Host-specific *Dactylogyrus* parasites revealing new insights on the historical biogeography of Northwest African and Iberian cyprinid fish

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**Abstract**

**Background:** Host specificity in parasites represents the extent to which a parasite’s distribution is limited to certain host species. Considering host-specific parasites of primarily freshwater fish (such as gill monogeneans), their biogeographical distribution is essentially influenced by both evolutionary and ecological processes. Due to the limited capacity for historical dispersion in freshwater fish, their specific coevolving parasites may, through historical host-parasite associations, at least partially reveal the historical biogeographical routes (or historical contacts) of host species. We used *Dactylogyrus* spp., parasites specific to cyprinid fish, to infer potential historical contacts between Northwest African and European and Asian cyprinid faunas. Using phylogenetic reconstruction, we investigated the origin(s) of host-specific *Dactylogyrus* spp. parasitizing Northwest African and Iberian cyprinid species.

**Results:** In accordance with hypotheses on the historical biogeography of two cyprinid lineages in Northwest Africa, Barbiní (*Luciobarbus*) and Torini (*Carasobarbus*), we demonstrated the multiple origins of Northwest African *Dactylogyrus*. *Dactylogyrus* spp. of *Carasobarbus* spp. originated from Asian cyprinids, while *Dactylogyrus* spp. of *Luciobarbus* spp. originated from European cyprinids. This indicates the historical Northern route of *Dactylogyrus* spp. dispersion to Northwest African *Luciobarbus* species rather than the Southern route, which is currently widely accepted for *Luciobarbus*. In addition, both Northwest African cyprinid lineages were also colonized by *Dactylogyrus marocanus* closely related to *Dactylogyrus* spp. parasitizing African *Labeo* spp., which suggests a single host switch from African Labeonini to Northwest African *Luciobarbus*. We also demonstrated the multiple origins of *Dactylogyrus* spp. parasitizing Iberian *Luciobarbus* species. One Iberian *Dactylogyrus* group was phylogenetically closely related to *Dactylogyrus* of Moroccan *Carasobarbus*, while the second was related to *Dactylogyrus* of Moroccan *Luciobarbus*.

**Conclusions:** Our study confirms the different origins of two Northwest African cyprinid lineages. It suggests several independent historical contacts between European Iberian *Luciobarbus* and two lineages of Northwest African cyprinids, these contacts associated with host switches of *Dactylogyrus* parasites.

**Keywords:** Cyprinids, *Dactylogyrus*, Historical biogeography, Host specificity, Iberia, Northwest Africa

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Background
Primary freshwater fish are supposed to be intolerant to salinity, and thus their dispersal is restricted to freshwater routes only. Because of such limited dispersion mechanisms, relationships between fish lineages may reflect relationships between different areas; therefore, freshwater fish are suitable for studies of historical biogeography [1]. Over evolutionary time, the diversity of parasite communities of such freshwater fish is shaped by coevolutionary and historical biogeographical processes (e.g. [2, 3]). However, over ecological time, parasite biogeography is also influenced by the temporal and spatial variability in ecological factors [4, 5].

Concerning freshwater fish, the biogeography of their helminth parasites was shown to reflect historical processes related to the current distribution of their hosts. For example, helminth diversity in Mexican freshwater fishes is determined by the historical and contemporary biogeography of their hosts [6]. The distribution of the metazoan parasites of the sturgeon fish (Acipenseridae) was shown to be in accord with the historical biogeographical routes of these fishes [7].

The host specificity of fish parasites (i.e. the extent to which a parasite's distribution is limited to certain host species) seems to be their most important characteristic, with the potential to reflect historical host-parasite associations and to indicate the historical biogeographical routes of hosts. McDowell [8] showed that parasites not coevolving with their galaxioid fish hosts (i.e. Galaxiidae and Retropinnidae) do not support a vicariance biogeography for galaxioid fish. However, if the host specificity of a parasite group is high, then the phylogenetic and biogeographical relationships between hosts and parasites may be mutually illuminating [1].

Gill monogeneans of the highly diversified genus *Dactylogyrus* Diesing, 1850 are species-specific to their cyprinid host species (with some rare exceptions). According to Šímková et al. [9], *Dactylogyrus* species often exhibit strict host specificity (i.e. they are specific to a single cyprinid species), congeneric host specificity (i.e. they are specific to congeneric cyprinid species), or phylogenetic host specificity (i.e. they are specific to phylogenetically closely related cyprinid species). The distribution of *Dactylogyrus* species on their cyprinid hosts reflects the evolutionary history of these fishes [10]. The evolution of *Dactylogyrus* lineages is associated with different cyprinid lineages, and the presence of the same *Dactylogyrus* species on the representatives of different cyprinid lineages (i.e. in cyprinid species with high divergence but living in the same biogeographical area) is only accidental [9, 10]. Little is known about *Dactylogyrus* of cyprinid species living in the Mediterranean region. However, some studies are suggesting that due to high host specificity, the endemism of *Dactylogyrus* parasites follows the endemism of their cyprinid host species. Such endemic *Dactylogyrus* were documented for *Luciobarbus* Heckel, 1843 from the Iberian Peninsula [11], for *Luciobarbus* from Northwest Africa [12], and for cyprinids living in Lake Mikri Prespa (northern Greece) [13, 14].

Cyprinids are primarily freshwater fish with their native distribution in Europe, Asia, Africa and North America. The different cyprinid lineages exhibit different biogeographical distributions across continents [15]. One of the lineages, the subfamily Cyprininae, was recently revised by Yang et al. [16] to include 11 tribes. Most representatives of this subfamily inhabit waters of southern Eurasia and Africa. Of the four evolutionary lineages (i.e. tribes) of Cyprininae present in Africa, two have been recognized in Northwestern Africa. The first lineage includes hexaploid genera of large-sized barbels (*Carasobarbus* Karaman, 1971, *Pterocapoeta* Günther, 1902 and *Labeobarbus* Rüppel, 1835) belonging to the tribe Torini (this tribe includes large-sized barbels from Asia and Africa). The second lineage is represented by tetraploid *Luciobarbus* belonging to the tribe Barbini (this tribe includes the taxa distributed in Eurasia and Northwest Africa). Different origins and different dispersal events from Eurasia to Africa were proposed for these lineages. The two genera which are widespread in Northwest Africa, *Carasobarbus* and *Luciobarbus*, have disjunct distributions. *Carasobarbus* is distributed in Northwest Africa and the Middle East, while *Luciobarbus* is distributed in West Asia, Northwest Africa, Greece and the Iberian Peninsula. The large-sized African hexaploids are not monophyletic like the Moroccan *Carasobarbus* cluster with Middle East *Carasobarbus*, suggesting that the diversification of African hexaploids preceded the separation between the Middle East and Northwest African hexaploids [16, 17]. Tsigenopoulos et al. [17] suggested that the large hexaploids invaded Africa through the land bridge between Africa and Asia (via the Arabian tectonic Plate) formed in the Middle Miocene (about 13 MYA). Using molecular calibration, they calculated that the splitting of the African hexaploids from their Asian ancestors and subsequently the beginning of the diversification of the African hexaploid lineage occurred in the Late Miocene. The genus *Luciobarbus* is paraphyletic, as the clade also includes the genus *Capeota* Güldenstädt, 1773. Concerning *Luciobarbus* species in Northwest Africa, they do not form a monophyletic group either, as two Northwest African species cluster with Iberian species [16, 18–20]. Concerning *Luciobarbus* in the Iberian Peninsula, three main hypotheses were proposed for their origin; some of them have direct implications for the origin of this genus in Northwest Africa. First, Banarescu [21] and
Almaça [22] proposed that the Iberian Peninsula was colonized from the North before the formation of the Pyrenees. Based on this hypothesis, barbels from the Iberian Peninsula are evolutionarily closer to European and African barbels than to Asian species. Secondly, Doadrio [23] proposed that Dactylogyrus colonized Iberia from Africa via southern Spain at the Miocene-Pliocene boundary (about 5 MYA) after the Messinian salinity crisis of the Mediterranean Sea. Following this hypothesis, barbels from the Iberian Peninsula are phylogenetically closer to Asian and North African barbels than to those of central Europe. Thirdly, Bianco [24] proposed that the distribution of Luciobarbus be explained by the freshwater phase (the so-called Lago Mare phase) of the Mediterranean Sea, which supposedly followed the Messinian salinity crisis. Following this hypothesis, Iberian barbels are more related to those of the Balkans than to central European species. However, this third hypothesis has been rejected by many authors by both geological data and the estimation of the time of diversification of freshwater fish species [25] according to the finding of fossils preceding the given geological period. Tsiagenopoulos et al. [26] and Yang et al. [16] showed that most Luciobarbus species from Northwest Africa are more closely related to Luciobarbus from the Middle East than to Luciobarbus from the Iberian Peninsula.

The aim of this study was to reconstruct the phylogeny of gill parasites of the genus Dactylogyrus, monogeneans specific to cyprinid fish species, to (i) investigate the phylogenetic position of African Dactylogyrus parasites in relation to European and Asian Dactylogyrus lineages with a special focus on the origin(s) of Dactylogyrus parasitizing Northwest African and Iberian cyprinid fish species, and (ii) infer potential scenarios of the Dactylogyrus colonization of Northwest African and Iberian cyprinids in relation to their historical biogeography.

**Methods**

**Dactylogyrus species**

For this study, Dactylogyrus species were sampled from cyprinid species in Morocco and the Iberian Peninsula. Other Dactylogyrus spp. collected from cyprinid species sampled in Europe (the Balkan Peninsula, including Greece and Bosnia and Herzegovina, and central Europe, represented by the Czech Republic) and Africa (Senegal) were included in this study. These Dactylogyrus spp. were selected to recover representatives parasitizing different cyprinid lineages and also to include species potentially phylogenetically related to the Dactylogyrus spp. collected in Northwest Africa and the Iberian Peninsula. In addition, Dactylogyrus species from Asian cyprinid species, for which molecular data (i.e. the sequences of 28S DNA) were available in GenBank, were included in the analyses. The list of studied Dactylogyrus species, their host species, locality of collection, and accession numbers are presented in Table 1.

In the field, Dactylogyrus species were removed from fish gills during fish dissection (following Ergens & Lom [27]), placed on slides, covered with a coverslip, and fixed in a mixture of glycerine and ammonium picrate (GAP). The identification was performed on the basis of the size and shape of the sclerotized parts of the attachment organ, the haptor, and the sclerotized parts of the reproductive organs, following the original descriptions [11, 12, 28–32]. Morphological examination was performed using an Olympus BX51 light microscope equipped with phase contrast and differential interference contrast. Some specimens of each Dactylogyrus species were bisected; one-half of the body (usually the anterior one with reproductive organs) was mounted on a slide for species identification, and the other was individually preserved in 96% ethanol for DNA extraction.

**DNA extraction, amplification and sequencing**

Dactylogyrus species collected from cyprinids in Africa and Europe were sequenced to obtain partial sequences of 28S rDNA and partial sequences of 18S rDNA and the ITS1 region. Dactylogyrus specimens were individually removed from ethanol and dried by using a vacuum centrifuge. Genomic DNA extraction was performed following a standard protocol (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany). Partial 28S rDNA was amplified using the forward primer C1 (5′-ACC CCG TGA ATT TAA GCA-3′) and the reverse primer D2 (5′-TGG TCC GTG TTT CAA GAC-3′) [33]. PCR followed the protocol included in Šimková et al. [34]. Partial 18S rDNA and the entire ITS1 region were amplified in one round using the primers S1 (5′-ATT GTA ACT-3′) and IR8 (5′-GCT AGC TGC GTT CTT CAT CGA-3′) [35] that anneal to 18S and 5.8S rDNA, respectively. Each amplification reaction for partial 18S rDNA and the ITS1 region was performed in a final volume of 15 μl, containing 1.5 U of Taq polymerase, 1× buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 μM of each primer, and 2.5 μl of DNA (20 ng/μl). PCR was carried out using the following steps: 2 min at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 53 °C and 1 min 30 s at 72 °C, and 10 min of final elongation at 72 °C. The PCR products were checked on 1.5% agarose gel, purified using ExoSAP-IT kit (Ecoli, SK) following a standard protocol and directly sequenced using the PCR primers and BigDye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA). Sequencing was carried out using an ABI 3130 Genetic Analyser (Applied Biosystems). Sequences were analysed
| Dactylogyrus species | Cyprinid host species | Cyprinid subfamily | Cyprinid tribe | Country of collection | GenBank ID (28S rDNA) | GenBank ID (18S rDNA with ITS1) |
|---------------------|----------------------|-------------------|---------------|----------------------|----------------------|-------------------------------|
| *D. bicornis* Malewitzkaja, 1941 | *Rhodeus meridionalis* Karaman, 1924 | Acheilognathinae | – | Greece | KY629345 | – |
| *D. labei* Musselius & Gussev, 1976 | *Catla catla* (Hamilton, 1822) | Cyprininae | Labeonini | India | JK566720 | – |
| *D. quanfami* Ha Ky, 1971 | *Cirrhinus molitorella* (Valenciennes, 1844) | Cyprininae | Labeonini | China | EF100536 | – |
| *D. lenkoranoides* El Gharbi, Renaud & Lambert, 1992 | *Luciobarbus guiraonis* (Steindachner, 1866) | Cyprininae | Barbini | Spain | KY629346 | – |
| *D. bocageii* Alvarez Pellitero, Simón Vicente & González Lanza, 1981 | *Luciobarbus bocagei* (Steindachner, 1864) | Cyprininae | Barbini | Portugal | KY629347 | – |
| *D. balistae* Simón Vicente, 1981 | *Luciobarbus bocagei* | Cyprininae | Barbini | – | KY629344 | – |
| *D. mascomai* El Gharbi, Renaud & Lambert, 1992 | *Luciobarbus guiraonis* | Cyprininae | Barbini | Spain | KY629348 | – |
| *D. linstowoides* El Gharbi, Renaud & Lambert, 1992 | *Luciobarbus guiraonis* | Cyprininae | Barbini | Spain | KY629349 | KY629329 |
| *D. legionensis* González Lanza & Alvarez Pellitero, 1982 | *Luciobarbus guiraonis* | Cyprininae | Barbini | Spain | KY629350 | KY629330 |
| *D. andalousiensis* El Gharbi, Renaud & Lambert, 1992 | *Luciobarbus sclateri* Günther, 1868 | Cyprininae | Barbini | Portugal | KY629351 | KY629331 |
| *D. zatensis* El Gharbi, Birgi & Lamberti, 1994 | *Carasobarbus fritschii* Günther, 1874 | Cyprininae | Torini | Morocco | KY629352 | KY629335 |
| *D. volutus* El Gharbi, Birgi & Lamberti, 1994 | *Carasobarbus fritschii* | Cyprininae | Torini | Morocco | KY629353 | KY629334 |
| *D. kulindrii* El Gharbi, Birgi & Lamberti, 1994 | *Carasobarbus fritschii* | Cyprininae | Torini | Morocco | KY629354 | KY629336 |
| *D. marocanus* El Gharbi, Birgi & Lamberti, 1994 | *Carasobarbus fritschii* | Cyprininae | Torini | Morocco | KY629355 | KY629333 |
| *D. scorpius* Rahmouni, Réhulková & Šimková, 2017 | *Luciobarbus rifensis* Doadrio, Casal-Lopéz & Yahyaoui, 2015 | Cyprininae | Barbini | Morocco | KY53860 | KY578023 |
| *D. benhoussai* Rahmouni, Réhulková & Šimková, 2017 | *Luciobarbus musculum* (Pellegrin, 1924) | Cyprininae | Barbini | Morocco | KY53862 | KY578025 |
| *D. varius* Rahmouni, Réhulková & Šimková, 2017 | *Luciobarbus magrebenensis* Doadrio, Perea & Yahyaoui, 2015 | Cyprininae | Barbini | Morocco | KZ53863 | KY578026 |
| *D. fatiphihallus* Rahmouni, Réhulková & Šimková, 2017 | *Luciobarbus magrebenensis* | Cyprininae | Barbini | Morocco | KZ53861 | KY578024 |
| *D. atlantensis* El Gharbi, Birgi & Lamberti, 1994 | *Luciobarbus pallani* (Pellegrin, 1919) | Cyprininae | Barbini | Morocco | KY629356 | KY629337 |
| *D. fimbriphallus* El Gharbi, Birgi & Lamberti, 1994 | *Luciobarbus massaensis* (Pellegrin, 1922) | Cyprininae | Barbini | Morocco | KY629357 | KY629332 |
| Dactylogyrus sp. 1 | *Enteromius niokoloensis* (Daget, 1959) | Cyprininae | Smilogastrini | Senegal | KY629358 | – |
| *D. aspili* Birgi & Lamberti, 1987 | *Enteromius macrops* (Boulenger, 1911) | Cyprininae | Smilogastrini | Senegal | KY629359 | – |
| *D. leonis* Musilová, Réhulková & Gelnar, 2009 | *Labeo coubie* Rüppell, 1832 | Cyprininae | Labeonini | Senegal | KY629360 | – |
| *D. oligospirophallus* Paperna, 1973 | *Labeo coubie* | Cyprininae | Labeonini | Senegal | KY629361 | – |
| *D. brevicirrus* Paperna, 1973 | *Labeo parvus* Boulenger, 1902 | Cyprininae | Labeonini | Senegal | KY629362 | – |
| *D. senegalensis* Paperna, 1969 | *Labeo senegalensis* Valenciennes, 1842 | Cyprininae | Labeonini | Senegal | KY629363 | – |
| *D. titus* Guégan, Lambert & Euzet, 1988 | *Labeo senegalensis* | Cyprininae | Labeonini | Senegal | KY629364 | – |
| *D. falciatus* Guégan, Lambert & Euzet, 1988 | *Labeo coubie* | Cyprininae | Labeonini | Senegal | KY629365 | – |
| *D. vastator* Nybelin, 1924 | *Carassius gibelio* (Bloch, 1782) | Cyprininae | Cyprinini | Czech Republic | KY629366 | KY201103 |
using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI, USA), and new sequences were deposited in GenBank (see Table 1 for accession numbers). The sequences of other Dactylogyrus species parasitizing European and Asian cyprinid species were retrieved in GenBank (Table 1) and were used for phylogenetic analyses.

**Table 1** List of Dactylogyrus species, their cyprinid host species, cyprinid phylogeny, country of collection and GenBank accession numbers for sequences used in the phylogenetic analyses (Continued)

| Dactylogyrus species | Cyprinid host species | Cyprinid subfamily | Cyprinid tribe | Country of collection | GenBank ID (28S rDNA) | GenBank ID (18S rDNA with ITS) |
|----------------------|-----------------------|-------------------|---------------|-----------------------|-----------------------|-----------------------------|
| D. extensus Mueller & Van Cleave, 1932 | Carassius carpio (Linnaeus, 1758) | Cyprinidae | Cyprinini | Czech Republic | AY553629 | – |
| D. inexpectatus Ijimura in Gussev, 1955 | Carassius gibelio | Cyprinidae | Cyprinini | Czech Republic | AY969945 | – |
| D. anchoratus (Dujardin, 1845) | Carassius gibelio | Cyprinidae | Cyprinini | Czech Republic | KY201116 | KY201102 |
| Dactylogyrus sp. AC2012 | Carassius carpio | Cyprinidae | Cyprinini | India | JQ926198 | – |
| D. dyki Ergens & Lucky, 1959 | Barbus barbus (Linnaeus, 1758) | Cyprinidae | Barbini | Barbini | KY629367 | KY629338 |
| D. crivellius Dupont & Lambert, 1986 | Barbus pelagonius Valenciennes, 1842 | Cyprinidae | Barbini | Greece | KY629368 | KY629339 |
| D. petenyi Kastak, 1957 | Barbus balcanicus Kotlik, Tsigenopoulos, Rab & Berrebi, 2002 | Cyprinidae | Barbini | Greece | KY201110 | KY201096 |
| D. malleus Linstow, 1877 | Barbus barbus | Cyprinidae | Barbini | Barbini | KY201111 | KY201098 |
| D. vistulae Prost, 1957 | Luciobarbus albanicus (Steindachner, 1870) | Cyprinidae | Barbini | Greece | KY201114 | KY201100 |
| D. fallax Wagener, 1857 | Vimba vimba (Linnaeus, 1758) | Leuciscidae | – | Albania | KY629369 | KY629460 |
| D. cornu Linstow, 1878 | Vimba vimba | Leuciscidae | – | Czech Republic | KY629370 | KY629341 |
| D. borealis Nybelin, 1937 | Phoxinus sp. | Leuciscidae | – | Bosnia and Herzegovina | KY629372 | KY629343 |
| D. nanus Dogiel & Bychowsky, 1934 | Rutilus rutilus (Linnaeus, 1758) | Leuciscidae | – | Czech Republic | AY969942 | AJ564145 |
| D. sphyrna Linstow, 1878 | Rutilus rutilus | Leuciscidae | – | Czech Republic | AY969943 | AJ564154 |
| D. suecicus Nybelin, 1937 | Rutilus rutilus | Leuciscidae | – | Czech Republic | KY629373 | – |
| D. crucifer Wagener, 1857 | Rutilus rutilus | Leuciscidae | – | Czech Republic | KY629374 | AJ564120 |
| D. wunderi Bychowsky, 1931 | Abramis brama (Linnaeus, 1758) | Leuciscidae | – | Czech Republic | KY629375 | AJ564164 |
| D. cryptomeres Bychowsky, 1943 | Gobio gobio (Linnaeus, 1758) | Gobioninae | – | Czech Republic | AY969947 | – |
| D. lamellatus Achmerow, 1952 | Ctenopharyngodon idella (Valenciennes, 1844) | Xenocyprididae | – | China | AY307019 | – |
| D. hypophthalmichthys Akhmerow, 1952 | Hypophthalmichthys molitrix (Valenciennes, 1844) | Xenocyprididae | – | China | EF100532 | – |
| Dactylogyrus sp. (YY) | Hypophthalmichthys nobilis (Richardson, 1845) | Xenocyprididae | – | China | EF100538 | – |
| D. parabramis Akhmerow, 1952 | Megalobrama terminalis (Richardson, 1846) | Xenocyprididae | – | China | EF100534 | – |
| D. petruscheskyi Gussev, 1955 | Megalobrama amblycephala | Xenocyprididae | – | China | AY548927 | – |
| D. skeletalis Gussev, 1955 | Megalobrama amblycephala | Xenocyprididae | – | China | EF100535 | – |

* Morphologically identical D. bicornis was also found on Rhodesius amarus (Bloch, 1782) from the Czech Republic; the sequence data are not available
* Morphologically and genetically identical D. marocanus was also collected from Pterocapoeta maroccana, Luciobarbus ksibii, Luciobarbus zayanensis

**Phylogenetic analyses**

The first alignment included the partial 28S rDNA sequences of 55 Dactylogyrus species. Among them, 36 were newly sequenced for this study. The sequences of the other 19 Dactylogyrus species as well as the sequences of three species of the Dactylogyridae...
(Euryhalitrematoides pirulum) Plaisance & Kritsky, 2004, Euryhalitrematoides triangulovagina Yamaguti, 1968 and Aliatrema cribbi Plaisance & Kritsky, 2004 with accession numbers AY820618, AY820619 and AY820612, respectively), used as the outgroup in the phylogenetic analyses, were retrieved from GenBank. The second alignment included the partial 18S rDNA sequences and the ITS1 region of 26 Dactylogyrus species belonging to Dactylogyrus lineage III. D. vistulae Prost, 1957 and D. sphyryna Linstow, 1978 were used as the outgroup in the phylogenetic analyses based on the 18S rDNA and ITS1 sequences.

All sequences of a given dataset were aligned using ClustalW multiple alignments [36] in Bioedit v. 7.2.5 [37]. The phylogenetic analyses were performed using unambiguous alignments. Gaps and ambiguously aligned regions were removed from alignments using GBlocks v. 0.91 [38]. The best-fit DNA evolution model was determined using the Akaike’s information criterion (AIC) in JmodelTest 2.1.10 [39, 40]. Phylogenetic trees were inferred using minimum evolution (ME) analysis using PAUP* 4b10 [41], maximum likelihood (ML) analysis using PhyML 3.0 [42], and Bayesian inference (BI) analysis using MrBayes 3.2 [43]. Supports for internal nodes were determined for all trees left in the plateau phase using posterior probabilities of the phylogeny and its branches. The last character represents the distribution of host species with the following character states applied: southern Asia including Southeast Asia, a large part of Eurasia, Europe with only West Asia, the Iberian Peninsula, the Balkan Peninsula, Northwest Africa and West Africa. The distribution of cyprinid species follows Froese & Pauly [46].

**Results**

An unambiguous alignment including the 55 Dactylogyrus species analysed and three outgroup species spanned 544 positions. The TVM + I + G model was selected as the best-fit evolutionary model. The ME, ML and BI analyses provided phylogenetic trees with similar topologies. The BI tree is presented in Fig. 1, including bootstrap values resulting from ME and ML analyses and posterior probabilities resulting from BI analysis. The phylogenetic reconstructions revealed four Dactylogyrus lineages with D. bicornis in the basal position (Fig. 1). Dactylogyrus lineage I included two Dactylogyrus species parasitizing Asian Labeonini in the basal position, and the monophyletic group including 3 Dactylogyrus species parasitizing Iberian Luciobarbus (the tribe Barbini within Cyprininae) and Dactylogyrus species parasitizing Northwest African Carasobarbus fritschii (Günther, 1874) (the tribe Torini within Cyprininae) (Table 1, Fig. 1). The other three Dactylogyrus lineages (II, III and IV) formed a clade well supported by BI analysis but weakly supported by ME and unsupported by ML. Dactylogyrus lineage II included two groups of African Dactylogyrus. The first group included Dactylogyrus parasitizing small Enteromius Cope, 1867 species (Smilogastrini) collected in West Africa (the basal position of this group was weakly supported by PP resulting from BI analysis and BP resulting from ML analysis and unsupported by BP resulting from ME analysis). The second group included Dactylogyrus species parasitizing West African Labeo Cuvier, 1816 (Labeonini) with the nested position of a single Dactylogyrus species (D. marocanus El Gharbi, Birgi & Lambert, 1994) from Northwest African cyprinins of the tribes Barbini and Torini. Dactylogyrus lineage II also included Dactylogyrus species parasitizing Cyprinus carpio Linnaeus, 1758 and the complex of Carassius auratus (Linnaeus, 1758), two species of Asian origin recently widely distributed in Europe. Dactylogyrus lineage III included the species collected from Europe and parasitizing Leuciscinae species, Barbus species (Barbini, Cyprininae) with a European distribution, and the Northwest African Luciobarbus (Table 1). Phylogenetic relationships within Dactylogyrus lineage III...
were either weakly resolved or unresolved by phylogenetic analyses. However, the monophyletic group including *Dactylogyrus* parasitizing Northwest African *Luciobarbus* species and *D. andalousiensis* El Gharbi, Renaud & Lambert, 1992 parasitizing Iberian *Luciobarbus sclateri* Günther, 1868 was either well or moderately supported by our phylogenetic analyses. *Dactylogyrus* lineage IV included *D. cryptomeris* Bychowsky, 1943 parasitizing cyprinids of Gobioninae in the basal position and the well-supported monophyletic group of *Dactylogyrus* parasitizing Asian Xenocyprinae (Table 1, Fig. 1).

Because of the impossibility of reconstructing a reliable alignment when including *Dactylogyrus* species of highly diversified cyprinid lineages (i.e. because of the presence of many hypervariable regions and indels), we used only the representatives of *Dactylogyrus* lineage III
in subsequent phylogenetic analyses to resolve the phylogenetic relationships within this lineage. An unambiguous alignment including *Dactylogyrus* species of lineage III spanned 1072 positions. The GTR + I + G model was selected as the best-fit evolutionary model. The ME, ML and BI analyses provided phylogenetic trees with similar topologies. The BI tree is presented in Fig. 2, including bootstrap values resulting from ME and ML analyses and posterior probabilities resulting from BI analysis. The basal position of *D. andalousiensis* in relation to the monophyletic group of *Dactylogyrus* species parasitizing Moroccan *Luciobarbus* was well supported by PP resulting from BI analysis and BP resulting from ME analysis, and moderately supported by BP resulted from ML analysis. Three *Dactylogyrus* species parasitizing Iberian *Luciobarbus* species formed a monophyletic group with two *Dactylogyrus* parasitizing Balkan *Barbus* species and one *Dactylogyrus* parasitizing *Barbus* species with a wide European distribution. This cluster was well supported by all phylogenetic analyses.

The mapping of characters was performed in the phylogenetic reconstruction (BI tree) of 55 *Dactylogyrus* species. An unambiguous alignment (BI tree) of 55 *Dactylogyrus* species. An unambiguous alignment (BI tree) spanning 568 positions. The GTR + I + G model was selected as the best evolutionary model. The mapping as the character of cyprinid lineages (i.e. cyprinid subfamilies) onto the phylogenetic reconstruction (Fig. 3) showed that Acheilognathinae is the most plesiomorphic host group for *Dactylogyrus*. *Dactylogyrus* of the Cyprininae are included in three lineages. The Gobioninae, Xenocyprinae and Leuciscinae were likely colonized by *Dactylogyrus* from the Cyprininae. However, some Cyprininae were secondarily colonized by *Dactylogyrus* from the Leuciscinae. The mapping of the cyprinid distribution onto the phylogenetic reconstruction (Fig. 4) showed the Asian origin of *Dactylogyrus*. This mapping revealed (i) the multiple origins of Northwest African *Dactylogyrus*, and (ii) the phylogenetic relatedness between *Dactylogyrus* parasitizing the Cyprininae of Labeonini, Cyprinini, Torini and some of Barbini across different continents. Northwest
African *Dactylogyrus* parasitizing *Carasobarbus fritschii* (the tribe Torini within Cyprininae, see Fig. 5) are phylogenetically closely related to Asian *Dactylogyrus* species. *Dactylogyrus marocanus* is of African origin. Our mapping suggests that *D. marocanus* diverged within *Dactylogyrus* of African Labeonini and switched to Moroccan cyprinids (a morphologically and genetically identical form of this parasite was found in two species of Torini and two *Luciobarbus* species of Barbini). *Dactylogyrus* parasitizing Northwest African *Luciobarbus* are of European origin (Fig. 4). In addition, our analyses also showed the multiple origins of *Dactylogyrus* parasitizing Iberian *Luciobarbus*. 

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**Fig. 3** Mapping of fish lineages into the BI reconstruction of *Dactylogyrus* phylogeny. Characters for fish lineages: 1, Acheilognathinae; 2, Xenocyprinae; 3, Gobioninae; 4, Leuciscinae; 5, Cyprininae
The mapping of fish distribution onto Dactylogyris phylogeny demonstrated that one group of Dactylogyris parasitizing Iberian Luciobarbus (i.e. *D. mascomai* El Gharbi, Renaud & Lambert, 1992, *D. lenkoranoides* El Gharbi, Renaud & Lambert, 1992 and *D. bocageii* Alvarez Pellitero, Simón Vicente & González Lanza, 1981) and the group of Dactylogyris parasitizing Northwest African Torini probably originated from Asian cyprinids (most likely...
Labeonini). However, the other three Dactylogyrus of Iberian Luciobarbus are most probably of European origin. Whilst *D. liinstowoides* El Gharbi, Renaud & Lambert, 1992 and *D. legionensis* González Lanza & Alvarez Pellitero, 1982 form the monophyletic group with the European Dactylogyrus of Barbus species, *D. andalousiensis* is included in the monophyletic group of Northwest African Luciobarbus species within Dactylogyrus of lineage III (i.e. the lineage including Dactylogyrus of Leuciscinae and some Dactylogyrus species of Barbus-Luciobarbus group).
Discussion

The present study was focused on host-specific monogeneans of Dactylogyrus as a potential tool for inferring historical contacts among their cyprinid hosts in the Mediterranean region, which is characterized by a high degree of endemism among cyprinid species. As indicated by a previous study [11, 12] and confirmed by our study, endemic Mediterranean cyprinids harbour endemic Dactylogyrus fauna. We investigated the origin of host-specific Dactylogyrus parasitizing Northwest African and Iberian cyprinid hosts, hypothesizing that phylogenetic relationships between Dactylogyrus species may cast new light on the biogeographical history of this fish group.

Šimková et al. [10] reconstructed the phylogeny of Dactylogyrus parasitizing central European cyprinid species (also including some invasive or introduced species). They presented evidence for three Dactylogyrus lineages in central Europe: the first includes Dactylogyrus of the Cyprininae (tribe Cyprinini), originating from Southeast Asia and historically introduced into Southeast Europe; the second includes Dactylogyrus of the Rasborininae and Xenocyprininae (the fish species of both groups originating from Southeast Asia and introduced into Europe) and the Gobioninae; and the last, a very diversified lineage, includes Dactylogyrus of the Leuciscinae and European Barbus (Barbini within Cyprininae). Šimková et al. [10] showed that the phylogenetic relationships between Dactylogyrus lineages reflected the phylogenetic relationships between cyprinid lineages (recently represented by cyprinid subfamilies), except for the particular position of Dactylogyrus species parasitizing European Barbus species, which were nested within the highly diversified clade of Dactylogyrus parasitizing European Leuciscinae.

Herein, the phylogenetic position of Dactylogyrus parasitizing African cyprinids was evaluated for the first time. By our phylogenetic analyses, we showed that Dactylogyrus parasitizing the African cyprinids investigated in our study belong to three different lineages (I, II and III), which suggests their different origins and presumably also reflects the different histories of their cyprinid hosts. Dactylogyrus lineage II includes Dactylogyrus parasitizing the Cyprinini of Southeast Asian origin and West African Cyprininae (Labeonini investigated in our study), which suggests that West African cyprinids and their co-evolving Dactylogyrus originated from Asia (the basal position of D. aspili and Dactylogyrus sp. from small African Enteromius was not supported). This is in accordance with predictions on the origin of African cyprinid fauna [16, 25].

However, the situation concerning the origin of Northwest African cyprinids and their Dactylogyrus parasites is more complicated. Dactylogyrus marocanus, a single species infecting both Northwest African tribes of the Cyprininae, Torini and Barbini, was nested within Dactylogyrus lineage II. This parasite occurring on the representatives of two cyprinine lineages was previously reported in seven cyprinin species, mostly the representatives of Torini, by El Gharbi et al. [12] and also documented by our study. We showed a morphologically and genetically identical form of this species in Casarobarbus fritschii, Pterocapoeta marocanna Günther, 1902, Luciobarbus ksibii Boulanger, 1905 and L. zayanensis Doadrio, Casal-Lopéz & Yahyaoui, 2016. However, the abundance of D. marocanus was higher in two Torini species than in Luciobarbus species, suggesting that Torini are the main host species for its reproduction (see [9]). Dactylogyrus marocanus clusters within West African Dactylogyrus species parasitizing Labeo species, suggesting a single host-switch by Dactylogyrus to Northwest African Cyprininae from the group of Cyprininae achieving high diversification on the African continent. The attachment organ (haptor) of D. marocanus is of the same morphological type as that recognized for Dactylogyrus of West African Labeo, Dactylogyrus of small West African Enteromius, and two Dactylogyrus of Cyprinini of Southeast Asian origin i.e. D. inexspectatus Isjumova in Gussev, 1955 and D. anchoratus (Dujardin, 1845). In addition, D. marocanus is the only species with this type of haptor within the Dactylogyrus species parasitizing Northwest African cyprinids. This may suggest that haptor morphology, in this case, is a character shared by common ancestry. The similar morphology of the haptor in Dactylogyrus parasitizing phylogenetically closely related cyprinid species was previously demonstrated by Šimková et al. [9].

Our phylogenetic analyses using cyprinid-specific Dactylogyrus spp. confirmed the occurrence of different independent dispersal events from Asia (or Eurasia) to Africa concerning the Moroccan cyprinids belonging to hexaploid Torini (Casarobarbus fritschii and Pterocapoeta marocanna in our study) and tetraploid Barbini (Luciobarbus species), as was highlighted by the molecular phylogeny of cyprinid species [16, 17]. Middle East Casarobarbus and Northwest African Casarobarbus form a monophyletic group within the Labeobarbus clade, and Pterocapoeta occupies the basal position in this clade [16, 17]. Wang et al. [47] proposed that the group comprising the Casarobarbus lineage originated about 9.94 MYA in the Orient. The Casarobarbus lineage separated about 7.7 MYA. Tsigenopoulos et al. [17] dated the beginning of the diversification of the African hexaploid lineage to the Late Miocene following the closing of the seaway between the Mediterranean Sea and the Indian Ocean and the emergence of the Gomphotherium land bridge between Africa and Asia (the Arabian tectonic Plate) in the Middle Miocene. In the Tortonian stage, the Anatolian tectonic Plate (Asia Minor) was connected to the Arabian Plate to the east and was separated from Europe to the west, where
the Aegean Sea formed [48]; this explains the absence of Torini in Europe [17]. The phylogenetic position of Dactylogyrus parasitizing Carasobarbus fritschii within Dactylogyrus lineage I and the phylogenetic affinity between Dactylogyrus species parasitizing South Asian Labeonini and Dactylogyrus species parasitizing Northwest African cyprinid species is in line with the hypothesis of the origin and historical dispersion of Northwest African Torini. The molecular phylogeny of tribes belonging to Cyprininae showed Labeonini to be a sister group to the group including other tribes with Torini in the basal position [16]. This may suggest close phylogenetic relationships between Dactylogyrus of Torini and Labeonini. However, our study suggests the need for future phylogenetic studies to investigate also the position of Dactylogyrus of Asian and African representatives of Torini as well as Dactylogyrus parasitizing other cyprinid tribes to specify the origin of Dactylogyrus diversity in Northwest African Torini.

Concerning the Mediterranean diversity of cyprinids, there are three main hypotheses of their historical dispersion explaining their actual distribution. All suggest that the cyprinids originated in Asia and reached the Mediterranean peninsulas via three main routes, a northern route [21], a southern route via land bridges connecting continents [25], and dispersion through the Mediterranean Sea during its supposed freshwater phase at the end of the Messinian [24]. According to the northern dispersal scenario, cyprinids dispersed slowly via river captures, through Siberia, and then from northern into southern Europe, from the late Oligocene until the late Pliocene (35–1.7 MYA). The colonization of southern Europe occurred before the alpine orogeny during the Miocene, which separated freshwater connections between northern and southern Europe [49]. Concerning Luciobarbus, it is hypothesized that they spread through central Europe to the Iberian Peninsula and Northwest Africa, and that, afterwards, a second invasion of Barbus from Asia colonized central Europe, where Barbus replaced Luciobarbus (except in the Iberian Peninsula due to the ancient isolation of the Iberian Peninsula from the rest of the European continent). This hypothesis was rejected for Iberian Luciobarbus by Zardoya & Doadrio [18]. According to the southern route hypothesis, cyprinids dispersed from Asia through Asia Minor via land bridges (Asian-Anatolian-Iranian, 33 MYA, and the Gomphotherium land bridge, 19 MYA) to the Balkans and Northern Africa, and subsequently to the Iberian Peninsula [24]. In accordance with this scenario, it is supposed that Luciobarbus colonized the Iberian Peninsula from Africa via southern Spain [18, 23]. The Lago Mare dispersal scenario [24] assumes that after the Messinian salinity crisis (5 MYA) the Mediterranean Sea underwent a lacustrine phase allowing the dispersion of freshwater fishes. This scenario predicts higher phylogenetic affinity among species in Mediterranean areas. Although this hypothesis is still widely cited, it has been largely discredited, both by geological evidence and phylogenetic studies (e.g. [19, 25]).

Recent views on the historical dispersion of Luciobarbus are, however, ambiguous. On the basis of morphological characters, Iberian and North African barbels are closely related to central European species, supporting the northern route of dispersion [21, 22], whilst molecular phylogenetic studies and a lack of fossil records of Luciobarbus in central Europe support the southern route of Iberian Luciobarbus dispersion [18, 19, 26]. Our phylogenetic reconstruction using host-specific Dactylogyrus would suggest that the northern route represents the more plausible scenario explaining the historical dispersion of Luciobarbus in Northwest Africa. This scenario is supported by our phylogenetic analyses, which indicate that (i) Dactylogyrus species parasitizing Northwest African Luciobarbus have a clearly European origin, and (ii) the monophyletic group of Dactylogyrus including D. balistae Simón Vicente, 1981, D. legionensis and D. lintswoides parasitizing Iberian Luciobarbus form a well-supported clade with Dactylogyrus parasitizing European Barbus. In addition, the Iberian species D. andalousiensis occupies the basal position in the clade including the monophyletic group of Dactylogyrus species parasitizing Northwest African Luciobarbus (a finding well supported by BP and PP using the combined data of partial 18S rDNA and ITS1). Even though our sampling of Dactylogyrus parasites did not include Dactylogyrus representatives of Middle East cyprinids, we showed that Dactylogyrus species parasitizing Northwest African Luciobarbus, four of the Dactylogyrus species parasitizing Iberian Luciobarbus, Dactylogyrus species parasitizing Greek Luciobarbus, Dactylogyrus species parasitizing Balkan Barbus, and Dactylogyrus species parasitizing the widely distributed European Barbus barbus form together with Dactylogyrus parasitizing Leuciscinae the well-supported lineage III. This may suggest the common origin of Dactylogyrus parasitizing Luciobarbus/Barbus (Cyprininae) of different Mediterranean areas and Dactylogyrus of European Leuciscinae. Indubitably, there is a strong relationship between Dactylogyrus parasitizing Northwest African Luciobarbus (Barbini) and those parasitizing European cyprinids belonging to the subfamily Leuciscinae and the tribe Barbini of the subfamily Cyprininae, identified in our Dactylogyrus lineage III. However, in this case, there is a large discrepancy between the phylogenies of the hosts and Dactylogyrus parasites, and the relationships in lineage III rather point to historical host-switching events.

In our study, we showed the close phylogenetic relationships between (i) Dactylogyrus parasitizing Northwest African Torini and one group of Dactylogyrus
parasitizing Iberian *Luciobarbus* species and (ii) *Dactylogyrus* parasitizing Northwest African Barbini and the second group of *Dactylogyrus* parasitizing also Iberian *Luciobarbus* species. This revealed (i) multiple historical contacts between Iberian *Luciobarbus* and two different lineages of Northwest African cyprinids with different origins and historical dispersions, and subsequently (ii) two independent diversifications of *Dactylogyrus* in Iberian *Luciobarbus*. The exchange of fauna between the Iberian Peninsula and Northwest Africa is hypothesized for the beginning of the Messinian salinity crisis 5.96 MYA [50], which was initiated by the closing of the Betic and Rifian corridors in Spain and Morocco [51–53]. If this event was responsible for the common origin of *Dactylogyrus* parasitizing Iberian and Northwest African cyprinids, the origin and diversification of Iberian *Luciobarbus* seem to be older than predicted by the Lago Mare route of *Luciobarbus* dispersion. Mesquita et al. [54] suggested an even earlier differentiation of Mediterranean *Luciobarbus* lineages (7.3 MYA). At the end of the Messinian 5.33 MYA, all connections between North African and Iberian populations were closed by the formation of the Strait of Gibraltar [52]. However, Machordom & Doadrio [19] suggested that the Betic area was connected with the Kabili Mountains after its isolation from the Rifian area by the Betic-Kabilian plate in the Pliocene (3.3 MYA). Cahuzac et al. [55] proposed the existence of plates also between southern Spain and the Maghreb. These plates may potentially have served as the contact zones between Iberian and North African cyprinids and may alternatively have contributed to the common ancestry of *Dactylogyrus* parasitizing Iberian *Luciobarbus* and Northwest African Torini or Barbini. However, we failed to identify any reliable resource documenting historical contacts between Iberian *Luciobarbus* and the two Moroccan cyprinid lineages.

Mesquita et al. [54] identified three polytomic evolutionary lineages of Iberian *Luciobarbus*, potentially suggesting multiple speciation events which could likely explain the evidence of two lineages for Iberian *Dactylogyrus*. However, the different positions of the two Iberian *Dactylogyrus* lineages in the phylogenetic tree have no association with the evolution and recent distribution of Iberian *Luciobarbus* (according to [54]), *L. bocagei* (Steindachner, 1864) representing the Atlantic lineage, *L. guiraonis* (Steindachner, 1866) representing the Mediterranean lineage, and *L. sclateri* representing the South-Western and South-Eastern lineage. *Dactylogyrus bocageii*, previously reported as a species endemic to Spanish *Luciobarbus* [11], was present in all three Iberian *Luciobarbus* species investigated in our study and living recently in allopatry. As indicated before, *D. andalousiensis* is a single Iberian *Dactylogyrus* species with the basal position in the clade including the monophyletic group of *Dactylogyrus* species parasitizing the Northwest African *Luciobarbus*. This parasite was previously recorded on two *Luciobarbus* species, namely *L. sclateri* and *L. microcephalus* (Almaça, 1967), both restricted to southern Portugal and Spain [11], but representing different evolutionary lineages [54].

**Conclusions**

To our knowledge, this study is the first to investigate the origin and phylogenetic position of Northwest African and Iberian *Dactylogyrus*, monogenean parasites specific to cyprinid fish. The phylogenetic reconstruction of these host-specific monogeneans sheds new light on historical contacts between African and European (here Iberian) cyprinids, these contacts associated with host switches of *Dactylogyrus* parasites. More specifically, phylogenetic analyses using *Dactylogyrus* demonstrated different and independent dispersal events from Asia (or Eurasia) to Africa concerning two lineages of Moroccan cyprinids: (i) *Carasobarbus fritschii* and *Pterocapoeta maroccana* belonging to hexaploid Torini, and (ii) *Luciobarbus* species belonging to tetraploid Barbini. In addition, our study revealed that *Dactylogyrus* parasitizing Iberian *Luciobarbus* do not form a monophyletic group, i.e. we demonstrated close phylogenetic relationships between (i) *Dactylogyrus* parasitizing Northwest African Torini and one group of *Dactylogyrus* parasitizing Iberian *Luciobarbus* species, and (ii) *Dactylogyrus* parasitizing Northwest African Barbini and the second group of *Dactylogyrus* parasitizing also Iberian *Luciobarbus*. This suggests multiple historical contacts between Iberian *Luciobarbus* and Northwest African cyprinids with different origins and historical dispersions, and subsequently two independent diversification of *Dactylogyrus* in Iberian *Luciobarbus*.

**Acknowledgements**

We are grateful to Mária Luža Kociancová, Eva Řehulková, Tomáš Pakosta, and Jaroslav Cervenka for their help with the fish examination, parasite collection and fixation. We thank Radek Sanda (National Museum, Czech Republic) for help with fish sampling. We also thank Carla Sousa-Santos (ISP Instituto Universitário, Portugal), colleagues from Piscifactoría de El Palmar (Spain), Stamatis Zogaris (Hellenic Centre for Marine Research, Greece), Dejan Radolović (Republic Institute for Protection of Cultural, Historical and Natural Heritage, Bosnia and Herzegovina), Antoine Pariselle (IRD, France), and Ouafae Berrada Râhani (Mohammed V University in Rabat, Morocco) for arranging permits and assisting with fieldwork. We kindly thank Matthew Nicholls for English revision of the final draft.

**Funding**

This study was funded by the Czech Science Foundation (grant No. 15-19382S).

**Availability of data and materials**

The data supporting the conclusions of this study are included in this article. The newly generated sequences were submitted to the GenBank database under the accession numbers shown in Table 1.

**Authors’ contributions**

AS conceived the ideas and designed the study. AS, MB, IR and JV conducted the fieldwork and collected the data. AS, MB and IR analysed the data. AS wrote
the manuscript with assistance from JV. All authors read and approved the final manuscript.

Ethics approval
All applicable institutional, national, and international guidelines for the care and use of animals were followed. This study was approved by the Animal Care and Use Committee of the Faculty of Science, Masaryk University in Brno (Czech Republic).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 3 July 2017 Accepted: 5 November 2017

Published online: 28 November 2017

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