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ORIGINAL PAPER

The tapeworm *Atractolytocestus tenuicollis* (Cestoda: Caryophyllidea)—a sister species or ancestor of an invasive *A. huronensis*?

Ivica Králová-Hromadová · Jan Štefka · Eva Bazsalovicsová · Silvia Bokorová · Mikuláš Oros

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Abstract *Atractolytocestus tenuicollis* (Li, 1964) Xi, Wang, Wu, Gao et Nie, 2009 is a monozoic, non-segmented tapeworm of the order Caryophyllidea, parasitizing exclusively common carp (*Cyprinus carpio* L.). In the current work, the first molecular data, in particular complete ribosomal internal transcribed spacer 2 (ITS2) and partial mitochondrial cytochrome *c* oxidase subunit I (*cox*1) on *A. tenuicollis* from Niushan Lake, Wuhan, China, are provided. In order to evaluate molecular interrelationships within *Atractolytocestus*, the data on *A. tenuicollis* were compared with relevant data on two other congeners, *Atractolytocestus huronensis* and *Atractolytocestus sagittatus*. Divergent intragenomic copies (ITS2 paralogues) were detected in the ITS2 ribosomal spacer of *A. tenuicollis*; the same phenomenon has previously been observed also in two other congeners. ITS2 structure of *A. tenuicollis* was very similar to that of *A. huronensis* from Slovakia, USA and UK; overall pairwise sequence identity was 91.7–95.2 %. On the other hand, values of sequence identity between *A. tenuicollis* and *A. sagittatus* were lower, 69.7–70.9 %. *Cox*1 sequence, analysed in five *A. tenuicollis* individuals, were 100 % identical and no intraspecific variation was observed. Comparison of *A. tenuicollis cox*1 with respective sequences of two other *Atractolytocestus* species showed that the mitochondrial haplotype found in Chinese *A. tenuicollis* is structurally specific (haplotype 4; Ha4) and differs from all so far determined *Atractolytocestus* haplotypes (Ha1 and Ha2 for *A. huronensis*; Ha3 for *A. sagittatus*). Pairwise sequence identity between *A. tenuicollis cox*1 haplotype and remaining three haplotypes followed the same pattern as in ITS2. The nucleotide and amino acide (aa) sequence comparison with *A. huronensis* Ha1 and Ha2 revealed higher sequence identity, 90.3–90.8 % (96.9 % in aa), while lower values were achieved between *A. tenuicollis* haplotype and Ha3 of Japanese *A. sagittatus*—75.2 % (81.9 % in aa). The phylogenetic analyses using *cox*1, ITS2 and combined *cox*1+ITS2 sequences revealed close genetic interrelationship between *A. tenuicollis* and *A. huronensis*. Independently of a type of analysis and DNA region used, the topology of obtained trees was always identical; *A. tenuicollis* formed separate clade with *A. huronensis* forming a closely related sister group.

Note: Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers KC834609-KC834634.

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I. Králová-Hromadová (✉) · E. Bazsalovicsová · M. Oros
Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia
e-mail: hromadova@saske.sk

J. Štefka
Biology Centre ASCR, Institute of Parasitology, and Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

S. Bokorová
Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Introduction

Monozoic, non-segmented tapeworms of the order Caryophyllidea are parasites of cypriniform and siluriform fishes of Europe, North America, Africa, Asia and Australia that are characterised by monozoic body plan and only one set of reproductive organs. Caryophyllidean genus *Atractolytocestus* Anthony 1958 (family Lytocestidae) comprises three valid species, *Atractolytocestus huronensis* Anthony 1958; *Atractolytocestus sagittatus* (Kulakovskaya and Akhmerov 1965); and

Atractolytocestus tenuicollis (Li 1964) Xi et al. 2009, all parasitizing exclusively common carp (*Cyprinus carpio* L.). The first species described within the genus, and so far most intensively studied is *A. huronensis*. It was originally found in River Huron, Michigan, USA (Anthony 1958), and since then it has been reported in several other North American localities (Hoffman 1999). The species was introduced along with its carp host to Europe, where it has shown its invasive potential and successfully invaded several European countries, such as England (Chubb et al. 1996; Kirk et al. 2003), Hungary (Majoros et al. 2003), Slovakia and the Czech Republic (Oros et al. 2004), Germany (Kappe et al. 2006), Croatia (Gjurčević et al. 2009), and Romania (Bazsalovicsová et al. 2011; Oros et al. 2011). Second species of the genus, *A. sagittatus*, was originally described from carp from the Amur River basin in Russia (Kulakovskaya and Akhmerov 1965), later on it was also found in Caspian Sea Drainage (Demshin and Dvoryadkin 1981) and Japan (Scholz et al. 2001).

The third and so far the least known species of the genus, *A. tenuicollis*, was described as *Khawia tenuicollis* from carp from Lake Wulasuhai, Inner Mongolia, in China (Li 1964), but Xi et al. (2009) transferred it to the genus *Atractolytocestus* Anthony 1958 according to the morphological characteristics typical to the genus—conical scolex (bulboacuminate type), vitelline follicles continuous alongside uterus and ovary with postovarian vitelline follicles. *A. tenuicollis* has been accepted as a valid species in recent taxonomic and phylogenetic studies, as a part of complex systematic revision of the order (Oros et al. 2011; Scholz et al. 2011; Xi et al. 2013).

In the current work, the first molecular data on Chinese *A. tenuicollis* are provided. In particular, complete ribosomal internal transcribed spacer 2 (ITS2) and partial mitochondrial cytochrome *c* oxidase subunit I (*cox*1) were analysed. In order to evaluate molecular interrelationships within *Atractolytocestus*, the data on *A. tenuicollis* were compared with relevant data on two other congeners, *A. huronensis* and *A. sagittatus*.

Materials and methods

Parasite material

Tapeworms *A. tenuicollis* were found in the intestine of common carp (*C. carpio* L.) from Niushan Lake, Wuhan, in China in March 2009 and processed as described in detail by Oros et al. (2011). Live tapeworms were washed and immediately fixed with hot 4 % formalin for morphological studies and five specimens were fixed with ethanol (95–99 %) for DNA analyses. Parasites were identified according to the morphological characters determined by Xi et al. (2009), i.e. bulboacuminate scolex, numerous testes begin posterior to first vitelline follicles, vitelline follicles form an uninterrupted line alongside uterine coils and ovarian arms. Photomicrographs of morphologically analysed tapeworms (Fig. 1) were digitally captured with a Leica DFC 450C camera mounted on a Leica DM 5000B light microscope with differential interference contrast. Voucher specimens are deposited in the Helminthological collection of the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (collection no. C-635).

Newly obtained molecular data on *A. tenuicollis* were combined with data on recently published molecular structure of ribosomal ITS2 spacer and mitochondrial *cox*1 of *A. huronensis* from several European countries and USA, and *A. sagittatus* from Japan (Králová-Hromadová et al. 2010; Bazsalovicsová et al. 2011, 2012). The details on *Atractolytocestus* populations and comparative ITS2 and *cox*1 sequences are summarised in Tables 1 and 2.

DNA isolation, PCR protocol, sequencing

Genomic DNA was isolated using the QIAamp® DNA Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Conditions of PCR amplification were described in detail in Králová-Hromadová et al. (2010). Sequencing was performed using automatic genetic analyzer Applied Biosystems 3130xl (Applied Biosystems, Foster City, California, USA) and BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). The sequence alignment was performed using ClustalW (Thompson et al. 1994).

ITS2 amplification, cloning and sequencing

For amplification of complete ITS2 spacer, the 5.8S-2 (5′-GTCGATGAAGAGCGCAGC-3′; Králová-Hromadová et al. 2003) and ITS-2 (5′-AGGAGGCGAATCACTAT-3; Cunningham 1997) primers with annealing positions in the 5.8S and LSU rDNA, respectively, were applied. The PCR products were loaded on the 1.5 % agarose gel and purified using the Wizard PCR purification Kit (Promega, Madison, Wisconsin). Purified PCR products of ITS2 ribosomal spacer amplified from five individuals (AT CH1–AT CH5) were cloned into the pGEM®-T Easy vector (Promega) following the manufacturer's protocol. Three to five recombinant clones from each individual were purified with the Plasmid miniprep kit (Genomed, Löhne, Germany) and sequenced using universal primers T7 and Sp6 (AT CH1/1–5, AT CH2/1–3, AT CH3/1–3, AT CH4/1–5, AT CH5/1–5). The boundaries of both spacers were determined by sequence alignment using ClustalW (Thompson et al. 1994) according to the sequences of Slovak *A. huronensis* (Králová-Hromadová et al. 2010).

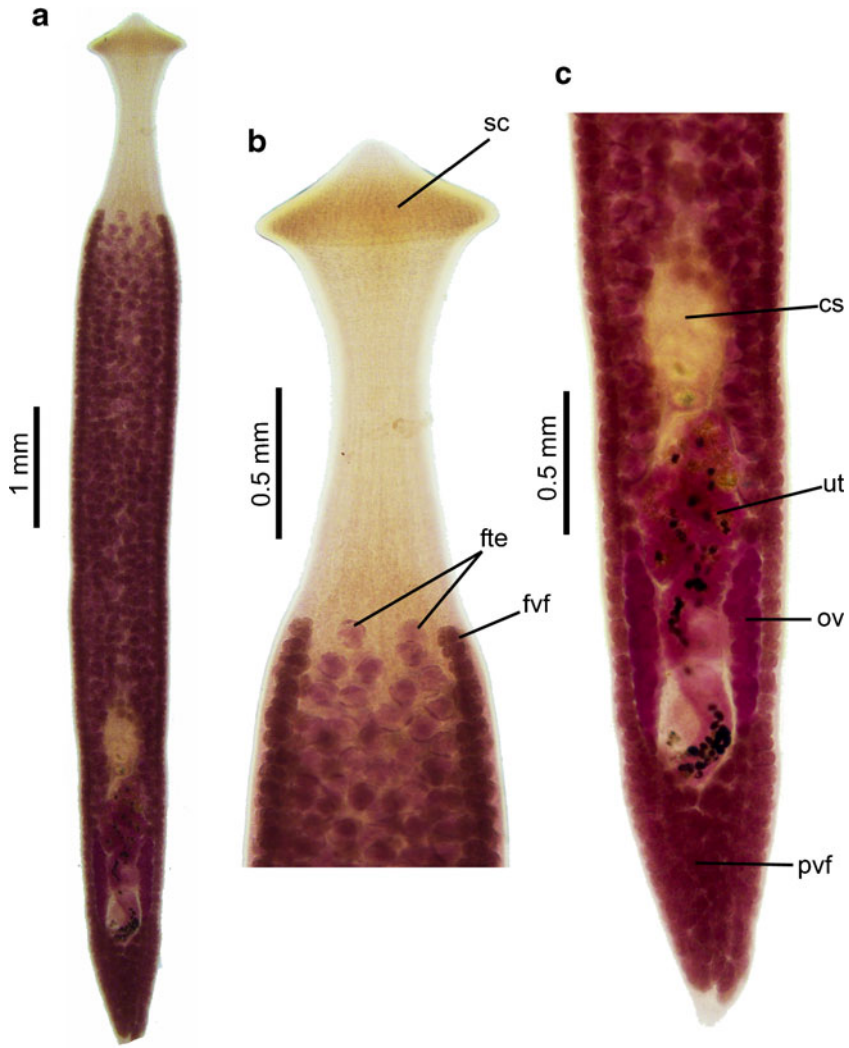

Fig. 1 Photomicrographs of *A. tenuicollis* from *Cyprinus carpio* in China. **a** Total view of the whole mounted worm; **b** anterior part of the body—note the bulboacumminate scolex and the distribution of first vitelline follicles and first testis; **c** posterior part of the body. Abbreviations: *cs*, cirrus-sac; *fte*, first testis; *fvf*, first vitelline follicles; *ov*, ovary; *pvf*, postovarian vitelline follicles; *sc*, scolex; *ut*, uterus

Partial cox1 amplification

Partial *cox*1 was amplified in all five *A. tenuicollis* individuals. The forward primer CFCYT2 (5′-ACTAAGTGTTTTCAAAA-3′), with annealing position in the tRNA-Trp (Tryptophane) and reverse primer CRCYT2 (5′-CCAAAAAACCAAAACAT-3′), annealing about 650 bp inside the *cox*1 gene, were originally designed by us and described in Bazsalovicsová et al. (2011). The ATG start codon was determined according to the flatworm mitochondrial code (Telford et al. 2000).

Phylogenetic reconstruction

*Cox*1 sequences were aligned by eye in the Seaview 4.2 (Gouy et al. 2010) and analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) approaches. The MP analysis was run in TNT 1.1 (Goloboff et al. 2000). ML was performed in PhyML 3.0 (Guindon et al. 2010) using HKY+I model of molecular evolution with parameters estimated from the data. The same model was used in BI run using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Two independent runs with four chains each were run for 10 million MCMC generations with sampling frequency of 10,000 steps. Twenty-five percent of the samples were discarded as burn in. Tracer 1.5 (Rambaut and Drummond 2005) was used to check convergence between runs. The molecular model was selected in jModeltest 2.1 (Darriba et al. 2012) using AIC criterion. Clade supports for MP and ML trees were obtained with 1000 bootstraps of the data. Sequences of *Caryophyllaeides fennica* and *Breviscolex orientalis* downloaded from GenBank (Accession nos. JQ034052.1 and JQ034055.1) were used as outgroups in the analyses.

*BEAST software (Heled and Drummond 2010) was used to reconcile *Atractolytocestus* species tree using both *cox*1 and ITS2 sequence data. The alignment of ITS2 was first trimmed by manually deleting numerous gap positions. Trimmed dataset was 607 bp long and contained 100 sequences (21 from China, 21 from Japan, 20 from Slovakia, 19 from UK and 19 from USA). Separate models were used

Table 1 Internal transcribed spacer 2 (ITS2) sequences of *A. tenuicollis*, *A. huronensis* and *A. sagittatus* applied in current study

Atractolytocestus species	Species code	Country of origin	Country code	Code of tapeworm/ nos of ITS2 clones	GenBank accession numbers
A. tenuicollis[a]	AT	China	CH	AT CH 1/1-5	KC834614-KC834618
				AT CH 2/1-3	KC834619-KC834621
				AT CH 3/1-3	KC834622-KC834624
				AT CH 4/1-5	KC834625-KC834629
				AT CH 5/1-5	KC834630-KC834634
A. huronensis[b]	AH	Slovakia	SK	AH SK 1/1-5	FJ475089-FJ475093
				AH SK 2/1-5	FJ475094-FJ475098
				AH SK 3/1-5	FJ475099-FJ475103
				AH SK 4/1-5	FJ475104-FJ475108
A. huronensis[c]	AH	United States	US	AH US 1/1-4	HM480456-HM480459
				AH US 2/1-5	HM480460-HM480464
				AH US 3/1-5	HM480465-HM480469
				AH US 4/1-5	HM480470-HM480474
A. huronensis[c]	AH	UK	UK	AH UK 1/1-2	HM064009-HM064010
				AH UK 2/1-4	HM064011-HM064014
				AH UK 3/1-3	HM064015-HM064017
				AH UK 4/1-5	HM064018-HM064022
				AH UK 5/1-5	HM064023-HM064027
A. sagittatus[d]	AS	Japan	JP	AS JP 1/1-4	JF424648-JF424651
				AS JP 2/1-4	JF424652-JF424655
				AS JP 3/1-5	JF424656-JF424660
				AS JP 4/1-4	JF424661-JF424664
				AS JP 5/1-4	JF424665-JF424668

Note—species codes, country codes and tapeworm codes/numbers of ITS2 clones correspond to that indicated on ESM, Supplement 2

[a] Original data

[b] Data published in Králová-Hromadová et al. 2010

[c] Data published in Bazsalovicsová et al. 2011

[d] Data published in Bazsalovicsová et al. 2012

Table 2 Cytochrome *c* oxidase (*cox*1) sequences of *A. tenuicollis*, *A. huronensis* and *A. sagittatus* used in the present study

Atractolytocestus species	Species code	Country of origin	Country code	*Cox*1 haplotype	Tapeworms codes and numbers	GenBank accessions numbers
A. tenuicollis[a]	AT	China	CH	Ha4	AT CH1—AT CH5	KC834609-13
A. huronensis[b]	AH	Slovakia	SK	Ha1	AH SK1—AH SK6	HM480475
A. huronensis[b]	AH	Hungary	HU	Ha1	AH HU1—AH HU6	HM480476
A. huronensis[b]	AH	Croatia	CR	Ha1	AH CR1—AH CR6	HM480477
A. huronensis[b]	AH	Romania	RO	Ha1	AH RO1—AH RO5	HM480478
A. huronensis[b]	AH	United States	US	Ha2	AH US1—AH US4	HM480480
A. huronensis[b]	AH	UK	UK	Ha2	AH UK1—AH UK6	HM480479
A. sagittatus[c]	AS	Japan	JP	Ha3	AS JP1—AS JP5	JF424669

Note—species codes, country codes, haplotype numbering and tapeworm codes correspond to those indicated on Fig. 2 and ESM, Supplement 1

[a] Original data

[b] Data published in Bazsalovicsová et al. 2011

[c] Data published in Bazsalovicsová et al. 2012

for the two genes. HKY+I was used for *cox*1. GTR+G with four gamma categories was selected for ITS2 using AIC criterion in jModeltest 2.1. *BEAST was run for 200 million MCMC iterations with parameters recorded every 20,000 steps. Ten percent of the samples were discarded as burn in. Lognormal relaxed clock were selected as the clock prior. Due to lack of fossil record there are no general molecular clock rate estimates available for cestodes, but the absolute values of rates or dating splits were not subject of this study. To avoid fixing relative rates between the two genes with ad hoc values, the rate for *cox*1 gene was fixed at 1 and the clock rate for ITS2 was allowed to vary using a uniform prior (0.1, 1.0E100) with initial value 1. Yule process was selected as the species tree prior. Two independent runs were made to check for convergence. Tracer 1.5 (Rambaut and Drummond 2005) was used to examine ESS values of parameters and convergence between runs. ESS values for all parameters were above 300 and traces showed good mixing.

Results

Ribosomal ITS2

Divergent intragenomic copies were detected in the ITS2 ribosomal spacer of *A. tenuicollis*. A total of 21 recombinant clones obtained from five tapeworm individuals yielded 16 different sequence types caused mainly by single nucleotide polymorphisms and varying numbers of short repetitive region $(TTGGT)_n$ (Table 3, microsatellite repeat 3). It was present either in three or in five repetitions, which differentiated ITS2 in two ITS2 variants (643 and 653 bp, respectively) (Table 3).

ITS2 structure of *A. tenuicollis* was similar to that of *A. huronensis* from Slovakia, USA and UK. Overall pairwise sequence identity was 92.0–95.2 % between *A. tenuicollis* and British *A. huronensis*, 91.7–94.8 % between *A. tenuicollis* and *A. huronensis* from USA, and 92.0–93.9 % sequence identity was determined between *A. tenuicollis* and *A. huronensis* from Slovakia. The most profound difference between *A. tenuicollis* and all geographic *A. huronensis* populations was in variation of number of repetitive regions GT (Table 3, microsatellite repeat 1) and AGCC (Table 3, repeat 4) .

ITS2 sequence structure of *A. tenuicollis* was more different from *A. sagittatus* than from *A. huronensis*. Structure and distribution of microsatellite motifs in *A. sagittatus* had substantial species-specific character and did not correspond to those found in *A. tenuicollis* and *A. huronensis*. Pairwise sequence identity between *A. tenuicollis* and *A. sagittatus* was 69.7–70.9 %.

Mitochondrial cox1

The two designed primers CFCYT2 and CRCYT2 amplified 672 bp of *cox*1 gene, covering ATG start codon and the following 5′ part of the gene, encoding for 224 amino acids of the protein. *Cox*1 sequence was identical for all five *A. tenuicollis* individuals.

Comparison of *A. tenuicollis cox*1 with respective sequences of two other *Atractolytocestus* species showed that the mitochondrial haplotype found in Chinese *A. tenuicollis* is structurally specific (marked as haplotype 4, Ha4) and differs from all so far determined *Atractolytocestus* haplotypes, in particular Ha1 (*A. huronensis* from Slovakia, Hungary, Croatia, Romania), Ha2 (*A. huronensis* from USA and

Table 3 Polymorphisms in repetitive motifs within ITS2 rDNA of *A. tenuicollis* from China and *A. huronensis* from Slovakia, United States (US), and United Kingdom (UK)

Atractolytocestus species	Country of origin	ITS2 variant[a]	Microsatellite repeat 1	Microsatellite repeat 2	Microsatellite repeat 3	Microsatellite repeat 4	Size of ITS2 variant (bp)
A. tenuicollis[b]	China	1	$(GT)_3$	TGT $(TGC)_2$	$(TTGGT)_3$	$(AGCC)_3$	643
		2	$(GT)_3$	TGT $(TGC)_2$	$(TTGGT)_5$	$(AGCC)_3$	653
A. huronensis[c]	Slovakia	1	$(GT)_1$	$(TGC)_3$ TGT $(TGC)_2$	$(TTGGT)_3$	$(AGCC)_1$	637
		2	$(GT)_1$	$(TGC)_6$	$(TTGGT)_2$	$(AGCC)_1$	632
		3	$(GT)_1$	$(TGC)_3$	$(TTGGT)_2$	$(AGCC)_1$	623
A. huronensis[d]	US	1	$(GT)_1$	$(TGC)_3$ TGT $(TGC)_2$	$(TTGGT)_3$	$(AGCC)_1$	637
		2	$(GT)_1$	$(TGC)_6$	$(TTGGT)_2$	$(AGCC)_1$	632
A. huronensis[d]	UK	1	$(GT)_1$	$(TGC)_3$ TGT $(TGC)_2$	$(TTGGT)_3$	$(AGCC)_1$	637
		2	$(GT)_1$	$(TGC)_6$	$(TTGGT)_2$	$(AGCC)_1$	632

[a] The number of ITS2 variants is specific and corresponds to the respective geographic population of the species

[b] Original data

[c] Data published by Králová-Hromadová et al. (2010)

[d] Data published by Bazsalovicsová et al. (2011)

UK) (Bazsalovicsová et al. 2011), and Ha3 (*A. sagittatus* from Japan) (Bazsalovicsová et al. 2012).

Pairwise sequence identity between *A. tenuicollis cox*1 haplotype and remaining three haplotypes followed the same pattern as in ITS2. The nucleotide and amino acid (aa) sequence comparison with *A. huronensis* Ha1 and Ha2 revealed 90.3–90.8 % (96.9 % in aa) sequence identity. Significantly lower values were achieved between *A. tenuicollis* Ha4 and Ha3 of Japanese *A. sagittatus*—75.2 % (81.9 % in aa).

To visualise the sample sizes of our data in phylogenetic analyses, all available sequences for the four *cox*1 haplotypes were included when building the trees. The MP method produced a single most parsimonious tree, identical in topology with the two other methods of phylogenetic reconstruction (Fig. 2). In accordance with the indices of sequence similarity the most basal split was between Japanese samples of *A. sagittatus* and the Chinese, US and European samples. The latter group formed two well-supported clusters separating Chinese *A. tenuicollis* from American and European *A. huronensis*. Despite very low sequence divergence between Ha1 and Ha2 of *A. huronensis* the clade separating USA and UK populations from continental Europe received relatively

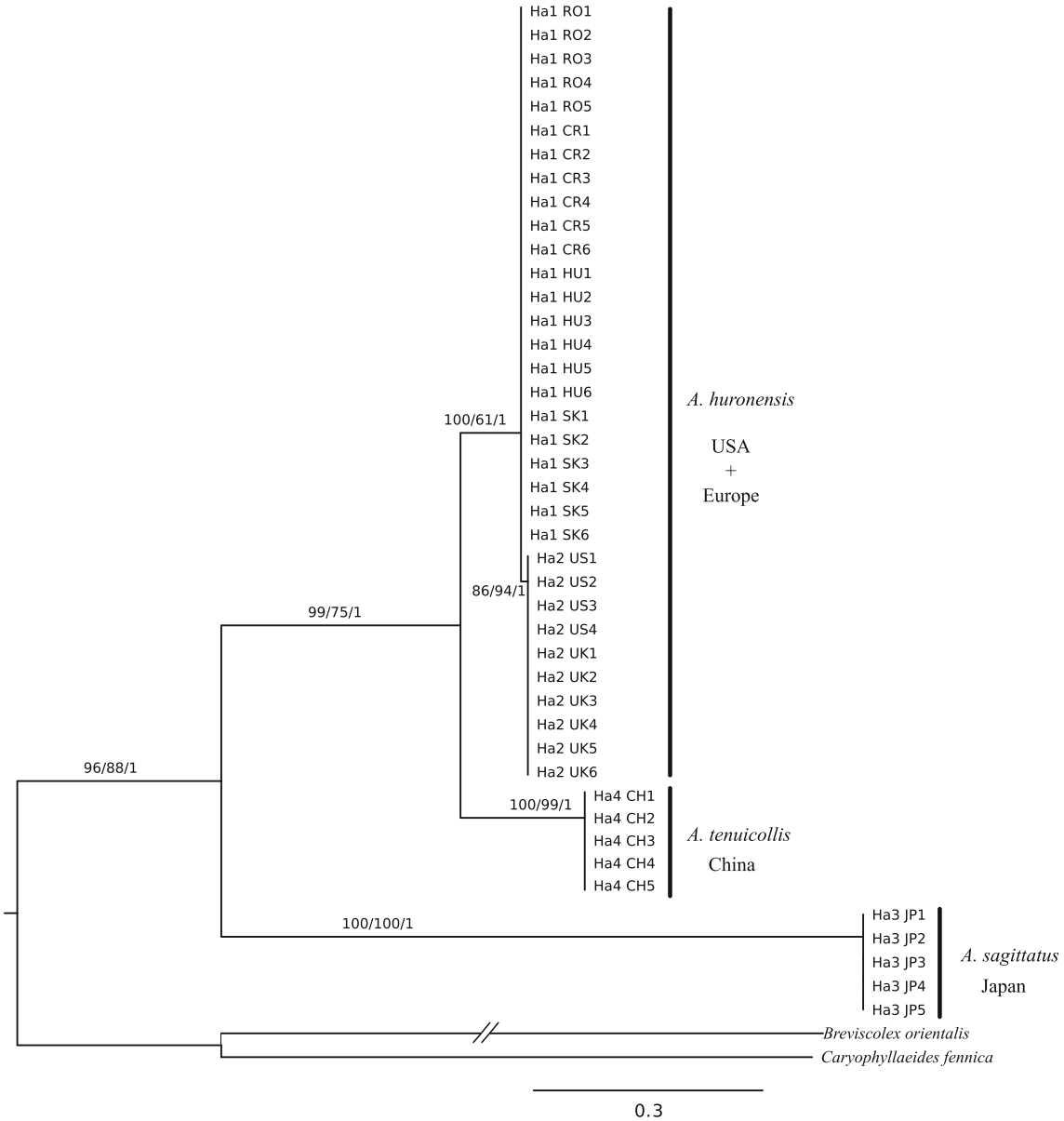

Fig. 2 Maximum Likelihood phylogeny of *Atractolytocestus* obtained using mitochondrial sequences. Clade supports (bootstraps and posterior probabilities) are as follows maximum parsimony/maximum likelihood/Bayesian inference. See Table 2 for details on species codes, country codes, haplotype numbering and individual codes

high support in all analyses. Bootstrap support was 86 and 94 % in MP and ML, respectively, and posterior probability was 1 in BI.

Joint analysis of mtDNA and ribosomal data

Species tree (Fig. 3) produced in the *BEAST analysis showed pattern congruent with mtDNA phylogenies in Fig. 2. Posterior probabilities of *A. huronensis* and of its inner clade separating UK and USA populations from continental Europe showed high values (1 and 0.95 respectively). The latter split was relatively well-supported despite disagreement between ITS2 and *cox*1 data caused by occurrence of intragenomic variants in ITS2. The clade separating *A. huronensis* and *A. tenuicollis* from *A. sagittatus* had lower but still relatively high posterior probability (0.88).

Individual gene trees (Electronic supplementary material (ESM), Supplements 1 and 2) showed values of posterior probabilities above 0.9 in all but one clade in *cox*1 and values equal or very close to 1 in all ITS2 clades. Similarly to earlier studies (Bazsalovicsová et al. 2011, 2012), some ITS2 variants were shared between the samples belonging to UK+US and European mtDNA clades. However, a general pattern of three clearly separated lineages (*A. sagittatus*, *A. tenuicollis* and *A. huronensis*) remained the same in both gene trees.

Discussion

Current paper provides new molecular data on *A. tenuicollis* and its comparison with relevant data on the only two congeners, *A. huronensis* and *A. sagittatus*. Molecular data along with phylogenetic analysis and statistical methods revealed interesting outcomes on current taxonomy and interrelationships within the genus *Atractolytocestus*.

Morphological and molecular studies on caryophyllidean family Lytocestidae (mainly those of *Khawia* and *Atractolytocestus*) revealed that conflict between morphological and genetic data represents a serious problem of the current taxonomy and systematics of the order Caryophyllidea (Scholz et al. 2011; Králová-Hromadová et al. 2012; current work). In a majority of tapeworms (e.g. *Bothriocephalus*, *Taenia*), an occurrence of sibling species or cryptic species complexes (morphologically similar or identical species with high molecular variation) should not be underestimated when envisaging their taxonomy (Verneau et al. 1997; Lavikainen et al. 2010). Controversially, monozoic tapeworms of the order Caryophyllidea are characterised by an opposite example; occurrence of morphologically dissimilar taxa (mainly recognised as different species) that display high genetic similarity.

This feature was studied in detail in caryophyllidean genus *Khawia*. *Khawia sinensis*, specific parasite of carp (*C. carpio*—Cyprininae), and *Khawia saurogobii*, parasite of gudgeons (*Saurogobio* spp.—Gobioninae) are morphologically clearly different species (Xi et al. 2009; Scholz et al. 2011) that displayed very high molecular sequence identity in ribosomal ITS2, SSU and LSU (ribosomal small and large subunit), and in mitochondrial *cox*1 and *nad*3 (nicotinamide dehydrogenase subunit III) (Scholz et al. 2011; Králová-Hromadová et al. 2012). In spite of this, taxonomic status of recently described *K. saurogobii* was not questioned. It is assumed that *K. saurogobii*, after switching to a new unrelated fish host, underwent morphological divergence as a result of ongoing sympatric speciation, but this process has not been accompanied by corresponding nucleotide changes (Scholz

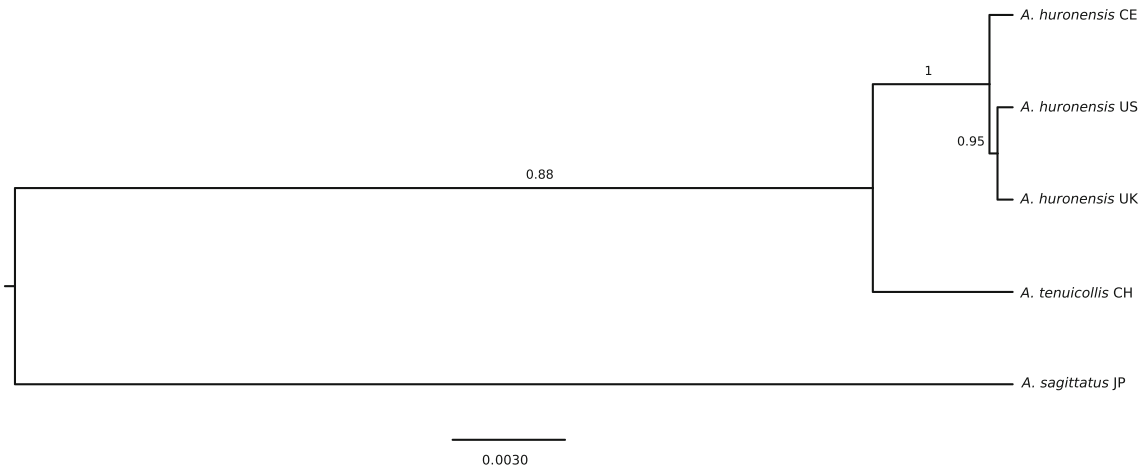

Fig. 3 Species tree of *A. tenuicollis* and *A. huronensis* obtained in *BEAST combining datasets of ITS2 and *cox*1. Posterior probabilities are provided above clades. Abbreviations of samples origin are as follows: Continental Europe (*CE*), United Kingdom (*UK*), USA (*US*), China (*CH*) and Japan (*JP*)

et al. 2011). Since sympatrically derived species are expected to show profound genetic similarity (Via 2001), striking molecular similarity of *K. saurogobii* and *K. sinensis* was explicable (Králová-Hromadová et al. 2012).

In genus *Atractolytocestus*, the first ambiguity was raised by Jones and Mackiewicz (1969), who claimed that *A. huronensis* is very similar to *A. sagittatus*; the same objection was later on claimed by Chubb et al. (1996). The reason which had let them to such a conclusion was similar morphology of both congeners, with the only significant morphological difference being in the body size, and variation in number and position of testes. In *A. sagittatus*, the number of testes considerably exceeds 100–200 (in some specimens reaching to several hundreds) (Scholz et al. 2001). In *A. huronensis*, the number of testes is significantly lower (up to 20) and, contrary to *A. sagittatus*, they are always posterior to the first vitelline follicles (Oros et al. 2004, 2011). Comparative molecular analysis of sequences of three DNA regions (ITS1, ITS2, *cox*1) has revealed conspicuous interspecific differences between the two *Atractolytocestus* species (Bazsalovicsová et al. 2012). Since differences between *A. huronensis* and *A. sagittatus* were significantly higher than well-known intraspecific variation (Bazsalovicsová et al. 2011), the validity of *A. sagittatus* was not questioned.

A. tenuicollis represent another example in family Lytocestidae where parallel morphological and molecular outcomes do not follow same conclusions. *A. tenuicollis* (originally described as *K. tenuicollis* (Li 1964)) was recently transferred to genus *Atractolytocestus* (Xi et al. 2009) because it possess typical morphological characteristics of the genus (Anthony 1958). However, *A. tenuicollis* resembles morphological markers of both congeners; similarly to *A.sagittatus*, it is characterised by numerous testes, although anteriormost testes begin posterior to first vitelline follicles is alike in *A. huronensis*. Current molecular data confirmed validity of *A. tenuicollis* which formed separate clade using all, *cox*1, ITS2 and combined *cox*1+ITS2 input data. According to sequence identity values and based on phylogenetic tree topologies, Chinese *A. tenuicollis* is evidently genetically more closely related to *A. huronensis* than to *A. sagittatus*.

With cumulating molecular and genetic data on *A. huronensis*, the question of its geographic and genetic origin has been raised (Jones and Mackiewicz 1969; Králová-Hromadová et al. 2010; Bazsalovicsová et al. 2011). First genetic data on *A. huronensis* were achieved by Jones and Mackiewicz (1969) who revealed triploid character of American specimens of the tapeworm, described morphologically abnormal sperm cells and concluded parthenogenesis to be a regular mode of reproduction of the species. Recently, triploidy was confirmed also in Slovak *A. huronensis* individuals, along with distinct intragenomic ITS1 and ITS2 variants and dispersed chromosomal loci of nucleolar organiser regions (multiple NORs) (Králová-Hromadová et al. 2010; Špakulová et al. 2011). These features are evidently well fixed within the species since intragenomic ITS variants were present besides Slovak also in British and American populations (Bazsalovicsová et al. 2011) and a parthenogenic mode of reproduction was further supported also by ultrastructural studies of spermatic cells of Slovak *A. huronensis* (Bruňanská et al. 2011). *A. huronensis* represents so far the only cestode species where triploidy, intragenomic ITS variants, parthenogenesis and multiple NORs were proven to be mutually linked.

Jones and Mackiewicz (1969) hypothesised that if triploidy in *A. huronensis* arose by genetic and not interspecific hybridization, then immediate ancestor of the triploid line may still exist in carp. According to the authors, *A. huronensis* is either an old, relatively stable member of the caryophyllidean complex, since the pace of evolution is slowed when meiosis and fertilisation is suppressed, or it represents new species, because polyploidy and parthenogenesis has a limited potential for long-term survival. Further, the authors concluded that if *A. huronensis* is an ancient species, it seems unlikely that organs of so little usefulness as sterile testes would have persisted at all and that there is a possibility that *A. sagittatus* with "many testes" might have been the diploid ancestor of *A. huronensis*. Since the third *Atractolytocestus* species (*A. tenuicollis*) was not questioned at that time, the hypothesis of Jones and Mackiewicz (1969) was very rational.

It can be hypothesised that *A. huronensis* (triploid parthenogen with few testes) emerged through genetic hybridization from a common ancestor (diploid sexual with many testes) that resided in Asia (China) for a long time. Latest molecular data on all three *Atractolytocestus* species (Králová-Hromadová et al. 2010; Bazsalovicsová et al. 2011, 2012; current work) strongly indicate that the sought-after species, sharing close ancestry with *A. huronensis*, is *A. tenuicollis*. The following so far achieved data on both *Atractolytocestus* species support this theory: (1) *morphology*; strikingly similar morphology of both species, with the most profound difference being in number of testes, numerous in *A. tenuicollis*, several in *A. huronensis*, however, posterior position of testes relative to the anteriormost vitelline follicles is common for both species (Oros et al. 2004; Xi et al. 2009); (2) *molecular data*; sequential differences between *A. huronensis* and *A. tenuicollis* and their phylogenetic relationships indicate evolutionarily close bonds (current data); (3) *karyology*; triploidy/ parthenogenesis have been well documented in *A. huronensis* (Jones and Mackiewicz 1969; Králová-Hromadová et al. 2010), contrary, two sets of chromosomes in *A. tenuicollis* observed in mitotically dividing cells point to its diploid character (M. Orosová and M. Oros, unpublished data); (4) *geographic distribution* of both species and *invasive character* of *A. huronensis* (details see below).

It is evident that common carp, as the specific host of *Atractolytocestus* spp., plays an important role in its introduction to novel territories. Common carp is the world's

oldest domesticated and most frequent aquaculture species whose domestication commenced over 4,000 years ago in China. It is supposed that the relatively continuous area of the wild carp ranged from the Black Sea and the River Danube drainage to the Far East, China and Japan during Neogene Period, and that it had been disrupted into western (Europe) and eastern (Asia) parts during the Ice Ages (Baruš and Oliva 1995). Throughout the history, carp has been cultured, wide-scale translocated and restocked; consequently the fish has spread to all continents except for Antarctica (Thai et al. 2004; Mabuchi et al. 2008).

Non-overlapping spatial distribution pattern and other biological features of *A. tenuicollis* and *A. huronensis* are consistent with characteristics of sexual and parthenogen conspecifics (see Pongratz et al. 2003 and references therein). The ancestral sexual population (represented here by state preserved in *A. tenuicollis*) used to be located in the distribution centre of the species, while parthenogens (here *A. huronensis*) are present at the margin of the distribution. It has been proposed that parthenogenetic descendant might fail to establish itself in the presence of sexual ancestor but it may have better colonising capacities which allow it to subjugate areas where sexuals have difficulties establishing a population.

A noteworthy feature of the triploid *A. huronensis* is its apparent ability to reproduce successfully and occupy new regions. As discussed by Jones and Mackievicz (1969), polyploid and parthenogenetic animals were often referred to exploit disturbed regions, like posglacial fringe areas. Even though the polyploidy and parthenogenesis are considered to have restrict potential for long-term survival, *A. huronensis* apparently represents a very successful exception. The species managed to colonise USA, Great Britain and continental Europe what was undoubtedly enhanced by intensive fish trade.

The pattern of virtually missing intrapopulation genetic variability in *A. huronensis*, where all individuals in each of the populations comprise a single mitochondrial *cox*1 haplotype, fits well their recent invasive origin and parthenogenetic way of reproduction. Although *A. tenuicollis* and *A. sagittatus* samples studied here comprised a single haplotype too, more specimens need to be sequenced to infer whether the extremely low level of mitochondrial diversity is a general feature of the genus and is inherent due to its biology.

Carp, as the specific host of *Atractolytocestus* spp. and China, as a very probable historic cradle of some caryophyllideans, play apparently an important role in spatial distribution and speciation processes within this genus. The conspicuous genetic similarity of *A. tenuicollis* with *A. huronensis* is very interesting and indicates that future studies of the genus might be very helpful. First of all, larger sample sets especially from the Asian area of distribution need scrutinising for morphological re-description and possible taxonomic re-evaluation. Besides, multilocus molecular markers, such as microsatellites or SNPs, may help to consolidate the taxonomic status of *A. tenuicollis* and its interrelationship with *A. huronensis*.

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