***Khawia abbottinae* sp. n. (Cestoda: Caryophyllidea) from the Chinese false gudgeon *Abbottina rivularis* (Cyprinidae: Gobioninae) in China: morphological and molecular data**

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Abstract: *Khawia abbottinae* sp. n. is described from the Chinese false gudgeon, *Abbottina rivularis* (Basilewsky) (Cyprinidae: Gobioninae), from the Yangtze River basin in China. The new species can be distinguished from the congeneric species mainly by the arrangements of the testes, which form two longitudinal bands (other congeneric species have the testes irregularly scattered throughout the testicular region) and their number (at maximum 85 testes versus at least 160 in the other *Khawia* spp.), and the morphology of the scolex, which varies from cuneiform to widely bulbate scolex, being separated from the remaining body by a short neck and possessing a smooth, blunt or rounded anterior margin. Other typical features of *K. abbottinae* are its small size (total length less than 1.5 cm) and body shape, with the maximum width at its first third. The distinct status of the new species was confirmed by molecular data (ssrDNA and ITS1 sequences). Phylogenetic analyses revealed a close relationship of the new species with *K. rossittensis* (Szidat, 1937) and *K. parva* (Zmееv, 1936), parasites of crucian carp and goldfish (*Carassius* spp.), but both species markedly differ from *K. abbottinae* in their morphology. Until now, five valid species of *Khawia* (*K. abbottinae*, *K. japonensis*, *K. rossittensis*, *K. saurogobii* and *K. sinensis*) have been reported from China.

Keywords: Cestoda, *Khawia*, *Abbottina*, Cyprinidae, Yangtze River, 18S rDNA, ITS1

Caryophyllideans represent a unique group among ‘true’ cestodes (Eucestoda) because they possess a non-strobilate and monozoic body, i.e. containing only a single set of male and female reproductive organs (Mackiewicz 1994, Oros et al. 2008). They are supposed to represent the most basal order of the Eucestoda, which would imply tapeworm evolution from simple monozoic to polyzoic, externally segmented (strobilate) forms (Olson et al. 2001, Waeschenbach et al. 2012). Molecular analyses carried out on caryophyllideans have detected some interesting phenomena, such as paralogous structure of nuclear ribosomal spacers and nuclear copies of mitochondrial genes (Brabec et al. 2012), triploid character of some species (Špakulová et al. 2011), divergent intragenomic ribosomal internal transcribed spacers (ITS1 and ITS2 rDNA) along with multiple rDNA loci and dispersed nucleolar organizer regions (NORs) (Kráľová-Hromadová et al. 2012). These findings make caryophyllideans an interesting model for studies of unusual molecular phenomena in parasitic flatworms (Neodermata).

Khawia Hsü, 1935 is a species-rich genus in the order Caryophyllidea, including 17 nominal species parasitizing cyprinid fishes in Europe, Asia, Africa and North America. Scholz et al. (2011) have recently revised the genus, and revealed that only seven species are valid: *Khawia sinensis* Hsü, 1935 (type species); *K. armeniaca* (Cholodkovsky, 1915); *K. baltica* Szidat, 1941; *K. japonensis* (Yamaguti, 1934); *K. parva* (Zmееv, 1936); *K. rossittensis* (Szidat, 1937); and *K. saurogobii* Xi, Oros, Wang, Wu, Gao et Nie, 2009.

Species of *Khawia* exhibit strict (oioxenous or stenoxenous) host specificity, infecting only one or few closely related fish hosts, except *K. armeniaca* that exhibits a wider spectrum of fish definitive hosts (several genera of barbels – Barbinae) and is widely distributed in Africa, Asia and Europe (Scholz et al. 2011).

During the ichthyoparasitological survey in China, caryophyllidean tapeworms were found in the Chinese false gudgeon, *Abbottina rivularis* (Basilewsky) (Cyprinidae, Gobioninae). Morphological characteristics comple-

mented by molecular data (partial sequences of *ssrDNA* and *ITS1*) indicate that these tapeworms represent a new species of *Khawia*. Therefore, this new species is described in the present paper and its phylogenetic relationships to other congeneric species are discussed.

MATERIALS AND METHODS

Between April 2007 and April 2011 several tens of tapeworms were found in the intestine of the Chinese false gudgeon *Abbottina rivularis* from water bodies of the middle and lower reaches of the Yangtze River basin (Niushan Lake in Wuhan, Yangtze River in Wuhan, Hubei Province, and Taihu Lake in Wuxi, Jiangsu Province) in China (Table 1). Tapeworms found were rinsed in saline solution prior to fixation. Specimens used for morphological studies, including observations with scanning electron microscopy (SEM) and histology, were fixed with hot (almost boiling) 4% formaldehyde solution (see Oros et al. 2010). After two weeks, formalin was replaced by 70–75% ethanol for storage before further processing. Specimens used for molecular studies were fixed and stored in 100% ethanol.

For light microscopical observations, specimens were stained with iron hydrochloric carmine, destained in 70% acid ethanol, dehydrated in an alcohol series, cleared in clove oil and mounted in Canada balsam as permanent preparations. Illustrations were made using a drawing attachment of a Leica DM 5000 B microscope with the use of Nomarski differential interference contrast. Measurements were taken using the Tsview 7.1 program (Tucsen, China) and LAS V3.8lnk program (Leica, Switzerland). Histological sections (cross, longitudinal and sagittal sections) were made from hot formalin-fixed specimens using standard protocols (thickness of sections 10–12 µm), stained with hematoxylin and eosin, and mounted in Canada balsam. Four specimens used for SEM observations were processed as described by Oros et al. (2010). Measurements are in micrometres (µm) unless otherwise indicated.

Genomic DNA of specimens from Taihu Lake (collection numbers TaiL201104, TaiL200401) was extracted using QIAamp DNA kit (QIAGEN). The small nuclear ribosomal RNA subunit (*ssrDNA* or 18S rDNA) was amplified by PCR using primer pairs WormA and WormB (Littlewood and Olson 2001). Each PCR reaction consisted of 20 pmol of each primer, 5 µl 10× Ex Taq buffer, 250 µM of each dNTP, 1 U of Takara Ex Taq™, 2 µl DNA template and nuclease-free water to 25 µl volume. Amplifications were performed on Eppendorf Mastercycler with an initial denaturation step of 95 °C for 3 min followed by 30 cycles of 95 °C for 1 min, 56 °C for 45 sec and 72 °C for 1 min, and final extension of 72 °C for 10 min.

Amplified target products were purified by Takara Agarose Gel DNA Purification Kit and cloned into pMD-18T vector system (Takara). One positive clone from each worm was selected randomly. In total, four clones were sequenced using vector universal primers M13+, M13-, and internal primers 600F, 1600R (Littlewood and Olson 2001, Scholz et al. 2011) on the ABI BigDye Terminator 3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer automated DNA sequencer (Applied Biosystems).

The internal transcribed spacer 1 (*ITS1*) of ribosomal DNA of tapeworm *Khawia* was amplified with primer pairs 18S-F (CGTCCCTGCCCTTTGTA) and 5.8S-R (GGCTGCGCTCTCATCGA), designed according to the previous sequences in GenBank. The amplification and sequencing were similar to

the above procedure, except for the annealing temperature having been reduced to 52 °C, and three clones of each species of *Khawia* available having been selected for sequencing.

The *ssrDNA* and *ITS1* sequences were assembled and inspected using SeqMan™ (DNASTAR, Inc.) and aligned with available sequences of caryophyllideans in GenBank using the program MEGA5.1 with default setting (Tamura et al. 2011). The ambiguously aligned positions were excluded from subsequent phylogenetic analyses based on minimum evolution (ME), maximum likelihood (ML) and Bayesian inference (BI) criteria.

The appropriate nucleotide substitution model was estimated in MEGA 5.0. The phylogenetic trees based on 18S rDNA were analyzed by ME with Tamura-Nei + G model and ML with TN93+G+I model methods in MEGA 5.0. BI analyses were conducted in MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003), employing TN93+G+I model, running two independent MCMCMC runs of four chains for one million generations and sampling tree topologies every 100 generations. Posterior probability values were used as support for the Bayesian topology. In the case of *ITS1* of *Khawia*, the phylogenetic trees were analyzed by ME using Tamura-Nei model, ML and BI using HKY+I model.

RESULTS

Khawia abbottinae sp. n.

Figs. 1, 2

Description (based on 15 whole-mounted specimens, five slides of cross-sections and five slides of sagittal and longitudinal sections, from *Abbottina rivularis* from Taihu Lake in Wuxi and from the Yangtze River in Wuhan): Caryophyllidea, Lytocestidae. Testes and ovary medullary, vitelline follicles cortical. Body elongate to almost spindle-shaped, with relatively narrow scolex and neck, maximum width at anterior third, then slightly narrowing continuously towards rounded posterior end. Total length of body 6.8–13.0 mm, maximum width 0.8–2.0 mm (n = 9). Surface uniformly covered with acicular filitriches. Inner longitudinal musculature well developed, formed by small bundles of muscle fibers. Osmoregulatory canals about 6–8 pairs, external to vitelline follicles.

Scolex afossate, widely bulbate to almost cuneiform, 0.4–0.6 mm long and 0.7–1.5 mm wide, wider than neck, with smooth, blunt or rounded anterior margin. Neck distinct, 0.5–0.8 mm wide (n = 10).

Testes medullary, 62–84 in number (n = 8), almost spherical, 118–228 × 105–180 (n = 30), distributed in two more or less regular lateral rows; anteriormost testes begin at short distance, 0.7–1.2 mm, posterior to anterior margin of scolex and 0.3–0.6 mm (n = 10) posterior to anteriormost vitelline follicles. Posteriorly, testes reach to anterior margin of cirrus-sac. Cirrus-sac small, oval to almost spherical, 285–470 × 220–360 (n = 6), length/width ratio 1.3 : 1 (n = 6). External and internal seminal vesicle absent. Male genital pore separated from female pore, open into common genital atrium on ventral surface.

Ovary reticulate, medullary, H-shaped, with narrow and long ovarian arms, almost equal in length, 0.7–1.9 mm

Fig. 1. *Khawia abbottinae* sp. n. from *Abbottina rivularis* in China. **A, B** – scolices; **C** – cross section of testicular region; **D** – terminal genitalia, ventral view, eggs illustrated only in sinistral loop; **E** – posterior part, ventral view; **F** – sagittal section of uterine region, note common genital atrium; **G** – longitudinal section of uterine region, note testes in two longitudinal rows and uterine glands, only lateral vitelline follicles visible; **H** – outline of mature specimen with two rows of testes, cirrus-sac and ovary; **I** – outline of immature specimen with two rows of testes. *Abbreviations:* ca – common genital atrium; cs – cirrus-sac; eg – eggs; im – inner longitudinal musculature; ov – ovary; sr – seminal receptacle; te – testes; ug – uterine glands; ut – uterus; va – vagina; vf – vitelline follicles.

long, posterior arms may slightly bent inwards; total width of ovary 0.5–0.7 mm (n = 6). Vagina tubular, slightly sinuous. Seminal receptacle elongate, dorsal to

ovarian isthmus, 100 long and 44 wide (n = 1). Vagina joins terminal part of uterus to form short utero-vaginal duct that opens posterior to male pore in common genital

Fig. 2. *Khawia abbottinae* sp. n. from *Abbottina rivularis* in China. Scanning electron micrographs (SEM). A–C – scolices; D, E – genital pores; F – acicular filitriches from the middle part of the body. Abbreviations: c – cirrus; ca – common genital atrium; mg – male gonopore; uvp – uterovaginal pore.

atrium. Vitellarium in cortical parenchyma. Vitelline follicles lateral and median, widely oval, $65\text{--}130 \times 53\text{--}120$ ($n = 30$), begin 0.4–0.6 mm posterior to anterior extremity, anterior to first testes, reach posteriorly to anterior margin of ovary, absent alongside ovarian arms; postovarian follicles numerous. Uterus strongly coiled, between ovary and cirrus-sac, never extends anterior to cirrus-sac; uterine glands present. Postovarian region short, 0.9–1.7 long. Eggs operculate, without formed oncosphere in uterus, oval, $31\text{--}44 \times 23\text{--}33$ ($n = 30$, intrauterine eggs on whole-mounts).

Type host: *Abbottina rivularis* (Basilewsky) (Cyprinidae, Gobioninae).

Type locality: Yangtze River in Wuhan (Baishazhou), Hubei Province ($30^{\circ}38'\text{--}30^{\circ}50'\text{N}$, $114^{\circ}15'\text{--}114^{\circ}28'\text{E}$), China.

Other localities: Niushan Lake in Wuhan (Liufang), Hubei Province ($30^{\circ}16'\text{--}30^{\circ}34'\text{N}$, $114^{\circ}43'\text{--}114^{\circ}59'\text{E}$), located at the middle reach of the Yangtze River basin, China; Taihu Lake in Wuxi (Meiliangwan), Jiangsu Province ($30^{\circ}93'\text{--}31^{\circ}48'\text{N}$, $119^{\circ}92'\text{--}120^{\circ}30'\text{E}$), located at the lower reach of the Yangtze River basin, China.

Site of infection: Anterior intestine.

Prevalence and intensity of infection: Taihu Lake – 31% ($n = 45$; intensity of infection 2–10); Niushan Lake – 12% ($n = 100$; 1–5); more detailed data in Table 1.

Deposition of specimens: Holotype (complete, whole-mounted specimen collected on 24 March 2009; host field number 241/09WU), two paratypes (whole-mounts, host field numbers 224/09WU and 238/09WU) and 16 vouchers (four whole-mounts, four cross sections and eight

sagittal and longitudinal sections) from the Yangtze River in Wuhan in the helminthological collection of the Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic (IPCAS C-629); 12 paratypes (ten whole-mounts and two cross sections) from the Yangtze River in Wuhan and Taihu Lake, in the Museum of Aquatic Organisms, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China (IHMM201207); two paratypes (whole-mounts, host field numbers 231/09WU and 236/09WU) and one voucher (cross section) from the Yangtze River in Wuhan, in the Natural History Museum London, UK (BMNH 2012.11.23.1–3); two paratypes (whole-mounts, host field numbers 230/09WU and 233/09WU) and one voucher (cross sections) from the Yangtze River in Wuhan, in the U.S. National Parasite Collection, Beltsville, Maryland, USA (USNPC 106204–106206); two paratypes (whole-mounts, host field numbers 38/10WU and 40/10WU) from the Taihu Lake in Wuxi, in the Natural History Museum, Geneva, Switzerland (MHNG-PLAT-82474–82476).

E t y m o l o g y: The species name is derived (as genitive) from the generic name of the definitive host.

Remarks. *Khawia abbottinae* sp. n. is placed in *Khawia* because it has the diagnostic characters of this genus (Scholz et al. 2011), including scolex lacking apical structures (afossate) with smooth anterior margin, cortical vitelline follicles and a relatively long uterine region, i.e. long distance between the cirrus-sac and ovary. The generic placement of the new species in *Khawia* is strongly supported by molecular data (Figs. 3, 4). The new species differs from the remaining species of *Khawia* in

Fig. 3. Phylogenetic relationships (Bayesian inference) within the Caryophyllidae based on the small nuclear ribosomal RNA subunit (ssrDNA) sequences with definitive host groups of the main clades indicated (orders and families of type hosts). Numbers at the branches (from left to right) are posterior probability for Bayesian inference (BI), bootstrap values of 1000 replicates for maximum likelihood (ML) and minimum evolution (ME). Scale bar = 0.01 substitutions per site. Sequences of *Khawia abbottinae* sp. n. in bold.

the following diagnostic characteristics (see Scholz et al. 2011 for morphological descriptions of all valid species and a key to their identification):

(1) *Arrangements of the testes and their number.* In *K. abbottinae*, the testes form two longitudinal bands throughout the whole testicular region (Fig. 1G,H,I), each band being usually formed by a single testis; none of the other species of *Khawia* have such an arrangement of the testes (in these species testes are distributed irregularly in the medulla, especially in its middle and posterior portion; e.g. Fig. 2 in Oros and Hanzelová 2007 and Figs. 1G,E and 7A in Scholz et al. 2011); in addition, the testes are fewer in the new species (at maximum 85), whereas the other species of *Khawia* possess 160–750 testes (Scholz et al. 2011).

(2) *Scolex morphology.* The scoleces of *K. abbottinae* vary from cuneiform to widely bulbate, and are separated from the remaining part of body by a short neck; they possess a smooth, bluntly ended or rounded anterior margin; none of congeneric species has a similar scolex (Oros and Hanzelová 2007, Oros et al. 2010, Scholz et al. 2011).

(3) Other typical features of *K. abbottinae* are its small size (total length less than 1.5 cm) and body shape. All species of *Khawia* are larger, *K. sinensis*, *K. armeniaca*, *K. baltica* and *K. parva* conspicuously so, with the maximum body length from 2 cm in *K. saurogobii* up to 11 cm in *K. sinensis*.

Khawia abbottinae most closely resembles *K. saurogobii* in the shape of the body, which also occurs in gudgeons (subfamily Gobioninae) in China and shares a very short scolex and neck, and vitelline follicles beginning just posterior to the neck (Xi et al. 2009a, Scholz et al. 2011). Both species can be differentiated from each other, besides the diagnostic characteristics listed above, by the shape of the ovary (inverted A-shaped in *K. saurogobii* versus H-shaped in *K. abbottinae*), the distribution of the vitelline follicles, which are absent alongside the whole uterine region in the former species, but present alongside the preovarian loops of the uterus in the latter, and by the shape of the scolex, which is cuneiform-flabellate in *K. saurogobii* (versus bulbate to cuneiform in *K. abbottinae*; see Xi et al. 2009a and Scholz et al. 2011).

In the shape of the body and scolex, *K. abbottinae* is somewhat similar to *Breviscolex orientalis* Kulakovskaya, 1962, a parasite of the spotted steed *Hemibarbus maculatus* Bleeker and Cherskii's thicklip gudgeon *Sarcocheilichthys czerskii* (Berg) (both Gobioninae) from the Amur River basin in Russia (Far East) and Japan (Kulakovskaya 1962, Wang 1984, Scholz et al. 2001). However, the latter cestode species belongs in the family Capingentidae, because it possesses paramuscular vitelline follicles (Mackiewicz 1994); it also differs from *K. abbottinae* in the shape of the ovary (*B. orientalis* has short and wide arms), the presence of vitelline follicles alongside the

Fig. 4. Phylogenetic relationships (Bayesian inference) within *Khawia* based on the internal transcribed spacer 1 (ITS1) of ribosomal DNA sequences. Numbers at the branches (from left to right) are posterior probability for Bayesian inference (BI), bootstrap values of 1000 replicates for maximum likelihood (ML) and minimum evolution (ME). Scale bar = 0.05 substitutions per site. Sequences of *Khawia abbottinae* sp. n. in bold.

ovary, a much longer postovarian region, indistinct neck, and more anterior position of the testes, which may begin even anterior to the first vitelline follicles (see Fig. 4 in Scholz et al. 2001).

Specimens of *K. abbottinae* studied ($n = 25$) are phenotypically rather uniform and only slight variation was observed in the shape of the scolex and ovary (Figs. 1A,B and 2A–C), which apparently reflects intraspecific variability.

To facilitate the differential diagnosis of species in the genus *Khawia*, a simplified key following Oros et al. (2010) and Scholz et al. (2011) is provided, with inclusion of the newly described species *K. abbottinae*.

- 1 Ovary H- or inverted A-shaped, with long, relatively narrow lateral arms 2
- Ovary butterfly-shaped, with short and wide lateral arms *Khawia armeniaca* (Cholodkovsky, 1915)
- 2 Scolex fimbriated, with lobes on the front edge 3
- Scolex cuneiform or flabellate, without fimbriae or lobes on its front edges 4
- 3 Scolex festoon-shaped, with deep folds, markedly wider than the neck; testes begin posterior to vitelline follicles *Khawia sinensis* Hsü, 1935
- Scolex cuneifimbriate, with cresases formed by small lobes on the front edge; testes begin almost at the same level as vitelline follicles *Khawia japonensis* (Yamaguti, 1934)
- 4 Scolex flabellate, with distinct wrinkles along the front edge; vitelline follicles present alongside ovarian arms *Khawia baltica* Szidat, 1941
- Scolex cuneiform, without distinct wrinkles; vitelline follicles absent alongside ovarian arms 5
- 5 Vitelline follicles absent alongside uterine loops *Khawia saurogobii* Xi, Oros, Wang, Wu, Gao et Nie, 2009.

- Vitelline follicles present alongside uterine loops ... 6
- 6 Ovary with posterior ovarian arms bent inwards or inverted A-shaped .. *Khawia rossittensis* (Szidat, 1937)
- Ovary H-shaped or posterior ovarian arms slightly bent inwards 7
- 7 Testes distributed irregularly in the medulla *Khawia parva* (Zmeev, 1936)
- Testes distributed in two more or less longitudinal bands *Khawia abbottinae* sp. n.

Molecular data. Nearly complete ssrDNA sequences for *K. abbottinae* (2101 base pairs long, GenBank Accession Nos. JX661268–JX661270) were obtained. Among the four clones sequenced, three haplotypes were detected with only 5 variable sites (i.e. 0.2% sequence variability). The ssrDNA of *K. abbottinae* was most similar to that of *K. parva*, *K. japonensis* and *K. rossittensis*, with the sequence identity of 99.5%, 99.4%, 99.1%, respectively. It showed 98.2% similarity with that of *K. sinensis* and *K. saurogobii*, 97.1% with *K. armeniaca* and 96.7% with *K. baltica* (Fig. 3). In the cladogram constructed using ME, ML and BI methods, *K. abbottinae* clustered within *Khawia*, forming a reasonably well supported clade comprised of *K. parva* and *K. rossittensis* (Fig. 3).

ITS1 sequences for *K. abbottinae* (1090–1099 bp long), *K. japonensis* (986–1010 bp), *K. rossittensis* (1089–1092 bp), *K. saurogobii* (979–981 bp) and *K. sinensis* (983–987 bp) were obtained and deposited in GenBank under accession numbers JX661271–JX661285. The sequence pairwise distance analysis (p-distance) showed sequence similarity of 93.7% between *K. abbottinae* and *K. rossittensis*. Similarity between *K. abbottinae* and *K. japonensis*, *K. saurogobii* and *K. sinensis* were 80.0%, 67.9% and 67.8%, respectively. The phylogenetic analysis based on ITS1 sequences, using ME, ML and BI criteria, revealed a similar topology with 100% support value as that inferred from ssrDNA sequences, except for the absence of data on *K. parva*. *K. abbottinae* formed a clade with *K. rossittensis*, whereas *K. japonensis* represented its sister group (Fig. 4).

Comparisons of the new sequences of *Khawia* species with those of selected caryophyllideans retrieved from the GenBank database have shown that *Khawia* spp. do not form a monophyletic assemblage because *K. baltica*, a specific parasite of tench [*Tinca tinca* (Linnaeus)], clustered with the species of *Caryophyllaeus* Gmelin, 1790 (family Caryophyllaeidae) (Fig. 3). In contrast, the remaining members of *Khawia*, including *K. abbottinae*, form a monophyletic, well supported clade, distant from the clades composed of species of the other genera, such as *Atractolytocestus* Anthony, 1958, *Caryophyllaeides* Nybelin, 1922 (both Lytocestidae), *Breviscolex* Kulakovskaya, 1962 (Capingentidae) and *Caryophyllaeus* (Fig. 4).

DISCUSSION

Khawia abbottinae is considered the eighth valid species of the genus, but the second one described from a gudgeon (Gobioninae). Except for *K. saurogobii*, all the remaining species of *Khawia* parasitize fishes of other subfamilies (Cyprininae and Barbinae). In its morphology, the new species resembles *K. saurogobii* from the Chinese lizard gudgeon in the body and, to some extent, scolex morphology, both species possessing a very short scolex and neck, with first vitelline follicles beginning immediately after the neck. However, other morphological characteristics, such as the arrangement of the testes and vitelline follicles, and shape of the ovary, are markedly different. In addition, molecular data provide strong evidence that these species parasitic in related hosts are not closely related phylogenetically.

Khawia abbottinae is a relatively common parasite of the Chinese false gudgeon (*Abbottina rivularis*), with an overall prevalence of 28% (Table 1). It has been found in several localities of the Yangtze River basin. The Chinese false gudgeon is small (maximum body length of 10 cm), benthopelagic cyprinid fish of the subfamily Gobioninae. It feeds on larvae of chironomids, oligochaetes and plant seeds, and is even considered as pest in fish farms in China, where it competes with the juveniles of commercial species (Yan and Chen 2007). It has a broad native range, being widely distributed from the Russian Far East in the north, through China, western Korea and Japan to North Vietnam (Yan and Chen 2007).

Phylogenetic analyses invariably placed *K. abbottinae* very close to *K. rossittensis* (sequences of the ssrDNA and ITS1) and *K. parva* (ssrDNA; ITS1 sequences not available), both species parasitizing crussian carp and goldfish (*Carassius* spp.) in the Palearctic Region, the former reported from Europe and Japan and the latter endemic to the Russian Far East (Oros and Hanzelová 2007, Scholz et al. 2011). Morphologically, all three taxa are dissimilar, *K. parva* differing from *K. abbottinae* in the shape of the body and its much bigger size, markedly different shape of the scolex, much more posterior position of the first vitelline follicles and testes, the latter being more numerous and irregularly arranged, and shape of the ovary, with the posterior arms bent inwards. *Khawia rossittensis* has a different shape of the body, which is longer and more slender and the posterior (postovarian) part of which is pointed, inverted A-shaped ovary, more numerous, irregularly arranged testes, and a different scolex (Scholz et al. 2011).

The recent revision of *Khawia* has revealed that four species occur in China, namely *K. japonensis*, *K. rossittensis*, *K. saurogobii* and *K. sinensis*. Tapeworms described from common carp by Li (1964) and Liu et al. (1995), i.e. *Tsengia cyprini* Li, 1964 and *T. neimongkuensis* Li, 1964, from Wulasuhai Lake in Inner Mongolia, and *T. xiamenensis* Liu, Yang et Lin, 1995 from Xiamen, China,

Table 1. Prevalence and intensity of infection of *Khawia abbottinae* sp. n. from *Abbottina rivularis* from the Yangtze River basin, China.

Month	Year	Locality	No. Fish examined	No. Fish infected	Prevalence	Intensity of infection
April	2007	Niushan Lake	100	12	12%	1–5
March	2009	Yangtze River	31	20	65%	1–6
March	2010	Taihu Lake	26	16	62%	1–10
April	2010	Taihu Lake	24	12	50%	1–8
April	2011	Taihu Lake	95	17	18%	2–7
Total			276	77	28%	1–10

were synonymized with *Khawia japonensis* and *K. sinensis*, respectively (for details see Scholz et al. 2011). *Khawia tenuicollis* Li, 1964, described from common carp in Wulasuhai Lake, was transferred to *Atractolytocestus* Anthony, 1958 by Xi et al. (2009b). Thus, *K. abbottinae* is the fifth species of the genus occurring in China.

Cheng (1994) proposed the name *Tsengia tangi* Cheng, 1994 for tapeworms found in goldfish [*Carassius auratus auratus* (Linnaeus)], and then transferred this species into *Caryophyllaeides*, as *Caryophyllaeides tangi* (see Yang 2007). However, this taxon was originally described in an unpublished PhD thesis and is thus invalid according to the section 9.7 in Article 9 of the International Code of Zoological Nomenclature (fourth edition, 1999, www.nhm.ac.uk/hosted-sites/iczn/code/).

In addition, tapeworms named as *T. tangi* are morphologically indistinguishable from *Khawia rossittensis*, with which they share the characteristics considered to be species-specific, i.e. a cuneiform scolex, first vitelline follicles situated just posterior to the scolex, testes beginning posterior to the first vitelline follicles, and the posterior ovarian arms bent inwards or joined together, thus making the ovary inverted A-shaped (Scholz et al. 2011). Based on the similar morphology and the identical fish host, the present authors considered that the tapeworms described by Cheng (1994) should be *K. rossittensis*.

A comparative study based on three-dimensional scanning electron micrographs (SEM) and outlines of the scolexes of 19 Palearctic species of caryophyllidean cestodes has revealed that the highest diversity in scolex morphology exists among species of *Khawia* (Oros et al. 2010). Despite slight variation in the morphology of the scolexes observed in live specimens, *K. abbottinae* is characterized by a widely bulbate to cuneiform scolex, wider than the neck, with smooth anterior margin.

The present molecular data inferred from nearly complete ssrDNA and ITS1 sequences clearly delineate *K. abbottinae* as a distinct species of *Khawia*. The topology of the tree inferred from ssrDNA sequences has also indicated that this genus, which belongs to the family Lytocestidae, is polyphyletic. Whereas all but one species form a monophyletic group, *K. baltica* represents a sister group of *Caryophyllaeus* of another family, Caryophyllaeidae.

Thus, the generic placement of *K. baltica* should be re-addressed, but molecular data on a more comprehensive data set of caryophyllidean cestodes should be available before any taxonomic action is taken (Brabec et al. 2012).

In addition, China hosts a great diversity of cyprinid fauna, and there are about 560 cyprinid species inhabiting in the rivers and lakes (Chen 1998). However, the parasite fauna of most cyprinids has not been well investigated. Recent descriptions of two new species of caryophyllideans (Xi et al. 2009a, the present paper) indicate that the cestode fauna of freshwater fishes in China is still insufficiently known, and future surveys of fish parasites will certainly discover more taxa.

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