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An alternative classification of trypanorhynch cestodes considering the tentacular armature as being of limited importance

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Abstract

Based on a light- and scanning electron microscopical study of scolex morphology, tentacles and surface structures of 31 trypanorhynch species, an alternative classification of the trypanorhynch cestodes, adults, plerocerci and postlarvae, is presented. The arrangement of the tentacular armature is no longer used as a distinguishing feature for four different superfamilies. Instead, the presence or absence of ciliated pits and prebulbular organs is used to define three superfamilies: Tentacularioidea Poche, 1926; Otobothrioidea Dollfus, 1942; and Eutetrarhynchoidea Guiart, 1927. A total of 12 families are defined by the characters: the presence/absence of blastocysts, the number of bothridia and the reduction of the rhyncheal apparatus, together with a new character, complete rows of tentacular hooks (homeoacanth and heteroacanth typica) *versus* rows of hooks partly reduced (heteroacanth atypica and poeciloacanth). Of the 19 families previously accepted, 10 are retained (Eutetrarhynchidae, Gilquiniidae, Lacistorhynchidae, Mixodigmatidae, Otobothriidae, Paranybeliniidae, Pterobothriidae, Shirleyrhynchidae, Sphyriocephalidae and Tentaculariidae, all *sensu nov*.); one family is reinstated (i.e. Aporhynchidae Poche, 1926 *sensu nov.*) and a new one is added (i.e. Pseudotobothriidae n. fam.). Advantages of this alternative classification of trypanorhynch cestodes are: (i) the resolution of incongruities and questions caused by the use of the tentacular armature to distinguish superfamilies; (ii) the criteria for the establishment of higher taxa, superfamilies and families are clearly defined; (iii) with the findings of new species with different character combinations, this system can be enlarged up to 4 superfamilies and 48 families without loosing its stability; and (iv) all existing genera are easily re-assigned to the superfamilies and families.

Introduction

Trypanorhynch cestodes are characterised by the possession of a scolex bearing four eversible tentacles armed with hooks and two or four bothridia, enabling these cestodes to move along and attach to the digestive tract of their final hosts which are elasmobranchs. In their life-cycles, these cestodes use invertebrates as first intermediate hosts, with teleosts and some invertebrates as second intermediate or paratenic hosts. The presence of the metacestodes (postlarvae and plerocerci) in the musculature of fishes decrease the commercial value of affected stocks (Deardorff et al., 1984).

Despite their world-wide distribution and importance for commercial fisheries, trypanorhynch cestodes are still a relatively poorly studied group. One reason for this could be their classification which complicates identification of many of the c. 200–250 generally accepted species. Wardle & McLeod (1952) stated (and were recently quoted by Campbell & Beveridge, 1994) that, taxonomically, the Trypanorhyncha are complex and considered to be the most chaotic and confused of the tapeworm groups. Carvajal & Campbell (1975) saw the general problems within trypanorhynch classification as follows: “The problems of defining natural affinities of trypanorhynchs are perplexing because of our grossly insufficient knowledge concerning life histories, larval development, host specificity, etc., and because the significance of morphometric characters must be weighed by each investigator. [. . .] As more species are discovered, it appears that the current method of trypanorhynch classification is becoming increasingly unstable because of the variability inherent in organisms”. Furthermore, within existing trypanorhynch classifications, some characters are used as major distinguishing features for different taxa at different systematic levels at one and the same time. There are only a few specific character combinations which are characteristic for a single taxon.

Campbell & Beveridge (1994) published a new classification of trypanorhynch cestodes, in which the tentacular armature of the postlarvae, plerocerci and adults was used as a major character for four superfamilies: Homeacanthoidea Dollfus, 1942, Heteracanthoidea Dollfus, 1942, Otobothrioidea Dollfus, 1942 and Poecilacanthoidea Dollfus, 1942. Additionally, in their identification key, Campbell & Beveridge (1994) used bothridial form, tentacle length, position of the pars bothridialis relative to that of the pars bulbosa, uterus morphology and the occurrence of a blastocyst (these precede the tentacular armature in the key), leading the user to the different superfamilies. However, this system has grouped some families together that share similar tentacle arrangements, but have few other morphological similarities in common.

During a study of trypanorhynchs from all four superfamilies, two species with a new character combination [*Poeciloacanthum oweni* Palm, 1995 and *Pseudotobothrium dipsacum* (Linton, 1897)], ciliated pits on the bothridia together with a poeciloacanthous/heteroacanthous typica armature, were found (Palm, 1995). Both show a strong morphological relationship to species of the Otobothriidae Dollfus, 1942 (Otobothrioidea Dollfus, 1942), although lacking the superfamily character, a heteroacanthous atypica armature. Following the most recent classification of Campbell & Beveridge (1994), which uses the tentacular armature as a major character at the superfamily level, the species have to be placed in two superfamilies Poecilacanthoidea Dollfus, 1942 and Heteracanthoidea Dollfus, 1942, neither of which are related to the family Otobothriidae.

In the present paper, such incongruities arising from the most recent Campbell & Beveridge system together with the usefulness of ciliated pits and prebulbular organs as systematic characters are discussed. As a result, an alternative classification is presented which considers the tentacular armature as being of no importance at the superfamily level and of limited importance at the family level.

Materials and methods

Light- and scanning electron microscopical studies of morphology, tentacles and surface structures of the scoleces of trypanorhynch cestodes belonging to 18 genera (a total of 31 species) resulted in the description of four new species (*Grillotia kovalevae*, *Poeciloacanthum oweni*, *Pseudogrillotia zerbiae*, *Pseudolacistorhynchus noodti*) and the first SEM studies of 15 previously known species (see Palm, 1995).

Fresh postlarvae, plerocerci and adults were collected from teleosts and elasmobranchs along the coasts of Brazil, Nigeria and the southern (Gulf of Mexico) coast of Mississippi, USA. Preserved postlarvae and plerocerci were obtained from collections in Brazil, England, Russia and the USA (Palm, 1995). Characteristics of the 31 trypanorhynch genera not examined by Palm (1995) were obtained from the literature, in the form of the original descriptions, redescriptions and Campbell & Beveridge (1994), with the latter having priority in cases of discrepancy.

The current classification

Based on the fundamental work of Dollfus (1942), Campbell & Beveridge (1994) grouped 46 genera and 19 families of trypanorhynchs within four superfamilies, the latter characterised by the possession of a specific kind of tentacular armature. These authors preserved Dollfus' classification scheme and retained his superfamily concept. The Homeacanthoidea has hooks on the tentacles which form quincunxes or continuous spiral rows (homeoacanth, after Dollfus, 1942); the Heteracanthoidea is characterised by hooks on the tentacles which form half spiral rows with a constant number of hooks (heteroacanth typica). The Otobothrioidea is similar to the latter superfamily but has extra hooks or rows of hooks interpolated on the external surface of the tentacle (heteroacanth atypica). Within the Poecilacanthoidea, a distinctive hook file or files form one or more chainettes on the external tentacle surface (poeciloacanth). Finally, using 13 different characters, Campbell & Beveridge (1994) arranged the families and superfamilies within a cladogram, showing their possible phylogenetic relationships.

An innovation in the classification of Campbell & Beveridge (1994) is the use of pattern isometry for interpretation of the characteristic armatures. The use of hook arrangements on the tentacle surfaces as the major distinguishing feature for trypanorhynch classification has the advantage that this character is similar for plerocerci (postlarvae) and adults within the same species (Richmond & Caira, 1991). Furthermore, hooks are hard structures and do not change their appearance due to different fixation and preparation methods. However, despite the precision of the Campbell & Beveridge system, some incongruities are present, and some questions arise.

Different kind of armatures are present within closely related species

Within one superfamily or family, some closely related species (as far as other morphological characters other than the tentacular armature are considered) can have different characteristic armatures. For example, the superfamily Otobothrioidea *sensu* Campbell & Beveridge (1994) has a heteroacanthous atypica armature and therefore contains the family Otobothriidae Dollfus, 1942. This family is additionally characterised by the possession of ciliated pits on the bothridial borders (Campbell & Beveridge, 1994). Palm (1995) described a new trypanorhynch species with ciliated pits, *Poeciloacanthum oweni* Palm, 1995, having a distinct single chainette on the external tentacle surface (Figures 1a–b), and thus exhibiting the features of a poeciloacanth. The species *Pseudotobothrium dipsacum* (Linton, 1897) (formerly *Otobothrium dipsacum* Linton, 1897) has a heteroacanthous typica armature (Figures 1c–d), without extra hooks or rows of hooks interpolated on the external side of the tentacle (Palm, 1995). According to the Campbell & Beveridge system, these two species have to be placed into two different superfamilies, the Poecilacanthoidea and Heteracanthoidea [*sensu* Campbell & Beveridge (1994)]. However, both species show a strong morphological relationship to most species of the Otobothriidae (Otobothrioidea), sharing the following suite of characters: the occurrence of a blastocyst, a craspedote scolex with appendix, two patelliform bothridia with horse-shoe-shaped ciliated pits (Figure 2a), a similar form of the bulbs (not *P. dipsacum*), a similar position of the pars bothridialis relative to the pars bulbosa, the origin of the retractor muscles in the anterior part of the bulbs, and the presence of three-fingered palmate microtriches on the bothridia and filamentous microtriches only on the rest of the scolex (see Palm, 1995). Considering morphological characters other than the tentacular armature for characterising the Otobothriidae, there are three different armatures, heteroacanthous typica, heteroacanthous atypica and poeciloacanthous, within a range of closely related species.

Have ciliated pits developed independently or do they characterise a monophyletic taxon?

Only a few species of trypanorhynchs have ciliated pits on the borders of the bothridia, thus presenting some evidence for a monophyletic origin. Based on this character, Linton (1890) established the genus *Otobothrium*, and Dollfus (1942) established the family Otobothriidae. Using the classification scheme of Campbell & Beveridge (1994), which relies on the tentacular armature as a major distinguishing character, species with ciliated pits would be separated into all four superfamilies (see above), thereby implying an independent development of this organ in several unrelated lineages.

Thus, we are confronted with conflicting evidence. Most species with ciliated pits have heteroacanthous atypica armatures and therefore belong to the Otobothrioidea (*sensu* Campbell & Beveridge, 1994). In contrast to this, two species *Pseudonybelinia odontacantha* Dollfus, 1966 and *Paranybelinia otobothrioides* Dollfus, 1966 have homeoacanthous armatures and consequently have to be placed within the Homeacanthoidea. The armature of *Pseudotobothrium dipsacum* is heteroacanthous typica (Heteracanthoidea), whereas *Poeciloacanthum oweni* has a poeciloacanthous armature and therefore belongs to the Poecilacanthoidea. However, the non-tentacular morphology of the latter species shows a strong relationship to the otobothriids (see above). Therefore, a monophyletic origin of trypanorhynch cestodes with ciliated pits is likely.

Unique position of the Tetrarhynchobothriidae within the Homeoacanthoidea

Members of the homeoacanth family Tetrarhynchobothriidae Dollfus, 1969 have several characteristics shared with those of the Eutetrarhynchidae Guiart, 1927, a family belonging to the Heteracanthoidea. Such characters are an elongated scolex with oval bothridia, long tentacles as well as tentacle sheaths and the occurrence of a blastocyst and prebulbular organs. For this reason the group was placed together with the Eutetrarhynchidae by Dollfus (1969). Furthermore, Schmidt (1986) synonymised *Tetrarhynchobothrium* Dollfus, 1969 with *Eutetrarhynchus* Pintner, 1913. Beveridge & Campbell (1988) re-assessed the family Tetrarhynchobothriidae, discussed in detail its similarity to the Eutetrarhynchidae, and placed the family in

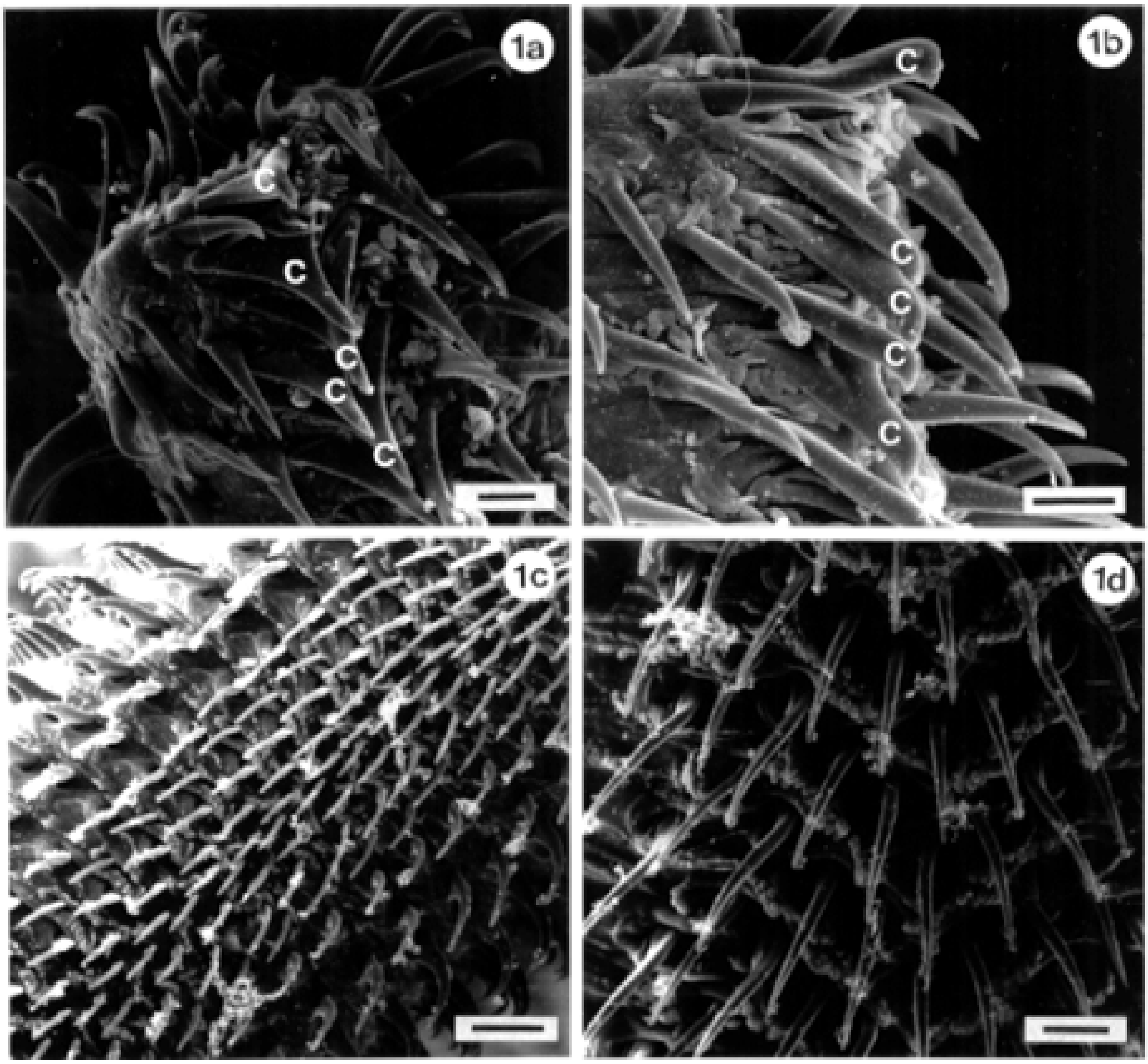

Figure 1. Trypanorhynch cestodes, scanning electron micrographs: a–b. External tentacle surface of *Poeciloacanthum oweni*, note the characteristic single chainette; c–d. External tentacle surface of *Pseudotobothrium dipsacum*, note the lack of interpolated hooks or rows of hooks. *Abbreviation*: C, chainette. *Scale-bars*: a, b, d, 10 μm; c, 20 μm.

the suborder Cystidea Guiart, 1927, although it has a homeoacanth tentacular armature like species of the Acystidea Guiart, 1927. In contrast to this, but consistent with their classification, the Tetrarhynchobothriidae is allocated to the Homeacanthoidea in Campbell & Beveridge (1994), mainly based on the homeoacanthous armature (see Campbell & Beveridge, 1994, p. 67). This interpretation is still open for discussion.

Two different kind of armatures on a single tentacle

In 1982, Dailey & Vogelbein described *Mixodigma leptaleum* Dailey & Vogelbein, 1982 as possessing a poeciloacanthous basal armature together with a heteroacanthous typica armature in the metabasal part of the same tentacle. According to the scheme of Dollfus (1942), who used only the metabasal armature, *M. leptaleum* would be considered as having a heteroacanthous typica armature. However, in the 1994 classification of Campbell & Beveridge, where the armature is

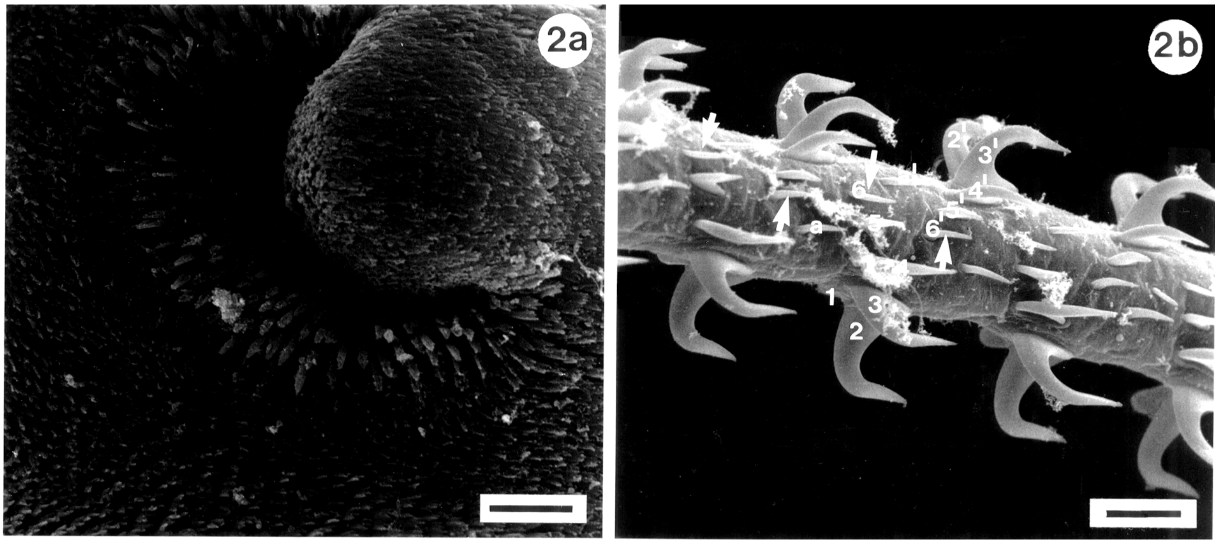

Figure 2. Trypanorhynch cestodes, scanning electron micrographs: a. Horse-shoe shaped ciliated pit of *Poeciloacanthum oweni*; b. External tentacle surface of *Otobothrium cysticum*, note the hooks marked with arrows, forming a file of alternating hooks along the tentacle. *Scale-bars*: 5 μm.

interpreted as possessing a chainette of reduced length, *M. leptaleum* is unequivocally a poeciloacanth. *M. leptaleum* is not alone in having different hook patterns on different parts of the tentacles; other examples are *Dasyrhynchus talismani* Dollfus, 1935 and *Pseudogrillotia peruviana* Escalante & Carvajal, 1984 (see Campbell & Beveridge, 1994, p. 67).

M. leptaleum has a strong resemblance to the Heteracanthoidea, especially to the Shirleyrhynchidae Campbell & Beveridge, 1994, with its metabasal armature, the presence of prebulbular organs, elongate bulbs and gland-cells around the retractor muscle, as well as its terminal genitalia (see Beveridge & Campbell, 1989). However, the Mixodigmatidae Dailey & Vogelbein, 1982 are placed together with the Gymnorhynchidae Dollfus, 1935 within the Poecilacanthoidea in the cladogram of Campbell & Beveridge (1994). Beside the distinct chainette on the external surface in the basal part of the tentacles, the two families have few characters in common. Thus, this implication of phylogenetic relationship between these two families on the basis of the poeciloacanthous armature can be considered doubtful.

Have prebulbular organs developed independently or do they characterise a monophyletic taxon?

Several trypanorhynch cestodes are known which have prebulbular organs around the tentacle sheaths at the entry to the bulbs. Campbell & Beveridge (1994) listed the following families belonging to all four superfamilies: Eutetrarhynchidae; Shirleyrhynchidae Campbell & Beveridge, 1994; (both Heteracanthoidea); Tetrarhynchobothriidae Dollfus, 1969 (Homeacanthoidea); Grillotiidae Dollfus, 1969 (Otobothrioidea); and Mixodigmatidae Dailey & Vogelbein, 1982 (Poecilacanthoidea). As a consequence, the prebulbular organs could not be used as a systematic character for defining higher taxa. However, considering the Tetrarhynchobothriidae to be closely related to the Eutetrarhynchidae and the species *Mixodigma leptaleum* to the Shirleyrhynchidae (see above), the enigmatic prebulbular organ characterises a large group of closely related species. Thus, it is concluded, although the function of this organ is still unclear, that prebulbular organs have developed only once within the trypanorhynchs.

Do intermediate forms of hook patterns exist?

Some trypanorhynchs are known which have features that indicate some relationships between the major groupings (tentacle armature types – currently at the superfamily level), and present a problem for the systematist. An example of such a worm is *Otobothrium cysticum* (Mayer, 1842). Its tentacular armature is considered to be heteroacanthous atypica (Dollfus, 1942; Campbell & Beveridge, 1994; Palm, 1995). However, in the metabasal part of the tentacle, the last principal hooks on the external tentacle surface could be interpreted as a file of alternating hooks (Figure 2b), thus fulfilling part of the criteria for a chainette (if all criteria for a chainette were met, the *O. cysticum* armature would be poeciloacanthous). As it is thought that a poeciloacanthous armature has evolved from a heteroacanthous atypical armature (Campbell & Beveridge, 1994), such a longitudinal hook arrangement might already represent a precursor of a poeciloacanthous hook pattern, with the principal hooks 6 and 6' thought to form a chainette. Thus, *O. cysticum* can be interpreted as an intermediate form between heteroacanthous atypica and poeciloacanthous species, supporting the idea of a relationship between *Poeciloacanthum oweni* Palm, 1995 and other otobothriid species (see above).

A further example of a trypanorhynch species, showing a tentacular armature that could be interpreted to be an intermediate form, is a species of *Pterobothrium* Diesing, 1850. Within this genus, species have a heteroacanthous atypica armature. Some species, such as *P. hawaiense* Carvajal, Campbell & Cornford, 1976 and *P. lintoni* (MacCallum, 1916), lack hooklets on the external tentacle surface; however, intercalary hooks are still present along the tentacle (Campbell & Beveridge, 1996). Using scanning electron microscopy, Palm (1995) described two specimens of a *Pterobothrium* species which he identified as *P. heteracanthum* Diesing, 1850. In the metabasal part of all tentacles both the hooklets on the external tentacle surface and the intercalary hooks were absent. Only in the basal part were some hooklets and 1-4 intercalary hooks present. Thus, considering the general morphology as well as the basal armature, the examined specimens clearly belong to a *Pterobothrium* species with the expected heteroacanthous atypica armature. However, the metabasal part is similar to a heteroacanthous typica armature.

Comments

These examples outlined above show that the tentacular armatures as defined by Dollfus (1942) and Campbell & Beveridge (1994) can split closely related species, as determined by other morphological characters, into different superfamilies. Additionally, the value of the presence of ciliated pits and prebulbular organs in relation to trypanorhynch classification appears to have been underestimated.

Carvajal & Campbell (1975) came to the conclusion that the complex oncotaxy is definitive for specific but not for familial determination. For this reason, a classification of superfamilies which uses the tentacular armature as of major importance splits morphologically similar species into different families or even superfamilies. Palm (1995) gave the main reason for this as a convergent development of tentacular armatures, caused by adaptation of the trypanorhynch tentacular apparatus to their sites of attachment in their final hosts. For example, a homeomorphous and homeoacanthous armature in combination with short tentacles is most favourable for attaching to the muscular tissue of the stomach wall, while the heteromorphous armature on long and slender tentacles is perfectly adapted to a connective tissue dominated intestinal wall (see Palm, 1995). Further support for this functional hypothesis is the dominance of worms with the former tentacle type in cephalopods, where they often occur encapsulated in the walls of stomach and caecum, the mesenteries and gonads, and in the ventral musculature of the mantle wall (Kinne, 1990). The mobile postlarvae of *Tentacularia coryphaenae* even occur in the water-filled mantle cavity, where they exist embedded (Kinne, 1990) or attached to the mantle tissue, a site from which they risk being expelled by turbulence. Cephalopods are voracious, versatile predators (Rodhouse & Nigmatullin, 1996) and should have as trophic opportunists a similar access to both types of trypanorhynchs.

Summarising the above, it is concluded that tentacular armatures are convergent within the trypanorhynchs. Thus, the tentacular armatures characterise superfamilies in an empirical system of trypanorhynchs which is unlikely to reflect their phylogeny.

The proposed classification

In the following section, an alternative classification of trypanorhynch cestodes is presented, which gives the tentacular armature no importance for superfamily and a reduced importance for family determination. Instead, the ciliated pits and prebulbular organs are integrated at a higher systematic level, solving the incongruities and unresolved questions discussed above. A total of six different characters are used to construct this alternative classification; ciliated pits on the bothridia; prebulbular organs; blastocysts; number of bothridia; rows of hooks on the tentacles; and rhyncheal apparatus. In addition, each of these can

Table I. Characters used for an alternative classification of trypanorhynch cestodes.

Character	Plesiomorphic	Apomorphic
Ciliated pits on the bothridia*	absent	present
Prebulbular organs	absent	present
Blastocyst*	absent	present
Number of bothridia*	4	2
Rows of hooks on tentacles	complete	partly reduced
Rhyncheal apparatus	present	absent

*Interpretation of character state in agreement with Campbell & Beveridge (1994).

be divided into primitive (plesiomorphic) and derived (apomorphic) states (Table I), thereby allowing phylogenetic interpretation of the order.

Superfamilies

The presence or absence of ciliated pits on the bothridia and the presence or absence of prebulbular organs is used to distinguish three superfamilies, the Otobothrioidea Dollfus, 1942, Tentacularioidea Poche, 1926 and Eutetrarhynchoidea Guiart, 1927 (Figure 3).

The Otobothrioidea is characterised by ciliated pits on the bothridial borders and the absence of prebulbular organs. The presence of ciliated pits is considered to be a synapomorphic character of this monophyletic taxon, relative to the absense of this character in all other trypanorhynchs. All species of the Otobothriidae Dollfus, 1942 as well as the Paranybeliniidae Schmidt, 1970 are included into the Otobothrioidea. For the species *Pseudotobothrium dipsacum* (Linton, 1897), the family Pseudotobothriidae Palm, 1995 is added to this superfamily.

The Eutetrarhynchoidea is characterised by the absence of ciliated pits on the bothridia and the possession of prebulbular organs, as defined by Pintner (1880). The presence of prebulbular organs is considered to be a synapomorphic character of this monophyletic taxon, relative to the absense of this character in all other trypanorhynchs. The Eutetrarhynchoidea includes the families Eutetrarhynchidae Guiart, 1927, Shirleyrhynchidae Campbell & Beveridge, 1994 and Mixodigmatidae Dailey & Vogelbein, 1982, as defined by Campbell & Beveridge (1994). Additionally the three genera, *Didymorhynchus* Beveridge & Campbell, 1988, *Zygorhynchus* Beveridge & Campbell, 1988 and *Tetrarhynchobothrium* Diesing, 1854, of the former Tetrarhynchobothriidae Dollfus, 1969 are added to the Eutetrarhynchidae.

The third superfamily, Tentacularioidea, contains trypanorhynchs having neither ciliated pits nor prebulbular organs. A character providing evidence for a monophyletic origin of this superfamily could not be demonstrated. Therefore, this superfamily must be considered paraphyletic. The Tentacularioidea contains all remaining genera listed by Campbell & Beveridge (1994) and Palm (1995) rearranged in six different families: Tentaculariidae Poche, 1926; Sphyriocephalidae Pintner, 1913; Gilquiniidae Dollfus, 1942; Aporhynchidae Poche, 1926; Pterobothriidae Pintner, 1931; and Lacistorhynchidae Guiart, 1927 (Figure 3).

Families

The following characters are used as criteria at the family level: the presence or absence of a blastocyst; the number of bothridia (two or four); the tentacular armature [complete rows of hooks (as exemplified by homeoacanthous and heteroacanthous typica armatures) or rows of hooks partly reduced (as exemplified by heteroacanthous atypica and poeciloacanthous armatures)]; and the reduction of the rhyncheal apparatus (see Palm, 1995). The penultimate character, rows of hooks complete or rows of hooks partly reduced, is new and refers to the metabasal armature. Rows of hooks are considered complete if the armature consists of only complete spirals or of only principal rows (respectively, homeoacanthous and heteroacanthous typica, as defined by Dollfus 1942). Rows of hooks are considered partly reduced if the armature includes any of the following: intercalary hooks, hooks or rows of hooks interpolated on the external surface (band of hooks), and a chainette. The common feature of these elements on the external tentacular armature is their interpretation as relicts of former complete spirals or principal rows.

In order to characterise the families, specific combinations of these characters are used. Of the 19 families previously accepted by Campbell & Beveridge (1994), 10 are retained (Eutetrarhynchidae Guiart, 1927; Gilquiniidae Dollfus, 1942; Lacistorhynchidae Guiart, 1927; Mixodigmatidae Dailey & Vogelbein, 1982; Otobothriidae Dollfus, 1942; Paranybeliniidae Schmidt, 1970; Pterobothriidae Pintner, 1931; Shirleyrhynchidae Campbell & Beveridge, 1994; Sphyriocephalidae Pintner, 1913; Tentaculariidae Poche, 1926 (all *sensu nov*.). One family is reinstated (Aporhynchidae Poche, 1926 *sensu nov*.) and

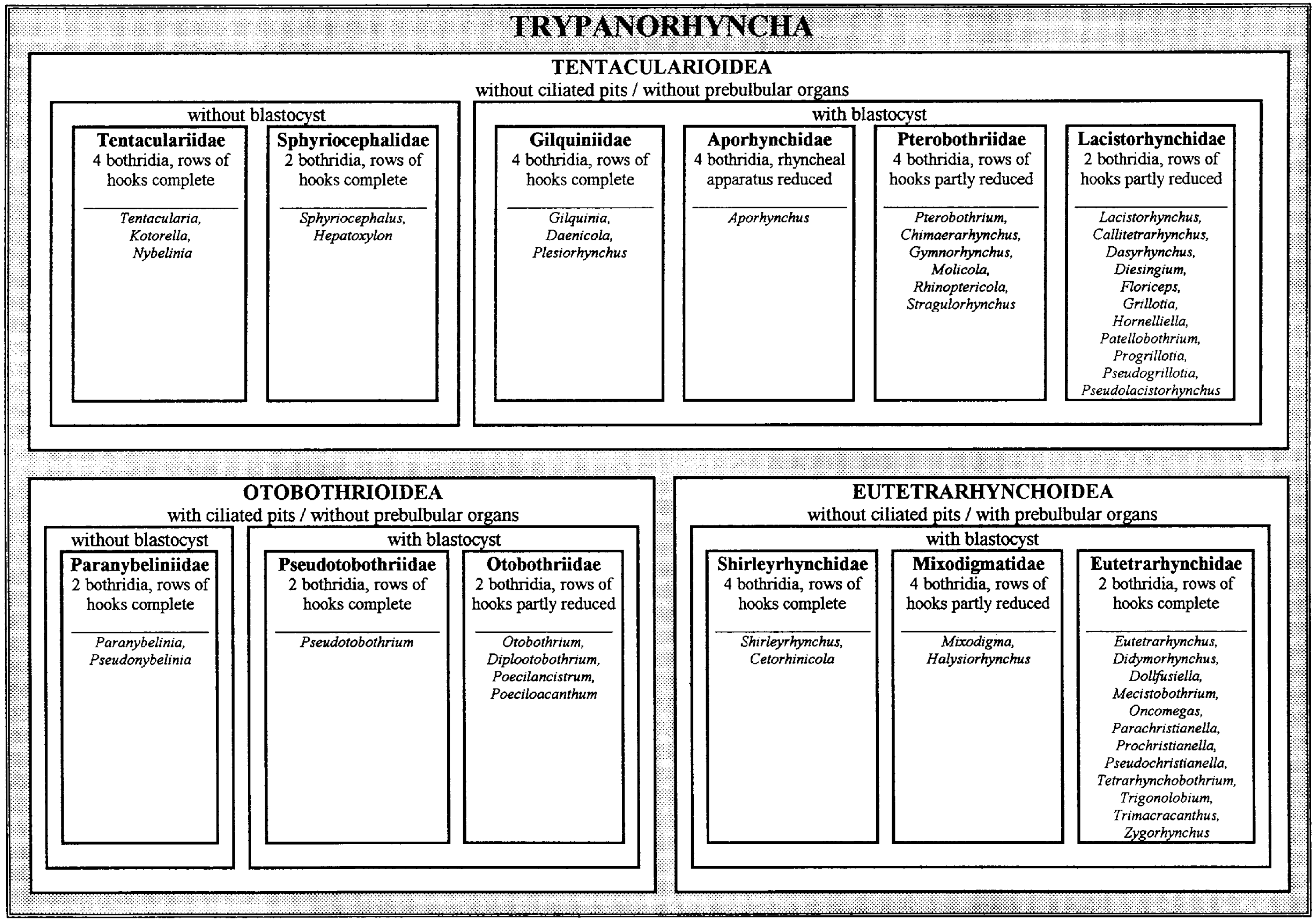

Figure 3. Alternative classification of trypanorhynch cestodes.

a new one is established (Pseudotobothriidae Palm, 1995). The following families are considered to be synonymous: Hepatoxylidae Dollfus, 1940 with Sphyriocephalidae; Tetrarhynchobothriidae Dollfus, 1969 with Eutetrarhynchidae; Gymnorhynchidae Dollfus, 1935, Molicolidae Beveridge & Campbell, 1989 and Rhinoptericolidae Carvajal & Campbell, 1975 with Pterobothriidae; and Dasyrhynchidae Dollfus, 1935, Grillotiidae Dollfus, 1960, Hornelliellidae Yamaguti, 1954 and Mustelicolidae Dollfus, 1969 with Lacistorhynchidae.

The proposed new classification of trypanorhynch cestodes, with definitions for superfamilies and families, is shown diagrammatically in Figure 3. Some genera are tentatively placed in superfamilies and families within the proposed classification, despite not knowing if they have a blastocyst (for example when the species description is based on adults). In these cases, assignment was based upon the general morphology, number of bothridia and kind of tentacular armature (see ‘Future studies’ below).

Comments on the proposed classification

The proposed alternative classification of trypanorhynch cestodes allows all known trypanorhynchs to be unequivocally placed on the basis of a defined combination of characters in superfamilies and families. Using evolutionary systematics, and no longer using the tentacular armature as the major character for superfamily separation, both the classification and identification of trypanorhynchs is simplified. Mayr & Ashlock (1991) pointed out that, for phenetics as well as for cladistics, a large number of characters should be used for classification. However, as the most usable characters show an enormous variability within the Trypanorhyncha, only six of them appear to be useful for definition of higher taxa. Thus, an evolutionary systematic approach was chosen.

Other strengths of this new classification are that incongruities in the existing classifications, caused by the use of the tentacular armature as a major character for distinguishing superfamilies, are omitted (Palm, 1995). Furthermore, with the finding of new species with new combinations of the chosen six characters or with increasing knowledge concerning misdetermined species (see ‘Future studies’ below), this new classification can be enlarged to up to four superfamilies and 48 families without losing its stability. It is also possible to determine all the information required using light microscopy.

The major difference between this alternative classification and the system of Campbell & Beveridge (1994) is that the tentacular armature is not used at the superfamily level, and, at the family level, the armature has only a third level of priority. However, in the present alternative scheme, the armature is limited to being described as rows of hooks complete (homeoacanthous and heteroacanthous typica) or rows of hooks partly reduced (heteroacanthous atypica and poeciloacanthous). Thus, the four established trypanorhynch armature types are still recognised as being valid, but they are used in a different way, and only at the family level. Additionally, it is still possible to use the tentacular armature for subfamily as well as for genera and species definition.

The family Tetrarhynchobothriidae (*sensu* Campbell & Beveridge, 1994) shows this difference very clearly: considering the armature to be of major importance, this family is allocated to the Homeacanthoidea by Campbell & Beveridge (1994), together with the other homeoacanth families Sphyriocephalidae, Hepatoxylidae, Tentaculariidae and Paranybeliniidae. In contrast to this, in the presented classification the genera belonging to this family (*sensu* Campbell & Beveridge, 1994) were assigned to the heteroacanthous typica family Eutetrarhynchidae.

In agreement with Campbell & Beveridge (1994), the presence and absence of blastocysts is no longer used to distinguish the two suborders Atheca and Thecaphora, originally proposed by Guiart (1927), and suborders within the Trypanorhyncha are no longer recognised. Mattis (1986) showed that the eutetrarhynchid *Prochristianella hispida* (Linton, 1890) (Eutetrarhynchoidea) develops a different type of blastocyst than the otobothriid *Poecilancistrum caryophyllum* (Diesing, 1850) (Otobothrioidea). Thus, the blastocysts are proposed to be convergent developments within the superfamilies of trypanorhynchs. Therefore, this character was used at the family level only.

The characteristics of the genitalia were not used to distinguish higher taxa within the trypanorhynchs, as they are characteristic only of adults. However, following the Campbell & Beveridge system, the character uterus linear/uterus a transverse sac appears to be useful to distinguish between families without blastocyst and those with blastocyst within the Tentacularioidea. Similarly, the muscles encircling the tentacle sheaths, yet lacking a prebulbular organ as present in several trypanorhynch genera, were not included in this alternative classification. This was because their relationship to these organs as well as their occurrence within the different trypanorhynch species could not be clarified.

Phylogenetic implications

The dendrogram constructed from the above proposed classification (Figure 4), compared with the dendrogram of Dollfus (1942) and the cladogram of Campbell & Beveridge (1994), shows very different phylogenetic relationships between the different families. For example, in this proposed classification, the Otobothrioidea is assumed to be a phylogenetically derived trypanorhynch group, which is split from all other forms by the development of ciliated pits. Otobothrioids have a plesiomorphic tentacular armature (*Pseudonybelinia odontacantha* Dollfus, 1966; *Paranybelinia otobothrioides* Dollfus, 1966; *Pseudotobothrium dipsacum* (Linton, 1897)) and an apomorphic (*Otobothrium* spp.) tentacular armature. A second phylogenetically derived group is the Eutetrarhynchoidea, having prebulbular organs as defined by Pintner (1880). Like the ciliated pits, these organs are characteristic developments within a single superfamily.

The development of poeciloacanthous armatures is indicated by the present system to have arisen independently several times: all three superfamilies have species with this type of armature. It is interesting to note that independent evolution of the poeciloacanthous feature “double-winged chainettes” has previously been proposed by Beveridge & Campbell (1989) based on the examination of oncotaxy and the anatomy of mature segments. Other remarkable similarities between the present alternative classification and the study of Beveridge & Campbell (1989) are that the Gymnorhynchidae (synonymous with Pterobothriidae in this classification) may be derived from a common ancestor with the Gilquiniidae, and the Myxodigmatidae may be derived from a common ancestor with the Shirleyrhynchidae, including both *Shirleyrhynchus* Beveridge & Campbell, 1988 and *Cetorhinicola* Beveridge & Campbell, 1988 (Figure 4). In their more recent work, however, Campbell & Beveridge (1994) indicated that these families were more distantly related.

Palm (1995, p. 204) demonstrated a reduction-row, a possible direction of development of the tentacular armature within the genus *Pterobothrium* Diesing, 1850. An important consequence of this was that an armature with spiniform principal hooks and lacking a band of hooks on the external tentacle surface represents a highly developed armature within the genus. Thus, within the present classification, the total reduction of tentacular hooks on the external tentacle surface of heteroacanthous atypica armatures is interpreted as a derived character.

It has yet to be proven by new findings, or with the help of molecular techniques, that the present interpretations of phylogenetic relationships are viable. However, this alternative classification is considered to be closer to a natural system than the proposed classifications proposed by Dollfus (1942) and Campbell & Beveridge (1994), as it no longer uses the hook arrangements on the tentacles (concluded to be convergent developments) as a major character for distinguishing superfamilies.

Future studies

Within the present classification, the importance of the character “with or without blastocyst” for distinguishing different trypanorhynch families causes problems if this feature is not known, due to the availability of adults only or metacestodes (plerocerci) already removed from their blastocyst. Such trypanorhynchs can, however, be assigned to a family based on other features. In many cases, assignment will be to one family only, but it is possible that a choice of two families will occur. In such cases, assignment should be based upon the general morphology, the number of bothridia and the kind of tentacular armature. To date there is a strong correlation between the presence/absence of blastocysts and the nature of tentacular armature. However, reassignment to another position within this classification is possible if increasing knowledge on the life-cycles of the species provides conflicting evidence.

This study shows that it is possible to assign all known Trypanorhyncha using a classification based on six different characters. However, in developing the present alternative classification, it was apparent that certain genera and organs remain poorly known. Therefore, it is suggested for future studies, that the following topics should be examined by trypanorhynch specialists: (i) the comprehensive characterisation of the surface ultrastructure of *Grillotia* Guiart, 1927, *Nybelinia* Poche, 1926 and *Otobothrium* Linton, 1890; (ii) the functional morphology of prebulbular organs; (iii) whether or not the muscular rings around the basal part of the tentacle sheaths are homologous to prebulbular organs; and (iv) the functional morphology of microtriches and other tegumental structures, including ciliated pits and sensory organs.

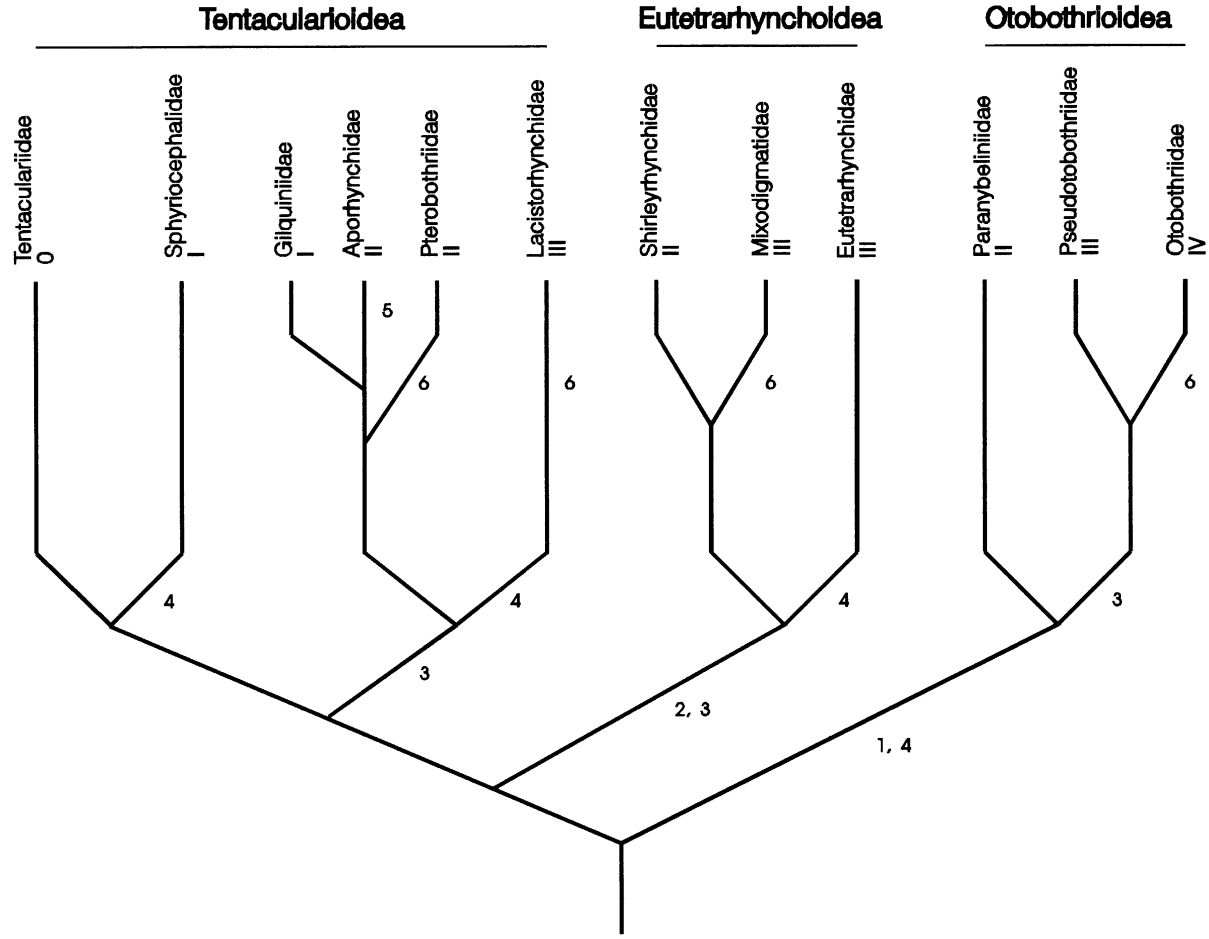

Figure 4. Dendrogram, showing a possible phylogenetic relationship between trypanorhynch families. *Characters*: 1. Ciliated pits; 2. Prebulbular organs; 3. Blastocyst; 4. Number of bothridia; 5. Reduction of the rhyncheal system; 6. Partial reduction of tentacular hooks. Roman letters I-IV = numbers of derived characters. The length of the branches does not contain cladistic information.

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