Integrating morphological and molecular approaches for characterizing four species of *Dactylogyrus* (Monogenea: Dactylogyridae) from Moroccan *Luciobarbus* species (Cyprinidae), with comments on their host specificity and phylogenetic relationships

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Abstract

~~Cyprinid fishes are known to harbour highly host-specific gill-associated parasites of *Dactylogyrus*. On the basis of morphological resemblance, Moroccan species of *Dactylogyrus* have been classified into four morphological types, with *Dactylogyrus* spp. parasitizing species~~

Commenté [D1]: But you are reporting and dealing with Dactylogyrus species from cyprinids from other genera as well.

Commenté [D2]: The abstract should include only what you did and what you found.

~~of *Luciobarbus* representing the largest group. These are characterized by an attachment organ with a cross-shaped (five radial) ventral bar and the male copulatory organ containing an accessory piece with a distal portion directed backwards along the circle of the curved copulatory tube.~~ High similarity in the morphology of sclerotized structures among these parasitic ~~parasite~~ species of *Dactylogyrus* makes identification difficult, even for specialists. In this paper, four previously known species of *Dactylogyrus* are characterized and/or illustrated under a reliable taxonomic framework integrating morphological and molecular evidence, and their phylogenetic relationships are investigated using molecular data. The species are as follows: *D. borjensis* from *Luciobarbus zayanensis*; *D. draaensis* from *Luciobarbus lepineyi*; *D. ksibii* from *Luciobarbus ksibi* and *Luciobarbus rabatensis*; and *D. marocanus* from *Carasobarbus fritschii, L. ksibi, L. zayanensis* and *Pterocapoeta maroccana*. Our results revealed intraspecific genetic variability among specimens of *D. ksibii* collected from two different hosts and geographically distant basins. Phylogenetic reconstruction showed that *Dactylogyrus* spp. parasitizing Moroccan cyprinids are representatives of three main lineages corresponding to morphological differences and host specificity. Our records of *D. marocanus* on *L. zayanensis* and *P. maroccana* increase the range of available host species i.e. eight species of four cyprinid genera representing two phylogenetic lineages (i.e. Barbinae and Torinae).

Introduction

Species of *Dactylogyrus* Diesing, 1850 are ectoparasitic flatworms (Monogenea, Platyhelminthes) occurring mainly on the gills of cyprinid fishes. With more than 900 nominal species (Gibson et al., 1996), *Dactylogyrus* represent the most speciose genus ~~within~~ of helminths. Recent studies on ~~Northwest~~ northwest African cyprinids have shown that the

Commenté [D3]: I don't know, but a lot of invertebrate groups are considered helminths. Are you sure about *Dactylogyrus* being the most speciose group?

biodiversity of this fish group is higher than previously estimated (e.g. Brahimi et al., 2018; Casal-López et al., 2015; Doadrio et al., 2016; Doadrio, Casal-López & Perea, 2016). In Morocco, 20 species belonging to four genera (i.e. *Carasobarbus* Karaman, 1971; *Labeobarbus* Rüppel, 1835; *Luciobarbus* Heckel, 1843 and *Pterocapoeta* Günther, 1902) are currently considered valid (Fricke, Eschmeyer & Van der Laan, 2020). Among these, *Luciobarbus* is the most diverse, with 15 species, some being only recently described (Brahimi et al., 2018; Casal-López et al., 2015; Doadrio et al., 2016; Doadrio, Casal-López & Perea, 2016). Descriptions of the new species may indicate that the diversity of their host-specific parasites, such as monogeneans of *Dactylogyrus*, is currently underestimated. To date, 17 *Dactylogyrus* spp. parasitizing 16 cyprinid species of three genera (*Carasobarbus, Labeobarbus* and *Luciobarbus*) have been recorded in Morocco. Of these, 11 species are restricted to a single host species, five occur on two to six species belonging to one host genus and one species parasitizes species of three host genera (El Gharbi et al., ~~Birgi & Lambert,~~ 1994; Rahmouni et al., 2017).

In 2015, a survey was initiated to determine the diversity of *Dactylogyrus* species parasitizing Moroccan cyprinids. A total of 13 cyprinid species were examined and 13 species of *Dactylogyrus* were collected and prepared for morphological and molecular analysis. The first paper stemming from this investigation included descriptions of four new *Dactylogyrus* species infesting three species of northern Moroccan *Luciobarbus* (Rahmouni et al., 2017). This study also demonstrated that an integrated morphological and molecular approach to species identification/description can reveal the presence of morphologically indistinguishable, but genetically distinct, species (or cryptic species) within *Dactylogyrus*. Rahmouni et al. (2017) reported two cryptic species (*Dactylogyrus benhoussai* and *Dactylogyrus varius* forma vulgaris) parasitizing allopatric species of *Luciobarbus* (*L. yahyaouii* [syn. *L. moulouyensis*] and *L.*

maghrebensis, respectively). On the other hand, they also identified three morphologically distinct, but genetically identical, forms of *D. varius*, demonstrating the usefulness of molecular markers for documenting levels of intraspecific morphological variability or, in extreme cases, to demonstrate that two or more monogenean morphospecies (morphotypes) represent a single species.

More recently, Šimková et al. (2017) used 10 species of *Dactylogyrus* parasitizing Moroccan cyprinids to infer potential historical contacts between northwest ~~Northwest~~ African, European and Asian cyprinid faunas. Using phylogenetic reconstruction, they suggested that *Dactylogyrus* species infecting Moroccan species of *Carasobarbus* were phylogenetically closely related to some *Dactylogyrus* spp. parasitizing Iberian *Luciobarbus* spp. and shared a common ancestor with southern and southeast ~~Southern and Southeast~~ Asian Labeonini hosts, while *Dactylogyrus* species infecting Moroccan *Luciobarbus* spp. formed a large group together with *Dactylogyrus* species parasitizing European *Luciobarbus* (including some Iberian and two known Balkan species), *Barbus* and leuciscid hosts.

Commenté [D4]: *Dactylogyrus* is a taxon (a genus), not a parasite. It's members are parasites however.

The present paper is a continuation of our research dealing with *Dactylogyrus* species from Moroccan cyprinids. Herein, an effort is made to characterize and/or illustrate four previously known species of *Dactylogyrus* under a reliable taxonomic framework integrating morphological and molecular evidence and their phylogenetic relationships investigated using molecular data.

Materials & Methods

Fish sampling

Two hundred specimens of six cyprinid species, namely *Carasobarbus fritschii* (Günther, 1874); *Luciobarbus ksibi* (Boulenger, 1905); *Luciobarbus lepineyi* (Pellegrin, 1939); *Luciobarbus*

rabatensis Doadrio, Perea & Yahyaoui, 2015; *Luciobarbus zayanensis* Doadrio, Casal-López & Yahyaoui, 2016; and *Pterocapoeta maroccana* Günther, 1902, were captured by means of gill nets or electro-fishing from eight localities in Morocco (Fig. 1, Table 1). The scientific names and classification of fishes used are those provided in Fricke et al. ~~, Eschmeyer & Van der Laan~~ (2020). Live fishes were kept in aerated holding tanks until processed for parasitological examination. The research was approved by the Ethics Committee of Masaryk University (approval number CZ01302). Field experiments were approved by the Haut Commissairiat aux Eaux et Forêts et à la Lutte contre la Désertification (Ministère de l'Agriculture, de la Pêche Maritime, du Développement Rural et des Eaux et Forêts, Royaume du Maroc) (N° 62 HCEFLCD/DLCDPN/CPC/PPC).

Parasite collection and fixation

Fishes were sacrificed by severing the spinal cord, after which the gill arches were removed via dorsal and ventral section and examined for monogeneans using a stereomicroscope. Specimens of *Dactylogyrus* were detached from the gills using fine needles and prepared following Řehulková (2018). Monogeneans, fixed with a mixture of glycerine and ammonium picrate (GAP) (Malmberg, 1957), were observed under an Olympus BX51 microscope equipped with phase contrast optics. Specimens were measured using ImageJ software (available at: http://rsb.info.nih.gov/ij/) following Rahmouni et al. (2017). Measurements, all in micrometers, are expressed as the mean followed in parentheses by the range and number (*n*) of structures measured. Numbering of hook pairs was adopted from Mizelle (1936). The male copulatory organ is henceforth abbreviated to MCO. Voucher specimens of monogeneans collected in the present study were deposited at the Muséum National d'Histoire Naturelle, Paris.

DNA isolation, amplification and sequencing

To guarantee identification of parasites collected for molecular analysis, specimens collected for DNA extraction were bisected into two parts, with one part transferred to an Eppendorf tube containing ethanol (95%) to preserve the DNA and the second half mounted in GAP for species identification. Individual parasites were dried using a vacuum centrifuge. Genomic DNA was extracted using the DNEasy extraction kit (Qiagen) following the manufacturer's instructions, the extracted DNA being concentrated to a final volume of 80µl. Partial 18S rDNA and the entire ITS1 region were amplified using the primers S1 (5'-ATTCCGATAACGAACGAGACT-3') and IR8 (5'-GCTAGCTGCGTTCTTCATCGA-3'), which anneal to the 18S and 5.8S rDNA sections, respectively (Šimková et al., 2003). PCR was carried out on a 30 µl volume containing 5 µl of DNA extract, 0.5 µM of each PCR primer, 1.5 U *Taq* DNA polymerase, 1 X PCR buffer, 0.1 mg/ml BSA, 1.5 mM $MgCl_2$ and 200 µM dNTPs. Amplification was achieved using the following steps: initial denaturation for 2 min at 94°C followed by 39 cycles of 94° C for 1 min, 53° C for 1 min and 72° C for 1 min 30 s, and a final extension at 72° C for 10 minutes. Partial 28S rDNA was amplified using the following primers: forward C1 (5'-ACCCGCTGAATTTAAGCA-3') and reverse D2 (5'-TGGTCCGTGTTTCAAGAC-3') (Hassouna, Michot & Bachellerie, 1984). PCR products were checked on 1% agarose TBE gel stained with Good View (SBS Genetech), visualized under UV light and documented using GBox F3 Bio Imaging System (Syngene). Successful PCR products were subsequently purified using the Exo SAP-IT kit (E-coli). Sequencing was undertaken using the same primers as for PCR on an ABI 3130 Genetic Analyzer (Applied Biosystems) using the Big Dye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems).

Sequence alignment and phylogenetic analysis

The DNA sequences obtained were analyzed using Sequencher software (Gene Codes Corp.). All sequences were aligned using Clustal W multiple alignments (Thompson, Higgins & Gibson, 1994) running in BioEdit version 7.2.5 (Hall, 1999). Genetic distances for each molecular marker (28S rDNA, 18S rDNA and ITS1), recorded as uncorrected p-distances between sequences of the different Moroccan species redescribed herein, were calculated using MEGA 7 (Tamura et al., 2013). For phylogenetic analysis, nine *Dactylogyrus* spp. ~~strictly~~ parasitizing Moroccan *Luciobarbus* and four species of *Dactylogyrus* parasitizing *C. fritschii* were included in this study. Eleven *Dactylogyrus* species parasitizing European *Barbus* and *Luciobarbus* species were also included in the phylogenetic analysis (see Table 2 for accession numbers). The midpoint method was used for rooting the phylogenetic tree. The phylogenetic analysis was performed using unambiguous alignment of combined sequences (28S rDNA, 18S rDNA and ITS1), with gaps and ambiguously aligned regions removed from the alignment using GBlocks version 0.91 (Talavera & Castresana, 2007). Phylogenetic analysis was performed using the maximum likelihood (ML) and Bayesian inference (BI) approaches in RAxML (Stamatakis, 2014) and MrBayes version 3.2.6 (Huelsenbeck & Ronquist, 2001), respectively. JModelTest version 2.1.10 (Guindon & Gascuel, 2003; Darriba et al., 2012) was employed to select the most appropriate model of DNA evolution using the Akaike information criterion (AIC). GTR+ I + G for 28S rDNA and ITS1, and TIM3ef + I + G for 18S rDNA, were selected as the best models of DNA evolution. Support values for internal nodes were estimated using a bootstrap resampling procedure with 1000 replicates (Felsenstein, 1985). The BI tree was constructed using four Monte Carlo Markov chains (MCMC) running under 200 0000 generations, with sampling tree

Commenté [D5]: You don't know this for sure.

Commenté [D6]: What is this? If it means that the root was arbitrarily assigned such that the tree was "balanced", such is not the case in your Figure 6. More importantly, why even show an arbitrarily assigned root, as it misleading and very unlikely to be real. You should delete this statement and the root on Fig. 6, both of which suggest to your readers the erroneous idea that the basal relationships you show in the figure are real.

Commenté [D7]: Is this an error?

topologies every 100 generations. The first 30% of the trees were discarded as "burn-in" according to the standard deviation split frequency value (<0.01). The posterior probabilities of the phylogeny and its branches were determined for all trees left in the plateau phase with the best ML scores.

Results

Six Moroccan cyprinid fish species were examined for monogeneans: *C. fritschii* (*n* = 40), *L. ksibi* (*n* = 15), *L. lepineyi* (n = 113), *L. rabatensis* (*n* = 9), *L. zayanensis* (n = 15) and *P. maroccana* (*n* = 3). In each species, the gills were infected with one or more species of *Dactylogyrus*. Four previously described species of *Dactylogyrus* ~~the genus~~ were found (*D. maroccanus*, *D. ksibii*, *D. borjensis* and *D. draaensis*) and these were taxonomically evaluated using an integrated approach combining both morphological and molecular analysis, thereby making them available for phylogenetic and coevolutionary analysis.

Subclass: Polyonchoinea Bychowsky, 1937

Order: Dactylogyridea Bychowsky, 1937

Family: Dactylogyridae Bychowsky, 1933

***Dactylogyrus marocanus* El Gharbi, Birgi & Lambert, 1994** (Fig. 2)

Type host: *Carasobarbus fritschii* (Günther, 1874) [syns. *Barbus* (*Labeobarbus*) *fritschii*; *B.* (*L.*) *paytonii*].

Type locality: Boulaouane Oum Er'Rabia Basin, Morocco.

Commenté [D8]: You are here combining two very different classifications. The Monogenea (an order) has two subordinate taxa, the Monopisthocotylea and the Polyopisthocotylea. On the other hand, the Class Monogenoidea has two subordinate taxa, the Heteronchoinea and Polyonchoinea. You cannot combine (and therefore confuse) the two classifications. I don't care which classification you choose to use, but combining them into some hybrid is not acceptable.

Commenté [D9]: By the way, many other synonyms. You might consider deleting such synonyms throughout the paper.

Other previously recorded hosts: *Carasobarbus harterti* (Günther, 1901) [syn. *Barbus* (*Labeobarbus*) *harteti*], *Labeobarbus reinii* (Günther, 1874) [syn. *Barbus* (*Labeobarbus*) *reinii*], *Luciobarbus ksibi* (Boulenger, 1905) [syn. *Barbus* (*Barbus*) *ksibi*], *Luciobarbus nasus* (Günther, 1874) [syn. *Barbus* (*Barbus*) *nasus*], *Luciobarbus setivimensis* (Valenciennes, 1842) [*Barbus* (*Barbus*) *setivimensis*] (El Gharbi et. al., ~~Birgi & Lambert,~~ 1994). Identification of *L. setivimensis* as host for *D. marocanus* is apparently erroneous (see Remarks for *D. ksibii*).

Other previously recorded localities: Oum Er'Rabia Basin (El Borj, Mechraa Ben Abbou); Tensift Basin, Zate River (Ait Ourir); Ksob Basin (Essaouira); Sebou Basin, Ouargha River (Ouazzane); Moulouya Basin (Mechraa Hammadi, Aklim); Morocco (El Gharbi et al., ~~Birgi & Lambert,~~ 1994).

Present hosts and localities: *Carasobarbus fritschii* (localities 1, 2, 3, 4, 7), *Luciobarbus ksibi* (localities 1, 3), *Luciobarbus zayanensis* Doadrio, Casal-López & Yahyaoui, 2016 (localities 4, 5), *Pterocapoeta maroccana* Günther, 1902 (locality 4).

Site on host: Gill lamellae.

Specimens deposited: Seven vouchers and two hologenophores (HELxxx) in the Muséum d'Histoire Naturelle, Paris.

Commenté [D10]: I assume from two different hosts. I note below that you sequenced specimens from 3 different hosts. What happened to the third hologenohore?

Representative DNA sequences: GenBank accession numbers KY629355 (28S rDNA), KY629333 (18S rDNA and ITS1).

Description

Description based on 10 specimens fixed in GAP. Body length 303 (263–348; *n* = 10); greatest width 80 (73–90; *n* = 10) at level of ovary. Anchors: total length 44 (41–50; n = 10); length to notch 22 (19–23; *n* = 10); inner root length 18 (15–20; *n* = 10); outer root length 2 (1–3; *n* = 10);

Commenté [D11]: I assume including the haptor. Right?

point length 10 (9–11; *n* = 10). One pair of needles located near hooks of pair V. Dorsal bar 24 (21–27; *n* = 10) long. Ventral bar reduced, 13 (12–15; *n* = 10) long. Hook lengths: pair I = 19 (18–20; *n* = 10); pair II = 19 (17–20; *n* = 10); pair III = 22 (20–25; *n* = 10); pair IV = 23 (21–26; *n* = 10); pair V = 18 (16–19; *n* = 10); pair VI =19 (16–24; *n* = 10); pair VII 18 (15–23; *n* = 10). MCO comprising basally articulated copulatory tube and accessory piece; total straight length 44 (38–57; *n* = 10); copulatory tube 47 (41–61; *n* = 10) long. Vagina lightly sclerotized, 5 (4–7; *n* = 10) long.

Commenté [D12]: These measurements should be deleted. They are arbitrary and cannot be repeated because they are based on soft points. For example, where is the dividing point between the anchor shaft and anchor point? Subjective!!

Commenté [D13]: Reduced from what?

Commenté [D14]: The MCO does not include the accessory piece. However, the copulatory complex includes both the MCO and accessory piece.

Commenté [D15]: What do you mean by "straight"? Did you measure along the curve(s) or was your measurement a straight-line distance between extreme points?

Molecular characterization

The partial 28S rDNA sequence of *D. marocanus* was 787 bp long, while the sequence including partial 18S rDNA, the entire ITS1 region and partial 5.8S rDNA was 972 bp long (478 bp corresponded to 18S rDNA, 483 bp corresponded to the ITS1 region and 11 bp corresponded to 5.8S rDNA). Seven specimens from three host species (*C. fritschii*, *L. ksibi*, and *P. maroccana*) were sequenced, with no intraspecific genetic variability between specimens parasitizing different host species found. Pairwise genetic distances between *D. marocanus* and other Moroccan *Dactylogyrus* spp. showed very high molecular divergence (Table 3).

Remarks

Dactylogyrus marocanus was originally described from the gills of six species belonging to three cyprinid genera (*Carasobarbus*, *Labeobarbus* and *Luciobarbus*) inhabiting five large river basins in Morocco (El Gharbi et. al., ~~Birgi & Lambert~~, 1994; see above). However, one of the host species recorded, *L. setivimensis*, was probably misidentified as the species only occurs naturally in coastal rivers in northeast Algeria (Soummam Basin) (Fricke et al., ~~Eschmeyer & Van de Laan,~~

2020). *Dactylogyrus marocanus* resembles several species belonging to the 'pseudanchoratus' species-group defined in Paperna (1979), in that it has anchors with a markedly elongated inner root, short outer root and a proximally swollen shaft (an attachment point for the anchor filament). It most closely resembles *Dactylogyrus longiphallus* Paperna, 1973 from African cyprinids in that it possesses a J-shaped copulatory tube and a rod-shaped accessory piece distally bifurcated to form two arms (one hook-shaped) that serve as a guide for the distal part of the copulatory tube. In addition, the MCO is markedly large in relation to the size of the haptoral structures in both species (compare with Plate XV, Figs. 1–11 in Paperna (1979)). However, *D. marocanus* is easily differentiated from other species belonging to the 'pseudanchoratus' species-group by the presence of the ventral bar. *Dactylogyrus marocanus* demonstrates a relatively low level of host specificity, having been recorded on eight host species of four genera from Morocco, including *L. zayanensis* and *P. maroccana* reported in our study. During the present survey, no morphological differences were found between specimens of this species collected from *C. fritschii, L. ksibii*, *L. zayanensis* and *P. maroccana*.

***Dactylogyrus ksibii* El Gharbi, Birgi & Lambert, 1994** (Figs. 3A, B)

Type host: *Luciobarbus ksibi* (Boulenger, 1905) [syn. *Barbus* (*Barbus*) *ksibi*]

Type locality: Ksob Basin (Essaouira); Morocco.

Other previously recorded hosts: *Luciobarbus magniatlantis* (Pellegrin, 1919) [syn. *Barbus* (*Barbus*) *magniatlantis*], *Luciobarbus setivimensis* (Valenciennes, 1842) [syn. *Barbus* (*Barbus*) *setivimensis*] (El Gharbi et. al., ~~Birgi & Lambert,~~ 1994). Identification of *L. setivimensis* as host for *D. ksibii* is apparently erroneous (see Remarks).

Commenté [D16]: See comments for the taxonomic summary of *D. marocanus*. Same apply here as in the taxonomic accounts of the remaining species.

Other previously recorded localities: Oum Er'Rabia Basin (Borj, Bounual); Tensift Basin, Ourika River (Ourika), Zate River (Ait Ourir); Mellah Basin (Khouribga); Bouregreg Basin, Grou River (Moulay Bouazza), Boulahmayl River (Aguelmouss); Morocco (El Gharbi et. al., ~~Birgi & Lambert,~~ 1994).

Present hosts and localities: *Luciobarbus ksibi* (Boulenger, 1905) (localities 1, 3), *Luciobarbus rabatensis* Doadrio, Perea & Yahyaoui, 2015 (locality 6).

Site on host: Gill lamellae.

Specimens deposited: Three vouchers and one hologenophore (HELxxx) (= *D. ksibii* 1 in our analyses) from *L. ksibi* (Ksob River), one voucher and one hologenophore (HELxxx) (= *D. ksibii* 2 in our analyses) from *L. ksibi* (Oum Er'Rabia River), and one hologehophore (HELxxx) (= *D. ksibii* 3 in our analyses) from *L. rabatensis* (Grou River) in the Muséum d'Histoire Naturelle, Paris.

Representative DNA sequences: GenBank accession numbers MN973812 (28S rDNA), MN974252 (18S rDNA and ITS1) for *D. ksibii* 1, MN973811 (28S rDNA), MN974251 (18S rDNA and ITS1) for *D. ksibii* 2, and MN973817 (28S rDNA), MN974250 (18S rDNA and ITS1) for *D. ksibii* 3.

Description

Description based on 20 specimens fixed in GAP. Body length 630 (580–670; $n = 20$); greatest width 128 (120–136; $n = 20$) at level of ovary. One pair of anchors located dorsally: total length 50 (45–53; $n = 20$); length to notch 43 (39–45; $n = 20$); inner root 20 (17–22; $n = 20$) long; outer root 7 (5–8; $n = 20$) long; shaft curved; point 10 (9–11; $n = 20$) long. One pair of needles located near hooks pair V. Dorsal bar broadly V-shaped, with slightly narrowed median part, 32 (29–35;

n = 20) long. Ventral bar cross-shaped, with five arms, 34 (32–36; *n* = 20) long, 27 (24–30; *n* = 20) wide. Hooks 7 pairs, similar in shape; each comprised of 2 subunits (proximal subunit expanded); filamentous hook (FH) loop extending to near level of termination of shank inflation; hook lengths: pair I = 26 (24–29; *n* = 20); pair II = 25 (23–26; *n* = 20); pair III = 31 (26–33; *n* = 20); pair IV = 31 (29–33; *n* = 20); pair V = 27 (25–30; *n* = 20); pair VI = 29 (27–31; *n* = 20); pair VII = 28 (26–30; *n* = 20). MCO comprising articulated copulatory tube and accessory piece; total straight length 30 (27–33; *n* = 20). Copulatory tube a loose coil following sinuous path; 76 (75–77; *n* = 13) long. Accessory piece proximally enclosing base of copulatory tube to form frill-belted capsule; medial portion with three processes: primary process distally articulated to the capsule by lightly sclerotized ligament; secondary process grooved, closely associated with wedge-shaped tertiary process, serving as a guide for distal part of the tube; distal portion recurved, elongated, following medial part of the copulatory tube. Vagina a wavy tube, with enlarged funnel-shaped opening, 63 (57–70; *n* = 20) long.

Molecular characterization

The partial 28S rDNA sequence of *D. ksibii* was 792 bp long, while the sequence including partial 18S rDNA, the entire ITS1 region and partial 5.8S rDNA was 978 bp long (478 bp corresponded to 18S rDNA, 489 bp corresponded to the ITS1 region and 11 bp corresponded to 5.8S rDNA). Nine specimens of *D. ksibii* from three different rivers (Ksob River, Oum Er'Rabia River and Grou River) were sequenced. Using molecular data, genetic variability was reported between specimens of *D. ksibii* parasitizing (i) *L. ksibi* collected from two different regions (Ksob River and Oum Er'Rabia River), and (ii) different host species (*L. ksibi* and *L. rabatensis*).

Pairwise distances calculated between *D. ksibii* and other *Dactylogyrus* species of Moroccan *Luciobarbus* are shown in Table 3.

Remarks

El Gharbi et. al., ~~Birgi & Lambert,~~ (1994) described *D. ksibii* from specimens collected on the gills of *L. ksibi* from the Ksob and Oum Er'Rabia Rivers, *L. magniatlantis* from the Tensift River (Tensift Basin) and *L. setivimensis* from the Boulahmayl (Bouregreg Basin) and Mellah (Mellah Basin) Rivers. However, ~~host~~ identification of the latter host species is probably erroneous (for the reason suggested above), with *L. rabatensis* apparently captured instead of *L. setivimensis* in the Boulahmayl River (Bouregreg Basin). All cyprinid specimens collected from the Bouregreg Basin during the present survey were identified as *L. rabatensis* (J. Vukic, pers. comm.). The Bouregreg Basin represents the current distribution area of this endemic Moroccan species (Casal-López et al., 2015). In the original description of *D. ksibii*, El Gharbi, et al. ~~Birgi & Lambert~~ (1994) showed four iconotypes for the MCO, without indicating which corresponded to the specimens of *D. ksibii* found on the type host species and locality (i.e. *L. ksibi*, Ksob River). In addition, these authors reported morphometric variation in the haptoral sclerites among specimens of *D. ksibii* parasitizing different host species and occurring in different localities. Rahmouni et al. (2017) suggested that these morphological differences in the haptoral sclerites could indicate that *D. ksibii* represented a complex of several morphologically similar species. In the present study, specimens morphologically identified as *D. ksibii* were found on the gills of *L. ksibi* (= type host) and *L. rabatensis*. Subsequent DNA analysis revealed that the specimens of *D. ksibii* consisted of three genotypes, one found on *L. ksibi* collected in the Ksob River, one on the same host species but from the Oum Er'Rabia River and one on *L. rabatensis* from the Grou

River (Table 2). A posteriori analysis of the specimens associated with the three genotypes revealed no morphological basis for splitting *D. ksibii* into two or three species (see Figs. 3A, B). Given that the level of morphological variation observed corresponds more to intraspecific variation, no new *Dactylogyrus* species are named at this time.

Dactylogyrus ksibii belongs to the group of congeners having a cross-shaped ventral bar (= 'carpathicus' type; El Gharbi et al., ~~Birgi & Lambert,~~ 1994). The MCO of *D. ksibii* most closely resembles that of *D. scorpius* Rahmouni, Řehulková & Šimková 2017, *D. benhoussai* Rahmouni, Řehulková & Šimková 2017 and *D. varius* Rahmouni, Řehulková & Šimková 2017, all three parasitizing species of Moroccan *Luciobarbus* and morphologically belonging to the 'scorpius' group (Rahmouni et al., 2017). *Dactylogyrus ksibii* differs from these three species by possessing a longer copulatory tube (76 vs 45 in *D. scorpius*; 76 *vs* 67 in *D. benhoussai*; 76 *vs* 65 in *D. varius*) and by details in the structure of the accessory piece. The accessory piece of *D. ksibii* is characterized by a medial part with a primary process closely associated with the secondary process (medial part with primary and secondary processes well defined/separated in *D. scorpius*, *D. benhoussai* and *D. varius*) and by a comparatively robust distal part (distal part smaller in *D. scorpius*, *D. benhoussai* and *D. varius*).

***Dactylogyrus borjensis* El Gharbi, Birgi & Lambert, 1994** (Fig. 4)

Type host: *Luciobarbus zayanensis* Doadrio, Casal-López & Yahyaoui, 2016, previously referred to as *Luciobarbus nasus* [syn. *Barbus* (*Barbus*) *nasus*] (see Remarks).

Type locality: Oum Er'Rabia Basin, El Borj; Morocco.

Other previously recorded hosts: *Luciobarbus magniatlantis* (Pellegrin, 1919), previously referred to as *L. nasus* [syn. *B.* (*B.*) *nasus*] (El Gharbi et al., ~~Birgi & Lambert,~~ 1994) (see Remarks).

Other previously recorded locality: Tensift Basin, Zate River (Ait Ourir); Morocco (El Gharbi et al., ~~Birgi & Lambert,~~ 1994).

Present host and localities: *Luciobarbus zayanensis* Doadrio, Casal-López & Yahyaoui, 2016 (localities 4, 5).

Specimens deposited: Three vouchers and two hologenophores (HELxxx) from *L. zayanensis* (El Borj) in the Muséum d'Histoire Naturelle, Paris.

Site on host: Gill lamellae.

Representative DNA sequences: GenBank accession numbers MN973819 (28S rDNA), MN974257 (18S rDNA and ITS1).

Description

Description based on 24 specimens fixed in GAP. Body length 624 (540–683; $n = 24$); greatest width 123 (100–130; $n = 24$) at level of ovary. One pair of anchors located dorsally: total length 47 (44–48; $n = 24$); length to notch 41 (40–42; $n = 24$); inner root 17 (15–19; $n = 24$) long; outer root 5 (4–6; $n = 24$) long; shaft curved, slightly swollen medially; point 12 (11–13; $n = 24$) long. Anchor filament conspicuous. One pair of needles located near hooks of pair V. Dorsal bar broadly V-shaped, with slightly rounded extremities, 36 (34–37; $n = 24$) long. Ventral bar cross-shaped, with five arms, 36 (34–38; $n = 24$) long, 28 (26–31; $n = 24$) wide. Hooks 7 pairs, similar in shape; each comprised of 2 subunits (proximal subunit expanded); FH loop extending to near level of termination of shank inflation; hook lengths: pair I = 25 (23–26; $n = 24$); pair II = 26

(25–28; *n* = 24); pair III = 31 (29–33; *n* = 24); pair IV = 31 (29–34; *n* = 24); pair V = 25 (23–27; *n* = 24); pair VI = 27 (26–30; *n* = 24); pair VII = 28 (26–30; *n* = 24). MCO complex, comprising articulated copulatory tube and accessory piece; total straight length 40 (36–42; *n* = 24). Copulatory tube a loose coil following sinuous path; 111 (107–120; *n* = 12) long. Accessory piece proximally enclosing base of copulatory tube to form frill-belted capsule; distal portion recurved, elongated, following medial part of copulatory tube; medial portion with three processes: primary process distally articulated to the capsule by lightly sclerotized ligaments; secondary process apically expanded into a wing like flap, closely associated with tertiary process serving as a guide for distal termination of the tube. Vagina a lightly sclerotized meandering tube, with disc-shaped opening, 92 (88–98; *n* = 24) long.

Molecular characterization

The sequence of partial 28S rDNA of *D. borjensis* was 792 bp long. The sequence including partial 18S rDNA, entire ITS1 region and partial 5.8S rDNA was 998 bp long (478 bp corresponded to 18S rDNA, 489 bp corresponded to ITS1 region, and 11 bp corresponded to 5.8S rDNA). Three specimens of *D. borjensis* from the Oum Er'Rabia River were sequenced, and no intraspecific variation between the specimens of this species was noted. Pairwise distances between *D. borjensis* and the other *Dactylogyrus* species from Moroccan *Luciobarbus* are shown in Table 3.

Remarks

Although the type specimens of *D. borjensis* ~~was~~ were not available (see Rahmouni et al., 2017). ~~, based~~ Based on the morphology of the haptoral and copulatory sclerites shown ~~showed~~ by El

Gharbi et al. ~~Birgi & Lambert~~ (1994), we consider our samples to be conspecific with this species. The original description is adequate except that these authors depicted the accessory piece of the MCO with the largest (secondary) medial process expanded into a tube through which the distal (recurved) part passes. As in other species of *Dactylogyrus* belonging to the "scorpius" group, the accessory piece is somewhat variable in this species, with the distal part bending inward to the right or left side of the medial part. This variability is likely a result of the compression used during preparation of individual worms for mounting. It appears likely that El Gharbi et al. ~~Birgi & Lambert~~ (1994) misinterpreted the distal part of the accessory piece as incorporated into the tubular part of the largest medial process, instead of lying under or above this part. In the present specimens of *D. borjensis*, the distal part of the accessory piece is not associated with any processes of the medial part. The largest process of the accessory piece is terminally extended to form wing like flap usually rolled in the shape of a sleeve, which serves as a guide for the distal part of the copulatory tube, and not for the distal part of the MCO ~~accessory piece~~ as it is depicted in the original description of *D. borjensis*.

Dactylogyrus borjensis is most similar to *D. falsiphallus* Rahmouni, Řehulková & Šimková, 2017, described on the gills of *L. maghrebensis* from the Lahdar and Sebou rivers (Sebou Basin) (Rahmouni et al., 2017), in the general morphology of the haptoral sclerites and MCO. It differs from *D. falsiphallus* by having relatively robust and evenly sclerotized distal part of the accessory piece (distal part reduced in its sclerotization into a long spike in *D. falsiphallus*). In addition, the MCO is measurably smaller in *D. falsiphallus*.

El Gharbi et al. ~~, Birgi & Lambert~~ (1994) listed *L. nasus* [syn. *B.* (*B.*) *nasus*] as the type host for this species. According to Doadrio et al. ~~Casal-López & Perea~~ (2016) the rheophilic *Luciobarbus* populations traditionally assigned to *L. nasus* (Günther, 1874) and *L. magniatlantis* (Pellegrin,

1919) comprise three species, each endemic for different basins in Morocco. *Luciobarbus nasus* is restricted to the Ksob Basin, *L. magniatlantis* to the Tensift Basin, and the recently described *L. zayanensis* to the Oum Er'Rabia Basin. Since the last basin represents the type locality for *D. borjensis*, we consider *L. zayanensis* as the type host of this species. The additional host record of *D. borjensis* on a population of *Luciobarbus* ~~population~~ species previously referred to *L. nasus* from Tensift Basin (El Gharbi et al., ~~Birgi & Lambert,~~ 1994) should be attributed to *L. magniatlantis*.

Commenté [D17]: Similarly, *Luciobarbus* is a taxon, not a host. Therefore, the population was of a species.

***Dactylogyrus draaensis* El Gharbi, Birgi & Lambert, 1994** (Fig. 5)

Type host: *Luciobarbus pallaryi* (Pellegrin, 1919) [syn. *Barbus* (*Barbus*) *pallaryi*].

Type locality: Draa Basin, Dadès River, Ait Oudinar; Morocco.

Other previously recorded locality: Draa Basin, Tidili River, Iouine River; Morocco (El Gharbi et. al., ~~Birgi & Lambert,~~ 1994).

Present host and locality: *Luciobarbus lepineyi* (Pellegrin, 1939) (locality 8).

Specimens deposited: Vouchers and hologenophores (HELxxx) in the Muséum d'Histoire Naturelle, Paris.

Site on host: Gill lamellae.

Representative DNA sequences: GenBank accession numbers: MN973816 (28S rDNA), MN974258 (18S rDNA and ITS1).

Description

Description based on 30 specimens fixed in GAP. Body length 790 (645–901; $n = 16$); greatest width 120 (110–125; $n = 30$) at level of ovary. Anchors: total length 51 (48–55; $n = 30$); length

to notch 39 (36–42; *n* = 30); inner root length 19 (17–21; *n* = 30); outer root length 5 (3–6; *n* = 30); point length 12 (11–14; *n* = 30). One pair of needles located near hooks of pair V. Dorsal bar 41 (34–45; *n* = 30) long. Ventral bar cross-shaped, with five arms, 51 (37–56; *n* = 30) long, 36 (27–45; *n* = 30) wide. Hook lengths: pair I = 29 (25–35; *n* = 27); pair II = 29 (25–32; *n* = 29); pair III = 31 (29–35; *n* = 25); pair IV = 31 (24–36; *n* = 29); pair V = 28 (24–33; *n* = 29); pair VI = 31 (27–38; *n* = 28); pair VII = 31 (28–36; *n* = 25). MCO complex, comprising articulated copulatory tube and accessory piece; total straight length 43 (40–52; *n* = 30); copulatory tube 116 (113–120; *n* = 25) long. Vagina 54 (47–60; *n* = 30) long.

Molecular characterization

The partial 28S rDNA sequence of *D. draaensis* was 792 bp long. The sequence including partial 18S rDNA, the entire ITS1 region and partial 5.8S rDNA was 998 bp long, of which 478 bp corresponded to 18S rDNA, 489 bp corresponded to the ITS1 region and 11 bp corresponded to 5.8S rDNA. Three specimens of *D. draaensis* from the Zouala Oasis were sequenced, with no intraspecific variability between specimens of this species noted. Pairwise distances between *D. draaensis* and the other *Dactylogyrus* species of Moroccan *Luciobarbus* are shown in Table 3.

Remarks

Dactylogyrus draaensis was originally described from the gills of *L. pallaryi* from two rivers in the Draa Basin (El Gharbi et al., ~~Birgi & Lambert,~~ 1994). The species is characterized by possessing a sinuous copulatory tube with the distal part supported by two auricle-like processes rising from the accessory piece. During the present survey, specimens provisionally identified as *D. draaensis* were collected from *L. lepineyi* in the Zouala Oasis. The drawings provided by El

Gharbi et al. ~~Birgi & Lambert~~ (1994) were unclear regarding the morphology of the medial part of the accessory piece, hence we cannot state with certainty that our specimens are conspecific with those of *D. draaensis* from the Draa Basin. Assignment of a new species name to our specimens is not made at this time and will depend on re-collection of *D. draaensis* from the type host in the type locality for comparison with our specimens.

Phylogenetic reconstruction

A concatenated sequence alignment (partial 28S, 18S rDNA and ITS1 combined) was used to construct a phylogenetic tree including 24 species, the alignment comprising 1458 aligned positions. Both phylogenetic analyses yielded similar tree topologies. The BI tree is presented in Figure 6, with bootstrap support values for ML and posterior probabilities for BI.

The phylogenetic reconstruction divided *Dactylogyrus* spp. parasitizing Moroccan cyprinids into three lineages. The first well-supported clade includes three species of *Dactylogyrus* (*D. kulindrii*, *D. volutus*, and *D. zatensis*) from Northwest African *C. fritschii* (Torinae) (Table 2). These species all have 'varicorhini' type of the anchors and bars: the anchors possess shaft turned into a point with a sharp-stepped narrowing from the inner side of the anchor; dorsal bar is saddle-shaped with posterior groove, usually giving the impression of butterfly wings; and ventral bar is V- or omega-shaped (El Gharbi et al, ~~Renaud & Lambert,~~ 1992; Pugachev et al., 2009). A sister relationship between *D. volutus* and *D. kulindrii* is also supported by morphological similarities in their MCOs, i.e. the copulatory tube is relatively wide in diameter and a simple accessory piece possesses two projections guiding the distal portion of the copulatory tube. The second clade, with several subclades, comprises species of *Dactylogyrus* collected from European and Moroccan species of *Barbus* and *Luciobarbus* (Table 2).

Commenté [D18]: See comment above concerning the root of the tree. You should eliminate the root from your figure 6.

Commenté [D19]: You have three lineages based on where you placed the root, but if the root occurred between the clade of 3 and the remaining terminal taxa, you would have two (one clade with four species (including *D. marocanus*) and one with remaining species). Further, you have no idea where the root might occur because you didn't use an outgroup. It just as likely could have occurred somewhere within the terminal clade (with many species). **Again, remove the root from your tree.**

Another point regarding your Figure 6. You have many branchings which lack significance. These should be collapsed.

Dactylogyrus andalousiensis El Gharbi, Renaud & Lambert, 1992 from Iberian *Luciobarbus sclateri* (Günther, 1868) (Portugal) was sister to a well-supported clade formed by nine species of *Dactylogyrus* parasitizing Moroccan *Luciobarbus*, all of which were characterized by i) a cross-shaped ventral bar with five extremities (= 'carpathicus' or 'barbus' type; see Pugachev et al., 2009), ii) an MCO possessing an accessory piece with the distal portion directed backwards along the circle of the curved copulatory tube (= 'chondrostomi' type; see Pugachev et al., 2009), a complex medial portion formed into ridge-shaped processes supporting the distal part of the copulatory tube and capsule-like proximal portion (= 'scorpion' subtype; Rahmouni et al., 2017). The basal position of *D. draaensis* in relation to the other *Dactylogyrus* spp. from Moroccan *Luciobarbus* was well supported by PP resulting from BI analysis and moderately/weakly supported by BP resulting from ML analysis. *Dactylogyrus borjensis* is sister to the well-supported clade formed by two weakly supported assemblages represented by *D. benhoussai* and *D. varius* clustered together and *D. scorpius* clustered with three genotypes of *D. ksibii*. All four *Dactylogyrus* species are morphologically very similar, both in the haptoral structures and the MCOs. Finally, *D. marocanus* formed a separated lineage supported by differing morphology.

Discussion

Since the anatomical details of dactylogyrids are generally poorly known, discrimination between species of *Dactylogyrus* relies chiefly on the morphometric characteristics of sclerotized structures of the haptor and reproductive organs. In recent years, however, molecular phylogenetic analysis has revealed hidden genetic variation and/or cryptic species within this genus. Rahmouni et al. (2017) reported two cryptic species of *Dactylogyrus* (*D. benhoussai* and *D. varius*) on two species of Moroccan *Luciobarbus* species (*L. yahyaouii* and *L. maghrebensis*,

Commenté [D20]: The reason being that too many descriptions of dactylogyrids coming out (particularly out of Africa are "anchor & hook" description, with nearly total exclusion of internal anatomy. "Anchor & hook" descriptions were good enough during 1930s-1960s, but are insufficient these days.

Commenté [D21]: This is nice, but we know that molecules and organisms do not evolve in the same way. As a result, molecular phylogenies may not provide an accurate picture of what happened historically.

501 respectively), while Benovics et al. (2018) revealed potential cryptic species complexes within
502 three species of *Dactylogyrus* (*Dactylogyrus rutili* Glaeser, 1965, *Dactylogyrus dyki* Ergens &
503 Lucký, 1959 and *Dactylogyrus ergensi* Molnar, 1964) parasitizing Balkan cyprinids. As different
504 ribosomal DNA molecular markers show differing rates of evolution, they are appropriate for
505 evaluating genetic divergence at different levels (Huelsenbeck & Ronquist, 2001). In the present
506 study, we applied genetic markers widely used for monogeneans, i.e. 18S rDNA, 28S rDNA and
507 the ITS1 region (Cunningham, 1997; Meinilä et al. 2002; Ziętara & Lumme, 2002). Molecular
508 characterization of *Dactylogyrus* species in our study showed that specimens identified
509 morphologically as *D. ksibii*, collected from the gills of two different hosts (*L. ksibi* and *L.*
510 *rabatensis*) and geographically distant basins, exhibited a low level of intraspecific variability
511 (0.8% for the combined 18S rDNA and ITS1 sequences). Molecular divergence observed
512 between specimens of *D. ksibii* may be explained by the large geographical distances between
513 parasites and hosts precluding gene flow between isolated populations. Our findings indicate that
514 specimens of *D. ksibii* reported by El Gharbi et al. ~~, Birgi & Lambert~~ (1994) from *Luciobarbus*
515 species inhabiting various river basins in Morocco need serious reinvestigation in the future
516 using morphological and molecular data.
517 Besides providing crucial taxonomic information on species, the sclerotized structures of the
518 haptor and reproductive organs are of particular interest in evolutionary studies focused on the
519 link between morphological and molecular interspecific similarities of *Dactylogyrus* spp. (e.g.
520 Benovics et al., ~~Kičinjaová & Šimková,~~ 2017; Benovics et al., 2018). In addition, as the
521 morphology of the attachment organ is usually viewed as the result of adaptive processes to the
522 host microenvironment, the morphological characteristics of the haptor may have the potential to
523 reflect the phylogeny and historical biogeographical routes of their hosts (e.g. Šimková et al.,

Commenté [D22]: It would be useful for you to provide hosts and localities for each of the three specimens of D. ksibii you sequenced. This would fit (and perhaps) support the rest of the sentence.

Commenté [D23]: Give a reference for this clause.

524 2006). On the basis of morphological resemblance, many *Dactylogyrus* species can be grouped
525 into morphological types, often derived from a single structure (Pugachev et al., 2009). Some of
526 these groups may be considered as phylogenetic units, members of one unit often parasitizing
527 closely related hosts. However, it is relatively difficult to determine to what extent
528 morphological features reflect a phylogenetic signal unless adaptive forces associated with the
529 possibility of host switching cannot be excluded.
530 Based on the shape of the ventral bar, species of *Dactylogyrus* parasitizing *Luciobarbus* spp. are
531 grouped into four morphological types, i.e. those with a rod-shaped, omega-shaped, inverted T-
532 shaped or cross-shaped ventral bar (see Pugachev et al., 2009). All Moroccan species of
533 *Dactylogyrus* that are host-specific to *Luciobarbus* spp. belong to the group with a cross-shaped
534 ventral bar, where the anterior arm is widely bifurcated and the posterior arm is more or less split
535 (= five radial type; 'carpathicus' or 'barbus' type in El Gharbi et al. ~~Birgi & Lambert~~ (1994) and
536 Pugachev et al. (2009), respectively). *Dactylogyrus* spp. with this type of ventral bar have also
537 been recorded on *Luciobarbus* spp. inhabiting the Balkan Peninsula (Řehulková et al.,
538 unpublished) and the region around the Caspian Sea (Pugachev et al., 2009), and on *Aulopyge*
539 *huegelii* Benovics, Kičinjaová & Šimková, 2017 in the Balkans (Benovics et al., ~~Kičinjaová &~~
540 ~~Šimková,~~ 2017). The majority of *Dactylogyrus* species (except *D. andalousiensis* and
541 *Dactylogyrus linstowoides*) reported on *Luciobarbus* spp. on the Iberian Peninsula have V- or
542 omega-shaped ventral bars. Inasmuch as the haptor has to be evolutionary adapted as far as
543 possible to the host microenvironment (e.g. Kearn, 1968; Šimková et al., 2001), the same
544 morphological type of haptor in Moroccan and Balkan-Caspian *Dactylogyrus* spp. may suggest
545 that *Luciobarbus* spp. inhabiting these regions share a common ancestor. This assumption is also
546 supported by Tsigenopoulos et al. (2003) and Yang et al. (2015), who showed that most

Luciobarbus spp. from Northwest Africa are more closely related to *Luciobarbus* spp. from the Middle East than to those ~~*Luciobarbus*~~ from the Iberian Peninsula. In addition, on the basis of phylogenetic reconstruction, Šimková et al. (2017) demonstrated that species of *Dactylogyrus* parasitizing ~~Northwest~~ northwest African *Luciobarus* have a European/west Asian origin. When looking at MCO morphology, the 'Moroccan' and 'Balkan-Caspian' species of *Dactylogyrus* with a cross-shaped ventral bar are characterized by different types of MCO, i.e. the 'chondrostomi' and 'kulwieci' types, respectively (Pugachev et al., 2009). However, the 'chondrostomi' group, characterized by an accessory piece with the distal portion directed backwards along the circle of the curved copulatory tube, is one of the most specious ~~numerous~~ groups within the Palaearctic ~~*Dactylogyrus* species~~ exhibiting different types of ventral bars (Pugachev et al., 2009). On the basis of detailed morphology of the accessory piece, it would appear that Moroccan species of *Dactylogyrus* form another subgroup within this group, named by Rahmouni et al. (2017) as the 'scorpius' subgroup. This subgroup is characterized by an accessory piece with a complex medial portion formed into ridge-shaped processes supporting the distal part of the copulatory tube and a relatively massive capsule-like proximal portion. It would be interesting to further investigate the degree of relatedness between Moroccan and other *Dactylogyrus* spp. within the 'chondrostomi' type of MCO, with the intention of answering the question of whether the similarity between MCOs is a result of homoplasy or shows a phylogenetic signal.

Concerning host specificity, *D. marocanus* exhibits an unusually broad host range that includes phylogenetically distant host species. Our records of *D. marocanus* on the gills of *L. zayanensis* and *P. maroccana* ~~and~~ increases the range of available host species such that the host range now includes eight species of four cyprinid genera representing two phylogenetic lineages, i.e. the

Commenté [D24]: What group? List it here rather than using the pronoun. The "chonrostomi group"? Be very careful with use of pronouns as it might be clear to you what you are referring to, but not so for your readers.

570 Torinae, including *Carasobarbus*, *Labeobarbus* and *Pterocapoeta*, and the Barbinae, including
571 *Luciobarbus*. El Gharbi et al. ~~, Birgi & Lambert~~ (1994) suggested that *C. fritschii* [syn.
572 *Labeobarbus fritschii*] represents the original host of *D. marocanus*; however, Šimková et al.
573 (2017) showed that *D. marocanus* is closely related to *Dactylogyrus* spp. parasitizing West
574 African species assigned to ~~of~~ *Labeo*, which suggests a host-switch from African labeonins
575 ~~Labeoninae~~ to *C. fritschii* and the other reported Northwest African cyprinid hosts. In terms of
576 morphology, *D. marocanus* is the only Moroccan *Dactylogyrus* species belonging to the
577 'pseudanchoratus' group (El Gharbi et al., ~~Birgi & Lambert,~~ 1994), which includes *Dactylogyrus*
578 spp. reported on cyprinids (mostly species of *Labeo*) from the wider equatorial region in Africa
579 (Paperna, 1979) and, interestingly, *Dactylogyrus* spp. parasitizing *Garra rufa* (Labeoninae) in
580 Iran (Gussev et al., ~~Jalali & Molnár,~~ 1993; Pugachev et al., 2009). The molecular phylogeny of
581 labeonins provided by Tang et al. ~~Getahun & Liu~~ (2009) showed that the Asian *G. rufa* and
582 African *Garra* spp. formed ~~two~~ sister groups, and that this Afro-Asian clade was nested within a
583 larger clade containing all other Asian *Garra* spp. In addition, their study supports an East Asian
584 origin of labeonins, and ~~in to~~ into-Africa dispersal events for the African species of *Garra* and
585 *Labeo*. If the phylogeny of highly host-specific parasites follows the phylogeny and historical
586 biogeography of their hosts, it would be interesting to analyze the phylogenetic relationship
587 between *Dactylogyrus* spp. parasitizing *G. rufa* and those of the 'pseudanchoratus' group
588 (including *D. marocanus*) parasitizing African labeonins. The close relationship between these
589 *Dactylogyrus* spp., supported by both morphological and molecular data, could point to their
590 common Asian origin.
591 Our phylogenetic analysis confirmed the results of Šimková et al. (2017), suggesting three
592 independent origins of *Dactylogyrus* spp. parasitizing Northwest African cyprinid fishes.

Commenté [D25]: Sister groups implies 2 taxa.

Dactylogyrus spp. from Moroccan *Luciobarbus* (Barbinae) hosts represent the largest (monophyletic) group in number of species. High similarity in morphology of the sclerotized structures in these parasites, together with high host specificity, could suggest rapid diversification following the geographical separation and diversification of *Luciobarbus* spp. The basal position of *D. andalousiensis* parasitizing Iberian *L. sclateri* in relation to the monophyletic group including *Dactylogyrus* spp. parasitizing Moroccan *Luciobarbus* spp. (Barbinae) ~~confirms~~ strongly supports (or strongly suggests that) a European origin for this group of parasites, as previously shown by Šimková et al. (2017). *Dactylogyrus* spp. parasitizing fishes of Moroccan Torinae (species of *Carasobarbus* and *Labeobarbus*) form the second largest group characterized by the 'varicorhini' morphological type of sclerotized structures (El Gharbi et al., ~~Birgi & Lambert,~~ 1994), probably originating from Asian cyprinids (Šimková et al., 2017). Mapping the evolutionary history of *D. marocanus* is difficult due to its low level of host specificity; however, morphological and molecular data suggest an affinity of this species to *Dactylogyrus* spp. parasitizing African labeonins (Šimková et al., 2017). The phylogenetic position of *Dactylogyrus guirensis* El Gharbi, Birgi et Lambert, 1994, the only Moroccan species of the 'guirensis' morphological type (El Gharbi et al., ~~Birgi & Lambert,~~ 1994), remains unresolved as no molecular data are available for this species at this time.

Commenté [D26]: You haven't confirmed anything. You have supported the idea but you did not "prove" or "confirm" anything. There could easily be other expanations.

Acknowledgements

We wish to thank Jasna Vukić from Faculty of Science, Charles University, Prague, for fish collection and determination. We are also grateful to Professor Abdelaziz Benhoussa from Mohamed V University of Rabat for the help in the fish collection. Thanks are also due to Mária

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616 collection and fixation. We kindly thank Kevin Roche for English revision of the final draft.
617

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