



Article Diversity and Phylogeny of *Gyrodactylus* spp. (Monogenea: Gyrodactylidae) across the Strait of Gibraltar: Parasite Speciation and Historical Biogeography of West Mediterranean Cyprinid Hosts

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Abstract: Knowledge on the diversity of parasitic flatworms of Western Mediterranean cyprinids is extremely scarce. In the present study, we parasitologically investigated 12 cyprinid species across the Strait of Gibraltar inhabiting watersheds in northwest Africa (Morocco) and Iberia (Portugal and Spain). Taxonomically relevant features of the attachment organ and sequences of the 18S rDNA and ITS regions were used for species delineation and to investigate their phylogenetic relatedness. Among the Gyrodactylus collected from Morocco and Spain, we identified specimens with an unusual T-shaped dorsal bar observed herein for the first time. In contrast, the membranous patch-like structure surrounding the twisted inner roots of hamuli and the median ridge of the ventral bar have been generally observed in Eurasian relatives. Our analyses suggest vicariant speciation of Gyrodactylus across the Strait of Gibraltar. We describe herein G. gibraltarensis sp. nov. from Iberian Luciobarbus graellsii; G. moroccensis sp. nov. from northwest African cyprinids, i.e., L. maghrebensis, L. rabatensis, L. rifensis, L. yahyaouii, and L. zayanensis; and finally, G. pseudomoroccensis sp. nov. from Moroccan L. ksibi, all possessing a new haptoral configuration. The genetic divergence and conservative morphologies in populations of G. moroccensis sp. nov. from five cyprinid species support its ongoing speciation in Northwest Africa. The West Mediterranean lineage was revealed to be monophyletic, with Eurasian species forming a sister group. Morphologically, West Mediterranean Gyrodactylus also appeared to be of Middle Eastern origin. Gyrodactylus spp. possessing an unusual T-shaped dorsal bar have most likely speciated, allowing for the appearance of a haptoral morphology that is restricted to the region across the Strait of Gibraltar. To conclude, viviparous Gyrodactylus reflect parasite speciation across the Strait of Gibraltar and the historical biogeography of cyprinids in the West Mediterranean.

Keywords: cyprinoids; Morocco; Iberian Peninsula; viviparous Monogenea; haptor morphology; Northwest Africa; Southwest Europe

1. Introduction

Parasitism is one of most successful modes of life, with almost every animal species potentially parasitized by at least one parasite species [1]. This mode has evolved independently in over 200 lineages throughout the animal tree of life alone [2], making parasites one of the best models for studying speciation processes due to their high potential for diversification and specialization [1]. As hosts represent the primary source of life for parasites, both are affected by reciprocal evolutionary interactions, and the diversification processes of one may influence those of the other [3]. From a parasitological perspective, it is generally accepted that sympatric speciation may occur when the isolation of parasite populations is maintained by intrinsic barriers independently of host speciation



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). events [4,5]. Inversely, allopatric speciation may appear when extrinsic barriers prevent parasite reproduction among geographically isolated host populations [5].

Monogenea, a group of over 5500 parasitic flatworm species [6] parasitizing mostly teleost fish, appear to be the most suitable candidates for studying host–parasite speciation patterns due to their direct life cycle that favors fast infection [7,8]. Previous studies devoted to identifying the processes of diversification in monogeneans have focused on fish taxa living in sympatry, which has biased conclusions in favor of sympatric speciation as the most common evolutionary scenario. This was demonstrated for oviparous dactylogyrid monogeneans in particular [5,9–11], with allopatric speciation known mainly in viviparous gyrodactylids (Gyrodactylidae van Beneden et Hesse, 1832) [12].

Gyrodactylids are ectoparasitic monogeneans that feed mostly on the epithelial cells of freshwater and marine teleost fish [13,14]. The extremely rare viviparity observed in most gyrodactylids with fully grown daughters in utero have made them the focus of intensive research [15]. A recent study by Boeger et al. [16] supported the monophyly of Gyrodactylidae and summarized the evolutionary features that most members of the family share, such as the loss of the Mehlis gland, the vitellarium, and characters related to the male copulatory organ (MCO) (see exceptions in [17]). Taxonomically, Gyrodactylidae is composed of 25 valid viviparous genera [16,17]. Pugachev et al. [18] classified four subfamilies within Gyrodactylidae for the Palearctic region. Gyrodactylinae include four genera recognized on Eurasian fishes: Gyrodactylus von Nordmann, 1832 (more than 300 spp.), Paragyrodactylus Gvosdev and Martechov, 1953 (3 spp.), Laminiscus Pálsson and Beverley-Burton, 1983 (3 spp.), and Gyrodactyloides Bychowski, 1947 (4 spp.). While the first two genera parasitize a wide range of widespread marine and freshwater fish taxonomically representing different families, the two remaining ones are strictly of marine origin and are, so far, limited to a few fish representatives like herring and salmonids [18–21]. Seven oviparous gyrodactylid genera are found exclusively on the African continent, of which six are specific to teleost fish [6,17,22].

Gyrodactylus, a cosmopolitan and hyper-diverse genus with an estimated 20,000 species worldwide [23], is one of the most valuable model organisms with respect to investigating continental affinities due to its high ability to switch hosts, a scenario which, however, considerably reduces its usefulness in studying phylogenetic inter-host relationships [24]. Nevertheless, with regards to the time scale and the dispersion routes of the hosts studied, *Gyrodactylus* monogeneans may provide information on the historical dispersion and/or contemporary contacts of their hosts due to host-switch [25]. Recently, gill-specific monogeneans of the genus *Dactylogyrus* Diesing, 1850 (Dactylogyridae) parasitizing cyprinoids have been applied to infer the historical biogeographical contacts of their hosts on the intercontinental scale [26–28], while *Gyrodactylus* have received less attention in this regard (e.g., [25]). With respect to *Gyrodactylus*, the limited number of parasitological surveys, especially in the West Mediterranean area, and the still ambiguous taxonomical status of many fish species in this region suggest the presence of much higher-than-expected diversity regarding species number, morphology, and genetics.

With more than 3000 spp. [29] divided into eleven subfamilies [30] and a pan-African distribution, Cyprinidae, *sensu* [30,31] form the most widespread fish group, from the Maghreb province of Northwest Africa to the South African coastal streams [32]. Torinae Karaman 1971 and Barbinae Bleeker 1859 are recognized as native to the Afro-European region of the West Mediterranean [33]. In this region, Torinae clusters three hexaploid and large-sized cyprinid genera, of which *Labeobarbus* Karaman, 1971 (~20 spp.) and the monospecific *Pterocapoeta* Günther, 1902 are both of the '*Labeobarbus*' clade (*Labeobarbus sensu lato*) [34–36]. Members of *Labeobarbus* are endemic to Northwest Africa and Southwestern Asia [37], whereas *Pterocapoeta* is restricted only to Morocco [34,38]. Members of Torinae are in fact the last invaders of the African continent to have crossed the land bridge between Africa and Asia via the Arabian tectonic plate in the Late Miocene (~13 MYA) [39]. Similarly, Barbinae (*Barbus sensu stricto*) is highly diversified with three genera including the paraphyletic *Luciobarbus* Heckel, 1843 (more than 35 spp.) [29,38], representing tetraploid

3 of 36

cyprinids [35,40,41]. They are present exclusively throughout the circum-Mediterranean area, including the Middle East, North Africa, the Iberian Peninsula, and the Balkan Peninsula (Greece) [41].

For the present study focused on gyrodactylid monogeneans occurring on both sides of the Strait of Gibraltar (Northwest Africa and Iberia), we targeted a range of cyprinids belonging to Luciobarbus and Pterocapoeta inhabiting West Mediterranean freshwater drainage systems located in Morocco, as well as Luciobarbus from Portugal and Spain. The West Mediterranean has been known for past dramatic geological and climate changes since the Cenozoic Era, like the Messinian Salinity Crisis (5.9-5.3 MYA) [42] and the opening of the Strait of Gibraltar (5.3 MYA) [43]. These events have considerably shaped the faunistic evolutionary history in the regions of North Africa and the Iberian Peninsula. These are separated by only the 13 km long Strait of Gibraltar [44]. Being physiologically intolerant to marine conditions, the present-day, wide distributional range of freshwater fishes in Northwest Africa, on the one hand, and confinement in Iberian watersheds, on the other hand, is the result of the paleogeographical evolution of the river basins, and of the history of hydrogeological networks in this area [41,44,45]. From the upper Miocene and Pliocene, ancient tectonic and paleogeographical crises [42] have had the greatest influence on the diversification of West Mediterranean lineages of *Luciobarbus* originating in the Middle East [46]. Currently, West Mediterranean provinces show the highest diversity of *Luciobarbus* species, these forming one of the main components of the freshwater ichthyofauna [41,47]. Interestingly, northwest African *Luciobarbus* spp. are paraphyletic, as two species from this region clustered with Iberian species [35,44,48,49], and they form the most diverse genus of Moroccan cyprinids, e.g., refs. [11,17]. Remarkably, these comprise both limnetic (abundant in upstream areas) and rheophilic species (abundant in downstream areas), like Luciobarbus ksibi (Boulenger, 1905) and Luciobarbus zayanensis Doadrio, Casal-López & Yahyaoui, 2016, respectively. Luciobarbus ksibi co-occur in the Kasab River Basin, while the presence of Luciobarbus yahyaouii Doadrio, Casal-Lopez & Perea, 2016 and L. zayanensis is limited to the Moulouya and Oum Er-Rbia drainage systems, respectively [38,49]. Luciobarbus massaensis (Valenciennes, 1842), Luciobarbus maghrebensis, Luciobarbus rabatensis Doadrio, Perea & Yahyaoui, 2015, and Luciobarbus rifensis Doadrio, Casal-Lopez & Yahyaoui, 2015, are all endemic to watersheds of Northern and Central Morocco [38,50,51]. From the Iberian ichthyogeographic system and out of at least seven endemic Luciobarbus spp. inhabiting South Mediterranean drainage systems [38], we studied Luciobarbus comizo (Steindachner, 1864), Luciobarbus bocagei (Steindachner, 1864), Luciobarbus graellsii (Steindachner, 1866), and Luciobarbus sclateri (Günther, 1868). Like their Northwest African counterparts, Iberian Luciobarbus were shown to be paraphyletic [44,48] due to the rapid block rotation of this system [52].

Using the phylogenetic reconstruction of dactylogyrid monogeneans, Simková et al. [28] showed multiple origins of *Dactylogyrus* spp. parasitizing cyprinids in Northwest Africa, reflecting different historical dispersal routes for Torinae and Barbinae, and revealed the historical northern route of Dactylogyrus spp. to Northwest Africa. The authors also demonstrated the multiple origins of Dactylogyrus spp. parasitizing Iberian Luciobarbus species, suggesting several independent historical contacts between Iberian Luciobarbus and two lineages of Northwest African cyprinids. These contacts were associated with host switches of Dactylogyrus parasites. Likewise, Benovics et al. [26] supported the Middle Eastern origin of *Dactylogyrus*, and evidenced multiple origins of endemic Southern European *Dactylogyrus* spp. from Barbinae [53]. So far, these studies remain the only ones using gill-specific monogeneans to elucidate historical contacts between West Mediterranean ichthyofauna. Knowledge on the morphological diversity of gyrodactylids of Northwest African cyprinids [54] and from the Iberian Peninsula [55] remains largely insufficient. To our knowledge, the available literature on gyrodactylid species from Iberian cyprinids is outdated and apparently inaccurate [55], and no genetic data are available for Gyrodactylus spp. from the West Mediterranean area. Meanwhile, Palearctic cyprinids and their *Gyrodactylus* communities have been extensively studied, and pertinent data about the

configuration of the haptoral sclerotized structures are fully available [18]. Additionally, hundreds of DNA sequences are available in the GenBank database.

Accordingly, the aim of this study was to investigate the taxonomic and genetic diversity of gyrodactylid fauna in the West Mediterranean, as well as the phylogenetic position of North African and Iberian *Gyrodactylus* spp. in relation to their congeners worldwide. Considering past geoclimatic events that were experienced by both sides of the Strait of Gibraltar, and which undoubtedly favored multiple dispersion and speciation events in susceptible freshwater fauna like cyprinids, we hypothesized herein multiple origins for West Mediterranean gyrodactylid monogeneans. In terms of parasite morphology, we expected the presence of the Eurasian morphotype of haptoral sclerites in the Northwest African and Iberian lineages due to the shared evolutionary history between eastern and western cyprinid hosts.

2. Material and Methods

2.1. Collection of Cyprinid Host Specimens and Their Gyrodactylid Monogeneans

A total of 128 fish specimens belonging to six cyprinid species restricted to the West Mediterranean were collected in Morocco, Portugal, and Spain between 2015 and 2017. Fish host species with their sample sizes, sampling locations, and indices of *Gyrodactylus* infection (prevalence and intensity of infection) are shown in Table 1. The sampling localities in Morocco and Iberia are shown in Figure 1. The fish specimens were identified in situ by scientific collaborators (listed in the acknowledgements). In the present study, the fish host nomenclature follows that of FishBase [38] and Eschmeyer's Catalog of Fishes [29]. According to FishBase [28], both L. ksibi and L. massaensis are synonyms for L. callensis (Valenciennes, 1842), and *P. maroccana* is a synonym for *Labeobarbus maroccanus* (Günther, 1902). The external body surface (scales and skin), fins, and gills of the cyprinid hosts were checked for the presence of viviparous gyrodactylid monogeneans using an MST-130 stereoscopic microscope (PZO Ltd., Warsaw, Poland). When present, parasite specimens were removed using surgical needles and mounted on slides with a mixture of glycerine and ammonium picrate (GAP) [56]. Gyrodactylid specimens were studied morphologically and genetically. A selected number of specimens were fixed only on slides using GAP (for morphology), and a selected number of specimens were bisected. The anterior part of the body containing the reproductive organ was stored in 96% ethanol for DNA extraction, and the posterior part of the body containing the haptoral sclerites was mounted on slides with GAP.



Figure 1. Map of sampling localities of cyprinoid fish hosts in Northwest Africa (Morocco) and the Iberian Peninsula (Portugal and Spain). Map was created using the simple Mapper tool (www. simplemapper.com, accessed on 30 April 2023) and manually edited (see details in Table 1).

Host Subfamily	Host Species	Total Hosts	Total Worms	Sampling Locality	Abbreviation on the Map	GPS Coordinates	Country	Gyrodactylus spp.	Prevalence (%)	Intensity of Infection
Torinae Karaman, 1971	Pterocapoeta maroccana Günther, 1902	03	14	Oum Er-Rbia River (El Borj)	M3	33°00′58.07″ N 05°37′48.06″ W	Morocco	<i>Gyrodactylus</i> sp. 5	33	1–6
Barbinae Bleeker, 1859	Luciobarbus bocagei (Steindachner, 1864)	15	03	Colares	P1	38°47′53.37″ N 09°26′14.16″ W	Portugal	<i>Gyrodactylus</i> sp. 1	7	3
			05					<i>Gyrodactylus</i> sp. 4	13	2–3
		10	08	Rio Ucera	S2	41°32′49.11″ N 03°04′32.50″ W	Spain	<i>Gyrodactylus</i> sp. 1	30	2–4
			11					<i>Gyrodactylus</i> sp. 4	60	1–3
	<i>Luciobarbus comizo</i> (Steindachner, 1864)	11	25	Peraleda de Zancejo, Rio Zujar	S1	38°27′12.02″ N 05°31′59.67″ W	Spain	<i>Gyrodactylus</i> sp. 3	27	5–14
	Luciobarbus graellsii (Steindachner, 1866)	12	10	upstream Mella, tributary of Materraña	S3	41°06′41.00″ N 00°08′05.00″ E	Spain	<i>G. gibraltarensis</i> sp. nov.	16	1–9
	<i>Luciobarbus yahyaouii</i> Doadrio, Casal-Lopez & Perea, 2016	09	04	Za River	M1	34°24′38.09″ N 02°52′29.1″ W	Morocco	<i>G. moroccensis</i> sp. nov.	11	1–2
		05	04	Meloulou River	M2	34°10′51.07″ N 03°31′59.06″ W		<i>Gyrodactylus</i> sp. 10	60	1–2
	Luciobarbus sclateri (Günther, 1868)	10	0	Torgal river, Mira basin	P2	37°38′16.76″ N 08°37′10.58″ W	Portugal	-	-	-
		10	10	Benahavis, Rio Guadalmina	S4	36°31′03.45″ N 05°02′25.07″ W	Spain	<i>Gyrodactylus</i> sp. 2	20	4–6
	<i>Luciobarbus ksibi</i> Doadrio, Perea and Yahyaoui, 2015	6	5	Ksob	M4	31°27′50.07″ N 09°45′25.03″ W	Morocco	G. pseudomoroccensis sp. nov.	45	1–3
		9	0	Oum Er-Rbia River (Chakouba)	M5	32°51′32.09″ N 05°37′18.09″ W		-	-	-
	Luciobarbus maghrebensis Doadrio, Perea and Yahyaoui,	05	02	Lahdar River	M6	34°15′30.01″ N 04°03′52.01″ W	Morocco	<i>G. moroccensis</i> sp. nov.	20	2
		05	28	Sebou River	M7	34°17′14.2″ N 06°33′14.08″ W			20	1–23

Table 1. List of West Mediterranean cyprinoid hosts collected between 2015 and 2017 and investigated in the present study.

Table 1. Cont.

Host Subfamily	Host Species	Total Hosts	Total Worms	Sampling Locality	Abbreviation on the Map	GPS Coordinates	Country	Gyrodactylus spp.	Prevalence (%)	Intensity of Infection
	Luciobarbus massaensis (Pellegrin, 1922)	11	4	Tamrhakht	M8	30°31′33.06″ N 09°38′53.06″ W	Morocco	<i>Gyrodactylus</i> sp. 11	45	2
	J. J		4					<i>Gyrodactylus</i> sp. 8	75	2
	<i>Luciobarbus rabatensis</i> Doadrio, Perea and Yahyaoui, 2015	11	18	Maleh River	M11	33°31′58.00″ N 06°37′39.06″ W	Morocco	<i>G. moroccensis</i> sp. nov.	72	1–9
	j i i i i j i i i i i i i i i i i i i i		22					<i>Gyrodactylus</i> sp. 7	27	1–17
	<i>Luciobarbus rifensis</i> Doadrio, Casal-Lopez & Yahvaoui, 2015	10	02	Tributary of Loukkos	M12	34°54′57.02″ N 05°32′17.02″ W	Morocco	<i>G. moroccensis</i> sp. nov.	10	2
	<i>,</i>		06					<i>Gyrodactylus</i> sp. 9	50	2
	<i>Luciobarbus zayanensis</i> Doadrio, Casal-López & Yahyaoui, 2016	06	03	Oum Er-Rbia River (El Borj)	M9	33°00′58.07″ N 05°37′48.06″ W	Morocco	<i>G. moroccensis</i> sp. nov.	13	2
	inity abai, 2010		04	Oum Er-Rbia River (El Borj)	M9	33°00′58.07″ N 05°37′48.06″ W		<i>Gyrodactylus</i> sp. 6	50	2
		09	0	Oum Er-Rbia River (Dar Oul Zidouh)	M10	32°18′54.00″ N 06°54′28.07″ W		-	-	-

2.2. Morphological Characterization and Multivariate Analysis of West Mediterranean Gyrodactylus

Measurements and microphotographs were taken using an Olympus BX51 phasecontrast microscope and Olympus Stream Image Analysis v. 1.9.3 software (Olympus, Tokyo, Japan). Drawings of the haptoral sclerotized parts of flattened specimens (hamuli, bars, marginal hooks, and MCO) were made using an Olympus BX51 microscope equipped with a drawing tube and edited with a graphic tablet compatible with Adobe Illustrator CS6 v. 16.0.0 and Adobe Photoshop v. 13.0 (Adobe Systems Inc., San Jose, CA, USA). The terminology used for the hard parts of gyrodactylid monogeneans follows refs. [15,18]. Infection indices were calculated for each *Gyrodactylus* species according to ref. [57] (Table 1). The type-material was deposited in the Helminthological collection of the Institute of Parasitology, Biology Centre of Academy of Sciences of the Czech Republic, České Budějovice (IPCAS) under the accession numbers IPCAS-M779-81. Following Ondračková et al. [58], principal component analysis (PCA) was performed on standardized morphometric data using PAST v. 4.11 [59] in order to visualize the position of the gyrodactylid specimens parasitizing Moroccan and Iberian cyprinid hosts that showed unusual haptoral sclerotized structures in morphological space. Measurements of 21 morphological characters of the haptoral sclerotized structures were considered.

2.3. Phylogenetic Reconstruction

To confirm the genus level of the collected gyrodactylid specimens, the nuclear noncoding gene 18S rDNA was amplified, while the internal transcribed spacer composed of the ITS1, 5.8S and ITS2 regions was amplified to confirm the conspecifity of the parasite samples. Indeed, the former marker has repeatedly been shown to be efficient in discriminating among Gyrodactylidae genera, e.g., ref. [57], while the ITS regions permit gyrodactylids to be subdivided into subgenera and species groups while also allowing for accurate species-level delineation [60–62]. Genomic DNA was isolated and amplified, and DNA sequences were obtained for the gyrodactylids targeted in this study, including so far undescribed species (Tables 1 and 2). Each tube containing a single gyrodactylid specimen preserved in 96% ethanol was dried using a vacuum concentrator (Eppendorf concentrator 5301, Hamburg, Germany). The genomic DNA was extracted using the DNeasy[®] Blood & Tissue Kit (Qiagen, Hilden, Germany) following the protocol for the purification of total DNA from animal tissues. The partial fragment of 18S rDNA was amplified using the primer pairs PBS18SF (5'-CGCGCAACTTACCCACTCTC-3') and PBS18SR (5'-ATTCCATGCAAGACTTTTCAGGC-3') [63]. The fragment spanning ITS1, 5.8S and ITS2 was amplified using the forward primer ITS1F (5'-GTTTCCGTAGGTGAACCT-3') [64], complementary to the sequence at the 3' end of the 18S rRNA gene, and the reverse primer ITS2 (5'-TCCTCCGCTTAGTGATA-3'), complementary to the sequence at the 5' end of the 28S rRNA gene [65]. Polymerase chain reactions (PCRs) for the 18S rDNA and ITS regions were performed in a final volume of 30 μ L, containing 1× PCR buffer (Fermentas, Bratislava, Slovakia), 1.5 mM of MgCl₂, 200 μ M of each dNTP, 0.5 μ M of each primer, 1 U of Taq Polymerase (Fermentas) and 5 μ L of template DNA. The PCRs were carried out using a Mastercycler ep gradient S (Eppendorf) in the following steps: (i) for the ITS region: an initial denaturation at 96 °C for 3 min, followed by 39 cycles of denaturation at 95 °C for 50 s, annealing at 52 $^{\circ}$ C for 50 s, and an extension at 72 $^{\circ}$ C for 50 s, with a final elongation at 72 °C for 7 min; and (ii) for the 18S region: an initial denaturation at 95 °C for 3 min, followed by 39 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 45 s and an extension at 72 °C for 1 min 30 s, with a final elongation at 72 °C for 7 min. The PCR products were electrophoresed on 1.5% agarose gels stained with Good View (SBS Genetech, Bratislava, Slovakia) and then purified using ExoSAP-IT reagents (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's protocol. The purified PCR products were sequenced directly in both directions using the PCR primers. For sequencing of the ITS region, one additional internal primer, ITSR3A (5'-GAGCCGAGTGATCCACC-3') [62], was used. Sanger sequencing was carried out using a BigDye[®] Terminator v3.1 Cycle

Sequencing Kit (Applied Biosystems by Life Technologies, Carlsbad, CA, USA; hereinafter Applied Biosystems) and an ABI 3130 Genetic Analyzer (Applied Biosystems). The obtained DNA sequences were assembled and edited using Sequencher software v. 5.0 (Gene Codes Corporation, MI, USA). Newly generated DNA sequences were checked using the NCBI Nucleotide Blast algorithm (nBLAST Search Tool, https://blast.ncbi.nlm.nih.gov/, accessed on 16 July 2023) to assess any similarities to available congeners, then deposited in GenBank under accession numbers (see Table 2). The uncorrected genetic *p*-distances among the newly generated sequences of the 18S rDNA and ITS regions were calculated separately using MEGA X [66].

The newly obtained sequences of the 18S rDNA and ITS regions were aligned separately using MAFFT v.7 [67], together with already published ones obtained from a representative selection of valid African and European gyrodactylid genera/species according to Boeger et al. [16] (Table 2). The final dataset (18S rDNA: 457 bp, ITS regions: 838 bp) containing a total of 63 DNA sequences from gyrodactylids was concatenated using MEGA 11 [66]. The best-fitting model of molecular evolution was selected for each gene dataset using ModelFinder [68]. According to the Bayesian information criterion (BIC), the TVM + F + I + G model was selected as the most appropriate evolutionary model for the 18S dataset, the TVM + F + I + G model for the ITS1 dataset, the TNe + G model for the 5.8S dataset, and the TVM + F + G model for the ITS2 dataset. Oviparous gyrodactylid species of the Palearctic region, represented by Gyrodactyloides bychowskii Albova, 1948, Ieredactylus rivuli Schelkle et al., 2011 and Laminiscus gussevi (Bychovsky et Polyansky, 1953) were used as outgroups (Table 2). Maximum likelihood (ML) trees were inferred using IQ-TREE v. 2.2.2.6 [69], employing the best fit substitution model (see above) and a sub-tree pruning and re-grafting (SPR) branch-swapping algorithm. Branch support (bootstrap support, BS) was estimated using ultrafast bootstrap approximation [70] with 1000 replicates. Bayesian inference (BI) analysis was performed using MrBayes v. 3.2.1 [71] with two independent Markov chain Monte Carlo (MCMC) simulations (six chains, 10⁶ generations, sampling frequency 100, 25% burn-in). The chain stationarity and parameter convergence were assessed using TRACER v. 1.7.1 [72], with effective sample sizes (ESS) > 200 for all parameters, and via the average standard deviation of split frequencies (<0.01). The post burn-in trees were summarized in a 25% majority rule consensus tree. The ML and BI trees were visualized using FigTree v. 1.4.4 [73].

Finally, clade A (see results section) of the ML tree based on the concatenated sequences of the 18S rDNA and ITS regions was visualized using TreeGraph v. 2.15 [74]. This step was performed in order to show the biogeographical distribution of *Gyrodactylus* spp. belonging to clade A together with patterns of haptoral sclerotized structures. The *Gyrodactylus* spp. investigated in this study and additional selected genetically closely related European and Middle East relatives were included. The morphological characters of the different parts of the attachment organ were selected following refs. [15,18].

Table 2. List of gyrodactylid species included in phylogenetic analyses based on sequences of the 18S rDNA gene and ITS regions. Monogenean species are grouped by host species, family, and geographical locality. The sequence indicated by "*" has no reference in the GenBank database. Fish host nomenclature follows FishBase [38] and Eschmeyer's Catalog of Fishes [29].

Gyrodactylid Species	Host Species	Host Family	Geographical Locality	18S rDNA	ITS Regions	Reference
Afrogyrodactylus girgifae	Brycinus nurse	Alestidae	Africa	HF548672	HF548671	[75]
Přikrylová et Luus-Powell, 2014	(Rüppell, 1832)					
Diplogyrodactylus martini	Polypterus senegalus	Polypteridae	Africa	HE858426	AM943008	[75]
Přikrylová, Matějusová, Musilová, Gelnar and	Cuvier, 1829					
Harris, 2009						
Gyrodactyloides bychowskii	Salmo salar	Salmonidae	Europe	AJ566379	AJ249348	[76]
Albova, 1948	Linnaeus, 1758					
Gyrodactylus alekosi	Clarias gariepinus	Clariidae	Africa	FR850683	FR850682	[77]
Prikrylova, Blazek and Vanhove, 2011	(Burchell, 1822)					
Gyrodactylus arcuatus	Gasterosteus aculeatus	Gasterosteidae	North America	JF836088	AF156668	[61,78]
Bychowsky, 1933	Linnaeus, 1758					
Gyrodactylus blicensis	Gymnocephalus cernua	Percidae	Europe	AJ407896	AJ407869	[62]
Glaser, 1974	(Linnaeus, 1758)				AJ407919	
Gyrodactylus brachymystacis	Brachymystax lenok	Salmonidae	Asia	JF836109	KP325622 *	[78]
Ergens, 1978	(Pallas, 1773)					
	Carassius auratus	Cyprinidae				
	(Linnaeus, 1758)					
	Oncorhynchus mykiss	Salmonidae				
	(Walbaum, 1792)					
Gyrodactylus carassii	Alburnus alburnus	Leuciscidae	Europe	AJ566377	AY278033	[62,79]
Malmberg, 1957	(Linnaeus, 1758)					
-	Carassius carassius	Cyprinidae				
	(Linnaeus, 1758)					
Gyrodactylus cernuae	Gymnocephalus cernua	Percidae	Europe	AJ407897	AJ407869	[62]
Malmberg, 1957	(Linnaeus, 1758)				AJ407919	
Gyrodactylus crysoleucas	Notemigonus crysoleucas	Leuciscidae	North America	KT149283	KT149287	[80]
Mizelle and Kritsky, 1967	(Mitchill, 1814)					
Gyrodactylus derjavinoides	Salmo salar	Salmonidae	Europe	JF836110	GQ368236	[78]
Malmberg, Collins, Cunningham and Jalali, 2007	Linnaeus, 1758		-			
Gyrodactylus ergensi	Sarotherodon galilaeus	Cichlidae	Africa	HF548668	FN394985	[75]
Přikrylová, Matějusová, Musilová et Gelnar, 2009	(Linnaeus, 1758)					

Table 2. Cont.

Gyrodactylid Species	Host Species	Host Family	Geographical Locality	18S rDNA	ITS Regions	Reference
<i>Gyrodactylus fossilis</i>	Misgurnus fossilis	Cobitidae	Europe	AJ407898	AJ407871	[76]
Lupu and Roman, 1956	(Linnaeus, 1758)		1		AJ407921	
Gyrodactylus gibraltarensis Rahmouni sp. nov.	Luciobarbus graellsii	Cyprinidae	Europe	OR773531	OR773480	This study
	(Steindachner, 1866)	••	-			-
Gyrodactylus gobiensis	Gobio gobio	Gobionidae	Europe	AJ566375	AY278041	[76]
Gloser, 1974	(Linnaeus, 1758)					
Gyrodactylus gobii	Gobio gobio	Gobionidae	Europe	AJ407900	AJ407873	[62]
Schulman, 1953	(Linnaeus, 1758)		-			
Gyrodactylus gracilihamatus	Abramis brama	Leuciscidae	Europe	AJ407901	AF484531	[76,81]
Malmberg, 1964	(Linnaeus, 1758)					
	Alburnus alburnus					
	(Linnaeus, 1758)					
Gyrodactylus azeezsaeedi	Squalius berak	Leuciscidae	Middle East	OR777687	OR773093	[82] this study
Rahmouni, 2023	Heckel, 1843					
Gyrodactylus hronosus	Alburnoides bipunctatus	Leuciscidae	Europe	AJ407902	AJ407876	[62]
Žitňan, 1964	(Bloch, 1782)				AJ407924	
Gyrodactylus jurajdai	Chondrostoma regium (Heckel, 1843)	Leuciscidae	Middle East	OR777685	OR773088	[82], this study
Rahmouni, 2023						
Gyrodactylus katharineri	Barbus barbus	Cyprinidae	Europe	AJ407903	AJ407878	[62]
Malmberg, 1964	(Linnaeus, 1758)				AJ407926	
Gyrodactylus laevis	Alburnoides bipunctatus	Leuciscidae	Europe	AJ407904	AY278036	[76]
Malmberg, 1957	(Bloch, 1782)					
	Phoxinus phoxinus					
	(Linnaeus, 1758)					
Gyrodactylus longoacuminatus	Carassius auratus	Cyprinidae	Europe	AJ407906	AJ407883	[76]
Žitňan, 1964	(Linnaeus, 1758)				AJ407930	
Gyrodactylus luciopercae	Perca fluviatilis	Percidae	Europe	AJ407907	AJ407885	[76]
Gussev, 1962	Linnaeus, 1758					
Gyrodactylus malalai	Oreochromis niloticus	Cichlidae	Africa	FR695485	FR695484	[75]
Přikrylová, Blažek et Gelnar, 2012	(Linnaeus, 1758)					
Gyrodactylus mediotorus	Notropis texanus	Gasterosteidae	North America	MW666777	MW666182	[83]
King, Marcogliese, Forest, McLaughlin and	(Girard, 1856)					
Bentzen, 2013						
Gyrodactylus moroccensis Rahmouni sp. nov.	Luciobarbus rabatensis	Cyprinidae	North Africa	OR773529	OR773478	This study
	Doadrio, Perea and Yahyaoui, 2015					

Table 2. Cont.

Gyrodactylid Species	Host Species	Host Family	Geographical Locality	18S rDNA	ITS Regions	Reference
	Luciobarbus rifensis			OR773528	OR773477	
	Doadrio, Casal-Lopez and Yahyaoui, 2015					
Gyrodactylus mhaiseni	Alburnus sellal	Leuciscidae	Middle East	OR777688	OR773082	[82], this study
Rahmouni, 2023	Heckel, 1843					
Gyrodactylus nigritae	Synodontis nigrita	Mochokidae	Africa	FR850687	FR850686	[77]
Přikrylová, Blažek and Vanhove, 2012	Valenciennes, 1840					
Gyrodactylus pseudomoroccensis Rahmouni sp. nov.	Luciobarbus ksibi (Boulenger, 1905)	Cyprinidae	North Africa	OR773530	OR773479	This study
Gyrodactylus rarus	Spinachia spinachia	Gasterosteidae	Europe	AY339776	AY338445	[84]
Wegener, 1910	(Linnaeus, 1758)					
Gyrodactylus rhodei	Rhodeus sericeus	Acheilognathidae	Europe	AJ567670	AJ407889	[76]
Žitňan, 1964	(Pallas, 1776)				AJ407933	
Gyrodactylus rugiensis	Pomatoschistus microps	Gobiidae	Europe	AY339762	AY338446	[84]
Glaser, 1974	(Krøyer, 1838)					
Gyrodactylus rugiensoides	Pomatoschistus minutus	Gobiidae	Europe	AY339763	AJ427414	[84,85]
Huyse and Volckaert, 2002	(Pallas, 1770)					
Gyrodactylus rutilensis	Rutilus rutilus	Leuciscidae	Europe	AJ566376	AJ407890	[76]
Glaser, 1974	(Linnaeus, 1758)				AJ407934	
Gyrodactylus rysavyi	Clarias anguillaris	Clariidae	Africa	FR850680	FR850679	[77]
Ergens, 1973	(Linnaeus, 1758)					
Gyrodactylus salaris	Salmo salar	Salmonidae	Europe	JF836111	AJ515912	[86]
Malmberg, 1957	Linnaeus, 1758					
	Oncorhynchus mykiss					
	(Walbaum, 1792)					
Gyrodactylus salmonis	Oncorhynchus mykiss	Salmonidae	Europe	JF836097	MN850542	[78,87]
Yin and Sproston, 1948	(Walbaum, 1792)					
Gyrodactylus sandai	Capoeta umbla	Cyprinidae	Middle East	OR777689	OR773089	[82], this study
Rahmouni, 2023	(Heckel, 1843)					
Gyrodactylus satanicus	Garra rufa	Cyprinidae	Middle East	OR777686	OR773091	[82], this study
Rahmouni, 2023	Heckel, 1843					
Gyrodactylus sedelnikovi	Barbatula barbatula	Nemacheilidae	Europe	AJ407911	AJ407891	[62]
Gvozdev, 1950	(Linnaeus, 1758)				AJ407935	
Gyrodactylus stephanus	Fundulus heteroclitus	Gasterosteidae	North America	JF836099	FJ845515	[78,88]
Müller, 1937	(Linnaeus, 1766)					

Table 2. Cont.

Gyrodactylid Species	Host Species	Host Family	Geographical Locality	18S rDNA	ITS Regions	Reference
Gyrodactylus synodonti	Synodontis nigrita	Mochokidae	Africa	FR850685	FR850684	[77]
Přikrylová, Blažek, Maarten and Vanhove, 2012	Valenciennes, 1840					
Gyrodactylus teuchis	Salmo trutta	Salmonidae	Europe	AJ407912	AJ249350	[76]
Lautraite, Blanc, Thiery, Daniel and Vigneulle, 1999	Linnaeus, 1758					
Gyrodactylus truttae	Salvelinus fontinalis	Salmonidae	Europe	JF836112	AJ132260	[65,78]
Gläser, 1974	(Mitchill, 1814)					
Gyrodactylus vimbi	Squalius cephalus	Leuciscidae	Europe	AJ407914	AJ407892	[62]
Shulman, 1954	(Linnaeus, 1758)		-		AJ407936	
Ieredactylus rivuli	Anablepsoides hartii	Cyprinodontiformes	Neotropical	JX840358	HQ738514	[89,90]
Schelkle et al., 2011	(Boulenger, 1890)		•			
Laminiscus gussevi	Mallotus villosus	Osmeridae	Europe	HF548679	HF548678	[75]
(Bychovsky et Polyansky, 1953)	(Müller, 1776)		1			
Macrogyrodactylus congolensis	Clarias gariepinus	Clariidae	Africa	HF548680	GU252716	[75,91]
(Prodhoe, 1957)	(Burchell, 1822)					
Macrogyrodactylus polypterid	Polypterus senegalus	Polypteridae	Africa	AJ567671	AJ567672	[76]
Malmberg, 1957	Cuvier, 1829	••				
Macrogyrodactylus simentiensis	Polypterus senegalus	Polypteridae	Africa	HF548682	HF548681	[75]
Přikrylová and Gelnar, 2008	Cuvier, 1829	, I				
Paragyrodactylus variegatus	Homatula variegata	Nemacheilidae	Asia	KF680220	KF680221	[19]
You, King, Ye and Cone, 2014	(Dabry de Thiersant, 1874)					
<i>Gyrodactylus</i> sp. 1	Luciobarbus bocagei	Cyprinidae	Europe	OR807824	OR807835	This study
	(Steindachner, 1864)		1			2
<i>Gyrodactylus</i> sp. 2	Luciobarbus sclateri	Cyprinidae	Europe	OR807825	OR807836	This study
	(Günther, 1868)		1			2
<i>Gyrodactylus</i> sp. 3	Luciobarbus comizo	Cyprinidae	Europe	OR807826	OR807837	This study
	(Steindachner, 1864)	51	1			2
<i>Gyrodactylus</i> sp. 4	Luciobarbus bocagei	Cyprinidae	Europe	OR807827	OR807838	This study
	(Steindachner, 1864)	51	1			2
<i>Gyrodactylus</i> sp. 5	Labeobarbus maroccanus	Cyprinidae	North Africa	OR807828	OR807839	This study
	(Günther, 1902)	71				5
<i>Gyrodactylus</i> sp. 6	Luciobarbus zayanensis	Cyprinidae	North Africa	OR807829	OR807840	This study
	Doadrio, Casal-López & Yahyaoui, 2016	71				5
<i>Gyrodactylus</i> sp. 7	Luciobarbus rabatensis	Cyprinidae	North Africa	OR807830	OR807841	This study
	Doadrio, Perea and Yahyaoui, 2015	71				5
<i>Gyrodactylus</i> sp. 8	Luciobarbus massaensis (Valenciennes, 1842)	Cyprinidae	North Africa	OR807831	OR807842	This study

Tabl	le 2.	Cont.
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Gyrodactylid Species	Host Species	Host Family	Geographical Locality	18S rDNA	ITS Regions	Reference
Gyrodactylus sp. 9	Luciobarbus rifensis Doadrio, Casal-Lopez and Yahyaoui, 2015	Cyprinidae	North Africa	OR807832	OR807843	This study
Gyrodactylus sp. 10	Luciobarbus yahyaouii Doadrio, Casal-Lopez & Perea, 2016	Cyprinidae	North Africa	OR807833	OR807844	This study
<i>Gyrodactylus</i> sp. 11	Luciobarbus massaensis (Valenciennes, 1842)	Cyprinidae	North Africa	OR807834	OR807845	This study

3. Results

The examination of 157 specimens of cyprinid fish hosts sampled in West Mediterranean watersheds revealed the presence of nearly 200 Gyrodactylus specimens. The collected monogenean specimens were identified as members of the genus Gyrodactylus based on the presence of up to two developing embryos in the uterus, 16 marginal hooks of the same type, a single pair of hamuli (anchors), and dorsal and ventral bars. A total of 72 gyrodactylid specimens parasitizing six northwest African cyprinid species of the genus Luciobarbus (L. ksibi, L. maghrebensis, L. rabatensis, L. rifensis, L. yahyaouii, and L. zayanensis) and Iberian L. graellsii exhibited an unusual morphotype of the attachment organ (Figure 2) compared to that observed in the remaining specimens found on the six cyprinid species sampled in Morocco (L. yahyaouii, L. massaensis, L. zayanensis, L. rabatensis, L. rifensis, and P. maroccana), three Luciobarbus spp. collected in Spain (L. bocagei, L. comizo and L. sclateri), and one collected in Portugal (L. bocagei) (see Table 1). Below, a special focus is placed on gyrodactylid specimens with an unusual haptor morphology. We describe G. gibraltarensis sp. Nov. from Iberian L. graellsii, G. moroccensis sp. Nov. from L. yahyaouii, L. maghrebensis, L. zayanensis, L. rabatensis, and L. rifensis, and finally, G. pseudomoroccensis sp. nov. from L. ksibi.



Figure 2. Micrographs showing the unusual morphology of haptoral sclerotized structures recovered in Gyrodactylus specimens found to parasitize Northwest African cyprinid fish hosts across the Strait of Gibraltar. Micrographs were taken from a parasite specimen of *Gyrodactylus moroccensis* sp. nov. parasitizing the Rabat barbel *Luciobarbus rabatensis* Doadrio, Perea & Yahyaoui, 2015 (type–host). (A) Haptoral part, general view; (B) detailed morphology of hamuli (H), dorsal (DB) and ventral bars (VB); (C) detailed morphology of marginal hooks.

3.1. Systematics and Molecular Characterization

The Life Science Identifier (LSID) for this publication is: urn:lsid:zoobank.org:pub: E55D7363-D7FD-4150-9EC5-1F3EB2926E46.

Gyrodactylus gibraltarensis Rahmouni sp. nov. (Figure 3)



Figure 3. Illustrations of haptoral parts of *Gyrodactylus gibraltarensis* sp. nov. from the Iberian barbel *Luciobarbus graellsii* (Steindachner, 1866) (type–host) (MCO not observed).

Zoobank registration: urn:lsid:zoobank.org:act:88464789-AB95-4F25-99D2-44EA94E81F59 Type–host: Iberian barbel *Luciobarbus graellsii* (Steindachner, 1866) (Cyprinidae) Type–locality: upstream Mella, tributary of Materraña, Spain (Table 1, Figure 1)

Type–material: one holotype and one paratype under the accession number IPCAS M-779 Site on the host: gill filaments

Etymology: The epithet "gibraltarensis" of the type species refers to the Strait of Gibraltar, which played an important role in the dispersal of freshwater fish to the Iberian Peninsula DNA sequences: 18S rDNA: OR773531, ITS regions: OR773480Description (morphometric measurements are given in Table 3)

		Gyra	odactylus moroccensis	sp. nov.		<i>Gyrodactylus</i> gibraltarensis sp. nov.	Gyrodactylus pseudomoroccensis sp. nov.
Cyprinid fish hosts	Luciobarbus maghrebensis	Luciobarbus yahyaouii	Luciobarbus zayanensis	Luciobarbus rabatensis *	Luciobarbus rifensis	Luciobarbus graellsii *	Luciobarbus ksibi *
Hamuli							
Length	47.6 (45.9–50.9) $_{10}$	48 (45.1–49.7) ₃	51.8 (49.9–54) ₃	$47.6 (45.9 - 50.9)_{10}$	44.9 (43.8–46) ₂	36.9 (35.3–40.8) ₄	37.1 (36.1–38.2) ₅
Outer root length	10.6 (8.8–12.5) ₁₀	11.7 (9.5–13.9) ₃	12.3 (11.1–13.4)3	10.6 (8.8–12.5) ₁₀	8.4 (8-8.9)2	8.5 (7.6–9.8)4	11.4 (10.6–12) ₅
Shaft length	37.6 (35.5–39) ₁₀	37.2 (36.5–37.9) ₃	40.5 (38.6–41.9)3	37.6 (35.5–39) ₁₀	36.7 (35.7–37.6) ₂	28.5 (27.1–30.7)4	27.3 (26–28.9) ₅
Point length	23 (20.1–25.1) ₁₀	22.5 (21.9–23.4)3	23.2 (22.6–23.6) ₃	23 (20.1–25.1) ₁₀	24.2 (22.8–25.5) ₂	17.4 (15.5–19.3) ₄	14.9 (14.3–15.4) ₅
Ventral bar							
Length	28.8 (28.6–29.4) ₁₀	29.5 (27.6–31.9) ₃	30.9 (29.1–33.6) ₃	28.8 (28.6–29.4) ₁₀	27.9 (27.6–28.3) ₂	21.2 (19.2–25.6) ₄	15.3 (14.7–16) ₅
Width	29.7 (27.7–31) ₁₀	26.7 (26.1–27.2) ₃	32 (31–32.5) ₃	29.7 (27.7–31) ₁₀	26.8 (26.2–27.3) ₂	21.6 (19.8–24.6) ₄	17.4 (16.5–18.4) ₅
Tips length	8 (6.3–9.4) ₁₀	6.1 (5.7–6.5) ₃	7.2 (6.9–7.6) ₃	8 (6.3–9.4) ₁₀	7.1 (5.9–8.3) ₂	2.9 (2.7–3.2) ₄	3.2 (2.4–4.1) ₅
Distance between tips	31.1 (29.2–32.4) ₁₀	27.9 (26.7–28.8) ₃	31.5 (30.8–32.2) ₃	31.1 (29.2–32.4) ₁₀	27.3 (26.7–27.9) ₂	23.2 (21.9–24) ₄	17.4 (16–18.1) ₅
Median width	7.5 (6.5–8.2) ₁₀	5.9 (5.4–6.2) ₃	7.5 (7–7.9) ₃	7.5 (6.5–8.2) ₁₀	5.3 (4.8–5.8) ₂	4.2 (3.6–5.4) ₄	5.3 (5.1–5.6) ₅
Membrane length	12.3 (11.1–13.5) ₁₀	14.7 (14.1–15.6) ₃	14.2 (13.7–14.5) ₃	12.3 (11.1–13.5) ₁₀	13 (12.5–13.5) ₂	11.6 (10.6–12.8) ₄	9 (8.3–9.6) ₅
Membrane width	15.3 (14.1–16.6) ₁₀	13.3 (11.2–15.2) ₃	18.7 (17.2–20.3) ₃	15.3 (14.1–16.6) ₁₀	15.8 (15.1–16.4) ₂	13.7 (13–15.2) ₄	10.4 (9.4–11.2) ₅
Dorsal bar							
Length	27.9 (26.7–28.5) $_{10}$	27.9 (26.8–29.1) ₃	31.5 (31.2–32) ₃	$27.9(26.7-28.5)_{10}$	27.3 (25.4–29.1) ₂	22.3 (20.5–24.2) ₄	19.9 (18.7–21.5) ₅
Median width	17.9 (16–19.4) ₁₀	16.9 (15.8–17.7) ₃	20 (19.7–20.6) ₃	17.9 (16–19.4) ₁₀	15.4 (14.3–16.5) ₂	10.8 (8.6–14) ₄	16.2 (15–17) ₅
Marginal hooks							
Length	29.5 (28.1–31.8) ₁₀	27.1 (26.7–27.5) ₃	31.3 (30.5–32) ₃	29.5 (28.1–31.8) ₁₀	25.5 (25.3–25.7)2	22.8 (21.5–23.4)4	22.1 (20.5–23.6) ₅
Filament loop length	10.6 (9.2–12.7) ₁₀	8.9 (8.3–10) ₃	13.2 (12.4–14.3)3	10.6 (9.2–12.7) ₁₀	11.5 (10.9–12) ₂	10.1 (9.6–10.6) ₄	8.2 (7.5–8.8) ₅
Handle length	24.1 (22.6–26.6)10	21.2 (20.4–21.8)3	24.9 (24.2–25.4)3	24.1 (22.6–26.6)10	20.4 (20.4–20.5)2	17.8 (17.3–18.6)4	17.6 (16.8–18.6)5
Sickle length to shaft attachment	6 (5–6.9) ₁₀	5.4 (5.2–5.5)3	6.2 (5.9–6.4)3	6 (5–6.9) ₁₀	5.1 (5–5.2) ₂	4.6 (4.2–5) 4	4.6 (4.4–4.8) ₅

Table 3. Morphometric parameters (in μm) obtained from specimens of *Gyrodactylus moroccensis* Rahmouni sp. nov., *G. gibraltarensis* Rahmouni sp. nov. and *G. pseudomoroccensis* Rahmouni sp. nov. Meristic data are presented as mean (min–max) with the number of specimens as subscripts. (*) refers to the type–host.

		Gy	rodactylus moroccensis	s sp. nov.		Gyrodactylus gibraltarensis sp. nov.	Gyrodactylus pseudomoroccensis sp. nov.
Sickle proximal width	5.1 (4.6–5.5) ₁₀	4.5 (4.2–4.8)3	5.1 (4.7–5.4)3	5.1 (4.6–5.5) ₁₀	4.2 (4.1–4.3) ₂	3.8 (3.3–4.5) ₄	3.7 (3.5–4) ₅
Sickle distal width	4.9 (4.4–5.2) ₁₀	5.3 (4.7–5.6) ₃	6.1 (5.6–6.4)3	4.9 (4.4–5.2) ₁₀	4.3 (4.2–4.4)2	4.2 (3.9–4.4) ₄	4.1 (4–4.4)5
Shaft length of sickle	4.9 (4.6–5.1) ₁₀	4.7 (4.1–5.2)3	5.3 (4.7–5.8)3	4.9 (4.6–5.1) ₁₀	4 (3.9–4.1) ₂	4.4 (3.7–5.5)4	3.7 (3.5–3.8) ₅
Point length of sickle	2.5 (2.1–2.7)10	2.6 (2.4–2.8)3	3.7 (3.4–3.9)3	2.5 (2.1–2.7) ₁₀	2.2 (2.2–2.3) ₂	1.8 (1.3–2.8)4	1.9 (1.8–2) ₅
Male copulatory organ (MCO) Length	16 (14.7–19.4) ₆	Not observed	17.7 ₁	16 (14.7–19.4) ₁₀	12.7 ₁	Not observed	Not observed
Width	17.2 (15.9–19.2) ₆	Not observed	17.61	17.2 (15.9–19.2) ₁₀	13.5 ₁	Not observed	Not observed
Pharynx Length	46.8 (43.5–54.9) ₁₀	38.8 (35.2–44.2) ₃	40.1 (38.6–42.9) ₁₀	40.1 (38.6–42.9) ₁₀	36.1 (35.7–36.5) ₂	_	_
Width	39.3 (37.6-44.5)10	38.7 (36–41.8)3	36.5 (30.6–40.3)10	36.5 (30.6–40.3)10	27 (25-4-28.7)2	_	_

The body fusiform has four main parts: a cephalic region, trunk, peduncle forming the anterior part, and the attachment organ (opisthaptor) located posteriorly. The body wall is thin and smooth. The cephalic region is bilobed, and each lobe has a visible sensillum and gland. There are no eyespots. The mouth (oral opening) is located ventrally. There is a large spherical pharynx with eight long, finger-like pharyngeal processes projecting forward in the anterior part. The esophagus is visible, branching into two simple blind-ended intestinal crura that extend beyond the uterus. There are excretory bladders present. They are viviparous, with up to two embryos in utero positioned ventrally. There is no vagina, and the MCO is not visible. The attachment organ is delineated from the rest of the body, bearing a single pair of well-developed hamuli. The anterior part of the hamuli has an additional arched, patch-like, membranous structure that is not connected and slightly sclerotized at the extremities, covering the twisted inner root edges inward, and there are folds present in the posterior part of the base. The hamuli shaft is slightly bowed and point long. The ventral (superficial) bar is of medium size, with blunt, well-developed lateral processes extending out of bar. The median part is relatively thick, with a membrane (shield) of medium size that is slightly trapezoid and almost 1/3 the length of the hamuli shaft, with a median ridge. The dorsal (deep) bar is T-shaped, lying between the hamuli, with the straight anterior part associated with attenuated extremities that are inserted into terminal plates. The dorsal bar is medially prolonged and narrower at the halfway point, ending in a swollen elongated portion positioned above the median ridge of the ventral bar. There are eight pairs of marginal hooks (sixteen units) of equal size, composed of a sickle associated with a filament loop (lamella). The sickle proper has a relatively robust shaft, rising forward from base and curving gradually. The point of the marginal hook sickle is positioned above the edge of the sickle toe. The sickle foot has a rounded heel. There is a well-developed sickle toe, which is finger-like and positioned downward, with a visible shelf leading to the toe at the same level of the hook heel. MCO is not observed.

Diagnosis

The gyrodactylid specimens found herein to parasitize L. graellsii were assigned to the newly described G. gibraltarensis sp. nov (Figure 3). The hamuli of G. gibraltarensis sp. nov., with a distinctive patch-like structure around the inner roots, are reminiscent of Gyrodactylus malmbergi Ergens 1961, parasitizing a range of Barbus spp. [18,92,93]. The representatives of Paragyrodactylus are also known mainly from Asian loaches (Nemacheilidae Regan, 1911), with additional membrane-like structures surrounding the hamuli roots [18]. Yet, G. gibraltarensis sp. nov. is easily distinguishable by the T-shaped dorsal bar and median ridge in the ventral bar membrane; features grouped in the newly described species only. The accessory portion around the hamuli and the T-shape of the dorsal bar differentiate G. gibraltarensis sp. nov. from a single gyrodactylid species found on the opposite side of the Strait of Gibraltar (Maghreb), namely Gyrodactylus nyingiae Shigoley, Rahmouni, Louizi, Pariselle and Vanhove, 2023, described from Luciobarbus pallaryi Pellegrin, 1919 and L. ksibi [54]. The median ridge of the ventral bar and its long lateral processes are characters which seem to be restricted to some Gyrodactylus spp. from Eurasian cyprinids [18]. More specifically, the median ridge was previously considered by Malmberg (1970) to define the G. katharineri group (subgenus G. Limnonephrotus Malmberg, 1964), with Gyrodactylus katharineri as the former species of the group. Furthermore, the marginal hooks of G. gibraltarensis sp. nov. seem to possess a similar shape to that exhibited by species of the G. katharineri group. With regard to the ventral bar, the above-described morphotype can be found, for instance, in Gyrodactylus barbi Ergens 1976, Gyrodactylus gobii Shulman, 1954, Gyrodactylus gobiensis Glaser, 1974 and Gyrodactylus tokobaevi Ergens & Karabekova, 1980 within the *G. katharineri* group in the Palearctic region [18]. *Gyrodactylus gibraltarensis* sp. nov. differs from members of the G. katharineri group by the T-shaped dorsal bar, as this structure in its congeners is of a common shape.

Molecular taxonomy

Fragments covering the ITS regions (ITS1 (455 bp), 5.8S (157 bp), ITS2 (420 bp)) and 18S rDNA (440 bp) were successfully sequenced for a single specimen of *G. gibraltarensis* sp. Nov. parasitizing *L. graellsii* sampled in Iberia (Spain). While no close hit to *G. gibraltarensis* sp. Nov. was found using sequences of the ITS regions, an nBLAST search indicated *G. gobiensis* (AJ566375) from *Gobio gobio* (Linnaeus, 1758) (Morava River, Czech Republic) [76], a member of the *G. katharineri* group [15] (see above), as its closest hit, with a 99% similarity based on sequences of 18S rDNA. According to sequences of the

with a 99% similarity based on sequences of 18S rDNA. According to sequences of the ITS regions, *G. gibraltarensis* sp. nov. from *L. graellsii* exhibited the smallest *p*-distances from its below-described northwest African congeners, *G. moroccensis* sp. nov. from *L. rabatensis* and *L. rifensis* (*p*-distance = 1.3%), and *G. pseudomoroccensis* sp. nov. from *L. ksibi* (*p*-distance = 5%). On the basis of sequences of 18S rDNA, *G. gibraltarensis* sp. nov. exhibited the smallest *p*-distances from each of *G. moroccensis* sp. nov. from *L. rifensis* and *G. pseudomoroccensis* sp. nov. from *L. ksibi* (*p*-distance = 0.4%), followed by that from *G. moroccensis* sp. nov. from *L. rabatensis* (*p*-distance = 0.8%) (see *p*-distances in Supplementary Material Table S1 in supplementary material and the phylogenetic section below).

Gyrodactylus moroccensis Rahmouni sp. nov. (Figure 4)



Figure 4. Illustrations of the haptoral parts (**A**) and male copulatory organ (MCO) with six (**B**) and eight spinelets (**C**) of *Gyrodactylus moroccensis* sp. nov. from the Rabat barbel *Luciobarbus rabatensis* Doadrio, Perea & Yahyaoui, 2015 (type–host).

Zoobank registration: urn:lsid:zoobank.org:act:D58EB381-CAB9-4DF5-9157-FCC55EB0EE18 Type–host: Rabat barbel *Luciobarbus rabatensis* Doadrio, Perea and Yahyaoui, 2015 (Cyprinidae)

Type–locality: Maleh River, Morocco (Table 1, Figure 1)

Additional hosts: Yahyaoui barbel *Luciobarbus yahyaouii* Doadrio, Casal-Lopez & Perea, 2016, Zayan Barbel *Luciobarbus zayanensis* Doadrio, Casal-López & Yahyaoui, 2016, Maghreb barbel *Luciobarbus maghrebensis* Doadrio, Perea and Yahyaoui, 2015, and Rifian barbel *Luciobarbus rifensis* Doadrio, Casal-Lopez & Yahyaoui, 2015 (all Cyprinidae)

Additional localities: Sebou River, Lahdar and Sebou drainage for *L. maghrebensis*, Za and Meloulou Rivers for *L. yahyaouii*, Oum Er-Rbia River for *L. zayanensis*, tributary of Loukkos for *L. rifensis*, all in Morocco (Table 2, Figure 1)

Type–material: one holotype and three paratypes under the accession number IPCAS M-779 Site on the host: fins for *L. maghrebensis*, *L. yahyaouii*, *L. zayanensis* and *L. rifensis*; gill filaments for *L. rabatensis*

Etymology: The epithet "moroccensis" of the type–species refers to its country of origin (Morocco)

DNA sequences: 18S rDNA: OR773528-29, ITS regions: OR773477-78

Description (morphometric measurements are given in Table 3)

The body fusiform has four main parts: a cephalic region, trunk, peduncle forming the anterior part, and an attachment organ (opisthaptor) located posteriorly. The body wall is thin and smooth. The cephalic region is bilobed, and each lobe has a visible sensillum and gland. There are no eyespots. The mouth (oral opening) is located ventrally. There is a large spherical pharynx with eight long, finger-like pharyngeal processes projecting forward in the anterior part. The esophagus is visible, branching into two simple blindended intestinal crura that extend beyond the uterus. Excretory bladders are present. It is viviparous, with up to two embryos in utero positioned ventrally. A vagina is absent. The MCO is visible in all the *G. moroccensis* sp. nov. specimens except in those collected from *L*. yahyaouii. The MCO is bulbous with a visible opening. It is positioned ventrally, close to the bifurcation of intestinal crura and garnished with a single large terminal spine followed by a row of six to eight spines of medium size. The attachment organ is delineated from rest of the body, bearing a single pair of well-developed hamuli. The anterior part of the hamuli has visible tendons and an additional arched, patch-like, membranous structure. This structure is not connected and slightly sclerotized at the extremities, covering twisted inner root edges inward, with folds present in the posterior part the of base. The hamuli shaft is slightly bowed and point long. The ventral (superficial) bar is of medium size, with blunt, well-developed lateral processes extending out of the bar. The median part is relatively thick, large in the sides, and can show a hollow membrane (shield) that is relatively short, slightly trapezoid, and almost 1/3 the length of the hamuli shaft, with striations and a median ridge. The dorsal (deep) bar is T-shaped, lying between the hamuli, with a straight anterior part, hollow medial part, and bifurcations near the attenuated extremities inserted into the terminal plates. It is medially prolonged with a constricted portion near the anterior branch, ending in a swollen elongated portion positioned above the median ridge of the ventral bar. There are eight pairs of marginal hooks (sixteen units) of equal size composed of a sickle associated with a filament loop (lamella). There is a sickle proper with a robust shaft rising forward from the base and curving gradually. The point of the marginal hook sickle is positioned above the edge of the sickle toe. The sickle foot has a rounded heel. There is a well-developed sickle toe, which is finger-like and positioned downward with a visible shelf leading to a toe at the same level the of hook heel.

Diagnosis

Although it is morphologically highly reminiscent of *G. gibraltarensis* sp. nov., Northwest African *G. moroccensis* sp. nov. (Figure 4), identified on five endemic Moroccan cyprinids, is distinguishable from the former Iberian species by the size of the haptoral sclerites, mainly the hamuli, which is comparatively shorter in *G. gibraltarensis* sp. nov. (Table 3, Figure 3). No obvious variability in the shape of the haptoral structures was evidenced between these two species. As a member of the *G. katharineri* group [15], *G. moroccensis* sp. nov. shares all of the morphological features characterizing this group, just like *G. gibraltarensis* sp. nov. (see above). It should be noted that, according to Shigoley et al. [54], the inner roots of hamuli exhibited by the former described species, *G. nyingiae* from *L. pallaryi*, are not twisted. Conversely, the hamuli of *G. moroccensis* sp. nov. seem similar to those in the *Gyrodactylus* specimen from *L. ksibi* assigned by Shigoley et al. [54] to *G. nyingiae*. The specimen included in [54], which was identified as *G. nyingiae* on *L.*

ksibi, may thus be either *G. moroccensis* sp. nov. or a so-far-undescribed species, but it definitely does not represent the same species as that found on *L. pallaryi*. We suspect the former possibility; Shigoley et al. [54] likely overlooked the T-shape of the dorsal bar due to the weak sample size they examined (or the structure of this character was damaged during fixation). The main difference between *G. moroccensis* sp. nov. and *G. nyingiae* is, indeed, the T-shaped dorsal bar in the former newly described species. Furthermore, the median ridge of the ventral bar was not mentioned in the description of *G. nyingiae*, but we believe that this structure was present, as illustrated by the micrographs provided by Shigoley et al. [54], which show a kind of fold in the posterior edge of the ventral bar.

Molecular taxonomy

Fragments covering the ITS regions (ITS1 (477 bp), 5.8S (157 bp), ITS2 (422 bp) were successfully sequenced for two specimens of G. moroccensis sp. nov. isolated from each of L. rabatensis and L. rifensis, and for a single specimen from L. maghrebensis, all sampled across Northwest African freshwater habitats (Morocco). The ITS sequences obtained from G. moroccensis sp. Nov. from L. rabatensis and L. maghrebensis were identical. However, a degree of weak intraspecific variability below the limit value usually considered for discriminating Gyrodactylus spp. [60,94,95] was found between the sequences representing *G. moroccensis* sp. Nov. from *L. rabatensis* and from *L. rifensis* (*p*-distance = 0.8%). We failed to obtain ITS sequences for *G. moroccensis* sp. Nov. from *L. yahyaouii* and *L. zayanensis*. No close hit to G. moroccensis sp. Nov. was found using the nBLAST search tool for ITS regions. Fragments of the 18S rDNA region (456 bp) were successfully sequenced for two specimens of G. moroccensis sp. Nov. isolated from each of L. rifensis and L. rabatensis. We failed to obtain 18S rDNA sequences for specimens of G. moroccensis sp. Nov. from L. yahyaouii, L. zayanensis, and L. maghrebensis. As obtained for the ITS regions, a level of weak intraspecific variability in the sequences of 18S rDNA representing G. moroccensis sp. Nov. was found at the cyprinid host level (*L. rabatensis* vs. *L. rifensis*, *p*-distance = 0.4%). The nBLAST search revealed G. katharineri (AJ407903) from Barbus barbus (Linnaeus, 1758) (Morava River, Czech Republic) [62] as the closest known hit to G. moroccensis sp. Nov. from L. rabatensis and L. rifensis, with a 95.06% similarity. Sequences of the ITS regions supported the distinction between G. gibraltarensis sp. nov. from Iberia and G. moroccensis sp. nov. from Northwest Africa, with the genetic divergence exceeding the limit value [60,94,95] (p-distance = 1.3%, see above, Table S1 in supplementary material and the phylogenetic section below).

Gyrodactylus pseudomoroccensis Rahmouni sp. nov. (Figure 5)



Figure 5. Illustrations of the haptoral parts of *Gyrodactylus pseudomoroccensis* sp. nov. from the Rabat barbel *Luciobarbus rabatensis* Doadrio, Perea & Yahyaoui, 2015 (type–host) (MCO not observed).

Zoobank registration: urn:lsid:zoobank.org:act:45ED3EAA-7F91-4370-ACDE-7B5AEA 155363

Type–host: *Luciobarbus ksibi* (Boulenger, 1905) (Cyprinidae) (synonym of *Luciobarbus callensis* (Valenciennes, 1842) according to Fishbase [38] but considered as a valid species on Eschmeyer's Catalog of Fishes [29])

Type–locality: Ksob River, Morocco (Table 1, Figure 1)

Type–material: one holotype and one paratype under the accession number IPCAS M-781Site on the host: fins

Etymology: The epithet "*pseudomoroccensis*" of the type–species refers to the morphological similarity to specific elements of the attachment organ (T-shaped dorsal bar) in the two new *Gyrodactylus* spp. described from Morocco.

DNA sequences: 18S rDNA: OR773530, ITS regions: OR773479

Description (morphometric measurements are given in Table 3)

The body fusiform has four main parts: a cephalic region, trunk, a peduncle forming the anterior part, and an attachment organ (opisthaptor) located posteriorly. The body wall is thin and smooth. The cephalic region is bilobed, and each lobe has a visible sensillum and gland. There are no eyespots. The mouth (oral opening) is located ventrally. There is a large spherical pharynx with eight long, finger-like pharyngeal processes projecting forward in the anterior part. The esophagus is visible, branching into two simple blindended intestinal crura that extend beyond the uterus. Excretory bladders are present. It is viviparous, with up to two embryos in utero positioned ventrally. A vagina is absent. The MCO is not visible. The attachment organ is delineated from the rest of the body, bearing a single pair of well-developed hamuli. The hamuli lack the chitinous patch-like structure of the inner roots, and the tips of the inner roots are straight with a hat-like cover and a groove-like portion present in the posterior part of the base. The hamuli shaft is slightly bowed and point long. The ventral bar has a relatively short lateral processes extending out of the bar, with a membrane of medium width ending in the median ridge. There is a dorsal T-shaped bar with median excavation, posteriorly traversing the ventral bar to end in an inflated portion. There are sixteen similar marginal hooks with a sickle proper attached to a filament that extends almost 2/3 of the handle length. There is a sickle proper base with a globous heel and a finger-like downward toe positioned perpendicular to the short point formed by a curved shaft of medium thickness. MCO is not observed.

Diagnosis

Gyrodactylus pseudomoroccensis sp. nov. (Figure 5) is the third species of Gyrodactylus recognized in Northwest Africa, and is thus the fourth species to be found in the West Mediterranean region. Gyrodactylus pseudomoroccensis sp. nov. highly resembles the abovedescribed congeners, G. gibraltarensis sp. nov. from Iberia and Northwest African G. moroccensis sp. nov., due to the T-shaped dorsal bar and ventral bar with a median ridge. The latter feature places G. pseudomoroccensis sp. nov. in the G. katharineri group [15]. Compared to G. gibraltarensis sp. nov. and G. moroccensis sp. nov., G. pseudomoroccensis sp. nov. exhibits shorter hamuli lacking the membranous patch-like structure and shorter lateral processes of the ventral bar. It also exhibits straight inner roots covered by a hat posteriorly with a groove-like portion in the base; a feature that is missing in the two former species (see descriptions of G. gibraltarensis sp. nov. and G. moroccensis sp. nov.). The morphology of the hamuli in *G. pseudomoroccensis* sp. nov. described herein from *L.* ksibi is surprisingly different from that reported by Shigoley et al. [54] in a single specimen identified as G. nyingiae collected from L. ksibi inhabiting a nearby (almost identical) locality in Western Morocco. The main differences between these two species are in the shape of the inner roots of the hamuli, which are straight in *G. pseudomoroccensis* sp. nov. but appear rather twisted in G. nyingiae. The marginal hooks in the specimens of both species are of a common shape.

3.2. Morphological Delineation among Northwest African and Iberian Gyrodactylus

A principle component analysis (Figure 6A–C) was performed using the morphometric data obtained from the haptoral structures of the gyrodactylid specimens possessing unusual shapes of the haptoral sclerites. A total of 89.4% of the variation was explained by the first two PC axes (85.04% and 4.36%, respectively). The first PC axis (Figure 6A) mostly separated Gyrodactylus specimens from L. rabatensis and L. zayanensis (corresponding to different populations of G. moroccensis sp. nov.), as well as those from L. ksibi (corresponding to G. pseudomoroccensis sp. nov.) from Gyrodactylus specimens from each of L. maghrebensis (corresponding to G. moroccensis sp.) nov. and L. graellsii (corresponding to G. gibraltarensis sp. nov.). Along this axis, the gyrodactylid specimens from L. rifensis (corresponding to G. moroccensis sp. nov.) were well separated from the Gyrodactylus specimens from L. ksibi (corresponding to G. pseudomoroccensis sp. nov.) and from the two populations designated as G. moroccensis sp. nov. (from L. zayanensis and L. rabatensis). However, the population of *G. moroccensis* sp. nov. from *L. rifensis* was represented by only two specimens in our data set. At the cyprinid host level within Northwest Africa, the PC1 axis further allowed us to discriminate among the *Gyrodactylus* specimens from different hosts; however, there was an overlap between the specimens from L. yahyaouii, L. maghrebensis, and L. rifensis. The main morphometric changes along PC1 were associated with the total length of the hamuli and the length of their shafts, the length and width of the ventral bars, the distance between lateral processes, the total length of the dorsal bars, and finally the length of the marginal hooks and their handles (Figure 6B). The second PC axis (Figure 6A) differentiated Gyrodactylus populations corresponding to G. moroccensis sp. nov. from the specimens representing each of G. pseudomoroccensis sp. nov. and G. gibraltarensis sp. nov. The overall changes along PC2 were mostly related to the total length of the hamuli and the length of their roots, the length of each ventral bar, and the median of the dorsal bar (Figure 6C).

3.3. Phylogenetic Relationships

The phylogenetic tree based on the concatenated dataset of the 18S rDNA and ITS regions comprised a total of 64 sequences, of which 15 obtained from the West Mediterranean *Gyrodactylus* spp. were newly generated. These comprised ten sequences from Northwest African (Morocco) Gyrodactylus and five sequences from Iberian Gyrodactylus, with two and three sequences from Portugal and Spain, respectively. The phylogenetic tree was rooted with three gyrodactylid species from Europe and the Neotropics. The ML and BI trees showed identical topologies when considering the well-supported nodes, and the trees obtained through both analyses were fully resolved. The ML tree is presented in Figure 7, including the bootstrap values and posterior probabilities resulting from the ML and BI analyses, respectively. Overall, the phylogenetic reconstruction revealed a total of seven well-to-moderately supported clades (A–G). With regard to outgroups, clades A–C were restricted to *Gyrodactylus* spp., with long ITS1 sequences, in contrast to clades D–G, which clustered gyrodactylid representatives from distinct genera worldwide with short ITS sequences, in agreement with Cable et al. [96]. Clade A (BS = 99, PP = 1) included Gyrodactylus spp. studied herein from the West Mediterranean, Middle Eastern Gyrodactylus mhaiseni and Gyrodactylus sandai, and European G. katharineri. The West Mediterranean lineage of Gyrodactylus, including all the representatives from the Northwest African and Iberian regions (i.e., G. gibraltarensis sp. nov., G. moroccensis sp. nov., G. pseudomoroccensis sp. nov., and *Gyrodactylus* sp. 1–11) was monophyletic, whereas the *Gyrodactylus* from the two sides of the Strait of Gibraltar, i.e., from Northwest Africa and Iberia, were polyphyletic. The phylogenetic reconstruction showed a well-supported monophyletic group, including G. moroccensis sp. Nov. from L. rabatensis, G. moroccensis sp. Nov. from L. rifensis, and Iberian G. gibraltarensis sp. nov. from L. graellsii. Their congener G. pseudomoroccensis sp. nov. from Moroccan *L. ksibi* was at the basal position to the well-supported group (BS = 99, PP = 1) of undescribed *Gyrodactylus* species from Iberia. The well-supported clade B (BS = 100, PP = 1) grouped *Gyrodactylus* spp. parasitizing European freshwater, brackish, and marine gobids, and had a basal position to Gyrodactylus species in Clade A. Clade C showed moderate

to high support values (BS = 87, PP = 0.91) and included Eurasian and Middle Eastern *Gyrodactylus* spp. from a range of teleost fish taxa. The well-supported clade D (BS = 100, PP = 1) included the *Gyrodactylus* spp. found on gasterosteid fish in the Nearctic region and from brackish environments in the North-eastern Atlantic Ocean, and it had a basal position to clades A, B, and C. Clade E was similarly well-supported (BS = 100, PP = 1), grouping *Gyrodactylus* spp. from African cichlids. The well-supported clade F (BS = 92, PP = 0.95) was composed of a group of *Gyrodactylus* spp. parasitizing African siluriforms and a group of European *Gyrodactylus* spp. from cyprinids. The position of East Asian *Paragyrodactylus variegatus* as a sister species to the European *Gyrodactylus* spp. was only weakly supported by the ML analyses (BS = 76). Finally, clade G included three African gyrodactylid genera with high support values in the basal position of the tree (Figure 7).



Figure 6. Principal component analysis (PCA) based on 21 morphological characters of the haptoral sclerotized structures supporting the taxonomical differentiation of *Gyrodactylus gibraltarensis* sp. nov. from Iberia, *Gyrodactylus moroccensis* sp. nov., and *Gyrodactylus pseudomoroccensis* sp. nov. from Northwest Africa. (**A**) Plot of the PCA (two first axes) illustrating the distribution of *Gyrodactylus gibraltarensis* sp. nov. specimens (triangles), *Gyrodactylus moroccensis* sp. nov. (circles), and *Gyrodactylus pseudomoroccensis* sp. nov. (diamonds). Histograms of the factor loading of the characters contributing most to the variation along PC1 (**B**) and PC2 (**C**). Characters 1–4 are for hamuli, 5–11 are for the ventral bar, 12–13 are for the dorsal bar, and 14–21 are for marginal hooks: (1) anchor total length; (2) outer root length; (3) shaft length; (4) point length; (5) ventral bar total length; (6) ventral bar total width; (7) lateral processes length; (8) distance between lateral processes; (9) median width; (10) membrane length; (11) membrane width; (12) dorsal bar total length; (13) dorsal bar width at midpoint; (14) marginal hooks total length; (15) sickle length to shaft attachment; (16) sickle proximal width; (17) sickle distal width; (18) shaft length of sickle; (19) point length of sickle; (20) handle length; (21) filament loop length (terminology follows refs. [15,18]).



Figure 7. Maximum likelihood (ML) phylogram of *Gyrodactylus* spp. parasitizing distinct fish hosts worldwide based on sequences of the V4 region of the 18S rDNA (457 bp) and ITS regions (838 bp). Values above branches indicate bootstrap support (BS) from ML and posterior probabilities (PP) from BI analyses. Values below 60 (ML) and 0.80 (BI) are shown as dashes. Clades (**A–G**) refer to different *Gyrodactylus* spp. lineages.

3.4. Morphological Evolution of Gyrodactylus in the West Mediterranean Region

The maximum likelihood tree including only West Mediterranean *Gyrodactylus* spp. with their genetically closely related congeners *G. mhaiseni* and *G. sandai* from the Middle East and *G. katharineri* from Europe (i.e., members of clade A) was used to illustrate the structural evolution of the different parts of the haptoral sclerites in *Gyrodactylus* of the West Mediterranean (Figure 8). The projection of the geographical distribution onto the phylogenetic tree revealed two potential contacts between the North African and Iberian cyprinoids associated with the host switch of *Gyrodactylus* spp. In terms of morphology, we evidenced three main features found exclusively together in the West Mediterranean lineage, in addition to the median ridge of the ventral bar (character 1) found in all the species of clade A from Eurasia and the West Mediterranean. Conversely, the morphology related to the ventral bar was of two origins. While most of the *Gyrodactylus* spp. included in the analysis exhibited relatively short lateral processes of the ventral bar, *G. katharineri* from Europe and *G. sandai* from the Middle East possessed well-developed structures

of the ventral bar (character 2); a feature found to characterize two newly described northwest African and Iberian congeners, *G. moroccensis* sp. nov. and *G. gibraltarensis* sp. nov. The anterior part of the hamuli with twisted inner roots (character 3) was found in the *Gyrodactylus* spp. from West Mediterranean cyprinids, but not in the *G. pseudomoroccensis* sp. nov. from Northwest African *L. ksibi*, nor in the undescribed *Gyrodactylus* sp. 3 and *Gyrodactylus* sp. 4 from Iberia (Portugal). As already stated above (see species descriptions), the membranous, patch-like structure surrounding the tips of the hamuli (character 4) exclusively characterized *G. moroccensis* sp. nov. and *G. gibraltarensis* sp. nov. from the West Mediterranean. The dorsal bar with its typical T-shape (character 5) was reported for the first time in *Gyrodactylus*. This structure was found to characterize the three newly described West Mediterranean species only. No clear evolutionary pattern was noted for the other haptoral parts of the studied *Gyrodactylus* spp., such as the cup-like structure covering the anterior part of the hamuli, the presence of folds in the hamuli base, or the



Figure 8. Schematic representation of the haptoral sclerotized structures of *Gyrodactylus* spp. parasitizing Eurasian and West Mediterranean species that formed clade A in Figure 7 along the ML phylogram obtained using sequences of the 18S rDNA and ITS regions. The geographical (continental) distribution is mapped onto the ML tree. The drawings were edited using Adobe Illustrator CS6 (see methodology above). A specimen of *G. katharineri* was collected from *B. barbus* from France (see [97]). The specimens are not uniformly scaled. Yellow: Asia, including the Middle East; blue: Europe; red: Africa. Character 1: median ridge in the ventral bar; character 2: long lateral processes of the ventral bar; character 3: twisted inner roots of the hamuli; character 4: accessory portion of the hamuli, i.e., the membranous patch-like structure surrounding the inner roots; character 5: T-shaped dorsal bar.

4. Discussion

The present study focused on gyrodactylid communities found in West Mediterranean cyprinids and covering the freshwater of two distinct continents separated by the Strait of Gibraltar. The West European part, i.e., the Iberian Peninsula, is known for its relatively low species diversity of freshwater ichthyofauna compared to Eastern Europe due to its historical isolation [48]. Previous studies on parasites of endemic cyprinid hosts in the West Mediterranean [28,98–100] have documented the presence of endemic and host-specific dactylogyrid monogeneans in the North African and South European (Iberian) parts of the Mediterranean. In line with those studies and considering the historical dispersal routes of cyprinids to the West Mediterranean, we hypothesized that the Northwest African and Iberian cyprinids studied herein may also harbor distinctive gyrodactylid communities, and we expected that at least some Gyrodactylus spp. from the West Mediterranean would share their morphological characters with potential Eurasian ancestors. In terms of the diversity of *Gyrodactylus*, the most cosmopolitan and speciose ectoparasitic group in the Palearctic region thus far [18], the West Mediterranean region has not been extensively studied. To date, knowledge on *Gyrodactylus* spp. parasitizing African cyprinids is quite limited. Only a few Gyrodactylus species have been described—specifically, G. nyingiae from L. pallaryi and L. ksibi [54] from North African (Morocco) watersheds, and three species, namely Gyrodactylus ivindoensis Price and Gery, 1968, Gyrodactylus kyogae Paperna, 1973, and *Gyrodactylus paludinosus* Truter, Smit, Malherbe and Přikrylová, 2021, from cyprinids of the genus Enteromius Cope, 1867 have been found in the more southerly freshwaters in Africa [6,101]. Similarly, little is known about the parasite fauna of Iberian cyprinid hosts, and the data available so far can be considered old and most probably unreliable [55].

In this study, gyrodactylid monogeneans belonging to the genus Gyrodactylus were found to parasitize eight Northwest African and four Iberian cyprinid species belonging to West Mediterranean Torinae and Barbinae. All the Gyrodactylus specimens exhibited up to two developing embryos in the uterus, a single type of marginal hook, a single pair of hamuli, and dorsal and ventral bars. Moreover, a typical Gyrodactylus-like MCO of bulbous form garnished with a single apical spine and a row of spinelets was recognized in some specimens. Overall, the attachment organ in most of the gyrodactylid genera found in European freshwaters exhibited additional structures associated with the hamuli (Gyrodactyloides, Laminiscus, and Paragyrodactylus), and the hamuli further exhibited welldeveloped outer and inner roots (*Gyrodactyloides*, *Laminiscus*). The dorsal bar was missing in some genera (Laminiscus). Interestingly, the MCO retained its bulbous form, with a single apical spine supplemented by one or more rows of spinelets in the species from all the above-listed genera, as well as in *Gyrodactylus* [18,102]. The bulbous form of MCO also characterized other genera, like the Nearctic Gyrocerviceanseris Cone, Abbott, Gilmore & Burt, 2010, Fundulotrema Kritsky & Thatcher, 1977, and the African Macrogyrodactylus Malmberg, 1957. The marginal hooks have been shown to be highly diverse in the African continent, where species can harbor either a single type of marginal hook, as in the genera Afrogyrodactylus Paperna, 1968, Citharodactylus Přikrylová, Shinn et Paladini, 2017, and Gyrodactylus, or hooks with distinct morphologies, as in Diplogyrodactylus Přikrylová, Matějusová, Musilová, Gelnar and Harris, 2009, Macrogyrodactylus, Mormyrogyrodactylus Luus-Powell, Mashego et Khalil, 2003, and Tresuncinidactylus Přikrylová, Barson and Shinn, 2021 (see summary in [102]).

In this study, morphological characterization based on informative haptoral features combined with sequences of the 18S rDNA and ITS regions allowed for the identification of a total of 14 *Gyrodactylus* spp. on a range of West Mediterranean Barbinae and Torinae. These include a single species from *P. maroccana*, while the remaining thirteen species were recovered from *Luciobarbus* spp. across the Strait of Gibraltar, with five in Iberia and eight in Northwest Africa. Using an integrative approach combining morphological diagnoses and genetic data has become a common practice in monogenean species identification [60,100,103,104]. Such an approach also provides more accurate taxonomic support for *Gyrodactylus* spp., though each analysis separately has specific limitations [83]. DNA

segments such as the ITS regions and, to a lesser degree, the 18S rDNA region have been shown to be successful markers for *Gyrodactylus* species delineation, and for assessing intraspecific variability [59–61,105]. Moreover, in our study, the integrative approach mentioned above was supplemented using multivariate analyses of morphological data on the haptoral parts to discriminate between *Gyrodactylus* specimens of three newly described species and between populations of *G. maroccensis* sp. nov. found on five Northwest African cyprinids. The most relevant morphological traits for *Gyrodactylus* spp. delimitation are the shape, size, and proportions of the haptoral sclerites [15]. With regard to the impressive species richness of *Gyrodactylus* [23], weak morphological variability may confuse taxonomic identification [106].

In our study, microscopic examination of the collected Gyrodactylus specimens from Northwest Africa and Iberia revealed the presence of two distinct haptoral morphotypes. Surprisingly, one of these morphotypes was unusual and had not been documented in *Gyrodactylus* so far. This morphotype was found in *Gyrodactylus* specimens collected from Northwest African L. yahyaouii, L. maghrebensis, L. zayanensis, L. rabatensis, L. rifensis (described as G. maroceensis sp. nov.), and L. ksibi (described as G. pseudomoroceensis sp. nov.), and from Iberian L. graellsii (described as G. gibraltarensis sp. nov.). The first remarkable feature which was common to this morphotype was the T-shaped dorsal bar, with a prolonged portion lying between the shafts of the hamuli. The second character was the shape of the hamuli, which were supplemented by a membranous patch-like structure surrounding the twisted inner roots. This character was missing, however, in the Gyrodactylus specimens from *L. ksibi* corresponding to *G. pseudomoroceensis* sp. nov., where the hamuli roots were relatively strait and of the usual form commonly documented in Gyrodactylus species. The combination of a T-shaped dorsal bar with hamuli presenting with accessory portions was not previously observed in *Gyrodactylus*, and to our knowledge, this morphotype is so far limited to the West Mediterranean. Meanwhile, all of the *Gyrodactylus* specimens studied herein from opposite parts of the Strait of Gibraltar possessed a median ridge in the posterior edge of the ventral bar.

Regarding the West Mediterranean Gyrodactylus specimens with an unusual morphology, the *p*-distances calculated using sequences of the 18S rDNA and ITS regions supported the existence of three species, described herein as G. gibraltarensis sp. nov., G. moroccensis sp. nov.m and G. pseudomoroccensis sp. nov., since the genetic divergences in the sequences of the ITS regions were above the conventional limit value ($\geq 1\%$) that is usually considered for delineating Gyrodactylus spp. [60,94,95]. Moreover, morphometrical analyses also supported the genetic evidence for three *Gyrodactylus* spp. with an unusual morphology across the Strait of Gibraltar, with a single species distributed in Iberia and two species distributed in Northwest Africa. However, our morphometrical analyses also revealed a certain degree of population differentiation for generalist G. moroccensis sp. Nov. from L. maghrebensis, L. zayanensis, L. rabatensis, L. rifensis, and L. yahyaouii. The presence of distinct *Gyrodactylus* spp. across the Strait of Gibraltar since the reopening of the Strait at the beginning of the Pliocene and the refilling of the Mediterranean accounts for allopatric speciation driven by vicariance events, as already attributed to Mediterranean ichthyofauna [48]. Considering the endemism of the studied cyprinid hosts across the Strait of Gibraltar, we can assume a cospeciation scenario in the cyprinid-Gyrodactylus system, followed by diversification events in Northwest Africa and Iberia, as hypothesized for Balkan dactylogyrids [26]. Within the North African region, morphological and genetic divergences between *G. moroccensis* sp. nov. from *L. rabatensis* and *G. pseudomoroccensis* sp. nov. from L. ksibi indicate the successful coexistence of these two gyrodactylid species in overlapped niches in neighboring freshwater systems like the Oum Er-Rbia and Tensift basins [38,107]. Yet, past and current Gyrodactylus records from L. ksibi occurring in the Tensift basin in West Morocco are contradictory. Shigoley et al. [54] recently classified as G. nyingiae a single Gyrodactylus parasite from L. ksibi collected in a West Moroccan river (Oued Ksob, Tensift basin), together with visibly differentiated Gyrodactylus specimens from L. *pallaryi* sampled on the opposite side in Eastern Morocco (Oued Guir, Sud Atlas basin). The haptoral morphology exhibited by our specimens described as *G. pseudomoroccensis* sp. nov. from West Moroccan L. ksibi was likewise different from that found in the previously collected Gyrodactylus specimen from L. ksibi, despite the fact that they came from adjacent locations in the Ksob River. Micrographs provided by Shigoley et al. [54] clearly illustrate hamuli with twisted inner roots in *Gyrodactylus* specimens from *L. ksibi*; a feature which we found in *G. moroccensis* sp. nov. from five cyprinid species dispersed in most Moroccan drainage systems but not in in its congener *G. pseudomoroccensis* sp. nov. described from L. ksibi. This may indicate that, first, the previous assignment of Gyrodactylus of L. ksibi to G. nyingiae was most likely a mistake. Second, the generalist G. moroccensis sp. nov. can also parasitize L. ksibi in extended ecological niches. The lack of information regarding the morphology of the remaining haptoral parts of the parasite of *L. ksibi* studied by Shigoley et al. [54] makes further investigations necessary. The generalist G. moroccensis sp. nov. was found on a range of Northwest African *Luciobarbus* spp. This could be the result of host switching. The low intraspecific variability found in sequences of the ITS regions of G. moroccensis sp. nov. from L. rabatensis and L. rifensis and in the phylogenetically more conservative and slowly evolving 18S rDNA region, however, may indicate ongoing speciation in the North African freshwater systems. Unfortunately, genetic data for the populations of *G. moroccensis* sp. nov. collected from the remaining host species are still missing. Using PCA on the morphometric data, we also revealed at least the partial morphological differentiation of *G. moroccensis* sp. nov. populations. However, future sampling to obtain representative sample sizes for G. moroccensis sp. nov. populations from different cyprinid hosts and from different drainage systems in North Africa is necessary to investigate the potential ongoing speciation across Northwestern Africa. Interestingly, the intrapopulation variability in the 18S rDNA between the genetic variants of G. moroccensis sp. nov. was the same as the interspecific variability between G. gibraltarensis sp. nov. and one of the genetic variants of *G. moroccensis* sp. nov. or *G. pseudomoroccensis* sp. nov. The sequences of the ITS regions showed the highest similarity between the two analyzed genetic variants of G. moroccensis sp. nov. DNA sequences of the ITS regions also indicated a low variability (1.3%) between the morphologically similar and geographically isolated G. moroccensis sp. nov. and G. gibraltarensis sp. nov. on their respective cyprinid hosts currently living in allopatry in the North African and Southern European parts of the Mediterranean, respectively.

In this study, we investigated the phylogenetic positions of West Mediterranean Gyrodactylus and their relationships with representatives of several gyrodactylid genera worldwide. We included in the analyses DNA sequence data of *Gyrodactylus* from the Middle East, since it represents a putative region of the ancestral diversification of cyprinids prior to their dispersion into Europe and North Africa [35]. Overall, Gyrodactylus was shown to be paraphyletic in our study; a finding previously documented in refs. [75,76,78,108]. Herein, Gyrodactylus spp. from the West Mediterranean formed a monophyletic group, and two Gyrodactylus spp. from the Middle East and European G. katharineri had a basal position. This is in accordance with the phylogeography of their respective tetraploid and hexaploid hosts of Barbinae and Torinae, respectively, which reached Northwest Africa through independent dispersal events from Eurasia [35]. This also implies a Eurasian origin for the West Mediterranean lineage of Gyrodactylus spp., as previously suggested for one lineage of gill-specific dactylogyrid monogeneans [28]. However, in contrast to the multiple origins for each of the following: (i) *Dactylogyrus* spp. parasitizing North African cyprinids and (ii) Dactylogyrus spp. parasitizing Iberian Luciobarbus, the West Mediterranean *Gyrodactylus* lineage seems to have a single origin, which is likely Middle Eastern. Yet, our phylogenetic analyses indicated that within the West Mediterranean Gyrodactylus, species parasitizing Iberian Luciobarbus have a North African origin, and that the diversification of Gyrodactylus spp. in Iberian Luciobarbus seems to be related to historical contacts between West Mediterranean cyprinids (currently separated by the Strait of Gibraltar). On the basis of the phylogenetic relationships among Gyrodactylus spp. with unusual morphology of the haptor, those historical contacts of cyprinids were

likely associated with the North African–Iberian host switching of *Gyrodactylus*, followed by parasite speciation in Iberian *Luciobarbus* (especially concerning *G. gibraltarensis* sp. nov., which was morphologically highly similar to Northwest African *G. moroceensis* sp. nov., two species currently geographically isolated by the Strait of Gibraltar). Our phylogenetic study also indicated that the monophyletic group including four *Gyrodactylus* spp. (*Gyrodactylus* sp. 1–4) parasitizing Iberian *Luciobarbus* originated from historical contacts between West Mediterranean cyprinids currently living on each side of the Strait of Gibraltar. We further evidenced that *Gyrodactylus* spp. from European marine *Pomatoschistus* spp. (Gobiidae) form a sister group to the West Mediterranean lineage. This may support the ancestral freshwater lifestyle of soma gobiid populations during the opening of the Strait of Gibraltar (5.3 MYA) and the subsequent re-flooding of the Mediterranean basins during the Messinian salinity crisis [43].

We focused on the morphological characters of the haptor of *Gyrodactylus* spp. of the West Mediterranean lineage and their closest relatives (clade A). The presence of specific morphological characters of the haptor representing a new morphotype in Gyrodactylus spp. found in Northwest Africa and Iberia was positioned on the phylogenetic tree of clade A. Since the studied Gyrodactylus spp. were collected only from Morocco and Spain, and because this morphotype has not so far been reported in the Middle East, where parasitological data are still insufficient, future extensive parasite sampling is necessary to cover a larger geographical range in order to investigate the potential boundaries of the distribution of the unusual Gyrodactylus morphotype. All the Gyrodactylus spp. from West Mediterranean cyprinids, mostly represented by *Luciobarbus* spp., possessed the median ridge in their ventral bar membranes (character 1). The widespread European G. katharineri, known from a range of cyprinids including *B. barbus* and *G. sandai* from the cyprinid *Capoeta* umbla (Heckel, 1843) endemic to the Middle East, showed well-developed lateral processes of the ventral bar (character 2), while the next species, G. mhaiseni, parasitizing the leuciscid Alburnus sellal Heckel, 1843 from the same latter region, showed poorly developed lateral processes. Thus, the former character of the ventral bar appeared twice in clade A and was found in two newly described *Gyrodactylus* spp. from the West Mediterranean. In contrast, G. pseudomoroccensis sp. nov. possessed short lateral processes of the ventral bar. This finding may suggest that well-developed lateral processes are ancestral characters of the ventral bar originating in Gyrodactylus parasitizing cyprinids occurring in the Middle East. A large sample size is, however, necessary to re-examine this hypothesis.

Other West Mediterranean Gyrodactylus spp., including Moroccan as well as Iberian species, possessed short or poorly developed lateral processes of the ventral bar. In contrast to the evolution of the ventral bar, the twisted inner roots of the hamuli (character 3) were found in Iberian Gyrodactylus sp. 1–2, as well as in G. gibraltarensis sp. nov. and Moroccan G. moroccensis sp. nov., evolving through allopatric speciation across the Strait of Gibraltar. Likewise, the membranous structure associated with the hamuli (character 4) was present only in G. gibraltarensis sp. nov. and G. moroccensis sp. nov. To our knowledge, this character associated with the hamuli has never been recognized in West Mediterranean Gyrodactylus, while it has already been found in European Gyrodactylus, mainly from *Barbus* spp. [18,92,93]. Furthermore, we identified a morphological character of the haptor which was common to all three newly described Gyrodactylus spp. Specifically, the T-shaped dorsal bar (character 5) was present in Iberian G. gibraltarensis sp. nov. and Northwest African G. moroccensis sp. nov. and G. pseudomoroccensis sp. nov. The Lago Mare phase, which followed the Messinian salinity crisis, resulted in a close phylogenetic relationship between North African and Iberian Luciobarbus spp. [46]. Our study showed that Moroccan and Iberian Gyrodactylus from West Mediterranean cyprinids formed a wellsupported lineage that is phylogenetically closely related to Gyrodactylus representatives in the Middle East. Considering the morphological characters of the haptor, we suggest that the median ridge of the ventral bar (character 1) might be an ancestral character differentiating Palearctic Gyrodactylus from lineages occurring outside of this geographical range—for example, those occurring in the Nearctic region, where this feature has never

been reported [60]. This character preassembly evolved before Palearctic *Gyrodactylus* diverged from their Middle East ancestor and before they reached the West Mediterranean. The morphological homologies associated with the well-developed lateral processes of the ventral bar (character 2), as well as the structures of the inner roots of the hamuli (characters 3–4), and the T-shaped dorsal bar (character 5), may suggest either an inheritance from a common ancestor or an instance of convergent evolution. The former hypothesis is in line with the historical biogeography of Eurasian, African, and Iberian cyprinid hosts [39]. This can be supported by the fact that the accessory portion found in the hamuli (character 4) in the West Mediterranean lineage is also present in *G. malmbergi* of European Barbinae [18,92,93]. Unfortunately, genetic data are not available for *G. malmbergi*; therefore, this species was not included in the phylogenetic reconstruction.

The inheritance of morphological features from a common ancestor was previously suggested in gill flatworms of the genus Cichlidogyrus Paperna, 1960 (Dactylogyridae), parasitizing fast-radiating ichthyofauna of the African Great Lakes [3]. In the peri-Mediterranean and the Middle East, convergent evolution of the haptoral sclerites (ventral bar) has been documented in dactylogyrid monogeneans [26,53]. From an evolutionary perspective, the T-shaped dorsal bar (character 5) we recovered in West Mediterranean Gyrodactylus spp. most probably evolved in North Africa by means of prolongation of the median part of the dorsal bar, leading to an additional branch positioned between the hamuli. Then, historical contacts between cyprinids crossing the land bridge between North Africa and Iberia likely allowed for the successful host switch of ancestral *Gyrodactylus* with the unusual morphology from North African cyprinids to Iberian *Luciobarbus* and subsequent speciation (according to the morphology and genetic distances, ongoing speciation seems to have played a role after geographical isolation). Similar evolution of the membranous structure associated with the hamuli (character 4) can also be proposed; however, this character likely became lost during the evolutionary history of *Gyrodactylus* in Moroccan *Luciobarbus* spp., as evidenced in *G. pseudomoroccensis* sp. nov. Overall, we emphasize that the evolution of the morphological characters of the attachment organ needs to be more thoroughly investigated by adding genetic data from a wider range of Palearctic *Gyrodactylus* spp.

5. Conclusions

The Strait of Gibraltar and its neighboring freshwater realms have experienced drastic climate and geological changes that have shaped the present distribution of cyprinid fish in this region and have favored a high rate of endemism. To the best of our knowledge, little was previously known about the composition of the parasite fauna of cyprinids in the West Mediterranean, mainly that of viviparous monogeneans, and genetic information was still missing until now. The present research is the first to reveal the taxonomic and genetic diversity of Gyrodactylus communities in the West Mediterranean, as well as their phylogenetic position within congeners worldwide. Morpho-genetic characterization of monogenean specimens parasitizing a set of cyprinid hosts endemic to two regions separated by the Strait of Gibraltar supported the Eurasian origin of West Mediterranean Gyrodactylus lineages and indicated vicariant speciation across the Strait, as well as ongoing speciation in Northwest Africa. Nevertheless, we emphasize that the haptoral characters of Gyrodactylus should be meticulously featured, since the species are known for their inconspicuous morphological diversity, and that a wide range of *Gyrodactylus* members are required to resolve phylogenetic uncertainties and elucidate the evolutionary history of Gyrodactylus.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/d15111152/s1, Table S1: Matrix of pairwise genetic distances (*p*-distance) based on alignments of the 18S rDNA (457 bp) and ITS regions (838 bp). West Mediterranean *Gyrodactylus* spp. investigated in this study are indicated in bold. **Author Contributions:** A.Š. designed and supervised the study, co-organized the field trip, participated in parasite collection and the preparation of specimens, and provided scientific background in the field of monogenean fauna of North African cyprinid fish. C.R. designed the study, performed microscopical observations, identified the new species, and drew the hard parts. M.S. and M.B. isolated, amplified, and sequenced the DNA samples. M.S. and C.R. performed the phylogenetic analyses. C.R. wrote the manuscript. A.Š., C.R., M.S. and M.B. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data supporting the conclusions of this paper are included within the article. The type-material of the new species described in this study was deposited in the Helminthological collection of the Institute of Parasitology, Biology Centre of Academy of Sciences of the Czech Republic, České Budějovice (IPCAS) under the accession numbers IPCAS-M779–81. The genetic sequence data have been deposited in the GenBank database (see Table 2 for the accession numbers). Uncorrected *p*-distances from the DNA sequences associated with this manuscript are provided online as electronic supplementary materials.

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