

## On the morphology of *Opisthodiscus nigrivasis* (v. Mehely 1929) Odening, 1959 (Trematoda: Paramphistomidae) of *Rana ridibunda*

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### Summary

*Opisthodiscus nigrivasis*, recovered from *Rana ridibunda* frogs in Salamanca and León provinces (Spain) has been redescribed, with particular emphasis on the presence and characteristics of the oesophageal bulb, thus confirming Odening's work<sup>12</sup>.

As long as oesophageal bulb is present in all species of the subfamily Diplodiscinae, separation of the more allied genera (*Opisthodiscus* and *Pseudopisthodiscus*) requires attention to the situation of the vitellaria, genital porus and the position of the excretory ducts in connexion with the placing of the caeca.

According to the records of some French authors, born out by our findings, it seems that *Rana ridibunda* is the main definitive host used by *O. nigrivasis*, at least in an area of southeast France and in a great part of Norden and Central Spain.

**Key Words:** *Opisthodiscus nigrivasis*, oesophageal bulb, redescription.

### Resumen

A partir de ejemplares procedentes de *Rana ridibunda* de las provincias de León y Salamanca (España), se redescrive la anatomía de especímenes jóvenes y adultos de *Opisthodiscus nigrivasis*, con especial atención a la presencia y características del bulbo esofágico, órgano cuya existencia se confirma, en armonía con la descripción de Odening<sup>12</sup>.

Dado que este órgano existe en todos los géneros de Diplodiscinae, la separación entre sí de los géneros afines *Opisthodiscus* y *Pseudopisthodiscus* debe hacerse teniendo en cuenta otros aspectos morfológicos tales como la situación de las glándulas vitelógenas y el poro genital, así como la posición de los conductos excretores en relación con los ciegos.

Por la información recogida, parece que *Rana ridibunda* es el hospedador preferido de *O. nigrivasis* en una zona comprendida entre el Sur-Este de Francia y gran parte del Norte y Centro de España.

**Palabras Clave:** *Opisthodiscus nigrivasis*, bulbo esofágico, redescipción.

The species of *Diplodiscinae* Cohn, 1904 described from the amphibia of Europe are, according to our information, *Diplodiscus subclavatus* (Pallas 1760) Diesing, 1836; *Opisthodiscus diplodiscoides* v.

Mehely, 1929 (= *O. nigrivasis* v. Mehely, 1929) Odening, 1959). There have been difficulties in the differentiation of both genera, and also in that species, above all those of *Opisthodiscus*. The problem has in

some way solved itself since the work of Odening<sup>12</sup>, who supports the consideration of *O. diplodiscoides* as *species inquirenda*, while at the same time raising to the category of species that which was described by v. Mehely as a variety.

Nevertheless, on revision of the bibliography available on *O. nigrivasis* it should be pointed out that redescription can be useful in order to arrive at a better and more certain characterization of this species avoiding in this way miss descriptions like that of Lluch *et al*<sup>8</sup>. Therefore our work unites an attempt to establish new data while simultaneously confirming others, being based on various *R. ridibunda* flukes isolated in the provinces of León and Salamanca. Thus we also contribute to the knowledge of the helminth fauna of the Iberian amphibia, as yet only partially studied, as pointed out by Combes and Sarrouy<sup>4</sup>.

Together with this one, there are now several references to Diplodiscinae in the Iberian Peninsula. The first representative of this subfamily found in Spain was *D. subclavatus*, described by López-Neyra<sup>7</sup> in *Rana sculenta* in Granada. Combes and Gerbaux<sup>3</sup> discovered the presence of *O. nigrivasis* in *R. ridibunda perezii* at Isobol, in the Segre valley, Gerona province, and Combes and Sarrouy<sup>4</sup> discovered it again in the same host-species near the River Douro where it crosses Soria. Finally Simón-Vicente *et al*<sup>13</sup> described the life cycle of the species on *R. ridibunda* with *Ancylostomum fluviatilis* as first intermediary host.

#### Materials and Methods

The parasites were found in the cloaca of frogs from a stream at Garcirrey (Salamanca province) the same place where the life cycle was performed (Simón-Vicente *et al*<sup>13</sup>), and from various points of the rivers Orbigo and Bernesga (León province), caught between spring and late

summer.

In order to study them, they were observed fresh and in stained preparations, drawing being made with a *camera lucida* and/or projection screen.

Borax carmine stain were used on material previously fixed in hot 70° alcohol or in a physiological saline solution with 5% formalin.

*Opisthodiscus nigrivasis* (v. Mehely 1929)  
Odening, 1959

#### Characteristics.

Viewed alive, the body is whitish colour; and when not compressed between slide and coverslip appears slightly transparent. It is either conical or bell-shaped. It moves slowly, contracting itself, generally, in a dorso-ventral direction. The cuticle lacks spines.

The mouth opens into the pharynx and takes the form of a circular outwardly rolled process, situated at the anterior extremity of the body. At its posterior end, the pharynx form a pair of globular pharyngeal sacs, with muscular walls. Between the pharyngeal sacs, the pharynx opens into a long narrow oesophagus. At the hind end of the oesophagus there is a bulb, which when at rest is pear-shaped, yet when it moves up and down it acquires a more or less spherical shape and may double its diameter. The thickness of its muscular wall and the clarity of the circular fibres are both noticeable. At its base there is a very pronounced narrowing followed by an elongate longitudinal swelling with a weaker muscle structure, from which issue two narrow lateral branches, which are connected to the intestinal caeca by means of two fine-walled, funnel-shaped galleries of short diameter. The swelling behind the bulb is also capable of contracting itself, strengthening the bulb's movements and gives that organ the constitution and function of a double bulb (Figs. 1, 4, 6).

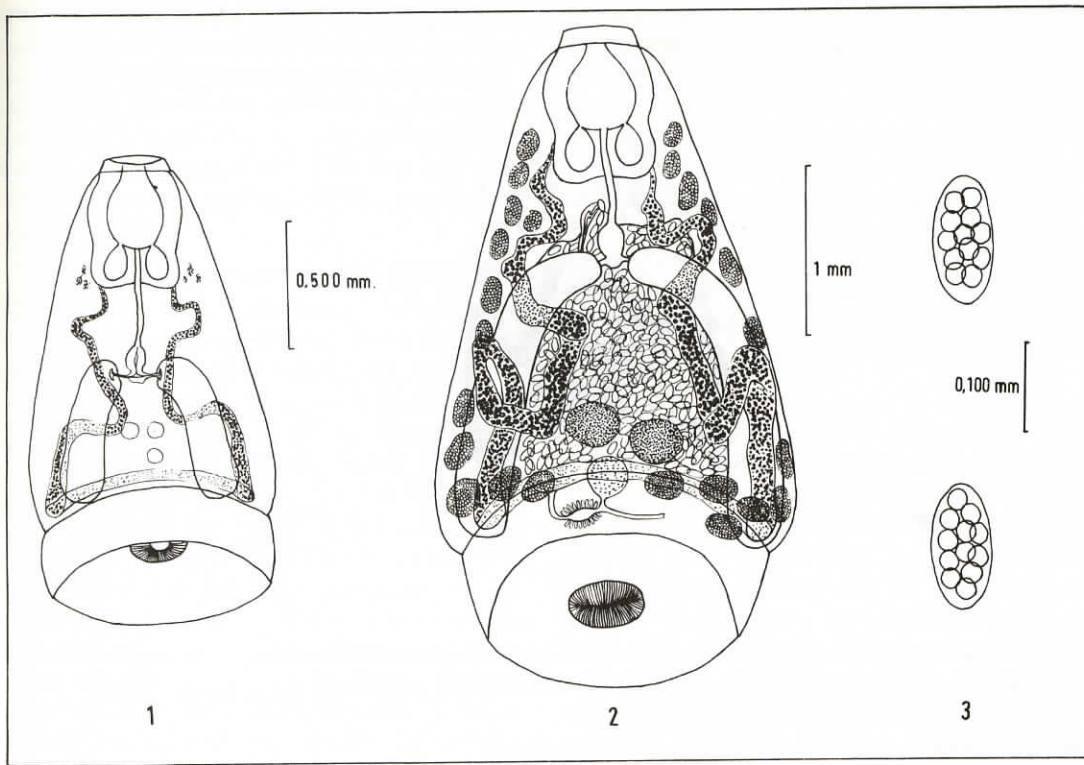


Fig. 1: Young *Opisthodiscus nigrivasis* experimentally obtained on *Rana ridibunda* following Simón Vicente and col. 1974.

Fig. 2: Adult *O. nigrivasis* from experimental infection.

Fig. 3: Ova in uterus.

The intestinal caeca are usually filled with a yellowish-brown substance, which makes their thick walls stand out even more than they otherwise would. In some specimens there occurs a slight asymmetry in the caecal branches.

The thick limbs of the excretory apparatus are the most visible organs of the parasite, most especially those specimens that are not very gravid, and which are neither stained nor fixed. That due to the blackish granules they contain, and to the movement of the grains by the occasional contractions of the excretory tubes. The strong pigmentation of this part of the excretory system becomes weaker, or actually disappears, when the parasites are fixed or stained. These excretory vessels

pass twice over the back of the caeca by the necessary bends, which trajectory we have observed very constantly (Figs. 1, 2, 5).

The large posterior sucker has a wide rim which, when directed downwards, forms a deep cup which has an acentrally-placed accessory sucker. Sometimes the rim of the posterior sucker is lifted up, leaving the central sucker prominent, and the parasite then resembles a spinning top or cone. The small central sucker and its surroundings are reddish of varying intensity, in specimens newly taken from the cloaca of a frog. On the interior surface of the posterior sucker striations and small buds are to be seen (Figs. 1,2,4,6).

The testes are arranged side by side,

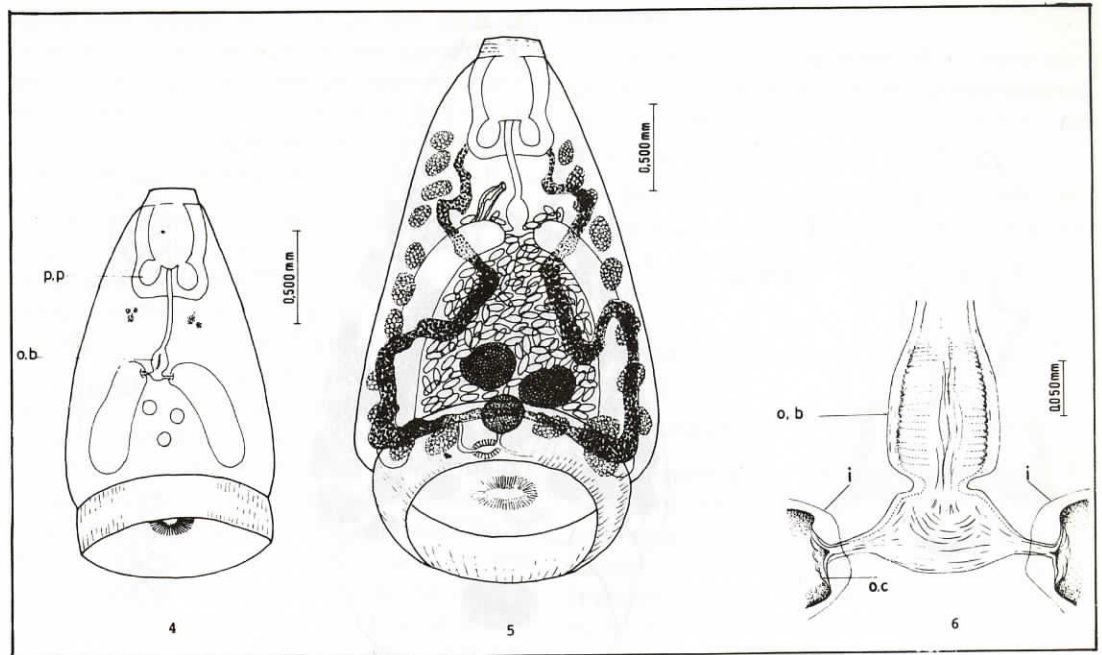


Fig. 4: Immature *O. nigrivasis* from a natural infection. The excretory system has not been represented.

p.p.-Pharyngeal pockets; o.b.-Oesophageal bulb.

Fig. 5: *O. nigrivasis*, adult from natural infected *Rana ridibunda*.

Fig. 6: Posterior region of oesophagus of *O. nigrivasis*: o.b.-Oesophageal bulb; o.c.-Oesophageal connection with the intestinal branches; i.-Intestine.

directly or obliquely, in front of the ovary. They lie near the ventral surface, whereas the ovary lies more dorsally. Beside the ovary, slightly lower down and perpendicular to it are Mehlis' gland and Laurer's canal, which follows a direction contrary to that of the oötype (Figs. 2, 5).

The uterus, being intercaecal, when full of eggs usually reaches beyond the caecal bifurcation forwardly and as far as the ends of the caeca hindwardly. The elliptical eggs (Fig. 3) contain numerous cells within a transparent envelope. Development is more advanced in the eggs which are in the loops of the uterus nearest to the genital pore.

The cirrus-sac is claviform, and relatively short and thin. Inside it there is a slightly undulated seminal canal or vesicle. The cirrus and the end of the uterus open together into the genital atrium, situated

in front of the esophageal bifurcation, between the bulb and the pharyngeal sacs, parallel to and on the right of the median line of the body. Depending on the stage of development of the fluke, the distance between the pore and the ends of the sacs is 0.060-0.227 mm; and up to 0.828 mm between the pore and the anterior extremity of the body. Its internal diameter is about 0.024 mm and it opens ventrally.

The vitellaria are follicular, placed laterally and often extracaecally. They extend from the pharyngeal sacs to the ends of the caeca, and in most flukes they are confluent in the median line posteriorly. There are about 38-45 follicles (Fig. 2).

### Discussion

A study of the displodiscine paramphistomes of León and Salamanca shows

that they possess morphological characteristics of the genus *Opisthodiscus*, and, on acquiring sexual maturity, they retain possession of two testes; and the genital pore lies always anteriorly to the oesophageal bifurcation, between the pharynx and the intestinal caeca, slightly off the median line of the body.

The specific characteristics coincide, as we shall see, with those set out by Odening<sup>12</sup> for *O. nigrivasis*, including the presence of an oesophageal bulb, and also broadly with those described by Dollfus<sup>5</sup> from two specimens from the Eastern Pyrenees, diagnosed by that author as *O. diplodiscoides*, but actually having the same characteristics as the *O. diplodiscoides nigrivasis* of v. Mehely<sup>10</sup>. Neither v. Mehely nor Dollfus mention the presence of an oesophageal bulb, nor is this feature given in Yamaguti's key<sup>14,15</sup> as a diagnostic character of the genus *Opisthodiscus*. Earlier, Cohn<sup>2</sup> did not describe it, nor did Skrjabin (1949) (cit. by Odening, 1959). On the other hand, Nasmark (1939), according to Odening, indicated the presence of the said organ in *Opisthodiscus* and Odening also found it in *O. nigrivasis* from Germany, noting that it is more highly developed in this species than in *Diplodiscus subclavatus*.

So in the differences of development presented by this organ lies one of the most frequent reasons for confusion when it comes to telling apart the genera and species of Diplodiscinae.

After repeated observations made on our material it was possible to specify certain interesting data concerning the arrangement of the oesophageal bulb of *O. nigrivasis*. Its rear part is not inserted in the intestinal branches, with no apparent structural change, as is shown in fig. 5b, by Odening<sup>12</sup>, but in the form explained above. In the morphological and cytochemical analyses, carried out with both optical and electron microscope, on the working of the digestive system of *Megalodiscus tem-*

*peratus* Bogitsh (1972) speaks of the oesophageal bulb of this diplodiscinae as a simple organ, surrounded by eight or ten layers of muscles, in contrast to the two layers observed in the oesophagus. According to this author, the transition between the oesophagus and the caecal region is abrupt and is brought about by a fine septated layer. A similar kind of work, based on *O. nigrivasis*, might establish interesting analogies and differences in the constitution of the oesophageal bulb in this genera.

We find ourselves in agreement with Odening's opinion that the dark "concretions" in the excretory canals of *O. nigrivasis* are neither constant nor of specific value, as their occurrence is of reversible physiological nature. So, in the field of specific description, the *Opisthodiscus* of León and Salamanca cannot be identified with Cohn's description<sup>2</sup> for *O. diplodiscoides*, but rather with Odening's<sup>12</sup> for *O. nigrivasis*, which, in its turn, agrees except in the presence of the bulb, with that of *O. diplodiscoides nigrivasis* given by v. Mehely in 1929<sup>10</sup>. The arguments put forward by Odening for not considering this species as a variety of *O. diplodiscoides* are, in our opinion, sufficient for the adopting of the denomination *O. nigrivasis* (v. Mehely, 1929) Odening 1959, the former being kept as a *species inquirenda* while its existence remains unproven.

A new reference to *Opisthodiscus diplodiscoides* Cohn, 1904, has been made by Matskasi<sup>9</sup>, but its validity can in no way be assured, since this author's work comes down exclusively to a histological study of the neurosecretory cells of the thus-named parasite, not embarking on details of specific identification. Nevertheless, in a drawing of the oesophageal area, which accompanies the work, the bulb is not shown, and its absence, is known, is one of the characteristics used as a basis of generic classification by Yamaguti<sup>14</sup>.

Dispensing with species *O. diplodiscoides*

Table 1

<p><i>Extreme measures (in mm) found in a group of five specimens from Garcirrey (Salamanca) (*).</i></p>	<p><i>Average measures (in mm) found in a group of thirteen full grown specimens from León (*).</i></p>	<p><i>Average measures (in mm) found in a group of eight small specimens from León (*).</i></p>
<p>Length: 2.6 - 5.3</p>	<p>Length: 4.431-4.063</p>	<p>Length: 2.114 - 2.260</p>
<p>Maximum width: 1.2. - 3.8</p>	<p>Maximum width: 2.406 - 2.242</p>	<p>Maximum width: 1.344 - 1.556</p>
<p>Oral sucker: 0.360 - 0.552</p>	<p>Pharynx (diverticles included): 0.980-0.714</p>	<p>Pharynx (diverticles included): 0.481-0.646</p>
<p>Oesophagus (length): 0.400-0.560 x 0.050-0.070</p>	<p>Oesophagus (length): 0.703 - 0.872</p>	<p>Oesophagus (length): 0.421</p>
<p>Pharyngeal diverticles: 0.550 - 0.560</p>	<p>Acetabulum: 1.987 x 1.209-2.361 x 1.449</p>	<p>Acetabulum: 0.959 x 0.751-1.352 x 0.966</p>
<p>Acetabulum: 1.5 - 2.5</p>	<p>Accessory sucker: 0.658 x 0.488-0.751 x 0.626</p>	<p>Accessory sucker: 0.399 x 0.327-0.471 x 0.399</p>
<p>Accessory sucker: 0.360 - 0.610</p>	<p>Ovary: 0.197 - 0.277</p>	<p>Ovary: 0.163 - 0.171</p>
<p>Genital pore: 0.024</p>	<p>Inferior testicle: 0.348 - 0.537</p>	<p>Inferior testicle: 0.217 - 0.250</p>
<p>Ovary: 0.230 - 0.414</p>	<p>Superior testicle: 0.556</p>	<p>Superior testicle: 0.217 - 0.239</p>
<p>Testicles: 0.276 - 0.414</p>	<p>Eggs (in uterus): 0.096 x 0.048-0.092 x 0.047</p>	<p>Eggs (in uterus): 0.089 x 0.044-0.092 x 0.049</p>
<p>Eggs (in uterus): 0.100-0.119 x 0.035-0.050</p>	<p>Oesophageal bulb: 0.150 x 0.134-0.179 x 0.157</p>	<p>Oesophageal bulb: 0.119 x 0.076</p>
<p>(*) Fresh specimens (not fixed)</p>	<p>(*) Fixed specimens</p>	<p>(*) Fixed specimens</p>
	<p>Mehlis' gland: 0.209 x 0.133</p>	<p>Mehlis' gland: 0.209 x 0.133</p>

des, amid the existing doubts, the oesophageal bulb, it would seem, is a common characteristic among all genera of Diplodiscinae of amphibia included in Yamaguti's key<sup>14</sup>. The differences between the two closest ones, *Opisthodiscus* Cohn, 1904 and *Pseudopisthodiscus* Yamaguti, 1958, are so defined that the situation of the vitellaria in *Pseudopisthodiscus americanus*, the only species in the said genus, is lateral, not forming a rear bridge between the caeca; the genital pore lies behind the intestinal bifurcation; and, according to Holl's description<sup>6</sup>, the main excretory ducts pass over the caeca three times, whereas they do so only twice in *Opisthodiscus nigrivasis*.

Considering now the definite-host factor, our findings bear out those of Combes and Gerbaux<sup>3</sup> made studying the characteristics of the helminthic fauna of amphibia in the Eastern Pyrenees. From among the conclusions drawn by these authors from their biogeographical analysis we should like to emphasize the fact proven by them (after comparing the helminth fauna of *Rana ridibunda perezi* with that of *R. temporaria* in distribution zones where both frogs live side by side) that there are certain parasites which are peculiar, or have a high degree of specificity, to only one of the two species of amphibia. This is the case of *O. nigrivasis*, found by them solely in *R. ridibunda perezi*, with a high frequency.

Bearing in mind the results of the already cited French authors, and our own, it is quite possible that *O. nigrivasis* has a wide distribution in the whole of Spain, following that of its favorite host, *R. ridibunda*, which, according to data collected by Nadal and others<sup>11</sup>, can live at an altitude of 700–800 meters in the plain and 1.000–1.100 meters in the Pyrenees in Catalonia, at Isobol.

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