# A NEW SPECIES OF FRESHWATER PLANARIAN FROM CHILE (PLATYHELMINTHES: TRICLADIDA), WITH A NOMENCLATURAL NOTE ON *GIRARDIA FESTAE* (BORELLI, 1898)

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Abstract. – A new species of triclad, Girardia canai (Paludicola, Dugesiidae), is described from the Mapocho River within the Región Metropolitana of Chile. The species has the following diagnostic characters: ventral testes; bursal canal forming a right angle at its opening into the genital atrium; epithelia of the atrium and bursal canal with intraepithelial nuclei; course of the sperm ducts very variable, generally asymmetric; the sperm ducts do not form intrapenial enlargements; instead, they change their histology abruptly, adopting a histological structure identical to that of the ejaculatory duct; the union of these slender ducts occurs within the penis papilla; penis papilla notably long. The intrapenial structure of this species is of a new morphological type, and is considered a main attribute for defining the species. The aforementioned features distinguish the new species from all other Girardia species. Particular emphasis is placed in the discussion on the comparison with G. tigrina (Girard), G. rincona (Marcus), G. longistriata (Fuhrmann) and G. microbursalis (Hyman). The nomenclature of Girardia festae (Borelli, 1898) is reviewed on the grounds that the commonly used spelling G. festai is an unjustified emendation.

*Girardia* Ball, 1974 (Paludicola: Dugesiidae) is a Neotropical genus of freshwater planarians encompassing more than 30 described species which are for the most part very closely related to one another and show only small morphological differences. The phylogenetic position of this genus within the Dugesiidae family was investigated recently by de Vries & Sluys (1991).

In 1986 planarians were collected from two streams, Río Mapocho and Estero Reñaca, near Viña del Mar, Chile, where there are previous records of *Girardia chilla* (Marcus, 1954) and *G. sanchezi* (Hyman, 1959). We were particularly interested in *G. sanchezi* since Hyman (1959) suggested that it could be conspecific with *G. anceps* (Kenk, 1930), for which we have already published a redescription (Cazzaniga & Curino 1987). However, the planarians we collected belong to a new species of the genus *Girardia*, as described below. Material and methods. — The specimens from both localities were collected by the authors together with Dr. Patricio Sánchez on 26 October 1986. They were found under stones, mainly in backwater areas of the rivers.

Before fixation in F.A.A. fluid (formaldehyde-ethyl alcohol-acetic acid), the specimens were placed on ice in order to induce muscular relaxation. The material was serially cut at 7–9  $\mu$ m intervals into sagittal, transverse and frontal sections which were then stained in hematoxylin and eosin.

# Girardia canai, new species Figs. 1-9

Diagnosis. — Girardia species with ventral testes; bursal canal forming a right angle at its opening into the genital atrium. Bursa copulatrix medium to large in size. Epithelia of the atrium and of the bursal canal with intraepithelial nuclei. Course of sperm ducts very variable, generally asymmetric. No intrapenial sperm vesicles. Instead, the sperm ducts change their histology abruptly, forming two slender ducts structurally identical to the ejaculatory duct with a tall epithelium of infranucleate cylindrical cells, pale cytoplasm and a very narrow lumen. The ducts fuse within the penis papilla and the ejaculatory duct ends terminally. The penis papilla is notably long.

*Distribution.* – Type locality: Mapocho River, in the vicinity of the town of Talagante, Región Metropolitana, Chile.

The other locality where they have been found is near the mouth of the Estero Reñaca, to the south of Concón, V Región, Chile.

*Type material.*—The type series includes 19 sets of serial sections deposited as follows.

Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN), Buenos Aires: Holotype: a set of sagittal serial sections on seven slides (MACN #33438). Paratype #1: a set of transverse serial sections on eight slides, specimen from the type locality (MACN #33439).

American Museum of Natural History (AMNH), New York: Paratypes #2 and 3: a set of transverse serial sections on nine slides, specimen from the type locality (AMNH #1595); a set of sagittal serial sections on 12 slides, specimen from Estero Reñaca (AMNH #1596).

Laboratorio de Ecología Acuática, Universidad Nacional del Sur, Bahía Blanca: Paratypes #4 to 19: ten sets of sagittal serial sections, four sets of transverse serial sections and one set of frontal serial sections. Thirteen specimens from the type locality and two from Estero Reñaca.

Description. — The fixed planarians are 9– 10 mm in length and 2–4 mm in width. The head has the typical triangular shape of the genus *Girardia*, with visible but not conspicuously large, rounded auricles. The ground pigmentation is light brown, with dark brown, almost black spots, the density of which is very variable. In some specimens the spots are very abundant and delimit a light longitudinal middorsal stripe; in others the spots are scarce and no stripe is apparent. The size and position of the eye cups conform to that described for all the species of *Girardia* from South America.

The body wall has a cylindrical to cubic epithelium underlain by a weakly developed subepidermal layer of circular muscles and a well-developed layer of longitudinal muscles; radial muscles were not discerned. The rhabdite-forming cells are located beneath the muscular layers. The marginal adhesive glands form a ring round the ventral surface; they are very developed, strongly eosinophilous, and their secretion is granular in appearance.

The digestive system is typical for triclads. The main anterior branch of the gut extends briefly past the level of the ocelli, but does not extend anteriorly to the brain. The pharynx is of the dugesiid type. The macroscopic aspect of the pharynx was not observed in the live material. No epidermal or subepidermal pigmentary cells are apparent in the histological sections.

The ovaries are ovoid in shape and located directly posterior to the brain branches, with a slight latero-medial displacement. The funnel-shaped oviducal tuba of each ovovitelline duct opens onto the lateral external face of the respective ovary. The oviducal tubae were filled with sperm in many of the specimens examined.

The ovovitelline ducts have an infranucleate cubic epithelium surrounded by a circular muscular layer. In transverse section, these ducts are surrounded by a layer of radially disposed nuclei, similar to that described for other *Girardia* and *Schmidtea* species (Marcus 1946, Schilt 1976).

The ovovitelline ducts run dorsally along the nerve cords up to shortly before the gonopore, where they curve upward and run toward the middle-sagittal plane; here they bend anteriorly and finally open into the bursal canal. In the majority of the speci-



Figs. 1–2. *Girardia canai*, new species. Sagittal reconstructions of the copulatory apparatus. BC: *bursa copulatrix*; BG: basophilous glands; BS: bursal canal; CG: cement glands; ED: ejaculatory duct; EDB: anterior branch of the ejaculatory duct; G: gonopore; MF: muscular fold; O: ovovitelloduct; S: sperm duct; SE: extrapenial seminal vesicle; SG: shell glands. Note that only the epithelia of S, EDB and ED are drawn. The diameter of S appears smaller than it in fact is, since the surrounding muscular coat is not depicted; EDB and ED do not have a muscular coat.

mens examined the ducts open separately, but very closely together, into the posterodorsal face of the bursal canal. In a few cases the openings are somewhat more separated. The posterior-most portion of the ovovitelline ducts shows a change in histological structure, being lined with a normal, and not infranucleate, epithelium.

The yolk glands have large polyhedrical cells with large nuclei. Their cytoplasm is eosinophilous and vacuolate. These glands occur mainly between and on the diverticula of the gut. In the majority of the specimens examined a variable number of these glands were observed to be present anteriorly to the ovaries.

The bursa copulatrix has a tall-celled epithelium with basal nuclei (Figs. 3, 4), the cells being thicker at the apical end than at the base. These cells frequently have a vacuole at their broader end, in the interior of which it is sometimes possible to observe masses of sperm in digestion. The size of the bursa copulatrix can vary greatly from



Figs. 3-5. Girardia canai, new species. 3-4, Sagittal sections of the holotype. 5, Sagittal section of paratype #4.

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small to very large, sometimes filling the entire height of the body. No correlation was found between size and sperm content.

The bursal canal originates from the caudo-upper part of the bursa, running in a smooth curve over the penial complex. At its posterior end it bends downward to form a right angle and opens into the common atrium. The shell glands open into the bursal canal immediately below the opening of the ovovitelline ducts. These glands are strongly eosinophilous and very developed. The distal portion of the bursal canal is displaced to the left in the majority of the specimens examined, opening into the laterodorsal portion of the common atrium; in only a few specimens is a sagittal course followed. The histological structure of the bursal canal (Fig. 7) shows a tall, cylindrical ciliate epithelium of nucleate cells, together with two muscle layers, one of which is subepidermal and circular and the other external and longitudinal. No other muscle layers or muscular additions are observed at the distal portion.

The testes were ventral in all of the studied specimens, extending from close behind the ovaries almost to the posterior end of the body. In only one of the specimens examined was a testicular follicle present anteriorly to one of the ovaries. As usual in the genus, the testes are at both sides of the nerve cords, with the exception of the pharynx and the genital complex zone, where they occur only on the outside. They are very abundant and show a continuous distribution.

The extrapenial seminal vesicles or false seminal vesicles are generally well developed, forming two wide and sinuous tubes full of sperm originating at the level of the anterior half of the pharynx.

Near the anterior face of the penis bulb or the origin of the papilla the extrapenial seminal vesicles abruptly become thinner and give rise to the sperm ducts. These ducts have a thin cavity and a cubic eosinophilous epithelium with normal intraepithelial nuclei and a well-developed circular muscle layer. The course of the ducts is extraordinarily variable from one specimen to another and even from one side of the same organism to the other. Of the 16 completely mature individuals examined, 11 showed asymmetry in their course. There is no evidence to suggest that this variation is due to different states of contraction. Three main schematic models can be described for the course of the sperm ducts:

- 1 The sperm duct runs upward and toward the bulb, curves both frontward and toward the middle of the body, penetrates into the bulb through the anterior-upper face and finally runs downward through the bulb (Fig. 1 on the right side of the specimen and Fig. 2 on the left side). This course clearly forms a loop similar to that described for *Girardia anceps*, though it never reaches such a dorsal position nor does it curve as far forward as it does in the latter species (Cazzaniga & Curino 1987).
- 2 The duct runs upward, curves toward the middle of the body, penetrates into the bulb through the lateral-upper face and then runs downward, the whole course running on the same transversal plane (Fig. 2 on the right side of the specimen), i.e., the ducts do not form a loop.
- 3 The duct does not run upward, or does so only slightly and then goes directly toward the bulb, entering through the lateral-inferior face (Fig. 1 on the left side). This course can be straight or sinuous, forming an "S" on a horizontal plane.

The asymmetry most frequently observed was a loop or type-1 course on one side and a type-3 course on the other side of the same specimen (Fig. 6).

In all cases the sperm ducts run a long way into the interior of the bulb without becoming true intrapenial seminal vesicles, i.e., they do not form intrabulbar enlargements. Instead, they transform into two

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Figs. 6–9. *Girardia canai*, new species. 6, Transverse section of paratype #1. 7, Enlargement of the bursal canal of the holotype to show the presence of two muscle layers, the internal layer being circular. 8, Frontal section of the penis of paratype #5, illustrating the structure of the anterior branches of the ejaculatory duct uniting in the penis papilla. 9, Enlargement of figure 1. CM: circular musculature; EB: cephalic branch of the ejaculatory duct; ED: ejaculatory duct; LM: longitudinal musculature; S: sperm duct.

slender ducts with the same histological structure as the ejaculatory duct (Figs. 8, 9).

The course of the intrabulbar portion of the sperm duct varies greatly. When it runs upward after entering the bulb the histological transformation occurs in the upper-posterior zone of the latter; in one of the specimens examined, the duct runs first upward and then anteriorly, forming an intrabulbar loop. In others the duct runs downward and even in a lateral direction, so that the histological transformation of the duct takes place at some point between the posterior half of the bulb and the base of the papilla. In the type-3 course the duct ends with a right-angled curve towards the papilla and immediately changes its structure.

The histological transformation of the sperm duct is abrupt. In the 16 mature specimens examined, the narrow lumen of the sperm ducts is reduced even further, the cubic epithelium replaced by a tall cylindrical epithelium of infranucleate cells with a pale cytoplasm (Fig. 8), and the circular muscle layer disappearing, with the exception of a few isolated fibers. On the basis of purely topographical considerations these modified intrapenial ducts would conventionally be called "sperm vesicles"; however, they have a very reduced lumen and upon uniting they give rise to the ejaculatory duct without undergoing any further modification in the histological structure. For these reasons we consider it more appropriate to denominate them "anterior branches of the ejaculatory duct."

The union of these two narrow ducts occurs at a point located somewhere between the base and two thirds of the length of the papilla (Fig. 9). The ejaculatory duct generally runs through the center of the papilla and its opening is terminal.

The penis bulb is hemispherical and short. The penis papilla is very long and in almost all the specimens examined reaches the posterior wall of the common atrium or even the gonopore (Figs. 3, 5), making it difficult to differentiate the male atrium from the common one. The epithelium of the papilla is cubic and shows intraepithelial nuclei; it is underlain firstly by a circular layer of muscles and then by a much thinner layer of longitudinal ones.

Basophilous glands extend from the periphery of the bulb to the ejaculatory duct into which they open, their degree of development varying greatly from one individual to another.

The epithelium of the atria has normal nuclei and varies from cubic to cylindrical. The atrial walls have a subepidermal circular muscle layer that is more developed than the longitudinal one. In five of the nine sagittally sectioned specimens, a fold with circular muscle fibers was observed on the ventral wall of the male atrium (Fig. 5). There can be no ring-shaped diaphragm since a corresponding fold has never been observed on the dorsal wall of the atrium.

The common atrium is surrounded by a ring of cement glands that open into it. They are very strongly eosinophilous, their secre-

tion is granular, and they extend a long way toward the posterior part of the body.

In only one specimen was a posterior pocket of the common atrium observed similar to that described for *G. tigrina* and *G. anceps* (Ball 1971, Cazzaniga & Curino 1987). In the rest of the material there was no evidence of evaginations in the posterior wall of the atrium.

*Etymology.* — The word *canai* means "friend" in Mapuche language and was chosen as the name for the new species in gratitude for the friendship accorded by Dr. Patricio Sánchez of the Universidad Católica de Santiago, who guided us in the search for the material.

### Discussion

The species described here can clearly be assigned to the *Girardia* genus by virtue of its triangular head, the presence of two muscle layers in the bursal canal with the subepidermal one circular and with no ectal reinforcement, the absence of a diaphragm in the ejaculatory duct, and the numerous ventral testes which extend along the length of the body.

The pigmentation of the outer pharyngeal wall is one of the autapomorphies proposed for defining *Girardia* (de Vries & Sluys 1991). This character has been described for many species, though it is quite variable intraspecifically, as demonstrated in *G. rincona, G. festae* or *G. tigrina* (du Bois Reymond-Marcus 1953; Marcus 1954, 1960; Kawakatsu et al. 1984; Ribas et al. 1989), and in the case of *G. anceps* the pharynx is fully depigmented (Cazzaniga & Curino 1987, Kawakatsu & Rovasio 1992). It is regrettable that no conclusive information on this point can be included for *G. canai*.

De Vries & Sluys (1991) presumed a close affinity among *Girardia* Ball, 1974, *Cura* Strand, 1942 and *Schmidtea* Ball, 1974. The relationship between these three dugesiid genera rests on a single synapomorphy, i.e., the presence of an angled bursal canal, instead of the plesiomorphic smoothly curved bursal canal. Ball (1980) suggested that it is possible to distinguish two groups within the *Girardia* genus, each encompassing roughly half of the known species. He rests his argument heavily on the curvature, either smooth or angled, of the bursal canal at its opening into the genital atrium. It appears that the course of the bursal canal, either smoothly curved or angled, is associated with the dorsal and ventral testes, respectively (Ball 1980). The unity of the genus and its affinities should thus be reconsidered in future analyses of additional characters.

With the exception of Beauchamp (1939), authors have accepted that the ventral or dorsal position of the testes is a useful taxonomical character (Ball 1971). De Vries & Sluys (1991) stated that the male gonads are unsuitable for inferring phylogenetic relationships between genera of Dugesiidae. Ball (1974) expressed a similar viewpoint, asserting however that the position of the testes may be useful for delimiting species groups.

The new species belongs to the group prevailing in southern South America that has ventral testes and an angled bursal canal. For this reason the species with dorsal testes and for the most part smoothly curved bursal canals (Ball 1980; Kawakatsu et al. 1983, 1992) will not be included in the discussion. Within this latter group is *Girardia chilla* (Marcus), known in central and southern Chile (Marcus 1954, Hyman 1959, Kawakatsu et al. 1984), and which has an enormous cavity in the penial bulb making it immediately distinguishable from *G. canai*.

Within the group of species with ventral testes, the conformation of the intrapenial ducts in the new species establishes a novel morphological model. In some species of *Girardia* such as *G. rincona* and *G. longistriata* the sperm ducts join almost immediately beyond the separate entrance to the penial bulb, where they form the ejaculatory duct. In other species the normal configuration is the formation of a bifid, more or

less broad seminal vesicle, as in *G. anceps*, *G. sanchezi*, *G. dorotocephala*, *G. tigrina*, or the occurrence of a unique intrabulbar vesicle from which the ejaculatory duct arises, as in *G. festae*, *G. nonatoi* and *G. arndti*. The new species is intermediate between the previously known forms without seminal vesicles and those in which the ejaculatory duct is short and limited to the penis papilla due to the formation of the intrapenial vesicle.

Girardia canai has no intrabulbar enlargements, but the union of the intrapenial ducts nevertheless occurs in the penis papilla. This difference is an important argument in favor of excluding the following species from the discussion: G. dimorpha (Böhmig) and G. sanchezi (Hyman), both from Chile, G. anceps (Kenk), G. arndti (Marcus), G. aurita (Kennell), G. dorotocephala (Woodworth), G. festae (Borelli), G. guatemalensis (Mitchell & Kawakatsu), G. nonatoi (Marcus).

Though G. tigrina (Girard) should also be excluded on the very same grounds, it is considered pertinent to include it in the discussion since it is a very variable and widespread species that serves a useful purpose in placing the description of the new species in perspective.

The diagnostic features of Girardia tigrina have been summarized by Kenk (1972). Most morphological descriptions referring to material from various parts of the world where the species is either autochthonous or has been introduced conform to these characters (for example, Ball 1971; Kawakatsu & Mitchell 1981; Ribas et al. 1989). Morphological variations mostly affect external pigmentation patterns, pharynx pigmentation, the occasional expansion of the intrabulbar sperm vesicles-uniting in a single cavity, or their contraction, appearing as tubular seminal vesicles, the position of the epithelial cell nuclei of the copulatory system, and some asymmetry in the course of the sperm ducts. Rather more stable characters are the short, conical penis papilla,

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the sharp angle of the bursal canal at its opening into the genital atrium and the presence of a well-developed blind diverticulum on the posterior wall of the common atrium (Ball 1971, Ribas et al. 1989).

*Girardia tigrina* is a species found in South America, and the descriptions given by Marcus (1946) and Kawakatsu et al. (1992) of specimens found in Brazil and Uruguay, respectively, agree with the characters already mentioned for the species. Other South American material with diverse characteristics also has been identified as *G. tigrina* but no explicit valuation of the described characters has been carried out.

For example, the musculature that surrounds the bursal canal provides a character of phylogenetic importance (Ball 1974, Sluys & de Vries 1988, de Vries & Sluys 1991). Girardia has the plesiomorphic condition of only two muscle layers, the subepidermal one of which is made up of circular muscle, as shown in Fig. 7. The specimens from southern Brazil (Kawakatsu et al. 1981, 1982, 1983, 1986) and Uruguay (Kawakatsu & Ponce de León 1990), however, are described as having a thin subepidermal layer of longitudinal muscle in the bursal canal and a variable number of muscle layers exhibiting diverse degrees of development.

Furthermore, the bursal canal of these south Brazilian planarians does not always form a sharp angle, some ectal reinforcements appear, the caudal diverticulum of the atrium wall is not always developed, the penis papilla is not always of a rounded conical form, and sometimes it is atypically long. All such divergences from the diagnostic characters of *G. tigrina* emphasize the need for a reassessment, since they would appear to separate some of the Brazilian planarians from *tigrina* or even from *Girardia*.

We consider that *G. canai*, new species, has the greatest morphological affinity with the following usually accepted species of *Girardia*, all of which have ventral testes, angled bursal canal and no seminal vesicles: G. rincona (Marcus), G. longistriata (Führmann), and G. microbursalis (Hyman).

Girardia rincona was originally described by Marcus (1954) for central Chile and redescribed by Kawakatsu et al. (1984) for Peru. This is the only species for which an epithelium of the ejaculatory duct similar to that of G. canai has been described, although it differs significantly in a number of other characteristics. The bursa copulatrix of G. rincona is even smaller than in the least developed cases of G. canai. Marcus (1954) and Kawakatsu et al. (1984) mention the presence of preovaric testicular follicles in G. rincona. In G. canai however, a testicular follicle was seen only once, exceptionally, in this position, on one side of a specimen. Both the G. rincona material from Chile and that from Peru show the extrapenial seminal vesicles running upward to the anterior dorsal side of the penis bulb, while in G. canai this condition does not appear, not even in fully developed individuals. In the new species the occurrence of sperm ducts that follow different, frequently asymmetric courses before entering the penial bulb is typical. In G. rincona the sperm ducts either unite in the penial bulb without giving rise to a bifid vesicle, as described by Marcus (1954), or give rise to a small bulbar cavity or seminal vesicle, as described by Kawakatsu et al. (1984), thereafter continuing in the form of a unique and narrow ejaculatory duct whose histology differs from that of the sperm ducts. In G. canai, on the other hand, the sperm ducts run along about half the length of the penial bulb and then undergo an abrupt histological transformation, acquiring the same characteristics as the ejaculatory duct, before uniting somewhere between the base of the papilla and a third of the way along to the opening. Kawakatsu et al. (1984) said that the penis papilla of the samples from Peru are accompanied by many eosinophilous glands that do not appear in G. canai. Finally, the penis papilla in G. rincona is short and conical whereas that in the new

species is significantly longer, probably the longest ever described for this group of species.

*Girardia longistriata*, from Colombia, has been briefly redescribed by Ball (1980). The greatest similarity is seen in the type of insunk epithelium that covers the ejaculatory duct, which is narrow and opens terminally but does not run centrally through the papilla. There are however several differences in the Colombian species: the dorsum has a different color pattern, the penis papilla is conical and very short, and the sperm ducts unite within the bulb rather than in the penis papilla as they do in *G. canai*. The initial course of the ovovitelline ducts is also different.

Girardia microbursalis from Connecticut, U.S.A., is known only through its original description (Hyman 1931), which lacks histological details and thus precludes an accurate comparison. From what can be seen it would appear to differ in the size of the bursa copulatrix (to which characteristic it owes its specific name) and in the site where the sperm ducts or "anterior branches of the ejaculatory duct" unite. Without a histological description there is no reliable way of telling whether or not a transformation of the type described in the new Chilean species occurs. Hyman (1939) cited new localities without giving any additional morphological data. Kenk (1974, 1989) suggested that it could be a synonym of G. tigrina, an opinion that was not sustained in the outcome of the ensuing discussion.

# A Note on the Nomenclature of Girardia festae

A South American freshwater planarian, now under *Girardia* Ball, 1974, was described by Borelli (1898) as *Planaria festae*, based on the name of Dr. Enrico Festa. This original spelling is correct under article 31 a(i) of the International Code of Zoological Nomenclature (International Trust for Zoological Nomenclature 1985; example: *podae* from Nicolaus Poda). Marcus (1946) suggested that "melhor seria *festai*" (*festai* would be better), and this comment was considered nomenclaturally valid by Kenk (1974). Du Bois Reymond-Marcus (1953) used the emendation for the explicit reason that *festae* "constitutes a lapsus, as it was chosen in honor of Dr. Enrico Festa." They thus changed the spelling to *Dugesia festai* on the grounds that Dr. Festa was male. This invalid emendation subsequently became common usage. The original orthography was not in fact erroneous but the correct formation of the first declension genitive in Latin.

Certainly, *festai* as an original spelling would also have been admissible (art. 31 a(ii); example *podai*), but there is no valid reason for accepting an emendation to Borelli's spelling of this species. *Girardia festai* (Marcus, 1946), based on an unjustified emendation, became a junior objective synonym of *G. festae* (Borelli, 1898). The following references are, therefore, correct subsequent spellings:

*Euplanaria festae*: Kenk (1930, explicit new generic combination), Beauchamp (1939). *Dugesia festae*: Hyman (1939, explicit new generic combination); whereas those that follow are incorrect (art. 33 c):

Dugesia festai: du Bois Reymond-Marcus (1953), Marcus (1960), Ball (1969, 1980), Mitchell & Kawakatsu (1972), Kawakatsu & Mitchell (1984a, 1984b), Kawakatsu et al. (1984), Kawakatsu & Rovasio (1992). Dugesia (Girardia) festai: Ball (1974, justified subgeneric assignation), Kenk (1989).

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