Scolex morphology of monozoic cestodes (Caryophyllidea) from the Palaearctic Region: a useful tool for species identification

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Abstract: A comparative study of the scoleces of caryophyllidean tapeworms (Cestoda: Caryophyllidea), parasitic in cypriniform fishes in the Palaearctic Region, was carried out using light and scanning electron microscopy. Three-dimensional pictures of the scoleces of 18 species of caryophyllidean cestodes of the Capingentidae (1 species), Caryophyllaeidae (7) and Lytocestidae (10), and outlines of the scoleces and anterior extent of the testes and vitelline follicles of 19 Palaearctic taxa were documented. Both species of Atractolytocestus Anthony, 1957 possess a bulbacoacuminate scolex, whereas species of Archigetes Leuckart, 1876 have fossate scoleces of the bothrioloculodiscate type, with loculi, bothrium-like depressions and an apical disc. Breviscolex orientalis Kulakovskaya, 1962, the only member of the Capingentidae, has a cuneiform scolex, as do both taxa of the lytocestid genus Caryophyllaedes Nybelin, 1922. The scoleces of two species of Caryophyllaeus Gmelin, 1790 are flabellate, whereas that of the congeneric C. fimbriceps Annenkova-Chlopina, 1919 is cuneicrispitate. Khawia Hsiü, 1935, the most specious Palaearctic genus, with seven taxa that we consider to be valid, has the highest diversity in scolex morphology: semi-bulbate, flabellate, cuneiform, cuneiformbibrate, truncated cuneiform-flabellate and festoon-like. Species of Monobothrium Nybelin, 1922 have either a digitiform scolex with widened posterior part or cuneiform, with lateral auricular extensions. Paracaryophyllaeus gotoi (Motomura, 1927) is characteristic in its possessing a bulbate scolex, whereas Paraglaridacris limnodrili (Yamaguti, 1934) has a fossate scolex of the bulbolocate type with bothrium-like depressions and feebly developed lateral loculi. Anterior extent of the testes and vitelline follicles and their mutual position show a somewhat higher variability than scolex shape, with intraspecific variation in some taxa, such as Atractolytocestus sagittatus (Kulakovskaya et Akhmerov, 1965), B. orientalis, Khawia armeniaca (Cholodkovsky, 1915) and K. sinensis Hsiü, 1935. Based on scolex morphology and relative position of the anterior testes and vitelline follicles, a key is provided to facilitate the routine identification of 20 Palaearctic caryophyllidean taxa.

Keywords: Cestoda, fish, comparative morphology, scanning electron microscopy, identification, Palaearctic Region

Caryophyllidean tapeworms (Platyhelminthes: Eucestoda) represent a widely distributed group of intestinal parasites of cypriniform and siluriform fishes occurring in all zoogeographical regions except the Neotropics (Mackiewicz 1994). They are unique among “true” cestodes of Eucestoda in their possession of a monozoic or monopleurid body type, which is astrobilate and consists of only a single set of male and female organs. Some caryophyllideans, especially those in carp and related cyprinids cultured in fishponds, may be pathogenic for their fish hosts (Bauer et al. 1973, Williams and Jones 1994, Oros et al. 2009).

Caryophyllideans possess a simple scolex, the morphology of which has been suggested to provide characteristics useful for generic classification (Mackiewicz 1972, 1981, 1994), and also in some cases for species identification (Kulakovskaya 1961, Dubinina 1987, Scholz 1989). However, the use of scolex morphology in identification can be limited because the scolces of these cestodes are highly motile and thus can change considerably in shape. In addition, the shape and size of the scolex depends to a great extent on processing methods, in particular fixation of worms.

Over the course of a long-term research project on the helminth parasites of freshwater fishes, extensive material of caryophyllideans was collected from fishes of the families Cyprinidae and Cobitidae (Cypriniformes) and Odontobutidae (Perciformes) in the Palaearctic Region. Since these cestodes were processed using the same method to ensure comparability of morphological and biometrical data, this procedure enabled us to perform a large-scale comparative study on scolex morphology. In addition, it
also made it possible to assess the use of scolex characters and the anteriormost extent of the testes and vitelline follicles for the differentiation of caryophyllidean taxa and to make a simple key to facilitate species identification.

MATERIALS AND METHODS

Newly collected specimens of 14 species (see the list below) for light (LM) and scanning electron microscopical (SEM) observations were isolated from the intestine of freshly killed fish hosts, rinsed in saline, fixed immediately with 4% hot (almost boiling) formaldehyde solution (= formalin) and transferred to 70% ethanol after 1–2 weeks. For light microscopy, specimens were processed by the methods of Scholz and Hanzelová (1998): stained with Schüberg’s hydrochlorid carmine, destained in 70% acid ethanol (i.e. ethanol with several drops of HCl), dehydrated through a graded ethanol series, clarified in clove oil, and mounted in Canada balsam as permanent preparations.

Drawings were made using a drawing attachment on an Olympus BX51 microscope with the use of Nomarski interference contrast. Measurements were taken using Olympus Image-Pro programme. For SEM studies, specimens were dehydrated through a graded ethanol series, followed by a graded amylacetate series, dried by critical-point method, sputtered with gold, etched through a graded ethanol series, followed by a graded amylacetate series, dried by critical-point method, sputtered with gold, and examined with a JEOL JSE 7401F microscope. Stubs with scolecites are deposited in the Laboratory of Helminthology of the Institute of Parasitology, České Budějovice, Czech Republic (acronym IPCAS; http://www.paru.cas.cz).

6. Monothorium wageneri Nybelin, 1922 – four specimens from Tinca tinca, Labe (Elbe) River, Czech Republic and four specimens from T. tinca (all hosts Cyprinidae), Bracciano Lake, Italy (IPCAS C-206/1).

7. *Paracaryophyllaeus gotoi* (Motomura, 1927) – one specimen from Cobitis taenia L. (Cobitidae), Latorica River, Slovakia (PISAS 458/04) and two specimens, Košteľ, Hungary (IPCAS C-218/1).

8. *Paragaliurus* limnordrīli (Yamaguti, 1934) – one specimen from Perccottus gleni Dybowski (Odontobutidae) (adventinal host), Latorica River, Slovakia (PISAS 28/05).

Family Lytocestidae


10. *Atractolytocestus sagittatus* (Kulakovskaya et Akhmedov, 1965) – 10 specimens from Cyprinus carpio, Suwa Lake and Biwa Lake, Japan (IPCAS C-340/1).

11. *Caryophyllaeides ergensi* Scholz, 1990 – eight specimens from Tribolodon hakonensis (Günther), Ogawara Lake, Japan (IPCAS C-217/1).

12. *Caryophyllaeides fennica* (Schneider, 1902) – eight specimens from Rutulus rutulus (L.), Tisa River, Slovakia (PISAS 18/04, 298/05a, b).

13. *Khavia armeniaca* (Cholodkovsky, 1915) – two specimens from Capoeta damascina (Valenciennes), Zayande (Zindeh) River at Esfahan, Iran (BMNH 1979.9.27.1–10), one specimen from Coregonus sp., Sevan Lake, Armenia (no voucher), one specimen from Barbus barbusculus (IPCAS C-48/4) and one specimen from B. gruppis (IPCAS C-48/2), Iraq.

14. *Khavia baltica* Szidat, 1941 – four specimens from Tinca tinca, Brehyně, Czech Republic (IPCAS C-42/1).

15. *Khavia japonensis* (Yamaguti, 1934) – five specimens from Cyprinus carpio, Biwa Lake, Hahima and Gifu, Japan (IPCAS C-348/1).

16. *Khavia parva* (Zmeev, 1936) – six specimens from Carassius carassius (L.) and C. auratus (L.), Amur River and Bolon Lake, Russia (IZSP 1461, 1462, 1463, 1466).

17. *Khavia rossittensis* (Szidat, 1937) – eight specimens from Carassius auratus, Tisa and Latorica Rivers, Slovakia (PI-SAS 43/02, 8/03, 81/05, 271/05, 554/05).

18. *Khavia saurogobii* (Szidat, 1941) – four specimens from Capoeta damascina (Valenciennes), Zayande (Zindeh) River at Esfahan, Iran (BMNH 1979.9.27.1–10), one specimen from Coregonus sp., Sevan Lake, Armenia (no voucher), one specimen from Barbus barbusculus (IPCAS C-48/4) and one specimen from B. gruppis (IPCAS C-48/2), Iraq.


No specimens of Caryophyllaeus straddarjensis Skrjabin, 1913, described from the barbel Schizothorax intermedius McClelland, 1842 from Turkest, Monothorium auriculatum Kulakovskaya, 1961 from Leciscus danilewskii (Kessler) from Ukraine, Archigetes brachyurus Mrazek, 1908 from tubificids (Annelida: Oligochaeta) and ctenid fish from the former Czechoslovakia, and A. cryptobothrius Wisniewski, 1928 from tubificids from Poland were available.

Terminology of scolex types is largely based on that proposed by Mackiewicz for caryophyllideans (1994 – see his figs. 5.1–5.21), but other morphological descriptors were used if necessary to better characterise scolex shape. In general, these additional terms are in common use in the literature.
RESULTS

Three-dimensional scanning electron micrographs (SEM) of the scoleces of 18 species of caryophyllidean cestodes of the Capingentidae (1 species), Caryophyllaeidae (7), and Lyctocestidae (10) are presented in Fig. 1. Outlines of the scoleces and anterior extent of the testes and vitelline follicles are shown in Figs. 2 and 3.

Both species of Atractolytocestus Anthony, 1958, A. huronensis and A. sagittatus, possess a bulbocunimate scolex (Fig. 1A–C), but they differ from each other in the length of the neck and mutual position and anterioriest extent of the testes and vitelline follicles (Fig. 2A–C). Species of Archigetes Leuckart, 1876 (only one species available and illustrated) possess fossate scoleces of the bothrioloculodiscate type, with loculi, bothrium-like depressions and an apical disc (Figs. 1D, 2D). Individual species can be distinguished by anterior extent of the testes and vitelline follicles (Fig. 2D; see also figures in Kennedy 1965). Breviscolex orientalis, the only member of the Capingentidae, has a cuneiform scolex (Figs. 1E, 2E), as do both taxa of the lytocestid genus Caryophyllaeidae Nybelin, 1922, C. ergensi and C. fennica (Figs. 1F, G, 2F, G). The scoleces of two species of Caryophyllaeus Gmelin, 1790, C. brachycollis and C. laticeps, are flabellate (Figs. 1H, J, 2H, J), whereas that of the congenic C. fimbriiceps is cuneicrissipitate (Figs. 1I, 1J).

The highest diversity in scolex morphology is found in species of Khawia Hsü, 1935, the most species Palaearctic genus with seven taxa we consider to be valid (unpublished data). Khawia armeniaca possesses a semi-bulbate scolex (Figs. 1K, 3A; no hot formalin-fixed specimens available), whereas that of K. baltica is flabellate (Figs. 1L, 3B; based on flattened specimens). Khawia japonensis has a cuneiformbricate scolex (Figs. 1M, 3C), whereas the scolex of K. sinensis is festoon-like (Figs. 1Q, 3G) and that of K. rossittensis is cuneiform (Figs. 1N, 3E). Khawia parva (Fig. 3D) has a cuneiform scolex expanded laterally (almost flabellate), but hot formalin-fixed material was not available. The scolex of K. saurogobii is truncated cuneiform-flabellate (Figs. 1O, 2F, G).

Monobothrium wageneri, for which hot formalin-fixed material was not available, has a digitiform scolex, widened posteriorly (Figs. 1R, 3I), whereas M. auriculatum (no material available) possesses a cuneiform scolex, with lateral auricular extensions (Fig. 3H; according to Kulakovskaya 1961). Paracaryophyllaeus gotoi is characteristic in possessing a bulbate scolex (Figs. 1S, 2K) and Paraglaridacris limnodrili has a fossate scolex of the bulbocululate type (Fig. 1T).

Anterior extent of the testes and vitelline follicles and their mutual position show a somewhat higher variability than scolex shape, with intraspecific variation in some taxa, such as A. sagittatus (Fig. 2B, C). Similar variation can be found in B. orientalis, K. armeniaca and K. sinensis (data not shown). However, this variation is low, species-specific in other taxa and thus enables good differentiation of individual species, including those of Caryophyllaeus (Fig. 2H–J) and Khawia (Fig. 3A–G). Unlike scolex shape, which is fairly similar, anterior extent of the testes and vitelline follicles is a suitable diagnostic feature of otherwise morphologically almost identical species of Atractolytocestus. In A. huronensis, the testes begin posterior to anterioriestmost vitelline follicles (Fig. 2A), whereas they begin anterior to the first vitelline follicles in A. sagittatus (Fig. 2B, C).

On the basis of the present data on scolex morphology and anterior extent of the testes and vitelline follicles, a preliminary key to facilitate routine identification of caryophyllideans from Palaearctic cyprinid and cobitid fishes is provided. However, reliable identification of some taxa may require other morphological characters, especially those related to the anterior extent of uterine loops, shape of the ovary, relative size of the cirrus-sac and other characteristics (see Scholz 1989, Protasova et al. 1990).

A preliminary key to the species of the Caryophyllidea from the Palaearctic Region based on scolex morphology and anterior extent of testes and vitelline follicles:

1a. Scolex fossate, i.e. with loculi or bothrium-like depressions (Fig. 1D, T) ................................................. 2
1b. Scolex afossate, i.e. without loculi or bothrium-like depressions (Fig. 1A–C, E–S) ............................................. 3

2a. Scolex bulboculate (Fig. 1T) ................................................. 4

2b. Scolex bothrioloculodiscate (Fig. 1D) .......................... Archigetes sieboldi Leuckart, 1878 Scolex slightly widened before neck region, with dorsal and ventral bothrium-like depressions and sublateral loculi, and with apical disc (Fig. 1D); testes begin anterior to vitelline follicles (Fig. 2D).

Remarks: Kennedy (1965) provided identification key to species of Archigetes; they are mainly differentiated by the anterior extent of the testes and vitelline follicles.

3a. Scolex bulbate or semi-bulbate (Fig. 1K, S) .......... 5
3b. Scolex bulbocunimate (Fig. 1A–C) .......................... 6
3c. Scolex cuneiform (Figs. 1E–G, N, 2H) ....................... 10
3d. Scolex truncated cuneiform-flabellate (Fig. 1O, P) ............................................. Khawia saurogobii Xi, Oros, Wang, Wu, Gao et Nie, 2009 Scolex short and markedly wider than neck, with shallow dorsal and ventral superficial grooves (Fig. 1O, P); vitelline follicles and testes begin immediately posterior to scolex, at approximately same level (Fig. 3F).

3e. Scolex with fimbriate anterior margin (cuneiformbricate, festoon-like or cuneicrissipitate) (Fig. 1I, M, Q) ................................................................. 11
3f. Scolex flabellate, with smooth anterior margin (Fig. 1H, J, L) .................................................................
Fig. 1. Scanning electron micrographs of the scoleces of Palaearctic species of Caryophyllidea. A – Atractolytocestus huronensis; B, C – Atractolytocestus sagittatus; D – Archigetes sieboldi; E – Breviscoles orientalis; F – Caryophyllaeides ergensi; G – Caryophyllaeides fennica; H – Caryophyllaeus brachycollis; I – Caryophyllaeus fimbriiceps; J – Caryophyllaeus laticeps; K – Khavia armeniaca; L – Khavia baltica; M – Khavia japonensis; N – Khavia rossittensis; O, P – Khavia saurogobii; Q – Khavia sinensis; R – Monobothrium wageneri; S – Paracaryophyllaeus gotoi; T – Paraglaridacris limnodrili. Originals. Scale bars: A, D, I, K, L, O, P, S = 0.25 mm; B, C, E–H, J, M, N = 0.5 mm; Q, R = 1 mm; T = 0.1 mm.
3a. Scolex digitiform (Fig. 1R)

3b. Scolex digitiform, widened posteriorly, with rounded lateral expansions (Fig. 1S); testes begin slightly wider than neck region; first vitelline follicles start at approximately same level, posterior to neck region (Fig. 3C).

4a. Anteriormost testes begin posterior to first vitelline follicles (Fig. 2K)

4b. Anteriormost testes and vitelline follicles begin at approximately same level, posterior to neck (Fig. 3A); Caryophyllaeides fennica (Cholodkovsky, 1915) Scolex semi-bulbate, slightly wider than neck, with oval to slightly wider than neck, with a few superficial grooves on dorsal and ventral margin (Fig. 1E); testes and first vitelline follicles begin just posterior to neck (Fig. 2E).

5a. Anteriormost testes begin posterior to first vitelline follicles (Fig. 2A)

5b. Anteriormost testes begin anterior to first vitelline follicles (Fig. 2B, C)

6a. Scolex slightly tapered anteriorly; neck absent (Fig. 1F, G)

6b. Scolex not tapered anteriorly; neck present (Fig. 1E, N)

7a. First testes and vitelline follicles begin at approximately same level, immediately posterior to neck region (Fig. 2E)

7b. First testes begin posterior to first vitelline follicles

8a. Scolex with anterior margin not curved, expanded laterally (Fig. 1N)

8b. Scolex with round anterior margin and prominent lateral expansions (Fig. 3D, H)

9a. Scolex short, with rounded lateral expansions; neck distinct (Fig. 3D); Khawia parva (Zmeev, 1936) Scolex cuneiform, with almost flabellate, short, laterally expanded, with distinct neck; anteriormost vitelline follicles begin far posterior to anterior edge of scolex; testes begin posterior to first vitelline follicles (Fig. 3D).

9b. Scolex with lateral expansions ("auricular"); neck indistinct (Fig. 3H)

10a. Scolex cuneicrispitate (flabellate with fimbriate anterior margin) (Fig. 1I)

10b. Scolex cuneiform, with fimbriate anterior margin, slightly wider than neck region (Fig. 1M; anteriormost vitelline follicles begin anterior to first testes (Fig. 2I).
Fig. 2. Line drawings of the anterior parts of the Palaearctic species of Caryophyllidea showing the anteriormost vitelline follicles (black) and testes (stippled). A – Atractolytocestus huronensis; B, C – Atractolytocestus sagittatus; D – Archigetes sieboldi; E – Breviscolex orientalis; F – Caryophyllaeides ergens; G – Caryophyllaeides fennica; H – Caryophyllaeus brachycollis; I – Caryophyllaeus fimбриiceps; J – Caryophyllaeus laticeps; K – Paracaryophyllaeus gotoi. Originals. Scale bars: A–C, E–J = 1 mm; D, K = 0.5 mm.
12a. Anterior margin of scolex with numerous, very shallow indentations; scolex about twice as wide as neck region (Fig. 1J). .................................................................

................. Caryophyllaeus laticeps (Pallas, 1781)
Scolex flabellate, anterior margin with numerous, shallow indentations; scolex about twice as long neck region (Figs. 1J, 2J); anteriormost vitelline follicles begin well posterior to front edge of scolex; testes begin well posterior to first vitelline follicles (Fig. 2J).

12b. Anterior margin of scolex with indentations; scolex less than twice as wide as neck region (Fig. 1L) ....

................................. Khawia baltica Szidat, 1942
Scolex flabellate, anterior margin with wrinkles, scolex less than twice as wide as neck region (Fig. 1L); anteriormost vitelline follicles begin well posterior to front edge of scolex; testes begin well posterior to first vitelline follicles (Fig. 3B).

Remarks: K. baltica and C. laticeps are somewhat similar in scolex morphology but they belong to different families (Lytocestidae and Caryophyllaeidae, respectively) and are markedly different in the morphology of genital organs, especially the extent of preovarian uterine loops (much longer in K. baltica compared to that in C. laticeps – see Scholz 1989) and the distribution of vitelline follicles alongside the lateral arms of the ovary (follicles present in the former species versus absent in C. laticeps) (Dubinina 1987, Scholz 1989, Protasova et al. 1990).

DISCUSSION

Scolex morphology and anterior extent of the testes and vitelline follicles of 20 species of caryophyllidean cestodes from cypriniform fishes (Cyprinidae and Cobitidae) in the Palaearctic Region that we consider to be valid were assessed as to their suitability for species identification. A comprehensive data set of three-dimensional characteristics of the scoleces of 18 taxa was provided for the first time using scanning electron microscopy (SEM). The main aim of the present study was to present comparative data on the scolex morphology of these cestodes based on the material processed by the same method (fixation with hot formaldehyde solution (= formalin) that ensures comparability of specimens collected from different hosts in different regions (see Appendix).

In the course of long-term studies on the helminth parasites of freshwater fishes throughout the Palaearctic Region (China, Czech Republic, Finland, Japan, Russia, and Slovakia), an extensive amount of caryophyllidean cestodes was collected and fixed in the same way. Evaluation of this material has revealed that all but one species have a high uniformity in the shape of their scoleces. Therefore, SEM pictures of scoleces of these tapeworms can be considered to document a specific characteristic of caryophyllidean species. The only exception is A. sagittatus from Japan, in which two morphotypes, differing in the shape of the scolex, but also in the size of the body and anteriormost extent of vitelline follicles, were observed.

Further studies are needed to confirm conspecificity of these morphotypes or possible existence of two sibling (cryptic) species. A preliminary analysis of ITS sequences indicated that paralogues are present in this species (D. Tietz and T. Scholz – unpublished data), but more data are necessary for the assessment of possible relationships between the presence of paralogues and different morphotypes (see also Kráľová-Hromadová et al. 2010).

A fairly different situation in scolex variability was observed among specimens from museum collections, which were not fixed with hot formalin. In these specimens, a wide variability in scolex shape and size was observed (e.g., in Khawia armeniaca and Monobothrium wagneri). It remains unclear whether such a higher degree of intraspecific variation reflects natural morphological variability of individual taxa or is mainly influenced by different processing and fixation methods. Based on our experience with several groups of fish cestodes and trematodes as well (Scholz et al. 2001), the latter option seems to be rather probable.

Besides scolex morphology, the present study assessed suitability of the comparative anterior extent of the testes and vitelline follicles for species identification. It has been documented that this feature is of taxonomic value (Kulakovskyay 1961, Kennedy 1965, Mackiewicz 1972, 2003, Dubinina 1987, Scholz 1989, Protasova et al. 1990), but this is the first assessment of its validity in a large sample of cestodes processed with the same method. Unlike scolex shape, the mutual position of the testes and vitelline follicles and their distance from the anterior edge of the scolex may vary to some extent. However, since most species exhibit relatively stable patterns in this characteristic, it thus can be used for identification of most caryophyllidean species from the Palaearctic Region.

An early attempt to propose scolex morphology as a useful tool for classification of caryophyllideans and the identification of species from North America was made by Hunter (1930). Additional scolex traits of use in identifying species parasitizing suckers (Cypriniformes: Catosomatidae) in the Nearctic Region were proposed by Calentine (1962), McCrae (1962), Mackiewicz (1963, 1968, 1972), and Mackiewicz and McCrae (1965). A more extensive comparison of scolex morphology in caryophyllideans showed their considerable morphological diversity and demonstrated dependence of the scolex shape and size on fixation method (Mackiewicz 1972, 1994, Chubb et al. 1987, Protasova et al. 1990).

Detailed information on the scolex morphology of the taxa parasitic in cypriniform fish in Central Europe was provided by Scholz (1989), who also used both light and scanning electron microscopy, as did Ibraheem and Mackiewicz (2006), who described the morphology of the scolex of Wenyonia viridis Woodland, 1923, a parasite of the mochokid catfish Synodontis schall (Bloch et Schneider) from Egypt. However, almost none of the descrip-
tions of caryophyllidean taxa contain scanning electron micrographs of their scoleces.

According to Mackiewicz (2003 – see Table II), two basic scolex types exist among caryophyllideans; 24 of the currently recognized genera (i.e., 59%) possess afossate scoleces and 17 genera (i.e., 41%) possess fossate scoleces. The present study has also demonstrated a great variety of scolex types in caryophyllidean cestodes from the Palaearctic Region, with several scolex types present within two most specious genera, i.e. Khawia Hsü, 1935 and Caryophyllaeus Gmelin, 1790. In these genera, species-specific differences exist mainly in the form or type of scolex margin.

No material was available for Monobothrium auriculatum, but the figure of its scolex was re-drawn from the original description (Kulakovskaya 1961) and the species was included in the key. In contrast, taxa of uncertain taxonomic status or doubtful validity, namely Caryophyllaeus syrdarjensis Skrjabin, 1913 described from the barbel Schizothorax intermedius McClelland from Turkestan,

Fig. 3. Line drawings of the anterior parts of the Palaearctic species of Caryophyllidea showing the anteriormost vitelline follicles (black) and testes (stippled). A – Khawia armeniaca; B – Khawia baltica; C – Khawia japonensis; D – Khawia parva; E – Khawia rossittensis; F – Khawia saurogobi; G – Khawia sinensis; H – Monobothrium auriculatum (according to Kulakovskaya 1961); I – Monobothrium wageneri. Originals except for H. Scale bars = 1 mm.
and two species of *Archigetes* reported mainly from tubificid oligochaetes (Kennedy 1965), were excluded from the present study.

There is virtually no key to all Palaearctic caryophyllidean cestodes because the most comprehensive ones, i.e. those of Kulakovskaya (1961), Dubinina (1987) and Protasova et al. (1990), are outdated and not accessible for everyone (all were written in Russian). In addition, placement of genera to individual families is based on the position of vitelline follicles in relation to the inner longitudinal musculature (Mackiewicz 1994). This requires preparation of good-quality cross-sections, which may impede routine identification. The key to Palaearctic caryophyllideans presented herein is aimed at facilitating routine identification, regardless of family. However, some taxa cannot be differentiated merely on the basis of their scolecæ and anteriormost vitelline follicles and testes and, therefore, other characteristics important for reliable identification of species must be considered. These include, among others, the shape of the ovary (species of *Caryophyllaeidae* and *Khawia*), the anterior extent of the uterus (*Caryophyllaeidae*), size and shape of the cirrus-sac (*Caryophylleaenus*), the number and position of testes (*Atractolytocestus*), and presence/absence of vitelline follicles along ovarian arms (*Khawia*). Despite certain limitations in differentiation of a few taxa possessing a similar scolex, such as *Atractolytocestus* and *Caryophyllaeidae*, the preliminary key may serve as a useful tool for identification of most Palaearctic caryophyllideans. We have found it a helpful tool for species identification and a partial step in the systematic revision of caryophyllidean tapeworms that is in progress.

**APPENDIX**

Many years ago, two of the present authors (T. S. and V. H.) attempted to use specimens fixed in different ways in studies on the taxonomy of cestodes parasitic in freshwater teleost fishes. Different fixatives, such as cold 4% formalin (or 10% formol), ethanol (70%) (including shaking the worms within a vial) and Berland’s fluid were used and tapeworms were fixed while flat or rolled up in alcohol (1965 – p. 441) who claimed “If specimens are allowed to relax for some time in water before fixation the shape of the scolex alters and the bothria become far less evident and may disappear”. Since 1995, the fixation method used by cestologists of the Natural History Museum in Geneva, Switzerland (C. Vaucher and A. de Chamberrier), fixation with hot 4% formalin, has been applied during studies on the systematics of proteocephalidean tapeworms (see Scholz and Hanzelová 1998, Scholz et al. 1998). This method is very simple, suitable even for field sampling, and it has provided by far the best results in our studies on cestodes of freshwater and marine teleost fishes. We have found that samples of tapeworms from the same hosts were always fairly uniform, homogeneous in shape of the body, including the scolex, notwithstanding worms’ size and state of maturity.

This fixation also made it possible to obtain comparable samples of tapeworms collected by different persons and from different hosts and regions. The tegument of these worms, including microtriches and armature of terminal genitalia, remains intact, thus making material suitable for SEM observations (see, e.g., Levron et al. 2008). In addition, tissues of the worms, including parenchymatous organs, are well preserved and thus suitable for staining as permanent mounts, SEM observations (see Scholz et al. 1998, Kuchta et al. 2008) and histological sections (de Chamberrier et al. 2008, 2009).

Suitability of this fixation method has already been documented by a number of taxonomic and morphological studies on proteocephalidean (see, e.g., de Chamberrier and Vaucher 1997, 1999, Scholz and Hanzelová 1998, de Chamberrier et al. 2007, 2008, 2009, de Chamberrier and Scholz 2008, Scholz et al. 2009) and bothriocephalidean cestodes (e.g., Kuchta et al. 2008, 2009a, b). A large-scale sampling of caryophyllidean cestodes in Africa, Europe, Asia (Japan and India) and North America, carried out by the present authors since 1995, has confirmed suitability of this fixation method also for caryophyllideans.

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