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Terminology of the sucker-like organs of the scolex of trypanorhynch cestodes

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Abstract

The literature associated with descriptions and definitions of the sucker-like attachment organs in trypanorhynchs, termed either bothria or bothridia, is reviewed. There are descriptions of 14 trypanorhynch species representing 10 families. In none of these trypanorhynchs was a membrane separating the attachment organ from the scolex parenchyma described, one of the definitions used to distinguish bothria from bothridia. Transmission electron microscopy of the bothria of the trypanorhynch species *Nybelinia queenslandensis* Beveridge & Jones, 1998 (Tentaculariidae) and *Otobothrium mugilis* Hiscock, 1954 (Otobothriidae) also failed to show any membranous structure separating the surface of the attachment organ from the cestode parenchyma. The sucker-like attachment organs of trypanorhynchan cestodes appear, therefore, to be bothria rather than bothridia. As a result, changes in the terminology of related features of the scolex are proposed here. Henceforth, the pars bothridialis should be referred to as the pars bothrialis and the bothridial pits should be referred to bothrial pits.

Introduction

The sucker-like organs of attachment borne on the scolex of cestodes belonging to the order Trypanorhyncha Diesing, 1863 have generally been described as ‘bothridia’ (Pintner, 1931, p. 804; Dollfus, 1942, p. 16; Wardle & McLeod, pp. 8, 288), with individual trypanorhynch families characterised by having either two or four bothridia (Dollfus, 1942) at the apex of the scolex. Joyeux & Baer (1961, p. 354) referred to these structures as ‘pseudobothridia’ without explanation of the use of the term, while Hyman (1951, p. 364) referred to them as ‘bothria’ because of their ‘weak muscularity’. More recently, Caira et al. (1999) have also applied the term ‘bothria’ to the attachment organs of trypanorhynch cestodes based on their histological structure, as defined by Fuhrmann (1931), who distinguished bothria from bothridia primarily by the presence of a delimiting membrane[1], which separates the bothridium from the scolex parenchyma, and its absence in bothria.

Pintner (1931) used a somewhat different definition of bothridium, considering any adhesive organ that was well defined externally as a bothridium. In his view, the paired muscular adhesive structures of both tetraphyllidean and trypanorhynchan cestodes constituted bothridia, while those of pseudophyllidean cestodes were bothria. Dollfus (1942, p. 16) provided a de-

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[1] It is recognised that the ultrastructural definition of a membrane and that used here will differ. Conn (1993) defined the structure underlying platyhelminth suckers as an interstitial matrix, a form of extracellular matrix. The interstitial matrix can be densely fibrous when viewed by electron microscopy. While this structure may not fulfil the modern cell biological criterion for membrane, the dictionary definition of a membrane is ‘any thin sheet of material’, and will suffice.

tailed comparison of the structure of bothria, bothridia and acetabula (=suckers), noting that the primary differences between bothria and bothridia were the separation of the internal aspect of the bothridium from the scolex parenchyma by a membrane in the bothridium and by the presence of a 'radial musculature', i.e. a series of muscles arranged perpendicular to the adhesive surface. According to Dollfus (1942), bothria lack a radial musculature, but this view is contradicted by descriptions by Rees (1941b, 1944) of the attachment structures of *Aporhynchus norvegicum* (Olsson, 1868) Nybelin, 1918 and *Grillotia acanthoscolex* Rees, 1944, which show prominent radial muscles in the adhesive structures. Hyman (1951), by contrast, distinguished bothridia from bothria primarily on the degree of development of their musculature. Joyeux & Baer (1961) contended that the difference between the two structures was the presence or absence of intrinsic musculature. This distinction, however, depends entirely on the presence of a delimiting membrane.

Distinction of the attachment structures based on muscle arrangement seems ill-advised in our opinion, for the primary distinctions are based on the degree of development of these different muscles and not necessarily on their presence or absence. The better definition remains the nature of the delimiting membrane of the attachment device, following Fuhrmann (1931), which appears to be clearly evident in bothridia and acetabula but entirely absent in bothria.

Pintner (1931) and Dollfus (1946) noted that, using the definition of Fuhrmann (1931), a histological examination was required to determine whether an individual cestode possessed bothria or bothridia. These authors consequently adopted the distinction of bothrium from bothridium suggested by Pintner (1931), that a bothridium is an adhesive organ the external surface of which is clearly delimited externally, thereby avoiding the necessity for transverse serial sections of the scolex of each species of cestode to determine whether bothridia or bothria were present. This pragmatic definition has subsequently been adopted in most reviews of trypanorhynch cestodes (eg.Wardle & McLeod, 1952; Campbell & Beveridge, 1994; Palm, 1997) with the notable exception of Hyman (1951). Traditionally, acceptable terminology for the adhesive organs of pseudophyllidean cestodes has been bothria, those of tetraphyllidean are bothridia, those of lecanicephalideans are bothridia or acetabula, and those of cyclophyllideans and proteocephalideans are acetabula (Hyman, 1951, p. 315; Wardle & McLeod, 1952, pp. 229, 559; Jensen et al., 2001). Thus the nomenclature of attachment organs is disputed only for the Trypanorhyncha and Diphyllidea. The argument over nomenclature for attachment organs of diphyllideans has been settled recently by Failex et al. (2001) in favour of bothria.

The problem of the differing definitions of bothria and bothridia exposed by Caira et al. (1999, p. 102), as it relates to the Trypanorhyncha, is significant when scoring morphological characters for phylogenetic analysis, but can potentially be resolved by reviewing the current state of knowledge of the histology of the attachment organs of these cestodes. If sufficient numbers of families of trypanorhynch cestodes have been examined and the sucker-like attachment organs found in them are bothria rather than bothridia, then there is a strong argument for changing the currently accepted terminology.

There are numerous light microscopical studies of the anatomy of trypanorhynch cestodes which include, incidentally, observations on the histology of the scolex and hence of the bothridia. An attempt is made here to catalogue such studies and to provide novel ultrastructural observations based on species from different subfamilies. From these observations, it should be possible to draw a general conclusion as to the nomenclature appropriate for the attachment organs of trypanorhynch cestodes.

Materials and methods

All literature available to the authors was examined for details of the scolex anatomy of trypanorhynch cestodes. Such descriptions are treated below by family, using the classification of Campbell & Beveridge (1994). In each case, the observations made are classified as being part of detailed histological studies or simple line drawings from taxonomic studies.

Transmission electron micrographs from the studies of Jones & Beveridge (1998) on *Nybelinia queenslandensis* Jones & Beveridge, 1998 and Jones (2000) on *Otobothrium mugilis* Hiscock, 1954 were also examined and the ultrastructural features of the bothridial region described for each species. Methods of preparation of the specimens have been described in the relevant papers cited herein.

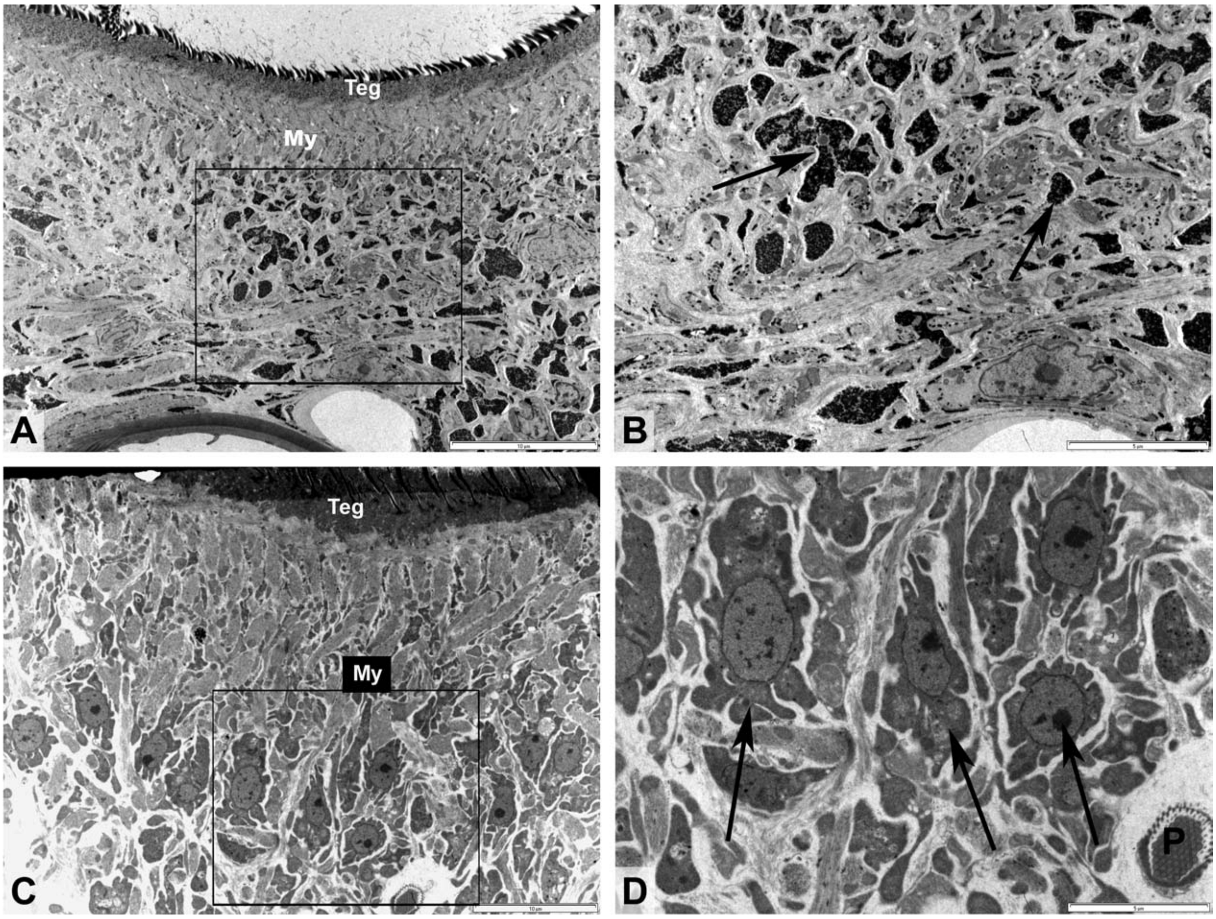

Figure 1. A,B. *Otobothrium mugilis*, transmission electron micrographs of the bothridium (B is an enlargement of A). The bothridial tissue merges imperceptibly with surrounding parenchymal tissue. The other margin of the bothridial tissue is apparent as a discontinuous band of insunken tegument cell bodies (arrows). C,D. *Nybelinia queenslandensis*, transmission electron micrographs of the bothridium (D is an enlargement of C). Glycogen-rich cells (arrowed) are present at the outer margin of the bothridium, but no delimiting membrane or extracellular matrix separates bothridial tissues from surrounding parenchyma. *Abbreviations*: My, muscles; Teg, tegument; P, protonephridial cell. *Scale-bars*: A,C, 10 μm; B,D, 5 μm.

Results

Descriptions of the scolex histology of trypanorhynch cestodes are presented on a family basis. Families are considered below in alphabetical order. Within families, taxa are presented in chronological order of the descriptions.

Family Eutetrarhynchidae Guiart, 1927

Pintner (1880) provided a detailed account of the histology of the scolex of *Eutetrarhynchus ruficollis* (Eysenhardt, 1829) Pintner, 1913, including an illustration of the attachment organs (plate 4, fig.1). In this species, the bothridia are not separated from the scolex parenchyma by a delimiting membrane.

Family Gilquiniidae Dollfus, 1942

Both Nybelin (1918, Figures 5,6) and Rees (1941b, figures 2–5) provided relatively detailed accounts of the bothridia of *Aporhynchus norvegicus* (as *Tetrabothrium norvegicum* in Nybelin, 1918), the former as light micrographs and the latter as detailed line drawings, noting that there was no separation of the bothridium from the parenchyma of the scolex other than a layer of circular muscles; no membranous delimitation of the bothridium was noted. Similarly, illustrations of the scolex of *Gilquinia squali* (Fabricius, 1794) Guiart, 1927 presented by Mackenzie (1965, figure 10) show no division between bothridia and scolex parenchyma.

Family Grillotiidae Dollfus, 1969

The study of the scolex of *Grillotia erinaceus* (van Beneden, 1858) Guiart, 1927 by Johnstone (1912, figure 4) indicated no separation of bothridia from scolex by a membranous structure, based on the detailed drawings of its histology. Likewise, Rees (1944, figure 6) described the musculature of *G. acanthoscolex* Rees, 1944 in considerable detail, including that of the bothridia. She noted radial muscles present, particularly in the lateral aspects of the bothridia, but no membranous division between the bothridium and the scolex parenchyma. The plerocercus of *G. heptanchi* (Vaullegeard, 1899) Dollfus, 1942 was described by Rees (1950), who noted that the muscles of the scolex extend into the bothridia, forming an ill-defined internal border to the bothridia. No delimiting membrane was observed.

Family Hepatoxylidae Dollfus, 1940

Among the most detailed contemporary accounts of the anatomy of the scoleces of trypanorhynch cestodes is that of Rees (1941a) on the histology of the bothridia of *Hepatoxylon trichiuri* Holten, 1802 (as *Dibothriorhynchus grossus* (Rudolphi, 1819) (Rees, 1941a, figures 22, 30). Her study indicated that the bothridia are not separated from the scolex parenchyma.

Family Lacistorhynchidae Guiart, 1927

The histology of *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931 was studied in detail by Rees (1988, figures 3B, 5C,D), who found no separation between bothridia and scolex parenchyma in this species.

Family Mixodigmatidae Dailey & Vogelbein, 1982

Caira et al. (1999, figure 58) published micrographs of the scolex of *Mixodigma leptaleum* Dailey & Vogelbein, 1982, showing the lack of any division between the bothridium and the scolex parenchyma.

Family Mustelicolidae Dollfus, 1969

In the description of *Bombycirhynchus sphyraenaicum* (Pintner, 1930) Pintner, 1931, a line drawing of a transverse section of the scolex included (Pintner, 1931 figure 27). Although not detailed, there is no indication of separation of the bothridia from the scolex parenchyma. Pintner's sections of *B. sphyraenaicum* (NHMV 2094) have been re-examined by the authors, and his observations confirmed.

Family Otobothriidae Dollfus, 1942

Pintner (1934, figure 8) illustrated the bothridia of *Poecilancistrium caryophyllum* (Diesing, 1850) Dollfus, 1929 (as *Tetrarhynchus caryophyllum*) as seen in a transverse section of the scolex. Although his figure is a relatively simple line drawing, there is no obvious separation between the bothridium and the scolex parenchyma. Jones (2000) described the ultrastructure of the scolex of *Otobothrium mugilis* (p. 30, figure 7) but did not address the structure of the bothridia. Nonetheless, his figure 7 illustrated a section through a bothridium without any evidence of a distinction between the bothridium and the underlying parenchyma. A re-examination of transverse sections of the material used by Jones (2000) revealed that the tegument of the bothridium is underlined by a thick muscle layer with little intervening extracellular matrix (Figure 1A,B). The muscle layer consists of myofibrils with two distinct orientations. The layer immediately beneath the tegument is designated longitudinal, as the myofibrils are cut almost in transverse section, indicating that the muscles spiral around the periphery of the scolex. The second, deeper layer appears to be radial, originating from a central point of the bothridium and running to the base of the tegument. Beneath this muscle layer, there lies a region of glycogen-rich cell bodies, consisting of tegumentary cytons and myocytons and their cellular processes, surrounded by an extensive extracellular matrix. No distinct partition separated the bothridial mass from the surrounding parenchyma.

Family Tentaculariidae Poche, 1926

In simple line drawings of transverse sections of the scolex of *Nybelinia syngenes* (Pintner, 1928) Dollfus, 1930 (as *Stenobothrium syngenes*), Pintner (1930, figures 9–10) illustrated bothridia with no separation from the scolex. Recently, Jones & Beveridge (1998, figure 15) have provided photomicrographs of transverse histological sections through the bothridia of *Nybelinia queenslandensis*, also showing no separation of bothridia from scolex parenchyma in this genus. Electron microscopy of transverse sections of the scolex of *N. queenslandensis* reveals that the tegument is lined by blade-like and elongate filamentous microtriches. The underlying parenchyma is more richly cellular than that underlying non-bothridial surfaces and the constituent cells include muscles, tegumentary cytons and possible neurosecretory cells (figure 1C,D). The tegumentary cytons form a band at the proximal extremity of this cellular mass and thus appear to mark the parenchymal margin of the bothridium. The musculature consists of an inner layer of longitudinal muscles and an outer region of slightly more diffuse radial muscles. The general parenchyma of the scolex is characterised by an extensive fibrillar extracellular matrix with little intervening cellular material.

Family Tetrarhynchobothriidae Dollfus, 1969

Pintner (1931, figure 26) presented line drawings of transverse sections through the scolex of *Tetrarhynchobothrium striatum* Wagener, 1854 (as *Tetrarhynchus striatus*) showing no separation of bothridia from the scolex parenchyma.

No data are apparently available for the families Dasyrhynchidae, Hornelliellidae, Molicolidae, Paranybeliniidae, Pterobothriidae, Rhinoptericolidae, Rhopalothylacidae, Shirleyrhynchidae or Sphyriocephalidae.

Discussion

The information available on the structure of sucker-like attachment organs, so-called in trypanorhynchs, is based on 14 species from 10 families. The quality of the information varies ranging from very simple line drawings of histological sections to extremely detailed drawings and to light micrographs. There are no published electron microscopical studies devoted to these structures and, consequently, observations on the fine structure of *O. mugilis* and *N. queenslandensis* are provided above to support the observations made by light microscopy. In all of the species reported, there is no evidence of any form of membranous structure separating the bothridium from the scolex parenchyma. In *A. norvegicus,* a layer of circular muscles separates the two, while in *N. queenslandensis*, the border is marked by a discontinuous layer of tegumentary cytons. However, in neither species is there a membranous structure. Dollfus (1942) included as a distinctive character of bothria, a series of radial muscles. However, Rees (1941a,b, 1944, 1988) identified such a series of muscles in several of the trypanorhynch species that she studied, thus indicating that this proposed distinguishing character may not be unique. Despite this minor difference, if the principal criterion of a bothridium is the presence of a distinctive membrane between it and the scolex parenchyma, then there is no evidence of any such structure in the species studied to date. The absence of a delimiting membrane in the range of families examined makes it doubtful that such a membrane will be found in the remaining families. If this inference is correct, then we have no evidence for continuing to refer to the attachment organs of trypanorhynchs as bothridia, and they should henceforth be called 'bothria'. The consequences of changing the terminology is that the term '*pars bothridialis scolecis'* used to describe the anterior part of the scolex (Dollfus, 1942) becomes '*pars bothrialis scolecis'* and the term 'bothridial pits' becomes 'bothrial pits'.

One point of potential controversy surrounds the attachment structures of *A. norvegicus*. In this species, the bothria are bound externally by a limiting layer of transverse muscle fibres rather than an extracellular matrix. Since the tissues that form muscles and lay down extracellular matrices in cestodes are the same (Conn, 1993), it might be argued that *Aporhynchus* spp. possess bothridia. This might suggest that bothridia-like structures have emerged independently in the evolution of the trypanorhynchs or, indeed, that *Aporhynchus* spp. are not trypanorhynchs.

The apparent lack of ultrastructural studies of the membranous boundaries of bothridia and acetabula suggests that the currently accepted terms should be treated with caution until adequate studies of the fine structure of these adhesive organs have been undertaken.

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