae, 1857

1. *Polycelis pantherina* Babalean, sp. nov.

Material examined

Specimen types and other specimens (Figs. 2 – 5):

Holotype (Fig. 2): Pp-H-Db-Ro-AFB, 08 March 2022, fixed in Beauchamp solution, deposited at Grigore Antipa Museum Bucharest

Paratype 1 (Fig. 3): Pp-P1-Db-Ro-AFB, 08 March 2022, fixed in Beauchamp solution, preserved in 75 ethanol, deposited at Naturalis Biodiversity Centre

Paratype 2 (Fig. 3): Pp-P2-Db-Ro-AFB, 08 March 2022, fixed in Beauchamp solution, preserved in 75 ethanol, deposited at Grigore Antipa Museum

Paratype 3 (Fig. 3): Pp-P3-Db-Ro-AFB, 08 March 2022, fixed in Beauchamp solution, preserved in 75 ethanol

Paratype 4: Pp-P4-Db-Ro-AFB, 08 March 2022, fixed in Beauchamp solution, preserved in 75 ethanol, deposited at Naturalis Biodiversity Centre

Paratype 5 (Fig. 4): Pp-P5-Db-Ro-AFB, 24 May 2022, fixed in absolute ethanol for histology, preserved in 75 ethanol, deposited at Grigore Antipa Museum

Paratype 6: Pp-P6-Db-Ro-AFB, 24 May 2022, fixed in absolute ethanol for histology, preserved in absolute ethanol, deposited at Naturalis Biodiversity Centre

Other specimens

Specimens collected on 14 October 2021 (Fig. 5), fixed in absolute ethanol for histology, preserved in absolute ethanol; 4 specimens deposited at Naturalis

All the sampled specimens were examined under binocular.

No sexual individuals were found. The asexual status was assessed by the absence of the copulatory area and of the genital opening, also by the absence of testes and ducts, usually visible through semi-transparent tegument and mesenchyme in other triclads.

Labels: Pp – *Polycelis pantherina*, H – holotype, P1 to P6 – paratype 1 to paratype 6, Db – Dăbuleni, Ro – Romania, AFB – Anda Felicia Babalean, coordinates: 43º48'32"N, 24º04'E

This new species is assigned to the genus Polycelis based on eyes disposition, according to Ehrenberg definition.

Diagnosis

Polycelis pantherina is characterized by the following features:

The specimens collected from the investigated population are asexual, reproduction by fission/fragmentation; small size: in fixed state, the smallest specimen – 0.3 mm, the largest – 5.5 mm.; colour: mottled, grey spotted with un-equal dark spots both in the blackish living specimens and in the grey fixed specimens; head with two mobile tentacles; 5 to 25 eyes on each half part of the head and anterior margins, depending on the size of the worm/worm fragment; paratypes 5 and 6 with no visible eyes, possible eyeless

Diagnoză

Indivizii colectați din populația investigată sunt asexuați, cu reproducere prin fisiune/fragmentare; talie redusă: cel mai mic individ – 0.3 mm în stare fixată, cel mai mare – 5,5 mm.; culoare: aspect pestriț-pătat, cu pete gri inegale atât la indivizii vii de culoare negricioasă cât și la indivizii fixați de culoare gri; capul cu două tentacule mobile; 5 până la 25 de ochi de fiecare parte a capului și marginilor anterioare ale corpului, în funcție de mărimea viermelui/fragmentului de vierme; paratipii nr. 5 și nr. 6 fără ochi vizibili, posibil lipsiți de ochi

Etymology – the name of this species is a word derived from the generic name Panthera and is treated as a noun in nominative singular standing in apposition to the generic name Polycelis. It alludes to the aspect of *Panthera pardus*.

Description of the specimen types - holotype and paratypes
Holotype – Fig. 2
Asexuate. Anterior margin of the head folded on ventral. Eyes of unequal size, placed at unequal distance one from each other; number of eyes visible on dorsal – 16 on the left side and 14 on the right side, visible on the ventral folded part – 10/11 on the left side and 7/8 on the right side. Body length – 3,2 mm to 3,3 mm including the folded ventral area. The pharynx located in the half posterior part of the body.
The paratypes – description included in the diagnosis.

Accompanying biocenosis – Gammaridae

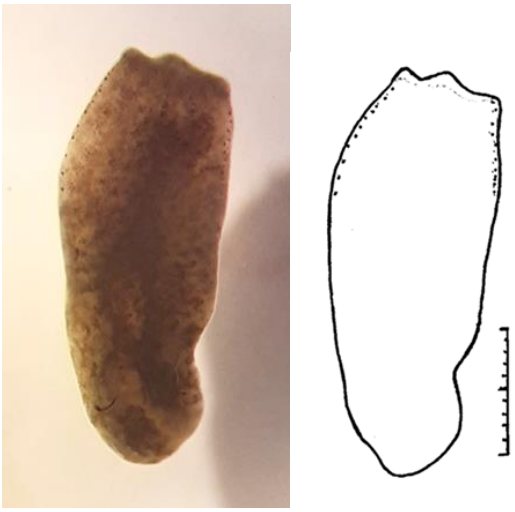

Figure 2. *Polycelis pantherina* holotype – photograph (left); drawing camera lucida (right), scale bar 1 mm

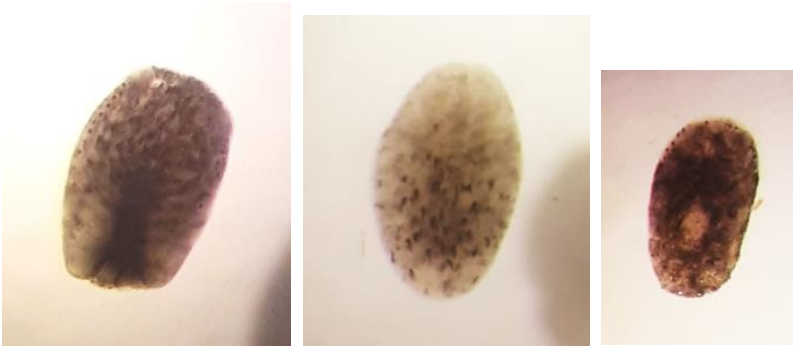

Figure 3. *Polycelis pantherina* – paratypes: paratype 1(left), paratype 2 (middle), paratype 3 (right)

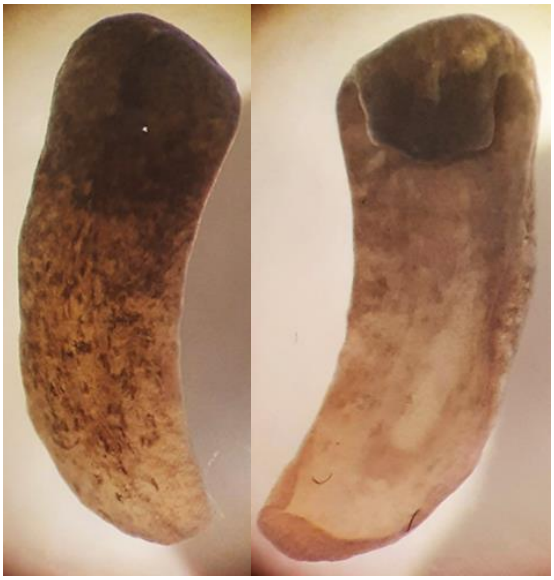

Figure 4. *Polycelis pantherina* paratype 5 - dorsal (left), ventral (right)

Figure 5. *Polycelis pantherina* - specimens collected on 14 October 2021

2. *Polycelis felina lomensis* Babalean, 2019

Emendation

Specimen types

Holotype: Pf7

Paratypes: paratype 1 – Pf2, paratype 2 – Pf3, paratype 3 – Pf4, paratype 4 – Pf5, paratype 5 – Pf6, paratype 6 – Pf8

Specimen types are deposited in author private collection at University of Craiova and will be shared (donated) between Grigore Antipa Museum Bucharest and Naturalis Biodiversity Center Leiden.
Labels: Pf7-H-VPR-Ro-AFB, Pf2-P1-VPR-Ro-AFB, and so on, where: H – holotype, P1 to P6 – paratype 1 to paratype 6, VPR – Valea Preajba River, Ro – Romania, AFB – Anda Felicia Babalean
The paper Babalean A.F. 2019. A new *Polycelis felina* subspecies (Platyhelminthes, Tricladida) in Romania, Edit. Sitech, Craiova, pp. 17 is deposited in Romanian National Library (Biblioteca Națională a României) and will also be deposited in: The Library of Grigore Antipa Museum Bucharest, The Library of University of Craiova, Academiei Library (Biblioteca Academiei) Bucharest, Researchgate, Zoological Record.

Elevation in rank

According to the higher classification of Sluys et al. (2009) and based on the presence of adenodactyls, the subspecies *Polycelis felina lomensis* is elevated to the species rank by reassigning to the genus Ijimia: *Ijimia lomensis*.

Discussions

The freshwater triclads display a wide range of reproductive strategies, varying from one group to another:
1) exclusive sexual reproduction – for instance in Dendrocoelidae (Rieger 1986)
2) a combined sexual and asexual reproduction, with asexuality by fission or by parthenogenesis, with a large variety of life cycles (see for instance Stocchino & Manconi 2013)
3) exclusive asexual reproduction by fission in natural conditions – for example in three Dugesia species (Stocchino & Manconi 2013)

With respect to the condition of exclusive asexual reproduction, D'Souza and Michiels (2010) consider that "purely clonal reproduction is likely to be restricted to only few species, for example ancient asexuals."

The question why asexual reproduction is so common among invertebrates and in planarians has been and is largely debated with emphasis on advantages (benefits) versus the disadvantages of asexual reproduction (see for instance D'Souza and Michiels 2010, Vila-Farré & Rink 2018 and included references).

Although there is a huge difference between fission and parthenogenesis, they are often treated under the general term of clonal reproduction.

The benefits versus disadvantages of clonal reproduction (by fission) can be resumed as follows:
a) "a complete lack of physiological ageing" (Vila-Farré & Rink 2018)
b) "colonizing new environments", a single specimen or fragment of a specimen can restore an entire population (Vila-Farré & Rink 2018)
c) ecological plasticity – "clones are of different ages, …., follow different ecological strategies and occupy different micro-niches" (D'Souza and Michiels 2010)
d) low material and energetic investment in producing testes and ovaries
Beveridge (1982), citing Kawakatsu, note that fragmentation could be entirely distinct from fission, "rather a pathological reaction to unfavourable conditions."

On the other hand, the clonal reproduction is sometimes considered as "an evolutionary dead-end." (Vila-Farré & Rink 2018 and included reference). Clonal reproduction is an evolutionary unfavourable process because determines the

accumulation of deleterious mutations which, eventually would lead to genome deterioration (D'Souza and Michiels 2010 and included reference). This disadvantage could be overcome by rare sex.

It is highly un-probably that purely asexual populations could occur in planarians. In relation with this issue, two judgements are found in D'Souza and Michiels (2010):

- "Occasional sex in presumably asexual species may arise" (Schurko & Logsdon 2008, Schurko et al. 2009 cited by D'Souza and Michiels 2010)
- "Sexual processes are often cryptic and proceed in an unpredictable fashion" (Hurst et al. 1992 cited by D'Souza and Michiels 2010)

It was demonstrated that clonal reproduction by fission produces "individuals with very high level of mosaicism and intraindividual genetic diversity" (Leria et al. 2019, Leria et al. 2020). Some of the mutations may be unfavourable, of no importance or favourable under different environmental conditions. If the clonal reproduction does generate populations with a high hidden genetic variability, then, the clonal reproduction should be seen as a speciation force, a speciation mechanism.

At this state of knowledge *Polycelis pantherina* may be considered a presumable asexual new species and most likely an asexual strain of a new Polycelis species. *Polycelis pantherina* differs from all other Polycelis species by the pantherine aspect in living and fixed state and by the reproductive biology. The panther-like aspect might have been induced by mutations during asexual cycles of reproduction.

CONCLUSIONS

Questions to be answered:

1) the origin and age of the new species and the speciation mechanism,
2) the phylogenetic relation with the other Polycelis species,
3) the conservation status of this new species, occupying an anthropized habitat,
4) more studies required to reveal the natural history of *Polycelis pantherina*.

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