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Molecular and morphological phylogeny of host-specific Dactylogyrus parasites (Monogenea) sheds new light on the puzzling Middle Eastern origin of European and African lineages

Michal Benovics1,2*, Farshad Nejat1, Asghar Abdoli3 and Andrea Šimková1

Abstract
Background: Freshwater fauna of the Middle East encompass elements shared with three continents—Africa, Asia, and Europe—and the Middle East is, therefore, considered a historical geographic crossroad between these three regions. Even though various dispersion scenarios have been proposed to explain the current distribution of cyprinids in the peri-Mediterranean, all of them congruently suggest an Asian origin for this group. Herein, we investigated the proposed scenarios using monogenean parasites of the genus Dactylogyrus, which is host-specific to cyprinoid fishes.

Methods: A total of 48 Dactylogyrus species parasitizing cyprinids belonging to seven genera were used for molecular phylogenetic reconstruction. Taxonomically important morphological features, i.e., sclerotized elements of the attachment organ, were further evaluated to resolve ambiguous relationships between individual phylogenetic lineages. For 37 species, sequences of partial genes coding 18S and 28S rRNA, and the ITS1 region were retrieved from GenBank. Ten Dactylogyrus species collected from Middle Eastern cyprinoids and D. falciformis were de novo sequenced for the aforementioned molecular markers.

Results: The phylogenetic reconstruction divided all investigated Dactylogyrus species into four phylogenetic clades. The first one encompassed species with the “varicorhini” type of haptoral ventral bar with a putative origin linked to the historical dispersion of cyprinids via the North African coastline. The second clade included the majority of the investigated species parasitizing various phylogenetically divergent cyprinid hosts. The morphological and molecular data suggest the ancestral diversification of the species of this clade into two groups: (1) the group possessing the haptoral ventral bar of the “cornu” type, and (2) the group possessing the “wunderi” type. Dactylogyrus diversification apparently occurred in the Middle East, which is indicated by the presence of species with all morphotypes in the region. The last two clades included species parasitizing cyprinids with an East Asian origin, and species possessing the “magnihamatus” type of ventral bar.

Conclusions: The molecular data suggest that some morphological characters of host-specific parasites may undergo convergent evolution in the divergent lineages, and therefore, to fully resolve the phylogenetic relationships.
among host-specific parasites, an integrative approach combining morphological and molecular data is still needed. In addition, our study indicates that parasite diversity in many regions is still under-explored, and thus we highlight the importance of studies of host-associated parasites, especially in the context of freshwater fish biogeography.

**Keywords:** Parasites, Platyhelminthes, Phylogeography, Historical dispersion, Cyprinoidei

**Background**

The geographical dispersion of host-specific parasites is strongly limited by the dispersal capabilities of their hosts. One of the most important events in the evolutionary history of parasites is cospeciation with their hosts, leading to the emergence of new species of parasites during the speciation of their hosts, which often occurs in allopatry [1]. However, such allopatric speciation of parasites may occur even prior to the divergence of their hosts, i.e., during the migration or translocation of hosts into new regions. Moreover, considering the faster life cycle of parasites compared to that of their hosts, parasites often tend to undergo sympatric speciation within a population of their respective hosts (usually referred to as intra-host duplication) [2–6].

One of the parasitic groups exhibiting a high degree of host specificity is ectoparasitic monogeneans parasitizing on fish. To attach themselves to gill apparatus, these parasites use a highly developed organ called the haptor. Monogeneans of the genus *Dactylogyrus* Diesing, 1850 are small-bodied common parasites of cyprinoid fishes (suborder Cyprinoidei, following the recent classification by [7]) [8–10]. *Dactylogyrus* is the most speciose genus among Platyhelminthes (more than 900 species, according to the last checklist compiled by [9]), which is most likely because of three major factors: the short developmental time of these parasites, the remarkably high host specificity in *Dactylogyrus*, and the numerosness of their common hosts. The haptor of *Dactylogyrus* parasites comprises one pair of anchor hooks, usually one dorsal and one ventral connective bar of various shapes, and seven pairs of marginal hooks (see [11] for morphotypes of haptoral sclerotized elements). Haptor morphometrics and the presence of specific sclerites apparently play an important role in the host specificity of *Dactylogyrus*, as the parasites of this genus require a high level of adaptation for the specific microhabitats provided by hosts ([12–14], reviewed in [10]). Some generalist species (e.g., *D. sphyrna, D. vastator*, and *D. vistulae*) have not developed a haptoral ventral connective bar. As this morphological feature is present in *Dactylogyrus* species of different phylogenetic lineages, it was hypothesized by Benovics et al. [15] that the secondary loss of the ventral connective bar may allow the parasite to infect a wider range of hosts. Concurrently, Šimková et al. [16] hypothesized that attachment organs with large-sized anchoral sclerites promote the colonization of several phylogenetically distant host species.

Following recent classification, Cyprinidae sensu stricto encompasses more than 1300 species [17] belonging to eleven morphologically diverse subfamilies (formerly tribes, as defined by [18]) with unequal distributions across Africa and Eurasia. Yang et al. [18] proposed five dispersion events to explain the present biogeographical distribution of these cyprinid lineages. At present, two subfamilies are recognized as native to the Afro- and Euro-Mediterranean: hexaploid, large sized Torinae (genera *Carasobarbus, Labeobarbus*, and *Pterocapseteta*) and highly diversified Barbinae (*Aulopyge, Barbus*, and *Lociobarbus*). Interestingly, while the phylogenetic relationships between *Barbus* and *Lociobarbus* are fully resolved and both genera are distinguishable on the basis of several autapomorphies (e.g., the number of pharyngeal teeth), *Lociobarbus* does not form a monophyletic group [18–22]. According to molecular phylogeny, the position of Middle Eastern *Capoeta* is nested within the lineage encompassing *Lociobarbus* species. Even though the diversity of cyprinids in the Middle East is not well documented (molecular data are especially scarce [23–25]), it appears that *Capoeta* represents the most speciose endemic genus [17]. Cyprinid fauna in the Middle East (Levant) is highly diversified in comparison to that in the Afro-Mediterranean and Euro-Mediterranean. The speciation of freshwater fauna was historically centered in the Mesopotamian Basin, where, before the Pliocene orogenesis, the Proto-Euphrates River maintained connection between the Black and Caspian Seas and allowed the mixing of African and Asian fish species [26, 27]. Therefore, the Middle East is considered to be a major biogeographical crossroad between biota of three continents, and the local fauna include cyprinoid genera also present in eastern Asia (e.g., *Garra* (Labeoninae) or *Schizothorax* (Schizothoracinae)) and Africa (i.e., *Luciobarbus* and *Carasobarbus*) [18, 28].

According to recent studies by Šimková et al. [29] and Benovics et al. [15, 30], endemic cyprinids of the peri-Mediterranean are parasitized by *Dactylogyrus* species belonging to at least three divergent phylogenetic lineages. The individual lineages are of uncertain origin and all aforementioned studies hypothesized their ancestry, which is most likely interconnected with the
phylogeography and historical dispersion of their cyprinid hosts. Reconstructions of Dactylogyrus phylogenies are commonly based on multiple molecular markers; such as partial genes coding ribosomal subunits (18S, 28S, 5.8S), and the internal transcribed spacer 1 (ITS1) [15, 16, 29–35]. The ribosomal subunits have been used to resolve phylogenetic relationships between divergent platyhelminth taxa for quite a long time (e.g. [36–39]) and represent slowly evolving parts of the genome under strong selection pressure. On the other hand, spliceosomal introns, such as ITS1, are generally composed of quasi-random sequences and due to their non-functional nature mutate much more rapidly over time (reviewed in [40]). The mechanisms of intron evolution are generally unknown; however, these regions are often used in the taxonomy of monogeneans, especially to investigate inter- and intraspecific variability (see [8, 30] for Dactylogyrus studies).

Since the majority of previous studies on Dactylogyrus phylogeny applied conservative markers for phylogenetic reconstruction (mainly due to aligning issues), it is tempting to assume that the inclusion of such variable non-coding segments may shed more light on the historical origin of Dactylogyrus parasites of cyprinids. Following the suggestion of Šimková et al. [29] that the Middle Eastern region may be considered a center of Dactylogyrus divergence predating the Messinian salinity crisis, the investigation of Middle Eastern endemic taxa may fill the gaps and resolve uncertain relationships between recent Dactylogyrus lineages. Unfortunately, prior to this study, the species diversity of Middle Eastern Dactylogyrus was largely under-explored and molecular data were missing. Thus, in the present study, we focused primarily on this region and, using an integrative approach combining molecular and morphological data, we investigated the phylogeography of Dactylogyrus parasites of peri-Mediterranean cyprinids. Accordingly, we discuss the potential scenarios of the dispersal of Dactylogyrus into Africa and Europe.

**Methods**

**Collection and selection of Dactylogyrus species**

For the purposes of this study, DNA sequences of cyprinid-specific Dactylogyrus species from Africa, Europe, and Eastern Asia were selected. The majority of sequences of partial genes coding small and large ribosomal subunits and the ITS1 segment were retrieved from GenBank (hereinafter abbreviated as 18S, 5.8S, 28S, and ITS1). In addition, new DNA sequences of the aforementioned genetic markers were obtained from Dactylogyrus species collected during the years 2018 and 2019 in Iran and Iraq, respectively (see Table 1 for accession numbers, host species, and countries of collection).

In the field, Dactylogyrus specimens were removed from the gills of fish hosts during standard parasitological dissection (according to [41]). The majority of specimens were mounted on a slide, covered with a coverslip, and fixed in a mixture of glycerine and ammonium picrate (GAP [42]) in order to expose taxonomically important morphological characters. At least five specimens of each newly collected Dactylogyrus species were selected and cut into two parts using fine needles, one half (usually the one with the copulatory organs) mounted on a slide and fixed for further morphological evaluation, the other half (usually the one with the haptor) fixed in 96% pure ethanol for the subsequent isolation of DNA. The sclerotized parts of the haptor (i.e., haptoral sclerites) and the reproductive organs (male copulatory organ and vaginal armament) were used for species determination, following Pugachev et al. [11]. Identification at species level was performed using an Olympus BX51 microscope (Olympus, Tokyo, Japan) equipped with phase-contrast optics.

**DNA extraction, amplification, and sequencing**

Bisected Dactylogyrus preserved in ethanol were dried using a vacuum centrifuge. The extraction of whole genomic DNA was performed using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the protocol provided by the manufacturer. Up to four genetic markers were used for Dactylogyrus. The partial 18S, entire ITS1, and partial 5.8S regions were amplified using the primers S1 (forward, 5′-TCCCTGACGAACTGACACT-3′) and Lig5.8R (reverse, 5′-GAGATCCTCGGACGTCGA-3′), which anneal to the segments of DNA coding 18S and 5.8S, respectively [43, 44]. Amplification reactions followed protocols optimized in Benovics et al. [15]. The partial 28S region was amplified using the forward primer C1 (5′-ACCGCTGAGTACATCTA-3′) and reverse primer D2 (5′-GGTTCCGTGTTTCAGAC-3′) [45], following the PCR protocol optimized by Šimková et al. [34]. The PCR products (~1000 bp for 18S, ITS1, and 5.8S, and ~800 bp for partial 28S) were checked on 1% agarose gel and purified using the ExoSAP-IT kit (Amplia, Bratislava, Slovakia) following the standard protocol. The purified products were directly sequenced using the same primers as for PCR and BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, USA). Sequencing was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA).

**Phylogenetic analyses and the mapping of the characters**

A DNA sequence alignment including 49 sequences, i.e., 48 Dactylogyrus species and one outgroup taxon Ancistrocephalus percae, was constructed by concatenating
Table 1  List of investigated *Dactylogyrus* species in this study with countries and hosts of collection

| Dactylogyrus species | HS | Ventral bar type | Region | Host | Subfamily | 18S+ITS1 | 28S |
|----------------------|----|-----------------|--------|------|-----------|---------|------|
| *D. achmowi*         | 4  | Sphynma         | Eastern Asia | Cyprinus carpio | Cyprininae | MF683071 | MF979966 |
| *D. affinis*         | 3  | Cornu           | Middle East | Barbus lacerta  | Barbiniae  | MZ031066* | MZ031054* |
| *D. anchoratus*       | 4  | Sphynma         | Eastern Asia | Carassius gibelo | Cyprininae | KY859795 | KY863555 |
| *D. andalousiensis*   | 2  | Rutili          | Iberia    | Luciobarbus comizo | Barbiniae | MN365672 | MN338207 |
| *D. atlasensis*       | 1  | Cornu           | Morocco   | Luciobarbus lepineyi | Barbiniae | KY629337 | KY629356 |
| *D. balistae*         | 3  | Sphynma         | Iberia    | Luciobarbus bocagei | Barbiniae | KY629344 | MN338205 |
| *D. balkanicus*       | 2  | Wunderi         | Europe    | Barbus prespensis | Barbiniae | KY201093 | KY201107 |
| *D. barbuli*          | 4  | Cornu           | Middle East | Luciobarbus xanthopterus | Barbiniae | MZ031074* | MZ031063* |
| *D. benhoussai*       | 1  | Cornu           | Morocco   | Luciobarbus yahyaoui | Barbiniae | MN974254 | MN973815 |
| *D. bocageii*         | 3  | Varicorhini     | Iberia    | Luciobarbus graellsii | Barbiniae | MN365675 | MN338210 |
| *D. benjesseni*       | 1  | Cornu           | Morocco   | Luciobarbus zayanensis | Barbiniae | MN974257 | MN973819 |
| *D. barborbarbi*      | 4  | Varicorhini     | Middle East | Carasobarbus luteus | Toriniae  | MZ031071* | MZ031060* |
| *D. carpathicus*      | 2  | Cornu           | Europe    | Barbus barbus | Barbiniae | KY201098 | KY201111 |
| *D. civellus*         | 2  | Cornu           | Europe    | Barbus prespensis | Barbiniae | KY201094 | KY201108 |
| *D. doadori*          | 2  | Varicorhini     | Iberia    | Luciobarbus gueroensis | Barbiniae | MN365682 | KY629346 |
| *D. draenensis*       | 1  | Cornu           | Morocco   | Luciobarbus lepineyi | Barbiniae | MN974258 | MN973816 |
| *D. dyki*             | 2  | Wunderi         | Europe    | Barbus barbus | Barbiniae | KY629338 | KY629367 |
| *D. falciformis*      | 4  | Sphynma         | Eastern Asia | Cyprinus carpio | Cyprininae | MZ031072* | MZ031061* |
| *D. falsiphalus*      | 1  | Cornu           | Morocco   | Luciobarbus maghrebensis | Barbiniae | MN974253 | MN973813 |
| *D. fimbriphalus*     | 2  | Cornu           | Morocco   | Luciobarbus lepineyi | Barbiniae | KY629332 | KY629357 |
| *D. formosus*         | 2  | Sphynma         | Eastern Asia | Carassius gibelo | Cyprininae | MG792869 | MG792984 |
| *D. golschaicus*      | 3  | Rutili          | Middle East | Barbus lacerta | Barbiniae | MZ031067* | MZ031055* |
| *D. gracilis*         | 3  | Rutili          | Middle East | Capoeta buhsei | Barbiniae | MZ031068* | MZ031056* |
| *D. guadianensis*     | 2  | Varicorhini     | Iberia    | Luciobarbus comizo | Barbiniae | MN365674 | MN338209 |
| *D. inexpectatus*     | 2  | Sphynma         | Eastern Asia | Carassius gibelo | Cyprininae | AJ564138 | AJ969945 |
| *D. kisbi*            | 2  | Cornu           | Morocco   | Luciobarbus kisbi | Barbiniae | MN974252 | MN973812 |
| *D. kulindrii*        | 2  | Varicorhini     | Morocco   | Carasobarbus fritschii | Toriniae  | KY629336 | KY629354 |
| *D. kulwici*          | 4  | Cornu           | Middle East | Luciobarbus xanthopterus | Barbiniae | MZ031075* | MZ031064* |
| *D. legionensis*      | 2  | Sphynma         | Iberia    | Luciobarbus graellsii | Barbiniae | MN365678 | MN338213 |
| *D. lenkorani*        | 4  | Varicorhini     | Middle East | Capoeta buhsei | Barbiniae | MZ031069* | MZ031057* |
| *D. lenkoranoides*    | 3  | Varicorhini     | Iberia    | Luciobarbus graellsii | Barbiniae | MN365676 | MN338211 |
| *D. linstowi*         | 4  | Wunderi         | Middle East | Luciobarbus capito | Barbiniae | MZ031073* | MZ031062* |
| *D. linstowoides*     | 2  | Wunderi         | Iberia    | Luciobarbus gueroensis | Barbiniae | KY629329 | KY629349 |
| *D. maileus*          | 2  | Wunderi         | Europe    | Barbus barbus | Barbiniae | KY201099 | KY201112 |
| *D. marocanus*        | 4  | Magniharumatus  | Morocco   | Carasobarbus fritschii | Toriniae  | KY629333 | KY629355 |
| *D. mascomai*         | 3  | Varicorhini     | Iberia    | Luciobarbus graellsii | Barbiniae | MN365680 | MN338215 |
| *D. omenti*           | 3  | Cornu           | Europe    | Aulopyge huegelli | Barbiniae | KY201091 | KY201105 |
| *D. petenyi*          | 2  | Wunderi         | Europe    | Barbus balanicus | Barbiniae | KY201097 | KY201113 |
| *D. prespensis*       | 1  | Wunderi         | Europe    | Barbus prespensis | Barbiniae | KY201096 | KY201110 |
| *D. pulcher*          | 4  | Magniharumatus  | Middle East | Capoeta capoeta | Barbiniae | MZ031070* | MZ031058* |
| *D. remi*             | 1  | Cornu           | Europe    | Luciobarbus graecus | Barbiniae | KY201101 | KY201115 |
| *D. romuli*           | 1  | Cornu           | Europe    | Luciobarbus albanicus | Barbiniae | KY201100 | KY201114 |
| *D. scorpis*          | 1  | Cornu           | Morocco   | Luciobarbus rifensis | Barbiniae | MN974256 | MN973818 |
| *D. vanus*            | 1  | Cornu           | Morocco   | Luciobarbus maghrebensis | Barbiniae | MN974255 | MN973814 |
| *D. vastator*         | 4  | Sphynma         | Eastern Asia | Carassius gibelo | Cyprininae | KY207446 | MZ031059* |
| *D. volutus*          | 1  | Vancorhini      | Morocco   | Carasobarbus fritschii | Toriniae  | KY629334 | KY629353 |
| *D. zatensis*         | 1  | Vancorhini      | Morocco   | Carasobarbus fritschii | Toriniae  | KY629335 | KY629352 |

HS = level of host specificity: (1) strict specialists, (2) intermediate specialists, (3) transitional generalists, (4) common generalists; 18S rDNA plus ITS and 28S rDNA = GenBank accession numbers of sequences of respective genetic loci. Newly generated sequences in this study are marked by asterisks (*)
partial genes for 18S and 28S rRNA, and the ITS1 region. Homolog sequences were aligned using the Fast Fourier transform algorithm in MAFFT [46] and ends were manually trimmed to unify their length. The data were treated as partitioned, and GTR (the general time-reversible evolutionary model) was applied for each partition. The shape parameter of the gamma distribution (G) and the proportion of invariable sites (I) were selected for each gene segment individually using PartitionFinder v.2 [47, 48]. Phylogenetic analyses using maximum likelihood (ML) were computed employing RaxML v.8.1.12 [49, 50]. The best ML tree was selected from 1000 iterations, and support for the branching pattern was validated through $5 \times 10^3$ pseudoreplicates. Phylogenetic analyses of Bayesian inference (BI) were carried out in MrBayes v.3.2 [51], and the resulting tree was constructed using the Metropolis-coupled Markov chain Monte Carlo algorithm. Four concurrent chains (one cold and three heated) ran for $5 \times 10^7$ generations, sampling trees every $10^5$ generations. The first 30% of trees were discarded as a relative burn-in period after checking that the standard deviation split frequency fell below 0.01. Results were checked in Tracer v.1.7.1 [52] to assess convergence. Posterior probabilities were calculated as the frequency of samples recovering particular clades.

The mapping of morphological characters onto the phylogenetic tree containing all investigated parasite species (resulting from the first phylogenetic analysis) was performed in Mesquite v.3.2 [53]. The character mapped onto the phylogenetic tree was the haptoral ventral connective bar, representing the most variable morphological character in Dactylogyrus, ranging from well-developed with five extremities up to completely absent (see [11, 16] for morphotypes).

Levels of host specificity in Dactylogyrus of Cyprinidae

Considering the delimitation of host specificity for Dactylogyrus by Šimková et al. [16] and taking into account the present classification of cyprinoids [7], the modified version of levels of host specificity is presented in Table 1. The Dactylogyrus species were divided into four categories: (1) strict specialists parasitizing single cyprinid species, (2) intermediate specialists parasitizing congeneric host species, (3) transitional generalists parasitizing cyprinid species belonging to a single subfamily, and (4) common generalists parasitizing species belonging to a single cyprinid family, i.e., in this case, to Cyprinidae. The level of host specificity for each investigated Dactylogyrus species was determined from the compilation of data from various sources, i.e., checklists ([9, 54–57] and references within), the determination key including original descriptions and/or host reports included in Pugachev et al. [11], and other studies reporting the presence of the investigated species [8, 15, 30–32, 35, 58–69]. All published reports were thoroughly evaluated, and potential misidentifications were not included. Publicly unavailable and/or non-traceable reports (e.g., master's or Ph.D. theses) were not taken into account.

Results

Host specificity in Dactylogyrus of Cyprinidae

The list of investigated Dactylogyrus species with their levels of host specificity is presented in Table 1. From 48 Dactylogyrus species, 12 species belonged to strict specialists, specific to a single host species. The majority of species with this narrow host specificity were from Northwest Africa. Sixteen species were intermediate specialists, i.e., parasitizing only cyprinid host species belonging to a single genus. Nine species were classified as transitional generalists parasitizing hosts from at least two different genera belonging to a single cyprinid subfamily (i.e., Barbinae, Cyprininae, or Torinae). Finally, eleven species were recognized as common generalists, i.e., species parasitizing multiple host species belonging to different subfamilies (within Cyprinidae).

Phylogenetic position of Middle Eastern Dactylogyrus species

The final alignment for phylogenetic reconstruction spanned 2075 nucleotide positions (445 positions for the partial gene coding 18S rRNA, 699 positions for the partial gene coding 28S rRNA, and 931 bp positions for the partial ITS1 region). The ML and BI analyses generated trees with congruent topologies. Therefore, only the BI tree is presented (Fig. 1), with values along nodes indicating posterior probabilities resulting from BI, and bootstrap support from ML analyses. The investigated Dactylogyrus species formed four major well-supported clades. The first clade (a) encompassed two Middle Eastern species (i.e., D. lenkorani and D. carassobarbus), three Dactylogyrus species host specific to North African Carassobarbus (i.e., D. kulindrii, D. volutus, and D. zatensis), and five species host specific to endemic Luciobarbus spp. of the Iberian Peninsula. The second clade (b) encompassed all Dactylogyrus species parasitizing European Barbus spp. and the Balkan Luciobarbus and Aulopyge, four species parasitizing endemic Iberian Luciobarbus spp., the majority of Dactylogyrus species parasitizing North African cyprinids, and eight Middle Eastern Dactylogyrus species. Six well- or moderately supported monophyletic groups were revealed within clade b, even though not all phylogenetic relationships between these groups were fully resolved by either BI or ML analysis. The phylogenetic analyses strongly support the phylogenetic proximity of Middle Eastern D. goktschaicus and D. linstowi (group b3); however, their positions within
clade b and their relationships to other groups were not resolved. The other two well-supported groups within clade b include three species of Iberian Luciobarbus spp. (i.e., *D. balistae*, *D. legionensis*, and *D. linstowioides*; group b1), and three species of Central European *Barbus* spp. (i.e., *D. petenyi*, *D. prespensis*, and *D. malleus*; group b2), respectively. Similarly to group b3 including two Middle Eastern species, the phylogenetic position of these two lineages within clade b was also not resolved. However, the basal position of *D. omenti* of the endemic Balkan *A. huegelii* to these three lineages (i.e., b1–3) was well/ moderately supported. Another well-supported group encompasses four European *Dactylogyrus* species parasitizing Balkan Luciobarbus spp. (*D. romuli* and *D. remi*) and *Barbus* spp. (*D. carpathicus* and *D. crivellius*), and *D. kulwieci* parasitizing Middle Eastern *Luciobarbus xanthopterus* (group b4). Also, the monophyly of *Dactylogyrus* species of North African cyprinids (blue species in group b4) was well supported by both analyses. Four Middle Eastern species (i.e., *D. affinis*, *D. gracilis*, *D. barbuli*, and *Dactylogyrus sp.*), and *D. andalousiensis* (a species endemic to the Iberian Peninsula) were revealed as phylogenetically close to the North African lineage. The last well-supported group was formed by two common
Dactylogyrus spp. of Barbus spp.—specifically, *D. balkanicus* and *D. dyki* (group b6). Even though the monophyly of these two species was strongly supported by both phylogenetic analyses, neither analysis resolved their position within clade b. Clade c included all investigated *Dactylogyrus* species specific to *Cyprinus carpio* and *Carassius gibelio* (putative of an East Asian origin). The last well-supported clade (d) included only two species—*D. marocanus* of North African *Carasobarbus fritschii*, and *D. pulcher* of Middle Eastern *Capoeta* spp.

**Diversity of haptoral elements in *Dactylogyrus* of Cyprinidae**

Out of the 17 basic morphological types of haptoral ventral bar (taken from [11]), five were present in the *Dactylogyrus* species of peri-Mediterranean cyprinids: the “cornu” double-cross-shaped type with five projections; the “rutili” cross-shaped with four projections; the “wunderi” three-armed triangular type; the “varicorhini” almost linear shape with V-shaped tubercle in the central part; and the small “magnihamatus” linear type (Fig. 2). A mapping of the morphological types of haptoral ventral connective bars into *Dactylogyrus* phylogeny is presented in Fig. 3. Six *Dactylogyrus* species parasitizing *C. carpio* and *C. gibelio*, and two *Dactylogyrus* species endemic to Iberian Peninsula (i.e., *D. balistae* and *D. legionensis*) had no ventral bar (clades c and d in Fig. 1). The most common type of ventral bar among the investigated species was the “cornu” type possessed by all *Dactylogyrus* species parasitizing Northwest African and Balkan *Luciobarbus* spp., two intermediate specialist species parasitizing European *Barbus* spp., *D. omenti*, and four Middle Eastern *Dactylogyrus* species (i.e. all species of subclade b4 plus *D. omenti* and the majority of species of subclade b5). The “varicorhini” type of ventral bar was possessed by 10 *Dactylogyrus* species, all belonging to clade A. The “wunderi” type of ventral bar was the most prevalent among the *Dactylogyrus* of European *Barbus* spp. (possessed by 2 species of clade b6 and 3 species of clade b2). This type of ventral bar is also present in Middle Eastern *D. linstowi* and Iberian *D. linstowoides*. The “rutili” type was only present in *D. gracilis*, *D. andaloensis*, and *D. goktschaicus*, even though in the last species this bar visibly differed in the width and shape of its projections compared to the former two species. The strongly miniaturized ventral bar of the “magnihamatus” type, in the shape of a thin line, was possessed by *D. marocanus* and *D. pulcher*—within our study, the only representatives of clade d.

**Discussion**

The present study was focused on the host specificity, phylogenetic relationships, and morphological diversity of *Dactylogyrus* parasites of Cyprinidae in three major regions in the peri-Mediterranean—North Africa, Southern Europe, and the Middle East. The Mediterranean is characterized by high diversity, especially that of endemic cyprinoid species, and encompasses several highly diversified, phylogenetic lineages of cyprinoids, each with a putatively different historical origin [18, 22, 70–74]. The phylogenetic relationships between *Dactylogyrus* parasites of North African and European cyprinoids were previously investigated over a relatively wide geographical scale by Šimková et al. [29] and Benovics et al. [15]. Even though both studies concluded that *Dactylogyrus* parasites, like their cyprinid hosts, are of multiple historical origins, there are still some uncertain relationships between present lineages of *Dactylogyrus*, and the inclusion of Middle Eastern species into phylogenetic reconstruction may shed more light into these uncertainties. Unfortunately, prior to the present study, publicly available molecular data on Middle Eastern *Dactylogyrus* species were almost nonexistent.

Compiling the data from 48 peri-Mediterranean *Dactylogyrus* species revealed that 25% of them are strict specialists, associated with only a single cyprinid host...
species. Nine of 12 strictly specific species are parasites of Northwest African Barbinae and Torinae (i.e., \textit{Luciobarbus} spp. and \textit{Carasobarbus fritschii}, respectively). Of the other three strictly specific species, two are associated with one of two Balkan \textit{Luciobarbus} spp., and \textit{D. prespensis} is specific to \textit{Barbus prespensis}. Both Balkan \textit{Luciobarbus} spp. and \textit{B. prespensis} are endemic to the Balkan Peninsula [75] and exhibit a limited distribution range. Therefore, we can assume that their respective host-specific parasite species cospeciated with their hosts.
after diversification in the respective regions. To date, only three works have been published investigating the diversity of endemic Dactylogyrus in the Afro-Mediterranean, these describing new Dactylogyrus species from North African cyprinids [65, 73, 76]. A total of 17 species are known from the Afro-Mediterranean and apparently more than half are strictly host specific. However, since data from this region are still scarce, as all three works are only from Morocco or Algeria, we can only assume that the diversity of Dactylogyrus is much higher than so far reported in the Afro-Mediterranean. Moreover, this may also imply that endemic hosts are under-explored and, therefore, that the host range of some endemic Dactylogyrus species may be wider than is actually documented, similarly as for D. omenti. This species was recently considered as a specialist of A. huegelii in the Balkans [31]; however, Koyee and Abdullah [62] reported D. omenti also from Middle Eastern L. xanthopterus. The validity of D. omenti was in their study also supported by the molecular data. In the present study, 33% of investigated species were reported as intermediate specialists, i.e., parasitizing congeneric host species. These species appear to be the most common in Europe, where the distribution ranges of congeneric cyprinids from two highly divergent genera (i.e., Barbus and Luciobarbus) often overlap. Therefore, Dactylogyrus parasites, due to their biological preconditions for host-switching (e.g., a free-living larval stage, or a sympatric distribution of phylogenetically related host species, further discussed in [15, 32]) are more likely to be present on two phylogenetically closely related hosts. The fraction of intermediate (congeneric) specialists in the peri-Mediterranean is higher in comparison to literature data compiled by Kuchta et al. [77], who reported that in Dactylogyrus parasitizing European cyprinoids strict specificity is less frequent than intermediate specificity. However, it is important to note that Leuciscidae (Dactylogyrus species diversity in all cyprinoids, including Leuciscidae, was analyzed by Kuchta et al. [77]) represent a far more diversified cyprinoid family in comparison to Cyprinidae [7, 75]. Highly diversified leuciscid taxa, whose distribution in the peri-Mediterranean is usually limited to a single river or lake system [75], more likely harbour endemic host-specific Dactylogyrus species [8, 30, 31, 78–80]. In our study, Dactylogyrus species parasitizing hosts belonging to phylogenetically close genera (i.e., genera from a single subfamily) represent 19% of Dactylogyrus species in the peri-Mediterranean. These species are especially common in the Middle East, which is the most likely related to the distribution of the divergent cyprinid genera (Barbus, Luciobarbus, Capoeta, and Carasobarbus) in this region. Since only three cyprinid genera besides Cyprinus and Carassius are natively present in Europe (monotypic Aulopyge, highly diversified Barbus, and Luciobarbus—all three belonging to Barbine)—all three belonging to Barbine) and their areas only rarely overlap, the possibilities for host-switching between species of different genera are limited and, therefore, rather rare. A notable exception is the northern part of the Iberian Peninsula belonging to the distribution range of southwest European endemic Barbus meridionalis, which often hybridizes with endemic Iberian B. haasi [81]. These Barbus species live on the Peninsula in sympathy with endemic Luciobarbus spp. [75, 82, 83], which putatively facilitates the host-switching of parasites between non-congeneric. Hybridization between Iberian Luciobarbus and Barbus (suggested by [83]) may be another factor further promoting the intergeneric host-switching of parasites. Thus, in Iberia, Dactylogyrus species common to Central European Barbus spp. are almost absent, and Dactylogyrus fauna of endemic B. haasi was completely replaced by endemic Dactylogyrus species which are typical for Luciobarbus spp. in the region (e.g., D. balistae, D. bocageii, or D. mascomai) [30, 84]. However, this one-way drift of parasite fauna has not yet been explained. The situation is different in the Middle East, where, in addition to Barbus and Luciobarbus, the presence of endemic genera is documented, i.e., Capoeta (phylogenetically closely related to Euro-Mediterranean Luciobarbus [20, 22, 85]), and Cyprinion, Scaphidionichthys and Semiplotosus (genera in the basal position to all other peri-Mediterranean Barbineae [18]). Therefore, the intergeneric host-switching of parasites in this region, where overlapping habitats of non-congeneric cyprinids are more common [23, 25, 86], is also reflected in the observed host specificity of endemic Dactylogyrus parasites.

Endemic generalist Dactylogyrus species were almost exclusively present on cyprinids in the Middle East (the exception was North African D. marocanus)—a region where the distribution ranges of native Barbineae and Torineae overlap. However, a slightly different trend is observable in multiple European regions—species, presumably specialists on representatives of Cyprininae (e.g. Cyprinus or Carassius), have been introduced together with their hosts into non-native regions in the Euro-Mediterranean, and, according to recent reports, have spread to endemic non-congeneric cyprinid hosts (such as D. achmerowi and D. falciformis on B. plebejus in the Apennine Peninsula [60], or D. vastator on A. huegelii in the Balkans [31], and B. plebejus in the Apennine Peninsula [60]), resulting in an increased host range.

The host range of an undetermined Dactylogyrus sp. collected from Iraq L. xanthopterus remains unknown, as this species is potentially new to science. Comparing only morphological data, Raisy and Ansari [66] reported specimens of this species from B. barbulus in Iran, which was in their study identified as D. akaraicus. Nonetheless,
the species reported by the authors does not morphologically match the original description by Mikailov [87], and therefore we concluded that the species in their study was misidentified. Despite this, we can still assume that the host range of *Dactylogyrus* sp. encompasses species of at least two genera (belonging to Barbinæ), and thus this *Dactylogyrus* species was categorized as an intermediate generalist.

In previous studies, Šimková et al. [29] and Benovics et al. [15] discussed the phylogenetic origin of the host-specific monogeneans of cyprinids. Both studies concluded that *Dactylogyrus* species parasitizing peri-Mediterranean cyprinids form three divergent phylogenetic lineages. Moreover, they also suggested congruently that the addition of Middle Eastern congeners into phylogenetic analyses of *Dactylogyrus* may shed more light into the phylogenetic relationships of these host-specific parasites, because the Middle East represents a putative region of the ancestral diversification of cyprinoids prior to their dispersion into Europe and Africa [18, 72, 88–92]. Following previous suggestions, herein we present the first phylogenetic reconstruction of *Dactylogyrus* including also molecular data on *Dactylogyrus* species parasitizing Middle Eastern cyprinids. Three previously revealed phylogenetic lineages are congruent with clades a, b, and c in our present study. In addition, we also propose a fourth lineage formed by *D. marocanus* and *D. pulcher*, in sister position to the clade of *Dactylogyrus of Cyprinus* and *Carassius*. Šimková et al. [29] suggested that this lineage should also include *Dactylogyrus* species of West African cyprinids (e.g., *Dactylogyrus* species parasitizing *Labeo* hosts). Unfortunately, multiloci molecular data for these species, described from labeonins in Senegal [93–95], are not yet publicly available. Since for the phylogenetic reconstruction in the present study we combined slowly evolving conservative regions (ribosomal subunits) with the rapidly evolving non-coding region (ITS1) in order to add more information to resolve the phylogenetic relationships between lineages within major clades, African *Dactylogyrus* species were omitted from the final dataset; therefore, we can only assume such phylogenetic relatedness from the previous work of Šimková et al. [29].

The monophyly of *D. pulcher* and *D. marocanus* is also supported by morphology; both species possess a haptoral ventral bar of the “magnihamatatus” type and anchor hooks with elongated inner roots (see [11] for morphology). The same morphological features are also shared by the *Dactylogyrus* species parasitizing West African *Labeo* (see [95, 96]), further supporting the phylogenetic proximity of *D. pulcher* and *D. marocanus* to congeners of widely distributed African cyprinids of *Labeo*. In addition, the similarity in haptoral features, especially the absence of a ventral connective bar, is also recognized in the *Dactylogyrus* of cyprinins of presumed West-Asian origin (e.g. *D. inexpectatus*, *D. anchoratus*, or *D. formosus* parasitizing *C. carpio* and/or *C. gibelio*). Therefore, considering the molecular data and the morphology of haptoral elements, which according to Šimková et al. [16], Vignon et al. [97], and Benovics et al. [15] are the optimal characters for resolving the phylogeny of monogenean parasites (especially *Dactylogyrus*), we can assume that species from clades c and d, and *Dactylogyrus* of African labeonins share a common ancestry with their hosts in eastern Asia. From all the above, we hypothesize that species of clade d (together with *Dactylogyrus* of African Labeonins) are descendants of the first colonization wave of *Dactylogyrus* into Africa occurring via the Gomphotherium land bridge around 19 MYA [90, 98–100]. This hypothesis is also supported by the discovery of the oldest *Labeo*-like fossils in Africa, which were found to be approximately 17 million years old [101].

According to our phylogenetic reconstruction, all investigated *Dactylogyrus* of Middle Eastern cyprinids belong to three phylogenetic clades (a, b, and d). The monophyly of the species within clade a is supported by both molecular data and morphology. The three North-west African species of *C. fritschii* and five Iberian species clustered with Middle Eastern *D. lenkorani* and *D. carassobarbi*—all species possessing the “varicorhini” type of haptoral connective ventral bar. The majority of the species within clade a do not exhibit a high level of host specificity. Even though African species parasitize only fish belonging to hexaploid Torines (*Carasobarbus* and *Labeobarbus* [73]) and Iberian species parasitize only fish from tetraploid Barbinæ (*Barbus* and *Luciobarbus* [30, 84]), Middle Eastern *Dactylogyrus* species have been reported from fish of both subfamilies (e.g. [55]). Due to the presence of *Dactylogyrus* parasitizing *Carasobarbus* and *Capoeta* within clade a, we can hypothesize that the historical origin of the species is associated with the historical dispersion of the common ancestor of large hexaploid torins (e.g., *Labeobarbus*, *Carasobarbus*, and *Pterocapoeta*) into Africa (route a, Fig. 4), after their former hexaploidization and divergence from the tetraploid lineage, which occurred in Western Asia during the Miocene [18, 85].

Considering the host range of *Dactylogyrus* species, we can assume that in contrast to the previously mentioned clade a, the historical origin of species belonging to clade b is associated with the dispersion of the Barbinæ into Europe and North Africa (route b, Fig. 4). This clade encompasses the rest of the investigated Middle Eastern *Dactylogyrus* species, the majority of *Dactylogyrus* of Northwest African cyprinids (*Luciobarbus*), and all *Dactylogyrus* species parasitizing European *Barbus* spp.
and *A. huegelii*, and six species of European *Luciobarbus* spp. (including four Iberian species and two Balkan species). While the morphology regarding the haptoral elements mostly supported the phylogenetic position of each individual species within the previous clades (a, c, and d), the species within clade b exhibit rather higher diversity in the attachment apparatus. The monophyly of all African species of clade b was well supported—these species include all specialists parasitizing *Luciobarbus* and possessing a haptoral ventral bar of the “cornu” type (see [65, 73, 76] for morphology). In the basal position to this clade were three *Dactylogyrus* species of Middle Eastern cyprinids (namely, *D. affinis*, *D. barbula*, and *Dactylogyrus* sp.), and *D. andalousiensis* of Iberian *Luciobarbus* spp. Even though the phylogenetic relationships between these basal Middle Eastern species were not fully resolved, their morphological similarities support their phylogenetic proximity to the Northwest African *Dactylogyrus* species of *Luciobarbus* (i.e., all possessing the connective ventral bar of the “cornu” type; for morphology, see the original descriptions by Bychowsky [102] and Gussev et al. [103], respectively, or [11]). The same type of connective ventral bar is recognized in the species from lineage b4, which encompasses *D. remi* and *D. romuli* of endemic Balkan *Luciobarbus*, two species parasitizing European *Barbus*, and *D. kulwieci* parasitizing Middle Eastern cyprinids. In addition to similarities in the haptoral elements, all these five species possess copulatory organs of similar shape, categorized by Řehulková et al. [67] as the type of the “Caspian” group (as opposed to the type of the “Moroccan” group possessed by *Dactylogyrus* of Northwest African and Middle Eastern cyprinids from clade b5). The presence of the “cornu” type of haptoral ventral bar in species of two *Dactylogyrus* phylogenetic lineages indicates historical divergence and two individual dispersion routes of an ancestor possessing this type of ventral bar into the peri-Mediterranean (red route b, Fig. 4). Other *Dactylogyrus* parasitizing European *Barbus* (i.e., lineages b2 and b6) possess a haptoral ventral bar of the “wunderi” type (see [11] for morphology), and therefore we can assume that they are also phylogenetically close, even though the molecular data did not support this. The same type of haptoral bar is also recognized in *D. linstowi* parasitizing Middle Eastern cyprinids. However, even though *D. goktschaicus* is, according to the phylogenetic reconstruction, close to *D. linstowi*, it possesses a haptoral ventral bar more resembling the “rutili” type, which is more common in the *Dactylogyrus* parasitizing leuciscids, and, in our dataset, also possessed by Middle Eastern *D. gracilis* and Iberian *D. andalousiensis* from clade b5 [11]. Nevertheless, there are measurable differences in the sclerotized parts of the haptor of *D. goktschaicus* in comparison to the latter two species from clade b5: (1) the ventral bar is, in general, larger in respect to other haptoral sclerites, and (2) the anterior and posterior projections of the ventral bar are comparatively wider in respect to the ventral bars of the other two species. Therefore, we can postulate two hypotheses. The first is that the haptoral ventral bar of the “rutili” type evolved convergently in *Dactylogyrus* parasitizing cyprinids, as the “rutili” type may represent a form of the ventral bar derived from either the “cornu” type by miniaturization of the posterior and anterior projections, or the “wunderi” type by enlargement of the posterior projection and the bifurcation of the anterior projection. The convergent evolution revealed by similarities in the haptoral elements recognized in some *Dactylogyrus* species of the two lineages of clade b is potentially also supported by Benovics et al. [15], who suggested two different historical dispersion routes for *Dactylogyrus* species in clade b—the southern route for species of clades b4 and b5 associated with the dispersions of North

**Fig. 4** Hypothesized historical dispersion routes of peri-Mediterranean *Dactylogyrus* species. The colors of lines, arrows, and letters correspond to the colors for lineages in Fig. 3.
African Luciobarbus and Balkan cyprinids (red routes b, Fig. 4), and the northern route for species of clades b1, b2, and b6 via Europe with ancestral Barbus hosts (blue route, Fig. 4) (proposed by [72]). The phylogenetic position of D. andalousiensis, which also possesses a ventral bar resembling the “rutili” type, but is endemic to the Iberian Peninsula, also supports the first hypothesis, i.e., the ventral bar evolved convergently in the Iberian Peninsula (illustrated by the orange route in Fig. 4). The second hypothesis is that the “rutili” type is, in fact, the ancestral state of the ventral bar for Dactylogyrus of clade b, and that the two derived types, i.e., “cornu” and “wunderi,” developed after the divergence from common ancestor, and during the historical dispersion of the two divergent Barbinae host lineages [72, 89]. In this case, regarding morphology, D. andalousiensis represents a slowly evolving species.

Conclusion
Fish of the highly diversified taxon Cyprinidae harbor a remarkably species-rich group of host-specific Dactylogyrus parasites. In general, the phylogenetic relationships between present Dactylogyrus lineages are concurrent with the phylogeny of their associated host lineages and are shaped by the historical dispersion of cyprinids in the peri-Mediterranean. However, the distribution of the Dactylogyrus lineages is influenced more by the diversity of divergent host lineages in the respective regions. The association of individual Dactylogyrus species (or lineages) with a particular dispersal event proposed for cyprinids may often be recognized at first sight by the morphological characters of the parasite attachment organ. However, the molecular data suggest that some morphological characters of host-specific parasites may undergo convergent evolution in the divergent lineages; therefore, to fully resolve the phylogenetic relationships among host-specific parasites, an integrative approach combining morphology and molecular data is still needed. Lastly, our study clearly indicates that parasite diversity in some biogeographical regions of fish distribution is still under-explored, and therefore we highlight the importance of studies of host-associated parasites, especially in the context of freshwater fish biogeography.

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References
1. Brooks DR, McLennan DA. Parascript: parasites and the language of evolution. Washington, DC: Smithsonian Institution Press; 1993.
2. Johnson KP, Adams RJ, Page RDM, Clayton DH. When do parasites fail to speciate in response to host speciation? Syst Biol. 2003;52:37–47.
3. Poulin R. Evolutionary Ecology of Parasites. 2nd ed. New Jersey: Prince- ton University Press; 2007.
4. Ronquist F. Phylogenetic approaches in coevolution and biogeography. Zool Scr. 1997;26:312–22.
5. Tan M, Armbruster JW. Phylogenetic classification of extant genera of fishes of the order Cypriniformes (Teleostei: Ostariophysi). Zootaxa. 2018;4476:006–39.
6. Benovics M, Desdevises Y, Vukčić J, Šandor R, Simková A. The phyloge- netic relationships and species richness of host-specific Dactylogyrus parasites shaped by the biogeography of Balkan cyprinids. Sci Rep. 2018;8:13006.
9. Gibson DI, Timofeeva TA, Gerasiev PI. A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol. 1996;35:3–48.
10. Šimková A, Morand S. Co-evolutionary patterns in congeneric monogeneans: a review of Dactylogyrus species and their cyprinid hosts. J Fish Biol. 2008;73:2210–27.
11. Pugachev ON, Gerasiev PV, Ergens R, Khotenowsky I. Guide to Monogenea of freshwater fish of Palearctic and Amur Regions. Milan: Ledizioni-Lenei Publishing, 2009.
12. Jarkovský J, Morand S, Šimková A. Reproductive barriers between congeneric monogenean parasites (Dactylogyrus: Monogenea): attachment apparatus morphology or copulatory organ incompatibility? Parasitol Res. 2004;92:95–105.
13. Šimková A, Desdevises Y, Gelinar M, Morand S. Co-existence of nine gill ectoparasites (Dactylogyrus: Monogenea): parasitising theroach (Rutilus rutilus L.): history and present ecology. Int J Parasitol. 2000;30:1077–88.
14. Turgut E, Shinn A, Wootten R. Spatial distribution of Dactylogyrus (Monogenan) on the gills of the host fish. Turk J Fish Aquatic Sci. 2006;6:63–98.
15. Benovics M, Vukíč J, Sanda R, Rahmouni I, Šimková A. Disentangling the evolutionary history of peri-Mediterranean cyprinids using host-specific gill monogeneans. Int J Parasitol. 2020;50:969–84.
16. Šimková A, Verneau O, Gelinar M, Morand S. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. Evolution. 2006;60:1023–37.
17. Frick E, Eschmeyer WN, Van der Laan R, editors. Eschmeyer’s catalog of Fishes, genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fieldchecklist.aspx. Accessed 15 Mar 2021.
18. Yang L, Sado T, Vincent Hirt M, Pasco-Viel E, Arunachalm M, Li J, Wang rutilus (Monogenan) on the gills of the host fish. Turk J Fish Aquatic Sci. 2006;6:63–98.
19. Benovics M, Vukíč J, Sanda R, Rahmouni I, Šimková A. Disentangling the evolutionary history of peri-Mediterranean cyprinids using host-specific gill monogeneans. Int J Parasitol. 2020;50:969–84.
20. Šimková A, Verneau O, Gelinar M, Morand S. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. Evolution. 2006;60:1023–37.
21. Frick E, Eschmeyer WN, Van der Laan R, editors. Eschmeyer’s catalog of fishes: genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fieldchecklist.aspx. Accessed 15 Mar 2021.
22. Yang L, Sado T, Vincent Hirt M, Pasco-Viel E, Arunachalm M, Li J, Wang rutilus (Monogenan) on the gills of the host fish. Turk J Fish Aquatic Sci. 2006;6:63–98.
23. Benovics M, Vukíč J, Sanda R, Rahmouni I, Šimková A. Disentangling the evolutionary history of peri-Mediterranean cyprinids using host-specific gill monogeneans. Int J Parasitol. 2020;50:969–84.
24. Šimková A, Verneau O, Gelinar M, Morand S. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. Evolution. 2006;60:1023–37.
25. Frick E, Eschmeyer WN, Van der Laan R, editors. Eschmeyer’s catalog of Fishes, genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fieldchecklist.aspx. Accessed 15 Mar 2021.
26. Yang L, Sado T, Vincent Hirt M, Pasco-Viel E, Arunachalm M, Li J, Wang rutilus (Monogenan) on the gills of the host fish. Turk J Fish Aquatic Sci. 2006;6:63–98.
27. Benovics M, Vukíč J, Sanda R, Rahmouni I, Šimková A. Disentangling the evolutionary history of peri-Mediterranean cyprinids using host-specific gill monogeneans. Int J Parasitol. 2020;50:969–84.
28. Šimková A, Verneau O, Gelinar M, Morand S. Specificity and specialization of congeneric monogeneans parasitizing cyprinid fish. Evolution. 2006;60:1023–37.
29. Frick E, Eschmeyer WN, Van der Laan R, editors. Eschmeyer’s catalog of Fishes, genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fieldchecklist.aspx. Accessed 15 Mar 2021.
30. Yang L, Sado T, Vincent Hirt M, Pasco-Viel E, Arunachalm M, Li J, Wang rutilus (Monogenan) on the gills of the host fish. Turk J Fish Aquatic Sci. 2006;6:63–98.
54. Djikanovic V, Paunovic M, Nikolic V, Simonovic P, Cakic P. Parasitofauna of freshwater fishes in the Serbian open waters: a checklist of parasites of freshwater fishes in Serbian open waters. Rev Fish Biol Fisheries. 2011;22:297–324.

55. Mhaisen FT, Abdul-Ameer KN. Checklists of freshwater fishes. Iran J Fish Sci. 2012;11:570–89.

56. Abdullah YS, Abdullah SMA. Some observations on fishes and their parasites of Darbandikhan lake, Kurdistan region in north Iraq. Eur Sci J. 2015;11:409–17.

57. Aydoğdu N, Kubilay A. Helminth fauna of Simav barbell, Barbus nilfe-rissi Turan, Kotetelat & Ekmecki, 2009 An endemic fish from Nižîfer river in Bursa (Turkey): new host and locality records. Bull Eur Assoc Fish Pathol. 2017;37:110.

58. Benovics M, Francová K, Volta P, Dlouhý V, Šimkova A. Helminth communities of endemic cyprinids of the Apennine Peninsula, with remarks on ectoparasitic monogeneans, and description of four new Dactylogyrus species. Parazitologia. 2021;53:1003–18.

59. Benovics M, Francová K, Volta P, Dlouhý V, Šimkova A. Helminth communities of endemic cyprinids of the Apennine Peninsula, with remarks on ectoparasitic monogeneans, and description of four new Dactylogyrus species. Parazitologia. 2021;53:1003–18.

60. Gutiérrez-Galindo JF, Lacasa-Millán MI. Study of the Monogenea of Barbus meridionalis species complex (Cyprinidae) parasitizing cyprinid gills. Pol J Environ Stud. 2019;28:1–13.

61. Rahmouni I, Řehulková E, Pariselle A, Rkhami OB, Šimková A. Four new species of Monogenea (Vermes) from African freshwater fishes. Peer J. 2021;9:e10867.

62. Šimková A, Desdevises Y, Gelnar M, Morand S. Morphometric correlates of host specificity in Dactylogyrus species (Monogenea). Int J Parasitol. 2020;50:771–86.

63. Stojanovski S, Hristovski N, Cakic P, Hristovski M. Fauna of Monogenean Trematoda – parasites of some cyprinid fishes from Lake Ohrid (Macedonia). Nat Monen Podgorica. 2005;6:41–70.

64. Stojanovski S, Hristovski N, Velkovka-Jordanoska L, Blazekovic-Dimovski D, Atanasov G. Parasite fauna of Chub (Squalius squalus Bonaparte, 1837) from Lake Ohrid (Fyrmacedonia). Acta Zool Bulg. 2012;14:119–22.

65. Machordom A, Berrebi P, Doardio I. Spanish barbel hybridization detected using enzymatic markers. Barbus melanodinas Risso X Barbus haasi Mertens (Osteichthyes, Cyprinidae). Aquat Living Res. 1990;3:295–303.

66. Gante HF. Diversification of Circum-Mediterranean Barbels. In: Grillo O, Venora G, editors. Changing biodiversity in changing environment: perspectives from the Mediterranean. Springer; 2013. p. 283–98.

67. Gante HF, Doardio I, Alves MJ, Dowling TE. Semi-permeable species boundaries in Iberian barbels (Barbus and Luciobarbus, Cyprinidae). BMC Evol Biol. 2015;15:111.

68. El-Gharbi S, Renaud F, Lambert A. Dactylogyrids (Platyhelminthes: Monogenea) of Barbus spp. (Teleostei: Cyprinidae) from Iberian Peninsula. Res Rev Parasitol. 1992;52:103–16.

69. Tsigenopoulos CS, Kasapis P, Berrebi P, Phynogenetic relationships of species Dactylogyridae. A preliminary report. Rev Zool Bot Africa. 1973;87:505–18.

70. Perea S, Böhme M, Zupančič P, Freyhof J, Šanda R, Özuluğ M, Abdoli A, Machordom A, Doardio I. Phylogenetic relationships and biogeographical patterns in Circum-Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. BMC Evol Biol. 2010;10:265.

71. Tang QY, Getahun A, Liu HZ. Multiple in–to–Africa dispersals of laboein fishes (Teleostei: Cyprinidae) revealed by molecular phylogenetic analysis. Hydrobiologia. 2009;632:261–71.

72. Yang L, Mayden RL. Phylogenetic relationships, subdivision, and biogeography of the cyprinid tribe Laboeini (sensu Rainboth, 1991) (Teleostei: Cyprinidae), with comments on the implications of lips and associated structures in the laboein classification. Mol Phylogenet Evol. 2010;54:254–65.

73. Guegan JF, Lambert A, Euzet L. Etude des Monogènes des Cyprinidae du genre Labeo en Afrique de l'Ouest — I. Genre Dactylogythus. J Syst Parasitol. 1985;18:252–63.

74. Kottelat M, Freyhof J. Handbook of European freshwater fishes. Conc: Publications Kottelat, 2007.

75. Šimková A, Desdevises Y, Gelnar M, Morand S. Morphometric correlates of host specificity in Dactylogyrus species (Monogenea). Int J Parasitol. 2020;50:771–86.

76. Dacius J, Ratnasinghe M, Berrebi P, Freyhof J, Kottelat M. Morphological and molecular approaches for characterizing four species of Dactylogythus (Monogenea: Dactylogyridae) from Moroccan cyprinids, with comments on their host specificity and phylogenetic relationships. Peer J. 2021;9:e10867.

77. Kuchta R, Řehulková E, Francová K, Scholz T, Morand S, Šimkova A. Diversity of monogeneans and tapeworms in cypriniform fishes across two continents: Int J Parasitol. 2020;50:771–86.

78. Dupont F, Lambert A. Study of the parasitic communities of Monoge-nea Dactylogyridae from Cyprinidae in Lake Mikri Prespa (Northern Greece). Description of three new species from endemic Barbus: Barbus cyclospis prespasensis Karaman, 1924. Ann Parasitol Hum Comp. 1986;35:597–616.

79. Stojanovski S, Hristovski N, Cakic P, Hristovski M. Fauna of Monogenean Trematoda – parasites of some cyprinid fishes from Lake Ohrid (Macedonia). Nat Monen Podgorica. 2005;6:41–70.
spp., with a description of *Dogielius sennarensis* n. sp. and a redescription of *Dogielius flosculus* Guégan, Lambert & Euzet, 1989. Helminthologia. 2018;55:306–21.

97. Vignon M, Pariselle A, Vanhove MPM. Modularity in attachment organs of African Cichlidogyrus (Platyhelminthes: Monogenea: Ancyrocephalidae) reflects phylogeny rather than host specificity or geographic distribution. Biol J Linn Soc. 2011;102:694–706.

98. Böhme M. Eine Weichschildkröte (Trionychidae) aus dem Untermiozän vom Dietrichsberg bei Vacha (Rhön). Mauritania (Altenburg). 1995;15:357–66.

99. Harzhauser M, Kroh A, Mandic O, Piller WE, Göhlich U, Reuter M, Berning B. Biogeographic responses to geodynamics: A key study all around the Oligo-Miocene Tethyan Seaway. Zool Anz. 2007;246:241–56.

100. Otero O. The oldest-known cyprinid fish of the Afro-Arabian Plate, and its paleobiogeographical implications. J Vertebr Paleontol. 2001;21:386–8.

101. Van Couvering JAH. Early records of freshwater fishes in Africa. Copeia. 1977;1977:163–6.

102. Bychowsky BE. Beitrag zur Kenntnis neuer monogenetischer Fischtrematoden aus dem Kaspesee nebst einigen Bemerkungen über die Systematic der Monopisthocotylea Fuhrmann, 1928. Zool Anz. 1933;105:17–38.

103. Gussev AV, Ali NM, Abdul-Ameer KN, Amin SM, Molnar K. New and known species of *Dactylogyrus* Diesing, 1850 (Monogenea, Dactylogyridae) from cyprinid fishes of the River Tigris. Iraq Syst Parasitol. 1993;25:229–37.

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