Cophylogenetic relationships between Dactylogyrus (Monogenea) ectoparasites and endemic cyprinoids of the north-eastern European peri-Mediterranean region

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Abstract

The study of host-parasite coevolution is one of the cornerstones of evolutionary biology. The majority of fish ectoparasites belonging to the genus *Dactylogyrus* (Monogenea) exhibit a high degree of host specificity. Therefore, it is expected that their evolutionary history is primarily linked with the evolutionary history of their cyprinid fish hosts and the historical formation of the landmass. In the present study, we used a cophylogenetic approach to investigate coevolutionary relationships between endemic Cyprinidae from selected regions in southern Europe and their respective *Dactylogyrus* species. A total of 49 *Dactylogyrus* species including

endemic and non-endemic species were collected from 62 endemic cyprinid species in the Balkan and Apennine Peninsulas. However, 21 morphologically identified *Dactylogyrus* species exhibited different genetic variants (ranging from two to 28 variants per species) and some of them were recognized as cryptic species on the basis of phylogenetic reconstruction. Phylogenetic analyses revealed several lineages of endemic and non-endemic *Dactylogyrus* species reflecting some morphological similarities or host affinities. Using distance-based and event-based cophylogenetic methods, we found a significant coevolutionary signal between the phylogenies of parasites and their hosts. In particular, statistically significant links were revealed between *Dactylogyrus* species of Barbini and their hosts belonging to the genera *Aulopyge*, *Barbus* and *Luciobarbus*. Additionally, a strong coevolutionary link was found between the generalist parasite *D. vistulae* and its hosts, and between *Dactylogyrus* species of *Pachychilon* and their hosts. Our cophylogenetic analyses suggest that host-switching played an important role in the evolutionary history of *Dactylogyrus* parasitizing endemic cyprinids in southern Europe. We propose that the high diversification of phylogenetically related cyprinid species in the Mediterranean area is a process facilitating the host switching of specific parasites among highly diverse congeneric cyprinids.

Introduction

Host-parasite coevolution plays an important role in the processes of parasite speciation and represents one of the most fascinating topics in evolutionary biology (Poulin, 2007). If the host specificity of the parasite is high (i.e. a parasite species restricted to a single host species or very few closely-related host species) it is tempting to assume that the evolution of parasitic organisms is associated with the evolution of their hosts (Ronquist, 1997). Hence, the Fahrenholz rule (Stammer, 1957, Brooks & McLennan, 1993) states that parasite phylogeny mirrors host phylogeny, and that cospeciation drives host-parasite coevolution. Congruent host-

parasite phylogenies has usually been inferred when the host-switching of parasites is impossible or highly improbable, such as in the case of chewing lice and pocket gophers, where parasite cospeciation likely resulted from an allopatric distribution of hosts and host-switching was supported only in the case of physical contact between two gopher species (Hafner & Nadler, 1988, Hafner et al., 1994, Page, 1996). However, the whole concept of the "Fahrenholz rule" has been re-evaluated and several studies have suggested that cospeciation is not always the predominant driver of parasite speciation during reciprocal host-parasite evolution. Host-switching (Klassen, 1992) and parasite duplication, i.e. parasite speciation within a host lineage (Johnson, Adams, Page & Clayton, 2003), play significant roles in parasite evolution, often resulting in incongruent host and parasite phylogenies (e.g. Desdevises, Morand, Jousson & Legendre, 2002, Šimková, Morand, Jobet, Gelnar & Verneau, 2004, Šimková, Serbielle, Pariselle, Vanhove & Morand, 2013, Mendlová, Desdevises, Civáňová, Pariselle & Šimková, 2012). Despite the fact that frequent host-switching during the evolutionary history of parasite taxa usually results in incongruent host-parasite phylogenies, a series of multiple host-switches followed by parasite speciation can generate trees with similar topologies (de Vienne, Giraud & Shykoff, 2007). Therefore, the independent estimation of the age of speciation events in host and parasite trees should also be taken into account when interpreting the outputs of cophylogenetic analyses.

Dactylogyrus Diesing, 1850 (Monogenea) are gill parasites generally exhibiting narrow host specificity and high morphological variability with respect to their attachment organ (termed haptor), putatively reflecting adaptations to their different host species or within-host microhabitats (Gibson Timofeeva & Gerasev, 1996, Šimková, Desdevises, Gelnar & Morand, 2000, 2001, Šimková, Verneau, Gelnar & Morand, 2006b, Šimková & Morand, 2008). In addition, *Dactylogyrus* currently represents the platyhelminth genus with the highest species diversity (more than 900 described species according to Gibson et al., 1996), certainly largely

74 underestimated as new species have recently been described (e.g. Aydogdu, Molnár, Emre &
75 Emre, 2015, Nitta & Nagasawa, 2016, Benovics, Kičinjaová & Šimková, 2017, Rahmouni,
76 Řehulková, Pariselle, Rkhami & Šimková, 2017). This high species richness in *Dactylogyrus*
77 is associated with their narrow host specificity toward a single host species or closely-related
78 species, and with a high diversity of their host species – primarily freshwater fish in the family
79 Cyprinidae (or Cyprinodei considering recent phylogenetic studies, e.g. Schönhuth, Vukić,
80 Šanda, Yang & Mayden, 2018). Previous studies have suggested that each cyprinid species
81 harbour at least one *Dactylogyrus* species (Dupont & Lambert, 1986, Gibson et al., 1996,
82 Moravec, 2001, Galli, Stefani, Zaccara & Crosa, 2002). In regards to host specificity, Šimková
83 et al. (2006b) classified five groups of *Dactylogyrus* species ranging from strict specialists,
84 living on a single host species, to generalists parasitizing host species from different
85 phylogenetic lineages. The high host specificity of *Dactylogyrus* (and other monogeneans) is
86 linked with their direct life cycle, where the larva (oncomiracidium) actively searches for a
87 suitable (specific) host and attaches directly to the gills or surface. Oncomiracidia are sensitive
88 to chemical cues from hosts which can either initiate the hatching of oviparous species, attract
89 larvae, or initiate larva deciliation (Buchmann & Lindenstrøm, 2002). The recognition of these
90 signals most likely requires specific parasite adaptation (Buchmann, 1999, Whittington &
91 Kearn, 2011).
92 Their narrow host specificity and expected host-parasite coevolution make monogeneans
93 potential proxies for the study of the evolution and dispersion of their hosts. Previous studies
94 (on *Lamellodiscus* Johnston & Tiegs, 1922 parasitizing Sparidae (Desdevises et al., 2002),
95 *Gyrodactylus* von Nordmann, 1832 parazitizing Gobiidae (Huyse, Audenaert & Volckaert,
96 2003, Huyse & Volckaert, 2005, Huyse, Oeyen, Larmuseau & Volckaert, 2017), *Cichlidogyrus*
97 Paperna, 1960 and *Scutogyrus* Pariselle & Euzet, 1995 parazitizing Cichlidae (Mendlová et al.,
98 2012), and *Thaparocleidus* Jain, 1952 parasitizing Pangasiidae (Šimková et al., 2013))

suggested that cophylogenetic patterns between monogeneans and their hosts are complex, involving less cospeciation than expected and a putatively high number of host switches, duplications, and losses. Frequent host-switching in these systems may be expected because of the active dispersion of the larvae and the capacity of adults to survive outside of the hosts for a short period of time (Brooks & McLennan, 1991, Bakke Cable & Harris, 2007), potentially allowing them to infect phylogenetically closely-related host species with similar ecological requirements.

In spite of the large interest in host-specific monogeneans, few phylogenetic and/or cophylogenetic studies have been performed for *Dactylogyrus*. In *Dactylogyrus* from central European cyprinids, intrahost duplication was inferred as a more widespread diversification process than host-switching (Šimková et al., 2004). Several coevolutionary scenarios were proposed by Benovics et al. (2017), Benovics, Desdevises, Vukić, Šanda and Šimková (2018), and Šimková, Benovics, Rahmouni and Vukić (2017) regarding *Dactylogyrus* and peri-Mediterranean endemic cyprinids, the last one hypothesizing that Iberian cyprinids harbour *Dactylogyrus* species originating from two different colonization events.

Southern European freshwater fauna is extremely rich in endemic cyprinid species (Kottelat & Freyhof, 2007). For instance, the Balkan Peninsula is considered a hotspot of endemic freshwater diversity and harbours 59 % of all European cyprinid species (Sušnik, Snoj, Wilson, Mrdak & Weiss, 2007, Abell et al., 2008, Albrecht & Wilke, 2008, Schultheiss, Albrechts, Bossneck & Wilke, 2008, Oikonomou, Leprieur & Leonardos, 2014), which have recently become the common interest of ichthyologists (e.g. Marková et al., 2010, Gante, 2011, Perea, Vukić, Šanda & Doadrio, 2016, Stierandová et al., 2016, Buj et al., 2017). According to Bianco (1990), the Balkans are divided into 4 ichthyogeographical districts, each identified by the presence of unique cyprinid species or lineages. One of them, the Padano-Venetian ichthyogeographic district, partially covers the Balkan Peninsula and also includes the north-

eastern part of the Apennine Peninsula; therefore, several cyprinid species are shared between these two geographical regions. In comparison to the species-rich Balkan Peninsula, only 14 endemic cyprinid species have been described from the Apennine Peninsula (Bianco, 1995). Since most of this Peninsula was below the sea level during most of the Miocene era, it is assumed that the Apennine ichthyofauna is of more recent origin in comparison to the ichthyofauna in other southern European Peninsulas (Steininger & Rögl, 1984). The northern part of the Apennine Peninsula is divided into two districts: the Tuscano-Latinum district which corresponds to the distribution of *Squalius lucumonis*, and the Padano-Venetian district which corresponds to the Po River basin during the last glacial maxima (Bianco, 1990). The native Apennine ichthyofauna has been significantly influenced and threatened by the introduction of numerous fish species (26 introduced species according to Bianco (1995)), which led to the simultaneous introduction of their non-native parasite species (such as *Dactylogyrus*, documented in Benovics et al., 2017). In general, Apennine cyprinids are phylogenetically more related to Balkan cyprinids than to central European or Iberian species (Perea et al., 2010), as cyprinid species from the northern part of the peri-Adriatic River system (confined to the Po River basin in Italy and the Soča River basin in Slovenia) and recent Balkan species show a low degree of molecular divergence (Sušnik, Snoj & Dovc, 2001, Perea et al., 2010). A possible explanation is that during the last glacial maximum, the Po basin expanded and connected the Italian and Balkan river systems, which led to the mixing of many primary native fish species (Waelbroeck et al., 2002, Stefani, Galli, Crosa, Zaccara & Calamari, 2004).

Since cophylogenetic patterns and processes between peri-Mediterranean cyprinids and their *Dactylogyrus* parasites are poorly known, we aimed to study the cophylogeny of these two groups in selected southern European regions and to elucidate the historical dispersion of endemic cyprinids using *Dactylogyrus* phylogeny. Therefore, the objectives of this study were (1) to reconstruct the evolutionary histories of Balkan and Apennine endemic cyprinids and

149 their endemic *Dactylogyrus* in order to clarify the relationships between recent lineages, and
150 (2) to investigate the speciation patterns of host specific *Dactylogyrus* and to assess whether
151 parasite phylogeny is linked to host phylogeny and the historical formation of the landmass, or
152 rather to the recent distribution and introduction of non-native species into the investigated
153 regions.
154

155 Material and Methods

156 Material collection and fixation

157 Between 2014 and 2017, 76 cyprinid species were sampled from 56 localities across the Balkan
158 and Apennine Peninsulas (Table 1). A fin clip was obtained from all fish individuals and
159 preserved in 96 % ethanol. Fish were dissected using standard methods described by Ergens
160 and Lom (1970). *Dactylogyrus* parasites were collected from the gills and nasal cavity, mounted
161 on slides, and fixed using a mixture of glycerine and ammonium picrate (GAP, Malmberg
162 1957). Species determination was performed according to the size and shape of the sclerotized
163 hard parts of the haptor and the reproductive organs (male copulatory organ and vaginal
164 armament) using Pugachev, Gerasev, Gussev, Ergens & Khotenowski (2009). Identification at
165 the species level was performed using an Olympus BX51 microscope equipped with phase
166 contrast optics. Several representatives of each collected *Dactylogyrus* species were bisected
167 using fine needles. One half of the body (containing the reproductive organs) was mounted on
168 a slide, while the other was individually preserved in 96 % ethanol for further DNA extraction.
169

170 DNA extraction, amplification, and sequencing

171 Bisected *Dactylogyrus* individuals preserved in ethanol were dried using a vacuum centrifuge.
172 DNA extraction was performed following the standard protocol (DNeasy Blood & Tissue Kit,
173 Qiagen, Hilden, Germany). For molecular analyses, four genetic markers commonly applied

for monogeneans were used. Partial 18S rDNA, the entire ITS1 region, and partial 5.8S rDNA were amplified using the primers S1 (forward, 5'-ATTCCGATAACGAACGAGACT-3') and IR8 (reverse, 5'-GCTAGCTGCGTTCTTCATCGA-3'), which anneal to the 18S and 5.8S rDNA regions respectively (Šimková, Plaisance, Matějusová, Morand & Verneau, 2003); PCR followed the protocol optimized in Benovics et al. (2018). Partial 28S rDNA was amplified using primers C1 (forward, 5'-ACCCGCTGAATTTAAGCA-3') and D2 (reverse, 5'-TGGTCCGTGTTTCAAGAC-3') following Hassouna, Michot & Bachallerie (1984); PCR followed the protocol optimized in Šimková, Matějusová and Cunningham (2006a). The PCR products were checked on 1 % agarose gel and purified using the ExoSAP-IT kit (Ecoli, Bratislava, SK) following the standard protocol. The purified products were directly sequenced using the PCR primers and BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Sequencing was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems).

For fish DNA extraction, fin clips were removed from the ethanol and dried, and the JETQUICK Tissue DNA Spin Kit (GENOMED) was applied following manufacturer's instructions. The complete mtDNA cytochrome *b* gene was amplified using primers GluF (forward, 5'-AACCACCGTTGTATTCAACTACAA-3') and ThrR (reverse, 5'-ACCTCCGATCTTCGGATTACAAGACCG-3') according to Machordom and Doadrio (2001a). The PCR reaction settings, amplification protocol, and PCR product purification followed Šanda et al. (2008). The sequencing of the cyprinid cytochrome *b* gene was carried out by the Macrogen Service Centre (Seoul, South Korea) using the PCR primers.

The new sequences for parasites and hosts obtained during this study were deposited in GenBank (see Tables 1 and 2 for accession numbers). For *Dactylogyrus*, whole sequences including the partial 18S rDNA and ITS1 regions were deposited in GenBank.

199 **Phylogenetic reconstruction**

200 DNA sequences of hosts and parasites were aligned using fast Fourier transform in MAFFT
201 (Katoh, Misawa, Kuma & Miyata, 2002). The new sequences of *Dactylogyrus* (Table 2) were
202 trimmed to concur with the length of sequences from GenBank (see Table 2).

203 Gaps and ambiguously aligned regions were removed from the alignment of *Dactylogyrus*
204 sequences using GBlocks v. 0.91 (Talavera & Kastresana, 2007). The most appropriate DNA
205 evolutionary model was determined using the Bayesian information criterion (BIC) with
206 jModelTest 2.1.10 (Guindon & Gascuel, 2003, Darriba, Taboala, Doallo & Posada, 2012).
207 Phylogenetic trees were inferred by means of Bayesian inference (BI) and Maximum
208 Likelihood (ML) using MrBayes 3.2 (Ronquist et al., 2012) and RaxML v8.1.X (Stamatakis,
209 2014), respectively. BI trees were constructed using the Metropolis-coupled Markov chain
210 Monte Carlo algorithm, with 2 parallel runs of 1 cold and 3 hot chains, 10^7 generations, and
211 trees sampled every 100 generations. 30 % of all saved trees were discarded as burn-in after
212 checking that the standard deviation split frequency value fell below 0.01. Convergence was
213 assessed using Tracer v.1.6 (Rambaut, Drummond, Xie, Baele & Suchard, 2018). Posterior
214 probabilities (PP) were calculated as the frequency of samples recovering any particular clade.
215 The clade support for ML trees (bootstrap support, BS) was assessed by 1000 bootstrap
216 pseudoreplicates.

217 The phylogenetic reconstruction of 49 *Dactylogyrus* species was based on concatenated partial
218 18S and 28S rDNA sequences. The resulting phylogram was rooted by *Dactylogyrus* species
219 from *Carassius gibelio* (Bloch, 1782) and *Cyprinus carpio* L., following Šimková et al. (2004).
220 Data were treated as partitioned and the optimal evolutionary model was selected for each
221 marker individually, including the alpha parameter of the gamma distribution (G) accounting
222 for rate heterogeneity across sites and/or the proportion of invariable sites (I). The phylogenetic
223 reconstruction of 76 cyprinid species based on the complete cytochrome *b* gene was rooted

following Mayden et al. (2009), using the outgroup comprising four representatives of the family Cobitidae (*Cobitis jadovaensis* Mustafić & Mrakovčić, 2008 (KP208162.1), *C. illyrica* Freyhof & Stelbrink, 2007 (KJ487484.1), *C. narentana* Karaman, 1928 (KP208170.1) and *C. elongata* Heckel & Kner, 1858 (EF672382.1)). Host sequence data were treated as codon partitioned and optimal evolutionary models were selected independently for each position within the codon, including both gamma distribution and the proportion of invariable sites.

Cophylogenetic analyses

The tanglegram connecting host and parasite phylogenetic trees via host-parasite associations was built with TreeMap 3.0b (Charleston, 2012). From many existing methods to investigate the congruence between parasite and host phylogenies (de Vienne et al., 2013), a distance-based method and an event-based method were used in the present study. ParaFit (Legendre, Desdevises & Bazin, 2002), implemented in CopyCat (Meier-Kolthoff, Auch, Huson & Göker, 2007), was used with patristic distances calculated for each host and parasite phylogeny, and 999 permutations to assess the statistical significance of global and individual coevolutionary links. The event-based analysis was performed with Jane 4.0 (Conow, Fielder, Ovadia & Libeskind-Hadas, 2010), which allows different costs to be set for each of the five coevolutionary events (i.e. cospeciation, duplication, duplication followed by host switch, loss, and failure to diverge where host speciation is not followed by parasite speciation). Eleven models with different event cost schemes were applied, using 500 generations and a population size of 50 as parameters of the genetic algorithm to assess the influence of each type of evolutionary event. The Jane 4.0 default model, TreeMap default model (Charleston, 1998), and TreeFitter default model (Ronquist, 1995) were included in our analyses following Deng et al. (2013). Each of these default models assumes that cospeciation has the lowest cost (i.e. is the most common evolutionary event). Several additional models were included in the

cophylogenetic analyses: TreeFitter models adjusted for host-switch and codivergence, respectively; a model with equal weights for coevolutionary events following Mendlová et al. (2012); and five models where each event is alternatively extremely penalized (cost set to 10, following Deng et al., 2013). To statistically test whether the global reconstruction cost was significantly lower than expected by chance, 500 randomizations were performed with the use of random parasite trees.

Results

Parasite phylogeny

Dactylogyrus parasites were collected from 62 cyprinid species (Table 1). A total of 49 *Dactylogyrus* species (Table 2) were identified on the basis of morphological markers (Pugachev et al., 2009). Genetic variability was observed among individuals of *Dactylogyrus* species collected from multiple host species and, therefore, all genetic variants were included in the final sequence alignment. The final 1,177 base-pair-long alignment of the 49 putative *Dactylogyrus* species included 138 concatenated sequences of 18S rDNA combined with partial 28S rDNA. The following optimal evolutionary models were selected: TrNef+I for the 441 bp-long partial 18S rDNA sequence alignment and TVM+I+G for the 736 bp-long partial 28S rDNA sequence alignment. BI and ML analyses generated trees with identical topologies (the BI tree is shown in Fig. 1). Morphological and molecular data suggested the presence of 10 potentially new species, labelled from *Dactylogyrus* sp. 1 to *Dactylogyrus* sp. 10. The phylogenetic reconstruction divided *Dactylogyrus* species into 3 well-supported groups (A, B and C in Fig. 1). The *D. rarissimus* group, which displayed a high level of intraspecific variability (12 genetic variants), formed a sister group to these three large clades, but the monophyly of *D. rarissimus* was not supported (PP = 0.49, BS = 51, respectively). The first clade (group A, PP = 0.98, BS = 76) included *D. erhardovae*, *D. cabelleroi* and *D. crucifer*.

These three species are common parasites of *Rutilus* spp. The second group (group B, PP = 1, BS = 74) comprised the majority of *Dactylogyrus* species. Within this group, *Dactylogyrus* species were divided into eight moderately to well-supported lineages. The monophyly of five *Dactylogyrus* species was not supported. Different genetic variants of *D. ergensi* collected from six *Chondrostoma* spp. clustered with *D. dirigerus* (a parasite of *Chondrostoma* spp.), *D. caucasicus* and *D. tissensis* (both parasites of *Alburnoides* spp., lineage 1). All four abovementioned species share a similar shape of male copulatory organ (see Pugachev et al., 2009 for morphology). Each of the four species *D. balkanicus, D. dyki*, *D. folkmanovae*, and *D. petenyi* contains morphologically similar but genetically different individuals (different genetic forms of the given *Dactylogyrus* species parasitized different host species). However, such different genetic forms of each abovementioned morphologically identified species did not form monophyletic groups. The well-supported lineage 3 (PP = 1, BS = 100) comprised all genetic variants of *D. dyki*, a common parasites of *Barbus* spp. in Europe, but also included individuals of *D. balkanicus* resulting in the paraphyly of both species. Both *Dactylogyrus* species from *Luciobarbus* (*Dactylogyrus* sp. 2 and *Dactylogyrus* sp. 3) formed the well-supported lineage 4. Two potentially new species collected from *C. knerii* and *S. tenellus* (*Dactylogyrus* sp. 4 and *Dactylogyrus* sp. 5, respectively) clustered with *D. nanoides* from *Squalius* spp. and *D. rysavyi*, a known parasite of *Alburnoides* spp. (but collected only from *A. thessalicus* in this study). The phylogenetic proximity of the four abovementioned species (lineage 5) was well supported by BI, but only weakly by ML (PP = 0.99, BS = 56). Lineage 6 exclusively comprised potentially new *Dactylogyrus* species collected from *Telestes* spp. (*Dactylogyrus* sp. 6, *Dactylogyrus* sp. 7 and *Dactylogyrus* sp. 8). The monophyly of all *D. petenyi* genetic variants was not supported (lineage 7). Lineage 8 within group B was formed by *Dactylogyrus* species from *Pachychilon* spp. (PP = 1, BS = 95). The third well-supported group (group C, PP = 1, BS = 91) included *D. alatus, D. sphyrna* and *D. vistulae*. Finally, all

28 genetic variants of *D. vistulae* collected from 25 cyprinid species from 7 genera formed a well-supported clade (PP = 1, BS = 100).

Host phylogeny

The alignment of complete cytochrome *b* sequences was used for phylogenetic analyses of cyprinid hosts. All investigated cyprinid species were included in the phylogenetic reconstruction. Five species (*Barbus peloponnesius, B. prespensis, S. prespensis, S. squalus* and *S. vardarensis*) showed interpopulation variability (each cyprinid species was reported in two localities). Two haplotypes for each of these five species were included in the analyses. In contrast, five species (*Alburnus neretvae*, *Chondrostoma vardarense, Pachychilon pictum, Pelasgus thesproticus* and *S. tenellus*) exhibited no interpopulation variability and, therefore, only one haplotype from each of these species was included in the analyses. The final alignment contained 85 sequences with 1140 unambiguous nucleotide positions. GTR+I+G was selected as the best evolutionary model for each position within the codon. Both BI and ML analyses yielded trees with congruent topologies and, therefore, only the phylogram resulting from BI was used (Figure 2). Except for *Barbus* and *Telestes* (Bonaparte, 1837), all cyprinid genera formed a well-supported monophyletic group (PP = 1, BS = 100). In general, phylogenetic relationships between the respective leuciscin clades (genera) were in congruence with the molecular phylogeny proposed by Perea et al. (2010) (e.g. *Telestes* formed a well-supported monophyletic group with *Phoxinellus* Heckel, 1843 and *Chondrostoma* Agassiz, 1832; *Delminichthys* Freyhof, Lieckfeldt, Bogutskaya, Pitra & Ludwig, 2006 and *Pelasgus* Kottelat & Freyhof, 2007 formed a well-supported group, and the *Phoxinus* Rafinesque, 1820 clade displayed a basal position to other leuciscins). The tribe Barbini formed a strongly supported group in sister position to leuciscins. However, the monophyly of the genus *Barbus* was only

weakly supported by both analyses (PP = 0.68, BS = 56). According to the present dataset, *A. huegelii* is phylogenetically closer to the clade comprising *Luciobarbus* spp.

Cophylogeny

BI phylogenetic reconstructions were used for cophylogenetic analyses (Figure 3). The distance-based analysis using ParaFit yielded a highly significant ($P < 0.001$) overall cophylogenetic structure. Out of 138 host-parasite individual links, 65 contributed significantly to the global cophylogenetic structure ($P < 0.05$). Significant links ($P < 0.05$) were inferred between the representatives of group C (*D. alatus, D. sphyrna* and *D. vistulae* or their genetic variants, Figure 1) and their leusiscin host species, and between *Dactylogyrus* representatives belonging to lineage 8 (*D. martinovici*, *D. petkovici* and *Dactylogyrus* sp. 10) and their *Pachychilon* hosts. Highly significant individual links ($P < 0.001$) were found between representatives of the genera *Barbus* and *Luciobarbus* and the monotypic *Aulopyge* and their *Dactylogyrus* spp. (or genetic forms of these *Dactylogyrus*): "*D. balkanicus*", *D. crivellius*, "*D. dyki*", "*D. petenyi*", "*D. prespensis*" from *Barbus*, undescribed *Dactylogyrus* sp. 2 and *Dactylogyrus* sp. 3 from *Luciobarbus* spp., and *D. omenti* from *A. huegelii*. Subsequent analysis performed using the same number of permutations (999) and focused only on this group supported the initial significant cophylogenetic structure ($P < 0.05$).

Applying different cost schemes, Jane produced reconstructions with similar proportions of coevolutionary events (Table 3). Global costs using each scheme were all statistically significant ($P < 0.01$). In general, it appears that *Dactylogyrus* speciation is primarily driven by duplication followed by host-switching, which was an important component in 8 of the 11 models tested. The lowest total cost was produced by the host-switch-adjusted TreeFitter model. The duplication-prohibited model and host-switch-prohibited model (also suggesting the importance of host switching in the evolution of *Dactylogyrus*) resulted in a high number of

loss events and represented the scenarios with the highest total costs. Setting the duplication cost to zero and equalizing the costs of the other events (codivergence adjusted TreeFitter model) or extremely penalizing cospeciation cost (cospeciation prohibited model) resulted in a higher occurrence of duplication events compared to cospeciation events in contrast to a relatively low occurrence of duplication events within each of the other models. Additionally, no losses were inferred in these models (models 4, 6 and also 9, Table 3). A high number of cospeciations were inferred in models with the cospeciation cost set to zero or in models with a high penalization of duplication, host switching, or failure to diverge. A low occurrence of duplication events was found either when cospeciation was not penalized (TreeMap default model), or when failure to diverge or duplication were highly penalized (FTD prohibitive model and duplication prohibitive models, respectively). In the latter model, a remarkably high number of losses were inferred (such as in the case of the host-switch prohibited model).

Applying the same cost schemes with the same number of generations and population size on a selected subgroup of cyprinids belonging to the Barbini tribe and their respective specific *Dactylogyrus* spp., between which a strong cophylogenetic signal was initially detected, resulted in only five schemes yielding cophylogenetic scenarios with statistically significant global costs (tested on 500 randomizations, Table 4). Three of these five models (schemes 1, 3 and 6) were set to expect duplication followed by host-switching as the least probable coevolutionary event simulating the allopatric speciation of hosts where the host-switching of parasites between new lineages is unlikely (an example of a cophylogenetic scenario from this subsequent dataset is presented in Figure 4). Nevertheless, in the majority of scenarios, duplication followed by host-switching was the most common coevolutionary event. This event was omitted only in the case of its extremely high penalization, modelling the scenario where physical contact between congeneric host species should be completely excluded. Equalizing all event costs, or highly penalizing other coevolutionary events when compared to duplication

followed by host-switching resulted in the same proportions of coevolutionary events. However, the results of all these cost schemes were not statistically significant.

Discussion

Phylogeny of *Dactylogyrus*

Following the former phylogenetic study by Benovics et al. (2018) focussed on 53 *Dactylogyrus* species parasitizing endemic cyprinids in the Balkans, this work is the first wide-ranging study focusing on the cophylogenetic relationships between endemic cyprinids of the peri-Mediterranean and their specific parasites. In the present study, a large dataset of 76 endemic cyprinid species covering 95 % of the known cyprinid diversity of the whole north-eastern peri-Mediterranean region (Balkan and Apennine Peninsulas) was used. A total of 49 morphologically identified *Dactylogyrus* species were recognized, representing 139 genetic variants. In the majority of host-parasite associations, *Dactylogyrus* species were specific to a single cyprinid species or to a group of congeneric cyprinids. For many *Dactylogyrus* species parasitizing more cyprinid species, i.e. generalists, different genetic variants of morphologically identical *Dactylogyrus* species were reported. In the majority of cases even these genetic variants exhibited host specificity.

The phylogenetic position of *D. rarissimus* is in congruence with the findings of Benovics et al. (2018), where this species represented a sister group to other *Dactylogyrus* from leuciscins. However, the monophyly of this taxon was only weakly supported by ML analysis and unsupported by BI. In contrast to the previous study by Benovics et al. (2018), our results suggest the monophyly of three *Dactylogyrus* species common to *Rutilus* spp. (*D. caballeroi, D. crucifer* and *D. erhardovae*, group A). The monophyly of the former two species was also suggested by Šimková et al. (2004).

Group B, recognized from phylogenetic reconstruction, contained several well-to-moderately supported clades. However, several *Dactylogyrus* species, formerly recognized on the basis of morphology, were not phylogenetically supported as monophyletic. These species include *D. ergensi*, *D. folkmanovae*, *D. dyki*, *D. balkanicus* and *D. petenyi*. The monophyly of *D. ergensi* was not supported, as *D. caucasicus* collected from *Alburnoides* spp. was included in the well-supported group comprising all *D. ergensi* individuals. However, two well supported groups that follow the biogeographical distribution of cyprinid hosts were formed by *D. ergensi* individuals (Figure 1). *Dactylogyrus ergensi* lineage 1, a sister group to *D. caucasicus*, included individuals found on *Protochondrostoma genei*, *S. lucumonis* and *S. squalus*, all cyprinid species native to the central/northern Adriatic and neighbouring Padano-Venetian ichthyogeographic districts (Bianco, 1990). The other clade, *D. ergensi* lineage 3, contained the genetic forms of *D. ergensi* collected from *C. ohridana* and *C. vardarense*, both endemic to the southern Balkans, specifically to the Albanian and north-eastern Aegan ichthyogeographic districts (Kottelat & Freyhof, 2007). The present data suggest that *D. ergensi* encompasses several species. In fact, the morphometric variability in the shape and size of the male copulatory organ of *D. ergensi* from the *Chondrostoma* spp. in different regions of Europe was reported in its original description by Gussev (1966). Later, Lambert (1977) proposed the splitting of *D. ergensi* by separating *D. toxostomi* (parasitizing *C. toxostoma*), but its taxonomic status was not considered valid since measurements of the sclerotized parts of the attachment organ and male copulatory organ overlapped with *D. ergensi* individuals (Pugachev et al., 2009). Therefore, on the basis of the present molecular data we can conclude that *D. ergensi*, originally described as a parasite of *Chondrostoma* spp. (although its presence was also documented on *Squalius* spp. in the Apennines), is in fact a species complex. Our results also suggest that *D. caucasicus* evolved from *D. ergensi* by host switching to the phylogenetically distant *Alburnoides* Jeitteles, 1861 species (Perea et al., 2010, Schönhuth et al., 2018), since

422 both of these *Dactylogyrus* species have a similar shape with respect to the male copulatory
423 organs (see Pugachev et al., 2009).
424 The previous phylogenetic reconstruction of *Dactylogyrus* performed by Šimková et al. (2004)
425 was focused on the species parasitizing central European cyprinids. Our study confirmed most
426 of the phylogenetic relationships between *Dactylogyrus* species previously suggested in their
427 study. For example, the sister species *D. minor* and *D. parvus* parasitizing *A. alburnus* L. in
428 Central Europe were also found on *A. scoranza* in the Balkans. *Dactylogyrus izjumovae*, *D.*
429 *differmis* and *D. differmoides*, all parasites of *Scardinius erythrophthalmus* L. in Central
430 Europe, formed a monophyletic group also reported in the phylogenetic reconstruction of
431 *Dactylogyrus* parasitizing endemic Balkan cyprinids, more specifically *S. plotizza* and *S.*
432 *dergle*. Congruency was also reported in the sister position of *D. prostae* of the clade formed
433 by *Dactylogyrus* from *Scardinius* Bonaparte, 1837. The present results suggest that *D. nanoides*
434 is phylogenetically closer to the new *Dactylogyrus* species from *Chondrostoma knerii* and *S.*
435 *tenellus* (*Dactylogyrus* sp. 4 and sp. 5 respectively) and to *D. rysavi* rather than to *D.*
436 *folkmanovae* (as was shown in the phylogenetic reconstruction of *Dactylogyrus* parasitizing
437 Central European cyprinids by Šimková et al., 2004). However, *D. folkmanovae* collected from
438 seven *Squalius* species appears to be paraphyletic, as its representatives clustered with other
439 *Dactylogyrus* from leuciscins species including *D. prostae* and *D. vranoviensis* parasitizing
440 *Squalius*, which also suggests the existence of a *D. folkmanovae* morphotype species complex.
441 The phylogenetic position of *D. borealis* is very interesting, as this species is host specific only
442 of representatives of the genus *Phoxinellus* in the Balkans and Central Europe. According to
443 Šimková et al. (2004) *D. borealis* is phylogenetically proximal to *Dactylogyrus amphibothrium*
444 Wagener, 1857 and *Dactylogyrus hemiamphibothrium* Ergens, 1956, both parasitizing
445 *Gymnocephalus cernuus* L. (Percidae) in the Czech Republic. However, considering only
446 *Dactylogyrus* of cyprinids (more specifically only leuciscins in our study), *D. borealis* clusters

447 together with *Dactylogyrus* spp. of *Pachychilon* Steindachner, 1882 (*Dactylogyrus* lineage 8),
448 which is endemic in the Balkans and represents the ancestral cyprinid lineage in this region.
449 The high molecular diversity among *Dactylogyrus* individuals collected from three *Telestes*
450 species (*T. karsticus*, *T. muticellus* and *T. metohiensis*) suggests the existence of three new
451 *Dactylogyrus* species (*Dactylogyrus* sp. 6, sp. 7 and sp. 8 respectively, representing
452 *Dactylogyrus* lineage 6). Extrapolating from the branch lengths and molecular similarity, we
453 can postulate that these species are of recent origin, diverging probably by cospeciation with
454 the *Telestes* genus (see phylogeny in Ketmaier et al., 2004, Perea et al., 2010, Schönhuth et al.,
455 2018). On the basis of the shape and size of sclerotized elements of the haptor and copulatory
456 organs, these three potentially new species greatly resemble *D. nanus* and *D. suecicus,*
457 belonging together with *D. rutili* to the clade which is sister to the clade including three new
458 *Dactylogyrus* species parasitizing *Telestes*. *Dactylogyrus nanus*, *D. rutili* and *D. suecicus* are
459 common parasites of *Rutilus*, the cyprinid species which is phylogenetically closely related to
460 *Telestes* (Perea et al., 2010, Schönhuth et al., 2018, and also supported by our results, see
461 below).
462 The group C, also recognized in previous phylogenetic studies (Šimková et al., 2004, Benovics
463 et al., 2018), was strongly supported in the present study. It comprises *D. alatus*, *D. sphyrna*
464 and *D. vistulae*, which all possess large haptoral anchor hooks ('sphyrna' morphotype) and miss
465 a ventral connective bar except for *D. alatus*, which has a thin 'phoxini' type ventral connective
466 bar (Pugachev et al., 2009). Šimková et al. (2004) also suggested that *Dactylogyrus similis*
467 Wagener, 1909, morphologically close to *D. sphyrna* and *D. vistulae*, is included in this group,
468 but this species was not found on endemic cyprinids of the north-eastern peri-Mediterranean
469 region. While *D. alatus* and *D. sphyrna* were collected from two *Alburnus* Rafinesque, 1820
470 and three *Rutilus* Rafinesque, 1820 species, *D. vistulae* used a wide range of host species
471 representing different genera and exhibiting a wide biogeographical distribution. The basal

position of *D. vistulae* individuals from *C. vardarense* and its sister position to individuals from *A. thessalicus* suggest that this species originated from the north-western Aegan ichthyogeographic district and further dispersed through host switching onto geographically adjacent cyprinid lineages (extrapolating from the results of cophylogenetic analyses). To investigate the true origin of *D. vistulae* we suggest that the representatives from Central European cyprinids (e.g. *Squalius cephalus* L. or *Chondrostoma nasus* L.), in which molecular variability was also observed (Šimková et al., 2004), should be included in future studies, based on population genetic markers to be developed.

Phylogeny of Cyprinidae

The phylogenetic reconstruction of the north-eastern peri-Mediterranean cyprinids obtained in this study is in general agreement with the molecular phylogeny proposed by Perea et al. (2010) and Schönhuth et al. (2018). The only incongruences concern the relationship between *Alburnoides* and *Tropidophoxinellus* Stephanidis, 1971, sister groups in Schönhuth et al. (2018). Our study supports the phylogenetic proximity of *Alburnus*, *Scardinius* and *Tropidophoxinellus*, which was previously hypothesized (e.g. Brito, Briolay, Galtier, Bouvet & Coelho, 1997, Briolay, Galtier, Brito & Bouvet, 1998, Zardoya & Doadrio, 1999, Perea et al. 2010). Interestingly, all three genera harbour *Dactylogyrus* from different evolutionary lineages. While *Alburnus* spp. are parasitized by *D. alatus*, *D. minor*, *D. parvus* and *D. rarissimus* (the last is a common species on *Rutilus* spp. and *Telestes* spp. and rare on *Pelasgus* spp.), *Scardinius* and *Tropidophoxinellus* harbour host-specific *Dactylogyrus* spp. (*D. difformis, D. difformoides, D. izjumovae* and *Dactylogyrus* sp. 9). The phylogenetic relationships within the *Alburnoides* clade follow the biogeographical distribution of *Alburnoides* species: a clade formed by *A. ohridanus*, *A. prespensis, A. devolli* and *A. fangfangae* comprises species distributed in the Albanian ichthyogeograpical district (Kottelat

497 & Freyhof, 2007), and a second clade is formed by *A. strymonicus* and *A. thessalicus* from the
498 Aegan district. The position of *A. economoui* was unresolved; however, Stierandová et al.
499 (2016) and Schönhuth et al. (2018) suggested that this species is closely related to the
500 'Albanian' group within the *Alburnoides* clade. A similar pattern was observed for
501 *Chondrostoma* spp., where two groups were recognized: the first comprises central Adriatic
502 species (*C. knerii* and *C. phoxinus*), and the second is formed by species from the southern
503 Balkans (*C. ohridana* and *C. vardarensis*). We could not resolve the phylogenetic position of
504 *Protochondrostoma genei*, the only representative of this monotypic genus, which is distributed
505 strictly in the Apennine Peninsula. According to Perea et al. (2010), this species should be in a
506 sister position to four other Balkan, Apennine and Iberian genera recently described from
507 *Chondrostoma s.l.* (*Achondrosotma, Iberochondrostoma, Parachondrostoma* and
508 *Pseudochondrostoma*).
509 The genus status of *Pachychilon* was highly controversial in the past and its representatives
510 shifted between various genera until this genus was established and species placed within
511 *Pachychilon* confirmed using osteological data (Soric, 1992). The genus *Pachychilon* was also
512 later supported by molecular data (Zardoya, Economidis & Doadrio, 1999). According to Levy,
513 Doadrio, and Almada (2009), this taxon represents one of the oldest lineages in the Balkans,
514 which diverged from other leuciscins approximately 43 Mya. Kottelat and Freyhof (2007)
515 suggested that *Pachychilon* contains only two species distributed in the western Balkans, and
516 that the distribution range of *Pachychilon* species is delimited by the area of the Albanian
517 ichthyogeographic district (Bianco, 1990). The phylogenetic position of the *Pachychilon* clade
518 and its high degree of endemism is likely reflected in its specific parasite fauna. In total, six
519 *Dactylogyrus* species were found on *Pachychilon* spp., each exhibiting a narrow host specificity
520 and belonging to three phylogenetic lineages (Benovics et al., 2018, present results).

521 The present study only weakly supports the monophyly of *Barbus*. The relationships within the
522 *Barbus* clade are in contrast to those found by Yang et al. (2015), being, in general, more
523 congruent with the phylogeny proposed by Gante et al. (2011). However, it is important to take
524 into account that both studies included different sets of species. The phylogenetic position of
525 *A. huegelii* appears uncertain in different studies, including this one. Yang et al. (2015)
526 suggested that *A. huegelii* occupied a sister position to the *Barbus* lineage, whilst Gante et al.
527 (2011) supported that this species is in basal position relatively to to a group comprising *Barbus*
528 and *Luciobarbus* species. In the present study, *A. huegelii* was phylogenetically closer to the
529 *Luciobarbus* clade, although its position was only moderately supported. The relationships
530 among *Barbus* and *Luciobarbus* species should be more deeply investigated, by including
531 additional *Luciobarbus* representatives as well as African and Middle Eastern *Capoeta,* in order
532 to attempt to resolve the phylogenetic uncertainty between these clades.
533
534 **Cophylogenetic host-parasite relationships**
535 In spite of their direct life cycle and narrow host specificity, previous cophylogenetic studies of
536 monogeneans and their fish hosts suggested that cospeciation is a rare event, much less common
537 than host switching and intra-host speciation (e.g. Desdevises et al., 2002, Zietara & Lumme,
538 2002, Huyse et al., 2003, Šimková et al., 2004, 2013, Mendlová et al., 2012, Messu Mandeng
539 et al., 2015).
540 It has been hypothesised that during evolutionary time monogeneans developed very
541 specialized haptors specifically to attach to (generally one) well-defined host species (Sasal,
542 Trouvé, Müller-Graf & Morand, 1999, Šimková et al., 2001, Jarkovský, Morand, Šimková &
543 Gelnar, 2004). For example, Šimková et al. (2001) found a positive correlation between the size
544 of *Dactylogyrus* anchor hooks and the size of their host species. Such highly adapted attachment
545 organs would make the switch to a different host species very difficult, and even unlikely (but

546 that may depend on the intraspecific variability of the sclerified pieces in this organ, see Kaci-
547 Chaouch et al. 2008). However, some *Dactylogyrus* species, such as *D. vistulae*, parasitize
548 phylogenetically distant hosts, from small-sized (e.g. *Alburnoides* spp. or *Phoxinellus* spp.) to
549 large-sized species (e.g. *Chondrostoma* spp., *Squalius* spp. or *Telestes* spp.), displaying only
550 minor morphological variability in their haptoral sclerites (Benovics, unpublished data). This
551 species clusters among the largest *Dactylogyrus* species (see Pugachev et al., 2009 for
552 morphology), exhibiting also large anchor hooks, which suggests that monogenean species
553 developing large attachment structures as an adaptation to large-sized hosts can host-switch to
554 smaller-size hosts.
555 According to our results, host-switching clearly appears to be the main coevolutionary event
556 inferred from the cophylogenetic reconstructions of *Dactylogyrus* and their hosts, followed by
557 cospeciation (Table 3). Host-switches likely result here from the sympatric distribution of
558 phylogenetically distant cyprinid species linked to the historical shift of the landmass and/or
559 from the more recent human induced introduction of non-native cyprinid species into the
560 Balkans and Apennines. In the present study, intra-host speciation (i.e. duplication) is suggested
561 to be a rather rare coevolutionary event. This is in contrast to previous cophylogenetic studies
562 on dactylogyrids, where intra-host duplication was the most commonly inferred coevolutionary
563 event (e.g. *Dactylogyrus* by Šimková et al., 2004, *Cichlidogyrus* and *Scutogyrus* on cichlids by
564 Mendlová et al., 2012, or *Thaparocleidus* on pangasiids by Šimková et al., 2013). This may be
565 explained by the fact that these studies included either a limited number of host species from
566 the investigated area or a high number of representatives from phylogenetically distant host
567 species where host-switching was highly improbable, in contrast to our study where highly
568 diversified groups of phylogenetically close and/or sympatric cyprinid species were included.
569 This suggest that host-switching is the primary cause of speciation in *Dactylogyrus*, followed
570 by intra-host speciation only if host-switching is not possible due to geographical isolation or

phylogenetic divergence (then presenting too large differences in parasites' microhabitat) among fish species living in sympatry.

In the present study, a statistically significant overall cophylogenetic structure was inferred among *Dactylogyrus* and their Cyprinidae hosts. The significant global fit computed with ParaFit relies on 47 % significant individual host-parasite links. Among these individual associations, the most significant were found between cyprinids of the Barbini tribe and their *Dactylogyrus* spp. All these *Dactylogyrus* species are genus-specific and their phylogenetic relationships followed the evolutionary history of barbels. However, this *Dactylogyrus* group is potentially subjected to cospeciation, as suggested in testing different cost schemes and reconstructing scenarios from phylogenetic trees topologies and the divergences of lineages. Cophylogenetic analyses considering only fish in Barbini and their *Dactylogyrus* species confirmed this significant cophylogenetic structure and suggested scenarios strongly implying duplication events in the evolutionary history of *Dactylogyrus* from Barbini. This intimate coevolutionary history between 'barbels' and their specific *Dactylogyrus* linages could be related to the fact that Barbini belong to the ancestral Cyprinidae lineage (Machordom & Doadrio, 2001b, Yang et al., 2015). We can hypothesize that during this long evolutionary period several *Dactylogyrus* species (i.e. *D. balkanicus, D. dyki, D. crivellius*) specialized on barbels, as supported by their specific distribution on European *Barbus* and the strong cophylogenetic structure between *Dactylogyrus* and Barbini in the Balkan and Apennine Peninsulas (Figure 4). However, two species, *D. petenyi* and *D. prespensis* (representatives of *Dactylogyrus* lineage 7 in our phylogenetic reconstruction), likely colonized their host via a recent host-switching from phylogenetically distant cyprinid taxa, followed by fast speciation on endemic barbels.

A strong cophylogenetic signal was also inferred between *D. alatus* and *D. sphyrna*, each with their respective hosts. In central Europe, these two species parasitize hosts from two or more

596 cyprinid genera (Moravec, 2001), while in southern European peninsulas they use only
597 *Alburnus* spp. and *Rutilus* spp., respectively. Frequent host-switching in the evolutionary
598 history of these *Dactylogyrus* species, inferred by the event-based analyses in Jane, suggest that
599 these species originally parasitized *Alburnus* and *Rutilus*, and subsequently switched to other
600 cyprinid genera in central Europe, where the number of phylogenetically related (congeneric)
601 cyprinid species living in sympatry is lower when compared to the diversity of congeneric
602 endemic cyprinids in southern Europe (Kottelat & Freyhof, 2007).
603 The cophylogenetic history of *Pachychilon* and their *Dactylogyrus* parasites reconstructed in
604 this study is noteworthy. Despite the fact that all *Dactylogyrus* species are genus or species-
605 specific, they do not form a monophyletic group. Three of the six *Dactylogyrus* species from
606 *Pachychilon* spp. found in this study formed a clade within group B (lineage 8), and a strong
607 cophylogenetic signal was observed exclusively between these species and their representative
608 *Pachychilon* hosts. This suggests that *D. petkovici* and the common ancestor of *D. martinovici*
609 and *Dactylogyrus* sp. 10 originated from an intra-host duplication during the evolutionary
610 history of *Pachychilon*, and that *Dactylogyrus* sp. 10 with *D. martinovici* originated from
611 cospeciation during the divergence of *Pachychilon* species. Additionally, *D. rosickyi* is
612 phylogenetically close to *Dactylogyrus* species from *Barbus* spp., which suggests a more recent
613 host switch of parasites between these phylogenetically distant cyprinid taxa. *Dactylogyrus*
614 *rosickyi* was collected only from *P. pictum* in the Aoos River (north-western Greece, a tributary
615 of the Adriatic Sea), where the occurrence of *Barbus* species (*B. prespensis*) was also
616 documented, and this *Dactylogyrus* species was not present on *P. pictum* in Lake Ohrid. *D.*
617 *rosickyi* was originally described by Ergens (1970) from Lake Skadar, which is a part of the
618 ancient Dessaretes Lake system (Albrecht & Wilke, 2008). This system potentially represent
619 the area of *D. rosickyi* origin were took place the initial transfer between ancestral *Barbus*
620 lineages and *Pachychilon* spp.

Figure 1. Phylogenetic tree of 139 haplotypes from 49 *Dactylogyrus* species collected in the Balkan and Apennine Peninsulas reconstructed by Bayesian inference (BI). The tree is based on concatenated partial 18S rDNA and partial 28S rDNA sequences. Values among branches indicate posterior probabilities from BI and bootstrap values from ML analyses. Values below 0.80 (BI) and 50 (ML) are shown as dashes. Branch lengths represent the number of substitutions per site. Letters in boxes (A-C) and numbers in the coloured areas (1-8) represent specific and well supported lineages described in the Results section. Numbers of genetic variants within each collapsed group are shown in brackets.

Figure 2. Phylogenetic tree of 85 haplotypes belonging to 76 endemic cyprinid species from the Balkan and Apennine Peninsulas, reconstructed by Bayesian inference (BI). The tree is based on 1140 bp long complete cytochrome *b* sequences and rooted using four representatives of the family Cobitidae. Values among branches indicate posterior probabilities from BI and bootstrap values from ML analyses. Values below 0.60 (BI) and 50 (ML) are shown as dashes. Branch lengths represent the number of substitutions per site. Coloured areas represent clades comprising individual genera.

Figure 3. Tanglegram showing the associations between Cyprinidae (left) and their *Dactylogyrus* parasites (right). Phylogenetic trees were reconstructed by Bayesian inference (Figures 1 and 2). Coloured lines represent statistically significant links computed with ParaFit (green $p < 0.05$; red $p < 0.001$). Each bracket represents the haplotypes belonging to one *Dactylogyrus* species. Cyprinid taxa without *Dactylogyrus* are shown in grey.

Figure 4. One of the optimal cophylogenetic scenario between representatives of the ribe Barbini and their specific *Dactylogyrus* species constructed with Jane 4.0 (11 cospeciations,

1 duplication, 18 duplications followed by host switch, 4 losses and 0 failure to diverge). Black branches represent the host phylogeny and blue branches represent the parasite phylogeny.

Table 1. List of cyprinid species including localities of their collection and accession numbers for complete cytochrome *b* sequences deposited in GenBank. LocID = codes used in all tables and figures for specific localities, N = number of processed fish individuals, NP = number of collected *Dactylogyrus* species. Cyprinid species without *Dactylogyrus* are shown by cross symbol (†). New sequences obtained in this study are shown by asterisks (*). Dashes represent sequences not used in the analyses.

Table 2. List of all collected *Dactylogyrus* species and their cyprinid hosts. Codes representing collection localities and GenBank accession numbers are included. New sequences obtained in this study are shown by asterisks (*).

Table 3. Outputs of cophylogenetic analyses calculated using 11 models with different cost schemes. Total costs represent the sum of inferred numbers of each evolutionary event multiplied by their respective costs. P-values were computed using 500 random reconstructions.

Table 4. Outputs of cophylogenetic analyses calculated using 11 models with different cost schemes applied to subset of cyprinids from the tribe Barbini and their respective *Dactylogyrus* species. Total costs represent the sum of inferred numbers of each evolutionary event multiplied by their respective costs. P-values were computed using 500 random reconstructions, n.s. – no significant scenario.

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References

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Contreras Balderas, S., Bussing, W., Stiassny, M. L. J., Skelton, P., Allen, G. R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T. J., Wikramanayake, E., Olson, D., López, H. L., Reis, R. E., Lundberg, J. G., Sabaj Pérez, M. H. & Petry, P. (2008). Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience*, 58, 403–414. doi: 10.1641/B580507

Albrecht, C. & Wilke, T. (2008). Ancient Lake Ohrid: biodiversity and evolution. *Hydrobiologia*, 615, 103–140. doi: 10.1007/978-1-4020-9582-5_9

Aydogdu, A., Molnár, K., Emre, Y. & Emre, N. (2015). Two new *Dactylogyrus* species (Monogenea: Dactylogyridae) *D. angorae* and *D. turcicus* from *Capoeta angorae* and *C. mauricii* endemic fishes in Turkey. *Acta Parasitologica*, 60, 130–133. doi: 10.1515/ap-2015-0017

Bakke, T. A., Cable, J. & Harris, P. D. (2007). The biology of gyrodactylid monogeneans: the "Russian Doll-killers". *Advances in Parasitology*, 64, 161–376. doi: 10.1016/S0065-308X(06)64003-7

Benovics, M., Desdevises, Y., Vukić, J., Šanda, R. & Šimková, A. (2018). The phylogenetic relationships and species richness of host-specific *Dactylogyrus* parasites shaped by the biogeography of Balkan cyprinids. *Scientific Reports*, 8, 13006. doi: 10.1038/s41598-018-31382-w

Benovics, M., Kičinjaová, M. L. & Šimková, A. (2017). The phylogenetic position of the enigmatic Balkan *Aulopyge huegelii* (Teleostei: Cyprinidae) from the perspective of host-specific *Dactylogyrus* parasites (Monogenea), with a description of *Dactylogyrus omenti* n. sp. *Parasites & Vectors*, 10, 547. doi : 10.1186/s13071-017-2491-z

706 Bianco, P. G. (1990). Potential role of the paleohistory of the Mediterranean and Parathethys
707 basins on the early dispersal of Euro-Mediterranean freshwater fishes. *Ichthyological*
708 *Exploration of Freshwaters*, 1, 167–184.
709 Bianco, P. G. (1995). Mediterranean endemic freshwater fishes of Italy. *Biological*
710 *Conservation*, 72, 159–170. doi: 10.1016/0006-3207(94)00078-5
711 Briolay, J., Galtier, N., Brito, R. M. & Bouvet, Y. (1998). Molecular Phylogeny of Cyprinidae
712 inferred from cytochrome *b* DNA sequences. *Molecular Phylogenetics and Evolution*,
713 9, 100–108. doi: 10.1006/mpev.1997.0441
714 Brito, R. M., Briolay, J., Galtier, N., Bouvet, Y. & Coelho, M. M. (1997). Phylogenetic
715 relationships within the genus *Leuciscus* (Pisces, Cyprinidae) in Portuguese freshwaters,
716 based on mitochondrial cytochrome *b* sequences. *Molecular Phylogenetics and*
717 *Evolution*, 8, 435–442. doi: 10.1006/mpev.1997.0429
718 Brooks, D. R., & McLennan, D. A. (1991). *Phylogeny, Ecology, and Behavior. A research*
719 *program in comparative biology*. Chicago, IL: University of Chicago Press. doi:
720 10.2307/1382264
721 Brooks, D. R & McLennan, D. A (1993). *Parascript: parasites and the language of evolution*.
722 Washington, DC: Smithsonian Institution Press. doi: 10.1016/0169-4758(94)90032-9
723 Buchmann, K. (1999). Immune mechanisms in fish skin against monogeneans – a model. *Folia*
724 *Parasitologica*, 46, 1–9.
725 Buchmann, K. & Lindenstrøm, T. (2002). Interactions between monogenean parasites and their
726 fish hosts. *International Journal for Parasitology*, 32, 309–319. doi: 10.1016/S0020-
727 7519(01)00332-0
728 Buj, I., Marčić, Z., Čaleta, M., Šanda, R., Geiger, M. F., Freyhof, J., Machordom, A. & Vukić,
729 J. (2017). Ancient connections among European rivers and watersheds revealed from

730 the evolutionary history of the genus *Telestes* (Actinopterygii; Cypriniformes). *PloS*
731 ONE, 12, e0187366. doi: 10.1371/journal.pone.0187366
732 Charleston, M. A. (1998). Jungles: a new solution to the host/parasite phylogeny reconciliation
733 problem. *Mathematical Biosciences*, 149, 191–223.
734 Charleston, M. A., (2012). TreeMap 3b. A Java program for cophylogeny mapping. Available
735 from: http://sydney.edu.au/engineering/it/~mcharles/.
736 Conow, C., Fielder, D., Ovadia, Y. & Libeskind-Hadas, R. (2010). Jane: a new tool for the
737 cophylogeny reconstruction problem. *Algorithms for Molecular Biology*, 5, 16. doi:
738 10.1186/1748-7188-5-16
739 Darriba, D., Taboala, G. L., Doallo, R. & Posada, D. (2012). JModelTest2: more models, new
740 heuristics and parallel computing. *Nature Methods*, 9, 772. doi: 10.1038/nmeth.2109
741 Deng, J., Yu, F., Li, H., Gebiola, M., Desdevises, Y., Wu, S. & Zhang, Y.-Z. (2013).
742 Cophylogenetic relationships between *Anicetus* parasitoids (Hymenoptera: Encyrtidae)
743 and their scale insect hosts (Hemiptera: Coccidae). *BMC Evolutionary Biology*, 13, 275.
744 doi: 10.1186/1471-2148-13-275
745 Desdevises, Y., Morand, S., Jousson, O. & Legendre, P. (2002). Coevolution between
746 *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): the study of a
747 complex host-parasite system. *Evolution*, 56, 2459–2471. doi: 10.1111/j.0014-
748 3820.2002.tb00171.x
749 Dupont, F. & Lambert, A. (1986). Study of the parasitic communities of Monogenea
750 Dactylogyridae from Cyprinidae in Lake Mikri Prespa (Northern Greece). Description
751 of three new species from endemic *Barbus*: *Barbus cyclolepis prespensis* Karaman,
752 1924. *Annales de Parasitologie Humaine et Comparée*, 6, 597–616.
753 Ergens, R. & Lom, J. (1970). *Causative Agents of Fish Diseases*. Prague, CZ: Academia.

754 Ergens, R. (1970). Parazitofauna ryb iz teritorii Cernogorii. I. Polyonchoinea (Monogenoidea)
755 nekotoryh ryb Skadarskogo ozera i bolsogo Chernogo ozera. *Poljoprivreda i Šumarstvo*,
756 16(1–2), 149–186.
757 Galli, P., Stefani, F., Zaccara, S. & Crosa, G. (2002). Occurrence of Monogenea in Italian
758 freshwater fish (Po river basin). *Parassitologia*, 44, 189–197.
759 Gibson, D. I., Timofeeva, T. A. & Gerasev, P. I. (1996). A catalogue of the nominal species of
760 the monogenean genus *Dactylogyrus* Diesing, 1850 and their host genera. *Systematic*
761 *Parasitology*, 35, 3–48. doi: 10.1007/BF00012180
762 Gante, H. F. (2011). Diversification of Circum-Mediterranean Barbels. In: O. Grillo & G.
763 Venora (Eds.), *Changing Biodiversity in Changing Environment* (pp. 283–298). Rijeka:
764 Intech. doi: 10.5772/24639
765 Guindon, S. & Gascuel, O. (2003). A simple, fast and accurate algorithm to estimate large
766 phylogenies by maximum likelihood. *Systematic Biology*, 27, 1759–1767. doi:
767 10.1080/10635150390235520
768 Gussev, A. V. (1966). Some new species of the genus *Dactylogyrus* from the European
769 freshwater fishes. *Folia Parasitologica*, 23, 250–255. (In Russian)
770 Hafner, M. S. & Nadler, S. (1988). Phylogenetic trees support the coevolution of parasites and
771 their hosts. *Nature*, 332, 258–259. doi: 10.1038/332258a0
772 Hafner, M. S., Sudman, P. D., Villablanca, F. X., Spradling, T. A., Demastes, J. W. & Nadler,
773 S. (1994). Disparate rates of molecular evolution in cospeciating hosts and parasites.
774 *Science*, 265, 1087–1090.
775 Hassouna, N., Michot, B. & Bachellerie, J. P. (1984). The complete nucleotide sequence of
776 mouse 28S rRNA gene. Implications for the process of size increase of the large subunit
777 rRNA in higher eukaryotes. *Nucleic Acids Research*, 12, 3563–3583.

778 Huyse, T., Audenaert, V. & Volckaert, F. A. M. (2003). Speciation and host-parasite
779 relationships in the parasite genus *Gyrodactylus* (Monogenea, Platyhelminthes)
780 infecting gobies of the genus *Pomatoschistus* (Gobiidae, Teleostei). *International*
781 *Journal for Parasitology*, 33, 1679–1689. doi: 10.1016/S0020-7519(03)00253-4. 23
782 Huyse, T., Oeyen, M., Larmuseau, M. H. D. & Volckaert, F. A. M. (2017). Co-phylogeographic
783 study of the flatworm *Gyrodactylus gondae* and its goby host *Pomatoschistus minutus*.
784 *Parasitology International*, 66, 119–125. doi: 0.1016/j.parint.2016.12.008
785 Huyse, T., Volckaert, F. A. M. (2005). Comparing host and parasite phylogenies: *Gyrodactylus*
786 flatworms jumping from goby to goby. *Systematic Biology*, 54, 710–718. doi:
787 10.1080/10635150500221036
788 Jarkovský, J., Morand, S., Šimková, A. & Gelnar. M. (2004). Reproductive barriers between
789 congeneric monogenean parasites (*Dactylogyrus*: Monogenea): attachment apparatus
790 morphology or copulatory organ incompatibility? *Parasitology Research*, 92, 95–105.
791 doi: 10.1007/s00436-003-0993-4
792 Johnson, K. P., Adams, R. J., Page, R. D. M & Clayton, D. H. (2003). When do parasites fail
793 to speciate in response to host speciation? *Systematic Biology*, 52, 37–47. doi:
794 10.1080/10635150390132704.
795 Kaci-Chaouch, T., Verneau, O., Desdevises, Y., 2008. Host specificity is linked to intraspecific
796 variability in the genus *Lamellodiscus* (Monogenea). *Parasitology* 135, 607–616.
797 Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002). MAFFT: a novel method for rapid
798 multiple sequence alignment based on Fourier transform. *Nucleic Acids Research*, 30,
799 3059–3066.
800 Ketmaier, V., Bianco, P. G., Cobolli, M., Krivokapic, M., Caniglia, R. & de Matthaeis, E.
801 (2004). Molecular phylogeny of two lineages of Leuciscinae cyprinids (*Telestes* and

802 *Scardinius*) from the peri-Mediterranean area based on cytochrome *b* data. *Molecular*
803 *Phylogenetics and Evolution*, 32, 1061–1071. doi: 10.1016/j.ympev.2004.04.008
804 Klassen, G. J. (1992). Coevolution: A history of the macroevolutionary approach to studying
805 host parasite associations. *Journal of Parasitology*, 78, 573–587.
806 Kottelat, M. & Freyhof, J. (2007). *Handbook of European Freshwater Fishes*. Cornol, CH:
807 Publications Kottelat.
808 Lambert, A. (1977). Les monogènes Monopisthocotylea parasites des poissons d'eau douce de
809 la France méditeranéenne. *Bulletin du Muséum National d'Histoire Naturelle* 3e sér.,
810 429, *Zoologie*, 299, 177–214.
811 Legendre, P., Desdevises, Y. & Bazin, E. (2002). A statistical test for host-parasite coevolution.
812 *Systematic Biology*, 51, 217–234. doi: 10.1080/10635150252899734
813 Levy, A., Doadrio, I. & Almada, V. C. (2009). Historical biogeography of European leuciscins
814 (Cyprinidae): evaluating the Lago Mare dispersal hypothesis. *Journal of Biogeography*,
815 36, 55–65. doi: 10.1111/j.1365-2699.2008.01969.x
816 Machordom, A. & Doadrio, I. (2001a). Evidence of a Cenozoic Betic-Kabilian connection
817 based on freshwater fish phylogeography (*Luciobarbus*, Cyprinidae). *Molecular*
818 *Phylogenetics and Evolution*, 18, 252–263. doi: 10.1006/mpev.2000.0876
819 Machordom, A. & Doadrio, I. (2001b). Evolutionary history and speciation modes in the
820 cyprinid genus *Barbus*. *Proceedings of the Royal Society London B*, 268, 1297–1306.
821 Malmberg, G. (1957). Om forekomsten av *Gyrodactylus* pa svenska fiskar. Skrifter Utgivna av
822 Sodra Sveriges Fiskeriforening. *Arsskift*, 1956, 19–76.
823 Marková, S., Šanda, R., Crivelli, A., Shumka, S., Wilson, I. F., Vukić, J., Berrebi, P. & Kotlík,
824 P. (2010). Nuclear and mitochondrial DNA sequence data reveal the evolutionary
825 history of *Barbus* (Cyprinidae) in the ancient lake systems of the Balkans. *Molecular*
826 *Phylogenetics and Evolution*, 55, 488–500. doi: 10.1016/j.ympev.2010.01.030

827 Mayden, R. L., Chen, W.-J., Bart, H. L., Doosey, M. H., Simmons, A. M., Tang, K. L., Wood,
828 R. M., Agnew, M. K., Yang, L., Vincent Hirt, M., Clements, M. D., Saitoh, K., Sado,
829 T., Miya, M. & Nishida, M. (2009). Reconstructing the phylogenetic relationships of
830 the earth's most diverse clade of freshwater fishes – order Cypriniformes
831 (Actinopterygii: Ostariophysi): A case study using multiple nuclear loci and
832 mitochondrial genome. *Molecular Phylogenetics and Evolution*, 51, 500–514. doi:
833 10.1016/j.ympev.2008.12.015
834 Meier-Kolthoff, J. P., Auch, A. F., Huson, D. H., Göker, M. (2007). CopyCat: cophylogenic
835 analysis tool. *Bioinformatics*, 23, 898–900. doi:10.1093/bioinformatics/ btm027.
836 Mendlová, M., Desdevides, Y., Civáňová, K., Pariselle, A. & Šimková, A.
837 (2012). Monogeneans of West African cichlid fish: evolution and cophylogenetic
838 interactions. *PLoS One*, 7(5), e37268. doi:10.1371/journal.pone.0037268
839 Messu Mandeng, F. D., Bilong Bilong, C. F., Pariselle, A., Vanhove, M. P. M., Bitja Nyon, A.
840 R. & Agnèse, J.-F. (2015). A phylogeny of *Cichlidogyrus* spp. (Monogenea,
841 Dactylogyridea) clarifies a host-switch between fish families and reveals an adaptive
842 component to attachment organ morphology of this parasite genus. *Parasites & Vectors*,
843 8, 582. doi: 10.1186/s13071-015-1181-y
844 Moravec, F. (2001). *Checklist of the Metazoan Parasites of Fishes of Czech Republic and*
845 *Slovak Republic (1873-2000).* Prague, CZ: Academia.
846 Nitta, M. & Nagasawa, K. (2016). A new species of *Dactylogyrus* (Monogenea:
847 Dactylogyridae) parasitic on an endangered freshwater fish, *Rhodeus atremius atremius,*
848 endemic to Japan. *Parasitology International*, 65, 483–487. doi:
849 10.1016/j.parint.2016.06.014
850 Oikonomou, A., Leprieur, F. & Leonardos, I. D. (2014). Biogeography of freshwater fishes of
851 the Balkan Peninsula. *Hydrobiologia*, 738, 205–220. doi: 10.1007/s10750-014-1930-5

852 Page, R. D. M. (1996). Temporal congruence revisited: comparison of mitochondrial DNA
853 sequence divergence in cospeciating pocket gophers and their chewing lice. *Systematic*
854 *Biology*, 45, 151–167.
855 Perea, S., Böhme, M., Zupančič, P., Freyhof, J., Šanda, R., Özuluğ, M., Abdoli, A. & Doadrio,
856 I. (2010). Phylogenetic relationships and biogeographical patterns in Circum-
857 Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both
858 mitochondrial and nuclear data. *BMC Evolutionary Biology*, 10, 265. doi:10.1186/1471-
859 2148-10-265
860 Perea, S., Vukić, J., Šanda, R. & Doadrio, I. (2016). Ancient mitochondrial capture as factor of
861 promoting mitonuclear discordance in freshwater fishes: a case study in the genus
862 *Squalius* (Actinopterygii, Cyprinidae) in Greece. *PloS ONE*, 11, e0166292.
863 doi:10.1371/journal.pone.0166292
864 Poulin, R. (2007). *Evolutionary Ecology of Parasites* (2nd ed.). Princeton, NJ: University Press.
865 Pugachev, O. N., Gerasev, P. I., Gussev, A. V., Ergens, R. & Khotenowsky, I. (2009). *Guide to*
866 *Monogenoidea of Freshwater Fish of Palearctic and Amur Regions.* Milan, IT:
867 Ledizione-Ledi Publishing.
868 Rahmouni, I., Řehulková, E., Pariselle, A., Rkhami, O. B. & Šimková, A. (2017). Four new
869 species of *Dactylogyrus* Diesing, 1850 (Monogenea: Dactylogyridae) parasitising the
870 gills of northern Moroccan *Luciobarbus Heckel* (Cyprinidae): morphological and
871 molecular characterisation. *Systematic Parasitology*, 94, 575–591. doi:
872 10.1007/s11230-017-9726-4
873 Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. (2018). Posterior
874 summarization in bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67, 901–
875 604. doi:10.1093/sysbio/syy032

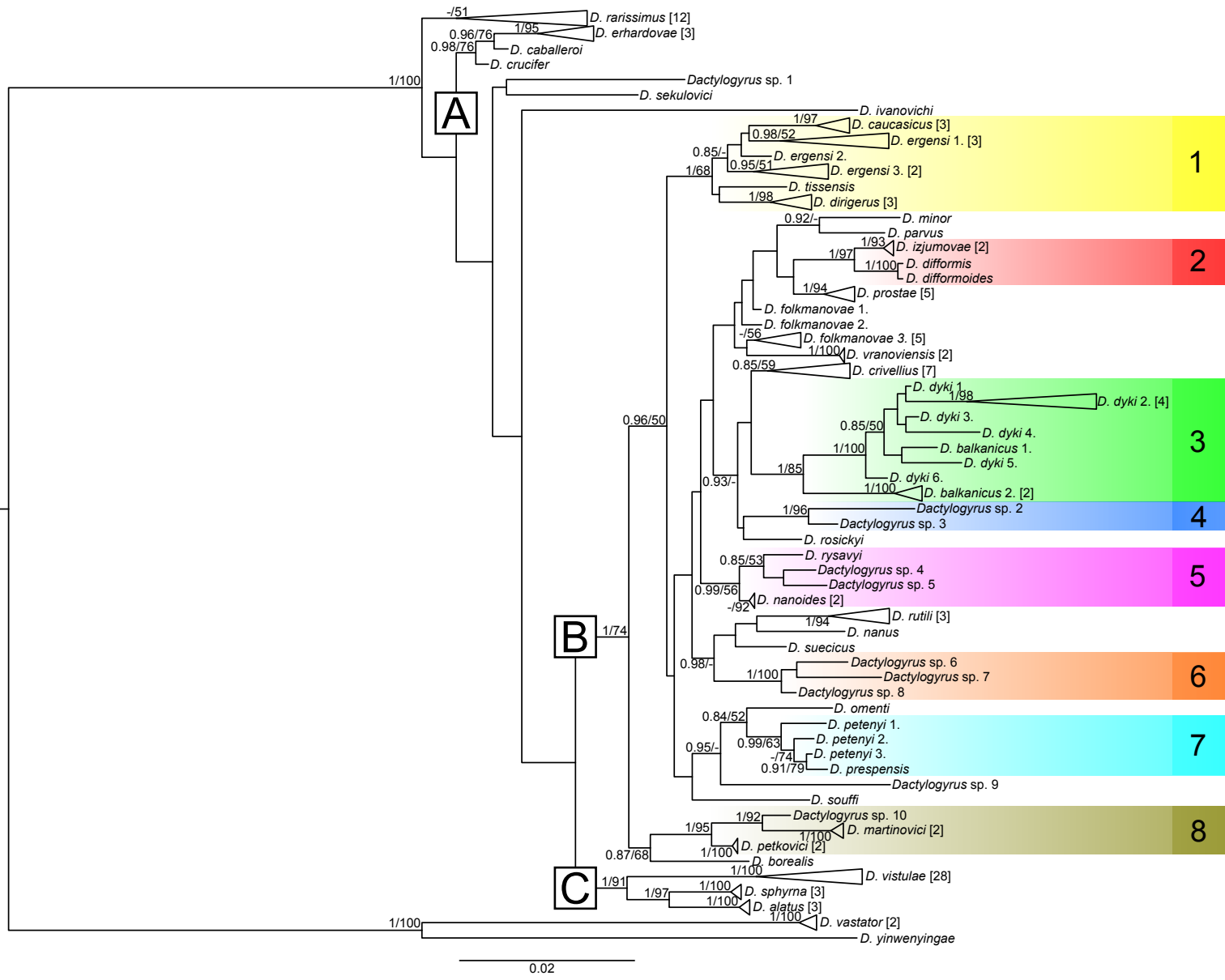
876 Ronquist, F. (1995). Reconstructing the history of host-parasite associations using generalized
877 parsimony. *Cladistics*, 11, 73–89. doi: 10.1111/j.1096-0031.1995.tb00005.x
878 Ronquist, F. (1997). Phylogenetic approaches in coevolution and biogeography. *Zoologica*
879 *Scripta*, 26, 312–322. doi: 10.1111/j.1463-6409.1997.tb00421.x
880 Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna S., Larget, B.,
881 Liu, L., Suchard, M. A. & Hulsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian
882 phylogenetic inference and model choice across large model space. *Systematic Biology*,
883 61, 539–542. doi: 10.1093/sysbio/sys029
884 Sasal, P., Trouvé, S., Müller-Graf, C. & Morand, S. (1999). Specificity and host predictability:
885 a comparative analysis among monogenean parasites of fish. *Journal of Animal*
886 *Ecology*, 68, 437–444. doi: 10.1046/j.1365-2656.1999.00313.x
887 Schönhuth, S., Vukić, J., Šanda, R., Yang, L. & Mayden, R. L. (2018). Phylogenetic
888 relationships and classification of the Holarctic family Leuciscidae (Cypriniformes:
889 Cyprinoidei). *Molecular Phylogenetics and Evolution*, 127, 781–799.
890 doi:10.1016/j.ympev.2018.06.026
891 Schultheiss, R., Albrecht, C., Bossneck, U. & Wilke, T. (2008). The neglected side of speciation
892 in ancient lakes: phylogeography of an inconspicuous mollusc taxon in lakes Ohrid and
893 Prespa. *Hydrobiologia*, 615, 141–156. doi: 10.1007/s10750-008-9553-3
894 Soric, V. (1992). Osteology and taxonomic status of the genus *Pachychilon* Steind., 1882
895 (Pisces, Cyprinidae). *Bios (Macedonia, Greece)*, 1, 49–67.
896 Stamatakis, A. (2014). RaxML version 8: A tool for phylogenetic analysis and post-analysis of
897 large phylogenies. *Bioinformatics*, 30, 1312–1313. doi: 10.1093/bioinformatics/btu033
898 Stammer, H. J. (1957). Gedanken zu den parasitophyletischen Regeln und zur Evolution der
899 Parasiten. *Zoologischer Anzieger*, 159, 255–267.

900 Stefani, F., Galli, P., Crosa, G., Zaccara. S, & Calamari, D. (2004). Alpine and Apennine
901 barriers determining the differentiation of the rudd (*Scardinius erythrophthalmus* L.) in
902 the Italian peninsula. *Ecology of Freshwater Fish*, 13, 168–175. doi: 10.1111/j.1600-
903 0633.2004.00060.x
904 Steininger, F. F. & Rögl, F. (1984). Paleogeography and palinspastic reconstruction of the
905 Neogene of the Mediterranean and Paratethys. In J.E. Dixon & A. H. Robertson (Eds.),
906 *The Geological Evolution of the Eastern Mediterranean* (pp. 659–668). Oxford:
907 Blackwell. doi: 10.1144/GSL.SP.1984.017.01.52
908 Stierandová, S., Vukić, J., Vasil'eva, E. D., Zogaris, S., Shumka, S., Halačka, K., Vetešník, L.,
909 Švátora, M., Nowak, M., Stefanov, T., Koščo, J. & Mendel, J. (2016). A multilocus
910 assessment of nuclear and mitochondrial sequence data elucidates phylogenetic
911 relationships among European spirlins (*Alburnoides*, Cyprinidae). *Molecular*
912 *Phylogenetics and Evolution,* 94, 479–491. doi: 10.1016/j.ympev.2015.10.025
913 Sušnik, S., Snoj, A. & Dovč, P. (2001). Evolutionary distinctness of grayling (*Thymallus*
914 *thymallus*) inhabiting the Adriatic river system, as based on mtDNA variation.
915 *Biological Journal of the Linnaean Society*, 74(3), 375–385. doi: 10.1111/j.1095-
916 8312.2001.tb01399.x
917 Sušnik, S., Snoj, A., Wilson, I. F., Mrdak, D. & Weiss, S. (2007). Historical demography of
918 brown trout (*Salmo trutta*) in the Adriatic drainage including the putative *S. letnica*
919 endemic to Lake Ohrid. *Molecular Phylogenetics and Evolution*, 44, 63–76. doi:
920 10.1016/j.ympev.2006.08.021
921 Šanda, R., Vukić, J., Choleva, L., Křížek, J., Šedivá, A., Shumka, S. & Wilson, I. F. (2008).
922 Distribution of loach fishes (Cobitidae, Nemacheilidae) in Albania, with genetic
923 analysis of populations of *Cobitis ohridana*. *Folia Zoologica*, 57, 42–50.

924 Šimková, A., Benovics, M., Rahmouni, I. & Vukić, J. (2017). Host-specific *Dactylogyrus*
925 parasites revealing new insights on the historical biogeography of Northwest African
926 and Iberian cyprinid fish. *Parasites & Vectors*, 10, 589. doi:10.1186/s13071-017-2521-
927 x
928 Šimková, A., Desdevises, Y., Gelnar, M. & Morand, S. (2000) Co-existence of nine gill
929 ectoparasites (*Dactylogyrus*: Monogenea) parasitising the roach (*Rutilus rutilus* L.):
930 history and present ecology. *International Journal for Parasitology*, 30, 1077–1088.
931 doi: 10.1016/S0020-7519(00)00098-9
932 Šimková, A., Desdevises, Y., Gelnar, M. & Morand, S. (2001). Morphometric correlates of host
933 specificity in *Dactylogyrus* species (Monogenea) parasites of European cyprinid fish.
934 *Parasitology*, 123, 169–177. doi: 10.1017/S0031182001008241
935 Šimková, A., Matějusová, I., Cunningham, C. O. (2006a). A molecular phylogeny of the
936 Dactylogyridae sensu Kritsky & Boeger (1989) (Monogenea) based on the D1-D3
937 domains of large subunit rDNA. *Parasitology*, 133, 43–53. doi:
938 10.1017/S0031182006009942
939 Šimková, A. & Morand, S. (2008). Co-evolutionary patterns in congeneric monogeneans: a
940 review of *Dactylogyrus* species and their cyprinid hosts. *Journal of Fish Biology*, 73,
941 2210–2227. doi: 10.1111/j.1095-8649.2008.02064.x
942 Šimková, A., Morand, S., Jobet, E., Gelnar, M. & Verneau, O. (2004). Molecular phylogeny of
943 congeneric monogenean parasites (*Dactylogyrus*): a case of intrahost speciation.
944 *Evolution*, 58, 1001–1018. doi:10.1111/j.0014-3820.2004.tb00434.x
945 Šimková, A., Plaisance, L., Matějusová, I., Morand, S. & Verneau, O. (2003). Phylogenetic
946 relationships of the Dactylogiridae Bychowsky, 1933 (Monogenea: Dactylogyridae):
947 the need for the systematic revision of the Ancyrophalinae Bychowsky, 1937.
948 *Systematic Parasitology*, 54, 1–11.

949 Šimková, A., Serbielle, C., Pariselle, A., Vanhove, M. P. M. & Morand, S. (2013). Speciation
950 in *Theparocleidus* (Monogenea: Dactylogyridae) parasitizing Asian pangasiid catfishes.
951 *BioMed Research International*, 2013, 1–14. doi: 10.1155/2013/353956
952 Šimková, A., Verneau, O., Gelnar, M., & Morand, S. (2006b). Specificity and specialization of
953 congeneric monogeneans parasitizing cyprinid fish. *Evolution*, 60, 1023–1037. doi:
954 10.1111/j.0014-3820.2006.tb01180.x
955 Talavera, G. & Castresana, J. (2007). Improvement of phylogenies after removing divergent
956 and ambigously aligned blocks from protein sequence alignments. *Systematic Biology*,
957 56, 564–577. doi: 10.1080/10635150701472164
958 de Vienne, D. M., Giraud, T. & Shykoff, J. A. (2007). When can host shifts produce congruent
959 host and parasite phylogenies? A simulation approach. *Journal of Evolutionary Biology*,
960 20, 1428–1438. doi:10.1111/j.1420-9101.2007.01340.x
961 de Vienne, D. M., Refreiger, G., Lopez-Villavicencio, M., Tellier, A., Hood, M. E. & Giraud,
962 T. (2013). Cospeciation vs. host-shift speciation: methods for testing, evidence from
963 natural associations and relation to coevolution. *New Phytologist*, 198, 347–385. doi:
964 0.1111/nph.12150
965 Waelbroeck, C., Labeyrie, L., Michel, E., Duplessy, J. C., McManus, J. F., Lambeck,
966 K., Balbon, E. & Labracherie, M. (2002). Sea-level and deep water temperature
967 changes derived from benthic foraminifera isotopic records. *Quaternary Science*
968 *Reviews*, 21, 295–305. doi: 10.1016/S0277-3791(01)00101-9
969 Whittington, I. A. & Kearn, G. C. (2011). Hatching strategies in monogenean (platyhelminth)
970 parasites that facilitate host infection. *Integrative and Comparative Biology*, 51, 91–99.
971 doi: 0.1093/icb/icr003
972 Yang, L., Sado, T., Vincent Hirt, M., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X.,
973 Freyhof, J., Saitoh, K., Simons, A. M., Miya, M., He, S. & Mayden, R. L. (2015).

974 Phylogeny and polyploidy: resolving classification of cyprinine fishes (Teleostei:
975 Cypriniformes). *Molecular Phylogenetics and* Evolution, 85, 97–116. doi:
976 10.1016/j.ympev.2015.01.014
977 Zietara, M. S. & Lumme, J. (2002). Speciation by host switch and adaptive radiation in a fish
978 parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution*, 56, 2445–2458.
979 doi: 10.1111/j.0014-3820.2002.tb00170.x
980 Zardoya, M. S. & Doadrio, I. (1999). Molecular evidence on the evolutionary and
981 biogeographical patterns of European cyprinids. *Journal of Molecular Evolution*, 49,
982 227–237.
983 Zardoya, M. S., Economidis, P. S. & Doadrio, I. (1999). Phylogenetic relationships of Greek
984 Cyprinidae: molecular evidence for at least two origins of the Greek cyprinid fauna.
985 *Molecular Phylogenetics and Evolution*, 13, 122–131.
986


-/51
D. rarissimus [12]
1/95
D. erhardovae [3]
0.96/76
0.98/76
D. caballeroi
D. crucifer
1/100
A
Dactylogyrus sp. 1
D. sekulovici
D. ivanovichi
1/97
D. caucasicus [3]
0.98/52
D. ergensi 1. [3]
0.85/-
D. ergensi 2.
0.95/51
D. ergensi 3. [2]
1/68
D. tissensis
1/98
D. dirigerus [3]
1
0.92/-
D. minor
D. parvus
1/93
D. izjumovae [2]
1/97
1/100
D. difformis
D. difformoides
2
1/94
D. prostae [5]
D. folkmanovae 1.
D. folkmanovae 2.
-/56
D. folkmanovae 3. [5]
1/100
D. vranoviensis [2]
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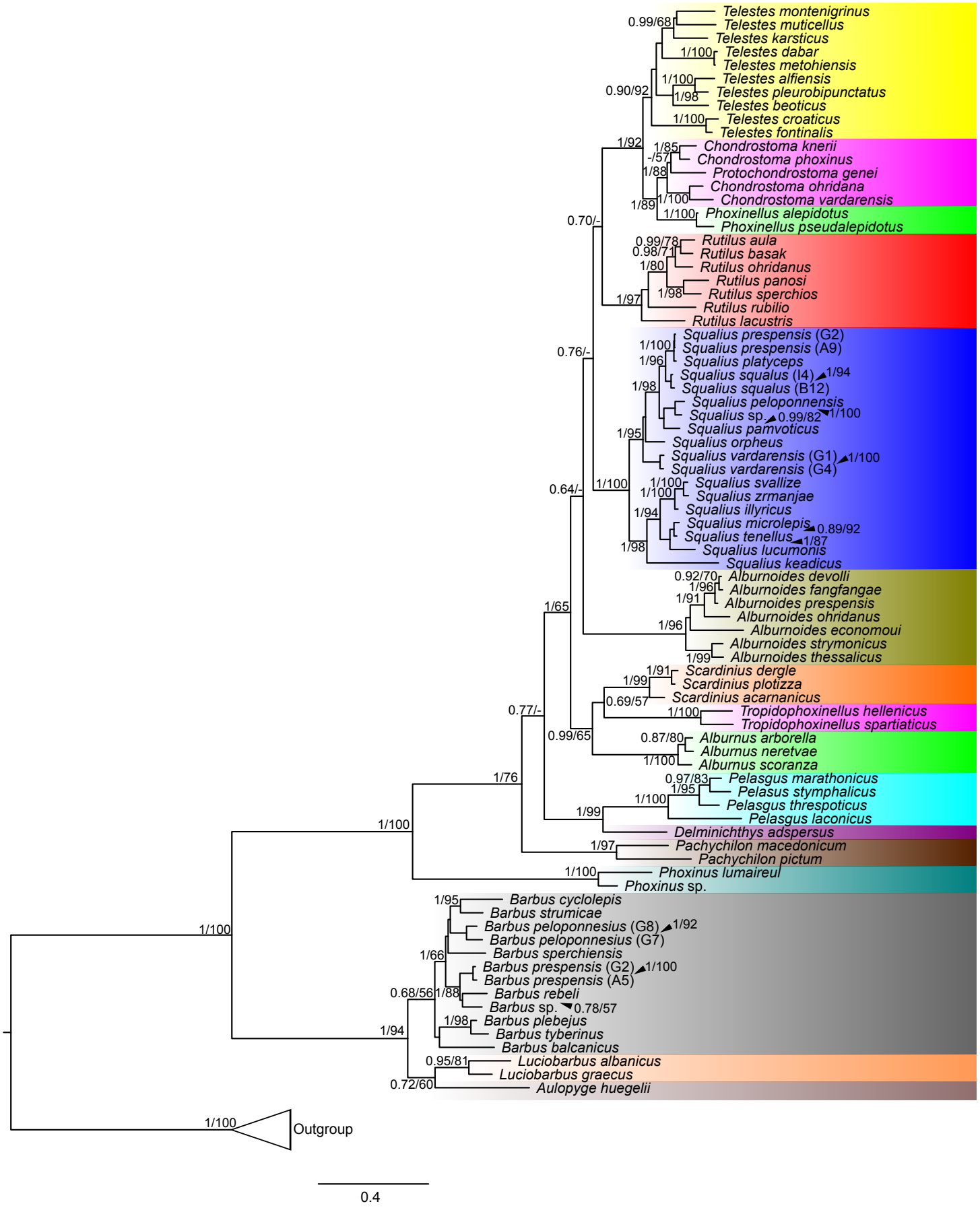
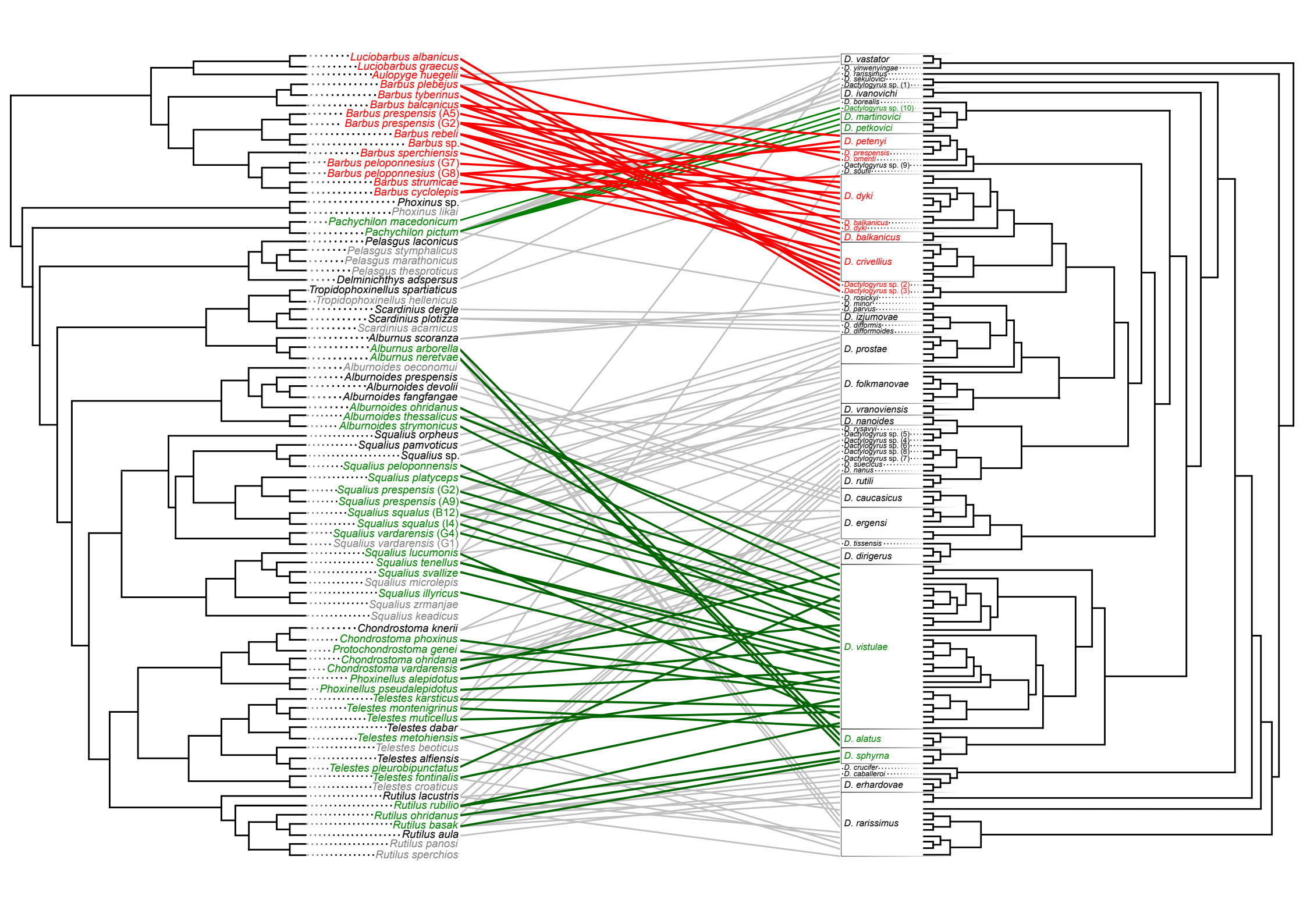
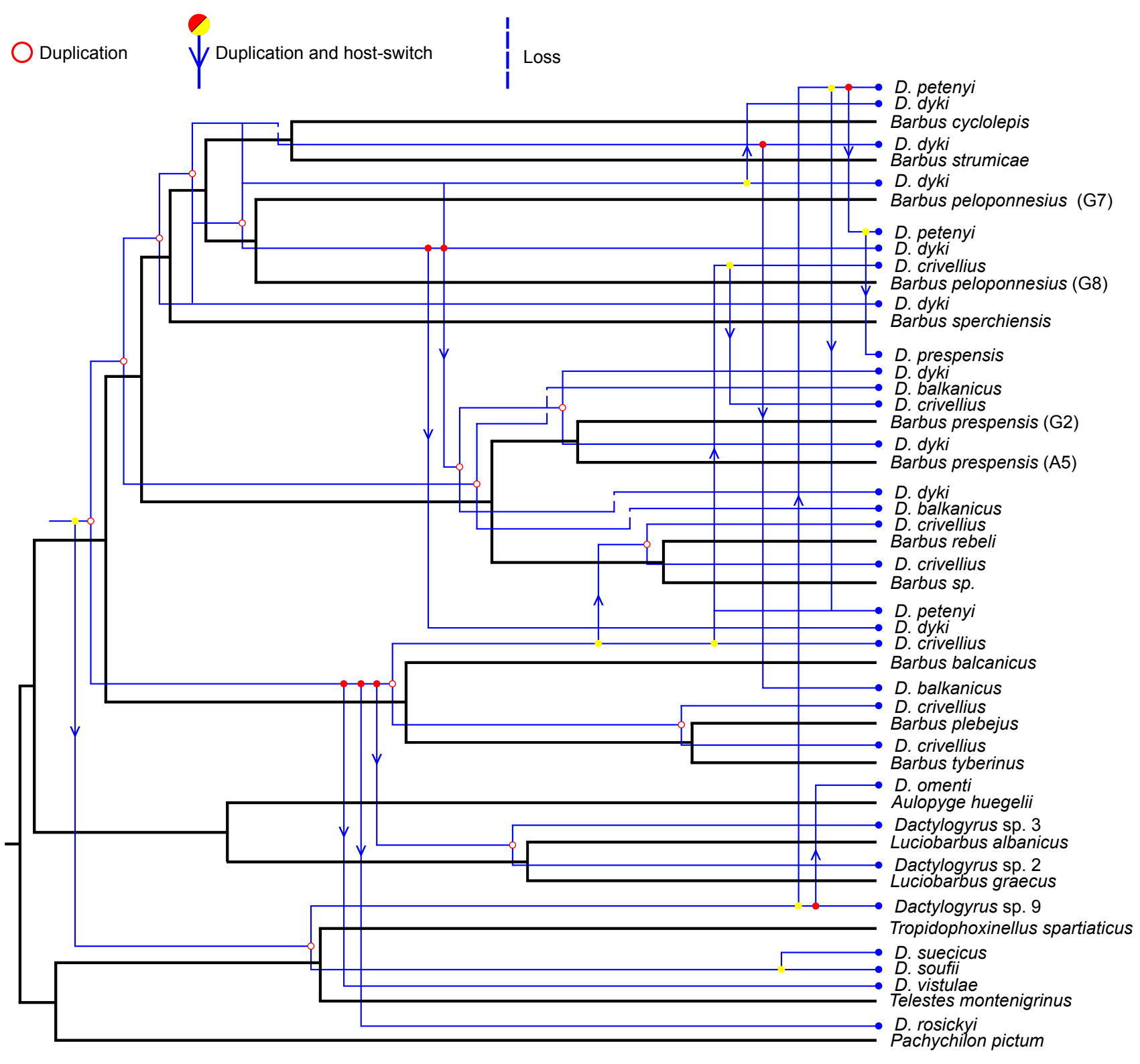
D. crivellius [7]
D. dyki 1.
1/98
D. dyki 2. [4]
0.96/50
D. dyki 3.
0.85/50
D. dyki 4.
1/100
D. balkanicus 1.
D. dyki 5.
1/85
D. dyki 6.
0.93/-
1/100
D. balkanicus 2. [2]
3
1/96
Dactylogyrus sp. 2
Dactylogyrus sp. 3
4
D. rosickyi
D. rysavyi
0.85/53
Dactylogyrus sp. 4
Dactylogyrus sp. 5
5
0.99/56
D. nanoides [2]
-/92
D. rutili [3]
1/94
D. nanus
B
1/74
D. suecicus
0.98/-
Dactylogyrus sp. 6
1/100
Dactylogyrus sp. 7
Dactylogyrus sp. 8
6
D. omenti
0.84/52
D. petenyi 1.
0.99/63
D. petenyi 2.
-/74
D. petenyi 3.
0.95/-
0.91/79
D. prespensis
7
Dactylogyrus sp. 9
D. souffi
1/92
Dactylogyrus sp. 10
1/95
D. martinovici [2]
1/100
D. petkovici [2]
1/100
0.87/68
D. borealis
8
1/100
D. vistulae [28]
C
1/91
1/100
D. sphyrna [3]
1/97
1/100
D. alatus [3]
1/100
D. vastator [2]
1/100
D. yinwenyingae
0.02


Telestes montenigrinus
Telestes muticellus
Telestes karsticus
Telestes dabar
Telestes metohiensis
Telestes alfiensis
Telestes pleurobipunctatus
Telestes beoticus
Telestes croaticus
Telestes fontinalis
Chondrostoma knerii
Chondrostoma phoxinus
Protochondrostoma genei
Chondrostoma ohridana
Chondrostoma vardarensis
Phoxinellus alepidotus
Phoxinellus pseudalepidotus
Rutilus aula
Rutilus basak
Rutilus ohridanus
Rutilus panosi
Rutilus sperchios
Rutilus rubilio
Rutilus lacustris
Squalius prespensis (G2)
Squalius prespensis (A9)
Squalius platyceps
Squalius squalus (I4)
Squalius squalus (B12)
Squalius peloponnensis
Squalius sp.
Squalius pamvoticus
Squalius orpheus
Squalius vardarensis (G1)
Squalius vardarensis (G4)
Squalius svallize
Squalius zrmanjae
Squalius illyricus
Squalius microlepis
Squalius tenellus
Squalius lucumonis
Squalius keadicus
Alburnoides devolli
Alburnoides fangfangae
Alburnoides prespensis
Alburnoides ohridanus
Alburnoides economoui
Alburnoides strymonicus
Alburnoides thessalicus
Scardinius dergle
Scardinius plotizza
Scardinius acarnanicus
Tropidophoxinellus hellenicus
Tropidophoxinellus spartiaticus
Alburnus arborella
Alburnus neretvae
Alburnus scoranza
Pelasgus marathonicus
Pelasus stymphalicus
Pelasgus threspoticus
Pelasgus laconicus
Delminichthys adspersus
Pachychilon macedonicum
Pachychilon pictum
Phoxinus lumaireul
Phoxinus sp.
Barbus cyclolepis
Barbus strumicae
Barbus peloponnesius (G8)
Barbus peloponnesius (G7)
Barbus sperchiensis
Barbus prespensis (G2)
Barbus prespensis (A5)
Barbus rebeli
Barbus sp.
Barbus plebejus
Barbus tyberinus
Barbus balcanicus
Luciobarbus albanicus
Luciobarbus graecus
Aulopyge huegelii
Outgroup
0.4


Luciobarbus albanicus
Luciobarbus graecus
Aulopyge huegelii
Barbus plebejus
Barbus tyberinus
Barbus balcanicus
Barbus prespensis (A5)
Barbus prespensis (G2)
Barbus rebeli
Barbus sp.
Barbus sperchiensis
Barbus peloponnesius (G7)
Barbus peloponnesius (G8)
Barbus strumicae
Barbus cyclolepis
Phoxinus sp.
Phoxinus likai
Pachychilon macedonicum
Pachychilon pictum
Pelasgus laconicus
Pelasgus stymphalicus
Pelasgus marathonicus
Pelasgus thesprotícus
Delminichthys adspersus
Tropidophoxinellus spartiaticus
Tropidophoxinellus hellenicus
Scardinius dergle
Scardinius plotizza
Scardinius acarnicus
Alburnus scoranza
Alburnus arborella
Alburnus neretvae
Alburnoides oeconomui
Alburnoides prespensis
Alburnoides devolii
Alburnoides fangfangae
Alburnoides ohridanus
Alburnoides thessalicus
Alburnoides strymonicus
Squalius orpheus
Squalius pamvoticus
Squalius sp.
Squalius peloponnensis
Squalius platyceps
Squalius prespensis (G2)
Squalius prespensis (A9)
Squalius squalus (B12)
Squalius squalus (I4)
Squalius vardarensis (G4)
Squalius vardarensis (G1)
Squalius lucumonis
Squalius tenellus
Squalius svallize
Squalius microlepis
Squalius illyricus
Squalius zrmanjae
Squalius keadicus
Chondrostoma knerii
Chondrostoma phoxinus
Protochondrostoma genei
Chondrostoma ohridana
Chondrostoma vardarensis
Phoxinellus alepidotus
Phoxinellus pseudalepidotus
Telestes karsticus
Telestes montenigrinus
Telestes muticellus
Telestes dabar
Telestes metohiensis
Telestes beoticus
Telestes alfiensis
Telestes pleurobipunctatus
Telestes fontinalis
Telestes croaticus
Rutilus lacustris
Rutilus rubilio
Rutilus ohridanus
Rutilus basak
Rutilus aula
Rutilus panosi
Rutilus sperchios
D. vastator
D. yinwenyingae
D. rarissimus
D. sekulovici
Dactylogyrus sp. (1)
D. ivanovichi
D. borealis
Dactylogyrus sp. (10)
D. martinovici
D. petkovici
D. petenyi
D. prespensis
D. omenti
Dactylogyrus sp. (9)
D. soufli
D. dyki
D. balkanicus
D. dyki
D. balkanicus
D. crivellius
Dactylogyrus sp. (2)
Dactylogyrus sp. (3)
D. rosickyi
D. minor
D. parvus
D. iziumovae
D. difformis
D. difformoides
D. prostae
D. folkmanovae
D. vranoviensis
D. nanoides
D. rysavyi
Dactylogyrus sp. (5)
Dactylogyrus sp. (4)
Dactylogyrus sp. (6)
Dactylogyrus sp. (8)
Dactylogyrus sp. (7)
D. suecicus
D. nanus
D. rutili
D. caucasicus
D. ergensi
D. tissensis
D. dirigerus
D. vistulae
D. alatus
D. sphyrna
D. crucifer
D. caballeroi
D. erhardovae
D. rarissimus


Duplication
Duplication and host-switch
Loss
D. petenyi
D. dyki
Barbus cyclolepis
D. dyki
Barbus strumicae
D. dyki
Barbus peloponnesius (G7)
D. petenyi
D. dyki
D. crivellius
Barbus peloponnesius (G8)
D. dyki
Barbus sperchiensis
D. prespensis
D. dyki
D. balkanicus
D. crivellius
Barbus prespensis (G2)
D. dyki
Barbus prespensis (A5)
D. dyki
D. balkanicus
D. crivellius
Barbus rebeli
D. crivellius
Barbus sp.
D. petenyi
D. dyki
D. crivellius
Barbus balcanicus
D. balkanicus
D. crivellius
Barbus plebejus
D. crivellius
Barbus tyberinus
D. omenti
Aulopyge huegelii
Dactylogyrus sp. 3
Luciobarbus albanicus
Dactylogyrus sp. 2
Luciobarbus graecus
Dactylogyrus sp. 9
Tropidophoxinellus spartiaticus
D. suecicus
D. soufii
D. vistulae
Telestes montenigrinus
D. rosickyi
Pachychilon pictum