Conflict between morphology and molecular data: a case of the genus *Caryophyllaeus* (Cestoda: Caryophyllidae), monozoic tapeworms of cyprinid fishes

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Abstract: Molecular analysis of an extensive specimen collection of morphologically distinct tapeworms of the genus *Caryophyllaeus* Gmelin, 1790 (Cestoda: Caryophyllidae), parasites of cyprinid fishes in the Palaearctic Region, brought evidence of host-related plasticity in critical morphological characters widely used for species circumscription and classification of these tapeworms. The results obtained here do not support the traditionally used morphological concept of species-defining characters of the order Caryophyllidae Carus, 1863, especially due to high morphological plasticity of the scolex and the anterior end of the body. Several morphotypes within both *Caryophyllaeus laticeps* (Pallas, 1781) and *Caryophyllaeus brachycollis* Janiszewska, 1953 generally corresponding to different hosts and geographical regions, were recognised likely suggesting host- and geography-related intraspecific morphological variability. Genetic data confirmed euryxenous host specificity of both species, most profoundly *C. laticeps*, but did not support the existence of cryptic species. In fact, some of the fish hosts may harbour both of the congeneric species including several of their respective morphotypes. The pattern of morphological and genetic divergence observed in both cestode species studied indicates a scenario of possible host range extensions and subsequent parasite diversification. If molecular sequence variability and host-related morphological polymorphism are confirmed in other groups of monozoic cestodes, it may complicate species identification and straightforward classification of these parasites.

Keywords: scolex morphology, morphological plasticity, *Caryophyllaeus laticeps*, *Caryophyllaeus brachycollis*, mitochondrial haplotypes, *cox1*, *lsrDNA*, morphotypes

Classification, taxonomy and species identification of parasites have been largely, if not exclusively, based on morphological characteristics. Monozoic fish cestodes of the order Caryophyllidae Carus, 1863 seem to represent the most basal group of ‘true’ tapeworms (Eucestoda) (Olson et al. 2001, Waeschenbach et al. 2012, Caira et al. 2014). Subtle differences in shape and size of the scolex and the anteriormost extent of vitelline follicles and testes play the central role in species identification of some groups of caryophyllideans (Dubinina 1987, Oros et al. 2010, Scholz et al. 2011).

As part of a large-scale survey of monozoic tapeworms parasitising cyprinids, we collected an extensive material of *Caryophyllaeus laticeps* (Pallas, 1781) from a wide spectrum of cyprinid fishes throughout Europe and processed it using the standardised methodology (Oros et al. 2009, 2010). In addition, samples of a less frequently found congeneric species, *Caryophyllaeus brachycollis* Janiszewska, 1953, were also included into present study.

Within the populations of these tapeworms we found several morphotypes, often specific to different fish hosts, some of which were supposed to represent new species, e.g. specimens from *Chondrostoma* Agassiz and *Vimba* Fitzinger.

Sequences of two genes (partial mitochondrial cytochrome *c* oxidase subunit I – *cox1*, and partial large subunit of the ribosomal DNA – *lsrDNA*), which were successfully used in previous phylogenetic studies on caryophyllideans (Bazsalovicsová et al. 2011, Scholz et al. 2011, Brabec et al. 2012), were obtained to assess the relationships among the individual *C. laticeps* and *C. brachycollis* morphotypes from different hosts and regions. The results of molecular analysis were confronted with main morphological discriminative characters of individual morphotypes. The reliability of the traditional morphological criteria for caryophyllidean species circumscription and systematics is also discussed.

MATERIALS AND METHODS

Parasites

Table 1 summarises details on *Caryophyllaeus laticeps* and *C. brachycolis* specimens analysed in the present work. Tapeworms studied were obtained by dissection of freshly killed fish; they were divided into three parts: the anterior part with the scolex and the posterior part with terminal genitalia were fixed with hot 4% formalin for morphological studies, whereas the middle part was fixed in molecular-graded ethanol (95–99%) for DNA analyses. All specimens studied could be assigned to one of seven morphotypes, distinguished mainly by the shape of the

body, scolex and neck, and position of the anteriormost testes and vitelline follicles (Table 2). A detailed morphological characterisation of all morphotypes along with redescription of *C. laticeps* and *C. brachycolis* will be presented in separate papers.

DNA isolation, PCR amplification, sequencing

Genomic DNA was isolated using the QIAamp® DNA Kit (QIAGEN, Hilden, Germany). For PCR amplification, a total volume of amplification mixture was 20 µl and contained 10–20 ng of genomic DNA, 20 pmol of each of the forward and reverse primers, 0.2 mM of each of the deoxynucleotide triphosphate (Fermentas UAB, Vilnius, Lithuania), 0.5 U of *Taq*

Table 1. List of *Caryophyllaeus* specimens (code, host, locality) and genes (mitochondrial *cox1*; nuclear *lsrDNA*) analysed.

Sample code ¹	Fish host	Country of origin	Locality	<i>cox1</i> Acc. No.	<i>cox1</i> haplotype	<i>lsrDNA</i> Acc. No.	Cestode species	Morphotype
SK-SC-1	<i>Squalius cephalus</i>	Slovakia	Myslava Stream	KF051109	Ha1	KF051154	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-SC-2	<i>S. cephalus</i>	Slovakia	Myslava Stream	KF051110	Ha1	KF051138	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-SC-3	<i>S. cephalus</i>	Slovakia	Myslava Stream	KF051111	Ha1	KF051155	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-SC-4	<i>S. cephalus</i>	Slovakia	Myslava Stream	KF051105	Ha1	KF051156	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-SC-5	<i>S. cephalus</i>	Slovakia	Myslava Stream	KF051112	Ha1	KF051140	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-SC-6	<i>S. cephalus</i>	Slovakia	Myslava Stream	KF051113	Ha1	KF051157	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-SC-7	<i>S. cephalus</i>	Slovakia	Myslava Stream	KF051106	Ha1	KF051158	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-BS-1	<i>Ballerus sapa</i>	Slovakia	Danube River	KF051114	Ha2	KF051159	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
SK-BS-2	<i>B. sapa</i>	Slovakia	Danube River	KF051115	Ha2	KF051160	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
SK-BS-3	<i>B. sapa</i>	Slovakia	Danube River	KF051117	Ha2	KF051162	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
SK-BS-4	<i>B. sapa</i>	Slovakia	Danube River	KF051118	Ha2	KF051163	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
SK-AB-1	<i>Abramis brama</i>	Slovakia	Latorica River	KF051121	Ha3	KF051166	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 2
SK-BB-1	<i>Ballerus ballerus</i>	Slovakia	Latorica River	KF051122	Ha4	KF051167	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 2
SK-AB-2	<i>A. brama</i>	Slovakia	Latorica River	KF051123	Ha5	KF051139	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 2
SK-AB-3	<i>A. brama</i>	Slovakia	Tisa River	KF051100	Ha6	KF051168	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 2
SK-AB-4	<i>A. brama</i>	Slovakia	Danube River	KF051131	Ha7	KF051169	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
BG-VM-1	<i>Vimba melanops</i>	Bulgaria	Arda River	KF051107	Ha8	KF051142	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-2	<i>V. melanops</i>	Bulgaria	Arda River	KF051108	Ha9	KF051143	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-3	<i>V. melanops</i>	Bulgaria	Arda River	KF051102	Ha8	KF051144	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-4	<i>V. melanops</i>	Bulgaria	Arda River	KF051092	Ha10	KF051145	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-5	<i>V. melanops</i>	Bulgaria	Arda River	KF051103	Ha8	KF051146	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-6	<i>V. melanops</i>	Bulgaria	Arda River	KF051104	Ha11	KF051141	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-7	<i>V. melanops</i>	Bulgaria	Arda River	KF051093	Ha12	KF051147	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-8	<i>V. melanops</i>	Bulgaria	Arda River	KF051094	Ha12	KF051148	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-9	<i>V. melanops</i>	Bulgaria	Arda River	KF051095	Ha12	KF051149	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-10	<i>V. melanops</i>	Bulgaria	Arda River	KF051096	Ha10	KF051150	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-11	<i>V. melanops</i>	Bulgaria	Arda River	KF051097	Ha12	KF051151	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-12	<i>V. melanops</i>	Bulgaria	Arda River	KF051098	Ha8	KF051152	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-VM-13	<i>V. melanops</i>	Bulgaria	Arda River	KF051099	Ha12	KF051153	<i>C. laticeps</i>	<i>C.l.</i> morphotype 2
BG-BC-1	<i>Barbus cyclolepis</i>	Bulgaria	Arda River	KF051124	Ha1	KF051170	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
RU-AB-1	<i>A. brama</i>	Russia	Volga River	KF051126	Ha13	KF051176	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
RU-AB-2	<i>A. brama</i>	Russia	Volga River	KF051127	Ha14	KF051177	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
RU-BS-1	<i>B. sapa</i>	Russia	Volga River	KF051132	Ha15	KF051173	<i>C. laticeps</i>	<i>C.l.</i> morphotype 1
RU-BB-1	<i>Blicca bjoerkna</i>	Russia	Volga River	KF051133	Ha16	KF051174	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 2
RU-AB-3	<i>A. brama</i>	Russia	Rybinsk WR	KF051134	Ha17	KF051175	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 2
AT-CN-1	<i>Chondrostoma nasus</i>	Austria	Drau River	KF051128	Ha18	KF051176	<i>C. laticeps</i>	<i>C.l.</i> morphotype 4
AT-CN-2	<i>C. nasus</i>	Austria	Drau River	KF051129	Ha19	KF051179	<i>C. laticeps</i>	<i>C.l.</i> morphotype 4
AT-CN-3	<i>C. nasus</i>	Austria	Drau River	KF051130	Ha20	KF051180	<i>C. laticeps</i>	<i>C.l.</i> morphotype 4
FR-BM-1	<i>Barbus meridionalis</i>	France	Cassole River	KF051135	Ha21	KF051137	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
FR-BB-1	<i>Barbus barbus</i>	France	Arges River	KF051136	Ha22	KF051181	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-BM-1	<i>B. meridionalis</i>	Slovakia	Myslava Stream	JQ034064 ²	Ha23	JQ034120 ²	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 1
SK-AB-5	<i>A. brama</i>	Slovakia	Tisa River	JQ034078-83 ²	Ha24–26	JQ034124 ²	<i>C. brachycolis</i>	<i>C.b.</i> morphotype 2
SK-BS-5	<i>B. sapa</i>	Slovakia	Latorica River	JQ034077 ²	Ha27	JQ034122 ²	<i>C. laticeps</i>	<i>C.l.</i> morphotype 5
SK-AB-6	<i>A. brama</i>	Slovakia	Tisa River	JQ034070-71 ²	Ha28–29	JQ034123 ²	<i>C. laticeps</i>	<i>C.l.</i> morphotype 5
SK-CC-1	<i>Cyprinus carpio</i>	Slovakia	Tisa River	JQ034066-68 ²	Ha30–32	JQ034121 ²	<i>C. laticeps</i>	<i>C.l.</i> morphotype 3

Abbreviations: *C.b.* – *Caryophyllaeus brachycolis*; *C.l.* – *Caryophyllaeus laticeps*; WR – water reservoir; ¹sample codes comprise international country code-initials of host-sample number; ²sequences published by Brabec et al. (2012).

Table 2. Main distinguishable features among morphotypes of *Caryophyllaeus laticeps* (CL) and *Caryophyllaeus brachycollis* (CB).

Morphotype	Fish host (genera)	Body	Scolex shape	Neck	First testes	Vitelline follicles (VF)
CL 1 ¹	<i>Abramis, Ballerus</i>	slender	flabellate to spatulate	long, slender	posterior to VF	small, reach to ovary
CL 2	<i>Vimba</i>	robust	spatulate	short, wide	close to VF	anterior to gonopore
CL 3	<i>Cyprinus</i>	slender	cuneicrispate	widened anteriorly	posterior to VF	small, reach to ovary
CL 4	<i>Chondrostoma</i>	large, robust	spatulate	long, wide	posterior to VF	small, reach to ovary
CL 5	<i>Abramis, Ballerus</i>	slender	flabellate	moderate	posterior to VF	large, anterior to gonopore
CB 1 ²	<i>Barbus, Squalius</i>	robust	flabellate	short, wide	same level as VF	small, reach to ovary
CB 2	<i>Abramis, Ballerus, Blicca</i>	slender	flabellate to spatulate	moderate	posterior to VF	small, reach to ovary

¹considered to be a 'nominotypical' morphotype described as *Taenia laticeps* by Pallas (1781) and redescribed by Fuhrmann (1931); ²considered to be a 'nominotypical' morphotype described by Janiszewska (1953).

DNA polymerase (Invitrogen, Carlsbad, CA, USA) with corresponding reaction buffer and 1.5 mM MgCl₂. The PCR amplifications were run as follows: 5 min at 94°C as an initial step; then 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. The final step was 10 min at 72°C. The PCR products were loaded on the 1% agarose gel and purified either using the Wizard PCR purification Kit (Promega, Madison, WI, USA) or with exonuclease I and shrimp alkaline phosphatase (Werle et al. 1994). Sequencing was performed using automatic genetic analyser Applied Biosystems 3130xl (Applied Biosystems, Foster City, California, USA) and BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems).

For amplification and sequencing of the partial *cox1* gene, the primers CFCYT2 and CRCYT2, specifically designed for caryophyllidean cestodes by Bazsalovicsová et al. (2011), were used. They amplified 657 bp (219 amino acids) of the *cox1* gene, including the start codon, which was determined according to the echinoderm mitochondrial code (Telford et al. 2000).

Partial lsrDNA gene (domains D1–D3) was amplified using the LSU5 and 1500R primers originally described in Olson et al. (2003). The length of the amplified D1–D3 lsrDNA region in *Caryophyllaeus* specimens varied from 1576 to 1623 bp. The amplified products were sequenced using two amplification primers together with four internal primers 300F, 400R, 900F and ECD2 (Olson et al. 2003). Nucleotide sequence data originally reported in this paper are available in the GenBank™, EMBL and DDBJ databases (see Table 1 for accession numbers).

Phylogenetic analyses

Contiguous sequences were assembled and inspected for errors using Geneious (Geneious version 6.0.5 created by Biomatters; available from <http://www.geneious.com/>). Alignments were created using the E-INS-i algorithm of the program MAFFT (Katoh and Standley 2013) and ambiguously aligned positions were manually excluded from the lsrDNA alignment thus creating a 1578 bp long data set. Interrelationships among *Caryophyllaeus* species were evaluated by analysing the D1–D3 lsrDNA and *cox1* data parts as a concatenated data set by maximum likelihood (ML) and Bayesian inference (BI) methods. Best ML estimate resulted from 10 ML searches in the program Garli ver. 2.0 (Zwickl 2006), employing partitioned scheme (lsrDNA) (*cox1* 1st) (*cox1* 2nd) (*cox1* 3rd) and models (HKY + I) (TrN + I) (F81 + G) (TrN + G) chosen according to the BIC criterion in PartitionFinder 1.1.0 (Lanfear et al. 2012). ML nodal support was computed by running 5 searches on each of the 500 bootstrap resamples in Garli.

BI trees were constructed using MrBayes ver. 3.2 (Ronquist et al. 2012), running 4 independent MCMCMC runs of 4 chains

for 8 million generations and sampling tree topologies every 1000th generation. Analogously to ML analyses, the (lsrDNA) (*cox1* 1st, *cox1* 2nd) (*cox1* 3rd) partitioning scheme and (HKY + I) (HKY + G) (GTR + G) model were chosen and employed. Burn-in period was set to 2.5 million generations according to the standard deviation of split frequencies values (< 0.01) and by checking for MCMC convergence using AWTY (Nylander et al. 2008).

RESULTS

Structure and variation of *cox1* and lsrDNA

In total, 32 different *cox1* haplotypes (Ha) were determined in all specimens of *Caryophyllaeus* Gmelin, 1790 analysed. Out of them, 13 and 19 Ha were found exclusively in *C. brachycollis* (Ha1, 3–6, 16, 17, 21–26) and *C. laticeps* (Ha2, 7–15, 18–20, 27–32), respectively (Table 1). In the *C. laticeps* clade, each host and/or geographic population was characterised by specific *cox1* haplotype/haplotypes, none of which was shared among populations.

The specimens from the Danube River (Slovakia) from white-eye bream *Ballerus sapo* (Pallas) possessed only single *cox1* haplotype (Table 1, Ha2), whereas the remaining *C. laticeps* tapeworms from Bulgaria (Arda River), Russia (Volga River) and Austria (Drau River) were characterised by multiple haplotypes and pairwise sequence divergence among their individuals ranged between 0–0.9%, 0.2% and 0.5–0.6%, respectively. The overall intraspecific *cox1* sequence differences of *C. laticeps* ranged between 0.2% and 16.9%.

A very similar situation was observed in *C. brachycollis*. Each host/geographic population was characterised by specific haplotype/haplotypes with just one exception: Ha1 was detected, in addition to a homogeneous population of *C. brachycollis* from chub *Squalius cephalus* (Linnaeus) in Slovakia (Myslava Stream), also in a specimen from Bulgaria from barbel *Barbus cyclolepis* Heckel (Arda River) (Table 1).

Nucleotide sequence comparison of D1–D3 lsrDNA revealed 0.1–3.1% sequence divergence between both *Caryophyllaeus* species. Intraspecific sequence differences were 0–0.4% in *C. laticeps* and 0–0.5% in *C. brachycollis*.

Fig. 1. Maximum likelihood analysis of phylogenetic relationships of *Caryophyllaeus* species based on the concatenated analysis of *lsrDNA* and *coxI* data. Nodal values depict bootstrap support above 50, and Bayesian Inference posterior probabilities.

Phylogenetic relationships

Sequence analyses of *Caryophyllaeus* specimens (Fig. 1) agreed on a single phylogenetic scenario: the existence of two statistically well-supported *Caryophyllaeus* clades that correspond to the two historically established congeneric species, *C. laticeps* and *C. brachycollis*. Each of these two clades further divided into several monophyletic lineages that receive weaker statistical supports, but generally not contradict the morphotype groups as revealed by the current morphological re-evaluation (Table 2). Therefore, the majority of the morphotypes of *C. laticeps* (2, 3, 4 and 5) can be viewed as cohesive genetic clusters that are correlated first with geography and nested within that, occasionally with a particular host.

In contrast, *C. brachycollis* morphotype 1 and *C. laticeps* morphotypes 1 and 5 belong to those groupings not found on the resulting phylogram as monophyletic lineages. However, the branching pattern that separates the unrelated lineages of both *C. brachycollis* morphotype 1 and *C. laticeps* morphotype 1 representatives, respectively, does not receive any statistical support in either of the cases, which also means that the monophyly of *C. brachycollis* and *C. laticeps* morphotypes 1 may not be expelled on the basis of the present data.

A slightly different situation can be observed with *C. laticeps* morphotype 5. Again, the split of these tapeworms into two evolutionary distinct lineages is not statistically supported, but both of these lineages form relatively long branches, a situation not found elsewhere on the tree. The remaining *C. laticeps* morphotypes parasitising species of the genera *Vimba* Fitzinger, *Cyprinus* Linnaeus and *Chondrostoma* Agassiz (morphotypes 2–4) reflect the definitive hosts and geographical distribution of possibly subpopulations of this tapeworm species.

DISCUSSION

The present study brings a DNA-based evidence of host-related plasticity of morphological characters currently employed to circumscribe and classify species of monozoic fish tapeworms. The results obtained here suggest that delimiting species of *Caryophyllaeus* using solely morphological characteristics may be misleading. Morphology of the scolex plays the key role in identification of caryophyllidean species, being quite diverse (Mackiewicz 1972, Oros et al. 2010, Scholz et al. 2011, Ash et al. 2012). However, molecular markers do not provide convincing evidence that the morphotypes of both *Caryophyllaeus* congeners from different fish hosts and geographical regions with markedly different scolex morphology represent individual species. Genetic data support the conclusion of euryxenous host specificity of both species, especially of the geographically widely distributed *C. laticeps*, and do not confirm the existence of cryptic species. In fact, several of the definitive hosts may harbour simultaneously both of the congeneric species and sev-

eral of their respective morphotypes, such as freshwater bream infected with *C. laticeps* as well as *C. brachycollis* (Fig. 1). The co-existence of divergent parasite lineages in the same fish host and the higher than previously expected species diversity in freshwater bream indicate that some factors related to transmission of developmental stages of parasites may play a key role. Blasco-Costa et al. (2010), who observed a similar phenomenon in haploporid trematodes in sympatric mullets in the Mediterranean, present two hypotheses on the action of factors linked to the effect of the enhanced encounter of larval stages from molluscs with the definitive hosts.

Genetic markers represent a powerful tool for studies on intraspecific variability and made it possible to unravel cryptic species (genetically distinct but morphologically very similar species) in many taxonomic groups, including helminths (Nadler and Pérez-Ponce de León 2011). However, morphology still has, and undoubtedly always will play, a key role in species circumscription and a higher-level classification, even though the importance of individual morphological characteristics, which have been previously used as differential criteria, may change. In the present study, morphologically well-defined populations of *Caryophyllaeus* species from different hosts appeared to represent only different morphotypes of presumably polymorphic species with a wide spectrum of definitive hosts.

It is evident that morphology alone may not yield adequate taxonomic resolution and can provide misleading answers to basic questions about parasite diversity and host-parasite relationships (Nadler and Pérez-Ponce de León 2011). The advent of molecular systematics brought new characters for circumscribing parasite species in nature, addressing concerns that morphology alone may be insufficient to identify all species unequivocally, and providing further advantages for studying parasite systematics and evolution (Nadler 1990, McManus and Bowles 1996).

Even though discovery of cryptic species has been a more frequently found scenario in parasitology, there are cases where the exact opposite occurred, e.g. when putatively separate species showed very high levels of molecular similarity, thus indicating conspecificity. This was the case of *Ostertagia* Ransom, 1907 nematodes of reindeer in Scandinavia (Dallas et al. 2000) and *Lamelodiscus* Johnston et Tiegs, 1922 monogeneans of sparid fishes in the Mediterranean (Desdevises et al. 2000). In such cases, molecular tools may contribute to a better understanding of biodiversity and parasites' phenotypic plasticity, including host-induced morphological changes. High phenotypic plasticity of *Siphomutabilis* Miller et Cribb, 2013 digeneans from Indo-Pacific caesionines represents another example where DNA sequence data are able to reveal relationships not easily recognised from morphology alone (Miller and Cribb 2013).

The disagreement between morphological and molecular data is apparently the case of some representatives of the order Caryophyllidea, where conflict between mor-

phological and genetic data represents one of the most important problems in their taxonomy and systematics. This problem was faced for the first time in the caryophyllidean genus *Khawia* Hsü, 1935 (family Lytocestidae). Type species of the genus, *Khawia sinensis* Hsü, 1935, which is a specific, widely distributed parasite of carp (*Cyprinus carpio* Linnaeus – Cyprininae), and *K. saurogobii* Xi, Oros, Wang, Wu, Gao et Nie, 2009, a parasite of gudgeons (*Saurogobio* spp. – Gobioninae) known from a relatively small area in China, were found to be morphologically clearly different species (Xi et al. 2009, Scholz et al. 2011). However, they are almost indistinguishable in their ribosomal internal transcribed spacer 2 (ITS2), ssrDNA, lsrDNA, and mitochondrial *cox1* and nicotinamide dehydrogenase subunit III (*nad3*) sequences (Scholz et al. 2011, Králová-Hromadová et al. 2012). It was assumed that *K. saurogobii*, after switching to a new, unrelated fish host, may have undergone morphological divergence as a result of ongoing sympatric speciation, but this process has not been accompanied by corresponding nucleotide changes in the standard genes used as informative markers in our analyses (Scholz et al. 2011).

In contrast to *K. saurogobii*, *C. laticeps* has a very large distribution area, which involves Europe, spanning in north-southern direction from Sweden (Chubb 1982) towards Turkey, and in west-eastern direction from Britain towards Russia (Protasova et al. 1990). High morphological plasticity of different host populations of this species probably reflects the effect of individual fish hosts and, possibly, their geographical origin. The present molecular data provide strong support to consider *C. laticeps* as a euryxenous parasite of a wide spectrum of fishes of different genera and subfamilies, i.e. cyprinid fishes of the subfamilies Leuciscinae, such as freshwater bream *Abramis brama* (Linnaeus), zope *Ballerus ballerus* (Linnaeus), white-eye bream *Ballerus sapo*, white bream *Blicca bjoerkna* (Linnaeus), vimba bream *Vimba vimba* (Linnaeus), roach *Rutilus rutilus* (Linnaeus), common nase *Chondrostoma nasus* (Linnaeus), and Cyprininae, such as crucian carp *Carassius carassius* (Linnaeus), common carp *Cyprinus carpio* (Protasova et al. 1990, Oros and Hanzelová 2009). Such an extraordinarily wide range of definitive fish hosts of *C. laticeps* may stand behind its phenotypic plasticity.

The pattern of morphological and genetic divergence, observed in both cestode species studied, is mostly concordant, with some exceptions involving paraphyly. It may indicate a scenario of possible host range extensions (colonisations) by the parasites within a particular geographical area and subsequent diversification. The limited data set did not allow us to determine an ancestral host, but the host associations of *Caryophyllaeus* suggest that the abramine lineage seems to be an important host group.

Another species of the genus, *C. fimbriceps* Annenkova-Chlopina, 1919 from common carp *C. carpio*, was

differentiated from *C. brachycollis* and *C. laticeps* mainly by its scolex morphology, anterior extent of vitelline follicles and large size of cirrus-sac (see Dubinina 1987, Scholz 1989, Protasova et al. 1990). As evidenced by the present study, *Caryophyllaeus* tapeworms from common carp were conspecific with *C. laticeps* and represent the morphotype 3 of this species. They have a wide scolex similar to *C. fimbriceps*, but their cirrus-sac is small and vas deferens is not surrounded by vitelline follicles on the ventral and dorsal side. Therefore, taxonomic status and validity of *C. fimbriceps*, which was previously often reported as a pathogenic parasite of common carp in fishponds of eastern Europe and the former Soviet Union (Bauer et al. 1973), have to be critically reviewed.

Caryophyllideans are generally known to have very specific morphological characteristics (monozoic body with only one set of reproductive organs) and also exceptional molecular and genetic features, such as ribosomal ITS paralogues, polyploidy, multiple ribosomal loci and NUMTs (nuclear copies of mitochondrial DNA) (Králová-Hromadová et al. 2010, 2012, Orosová et al. 2010, Bazsalovicsová et al. 2011, 2012, Brabec et al. 2012). However, the phenomenon of NUMTs, as described by Brabec et al. (2012) in Slovak *C. laticeps* individuals from freshwater bream *A. brama* and common carp *Cyprinus carpio*, and Slovak *C. brachycollis* specimen from freshwater bream *A. brama* (see Fig. 1), was not noticed in any specimens analysed in the present study.

As indicated by this study and based on evaluation of numerous materials, several genera of caryophyllidean tapeworms are pending revision, which should include morphological and genetic evaluation of well-fixed fresh materials from different hosts and geographical regions. Based on our previous experience with this cestode group, it is evident that combined methodological approach needs to be applied in their systematic studies. Molecular taxonomy alone is definitely not a panacea for resolving the systematics of caryophyllideans, but in combination with morphology-based taxonomy, it is a suitable tool for a better understanding of their diversity.

Acknowledgements. The authors are grateful to the following colleagues for help of collection of *Caryophyllaeus* cestodes or for providing some specimens: Anirban Ash (India), Franz Jirsa (Austria), Diana Kirin (Bulgaria), Larisa Poddubnaya (Russia), Andrea Šimková and Martina Dávidová (Czech Republic). Helpful suggestions of two anonymous reviewers are also much appreciated. This work was supported by the Slovak Research and Development Agency under contract APVV-0653-11, project of the Grant Agency of the Slovak Republic (VEGA 2/0129/12), project ‘Centre of Excellence for Parasitology’ (Code ITMS: 26220120022), based on the support of Operational Programme ‘Research & Development’ funded from the European Regional Development Fund (rate 0.1). Financial support of the Czech Science Foundation (project No. 505/12/G112) and the Institute of Parasitology (RVO: 60077344) is also acknowledged.

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Received 26 November 2013

Accepted 21 February 2014