

Research article

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A new species of *Gyrodactylus* (Monogenea, Gyrodactylidae), an ectoparasite from the endemic *Iranocichla hormuzensis* (Teleostei, Cichlidae), the only Iranian cichlid

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Abstract. *Iranocichla hormuzensis* occupies a biogeographically peculiar position. This endemic of southern Iran is the only Iranian cichlid. While it is phylogenetically related to African oreochromine members of the cichlid family, it remains unclear how it has dispersed into its current range. It is one of the many lasting enigmas of cichlid biogeography. Monogenean fish parasites may provide useful additional information in such cases. Therefore, *I. hormuzensis* was examined for these flatworms. A gyrodactylid parasite is reported and compared to congeners from the Palearctic and from cichlids. In this way, we verify whether it shows affinities to parasites from fishes that are either biogeographically or phylogenetically close to *Iranocichla hormuzensis*. The species is new to science and is described as *Gyrodactylus jalalii* sp. nov. This is the first description of a parasite infecting *I. hormuzensis*. Because of the fixation method or age of the material, DNA could not be isolated. Due to the lack of genetic data, no conclusions can be drawn on its phylogenetic positioning. Indeed, *Gyrodactylus* phylogeny cannot be

inferred from morphological characteristics alone. Moreover, the congeners phenotypically reminiscent of the new species belong to a *Gyrodactylus* clade which is highly diverse in geographic range and host choice. Hence, there is no evidence linking the new species to an exclusively African or cichlid-bound *Gyrodactylus* lineage.

Keywords. *Gyrodactylus jalalii* sp. nov., Oreochromini, Platyhelminthes, Perciformes, species description.

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Introduction

The fascination and scientific interest that cichlids raise in many scientists and hobbyists is well reviewed by Barlow (2000). This author aptly shows the importance of cichlid fishes in a wide range of fields in evolutionary biology, from ecology to behavior and from developmental biology to speciation. Numbering about 1350 described species, with hundreds remaining to be discovered (Nelson 2006), these “model” fishes are the most species-rich vertebrate family worldwide (Kocher 2004). Counting only described species, Cichlidae is the largest non-ostariophysan freshwater fish family (Kullander 2003). Its species richness reaches its peak in the more than 900 African representatives. Some relatives to African cichlids occur in the Middle East (belonging to *Astatotilapia* Pellegrin, 1904; *Oreochromis* Günther, 1889; *Sarotherodon* Rüppell, 1852; *Tilapia* Smith, 1840 and *Tristramella* Trewavas, 1942) (Werner & Mokady 2004) and one in Iran. The latter is *Iranocichla hormuzensis* Coad, 1982, the only representative of the monotypic *Iranocichla* Coad, 1982. The species was first reported by Behnke (1975) and Saadati (1977) before being formally described (Coad 1982). This southern Iranian endemic cichlid is found in mostly saline riverine systems draining into the Strait of Hormuz (Persian Gulf). The question rises how it arrived in this biogeographically isolated range.

Most cichlid species in the Middle East have congeners among the African “tilapiine” (*Oreochromis*, *Tilapia* and *Sarotherodon*: Schwarzer *et al.* 2009) or haplochromine (*Astatotilapia*: Werner & Mokady 2004) cichlids. *Tristramella* and *Iranocichla*, however, are endemic genera. Phylogenetically, they are also placed in Oreochromini, one of the tribes into which “tilapiine” cichlids were split by Schwarzer *et al.* (2009). Thus, they are closely affiliated to African cichlids. Middle-Eastern cichlids at present only occur in the Levant and these Iranian systems. This fact might indicate a relict-type consequence of a recent (Pleistocene to Recent) aridification event and decrease in temperature (overview in Coad 1982). Furthermore, the Persian Gulf was a river valley between 90,000 and 10,000 years ago. See also Arndt *et al.* (2003) for a discussion on how the low level of the Mediterranean during Pleistocene episodes is suggested to have allowed freshwater dispersal from the Nile to the Levant. Although those conditions would have allowed dispersal of cichlids, it is also possible that the marine environment would not represent a barrier to the spread of these animals, anyway (see references below). Indeed, occupying mostly saline streams shows that *I. hormuzensis* is salt-tolerant (Coad 1982). Moreover, several “tilapiines” are known to disperse between rivers through brackish coastal waters (Nelson 2006). Conversely, Murray (2001) favors marine dispersal through the Tethys Sea/Indian Ocean to coastal dispersal in view of the absence of cichlids along the rest of the region’s coastlines.

Either way, both freshwater and marine dispersal pathways could explain the presence of this cichlid in waters draining into the Strait of Hormuz. Be it on a local or a global scale, the history behind current cichlid distribution across continents has not been unequivocally unraveled. The roles of intercontinental

dispersal, on the one hand, and of vicariance (following the break-up of Gondwana) and intra-continental freshwater dispersal, on the other hand, are still highly debated. A potential source of information that remains largely unexplored is the (monogenean) parasite fauna of cichlid species (reviewed in Pariselle *et al.* 2011). Many parasites have a close association with their host and a shorter generation time than their host, hence providing an alternative perspective on host evolution. Ectoparasitic monogenean flatworms often do not tolerate substantial changes in salinity, as indicated by their distribution and phylogenetic patterns worldwide (although there are exceptions, e.g., this is less the case in Gyrodactylidae, certainly when infecting euryhaline hosts, see below). One might therefore hypothesize that certain freshwater monogeneans rarely survive dispersal through barriers represented by marine environments. Hence, the phylogenetic relationships within a lineage of monogenean ectoparasites reflect both continental patterns and host phylogeny. Freshwater fish hosts most likely retain their monogenean assemblage when dispersing through continental surface waters. Conversely, it was suggested that most freshwater fishes might lose their autochthonous parasites during marine dispersal events. This would allow subsequent recolonisation by “local” parasite species once arriving in freshwater at the other end of a marine barrier (Pariselle *et al.* 2011).

Here, a *Gyrodactylus* species parasitizing *I. hormuzensis* is described. We analyze its affinity to congeners infecting other cichlids and to species known from fishes of the same region. Because of the ability to switch hosts, gyrodactylids are expected to mainly mirror continental affinities rather than the relationships between its host species (Boeger *et al.* 2003), depending of course on the time-scale and the dispersal routes used. The result might hence shed light on the pathway cichlids used to reach southern Iran.

Material and Methods

Eight host fishes (fixed and stored in ethanol or in formaldehyde) were retrieved from collections of the Royal Museum for Central Africa (Tervuren, Belgium) (MRAC B2-28-P-1-8). Branchial arches, body, fins and recipient were inspected for parasites under a Wild M8 stereomicroscope. Monogeneans were removed with a dissection needle. They were treated with 10% sodium dodecyl sulphate for a couple of minutes, before being fixed using ammonium picrate-glycerine (Malmberg 1957) and mounted on a slide under a cover-slip. Measurements were adapted from Shinn *et al.* (2004) using a Zeiss Axio Imager Z1 microscope at a magnification of 100 x (oil immersion, 10 x ocular) under differential interference contrast, with an AxioCamMR3 camera and AxioVision v.4.2.8 software. For drawings, some specimens were stained with Gomori’s trichrome and mounted in Canada balsam for study of their soft anatomy; other specimens were cleared and mounted in Hoyer’s or Gray and Wess’ media for study of their sclerotized structures (all solutions prepared as in Humason, 1979). Illustrations were prepared with the aid of a digital camera (5 megapixels) and a projector attached to an Olympus BX51 microscope equipped with phase contrast. Taxon and author names in this study follow Eschmeyer (2012) for hosts and MonoDb (<http://www.monodb.org>) for Monogenea.

Results

A single (incomplete and therefore unfortunately unidentified) ancyrocephaline monogenean (Dactylogyridae, Ancyrocephalinae) was recovered. All other monogeneans found on the *I. hormuzensis* specimens belong to *Gyrodactylus*.

Class Monogenea Van Beneden, 1858
Subclass Polyonchoinea Bychowsky, 1937
Order Gyrodactylidea Bychowsky, 1937
Family Gyrodactylidae Van Beneden & Hesse, 1863
Subfamily Gyrodactylinae Van Beneden & Hesse, 1863
Genus *Gyrodactylus* von Nordmann, 1832

***Gyrodactylus jalalii* sp. nov.**

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Figs 1-2

Etymology

The species epithet, *jalalii*, honours prof. dr. Behiar Jalali Jafari (1953-2010) (obituary in Shamsi 2010). He was a researcher in aquatic animal health and fish parasitology at the Veterinary Department of the Islamic Azad University (Iran) and a keen student of monogeneans. The authors express the hope that this patronym might serve as an indication for the respect and appreciation this kind man enjoyed from his colleagues.

Type material examined

Thirty-one specimens, twenty of which (ethanol-preserved) were used for measurements. The holotype (MNHN HEL301) and paratypes (MNHN HEL302–HEL305) are deposited in the Muséum National d'Histoire Naturelle (Paris, France). Paratypes are deposited in the Natural History Museum (London, United Kingdom) (NHMUK 2012.9.10.1–2012.9.10.2), the Royal Museum for Central Africa (Tervuren, Belgium) (MRAC MT: 37711–37713), the Harold W. Manter Laboratory of Parasitology (Lincoln, Nebraska) (HWML-49758) and the United States National Parasite Collection (Beltsville, Maryland) (USNPC 106050).

Type host

Iranocichla hormuzensis Coad, 1982 (Teleostei, Perciformes, Cichlidae).

Type locality

Mehran River, Persian Gulf Basin (2009).

Infection site

Gill filaments, fins, eye.

Description

(measurements in micrometres (µm) and angles in degrees (°); average ± standard deviation, followed by range and number of measurements in parentheses).

Body (Fig. 1A) fusiform, 361.7 ± 64.4 (294.7-528.9; $n = 13$) long; greatest width 128.1 ± 21.3 (98.8-164.0; $n = 13$). Two head organs provided with single spicule each. Cephalic glands lateral to pharynx. Pharynx composed of two tandem, muscular bulbs. Oesophagus short. Digestive glands lateral to oesophagus. Caeca two, non-confluent, reaching level of germarium. Male copulatory organ (MCO) (Fig. 1B) armed with a broad-based, robust, recurved, apical spine 5.7 ± 1.1 (4.5-7.6; $n = 8$) long, 5-7 smaller flanking spines in a single row, becoming more slender from the terminal over the subterminal towards the median ones (terminology of García-Vásquez *et al.* 2007). Testis dorsal to germarium.

Germarium immediately posterior to uterus. Uterus with up to 2 embryos. Unicellular glands lateral to terminations of caeca, posterior to germarium. Haptor elongate (Fig. 1A, D). Anchor (hamulus) (Fig. 1E) 79.9 ± 4.4 (70.4-86.3; $n = 20$) long; point 33.9 ± 2.3 (28.3-38.4; $n = 19$) long; base (superficial root) 28.2 ± 3.9 (19.7-33.1; $n = 20$) long; deep root knob-like; groove proximally at the base of the anchor, serving as articulation to superficial (ventral) bar; shaft 49.5 ± 2.2 (45.6-55.0; $n = 19$) long, proximally 10.2 ± 0.8 (8.7-11.9; $n = 20$) wide, distally 5.7 ± 0.8 (4.5-7.4; $n = 19$) wide; point sharply curved, with aperture 30.5 ± 2.5 (26.2-35.4; $n = 20$), aperture angle 42.7 ± 3.2 (36.9-47.3; $n = 19$), inner aperture angle 46.9 ± 5.3 (30.1-53.3; $n = 19$); inner curve length 1.9 ± 0.8 (0.7-3.3; $n = 18$) with point curve angle 9.5 ± 4.5 (4.2-22.9; $n = 18$). Ventral bar (Fig. 1D) 33.5 ± 2.5 (29.8-37.7; $n = 20$) wide, 44.1 ± 3.9 (36.4-49.8; $n = 20$) long; anterior bilateral processes slender, pronounced and 6.0 ± 1.1 (4.2-7.8; $n = 20$) long with process to mid-length 10.4 ± 1.7 (7.5-14.8; $n = 20$). Median portion of ventral bar

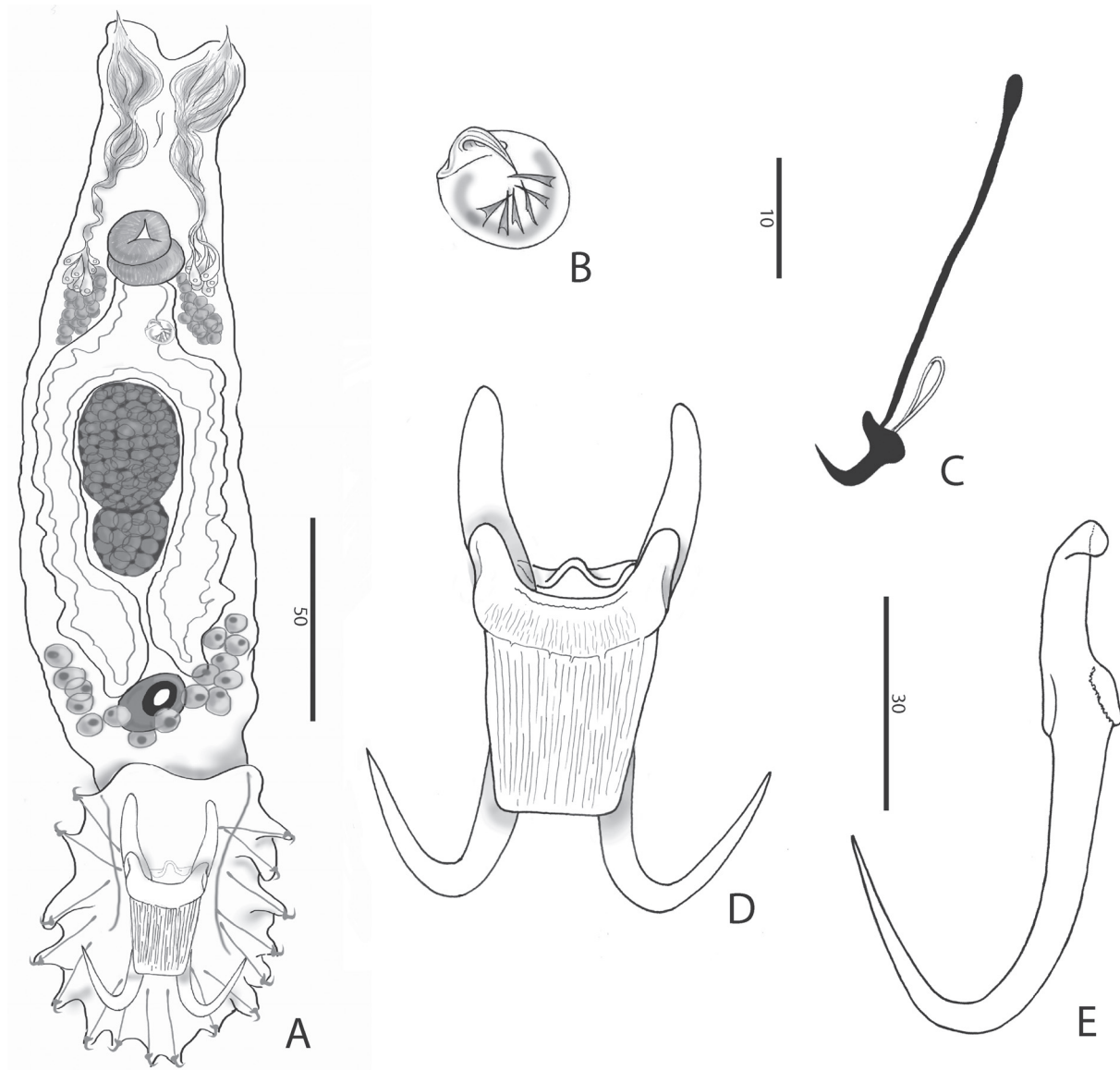


Fig. 1. *Gyrodactylus jalalii* sp. nov. **A.** whole mount. **B.** male copulatory organ. **C.** marginal hook. **D.** anchor-bar complex. **E.** anchor. Scale bars represent 50 μ m (whole mount), 10 μ m (marginal hook, MCO) or 30 μ m (anchor, anchor-bar complex).

8.3 ± 1.2 (5.7-9.8; *n* = 20) long; shield (ventral bar membrane) 25.4 ± 3.3 (19.8-30.1; *n* = 20) long, subrectangular, clearly striated in much the same way as bar proper. Deep (dorsal) bar 23.0 ± 2.8 (18.9-29.9; *n* = 20) wide, medially constricted and at connection to deep root of anchors. Marginal hook (Figs 1C, 2A, B) 31.8 ± 4.1 (25.8-43.5; *n* = 20) long, shank with small distal bulb, 26.9 ± 4.1 (22.1-40.0; *n* = 20) long; sickle (hooklet) 5.4 ± 0.3 (4.5-6.1; *n* = 20) long, 4.3 ± 0.4 (3.6-5.1; *n* = 20) wide proximally, 4.5 ± 0.5 (3.8-5.3; *n* = 20) distally; toe depressed, 2.0 ± 0.4 (1.4-3.0; *n* = 20) long; convex platform; concave base; round keel; point of sickle proper as long as shaft, forming an angle of about 90° from each other; aperture 5.2 ± 0.5 (4.5-6.4; *n* = 20); instep/arch height 0.6 ± 0.1 (0.4-0.8; *n* = 20).

Remarks

In comparison with congeners parasitizing cichlids, the striated ventral bar proper and shield, as well as the conspicuous ventral bar processes, seem most reminiscent to *G. zimbae* Vanhove, Snoeks, Huyse & Volckaert, 2011. However, the anterolateral processes of the ventral bar of *G. zimbae* are more ear-shaped. In *G. zimbae*, the ventral bar shield is slender and rounded and the hooklet lacks an arched base (*versus* subrectangular shield and concave hooklet base in *G. jalalii* sp. nov.). Other cichlid *Gyrodactylus* with relatively large ventral bar processes include *G. shariffi* Cone, Arthur & Bondad-Reantaso, 1995 and *G. yacatli* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011. Just like in *G. jalalii* sp. nov., point and shaft of their hooklet sickle are at a right angle. These species are easily distinguished from *G. jalalii* sp. nov. by the smaller size of their haptoral sclerites and the ventral bar in particular (e.g., anchor 47.5 and 48.4 long, ventral bar shield 14.4 and 8.5 long, in *G. shariffi* and *G. yacatli* respectively) (García-Vásquez *et al.* 2011). It should be noted, however, that these two species were described from cultured *Oreochromis niloticus* (Linnaeus, 1758) in the Philippines, resp. Mexico. The authors describing *G. yacatli* consider accidental infection or host switch a more likely scenario than an African origin (García-Vásquez *et al.* 2011). Hence, *G. zimbae* seems to be the most comparable cichlid parasite whose natural distribution is certainly African.

Comparison to Palearctic congeners followed Pugachev *et al.* (2009). The rather large ventral bar processes, in combination with the length of the marginal hooks, and MCO armed with one large apical spine and one row of smaller spines of similar size, resemble the morphology of *G. ophiocephali* Gussev, 1955 from *Channa argus* (Cantor, 1842) (Perciformes: Channidae) and *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes, Cyprinidae), and to *G. tokobaevi* Ergens & Karabekova, 1980 from *Gymnodiptychus*

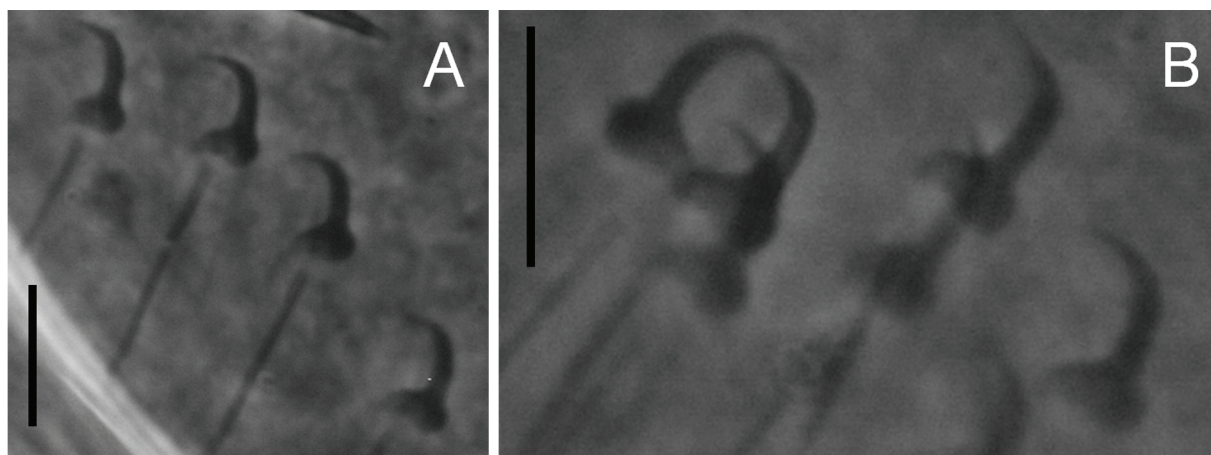


Fig. 2. A. micrograph detailing the marginal hooks of *Gyrodactylus jalalii* sp. nov. (photographed *in utero*, with an Olympus BX50 microscope, using phase contrast, and Olympus DP-soft v.3.2 software). B. *idem*, detail. Scale bars represent 8 µm.

dybowski (Kessler, 1874) (Cypriniformes, Cyprinidae). However, in *G. ophiocephali* and *G. tokobaevi*, the processes are longer than the ventral bar proper (median length, *i.e.*, without the shield), which is not the case in *G. jalalii* sp. nov. Elongate antero-lateral processes, albeit not longer than the ventral bar proper, are also found in *G. hrabei* Ergens, 1957 and *G. mariannae* Winger, Hansen, Bachmann & Bakke, 2008, parasites of *Cottus* Linnaeus, 1758 spp. (Scorpaeniformes, Cottidae). These species, however, have a comparatively shorter anchor root than *G. jalalii* sp. nov. The longitudinal striae on the ventral bar shield as well as an overlap in size of anchor and marginal hook are reminiscent of *G. lotae* Gussev, 1953 from *Lota lota* (Linnaeus, 1758) (Gadiformes, Lotidae). This species can be distinguished from *G. jalalii* sp. nov. because the new species has blunter and larger ventral bar processes, and a marginal hook sickle toe which joins smoothly into the platform, whereas this transition leaves a sharp “bump” in the platform in *G. lotae*.

Discussion

Just as Barlow (2000) praises cichlids, early students of *Gyrodactylus* expressed their high expectations for the scientific interest of these flatworms, as they recognized the species richness of this genus and the amount of work and skill it would take to adequately study it (von Nordmann 1832; Van Beneden & Hesse 1863). Given this scientifically challenging position of both cichlids and gyrodactylids, *G. jalalii* sp. nov. was described, a monogenean ectoparasite of the only Iranian cichlid *I. hormuzensis*. To the best of our knowledge, it is the first parasite to be formally described from this fish species. It brings the total number of *Gyrodactylus* species described from cichlid hosts to 15 (Paperna 1979; Boeger & Popazoglo 1995; Christison *et al.* 2005; Přikrylová *et al.* 2009, 2012; García-Vásquez *et al.* 2011; Vanhove *et al.* 2011).

Jalali *et al.* (2001) observed that the highly distinct zoogeographical regions of Iran and the high degree of endemism of its freshwater fishes gave rise to a diverse monogenean fauna. These authors did not mention *Gyrodactylus* in their overview. Indeed, Jalali *et al.* (2005) noted that representatives of this genus from the endemic freshwater fishes of the country are basically unknown and undescribed. They did not report gyrodactylids from *Iranocichla*. This cichlid seems distributed mostly in the Mesopotamian faunal region of Iran, from which Jalali *et al.* (2001) reported a rather unexplored, endemic and specific monogenean fauna, containing both Palearctic and African elements. For this reason, *G. jalalii* sp. nov. was compared to Palearctic and African congeners. Morphological similarities are unsurprisingly apparent (see above). Haptor morphology alone does not allow reliable phylogenetic inferences for species of *Gyrodactylus* (Ziętara & Lumme 2004). Unfortunately, we were unsuccessful in amplifying DNA from *G. jalalii* sp. nov. Hence, genetic data are at present unavailable for the new species, as is the case for several congeners used in this comparison. However, it is noteworthy that some of the aforementioned species show affinities in molecular phylogenetic reconstructions. Indeed, *G. mariannae* is close to *G. hrabei*, which is genetically similar to *G. flesi* Malmberg, 1957 (Winger *et al.* 2008). This species clusters with *G. lotae* and *G. zimbae*, among other species, in a *Gyrodactylus* clade containing representatives from various subgenera as defined by Malmberg (1970) and from many continents and host taxa (Ziętara & Lumme 2004; Vanhove *et al.* 2011). Despite the need for genetic data, assuming, that *G. jalalii* sp. nov. is indeed related to this lineage, this fact alone does not make it a very suitable candidate to provide information on biogeographical dispersal pathways of cichlid fishes. Indeed, several species also belonging to this diverse clade have been observed to show a broad salinity tolerance (*G. branchialis* Huyse, Malmberg & Volckaert, 2004 and *G. ostendicus* Huyse & Malmberg, 2004; Huyse *et al.* 2006) or the ability to switch infection sites (*G. arcuatus* Bychowsky, 1933; Raeymaekers *et al.* 2008) or hosts (*G. arcuatus*: Huyse *et al.* 2003, 2006). However, while there is hence no proof that *G. jalalii* sp. nov. belongs to an exclusively African or cichlid-infecting lineage, this broad ecological spectrum enhances the potential of these parasites as biogeographical markers, conditional to the availability of molecular data.

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