Review

Romanian Dendrocoelidae Hallez, 1892 (Platyhelminthes, Tricladida, Dendrocoelidae) Revisited, a Tribute to Radu Codreanu and Doina Balcesco

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Review

Romanian Dendrocoelidae Hallez, 1892 (Platyhelminthes, Tricladida, Dendrocoelidae) Revisited, a Tribute to Radu Codreanu and Doina Balcesco

Anda Felicia Babalean

Department of Biology and Environmental Engineering, Faculty of Horticulture, University of Craiova, 13 A. I. Cuza Street, Craiova, Romania; anda.babalean@ucv.ro

Simple Summary

The European Dendrocoelidae are an important group of freshwater flatworms. From the first described species Dendrocoelum lacteum by Müller in 1774, the current account comprises nearly 100 species, many of them endemic—with a geograph-ical range restricted to a small area; blind, inhabiting dark environments. The species were historically included in a system of 9 units (taxa) difficult to be sharply separated on their morphology, one or more character being shared by some units. The classical literature operates with the taxa Polycladodes, Dendrocoelum, Dendrocoelides, Paradendrocoelum, Pal-aeodendrocoelum, Eudendrocoelum, Neodendrocoelum, Bolbodendrocoelum, Apodendrocoelum. The endemicity and the diversity of the morphological characters make it difficult to achieve a clear understanding of the taxonomic diversity and natural history of the group—How many species? What is their history? When did they evolve? To what extent are they related? The Dendrocoelidae of Romania are part of the European freshwater diversity. A major deficit in their knowledge is the lack of the type-specimens, those specimens on which the species description was made. The Romanian Dendrocoelidae are a missing piece of a blurred, un-resolved puzzle. It is expected that the addressed questions will be answered by combining the classical morphology with the modern genetic tools.

Abstract

The paper presents the current state of knowledge on the Romanian Dendrocoelidae as part of the European/Palearctic Dendrocoelidae, with emphasis on the contribution of the Romanian zoologists Radu Codreanu and Doina Balcesco. The main objective of the work was to find the gaps in knowledge for future alignment with current standards. The topics of the article include: the species inventory; a short historical perspective over the classical phylogenetic system with an overview of the morphological characters used in the systematic of the group. The study analyses the arguments (and hypotheses) brought by Codreanu, Balcesco and other authors regarding the phylogenetic value of some characters: a) the position of the oviducts between the male atrium and the bursal canal (typical for *Paradendrocoelum*); b) the eyes and the penial flagellum in relation with the palaeogeographical context governed by the Quaternary Glaciation; c) the point of view of Codreanu and Balcesco on the origin and composition of the actual Romanian Dendrocoelidae fauna. The major key finding is that the Dendrocoelidae species on the Romanian territory should be reinvestigated in an integrative way. Specific future research needs, and future directions of study are suggested.

Keywords: Dendrocoelidae; morphological characters; palaeogeographical conditions; natural history

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1. Introduction

The Dendrocoelidae Hallez, 1892 is an important family of triclad freshwater worms belonging to suborder Continenticola [1]. The main characteristic of this family is the alternative disposition of the longitudinal and circular muscular layers in the pharynx [1,2]. The family Dendrocoelidae consists of 22 genera with a large distribution, out of which only *Dendrocoelum* and *Polycladodes* are present in the Romanian fauna [1,3]. The knowledge on European Dendrocoelidae began with the description of *Dendrocoelum lacteum* by Müller in 1774. A survey of the available literature [2–11] brought the current count, up to 100 Palearctic species. The species once described, they were included into a system of supraspecific taxa, either at the rank of genus or subgenus: *Dendrocoelum*, *Dendrocoelides*, *Eudendrocoelum*, *Neodendrocoelum*, *Bolbodendrocoelum*, *Paradendrocoelum*, *Apodendrocoelum*, *Palaeodendrocoelum*, *Polycladodes* (see paragraph 3). The most recent literature recognizes only the genera *Dendrocoelum* and *Polycladodes* [1,11], *Dendrocoelum* s.l. being the reunion of the remaining taxa. In this paper, I use the term Dendrocoelidae for the only two genera present in the Romanian fauna, *Dendrocoelum* and *Polycladodes*.

The Dendrocoelidae of Romania were studied by several scientists, especially from a faunal and systematic point of view. The faunal approach was served by de Beauchamp, del Papa, Codreanu, Codreanu and Balcesco, Stocchino and coauthors [3,12–21] who identified and described new species. The systematics of the whole Palearctic Dendrocoelidae have been a matter of prolonged debate, with the revisions of many zoologists, for instance, Komárek, Stanković and Komárek, Kenk, de Beauchamp, Reisinger and Gourbault [5]. Despite all the morphologically based revisions, the phylogenetic systematic remains unresolved, but it is expected to be continuously reshaped by modern tools as already prospected elsewhere [31]. Significant research and research programmes using modern tools (barcoding, multilocus phylogenetic, genomic analyses, etc.) as well combined molecular and morphological analyses, explored and unravelled many conundrums of the Tricladida taxonomic biodiversity, evolution and evolutionary dynamics, speciation patterns, systematic, phylogeny at lower and higher level [11,22–37].

The aim of this paper is a general view on the Romanian Dendrocoelidae as part of the European/Palearctic group, with emphasis on the historical contribution of Radu Codreanu and Doina Balcesco. Their effort to learn about this group of worms is part of the historical effort to learn about the entire European/Palearctic group. Therefore, even though only the genera *Dendrocoelum* and *Polycladodes* are now recognized, the analysis and discussions on former *Dendrocoelum* s.l. divisions are considered by author, mandatory for this paper.

2. Methodology

The review of the literature was done using various databases: Romanian libraries, MNHN library (Muséum National d Histoire Naturelle Paris), Google and Google Scholar, Researchgate, BHL (Biodiversity Heritage Library), EurekaMag, NHBS, etc. Key words related to the topic of the article were used: *Dendrocoelidae*, *Dendrocoelum species*, systematic, planarian molecular phylogeny, etc. The Refference final list of all accessed articles was the starting point for new search, by article and journal title. Some articles were kindly provided to me by group specialists and colleagues.

3. Species Inventory

The species inventory—Table 1—follows, in part, the system of Sluys et al. for the genera *Dendrocoelum* and *Polycladodes*, and Gourbault for the *Dendrocoelum* s.l. subgenera [1,2]

Table 1. Species inventory.

Species name	Original genus/subgenus	Type locality / loc. In Romania	Primary references	Literature source	Collection holding

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(System: Gourbault 1972 and Sluys et al. 2009)	(in primary reference)		(species original description)	(used by author)	type specimens
1. *D. (Dendrocoelides) sphaerophallus* (de Beauchamp, 1929)	*Dendrocoelides*	Pui (Hunedoara-Romania)	Beauchamp 1929	Beauchamp 1929 [12]	?
2. *D. (Dendrocoelides) chappuisi* de Beauchamp, 1932	*Dendrocoelum (Dendrocoelides ?)*	Babadag-Tulcea, Romania	Beauchamp 1932	Beauchamp 1932 [13]	?
3. *D. (Dendrocoelides) clujanum* Codreanu, 1943	*Dendrocoelum (Dendrocoelides)*	Cluj, Romania	Codreanu 1943	Codreanu 1943 [16]	?
4. *D. (Dendrocoelides) racovitzai* de Beauchamp, 1949	*Dendrocoelum (Dendrocoelides)*	Cloșani, Romania	Beauchamp 1949	Beauchamp 1949 [14]	?
5. *D. (Dendrocoelides) banaticum* Codreanu & Balcesco, 1967	*Dendrocoelum (Dendrocoelides)*	Oravița, Romania,	Codreanu & Balcesco, 1967a	Codreanu & Balcesco, 1967a, b [18,19]	?
6. *D. (Dendrocoelides) atriostrictum* Codreanu & Balcesco, 1967	*Dendrocoelum (Dendrocoelides)*	Reșița, Semenic Mt., Romania	Codreanu & Balcesco, 1967a	Codreanu & Balcesco, 1967a, b [18,19]	?
7. *D. (Dendrocoelides) debeauchampianum* Codreanu & Balcesco, 1967	*Dendrocoelum (Dendrocoelides)*	Orșova, Romania	Codreanu & Balcesco, 1967a	Codreanu & Balcesco, 1967a, b [18,19]	?
8. *D. (Dendrocoelides) tismanae* Codreanu & Balcesco, 1967,	*Dendrocoelum (Dendrocoelides)*	Tismana, Romania	Codreanu & Balcesco, 1967b	Codreanu & Balcesco, 1967b [19]	?
9 *D. (Dendrocoelides) stenophallus* Codreanu & Balcesco, 1967	*Dendrocoelum (Dendrocoelides)*	Sohodol, Romania	Codreanu & Balcesco, 1967b	Codreanu & Balcesco, 1967b [19]	?
10. *D. (Dendrocoelides) orghidani* Codreanu & Balcesco, 1967	*Dendrocoelum (Dendrocoelides)*	Lipova—Poiana Ruscă Mt., Romania	Codreanu & Balcesco, 1967c	Codreanu & Balcesco, 1967c [20]	?
11. *D. (Dendrocoelides) polymorphum* Codreanu & Balcesco, 1967	*Dendrocoelum (Dendrocoelides)*	Agigea, Romania	Codreanu & Balcesco, 1967c	Codreanu & Balcesco, 1967c [20]	?

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12. *D. (?Paradendreocoelum?) alexandrinae* Codreanu & Balcesco, 1970,	*Dendrocoelum (Paradendrocoelum)*	Vama Buzăului, Romania	Codreanu & Balcesco, 1970	Codreanu & Balcesco, 1970 [21]	?
13. *D. (Apodendrocoelum) brachyphallus* (de Beauchamp, 1929)	*Dendrocoelides*	Vaşcău, Romania,	Beauchamp 1929	Beauchamp 1929 [12]	?
14. *D. (Apodendrocoelum) lipohallus* (de Beauchamp, 1929)	*Dendrocoelides*	Turda, Romania,	Beauchamp 1929	Beauchamp 1929 [12]	?
15. *D. (Palaeodendrocoelum) romanodanubialis* (Codreanu, 1949-1950),	*Palaeodendrocoelum*	Iron Gates on Danube, Romania	Codreanu 1950	Codreanu 1950 [17]	?
16. *D. (Palaeodendrocoelum) getticum* Codreanu & Balcesco, 1970	*Dendrocoelum (Palaeodendrocoelum)*	Bucharest, Romania,	Codreanu & Balcesco, 1970	Codreanu & Balcesco, 1970 [21]	?
17. *D. (Eudendrocoelum) botosaneanui* del Papa, 1965	*Dendrocoelum (Eudendrocoelum)*	Anina, Romania,	Del Papa 1965	Gourbault 1972 [2]	?
18. *Polycladodes album* Steinmann, 1910,	*Polycladodes*	? Dobrogea, Romania	Steinmann 1910	Gourbault 1972 [2]	?
19. *Polycladodes voinovi*, Codreanu, 1929	*Polycladodes*	Sinaia, Romania,	Codreanu 1929	Codreanu 1929 [15]	?
20. *Polycladodes affine* (Codreanu & Balcesco, 1970),	*Dendrocoelum (Polycladodes)*	Type locality—unspecified in South Făgăraș Mt., Romania,	Codreanu & Balcesco, 1970	Codreanu & Balcesco, 1970 [21]	?
21. *Dendrocoelum obstinatum* Stocchino	*Dendrocoelum*	Movile Cave -Dobrogea, Romania	Stocchino et al. 2017	Stocchino et al. 2017 [3]	Naturalis Biodiversity Center,

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& Sluys, 2017, Romania, Dobrogea					University of Sassari

The deposition of the specimen-types of the species authored by Codreanu and Balcesco is not recorded in any publication, suggesting loss or private property, thus, no material for further comparative studies. The search for the histological slides could require significant effort. This should be the subject of a separate study that may reveal either the type-specimens or the need of a recollection strategy and protocols for neotype designation.

4. The Classical Phylogenetic System, the Morphological Types and Taxa

With the increase in the number of species, the systematics of Dendrocoelidae has become complicated and underwent several changes, according to the point of view of different authors. Historically, species have been grouped into several morphological types defined by sets of morphological characters, the morphological types corresponding to so many supraspecific taxa, at the genus or subgenus rank. The taxa morphologically established were: *Dendrocoelum* (s.l. and s.str.), *Polycladodes*, *Dendrocoelides*, *Eudendrocoelum*, *Neodendrocoelum*, *Paradendrocoelum*, *Bolbodendrocoelum*, *Apodendrocoelum* and *Palaeodendrocoelum*. The separation between them can be better discussed and understood from a historical perspective, as presented by Gourbault [2].

Dendrocoelum

This was created at the genus rank by Oersted, 1844, for the species *lacteum* which is the type species. The systematics of Gourbault recognises the genus *Dendrocoelum* (*Dendrocoelum* s.l.) separated into eight subgenera including the subgenus *Dendrocoelum* (*Dendrocoelum* s. str.) with two species: the occulated *D. lacteum* and the anophthalmic *D. infernale*. [2]. The distinctive character of the subgenus *Dendrocoelum* (*Dendrocoelum* s. str.) is the presence of a true flagellum which is defined as a long structure with longitudinal muscles covered by a vacuolated epithelium, derived from and attached to the inner epithelium of the penial papilla; it can be invaginated (inverted) or everted out of the seminal vesicle [5,9]. Other characters include: a globular penis with a large seminal vesicle, a short male atrium, the communication between the male atrium and the common atrium through an orifice and the opening of the common oviduct into the male atrium (Figure 1)

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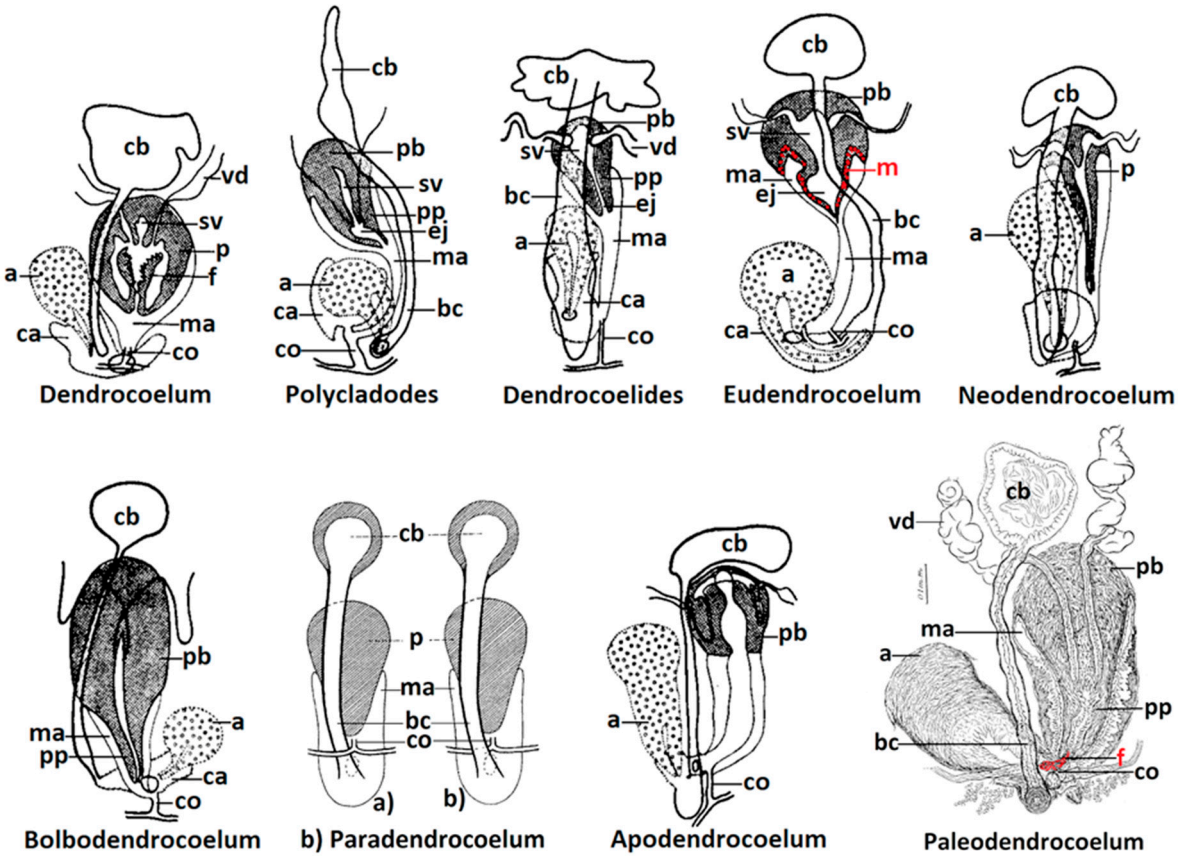

Figure 1. The morphological types corresponding to the supraspecific taxa *Dendrocoelum* s.str., *Polycladodes*, *Dendrocoelides*, *Eudendrocoelum*, *Neodendrocoelum*, *Bolbodendrocoelum*, *Apodendrocoelum* (adapted from Gourbault 1972 [2], (p. 43, Figure 7)), *Paradendrocoelum* (adapted from Kenk 1930 [38] (p. 56, Figure 2) and *Palaeodendrocoelum* (adapted from Codreanu 1950 [17] (p. 611, Figure 3, scale bar 0,1 mm). Abbreviations: a—adenodactyl, bc—bursal canal, ca—common atrium, cb—copulatory bursa, co—common oviduct, ej—ejaculatory duct, f—flagellum, m—supplementary muscular circular layer at the base of penis bulb, ma—male atrium, p—penis, pb—penis bulb, pp—penis papilla, sv—seminal vesicle, vd—vas deferens.

Polycladodes

Polycladodes was created by Steinmann in 1910, at the genus level for the pluri-occulated species *Polycladodes alba* (type species). The distinctive character of *Polycladodes* is a supplementary longitudinal muscular layer into the external area of the pharynx, a character identifying the current genus rank [1]. Other morphological characters extracted from Gourbault for *Polycladodes* are the lack of a flagellum, a long and tubular male atrium, the communication between the male atrium and the common atrium through a short duct with no sphincter, the opening of the common oviduct into a short area at the meeting place of the male atrium and the common atrium [2], (Figure 1). Gourbault included four species in *Polycladodes*: the pluri-occulated *P. album*, with 15-30 eyes and the anophthalmic *P. caecum*, *P. voinovi* and *P. affine* [2].

Dendrocoelides

This taxon was created by de Beauchamp in 1919, at the genus level, for the species *Dendrocoelides regnardi*. The distinctive character is the lack of flagellum, opposed to that in *Dendrocoelum* s.str. (Figure 1).

Gourbault considered *Dendrocoelides* at the subgenus rank and included in it 28 species out of which only two are occulated—*Dl. lescherae* in France and *Dl. vaillanti* in Algeria. Four species of this group (*Dl. lescherae, Dl. mrazeki panonicum, Dl. stenophalus* and *Dl. racovitzai*) possess invaginable penis papilla, that is a flagelliform papilla, thus, showing affinity with *Dendrocoelum* [2].

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Eudendrocoelum

Eudendrocoelum was created by Komarek in 1926, at the genus level, to include three species *lacteum, infernale* and *subterraneum*. The distinctive character is the presence of a supplementary muscular circular layer starting at the base of the penis bulb, which thins along the wall of the penis papilla (Figure 1). In 1932, de Beauchamp separated the species *lacteum* and *infernale* into the genus *Dendrocoelum*. The shift of these two species from one taxon to another shows a clear intersection of *Eudendrocoelum* with *Dendrocoelum***.** Gourbault, with respect to the flagellum, included in *Eudendrocoelum* a mixture of 10 species: (i) flagellum absent in some species, showing affinity with *Dendrocoelides* and (ii) a flagelliform structure, invaginated or not, present in some species: *E. sollaudi, remyi, gineti* and *tubuliferum*, showing affinity with *Dendrocoelum*. Most species are unpigmented and anophthalmic. Only one species, *D. (E.) parvioculatum,* has both eyes and a demi-invaginated penis papilla [2].

Neodendrocoelum

This taxon was created by Komárek in 1926. The diagnosis given by Gourbault (1972) for *Neodendrocoelum* at the subgenus rank includes the great development of penis and adenodactyl (Figure 1), the histology of the oviducts and the glands of the genital pore. Gourbault included in *Neodendrocoelum* 13 occulated species, most of them pigmented with a species-specific dorsal pattern, distributed mainly in former Yugoslavia, in Lake Ohrid and tributaries [2]. Absent in Romania.

Bolbodendrocoelum

This taxon was created by de Beauchamp in 1932, at the subgenus level for the anophthalmic species *agile* [2]. Monospecific. The distinctive character is the overdeveloped penis bulb (Figure 1). Absent in Romania.

Paradendrocoelum

This subgenus was created by Kenk in 1930 for the species *cavaticum,* based on the position of the oviducts between the male atrium and the bursal canal (Figure 1). Six hypogeic, anophthalmic species—*cavaticum*, *spelaeum*, *infernale*, *tubuliferum*, *hankói* and *carpathicum* were assigned by Kenk to *Paradendrocoelum* [38], one Romanian species—*P. alexandrinae* was also assigned by Codreanu & Balcesco to *Paradendrocoelum* [21].

Apodendrocoelum

Apodendrocoelum was created by de Beauchamp in 1932, at the subgenus rank. The distinctive character is the greatly reduced penis papilla (Figure 1). *Apodendrocoelum* contains four anophthalmic species: *A. brachyphallus*, *A. lipophallus* in Romania, *A. puteale*, and one species with insufficient diagnosis, in Germany [2].

Palaeodendrocoelum

This genus was created by Codreanu in 1949-1950, based on external morphology (pigmentation, eyes, adhesive organ) combined with genital characters. The diagnosis given by Codreanu [17] included: small size 9 mm x 1 mm, specific pigmentation, numerous eyes [14–31], with a particular disposition, differentiated and infra-nucleated adhesive organ, a non-invaginated flagellum, male and common atrium completely separated and the position of the oviducts between the male atrium and the bursal canal showing affinity with *Paradendrocoelum* (Figure 1).

A group of interest is represented by the species of Lake Ohrid. Kenk presented a group of 17 occulated *Dendrocoelum* species, authored by Müller, Stanković, Stanković & Komárek, and Kenk [5]. Of these species, only two bear a true flagellum (*D. lacteum, D. cruciferum*); most of the others have various types of penial papilla—inversible, invertible, pseudoflagellum, etc. [5]. Kenk showed the

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penis papilla variability which he attributed to physiological state, fixatives or even intrapopulational variability. Some of these species have a circular muscular layer at the base of the penis bulb (the wall of the penis papilla) [5], showing affinity with *Eudendrocoelum*. Nearly 10 of these 17 species were considered as belonging to *Neodendrocoelum* by Gourbault, including *D. cruciferum*, a species with a true flagellum [2].

5. The Morphological Characters of Dendrocoelidae and Their Phylogenetic Value

The characters used in the old and more recent literature (for species diagnosis and for the system) concern both external and internal morphology.

External morphology considers body pigmentation, eyes and adhesive organs. Internal morphology concerns a multitude of characters, some of them already presented: pharynx musculature; position of the oviducts; the dorsal/ventral position of the testes; the communication of the vas deferens with the penis, the adenodactyl; the characteristics of the copulatory apparatus—location, the penis bulb (arrangement of the musculature, degree of development), the shape and size of the seminal vesicle, the type of flagellum when present; the degree and way of separation of the male and common atria; the bursal canal; the size ratio penis: adenodactyl; the development and disposition of the eosinophilic glands, etc.

The phylogenetic value of the morphological characters is visible especially when they are correlated with other aspects, for instance, the paleogeographic conditions and the geological ''moment'' of appearance, that is their oldness. For the systematic phylogeneticists, this is a complex work, requiring the establishment of the synapomorphies and autapomorphies [39,40].

The taxonomic value of some characters:

1) The position of the oviducts between the male atrium and the bursal canal was used to establish the taxon *Paradendrocoelum*.

The taxonomic value of this character is seen differently by different zoologists:

A) as invalid generic character.

Gourbault considered that the genus *Paradendrocoelum* cannot be preserved because Codreanu showed evidence of the variability of this character "dans une même espèce" [2], but no information about the species, journal and year of publication was given.

De Beauchamp considered the character as a secondary one, later in Dendrocoelidae evolution; thus, only a specific character and not a generic one [17].

B) as a valid generic character.

For two reasons, Codreanu considered this character to be valuable:

a) this character is present in a group of hypogeic European species including *cavaticum sollaudi, hankoi, spelaeum* and one unnamed sp. (affinities between species), in opposition to the group *sphaerophalus, carpathicum, tubuliferum* and *infernale*, species considered of evident different origins [17]

b) as this character is present in all the Holarctic genera *Dendrocoelopsis*, *Amyadenium*, *Miodendrocoelum* etc., it has the meaning of a primitive (primary) character, first in Dendrocoelidae evolution [17].

2) The taxonomic value of the eyes and the biogeographical context (Figure 2 Map)

Hypotheses and arguments:

A) Kenk and de Beauchamp considered that the eyes have no generic value; they disappear in the hypogeic species which are closely related. The lack of the eyes is seen as a regressive adaptative convergence in relation to subterranean life [17].

Examples of related occulated and anophthalmic species belonging to the same genus are given to support this theory: the pluri-occulated *Polycladodes alba* and the anophthalmic *Polycladodes affine* [17] and *Polycladodes voinovi*; the occulated *Dendrocoelum lacteum* and the anophthalmic *Dendrocoelum infernale* [17]; the pluri-occulated *Palaeodendrocoelum romanodanubialis* and the anophthalmic *Palaeodendrocoelum getticum*.

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B) A second group of theories gives generic value to eyes. Species of Tertiary origin, relics in the actual fauna are occulated; the anophthalmic *Dendrocoelides* are the result of the Quaternary glaciation. These theories are supported by Beclemișev, Stanković and Codreanu [17].

a) The severe Glaciation in N and Central Europe was the driving force for speciation. Most epigeic Dendrocoelidae disappeared. Some species underwent a subterranean migration followed by adaptative loss of eyes. It is the case of the Romanian *Dendrocoelides* anophthalmic species. After migration into the subterranean and adaptative eye loss, a process of diversification followed at the level of the copulatory apparatus (evolutive divergences), giving numerous anophthalmic endemic species, distinct at the level of genitalia [18].

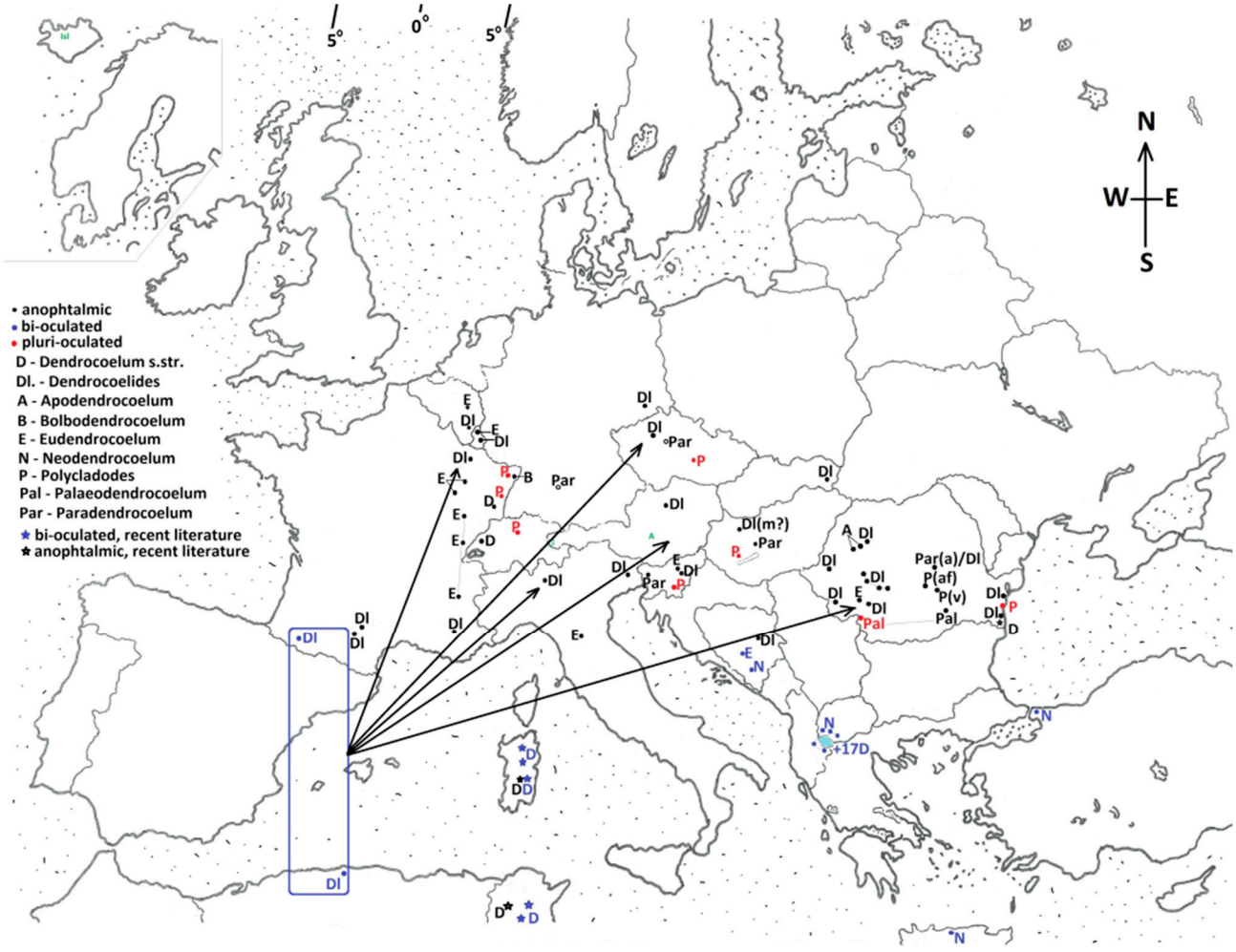

Figure 2. The geographical distribution of the actual Dendrocoelidae and the biogeographical (paleo-geographical) context supporting the hypothesis of *Dendrocoelides* evolution and diversification, determined by the Quaternary Glaciation.

b) Attenuated Glaciation in S Europe determined the survival of some species (Tertiary species) in lakes, springs and rivers, as tertiary relics. *Palaeodendrocoelum romanodanubiale* is a relic. *Palaeodendrocoelum* has the genus rank [17].

The age/oldness of the morphological characters in geological time is essential in tracing phylogenies. Regarding the eyes and copulatory apparatus, one question arises: which is the primary character (the primitive character), the eyes or the copulatory apparatus? This question generated two hypotheses:

Eyes first?

Codreanu considered the eyes as the primitive character [17], while the lack of eyes means the loss of this character. In this situation, the genus/subgenus *Dendrocoelum* s.str. should be considered

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the starting point in evolution. Such an evolutionary pattern involves the loss of the penial flagellum (*Dendrocoelum* s.str. has a more complex copulatory apparatus, by the presence of the flagellum).

Copulatory apparatus first?

Gourbault considered only the penis important to establish the systematic position [2]. The subgenus *Dendrocoelides* (most species anophthalmic), having the simplest penis, represents the starting point in evolution, while the subgenus *Dendrocoelum* s.str. has the highest evolutionary position due to the penial flagellum which is considered an evolutionary acquisition.

6. Discussion

The review of the literature reveals the gaps in knowledge.

The Dendrocoelidae fauna of Romania consists of 21 species. Of these, one species has a wide European distribution—*Polycladodes album*, the rest being endemic. More than half of the species (thirteen species) were authored by Codreanu and Codreanu & Balcesco [15–21], species for which the deposition of the specimen-types is unknown. The diagnosis of some species is incomplete, with no figurative reconstruction of the copulatory apparatus, including *Dendrocoelum (Polycladodes) affine*, *Dendrocoelum (Paradendrocoelum) alexandrinae* and *Dendrocoelum (Palaeodendrocoelum) getticum* [21]. These deficiencies impair the knowledge on the whole group.

Some of the morphological types presented are very distinct and unmistakable, making possible a clear diagnosis of the taxa—*Bolbodendrocoelum* and *Apodendrocoelum*. For the rest of the taxa (regardless of the genus or subgenus rank), a clear diagnosis is not possible, as one character of a set of characters is shared by more morphological types/taxa. Many supraspecific taxa include a mixture of species with a mixture of morphological characters which make them very difficult to order. This could be a sound reason to abandon these taxonomic divisions, at least temporarily. Some species may not be good species, and some others may not belong to the genus they were originally attributed. The taxa *Paradendrocoelum* and *Palaeodendrocoelum* seem to be the most problematic.

Using the eyes and the penial flagellum, and based on the hypotheses brought by all authors cited in this paper, I suggest two main evolutionary patterns:

1) one evolutionary pattern (Figure 3) may consider three groups (taxa) of ancient origin: *Polycladodes* and *Paleodendrocoelum* (of Tertiary origin) and a *Dendrocoelum*-type ancestor (of un-known geological origin), bi-occulated and with penial flagellum of un-known type. The evolution of the latter *Dendrocoelum*-type ancestor may have followed two main directions:

1) a) flagellum preservation and its evolution in the various flagellar types found in the current fauna

1) b) the loss of the flagellum, character typical for Dendrocoelides. This type of ancestor (of pre-quaternary age) underwent Glaciation (see pages 8-9 of this paper, point 2) B)). Attenuated glaciation in some areas (S Europe, N Africa) determined the survival of two occulated species in France and N Africa; France and N Africa represent a margin of an ancient geographical range. Other *Dendrocoelides* species disappeared, others migrated into the subterranean where lost the eyes and suffered diversification, giving numerous endemic species distinct at the level of genitalia.

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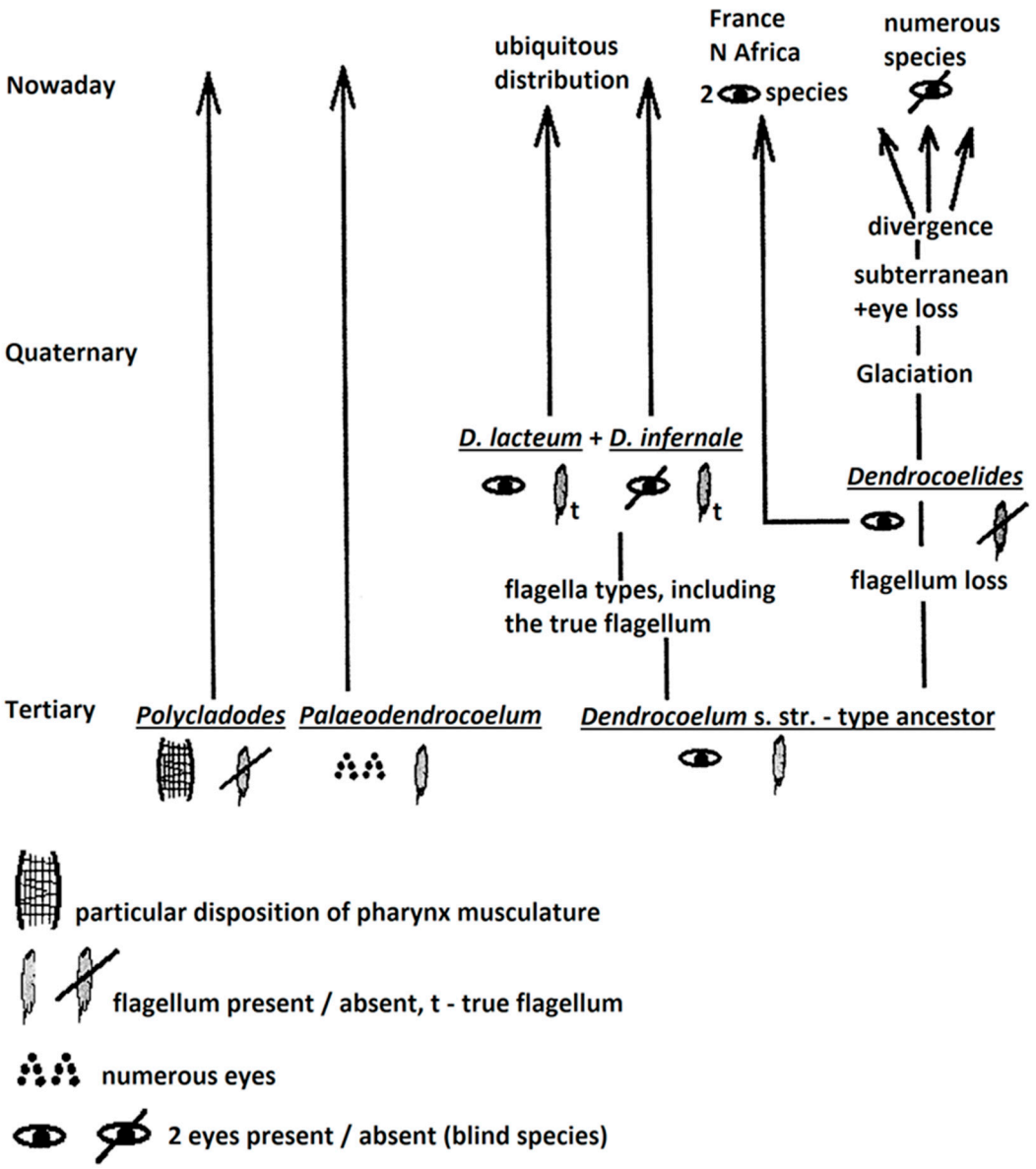

Figure 3. Evolutionary pattern with a *Dendrocoelum*-type ancestor.

2) a second model (Figure 4) may consider as well, three groups (taxa) of ancient origin, out of which *Polycladodes* and *Paleodendrocoelum* of Tertiary origin. The third group may be of the *Dendrocoelides*-type, occulated and without penial flagellum, following two evolutionary paths:

2) a) flagellum acquisition, giving various types of flagella

2) b) evolution induced by the Glaciation, with the preservation of the two occulated species in the current fauna (in France and N Africa), also with the subterranean radiation.

Both patterns imply the followings: the lack of the eyes means their loss; a possible independent dual/multiple origin of the penial flagellum—in *Palaeodendrocoelum* and the *Dendrocoelum*-type via *Dendrocoelides* ancestor (Figure 4). Amongst invertebrates, molecular data have indicated independent evolution (dual origin) in even more unexpected cases, for example, the dual origin of striated musculature [41].

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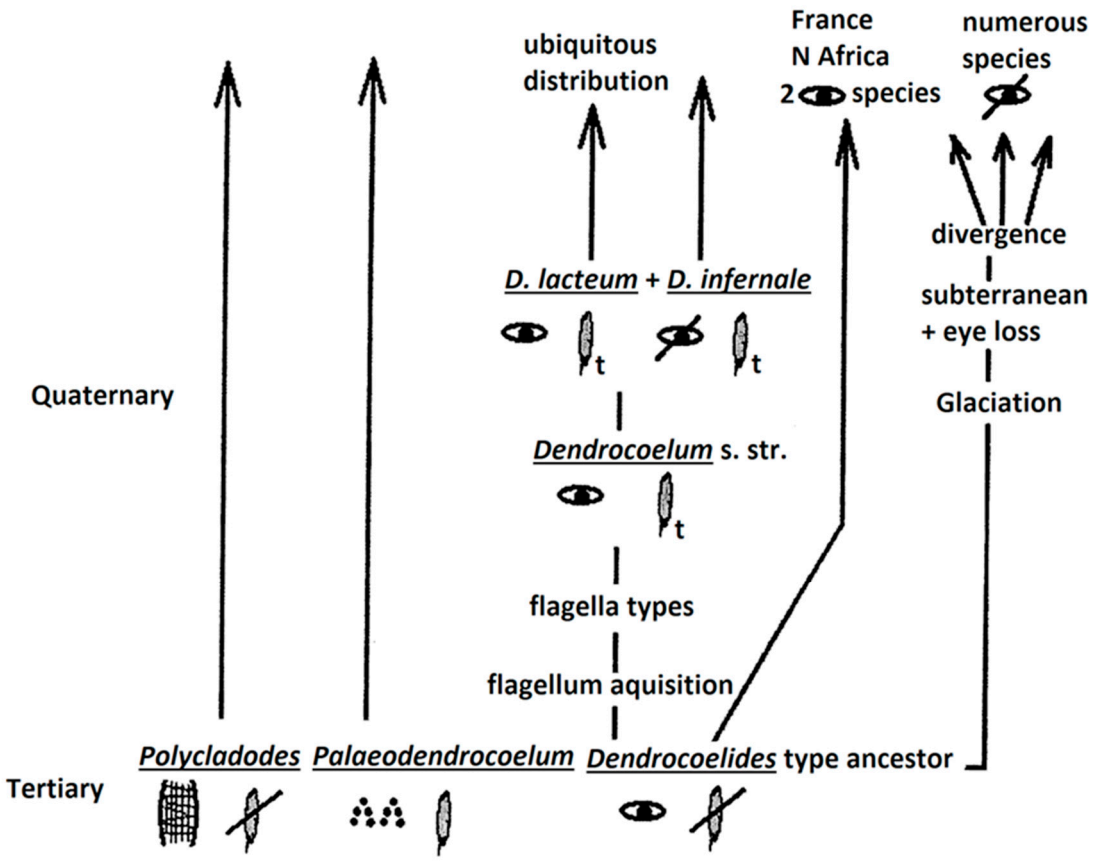

Figure 4. Evolutionary pattern with a *Dendrocoelides*-type ancestor.

Regarding the flagellar types, synthesised by Stocchino and coauthors [9], some observations can be made: the histological structure of the penial flagellum is not known in all species; most species (thirteen) possess a pseudoflagellum, of these, ten species are occulated and found in Lake Ohrid. Two species out of four possessing a completely inverted penial papilla are occulated and are found in Lake Ohrid. The phylogenetic value of the penial papilla types (the flagellar types) should answer the following issues: the variability of the penis shape indicated by Kenk [5]) (individual variability, variability in living and fixed worms); does the histological structure of the flagellum reflect a physiological state? (for instance, the vacuolated epithelium).

The natural history of this group is very difficult to be reconstructed on morphological grounds alone. The natural history may have had numerous morphological types lost during the geological times, not present in the actual fauna or not preserved in fossils. Additionally, it is completely unknown where and how many transitions from epigeic to hypogeic (and vice versa) occurred. The palaeogeographical evolution of European land, freshwater and brackish water could have taken place according to a model like the Glacial Sensitive Model (GSM) and 'Sea-Level Sensitive' dynamic model (SLS), models available for the marine island biogeography [42], thus shaping the speciation process.

7. Conclusions

The natural history and phylogenetic systematic of *Dendrocoelum* s.l. should be re-investigated. The integrative approach paleo-bio-geography—morphology—biological features—genetics may lead to a better understanding of the group and may bring surprising outcomes.

8. Future Directions of Study

Specific future research needs and questions

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The reinvestigation of the Romanian Dendrocoelidae should have different approaches and should aim to answer to the following questions:

1. the status of the describes species—some species may be synonymised; new species may be described.

2. the genus or subgenus level of *Paradendrocoelum* and *Palaeodendrocoelum*. In this regard, the species *Paradendrocoelum alexandrinae* and *Palaeodendrocoelum getticum* are of particular interest.

3. the anophthalmic species *Palaeodendrocoelum getticum*, *Polycladodes voinovi* and *Polycladodes affine* may be supportive for the validation of one of the hypotheses regarding the origin, the paleogeographic way of distribution (the migration route), also for clarifying the eye character status—homologous character (divergent evolution) or analogous character (convergent evolution)

4. the molecular support for timing of subterranean colonization, for eye/flagellum evolution hypotheses.

The Dendrocoelidae as freshwater flatworms in the era of genetics

The numerous modern genetic techniques, tools and protocols provide new and deeper insights into freshwater flatworms' knowledge. This group of worms is characterised by the presence throughout their lives of neoblasts. Neoblasts are population of adult pluripotent stem cells involved in many processes—regeneration, turn-over of specialized cells, responses to external insults [43]. The study of stem cells is focused on planarians (Dugesiidae) which became model system due to their high regenerative abilities. The literature in this field is impressive; numerous research articles and reviews bring into light a complex regulatory genetic network of a stem-cells system involved in developing, tissue and organ functioning, repairing various parts of a worm organism [43–49].

Dendrocoelidae must have neoblasts and a stem-cell system (lost or degraded in evolution) but, so far, very much un-explored. Few papers treat regeneration in *Dendrocoelum lacteum* alone [50] or in other summative and comparative contexts [49].

Two main topics seem to be important for *Dendrocoelides* geological evolution—the neoblasts and the eye loss. Most *Dendrocoelides* species of the actual fauna are endemic and cavernicolous. It is very well known and documented that the eye loss comes as an adaptation to dark environments in many invertebrate and vertebrate phyla, including planarians [51–53]. Regarding the eyes, one question should be answered: how does a cavernicolous anophthalmic species will react when exposed to light for a longer period—will it develop eyes? What is the meaning of the acquisition or loss of eyes in this group of worms characterised by the presence of neoblasts?

In recent years, there has been increasing awareness of the role of the environment in producing phenotypes. An inherited genome can respond not only to mutations to produce new phenotypes but also to numerous environmental factors involving epigenetic control (for instance DNA methylation) [54].

Other modern methodologies could bring new insights and outcomes in the study of this worms, like in other living beings, for various purposes and in different contexts—eDNA [55], genome skimming [56–58], microCT imaging [59–61].

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