Examination of the Validity and Systematic Position of Some Paramphistomids of Indian Ruminants

Dr. Ottó SEY
Department of Zoology, College of Education, Pécs


ABSTRACT. The author examined the validity and the occurrence of a group of species of amphistomes in India and came to the conclusion that Paramphistomum epiclitum Fischoeder, 1904 (synonyms: Cotylophoron indicum Stiles et Goldberger, 1910; P. thapari Price et McIntosh, 1953; Srivastavaia indica Singh, 1970), P. gracile Fischoeder, 1901 (synonyms: P. indicum Stiles et Goldberger, 1910; P. cervi of several Indian authors; P. ichikawai of Gupta and Nakhasi, 1977) and Orthocoe- lium gigantopharynx (Sقد, Kuntz, Anteson et Webster, 1964) (synonym: P. gotoi, of Tandon, 1955) are valid.

The examination of amphistomes of Indian ruminants has been made for more than a century, during which several papers and general works have dealt systematically with the problems of these helminths. Opinions, however, vary concerning the validity and occurrence of certain species in India.

The reason of these different opinions may lie in the absence of re-examination by newer methods of species, as the ones described earlier were mainly done on the basis of gross-morphological and anatomical features. Therefore recent descriptions of species by helminthologists of India have been done without clarifying the actual systematic position of some obscure species. Misinterpretation of features and nomenclature in case of histo-morphologically examined species led also to erroneous conclusions.

These circumstances did not only increase difficulties in identification but also disturbed the interpretation of the characteristic features of the amphistome fauna of East India.

The present paper comprises species Cotylophoron indicum Stiles et Goldberger, 1910; Paramphistomum cervi (Zeder, 1790); P. epiclitum Fischoeder, 1904; P. gotoi Fukui, 1922; P. ichikawai Fukui, 1922; P. thapari Price et McIntosh, 1953 and Srivastavaia indica Singh, 1970. This group of species includes a certain part of amphistomes which were often discussed.

Materials and Methods

In 1977/78 a collection of amphistomes was obtained by the author in different Indian slaughter houses (Allgahr, Bareilly, Delhi, Lucknow, Calcutta), research institutes (Indian Veterinary Research Institute, Izatnagar; Zoological Survey of India, Calcutta) and universities (Allahabad, Calcutta, Chandigarh, Gujarat, Guntur, Luthiana and Mathura). Besides these it was possible to examine several toto mounts and some median sagittal sections of amphistomes deposited in the Zoological Survey of India, Calcutta and the Zoological Department, Panjub University, Chandigarh.

Flukes removed from ruminants were fixed and preserved in 70 per cent alcohol or 2-3 per cent formalin. Median sagittal sections and toto mounts were prepared by the usual methods. About one hundred sections and some preparations of the type specimens of C. indicum,
Results and Discussion

After the examination of the species which constitute the scope of this paper, Paramphistomum epiclitum Fischoeder, 1904; Paramphistomum gracile Fischoeder, 1901 and Orthocoelium gigantopharynx (Schad, Kunz, Anteson et Webster, 1964) proved to be valid and the status of the rest will be discussed under the relevant species.

Paramphistomum epiclitum Fischoeder, 1904

This species was described from a South-Western Asian (Saigon) collection by FISCH-OEDER in 1904. In East India it was found for the first time by GUPTA (1963) in Pakistan and GUPTA and NAKHASI (1977) in India. In the course of the collection of the present test material, made along the Hindustanian Lowland, this species was found to be one of the commonest rumen flukes. C. indicum, P. thapari and S. indica are regarded as its synonyms.

From the point of view of justification of synonyms of P. epiclitum, indicated by the author is examination of validity and systematic position of C. indicum, seems to be essentially important.

STILES and GOLDBERGER (1910) based on the description of this species six immature specimens, and their histo-morphology was studied on cross sections. In the diagnosis of this species presented by STILES and GOLDBERGER (1910) specific features were emphasized: the absence of oesophageal thickening, less sharply delimited genital sucker (Fig. 1), much smaller genital atrium, post-bifurcal genital opening, in contrast to the then known other species of the genus, Cotylophoron cotylophorum (Fischoeder, 1901). There was no indication of the muscle elements of the acetabulum in the present sense. MAPLESTONE (1923) and FUKUI (1929) regarded it to be a synonym of C. cotylophoron. NÄSMARK (1937) himself did not examine the original type specimens but he was of the opinion that it is a valid species. His examinations were based on LOOSS' preparations collected in Sudan.

Having examined C. indicum of NÄSMARK (1937) it was obvious that it is not identical with C. indicum of STILES and GOLDBERGER (1910) (Fig. 2). It was PRICE and McINTOSH (1953) who examined median sagittal sections of C. indicum for the first time and they found that the acetabulum has d.e. 2 muscle layer (Fig. 3) and therefore they transferred this species to the genus Paramphistomum, named P. thapari Price et McIntosh, 1953. MUKHERJEE (1968) while describing the life cycle of C. indicum, has also found d.e. 2 muscle layer in the acetabulum but he was of the opinion that the presence of the genital sucker has greater importance than that of the d.e. 2 muscle layer and therefore he replaced the species to the genus Cotylophoron. BAER (1950) has also accepted validity and systematic position of C. indicum. GUPTA and GUPTA (1977) and GUPTA and NAKHASI (1977) have confirmed again the validity of this species.

Examination of a median sagittal and a cross sections of C. indicum from STILES and GOLDBERGER's (1910) original collection it was found that d.e. 2 muscle layer was present in its acetabulum, as it was established earlier by PRICE and McIntosh (1953). Otherwise, specimens, having such a structure of acetabulum, were found in a great number in our collection as well.

Fig. 1: Cross section of the genital opening of Cotylophoron indicum.
Fig. 2: Median sagittal section of C. indicum of Näsmark.
Fig. 3: Median sagittal section of acetabulum (dorsal half) of C. indicum.
Figs. 4-8: Median sagittal sections of genital openings (4 = Cotylophoron indicum; 5 = Paramphistomum epiclitum; 6 = P. gracile; 7 = P. cervi; 8 = P. gotoi, of Tandon).
Fig. 9: Median sagittal section of pharynx of P. gotoi, of Tandon (Figs. 1-2, 4-7 were taken with the same magnification).
As the structure of the genital sucker has always been an important argument as a generic feature therefore it is necessary to examine whether *C. indicum* has a *Cotylophoron* type of genital opening.

The *Cotylophoron* type of genital opening is characterized by the presence of a great number of radial muscle elements (Fig. 4) and, at the same time, by a fairly poor longitudinal musculature. In case of the genital opening of *C. indicum* the radial musculature is weak but the longitudinal one is strongly developed (Figs. 1, 5). Measurements of the genital opening of the species of the genus *Cotylophoron* are usually twice as bigger (Figs. 2, 4) than that of *C. indicum* (Figs. 1, 5). The genital opening clearly delineates from the surrounding tissues of the species *Cotylophoron*, in case of *C. indicum* it does not. Pars prostatica strongly developed in *C. indicum* (prostatic cells are situated in several rows /Figs. 1, 5/), while in *Cotylophoron spp.*, it is weak or moderately developed (Figs. 2, 4). Finally, there is as difference in the position of genital pore of the species of *C. indicum* and of *Cotylophoron spp.*

Accordingly, the structure of the genital opening of *C. indicum* reminds me rather of the *Epiclitum* than that of the *Cotylophoron* type and I presume that the differences mentioned above support this belief.

Beside the differences that can be discovered in the structure of the genital opening, the presence of a d.e. 2 muscle layer in the acetabulum also suggests that *C. indicum* should be excluded from the genus *Cotylophoron* (Price and McIntosh, 1953). Namely, the structure of the acetabulum of the *Cotylophoron* type seems to be constant for there are no literary data to report the presence of a d.e. 2 muscle layer.

In 1970 SINGH described a new genus and species, *Srivastavaia indica* whose validity should be examined in the light of the knowledge of histo-morphological details of *C. indicum*. The structure of the acetabulum of *S. indica* was seemingly interpreted erroneously because d.e. 2 muscle layer can be found in it according to the figure attached to the text of SINGH's paper. Namely, in case when a d.e. 2 muscle layer is absent the number of muscle units in d.e. 1 and v.e. muscle layers is usually the same. Accordingly, *S. indica* has *Paramphistomum* and not *Streptocoelium* type of acetabulum.

Due to a similarity of structure and measurement as well as to the presence of a well developed pars prostatica, all of which were expressed in connection with the genital opening of *C. indicum*, characterize *S. indica* as well.

SINGH (1970) himself made mention of the fact that *S. indica* showed some resemblances to *Paramphistomum thapari* (= *C. indicum*) especially in structure of the pharynx, genital opening, acetabulum and position of the excretory and Laurer's canals. Differences were emphasized, however, between these species in the number of muscle elements of d.e. 2 muscle layer and the development of sphincter papillae.

The structure of the acetabulum of *S. indica* was discussed above to point out that it is of *Paramphistomum* type that is the same as *P. thapari* (= *C. indicum*). The presence of a sphincter papillae mentioned by SINGH* (1970) in the genital opening seems to be a misinterpretation of a given histological structure because such a sphincter is not found in the genital atrium of *P. thapari* (= *C. indicum*) that the structure of the genital atrium of *S. indica* was compared with.

Laurer's and excretory canals, according to SINGH (1970) did not cross each other (p. 124) but there seems to be a controversy about two points. It was mentioned (p. 125) that these two canals show some resemblance in *S. indica* and *P. thapari* (= *C. indicum*) and later (p. 126) he wrote "Laurer's canal opens posterior to the excretory pore without crossing the excretory duct". On the one hand if there exists a resemblance in the position of these two canals in the species in question, then there is a crossing because in *P. thapari* (= *C. indicum*) these canals cross each other and on the other it is generally accepted that crossing exists not only in case when Laurer's canal passes through the excretory duct only but also when a crossing develops in the region of the excretory bladder. Accordingly, excretory and Laurer's canals cross each other in *S. indica*.

On the basis of the similarities of the histo-morphological features of *C. indicum* (= *P. thapari*) and *S. indica*, pronounced above I regard these species to be synonymous with *P. epiclitum*.

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*SINGH* did not examine the type specimens of *P. thapari* (= *C. indicum*) he had compared *S. indica* with an amphistome designated by himself to *P. thapari*.
Systematic position of species described under the name C. indicum, recovered in countries outside of East India (DINNIK et al. 1963, Uganda; DROŻDZ and MALCZEWSKI, 1987, Vietnam; KOTRLA and PROKOPIĆ, 1973, Cuba) is questionable and their re-examination is desirable.

**Paramphistomum gracile** Fischoeder, 1901

In their paper STILES and GOLDBERGER (1910) described another species, *Paramphistomum indicum* from Indian ruminants. In the diagnosis it was mentioned that it shows a close resemblance to *P. gracile* and they listed some anatomical features (length of oesophagus, position of reproductive organs, pore of Laurer’s canal) by which *P. gracile* and *P. indicum* can be differentiated. These features, however, are subject to more or less individual variations, depending on the circumstances of the prefixative treatment. I think NASMARK (1937) was right in that he regarded *P. indicum* to be a synonym of *P. gracile*. Before this *P. indicum* was lumped by MAPLESTONE (1923), together with other species into synonyms of *P. cervi*. It seems to be probable that the acceptance of MAPLESTONE’s opinion by Indian helminthologists (BHALERAO, 1935, THAPAR, 1956, MUKHERJEE and CHAUHAN, 1965) was the reason of the generally accepted belief that *P. cervi* is a widely distributed species in India. Recently, however, MUKHERJEE and CHAUHAN (1972) were of the opinion that *P. cervi* is not so common, if at all found, as other authors described it.

After histo-morphological examination of both the numerous samples collected by the author and samples presented by Indian authorities (MATHURA, IZATNAGAR, LUTHIANA), labelled as *P. cervi* proved to be either *P. epiclitum* (Fig. 5) or in majority *P. gracile* (Fig. 6) and *P. cervi* were not found in either of the samples. Otherwise, *P. cervi* is easily distinguishable from the former two species in question by the structure of their pharynx. *P. epiclitum* and *P. gracile* have a pharynx of Paramphistomum; *P. cervi* of the Liorchis type. Agreeing with MUKHERJEE and CHAUHAN (1972) the author is of the opinion that *P. cervi* is a very rare species or, what seems a more feasible answer to the problem, it is not found in India.

**Paramphistomum ichikawai** of GUPTA and NAKHASI (1977) should also be considered as a synonym of *P. gracile* because their characteristic features (structure of the genital atrium, and acetabulum) were not observed either on the slide (single, immature median sagittal sections) examined by the author or in figures attached to the text of the paper. *P. ichikawai* has a genital atrium of Ichikawai type which is characteristic only of its own. In the d. e. 2 muscle layer of its acetabulum there are 6-18 muscle units, loosely situated and both their arrangement and number are fairly constant (SEY, 1974). In GUPTA’s preparation the number of muscle units was 29.

*P. epiclitum* and *P. gracile* are species with a close resemblance both in gross-morphology and anatomical features. Pharynx and acetabulum of both of them are the same types without a specific structure. The most important differences can be found in the structure of the genital opening. It is Epiclitum in *P. epiclitum* (Fig. 5) and Gracile in *P. gracile* (Fig. 6). According to NASMARK (1937) the latter is characterized by the absence of both radial and circular musculature. Having examined several slides of some other species (*P. cervi*, *P. gotoi*) with this type of genital opening, it was found that weakly developed radial muscle elements, however, occurred and what is more, these muscle elements were also found in slide of *P. cervi* deposited in NASMARK’s (1937) collection (Fig. 7). Therefore the differences between these two types of genital openings are rather the bigger size of the genital opening of *P. epiclitum*, more strongly developed longitudinal muscles and the better developed pars prostatica, as opposed to the genital opening of the Gracile type, than the presence or absence of radial musculature in the latter type.

*P. gracile*, otherwise, was described for the first time by FISCHOEDER in 1901 from a collection derived from Ceylon.

**Orthocoelium giganthopharynx** (Schad, Kunz, Anteson et Webster, 1964)

TANDON (1955) described *Paramphistomum gotoi* Fukui, 1922 as a common parasite of bulalo in Lucknow. SCHAD et al. (1964) examined amphistomes of domesticated ruminants in North Borneo and they convincingly pointed out that *P. gotoi* of TANDON (1955) is identical with *O. giganthopharynx*. An examination of TANDON’s original slides or "P. gotoi"
(deposited in Zoological Survey of India, Calcutta) has conformed SCHAD et al.'s (1964) conclusions as to histological details of the muscular organs (Figs. 8, 9) of this species.

Finally, a histo-morphological note: in TANDON’s description there was mention of a so-called "muscular pad" at the bottom of the pharynx. It is not muscle in nature but a group of cells closely packed.

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Dr. SEY, O.
Department of Zoology
College of Education
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