Research Article

The diversity of *Khawia* tapeworms (Cestoda: Caryophyllidea) of barbels (Cyprinidae: Barbinae) in the western Palearctic: the existence of vicariant lineages and the description of a new species from the Iberian Peninsula

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Abstract

The caryophyllidean tapeworm *Khawia armeniaca* has long been regarded as an exceptionally widespread species within its genus, notable for its significant morphological variability. However, with the accumulation of molecular data from different fish hosts, *K. armeniaca* was suspected to represent a species complex. To clarify the true identity of these parasites, a comprehensive morphological and molecular study (using 18S, 28S and ITS2 ribosomal regions) of *K. armeniaca* tapeworms from barbels (Barbinae) across the Iberian Peninsula and the Middle East has been conducted. The results revealed two genetically distinct lineages within the *K. armeniaca* complex. The first lineage, found in *Arabibarbus grypus*, *Barbus lacerta*, *Capoeta birunii*, *Carassobarbus luteus*, *Luciobarbus barbulus*, *L. esocinus* and *L. kersin* in Iraq and Iran, is genetically congruent with *K. armeniaca* (Cholodkovsky, 1915), originally described from the Sevan khramulya (*Capoeta sevangi*) in Armenia. The second lineage, identified in *Luciobarbus bocagei* (type host), *L. comizo* and *L. guiraonis* from Portugal and Spain, is described as *Khawia iberica* n. sp. In addition to clear molecular divergence, *K. iberica* can be distinguished from *K. armeniaca* by notable morphological differences, including variations in the shape, structure and size of the ovary, the anterior extension of the vitelline follicles, the testes and several morphometric parameters.

Introduction

The order Caryophyllidea Van Beneden in Carus, 1863 represents a unique group of tapeworms, distinguished from other 'true tapeworms' within the subclass Eucestoda by a number of distinct characteristics, especially their monozoic body (i.e. non-proglottized, unsegmented). Phylogenetic analyses indicate that caryophyllidean tapeworms form the earliest diverging lineage within the Eucestoda (Waeschenbach et al., 2012). Tapeworms of the order Caryophyllidea primarily parasitize fish of the orders Cypriniformes and Siluriformes and are found across all zoogeographical regions, with the exception of the Neotropical region and Antarctica (Mackiewicz, 1972; Scholz and Oros, 2017).

Since its establishment in 1935, a total of 21 species have been assigned to the genus *Khawia* Hsü, 1935, which is among the most species-rich genera within the order Caryophyllidea. However, the validity of many of these species was reassessed in a comprehensive taxonomic revision by Scholz et al. (2011). This revision resulted in the synonymy of several taxa and, together with the reclassification of *K. baltica* by Barčák et al. (2017), resulted in a substantially reduced number of valid species. Currently, only seven species are recognized as valid (Table 1). Although the genus *Khawia* was historically classified under the family Lytocestidae due to the cortical distribution of vitelline follicles in the parenchyma, this morphology-based classification has recently been reassessed (Scholz et al., 2021) and the genus is currently placed in the family Caryophyllaeidae.

Species of the genus *Khawia* are found in Europe, Asia, Africa and North America (introduced with common carp), primarily in cypriniform fish. They typically infect one or a few closely related fish species and are thus considered host-specific (Scholz et al., 2011). A notable exception is *K. armeniaca*, which has been recorded in several species of barbels, particularly of

Table 1. List of species of *Khawia* Hsü, 1935 (Cestoda: Caryophyllidea) currently recognized as valid, with their synonyms and fish hosts

Species	Synonyms	Hosts
K. abbottinae Xi, Oros, Wang, Scholz et Xie, 2013	–	*Abbottina rivularis*
K. armeniaca (Cholodkovsky, 1915) Shulman, 1958 *sensu lato*	*Caryophyllaeus armeniacus* Cholodkovsky, 1915	various cyprinids
	Khawia barbi Rahemo and Mohammad, 2002	
	Khawia lutei Al-Kalak et Rahemo, 2003	
K. japonensis (Yamaguti, 1934) Hsü, 1935	*Caryophyllaeus japonensis* Yamaguti, 1934	*Cyprinus carpio*
	Bothrioscolex japonensis (Yamaguti, 1934) Szidat, 1937	
	Khawia iowensis Calentine et Ulmer, 1961	
	Khawia cyprini Li, 1964	
K. parva (Zmeev, 1936) Kulakovskaya, 1961	*Caryophyllaeus parvus* Zmeev, 1936	*Carassius gibelio*
K. rossittensis (Szidat, 1937) Markevich, 1951	*Bothrioscolex rossittensis* Szidat, 1937	*Carassius auratus*
	Bothrioscolex dubius Szidat, 1937	
K. saurogobii Xi, Oros, Wang, Wu, Gao et Nie, 2009	–	*Saurogobio dabryi*
K. sinensis Hsü 1935[a]	*Tsengia neimongkuensis* Li, 1964	*Cyprinus carpio, Hemibarbus barbus*
	Tsengia xiamenensis Liu, Yang et Lin, 1995	

[a]Type species.

the genera *Barbus, Capoeta, Carasobarbus* and *Luciobarbus. Khawia armeniaca* was first discovered in Lake Sevan, Armenia, and described as *Caryophyllaeus armeniacus* by Cholodkovsky (1915). The original description is relatively brief and lacks any illustrations, with the host species identified only as *Capoeta*. According to Popov (1924), this host was almost certainly *C. sevangi* (the taxonomy of the genus *Capoeta* herein follows the classification by Levin et al. (2012) and Zareian et al. (2018)). Furthermore, the type material of the new species has not been deposited and does not exist.

A more detailed description of *K. armeniaca* was provided by Popov (1924), who found the parasite in the intestines of *Varicorhinus capoeta sevangi* (= *C. sevangi*) and *Salmo ischchan* at the type locality (Lake Sevan) and the Hrazdan River (Zanga), which flows into the lake. Shulman (1958) later moved this species to the genus *Khawia*, although he mentioned this taxonomic change only in a footnote. Subsequently, Kulakovskaya (1961), in her extensive revision of the order Caryophyllidea in the USSR, also classified this species under the genus *Khawia*. Kulakovskaya (1961) assumed that this species was endemic to Lake Sevan, but Mikailov (1975) found it in Azerbaijan in *V. capoeta capoeta* (= *C. capoeta*), and Paperna (1964) reported it in Israel in *Barbus longiceps*. Williams et al. (1980) later documented its presence in Iran, providing a description with illustrations and were the first to suggest that *K. armeniaca* has a much broader range than previously thought.

Khawia armeniaca is the species with the widest geographical distribution within its genus, spanning three continents and two zoogeographical regions: the Palearctic and Afrotropical zones (Scholz et al., 2011). Recorded occurrences include the Transcaucasus, where the type locality is situated, southwestern Europe, and parts of Africa. This extensive distribution is also associated with a broad host range, encompassing 20 host species across eight genera of fish. This tapeworm species exhibits remarkable morphological variability in characteristics associated with the anterior part of the body, including the shape of the scolex, the anterior extent of the testes and vitelline follicles, the number of

vitelline follicles lateral to the ovary, and the number and extent of post-ovarian vitelline follicles (Oros et al., 2010; Scholz et al., 2011; Kibet et al., 2021). However, the authors note that these characteristics are not consistent within individual localities and cannot be used to unequivocally distinguish separate morphotypes. Therefore, these features are considered intraspecific variability within a morphologically polymorphic species, likely due to its broad host range and geographical distribution.

In the revision of the genus *Khawia*, Scholz et al. (2011) considered a typical feature of *K. armeniaca*, differentiating it from other congeners, a butterfly-shaped ovary with short, broad, almost oval lateral wings, a low number of post-ovarian follicles with few or no follicles alongside the posterior arms of the ovary, an oval to spherical cirrus sac, and a simple scolex with a smooth anterior margin.

Considering the remarkable host and geographical range of *K. armeniaca*, and high putative intraspecific morphological variability in important taxonomic features, the aims of this study were (1) to examine the magnitude of intraspecific morphological variability in *K. armeniaca* from different fish hosts from two distant geographical regions, (2) to assess genetic diversity among these specimens and (3) to evaluate the host range and geographical distribution of *K. armeniaca* in the western Palearctic.

Material and methods

Material collection

Tapeworms used in this study were obtained from 15 host species collected at 16 localities in seven countries (Table 2, Figure 1) by the present authors and their collaborators. The material originating prior to year 2011 was retrieved from the Helminthological collection of the Institute of Parasitology of the Biology Centre of the Czech Academy of Sciences in České Budějovice (IPCAS). Later material was collected from either freshly caught fish specimens or from fish specimens obtained from the market. Prior to parasitological dissection, live fish were kept in aerated holding

Table 2. List of specimens studied with their hosts, countries of origin and localities

Host species	Country	Locality	Coordinates		Year of collection
Arabibarbus grypus	Iran	1. Kashkan River, Mamulan	33.44228	47.93289	2022
	Iraq	2. Great Zab River, Aski Kalak	36.26851	43.64108	2011
Barbus lacerta	Iraq	3. Tabin River, Kani Shok	35.83361	45.10444	2021
Capoeta birunii	Iran	4. Zayanderud River, Champir	32.41955	51.12712	2022
Carasobarbus luteus	Iraq	5. Tabin River, Zahrzī	35.80889	45.02222	2021
Labeobarbus bynni[a]	Egypt	6. Nile River, Cairo	29.83897	31.29198	1971
Labeobarbus crassibarbis[a]	Ethiopia	7. Tana Lake	11.65120	37.32905	2007
Labeobarbus intermedius[a]	Ethiopia	7. Tana Lake	11.65120	37.32905	2006
Luciobarbus barbulus	Iraq	N/A			2001
	Iraq	8. Aw-e Shiler River, Sharbazher	35.76361	45.45333	2021
Luciobarbus bocagei	Portugal	9. Este River, Porto	41.37247	–8.70631	2009
	Portugal	10. Colares River, Sintra	38.79816	–9.43727	2016
	Spain	11. Orbigo River, Leon	42.50050	–5.87685	1973
Luciobarbus callensis[a]	Morocco	12. Azrou	33.46234	–5.24605	–
Luciobarbus comizo	Spain	13. Zújar River	38.45334	–5.53324	2017
Luciobarbus esocinus	Iraq	14. Great Zab River, Gwer	36.04818	43.49238	2011
Luciobarbus guiraonis	Spain	15. Magro River	39.35716	–0.66438	2016
Luciobarbus kersin	Iraq	2. Great Zab River, Aski Kalak	36.26851	43.64108	2011
Mastacembelus mastacembelus[b]	Iraq	14. Great Zab River, Gwer	36.04818	43.49238	2011

[a]Excluded due to poor condition.
[b]Postcyclic host.

barrels with river water from the collection site. Fish were anaesthetized and then sacrificed by severing the spinal cord. Fish were dissected using the standard method (Chervy, 2024), and intestinal tracts were examined under a stereomicroscope in order to collect *Khawia* specimens. The tapeworms collected by the present authors were rinsed, killed with boiling saline and then fixed in 70% ethanol for subsequent morphological and molecular analyses.

Due to poor quality which prevented reliable measurements (see Remarks to *K. armeniaca* below), some of the mounted specimens were excluded from the study, therefore reducing the available dataset to 11 host species from 14 localities and 4 countries (i.e. Iran, Iraq, Spain and Portugal). In total, 35 specimens were analysed morphologically and 22 molecularly.

### DNA amplification, sequence analysis and phylogenetic analyses

The extraction of genomic DNA was performed using a commercially produced extraction kit (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) following the manufacturer's protocol. Three genomic DNA regions were amplified: partial gene coding large ribosomal subunit (hereinafter referred to as 28S), partial gene coding small ribosomal subunit (18S) and entire ITS2 region (ITS2). A list of primers and thermocycling conditions are provided in Supplementary Table S1. Polymerase chain reactions (PCRs) were carried out in a total volume of 20 μL containing 10 μL Taq DNA PCR MasterMix Kit (Qiagen, Hilden, Germany), 0.5 μM of each primer, 8 μL nuclease-free water, and 1 μL of DNA template

(corresponding to approximately 20 ng of DNA). The PCR products were checked on 1% agarose gel and purified using EPPiC Fast (Amplia, Bratislava, Slovakia) following the standard protocol. Sequencing was then performed in both directions using PCR primers specific for each region. Commercial services provided by Macrogen Europe (Amsterdam, Netherlands) were used for Sanger sequencing of newly obtained amplicons.

In order to assess phylogenetic relationships and genetic diversity among *Khawia* spp. additional ortholog 18S and 28S sequences of *Khawia* spp. were retrieved from GenBank (accession numbers are included within phylogenetic trees). The sequence alignments for each genomic region were built using the Fast Fourier transform algorithm implemented in MAFFT software (Katoh et al., 2002), using the G-INS-i refinement method and then manually trimmed to unify the length of all sequences. Final alignment for phylogenetic tree reconciliation was built from concatenated DNA sequences of 18S and 28S, and concatenated ortholog sequences of *Atractolytocestus sagittatus* Anthony, 1958 (Caryophyllaeidae) were included and used for rooting of phylogenetic trees. Since ortholog 18S and 28S sequences were available only for six out of seven currently recognized *Khawia* species, non-complete data were discarded from the final alignment. The data were treated as partitioned, and a general time-reversible model (Lanave et al., 1984) was applied for each gene segment individually, also including a gamma distribution.

Phylogenetic trees were constructed using Bayesian inference (BI) and Maximum likelihood (ML) approaches in MrBayes 3.2 (Ronquist et al., 2012) and RAxML 8.1.12 (Stamatakis, 2006; 2014), respectively. BI analysis used the Metropolis-coupled Markov
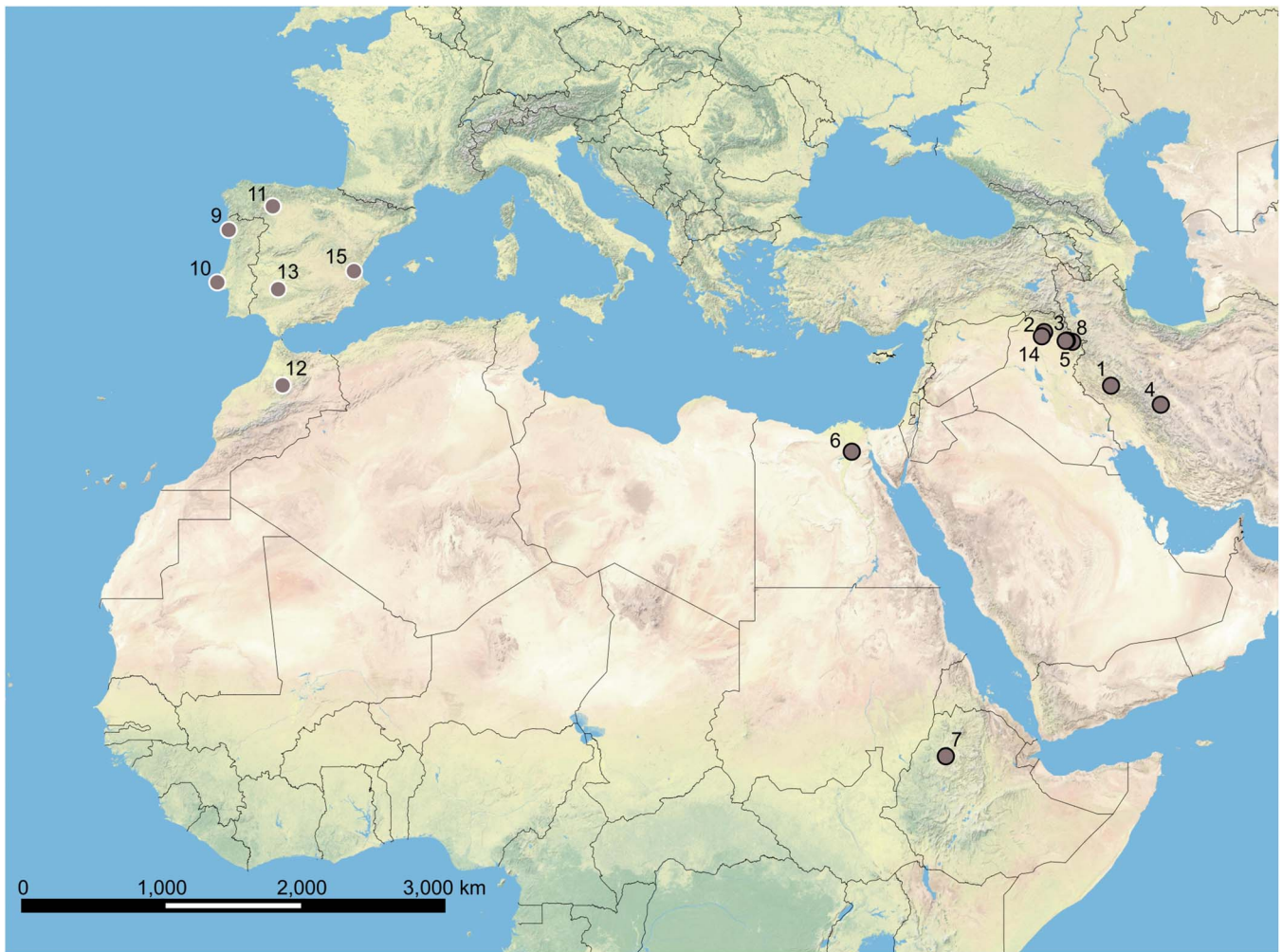
Figure 1. Collection sites of investigated '*Khawia armeniaca*' specimens. Numbers on the map correspond to locality numbers in Table 2

chain Monte Carlo algorithm with two parallel runs of one cold and three hot chains and was run for 10^6 generations, sampling trees every 100 generations. The initial 30% of all saved trees were discarded as a 'burn-in' period after checking that the standard deviation split frequency fell below 0.01. The convergence of the runs and the parameters of individual runs were checked using Tracer v. 1.7.1 (Rambaut et al., 2018). Posterior probabilities for each tree node were calculated as the frequency of samples recovering a given clade. The clade bootstrap support for ML trees was assessed by simulating 10^3 pseudoreplicates.

The ITS2 sequences were used to assess the intraspecific genetic variability among *a priori* delineated *K. armeniaca* populations. Uncorrected pairwise distances (*p*-distances) were calculated using the dist.dna function from the 'ape' package in R statistical environment, version 4.1.3 (R Core Team, 2022).

Morphological study

For light microscopy, specimens were stained with Mayer's carmine, dehydrated in an ethanol series, cleared with clove oil (eugenol), and mounted in Canada balsam. Line drawings were made using an Olympus BX51 microscope equipped with phase contrast and differential interference contrast (Olympus Inc., Tokyo, Japan). For scanning electron microscopy, scoleces

of selected specimens were processed as described by Oros et al. (2020) and examined with a Jeol JSEM 7401F electron microscope (Jeol Ltd., Tokyo, Japan). Voucher specimens and types of the new species are deposited in the IPCAS and Natural History Museum in London (NHMUK). To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (2012), details of the new cestode species have been submitted to ZooBank.

Results

Molecular study

The final alignment for assessing the phylogenetic relationships among investigated *Khawia* spp. built from 24 concatenated 18S and 28S sequences spanned 3,490 unambiguously aligned nucleotide positions (1,945 bp long for 18S and 1,545 bp for 28S). The ML and BI analyses generated trees with congruent topologies and therefore only the BI tree is presented in Figure 2. The *Khawia* species were divided into three well-supported clades, with two well-differentiated and strongly supported lineages formed by novel *Khawia* sequences within clade A.

In clade A, the lineage A1 encompassed all sequences from *Khawia* individuals collected from *Luciobarbus* species in the
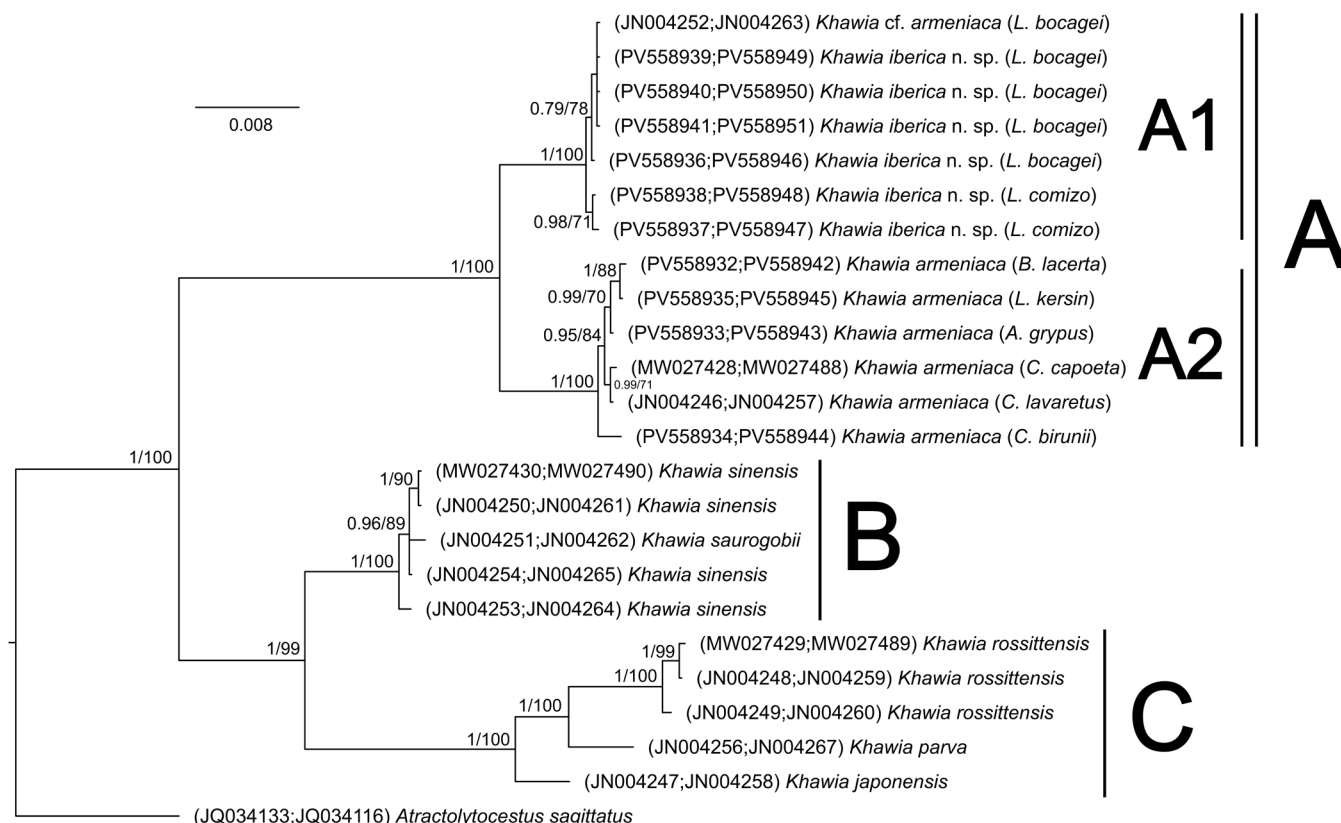
Figure 2. Phylogenetic tree of 23 concatenated sequences of seven *Khawia* species resulting from BI analysis. The tree is based on concatenated partial sequences of genes coding for 18S rDNA and 28S rDNA and is rooted with *Atractolytocestus sagittatus* as an outgroup. The numbers at the nodes represent posterior probabilities and bootstrap support values, resulting from BI and ML analyses, respectively. Specimen accession numbers (formatted as 18S;28S) and host species are provided in parentheses, when available.

Iberian Peninsula. It also included the sequences retrieved from GenBank, originating from a specimen of *Khawia* cf. *armeniaca* from Portugal. Lineage A2 in clade A included four sequences of *Khawia armeniaca* collected from cyprinids in the Middle East (i.e. from *Arabibarbus grypus, Barbus lacerta, Capoeta birunii* and *Luciobarbus kersin*) and two sequences retrieved from GenBank, from specimens collected from *C. capoeta* and *Coregonus lavaretus* in Lake Sevan (Armenia). Clade B included all sequences of *K. sinensis*, and a single sequence of *K. saurogobii*, nested within this group. The last clade C occupied a well-supported sister position to clade B and consisted of specimens of *K. parva, K. rossittensis* and *K. japonensis*.

The novel sequences of ITS2 region were used to assess intraspecific genetic variability among *Khawia* specimens initially recognized as *Khawia armeniaca*. A final sequence alignment included 18 sequences and spanned 807 unambiguously aligned nucleotide positions. Uncorrected *p*-distances ranged from 0.000 to 0.203 (Supplementary Figure 1). The highest genetic divergence was recorded between *Khawia* individuals from *L. bocagei* (Portugal – new species) and *C. birunii* (Iran – *K. armeniaca*).

Morphological study
Examination of stained specimens of both genetic lineages revealed some slight but consistent differences between specimens of individual clades, i.e. the 'true' *Khawia armeniaca* and another described below as new (Figures 3–8). The validity of both species

is supported by molecular data, their own unique host association and distribution range. Therefore, *Khawia armeniaca* is redescribed based on new material from Iraq and the new species from Spain and Portugal is described.

Redescription of Khawia armeniaca
(Cholodkovsky, 1915) Figures 3A–D, 4, 6A, 8C
Material studied: See Table 2.
Description: (based on total of 19 adult specimens from the following hosts: *A. grypus* – 5 specimens measured, *B. lacerta* – 3, *L. barbulus* – 1, *L. esocinus* – 2, *L. kersin* – 8; measurements in micrometres unless otherwise stated): Caryophyllidea, Caryophyllaeidae *sensu* Scholz et al. (2021). Body elongate, robust, rod-like, i.e. more or less of same width throughout (Figure 3A–D), 15–36 mm long, with maximum width 818–2446 at level of anterior vas deferens; width at level of cirrus sac 806–2,127, at level of ovarian isthmus 677–2,130. Posterior end of body widely rounded. Body surface covered with acicular fillitriches (filiform microtriches).

Scolex spatulate, dorsoventrally flattened, 917–2,175 wide, variable in shape (Figure 4), slightly (Figure 4A) to markedly (Figure 4C) wider than neck; neck 715–1,844 wide at level of anteriormost vitelline follicles. Anterior margin of scolex slightly convex, entire or with few, shallow notches (incisions); anterior part of dorsal and ventral surface of scolex slightly concave, with shallow longitudinal wrinkles (Figures 4, 6A). Inner longitudinal musculature formed by relatively few, irregular and small bundles of muscle fibres.
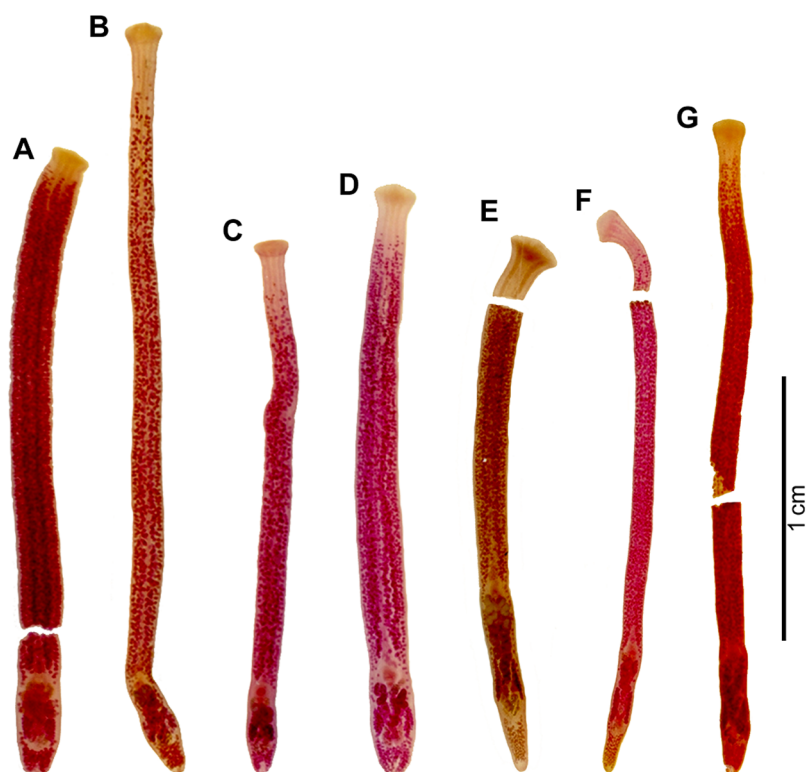
Figure 3. Whole, stained specimens of *Khawia armeniaca* s. str. (A–D) from various hosts in Iraq and *Khawia iberica* n. sp. (E–G) from various hosts in Spain. (A) *Luciobarbus esocinus*; (B) *Arabibarbus grypus*; (C) *Mastacembelus mastacembelus* (postcyclic host); (D) *L. kersin*; (E) *L. bocagei* (type host); (F) *L. comizo*; (G) *L. guiraonis*.
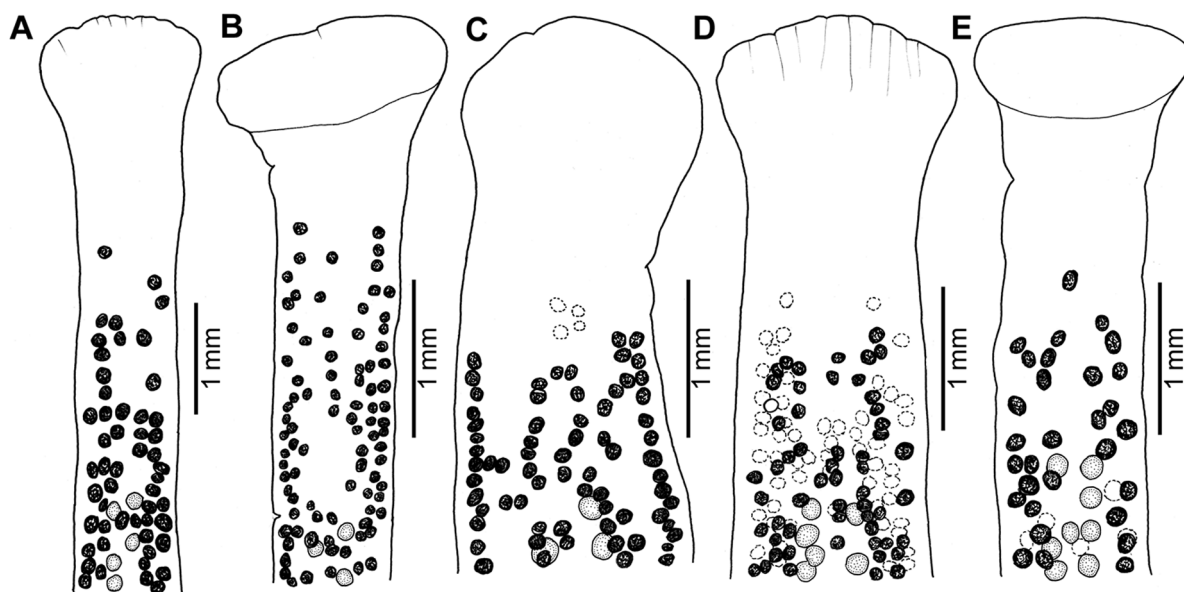
Figure 4. Morphological variation of scoleces of *Khawia armeniaca* s. str. from different hosts in Iraq. (A) *Arabibarbus grypus*; (B) *Barbus lacerta*; (C) *Carasobarbus luteus*; (D) *Luciobarbus kersin*; (E) *Mastacembelus mastacembelus* (postcyclic host). The image illustrates the relative position of anteriormost testes and vitelline follicles. Vitelline follicles (C, D) and testes (E) on the other (lower) side of the body are dotted (dotted open circles).

Osmoregulatory canals well developed, sinuous and anastomosing; number of canals varies greatly due to numerous anastomoses, but usually two wider canals situated medially on ventral and dorsal sides; narrower canals lateral or beneath median canals.

Testes medullary, subspherical to widely oval, variable in size, 85–335 long and 75–235 wide. Anteriormost testes begin posterior to anteriormost vitelline follicles (0.42–2.35 mm),

0.60–4.29 mm from anterior end of body (distance between first testes and anterior extremity represents 4.0–19.9% of total length of body) (Figure 4). First 10 testes occupy region 473–2,735 long. Posteriorly, testes reach cirrus sac.

Cirrus sac thick-walled, spherical to subspherical, 208–649 long and 252–658 wide. Width of cirrus sac represents 22–44% of body width. External seminal vesicle absent. Male genital duct opens
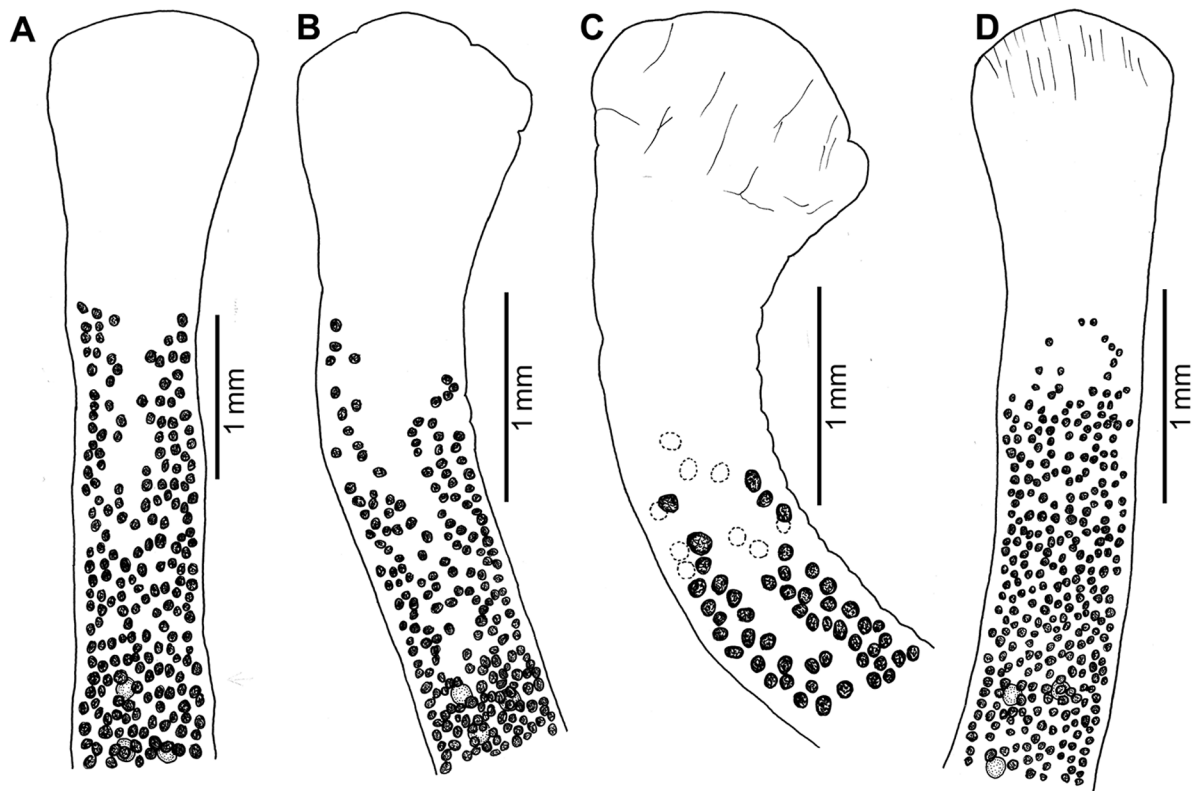
Figure 5. Morphological variation of scoleces of *Khawia iberica* n. sp. from different hosts in Spain. (A, B) *Luciobarbus bocagei* (type host); (C) *L. comizo*; (D) *L. guiraonis*. The image illustrates the relative position of anteriormost testes and vitelline follicles. Vitelline follicles on the other (lower) side of the body in C are dotted (dotted open circles).
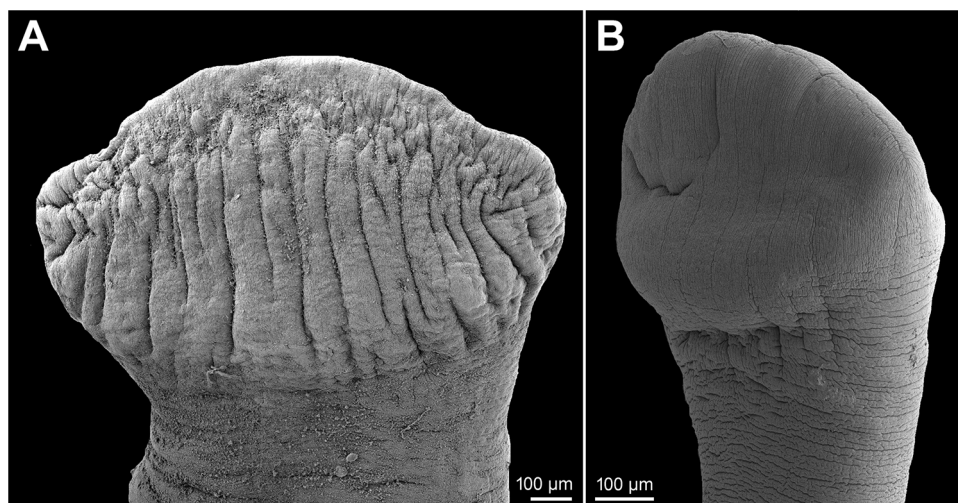

Figure 6. Scanning electron micrographs of scoleces. (A) *Khawia armeniaca* s. str. from *Luciobarbus kersin*, Iraq; (B) *Khawia iberica* n. sp. from *Luciobarbus bocagei*, Spain.

to genital atrium with female genital duct (uterovaginal duct), corresponding to 5.24 of Mackiewicz ([1994](#)).

Ovary follicular, grape-like, with deep lobes, H-shaped, with slightly concave (U-shaped) isthmus ([Figure 8C](#)). Ovary 614–1,730 wide at level of isthmus. Ovarian arms (wings) 528–1855 long and 212–658 wide, represent 30–55% of length of uterine region and 3.0–7.4% of total length of body. Length/width ratio of ovary 0.57–1.09. Ovarian isthmus pre-equatorial, equatorial to post-equatorial (ratio of length of ovarian arms anterior to isthmus to length of ovarian arms posterior to isthmus 0.41–2.52). Vagina

tubular, slightly sinuous, widened to form elongate, narrow seminal receptacle anterior to ovarian isthmus, joins with uterus to form uterovaginal canal, opens separately from gonopore into distinct genital atrium. Gonopore (opening of common genital atrium) situated 541–2,282 anterior to ovary.

Preovarian vitelline follicles numerous, cortical, variable in size, 58–190 long and 54–179 wide. First (anteriormost) vitelline follicles situated 577–2,603 from anterior margin of body (distance to anterior extremity represents 3.1–9.8% of total length of body), much anterior to first (anteriormost) testes; number of
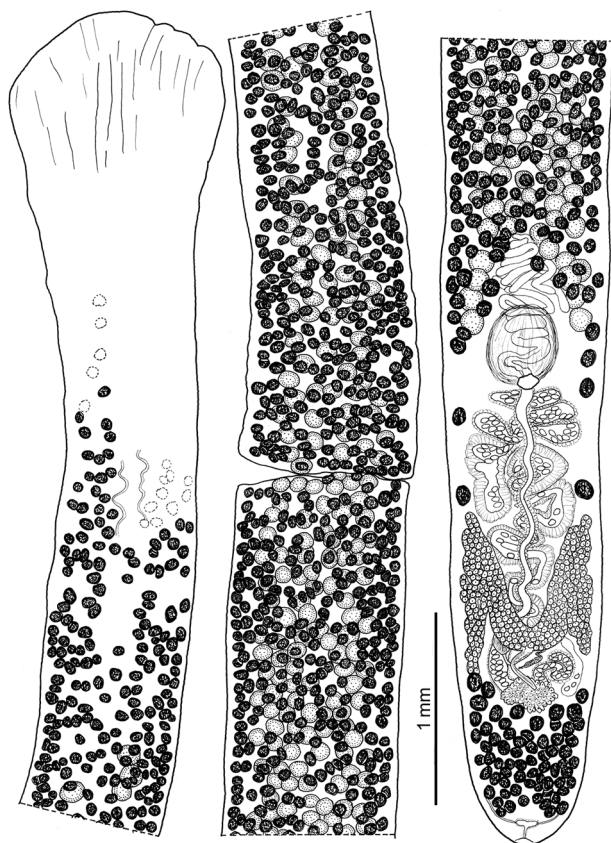
Figure 7. Line drawing of *Khawia iberica* n. sp., holotype from *Luciobarbus bocagei*, Spain. Note the elongated lobes of the ovary with a U-shaped isthmus. Vitelline follicles on the other (lower) side of the body are dotted (dotted open circles).

Figure 8. Line drawings of ovaries. (A) *Khawia iberica* n. sp. from *Luciobarbus bocagei* (type host), Spain; (B) *K. iberica* n. sp. from *L. comizo*, Spain; (C) *Khawia armeniaca* s. str. from *Arabibarbus grypus*, Iraq.
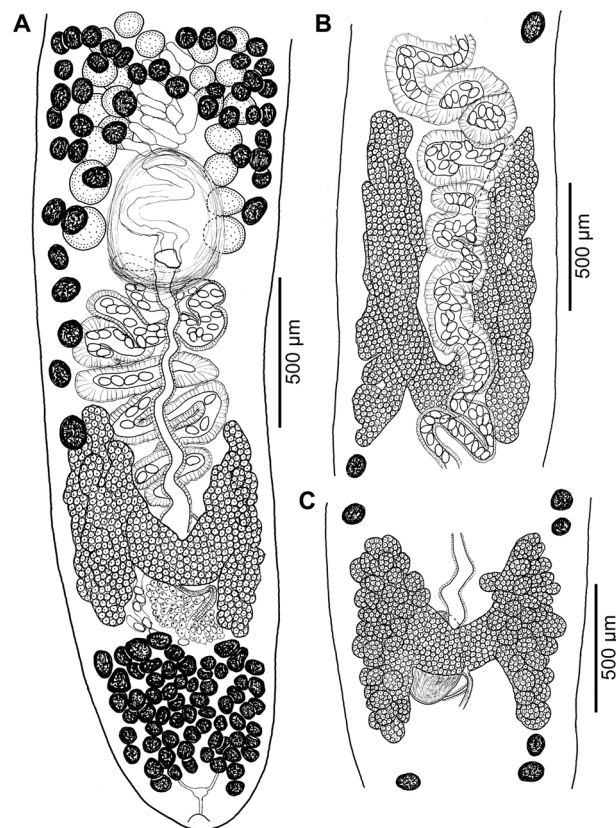
pretesticular vitelline follicles 7–194. Preovarian vitelline follicles reach posteriorly to level of cirrus sac, with few follicles alongside preovarian uterine region; follicles may reach to ovary; distance of last preovarian follicles to ovarian arms very variable, 0–585. Number of vitelline follicles alongside uterus 0–24; number of follicles alongside ovary 0–8. Post-ovarian vitelline follicles numerous, 58–172 in number; anteriormost post-ovarian follicles 0–79 posterior to ovary.

Uterus tubular, forms several preovarian loops, extending to posterior margin of cirrus sac. Uterine region 1.12–4.34 mm long, i.e. 2.8–6.9% of total length of body; region occupied by uterus between cirrus sac to ovary 442–2,142. Uterine glands well developed, absent only in most distal and proximal parts of uterus. Eggs operculate, without fully formed oncosphere *in utero*, 51–75 long and 31–45 wide.

Taxonomic summary

Type host: *Capoeta capoeta* (Güldenstädt, 1773).

Additional hosts (*confirmed genetically): *Arabibarbus grypus* (Heckel, 1843) (vouchers IPCAS C-48/2); *Barbus lacerta* Heckel, 1843 (IPCAS C-48/11); *Capoeta birunii* Zareian et Esmaeili, 2017; *Capoeta damascina* (Valenciennes, 1842); *Carasobarbus luteus* (Heckel, 1843) (IPCAS C-48/6); *Luciobarbus barbulus* (Heckel, 1847) (IPCAS C-48/4); *L. esocinus* Heckel, 1843 (IPCAS C-48/10); *L. kersin* Heckel, 1843 (IPCAS C-48/9); *Coregonus lavaretus* (Lavaretus, 1758); *Salmo ischchan* Kessler, 1877; *Silurus triostegus* Heckel, 1843, and *Mastacembelus mastacembelus* (Banks et Solander, 1794) (IPCAS C-48/12), four latter host species are atypical (postcyclic or accidental) hosts.

Site of infection: Anterior intestine.

Type locality: Lake Sevan, Armenia.

Distribution (* confirmed genetically): Armenia*, Azerbaijan, Georgia, Iran*, Iraq*, Israel, Turkey.

Records: Cholodkovsky (1915), Popov (1924), Dinnik (1933), Mikailov (1975), Williams et al. (1980), Rahemo & Al-Kalak (1993), Protasova et al. (1990); present study.

Type material: Does not exist.

Representative DNA sequences: See above and following provided from the host *B. lacerta*: 18S rDNA – PV558932; 28S rDNA – PV558942; ITS2 – PV558921. In addition, Scholz et al. (2011) provided sequences of tapeworms from *Coregonus lavaretus* (probably accidental host), Lake Sevan, Armenia (JN004246 – 18S rDNA; JN004257 – 28S rDNA; JN004235 – *nad3*; JN004224 – *cox*1).

Remarks: *Khawia armeniaca* is a common and widespread parasite of barbels in the Middle East. It has been found in a wide range of fish hosts (10 host species, excluding atypical hosts). The species exhibits morphological and biometrical variability, most likely reflecting intraspecific variability of individual parasite populations from different definitive hosts (Scholz et al., 2011). Furthermore, the specimens examined were fixed by different methods, including severe flattening, they are deformed or contracted, with the scolex unnaturally shortened, and some were obviously dead and therefore decomposed at the time of fixation, which may have affected their appearance and subsequent measurements.

Scholz et al. (2011) also reported *K. armeniaca* from the ripon barb, *Labeobarbus altianalis* (Boulenger, 1900), in Uganda;

the Niger barb, *Labeobarbus bynni* (Fabricius, 1775), in Egypt; *Luciobarbus callensis* (Valenciennes, 1842), in Morocco; and *Labeobarbus tropidolepis* (Boulenger, 1900), in Tanzania. However, the species affiliation of these tapeworms is unclear as no molecular data are available. It is possible that some of these specimens are another new species, especially the specimens from Tanzania and Uganda, which are very large and considerably exceed the maximum size of *K. armeniaca* and *K. iberica* n. sp. The species identification of '*K. armeniaca*' from barbels in Lake Tana is also uncertain and molecular data are needed.

Khawia iberica n. sp.

Figures 3E–G, 5, 6B, 7, 8A, B

Synonyms: *Khawia baltica* Szidat, 1942 of Chubb et al. (1997); *K. armeniaca* (Cholodkovsky, 1915) of Scholz et al. (2011) (*partim* – specimens from Portugal)

Material studied: See Table 2.

Description (based on total of 16 adult specimens from the following hosts: *Luciobarbus bocagei* – 11 specimens measured, *L. guiraonis* – 4, *L. comizo* – 1; measurements in micrometres unless otherwise stated; measurements of the holotype in brackets): Caryophyllidea, Caryophyllaeidae *sensu* Scholz et al. (2021). Body elongate, slender, rod-like (Figure 3E–G), i.e. more or less of same width throughout 13–29 mm [15.5 mm] long, with maximum width 787–1369 [937] at level of anterior vas deferens; width at level of cirrus sac 760–1,275 [854], at level of ovarian isthmus 689–1,264 [786]. Posterior end of body widely rounded posteriorly (Figures 7, 8A). Body surface covered with acicular fillitriches (filiform microtriches).

Scolex spatulate, dorsoventrally flattened, 860–2,200 [1,126] wide, slightly (Figure 5A, B, D) to markedly (Figure 5C) wider than neck; neck 656–1,266 [746] wide at level of anteriormost vitelline follicles. Anterior margin of scolex slightly convex, entire or with few, shallow notches (incisions); anterior part of dorsal and ventral surface of scolex slightly concave, with narrow longitudinal wrinkles (Figure 6B). Inner longitudinal musculature formed by relatively small bundles of muscle fibres. Osmoregulatory canals well developed, sinuous and anastomosing; number of canals varies greatly due to numerous anastomoses, but usually two wider canals situated medially on ventral and dorsal sides; narrower canals lateral or beneath median canals.

Testes medullary, subspherical to widely oval, 96–186 [106–123] long and 91–171 [91–108] wide. Anteriormost testes begin posterior to anteriormost vitelline follicles (0.90–5.92 mm [2.39 mm]), 1.62–7.50 mm [3.88 mm] from anterior end of body (distance between first testes and anterior extremity represents 7.6–31.9% [25.0%] of total length of body) (Figure 5). First 10 testes occupy region 170–1,798 [693] long. Posteriorly, testes reach cirrus sac (Figures 7, 8A).

Cirrus sac thick-walled, spherical to subspherical, 249–560 [264] long and 263–523 [263] wide. Width of cirrus sac represents 23–47% [28%] of body width (Figure 8A). External seminal vesicle absent. Male genital duct opens to joint genital atrium with female genital duct (uterovaginal duct), corresponding to 5.24 of Mackiewicz (1994) (Figures 7, 8A).

Ovary non-follicular, with deep lobes, H-shaped, with concave (U-shaped) isthmus (Figures 7, 8A, B). Ovary 446–1,048 [673] wide at level of isthmus. Ovarian arms (wings) 535–1639 [789] long and 184–477 [238] wide. Ovarian arms represent 29–58% [48%] of length of uterine region and 4.1–7.9% [5.1%] of total length of body. Length/width ratio of ovary 0.89–1.85 [1.17]. Ovarian isthmus usually post-equatorial (ratio of length of ovarian arms anterior to isthmus to length of ovarian arms posterior to isthmus 0.74–3.40 [1.74]. Vagina tubular, slightly sinuous, widened to form elongate, narrow seminal receptacle anterior to ovarian isthmus. Isthmus joins with uterus to form uterovaginal canal, opens separately from gonopore into distinct genital atrium (Figures 7, 8A). Gonopore (opening of common genital atrium) situated 516–2,282 [644] anterior to ovary.

Preovarian vitelline follicles numerous, cortical, variable in size, 58–133 [66–72] long and 50–125 [53–69] wide. First (anteriormost) vitelline follicles situated 900–3,321 [1487] from anterior margin of body (distance to anterior extremity represents 3.1–11.7% of total length of body), much anterior to first (anteriormost) testes; number of pretesticular vitelline follicles 57–627 [244] (Figure 5). Preovarian vitelline follicles reach posteriorly to level of cirrus sac, with few follicles alongside preovarian uterine region; follicles may reach to ovary; distance of last preovarian follicles to ovarian arms variable, 0–263 [41]. Number of vitelline follicles alongside uterus 0–25 [4]; number of follicles alongside ovary 0–7 [0]. Post-ovarian vitelline follicles numerous, 31–125 [125] in number; anteriormost post-ovarian follicles 0–543 [9] posterior to ovary (Figures 7, 8A).

Uterus tubular, forms several preovarian loops, extending to posterior margin of cirrus sac (Figures 7, 8A, B). Uterine region 1.56–4.77 mm [1.66 mm] long, i.e. 2.8–8.7% [4.2%] of total length of body; region occupied by uterus between gonopore to ovary 586–2,510 [651]. Uterine glands well developed, absent only in most distal and proximal parts of uterus. Eggs operculate, without fully formed oncosphere *in utero*, 53–64 [mean 53] long and 30–37 [mean 31] wide.

Taxonomic summary

Type host: *Luciobarbus bocagei* (Steindachner, 1864).

Additional hosts: *Luciobarbus comizo* (Steindachner, 1864); *Luciobarbus guiraonis* (Steindachner, 1866).

Site of infection: Anterior intestine.

Type locality: Colares, Portugal.

Additional localities: Este River, Portugal; Orbigo River, Spain; Magro River; Spain; Zújar River, Spain.

Type material: Holotype (IPCAS C-1014/1) and two paratypes (IPCAS C-1014/1) – whole-mounted specimens from *Luciobarbus bocagei* (host code LUBO 1), Colares, Portugal, collected by A. Šimková on 22 June 2016; one paratype – whole-mounted specimen from *L. bocagei* (LUBO 10), Colares, Portugal, collected by Andrea Vetešníková Šimková on 22 June 2016; one paratype (IPCAS C-48/6) from *L. bocagei* (PT 69), Este River near Porto, Portugal, collected by Tomáš Scholz on 21 September 2009; two paratypes (IPCAS C-1014/2) – whole-mounted specimens from *Luciobarbus comizo* (LUMI 1b, LUMI 3), Peraleda de Zancejo, Zújar River, Spain, collected by Kateřina Čermáková Vyčítalová on 25 June 2017; two paratypes (IPCAS C-1014/3) – whole-mounted specimens from *Luciobarbus guiraonis* (LUGU 4, LUGU 11), Magro River, Spain, collected by Andrea Vetešníková Šimková on 22 June 2016; and one paratype (NHMUK 2025.6.9.1) – whole-mounted specimen from *L. guiraonis* (LUGU 11), Magro River, Spain, collected by Andrea Vetešníková Šimková on 22 June 2016;.

Representative DNA sequences: 18S rDNA – PV558936; 28S rDNA – PV558946; ITS2 – PV558925

Etymology: The specific name refers to the recorded distribution of the species, which is Iberian Peninsula.

ZooBank registration (LSID): urn:lsid:zoo bank.org:act: 225DBA46-DAC4-49F9-8439-9FA0675D2AF8

Differential diagnosis: The new species differs from *K. armeniaca* in the structure, shape and size of the ovary and the anterior extension of the vitelline follicles and testes. The ovary of *K. iberica* n. sp. is compact and consists of elongated lobes (Figure 8A, B), whereas the ovary of *K. armeniaca* is follicular (Figure 8C). The ovary of the former species (*K. iberica* n. sp.) has longer, slender lateral arms (wings), and the isthmus is more concave (U-shaped) and located further back in most specimens (Figures 7, 8A, B).

Scholz et al. (2011) described the ovary of *K. armeniaca* sensu lato (*s. lat.*) as butterfly-shaped, but an ovary of this shape is almost exclusively typical for tapeworms from the Middle East, i.e. *K. armeniaca* sensu stricto (s. str.). A follicular ovary is also described in the first description of *K. armeniaca* by Cholodkovsky (1915).

The two species also differ in the anterior extension of vitelline follicles and testes (Figures 4, 5), especially in the distance between them, which is usually greater in *K. iberica* n. sp. (up to 2.5 times). In this species, the first (anteriormost) testes start at a greater distance from the anterior end compared to *K. armeniaca* (Figure 4) (usually twice as far).

In addition, the new species is a specific parasite of *Luciobarbus* spp. and has only been found in Portugal and Spain (the species identification of *Khawia* tapeworms from barbels in North Africa is uncertain).

Remarks: *Khawia iberica* was previously conflated with *K. armeniaca*, although Scholz et al. (2011) found a striking genetic divergence between specimens from Portugal (= *K. iberica* n. sp.) and those of *K. armeniaca* s. str. from the type locality (Lake Sevan in Armenia). The poor quality of the available material and the lack of molecular data on tapeworms from different host species and geographical regions prevented the proposal of a new species at that time. The new material, suitable for comparative molecular and morphological analysis, allowed us to distinguish the two species parasitising barbels in Europe and the Middle East.

Chubb et al. (1997) reported caryophyllidean tapeworms identified as *K. baltica* in *L. bocagei* in northwestern Portugal (Este River). However, a re-examination of vouchers (IPCAS C-48/6) confirmed that these tapeworms belong to *K. armeniaca* s. lat. (see Scholz et al., 2011), specifically to *K. iberica* n. sp.

At the beginning of the 21st century, two new species of *Khawia* were described from the yellow barbell, *Barbus luteus* (= *Carasobarbus luteus* [Heckel, 1843], a known fish host for *K. armeniaca* from the Tigris) at the same locality, the Tigris River in Iraq: *Khawia barbi* Rahemo et Mohammad (2002) and *Khawia lutei* Al-Kalak et Rahemo (2003). However, both species were inadequately described, based on poorly preserved material, and the original descriptions contained almost no morphometric data. Scholz et al. (2011) synonymized these species as they are morphologically indistinguishable from *K. armeniaca* and share the same host range and geographic distribution. The available molecular data fully support this synonymy.

Discussion

Previous studies documented remarkable intraspecific variability in *Khawia armeniaca* (see Scholz et al., 2011), although only a small number of properly fixed individuals had been investigated. The variability was apparent at both the morphological and the molecular level, suggesting the existence of two separate species with specific geographic distribution and host ranges. Nonetheless, previously available material was not sufficient to fully describe and delimit the new taxon, and therefore herein 35 specimens were morphologically analysed, and molecular analyses were performed on 22 specimens, in order to provide a proper taxonomic description.

The examined specimens were collected in Spain, Portugal, Iran and Iraq, with hosts including the cyprinid fish species of the genera *Arabibarbus*, *Barbus*, *Capoeta*, *Carasobarbus*, *Labeobarbus* and *Luciobarbus*, and *Mastacembelus mastacembelus*, which is considered to be an accidental or postcyclic host due to predation. Congruently with the aforementioned review, analysed specimens of *Khawia* were found to be clearly divided into two molecularly well-supported lineages, each associated with the different geographical region: the Iberian Peninsula lineage and the Middle Eastern lineage. Considering both morphological and molecular differences, the Middle Eastern lineage is identified as *Khawia armeniaca* s. str., corresponding to the original description and the genetically characterized isolate from the type locality (Lake Sevan, Armenia – see Scholz et al., 2011). The Iberian lineage, on the other hand, is considered a new species, *Khawia iberica* n. sp., based on molecular and morphological distinctiveness.

After the split of *K. armeniaca* s. lat. into two separated species, both taxa still exhibit a remarkably wide host range. In the case of *K. iberica* n. sp. this includes three endemic *Luciobarbus* species; however, it cannot be ruled out that this species may also infect other congeneric hosts in the region. The host range of *K. armeniaca* s. str. appears to be even wider, including 10 species belonging to six cyprinid genera. Other representatives of the genus *Khawia* generally exhibit much narrower host specificity (Scholz et al., 2011; Xi et al., 2013), which is typical for the majority of fish tapeworms. Notably, 61% of cestodes of bony fish utilize a single definitive host (Scholz and Kuchta, 2017). One of the few genera exhibiting similarly variable levels of host specificity is *Caryophyllaeus*; while *C. laticeps* has a broad host range encompassing more than 30 fish species, *C. balticus* is considered a specialist, occurring in only a single definitive host species (Scholz et al., 2011; Barčák et al., 2017; Kuchta et al., 2020).

As with many other parasites, the classification of the order Caryophyllidea historically relied primarily on morphological traits (Mackiewicz, 2003). However, molecular methods have introduced an invaluable tool for studying intraspecific variability and interspecific differences. In the case of the present study, the molecular data reinforced morphologically evident differences. These methods have also been instrumental in identifying so-called cryptic and sibling species – genetically distinct but morphologically nearly indistinguishable species (Nadler and Pérez-Ponce de León, 2011). For instance, cryptic or sibling species have recently been identified within the genera *Paracaryophyllaeus* (Caryophyllidea) and *Bothriocestus* (previously placed in *Bothriocephalus;* Bothriocephalidea) (Scholz et al., 2014; Choudhury and Scholz, 2020). The integration of molecular approaches into the study of parasite taxonomy and diversity has become almost indispensable. However, this approach was not used in earlier studies on *K. armeniaca*, such as those by Rahemo and Mohammad (2002), Al-Kalak and Rahemo (2003), and Kibet et al. (2021), which significantly complicated efforts to explore the true diversity of these tapeworms and their phylogenetic relationships.

The reliability of classical morphometric criteria has also been questioned in the study by Bazsalovicsová et al. (2014), which revealed substantial intraspecific plasticity in tapeworms of the genus *Caryophyllaeus*, members of the same family as *Khawia*. This study uncovered morphological plasticity that led to the division of *C. laticeps* and *C. brachycollis* into morphotypes, usually

typical of individual fish hosts. However, genetic analysis did not provide conclusive evidence that these morphotypes represented distinct species. As a result, these species are considered polymorphic parasites infecting a broad range of hosts (Barčák et al., 2017). The findings of Bazsalovicsová et al. (2014) highlight that species identification in the genus *Caryophyllaeus* based solely on morphology can be sometimes misleading due to their phenotypic plasticity. This observation is relevant to the present study, where morphology alone provided only weak evidence to differentiate the two genetically well-defined lineages because of high degree of intraspecific variability of both species. The observed differences in ovarian structure and the anterior part of the tapeworm's body may be considered manifestations of intraspecific variability.

It is clear that morphology alone may not provide adequate taxonomic resolution for closely related species (Nadler and Pérez-Ponce de León, 2011). This is particularly true for caryophyllidean tapeworms, which, compared to other orders, appear to lack prominent taxonomic traits. Based on the results of this study, several principles emerge as critical: proper and careful fixation methods, limiting tissue sampling for molecular purposes to the middle body segment (Chervy, 2024), and focusing morphological analyses on traits unaffected by fixation or the individual's age. This approach necessitates the use of only sexually mature, adult specimens. For widespread species and/or species with a broad host range, it is also important to include an appropriate number of specimens from different localities and/or host species. This will ensure that the data used cover the full range of potential variability, thereby increasing the reliability of the results.

The results of the phylogenetic analyses placed a clade encompassing *K. armeniaca* and *K. iberica* n. sp. in a basal position to the other congeners, consistent with phylogenetic trees presented by Scholz et al. (2011) and Scholz et al. (2021). Although *Khawia baltica* was identified as basal in the study by Scholz et al. (2011), this species was later reassigned to the genus *Caryophyllaeus* as *Caryophyllaeus balticus* by Barčák et al. (2017). Our phylogenetic analysis similarly places *K. iberica* n. sp. lineage as sister to *K. armeniaca*, aligning with the findings of Scholz et al. (2011). The resulting phylogenetic trees do not show significant intraspecific variability in ribosomal subunit (18S and 28S) sequences within either lineage at the molecular level, indicating that representatives of these lineages can be considered conspecific.

Within the *K. iberica* n. sp. lineage, analyses based on concatenated data of two ribosomal markers reveal an apparent genetic divergence between specimens from the host *Luciobarbus comizo* and those parasitizing *L. bocagei*. This separation is more pronounced in the ITS2 sequences, suggesting that even within *K. iberica* n. sp. conspecifics, there is host-dependent intraspecific structuring. *Khawia armeniaca* also exhibits a degree of structure, notably separating a single specimen from the host *Capoeta birunii* in Iran. This specimen originates from a highly isolated locality in comparison to other studied individuals, located approximately 400 km from the nearest other recorded occurrence. Furthermore, it is the only parasite recovered from this host species. To further investigate the differences between these isolates, additional material will be required.

From a geographic perspective, a key finding is the distribution of *K. iberica* n. sp., which, based on the available data, appears to be restricted to the Iberian Peninsula. These tapeworms, prior to this study considered to be *K. armeniaca*, have not been reported from other southern European peninsulas or elsewhere in Europe. This absence of findings cannot be simply attributed to insufficient

sampling in these regions. Such a restricted distribution suggests that the dispersal of these tapeworms to the Iberian Peninsula likely occurred via North Africa and Gibraltar, rather than through the Apennine or Balkan Peninsulas or Central Europe. While several records of '*K. armeniaca*' exist from North Africa (Scholz et al., 2011), no genetic data are available from this region, posing a major obstacle to understanding the presumed historical dispersal. The lack of genetic data also hinders the study of speciation and/or the origin of this species on the Iberian Peninsula and its subsequent dispersal. As such, it remains speculative how closely the Iberian *Khawia iberica* n. sp. resembles individuals from North Africa.

It is notable that the Iberian Peninsula harbours no other representatives of the order Caryophyllidea besides the genus *Caryophyllaeus* and the invasive genus *Atractolytocestus* Anthony, 1958 (Cordero del Campillo et al., 1994). The host range of *K. iberica* n. sp. based on the findings of this study appears to be restricted to the genus *Luciobarbus*, specifically the endemic and sympatric species *L. comizo, L. bocagei* and *L. guiraonis* (Kottelat and Freyhof, 2007; Gante et al., 2015; Froese and Pauly, 2025). The genus *Luciobarbus* is widely distributed across Europe, Asia and North Africa, with its historical dispersal being the subject of many biogeographical hypotheses (e.g. Doadrio, 1990; Oellermann and Skelton, 1990; Tsigenopoulos et al., 2010; Yang et al., 2015).

One such hypothesis (Doadrio, 1990; Perea et al., 2010) proposes that these fish dispersed to the Iberian Peninsula from North Africa. Given the sympatric occurrence of both the parasite and its host in North Africa and the Iberian Peninsula, it can be hypothesized (though not confirmed) that *K. iberica* n. sp. followed this dispersal route of its hosts. Nevertheless, a critical prerequisite for testing the hypothetical phylogeographic association of the two species is an analysis of the genetic diversity of the North African lineage of these tapeworms. If a close molecular phylogenetic relationship with *K. iberica* n. sp. is confirmed, this model would align with the known relationships of North African and Iberian *Luciobarbus* species (Machordom and Doadrio, 2001; Tsigenopoulos et al., 2003; Casal-López and Doadrio, 2018), supporting the reconstruction of the dispersal history of both host and parasite.

The occurrence of *K. armeniaca* s. lat. (or '*K. armeniaca*'; in previous literature referred to as *K. armeniaca*, nonetheless without molecular confirmation) in sub-Saharan Africa, specifically in Lake Tana, is remarkable and adds fish of the species *Labeobarbus intermedius* and *L. tsanensis* as definitive hosts (Scholz et al., 2011; Kibet et al., 2021). According to previous morphological data (see Scholz et al., 2011), the distribution of '*K. armeniaca*' also encompasses other equatorial African countries (i.e. Tanzania and Uganda), further highlighting the disjunctive distribution of this species (see also Figure 9). As was previously hypothesized (e.g. Tang et al., 2009; Yang and Mayden, 2010; Yang et al., 2015) cyprinids (specifically labeonins and labeonin-like species) migrated into Africa via the historical connections with the Middle East and then through the Nile basin dispersed into other river systems. As these fish species appear to be suitable hosts for *Khawia* cestodes, it is likely that their dispersal patterns are intertwined, considering the north-African presence of '*K. armeniaca*' in Egypt (Scholz et al., 2011). It is therefore reasonable to assume that the African '*K. armeniaca*' lineage will also be present in other eastern African countries. Nonetheless, understanding the phylogenetic relationship of this entirely separate lineage is again hindered by the lack of genetic data and the limited material available for morphological analysis in this study.
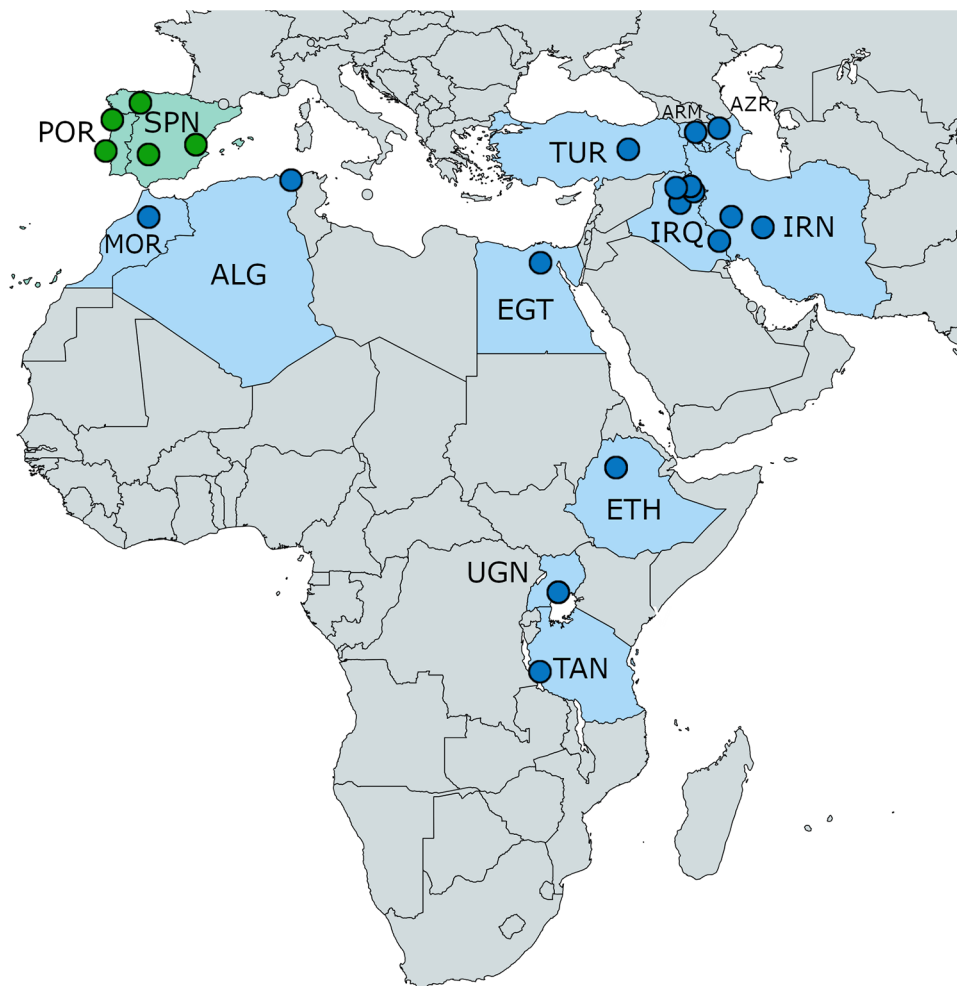
Figure 9. Distribution of *Khawia armeniaca* s. lat. (in blue colour) and *Khawia iberica* n. sp. (in green) based on Popov (1924), Özdemir and Sarieyyüpoğlu (1993), Abderrafik et al. (2010), Scholz et al. (2011) and present study. Abbreviations: ALG, Algeria; ARM, Armenia; AZR, Azerbaijan; EGT, Egypt; ETH, Ethiopia; IRQ, Iraq; IRN, Iran; MOR, Morocco; POR, Portugal; SPN, Spain; TAN, Tanzania; TUR, Turkey; UGN, Uganda.

References

Abderrafik M, Khedidja MB, Amel BTN, Eddine ZD, and Smail M (2010) Microscopie électronique à balayage des parasites des poissons du lac Oubeira-Algérie. *European Journal of Scientific Research* **48**, 129–141.

Al-Kalak SN, and Rahemo ZIF (2003) Morphological and histological studies on *Khawia lutei* sp. n. (Cestoda: Lytocestidae) a parasite of hemri, *Barbus luteus,* in Iraq. *Rivista di Parassitologia* **64**, 25–34.

Barčák D, Oros M, Hanzelová V and Scholz T (2017) A synoptic review of *Caryophyllaeus* Gmelin, 1790 (Cestoda: Caryophyllidea), parasites of cyprinid fishes in the Palaearctic Region. *Folia Parasitologica* **64**, 027.

Bazsalovicsová E, Králová-Hromadová I, Brabec J, Hanzelová V, Oros M and Scholz T (2014) Conflict between morphology and molecular data: A case of the genus *Caryophyllaeus* (Cestoda: Caryophyllidea), monozoic tapeworms of cyprinid fishes. *Folia Parasitologica* **61**, 347–354.

Casal-López M and Doadrio I (2018) The Messinian imprint on the evolution of freshwater fishes of the genus *Luciobarbus* Heckel, 1843 (Teleostei, Cyprinidae) in the western Mediterranean. *Journal of Biogeography* **45**, 1593–1603.

Cordero del Campillo M, Castañon L, and Reguera A (1994) *Índice Catálogo de Zooparasites Ibéricos. Segunda Edición*. Secretariado de Publicaciones, Universidad de León. 650.

Dinnik JA (1933) [Les vers parasitiques des poissons du lac de Sevan]. *Trudy Sevanskoi Ozernoi Stantsii* **4**, 105–132. In Russian, with French and Armenian summaries.

Doadrio I (1990) Phylogenetic relationships and classification of western Palaearctic species of the genus *Barbus* (Osteichthyes, Cyprinidae). *Aquatic Living Resources* **3**, 265–282.

Froese R and Pauly D (2025) *FishBase*. World Wide Web electronic publication.

Gante HF, Doadrio I, Alves MJ and Dowling TE (2015) Semi-permeable species boundaries in Iberian barbels (*Barbus* and *Luciobarbus*, Cyprinidae). *BMC Evolutionary Biology* **15**, 111.

Chervy L (2024) Laboratory techniques for the systematic and phylogenetic study of tapeworms (Cestoda) parasitic in bony fishes, amphibians and reptiles. *Folia Parasitologica* **71**, 001.

Cholodkovsky N (1915) Notes helminthologiques. *Annuaire de Museé Zoologique de l'Académie Impériale des Sciences de Saint Pétersbourg* **29**, 164–166.

Choudhury A and Scholz T (2020) *Ex uno plures*? Morphotype and lineage diversity of *Bothriocephalus* (Cestoda: Bothriocephalidea) in North American freshwater fishes. *Journal of Parasitology* **106**, 589–602.

Chubb J, Eiras JC and Saraiva A (1997) *Khawia baltica* (Cestoidea: Caryophyllidea) from *Barbus barbus bocagei* from some rivers in Northern Portugal. *Folia Parasitologica* **44**, 131–138.

International Commission on Zoological Nomenclature (2012) Amendment of articles 8, 9, 10, 21 and 78 of the international code of zoological nomenclature to expand and refine methods of publication. *Bulletin of Zoological Nomenclature* **69**, 161–169. doi:10.21805/bzn.v69i3.a8.161

Katoh K, Misawa K, Kuma KI and Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*. **30**, 3059–3066.

Kibet C, Wen-Ting Z, Huda S and Pin N (2021) Redescription of the monozoic cestode *Khawia armeniaca* (Cholodkovsky, 1915) Shulman, 1958 (Cestoda: Caryophyllidea) from cyprinid fish in lake Tana, Ethiopia. *Acta Hydrobiologica Sinica* **45**, 917–924.

Kottelat M and Freyhof J (2007) In *Handbook of European Freshwater Fishes*. Berlin: Kottelat, Cornol & Freyhof, 646.

Kuchta R, Řehulková E, Francová K, Scholz T, Morand S and Šimková A (2020) Diversity of monogeneans and tapeworms in cypriniform fishes across two continents. *International Journal for Parasitology* **50**, 771–786.

Kulakovskaya OP (1961) [Materials on the fauna of Caryophyllaeidae (Cestoda, Pseudophyllidea) of the Soviet Union.] *Parasitologicheskii Sbornik* **20**, 339–355. (In Russian.)

Lanave C, Preparata G, Saccone C and Serio G (1984) A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* **20**, 86–93.

Levin BA, Freyhof J, Lajbner Z, Perea S, Abdoli A, Gaffaroğlu M, Özuluğ M, Rubenyan HR, Salnikov VB and Doadrio I (2012) Phylogenetic relationships of the algae scraping cyprinid genus *Capoeta* (Teleostei: Cyprinidae). *Molecular Phylogenetics & Evolution* **62**, 542–549.

Mackiewicz JS (1972) Caryophyllidea (Cestoidea): A review. *Experimental Parasitology* **31**, 417–512.

Mackiewicz JS (1994) Order Caryophyllidea van Beneden in Carus, 1863. In Khalil LF, Jones A and Bray RA (eds.), *Keys to the Cestode Parasites of Vertebrates*. Wallingford: CAB International, 21–43.

Mackiewicz JS (2003) Caryophyllidea (Cestoidea): Molecules, morphology and evolution. *Acta Parasitologica* **48**, 143–154.

Machordom A and Doadrio I (2001) Evidence of a Cenozoic Betic-Kabilian connection based on freshwater fish phylogeography (*Luciobarbus*, Cyprinidae). *Molecular Phylogenetics & Evolution* **18**, 252–263.

Mikailov TK (1975) [Parasites of fish in the reservoirs of Azerbaydzhan (systematics, dynamics and origin).] *Baku: Elm*: 297. (In Russian.)

Nadler SA, and Pérez-Ponce de León G (2011) Integrating molecular and morphological approaches for characterizing parasite cryptic species: Implications for parasitology. *Parasitology* **138**, 1688–1709.

Oellermann LK and Skelton PH (1990) Hexaploidy in yellowfish species (*Barbus*, Pisces, Cyprinidae) from Southern Africa. *Journal of Fish Biology* **37**, 105–115.

Oros M, Scholz T, Hanzelová V and Mackiewicz JS (2010) Scolex morphology of monozoic cestodes (Caryophyllidea) from the Palaearctic Region: A useful tool for species identification. *Folia Parasitologica* **57**, 37–46.

Oros M, Uhrovič D, Choudhury A, Mackiewicz JS and Scholz T (2020) Scolex morphology of monozoic tapeworms (Caryophyllidea) from the Nearctic Region: Taxonomic and evolutionary implications. *Folia Parasitologica* **67**, 003.

Özdemir Y, and Sarıeyyüpoğlu M (1993) Some parasites of *Barbus capito pectoralis* caught in Keban Dam Lake. *Firat University Journal of Engineering Science* **5**, 114–126.

Paperna I (1964) The metazoan parasite fauna of Israel inland water fishes. *Bamidgeh, Bulletin of Fish Culture in Israel* **16**, 1–66.

Perea S, Böhme M, Zupančič P, Freyhof J, Šanda R, Özuluğ M, Abdoli A and Doadrio I (2010) Phylogenetic relationships and biogeographical patterns in circum Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. *BMC Evolutionary Biology* **10**, 1–27.

Popov TA (1924) [The anatomical structure of *Caryophyllaeus armeniacus* N. Cholodkowsky, 1915, and its position in the system]. *Proceedings of the Tropical Institute of Armenia, Yerevan,* **1**, 67–73. (In Russian)

Protasova EP, Kuperman BI, Roitman VA, and Poddubnaya LG (1990) [*Caryophyllid tapeworms of the fauna of USSR]. Moscow*: Nauka, *237*R. (In Russian.)

Rahemo ZIF and Al-Kalak SN (1993) A new record of a caryophyllaeid cestode, *Khawia armeniaca* from Iraq. *Acta Parasitologica Turcica* **17**, 74–78.

Rahemo ZIF, and Mohammad SA (2002) *Khawia barbi* sp. n. (Cestoda: Caryophyllidae) from the common freshwater fish, Barbus luteus from river Tigris, Mosul, Iraq. *Rivista di Parassitologia* **19**, 189–192.

Rambaut A, Drummond AJ, Xie D, Baele G and Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**, 901–904.

R Core Team (2022) *R: a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. https://www.R-project.org/

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across large model space. *Systematic Biology* **61**, 539–542.

Shulman SS (1958) [Zoogeographical analysis of parasites of freshwater fish of the USSR.] Basic Problems of the Parasitology of Fish. *Leningrad*: Izdatelstvo Leningradskogo Universiteta, 184–230. (In Russian)

Scholz T, Brabec J, Kráľová-Hromadová I, Oros M, Bazsalovicsová E, Ermolenko A and Hanzelová V (2011) Revision of *Khawia* spp. (Cestoda: Caryophyllidea), parasites of cyprinid fish, including a key to their identification and molecular phylogeny. *Folia Parasitologica* **58**, 197–223.

Scholz T, and Kuchta R (2017) A digest of fish tapeworms. *Vie et Milieu* **67**, 43–58.

Scholz T, and Oros M (2017) *Caryophyllidea van Beneden in Carus, 1863. Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth*: University of Kansas, Natural History Museum, Special Publication. Vol. 25. 47–64.

Scholz T, Oros M, Bazsalovicsová E, Brabec J, Waeschenbach A, Xi B-W, Aydoğdu A, Besprozvannykh V, Shimazu T, Králová-Hromadová I and Littlewood DTJ (2014) Molecular evidence of cryptic diversity in *Paracaryophyllaeus* (Cestoda: Caryophyllidea), parasites of loaches (Cobitidae) in Eurasia, including description of *P. vladkae* n. sp. *Parasitology International* **63**, 841–850.

Scholz T, Waeschenbach A, Oros M, Brabec J and Littlewood DTJ (2021) Phylogenetic reconstruction of early diverging tapeworms (Cestoda: Caryophyllidea) reveals ancient radiations in vertebrate hosts and biogeographic regions. *International Journal for Parasitology* **51**, 263–277.

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analyses and postanalysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.

Tang Q, Getahun A and Liu H (2009) Multiple in-to-Africa dispersals of labeonin fishes (Teleostei: Cyprinidae) revealed by molecular phylogenetic analysis. *Hydrobiologia* **623**, 261–271.

Tsigenopoulos CS, Durand JD, Unlu E and Berrebi P (2003) Rapid radiation of the Mediterranean *Luciobarbus* species (Cyprinidae) after the Messinian salinity crisis of the Mediterranean Sea, inferred from mitochondrial phylogenetic analysis. *Biological Journal of the Linnean Society* **80**, 207–222.

Tsigenopoulos CS, Kasapidis P and Berrebi P (2010) Phylogenetic relationships of hexaploid large-sized barbs (genus *Labeobarbus*, Cyprinidae) based on mtDNA data. *Molecular Phylogenetics & Evolution* **56**, 851–856.

Waeschenbach A, Webster BL and Littlewood DTJ (2012) Adding resolution to ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with large fragments of mtDNA. *Molecular Phylogenetics & Evolution* **63**, 834–847.

Williams JS, Gibson DI and Sadighian A (1980) Some helminth parasites of Iranian freshwater fishes. *Journal of Natural History* **14**, 685–699.

Xi B, Oros M, Wang G, Scholz T and Xie J (2013) *Khawia abbottinae* sp. n. (Cestoda: Caryophyllidea) from the Chinese false gudgeon *Abbottina rivularis* (Cyprinidae: Gobioninae) in China: Morphological and molecular data. *Folia Parasitologica* **60**, 141–148.

Yang L, and Mayden RL (2010) Phylogenetic relationships, subdivision, and biogeography of the cyprinid tribe Labeonini (*sensu* Rainboth, 1991) (Teleostei: Cypriniformes), with comments on the implications of lips and associated structures in the labeonin classification. *Molecular Phylogenetics & Evolution* **54**, 254–265.

Yang L, Sado T, Hirt MV, Pasco-Viel E, Arunachalam M, Li J, Wang X, Freyhof J, Saitoh K, Simons AM, Miya M, He S and Mayden RL (2015) Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics & Evolution* **85**, 97–116.

Zareian H, Esmaeili HR, Gholamhosseini A, Japoshvili B, Özuluğ M and Mayden RL (2018) Diversity, mitochondrial phylogeny, and ichthyogeography of the *Capoeta capoeta* complex (Teleostei: Cyprinidae). *Hydrobiologia* **806**, 363–409.