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Article

First Study on *Gyrodactylus* (Monogenea: Gyrodactylidae) in Morocco, with Description of a New Species from Cyprinids (Actinopterygii: Cyprinidae)

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Simple Summary: Monogenean flatworms are mainly parasitic in lower aquatic vertebrates including fish, anurans and chelonians. *Gyrodactylus* is one of the 23 genera of Gyrodactylidae,. With only 41 species described, the total number of *Gyrodactylus* species described from African freshwater fish still remains low. The known species represent only a fraction of the expected species richness of *Gyrodactylus* in Africa. In this study, we examined the gills of 738 cyprinid specimens. We isolated 26 *Gyrodactylus* individuals from these hosts. Twelve of these were morphologically characterized and proposed to belong to one single newly described species. In view of the importance of the cyprinid-monogenean system in studying the aquatic biodiversity and biogeography of North Africa, the present study is a substantial contribution to the parasite species inventory of these fishes.

Abstract: To date, 41 species of *Gyrodactylus* have been described from Africa. However, none of these have been reported in Morocco. After identifying and examining 738 cyprinid host specimens, 26 specimens belonging to *Gyrodactylus* were found to parasitize the gills of nine species of *Luciobarbus*, *Carasobarbus*, and *Pterocapoeta*. The current study describes in detail 12 specimens of *Gyrodactylus* isolated from the gills of *Luciobarbus pallaryi* (Pellegrin, 1919) and *Luciobarbus ksibi* (Boulenger, 1905). Based on morphoanatomical observations, the characterization of the specimens collected suggests a species of *Gyrodactylus* new to science, described here as *Gyrodactylus nyingiae* n. sp. The new species is different from previously described gyrodactylids infecting African cyprinid hosts because it has a longer hamulus total length, a longer hamulus root, a downward projecting toe, a trapezium shaped ventral bar membrane with slightly striated median portion and small rounded anterolateral processes. This study brings the total number of *Gyrodactylus* spp. found in African cyprinids to four.

Keywords: Cyprinidae; Ectoparasite; Gyrodactylidea; *Luciobarbus*; Monopisthocotylea; North Africa; Parasite; Platyhelminthes

1. Introduction

Morocco is considered to be one of the top producers of fisheries resources occupying the 13th place after Chile [1]. In 2018, the national fisheries production totaled a volume of 1,371,683 tons for a turnover of 11,579,544 thousand dirhams [1]. In freshwater, culture-based fisheries which are

projected to generate 13,000 tons of fish annually in Morocco, is the main source of fish protein. This production is based on the routine stocking of cultured organisms, mainly cyprinids, into lakes and reservoirs [2]. Biogeographers in the Maghreb region have often focused on ichthyofaunal studies because of its geographical position between the African and Eurasian plates. The primary freshwater fishes are a suitable subject for historical biogeography due to their limited dispersal that is strictly restricted to fluvial basins showing less capacity for trans-watershed dispersal [3]. However, the freshwater fish fauna of North Africa shows low diversity which could probably reflect a long period of isolation during the Cenozoic Era [3].

The high level of endemism of cyprinid fish in Morocco (20 endemic species) noted by [4] is linked to the geological and climatic history of the Mediterranean biome, which have led to the endemic status of many species (animal or plant) present in these zones [5,6]. Studies on freshwater fish parasites have increased globally due to the growing interest in developing fisheries and aquaculture as cheap sources of protein to sustain the rapidly growing human population, especially in some African communities [7]. For management of this resource, a thorough knowledge of the taxonomy, distribution, biology and ecology of parasites is of paramount importance [7].

The cyprinid host/parasite system constitutes a good model for studying evolutionary phenomena and determining speciation mechanisms. Previous studies [8] on representatives of the monogenean parasite *Dactylogyrus* Diesing, 1850 recorded 17 *Dactylogyrus* spp. on 17 cyprinid species from four genera (*Luciobarbus* Heckel, 1843; *Carasobarbus* Karaman, 1971; *Labeobarbus* Rüppel, 1835 and *Pterocapoeta* Günther, 1902) in Morocco. These studies revealed that these fishes arrived in Morocco through three different routes; from Europe, Asia and Sub-Saharan Africa [4,9]. Despite there being various studies on *Gyrodactylus* von Nordmann, 1832 in Africa, also on cyprinids, these often do not include species-level identifications (e.g. [10]) and only a few of its representatives have been identified to species level in the whole continent. Currently, there are over 400 valid species of *Gyrodactylus* described [11]. In African freshwater fishes, only 41 species of *Gyrodactylus* have been described [12]. The known species represent only a fraction of the expected *Gyrodactylus* spp. in Africa [13]. Only three *Gyrodactylus* species have been described from small African cyprinids, always with a host belonging to *Enteromius* Cope, 1867, namely *G. ivindoensis* Price & Gery, 1968 from *Enteromius* cf. *holotaenia* (Boulenger, 1904) in Gabon, *G. kyogae* Paperna, 1973 from *Enteromius neumayeri* (Fischer, 1884) and *Enteromius perince* (Rüppell, 1835) in Uganda and *G. paludinosus* Truter, Smit, Malherbe & Přikrylová, 2021 [12] from *Enteromius paludinosus* (Peters, 1852) in South Africa. In Morocco, no research has documented species of *Gyrodactylus* to date. Monogeneans belonging to *Gyrodactylus* are major pathogens in fishes as well as a major challenge in both fisheries and aquaculture. Gyrodactylids have an economic significance that outweighs that of any other monogenean family. In Norway, for example, the introduction of *Gyrodactylus salaris* Malmberg, 1957 into the salmon industry resulted in uncontrollable epidemics and mortalities, leading to massive economic losses [14].

Despite the numerous economic benefits a country may achieve from introduction of living organisms, they can also be detrimental to the native species [15]. In Lake Naivasha, Kenya, for example, common carp *Cyprinus carpio* Linnaeus, 1758 was thought to have reached the lake in 1999 during the heavy rains from juveniles that escaped in the Malewa River [16]. Parasitological studies on parasites of *C. carpio* in Lake Naivasha discovered it to be dominated by representatives of *Dactylogyrus* [17] with high prevalence of 99.3% [17]. Moroccan irrigation channels and reservoirs have also been stocked with non-native freshwater fish species such as the silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844), common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) which could pose a threat to the native fishes by providing a perfect opportunity for parasite transmission [18]. For this reason, it is important to have a baseline for the Moroccan native monogenean fauna of cyprinids. Therefore, and in view of the importance of the cyprinid-monogenean system in investigating the aquatic biodiversity and biogeography of North Africa, the present study aims to identify *Gyrodactylus* species infecting cyprinids across Morocco and contribute to the parasite species inventory of these fishes. The current

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study focuses on parasites belonging to *Gyrodactylus* isolated from the gills of *Luciobarbus pallaryi* (Pellegrin, 1919) and *Luciobarbus ksibi* (Boulenger, 1905).

2. Materials and Methods

2.1. Sample Collection

During September 2014 and June 2021, a total of 28 localities covering nine different watersheds in Morocco were sampled on five different occasions for cyprinid specimens as shown in Figure 1. The fish specimens were collected after getting the required permit from the Ministry of Water, Forestry and Desertification Control (sampling permit nº: 62 HCEFLCD/DLCDPN/CPC/PPC). These fish samples were collected using a backpack electrofisher (Samus-725G) or gill nets where the physico-chemical water parameters could not allow sampling using the electrofisher. Fish hosts were identified morphologically following [19], euthanized by severing their spinal cords and dissected immediately. The gills were fixed according to [20] and some fish specimens were frozen in a portable freezer and analyzed in the laboratory. The nomenclature and the classification of fishes are those provided in [21]. The map showing sampling localities (Figure 1) was created using QGIS v3.22.8 (QGIS Development Team 2022. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org, accessed on 20 January 2023).

2.2. Parasitological Examination

The fish samples were transported to the laboratory for parasitological examination. Monogeneans were isolated under a dissecting microscope (Wild Heerbrugg) from the gills (gill arches from the right side of the excised fish). With the aid of a fine needle, the parasites were picked out one by one and subsequently mounted on a glass slide then covered with a coverslip to 'flatten' the specimen. The slides were mounted according to [22]. For worms fixed in ethanol, Hoyer's chloral hydrate was used [23] while ammonium picrate glycerine was used for frozen parasites [24]. The glass slide is left to dry for 24 hours in a horizontal position before sealing the coverslip with Glyceel [25]. Type material was deposited in the collections of the Research Group Zoology: Biodiversity & Toxicology at Hasselt University (HU) (Diepenbeek, Belgium) (HU 838-841) and the Institut Scientifique of the Mohammed V University in Rabat (Rabat, Morocco) (ZA PPM 0101).

2.3. Identification of Representatives of Gyrodactylus

Gyrodactylus was distinguished from the other monogeneans as its members have a cylindrical body bearing two small cephalic lobes on the exterior part of the body, lack eyes and possess an opisthaptor armed with a single pair of hamuli linked by dorsal and ventral bars with 16 articulated marginal hooks (14 in members of *Dactylogyrus*, the other monogenean genus most common on Moroccan cyprinids) [26].

2.4. Infection Parameters

Infection parameters for members of *Gyrodactylus* were calculated according to [27] as follows in Table 2:

2.5. Morphological Characterization of Members of Gyrodactylus

Light microscopy using both phase and differential interference contrast approaches were used to study the shape and dimensions of sclerotized structures, which were viewed under a x100 oil immersion objective on a Leica DM2500 optical microscope using Las X software v3.6.0.20104 fitted with a Leica DMC4500 camera. The whole mount, attachment organ, and male copulatory organ (MCO) (when present) on each specimen were photographed. The haptoral morphometrics (26 point-to-point measurements) followed the measurements proposed by [28]; these were taken using ImageJ v1.53k software (available at http://imagej.nih.gov/ij). These measurements are given in micrometers (μm) as the mean, followed by the range in parentheses and number of structures (n) measured for

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each metric. The micrographs taken were used to draw taxonomically important structures using Inkscape v1.2.

2.6. Statistical Analyses

For statistical analysis, a Principal Component Analysis (PCA) was carried out in R Studio v4.1.0. The analyses included 19 measurements of the haptoral hard parts of hamuli and marginal hooks only. The MCO, ventral bar and dorsal bar measurements were excluded from the analysis due to the large number of missing data.

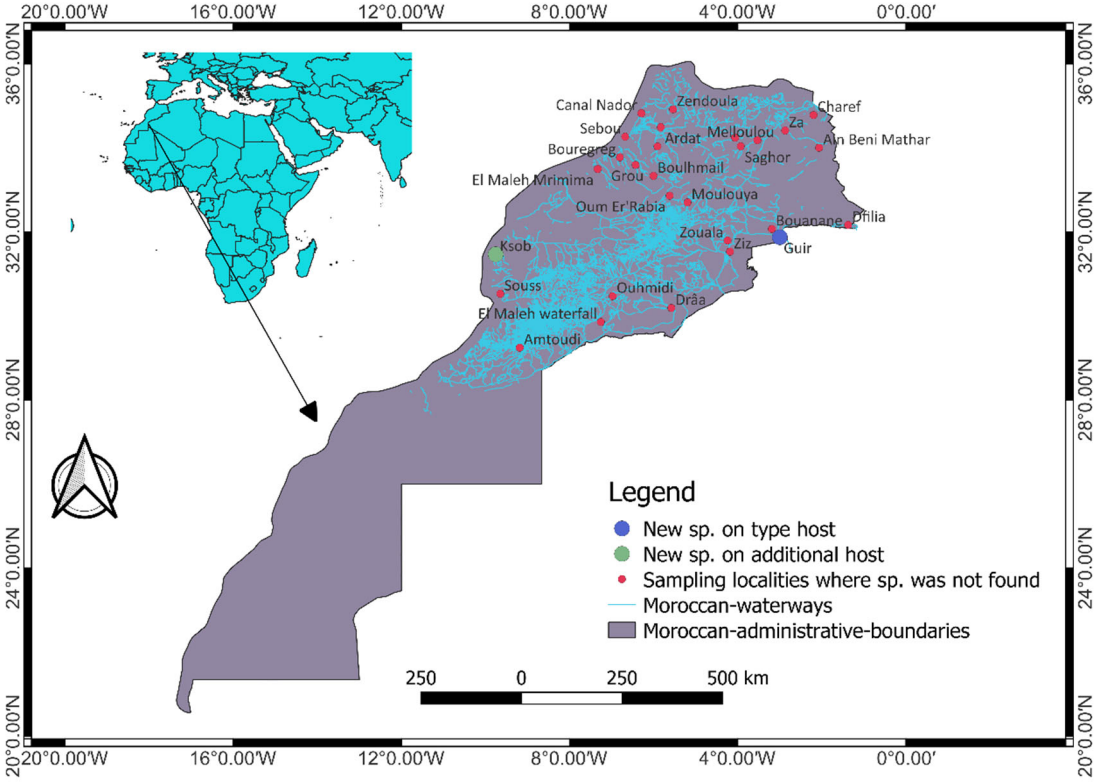

Figure 1. An overview of the sampling locations of cyprinid hosts examined for the presence of members of *Gyrodactylus*. The type host and additional host localities for the newly described *Gyrodactylus* species in the present study are depicted by blue and green circles respectively.

3. Results

3.1. Specimens Examined and Individuals of Gyrodactylus Isolated

A total of 738 fish specimens belonging to three genera (*Luciobarbus* Heckel, 1843, *Carasobarbus* Karaman, 1871 and *Pterocapoeta* Günther, 1902) were collected. Thirteen cyprinid fish species were identified and their gills examined for infection with species of *Gyrodactylus* (Table 1). Nine out of the 13 species were found to be infected with representatives of *Gyrodactylus* (n=26).

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Table 1. Cyprinid specimens collected, their localities and number of individuals belonging to *Gyrodactylus* infecting the hosts.

Host	Locality	Watershed	Coordinates		Nb. of hosts sampled	Nb. of specimens of *Gyrodactylus* isolated from the hosts
			Latitude	Longitude		
Luciobarbus pallaryi (Pellegrin, 1919)	Oued Guir	Ziz	31°52'12''N	003°0'00''W	157	14
	Oued Bouanane	Ziz	32°04'04''N	003°11'23.9'' W		
	Oued Dfilia	Ziz	32°9'48.892''N	001°22'37.4'' W		
Luciobarbus rabatensis Doadrio, Perea & Yahyaoui, 2015	Oued Grou	Bouregreg	33°35'28.0''N	006°25'49.6'' W	24	3
	Oued Bouregreg	Bouregreg	33°46'18.0''N	006°48'16.6'' W		
	Oued Boulhmail	Bouregreg	33°19'49.6''N	006°00'15.1'' W		
Luciobarbus maghrebensis Doadrio, Perea & Yahyaoui, 2015	Oued Lahdar	Sebou	34°14'32.7''N	004°03'53.9'' W	55	1
	Oued Saghor	Sebou	34°02'4.0'' N	003°55'45.5' W		
	Oued Ardat	Sebou	34°29'26.8''N	005°49'49.2'' W		
	Oued Beht	Sebou	34°01'55.5''N	005°54'43.2'' W		

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	Oued Sebou	Sebou	34°15'48.0"N	006°40'42.0" W		
	Canal Nador	Sebou	34°49'19.7"N	006°17'36.7" W		
Luciobarbus rifensis Doadrio, Casal-López & Perea 2015	Oued Zendoula	Loukkos	34°54'57.6"N	005°32'17.2" W	19	3
Luciobarbus guercifensis Doadrio, Perea & Yahyaoui, 2016	Oued Melloulou	Moulouya	34°10'51.7"N	003°31'59.6" W	4	0
	Oued Za	Moulouya	34°24'38.9"N	002°52'28.1" W		
Luciobarbus yahyaouii Doadrio, Casal-López & Perea 2016	Oued Za	Moulouya	34°24'38.9"N	002°52'28.1" W	62	0
	Oued Charef	Moulouya	34°46'44.0"N	002°11'56.0" W		
	Oued Melloulou	Moulouya	34°10'51.7"N	003°31'59.6" W		
	Ain Beni Mathar	Moulouya	34°00'00.3"N	002°03'58.6" W		
Luciobarbus zayanensis Doadrio, Casal-López & Yahyaoui, 2016	Oued Oum Er'Rabia	Oum Er'Rabia	32°51'32.8"N	005°37'18.9" W	25	1
	Oued Moulouya	Moulouya	32°41'55.4"N	005°11'51.2" W		
Luciobarbus lepineyi (Pellegrin, 1939)	Oued Ziz	Ziz	31°31'34.7"N	004°11'10.0" W	127	0

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	Oued Zouala	Ziz	31°47'31.9"N	004°14'43.5" W		
	Oued Dfilia	Ziz	32°9'48.892"N	001°22'37.4" W		
	Oued Drâa	Draa	30°11'12.24"N	005°34'47.34" W		
	Oued Ouhmidi	Draa	30°28'5.64"N	006°58'36.12" W		
	Oued El Maleh Mrimima	Draa	33°29′34.8′′N	007°19'58.1" W		
	Oued El Maleh Waterfall	Draa	29°51'108"N	007°15'23"W		
	Oued Amtoudi	Draa	29°14'32.42" N	009°11'8.71" W		
Carasobarbus moulouyensis (Pellegrin, 1924)	Oued Moulouya	Moulouya	32°41'55.4"N	005°11'51.2" W	44	1
Luciobarbus ksibi (Boulenger, 1905)	Oued Oum Er'Rabia	Oum Er'Rabia	32°51'32.8"N	005°37'18.9" W	40	1
	Oued Ksob	Ksob	31°27'50.7"N	009°45'25.3" W		
Luciobarbus massaensis (Pellegrin, 1922)	Oued Souss	Souss-Massa	30°31'33.6"N	009°38'53.6" W	21	1
Carasobarbus fritschii (Günther, 1874)	Oued Grou	Bouregreg	33°35'28.0"N	006°25'49.6" W	157	0
	Oued Boulhmail	Bouregreg	33°19'49.6"N	006°00'15.1" W		
	Oued Lahdar	Sebou	34°14'32.7"N	004°03'53.9" W		

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	Oued Oum Er'Rabia	Oum Er'Rabia	32°41'03.8"N	005°13'00.3" W		
	Oued Za	Moulouya	34°24'38.9"N	002°52'28.1" W		
	Oued Charef	Moulouya	34°46'44.0"N	002°11'56.0" W		
	Oued Ksob	Ksob	31°27'50.7"N	009°45'25.3" W		
	Oued Ardat	Sebou	34°29'26.8"N	005°49'49.2" W		
	Oued Beht	Sebou	34°01'55.5"N	005°54'43.2" W		
	Oued Sebou	Sebou	34°15'48.0"N	006°40'42.0" W		
Pterocapoeta maroccana Günther, 1902	Oued Oum Er'Rabia	Oum Er'Rabia	32°51'32.8"N	005°37'18.9" W	3	1
				Total	**738**	**26**

3.2. Infection Parameters

The infection parameters of examined hosts are as shown in Table 2.

Table 2. Prevalence, mean intensity and mean abundance of *Gyrodactylus* infecting cyprinid hosts, based on examination of the right-side gill arches only.

Locality	Species	H	N	*n*	P=(N/H)*100	M. I=n/N	M.A=n/H
Oued Guir	*Luciobarbus pallaryi*	157	1	14	0.64	14	0.09
Oued Bouregreg	*Luciobarbus rabatensis*	24	1	3	4.17	3	0.13
Oued Sebou	*Luciobarbus maghrebensis*	55	1	1	1.82	1	0.02
Oued Zendoula	*Luciobarbus rifensis*	19	1	3	5.26	3	0.16
Oued Moulouya	*Luciobarbus zayanensis*	25	1	1	4.00	1	0.04
Oued Moulouya	*Carasobarbus moulouyensis*	44	1	1	2.27	1	0.02
Oued Ksob	*Luciobarbus ksibi*	40	1	1	2.50	1	0.03
Oued Souss	*Luciobarbus*	21	1	1	4.76	1	0.05

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Oued Oum Er'Rabia	*massaensis Pterocapoeta maroccana*	3	1	1	33.33	1	0.33

H, number of examined hosts; N, number of infected hosts; n, number of individuals of *Gyrodactylus* in infected host; P, prevalence; M.I, mean infection intensity; M.A, mean abundance.

3.3. Characterization of a New Species of Gyrodactylus

All the isolated flatworms belonging to *Gyrodactylus* showed the diagnostic features of this genus: gyrodactylid monogeneans with opisthaptor having one pair of haptoral anchors surrounded up by 16 marginal hooks. The measurements are given in Table 3.

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

Type material: holotype (HU_838_IV.1.18) and five paratypes (HU_839_IV.1.19, HU_840_IV.1.20, HU_841_IV.1.21, ZA PPM 0101)
Type host: *Luciobarbus pallaryi* (Pellegrin, 1919) (Teleostei: Cyprinidae)
Other host: *Luciobarbus ksibi* (Boulenger, 1905) (Teleostei: Cyprinidae)
Type locality: Oued Guir (31°52'12''N, 003°0'00''W) (on type host)
Other locality: Oued Ksob (31°27'50.7''N, 009°45'25.3''W) (on *L. ksibi*)
Site of infection: Gill filaments
ZooBank registration: The Life Science Identifier (LSID) of the article is xxx. The LSID for *Gyrodactylus nyingiae* Shigoley, Rahmouni, Louizi, Pariselle & Vanhove n. sp. is xxx.
Studied material: 12 mounted individuals were measured; 11 of these were isolated from *L. pallaryi* and one from *L. ksibi*

Etymology: The species epithet honors Dr. Dorothy Wanja Nyingi, ichthyologist at the National Museums of Kenya and author of the first *Guide to Common Freshwater Fishes of Kenya*

Description: Elongated body. Male copulatory organ (MCO) observed in five specimens, spherical (Figure 2a, i; 3 ii), positioned posterior to the pharynx and armed with one principal spine and single row of 5-6 smaller spines (Figure 2a, i; 3 ii). Hamuli slightly slender with a pointed tip with a superficial root (Figure 2a, ii; 3 i). Anterior end where dorsal bar attaches on hamulus prominent, creating a notch between the root and dorsal bar attachment point. Dorsal bar simple and flexible. Ventral bar with small rounded anterolateral processes; trapezoid-like membrane with a slightly striated median portion (Figure 2a, iii). Marginal hook shaft approximately perpendicular to the base of the marginal hook sickle (Figure 2a, iv; 3 iii). Sickle point is slightly curved and perpendicular to the base with its tip in line with the distal end of the toe. Overlapping measurements (Table 3) and the similarity in the shape of the marginal hook sickle (Figure 4) suggest the worms infecting the two host species are conspecific.

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Table 3. Morphometric measurements of sclerotized parts of *Gyrodactylus nyingiae* n. sp. The number of structures measured is given in superscript.

Host	*Luciobarbus pallaryi* (n=1)	*Luciobarbus ksibi* (n=1)	Both host species combined
Total body length	386.8 (278.3-456)^{5}	443.7	396.3 (278.3-456)^{6}
Total body width	133 (115.8-145.9)^{6}	158	136.6 (115.8-158.4)^{7}
Hamulus total length	76.5 (65.9-88.2)^{10}	75.3	76.4 (65.9-88.2)^{11}
Hamulus sickle length	47.6 (42.5-54.8)^{8}	45.1	47.4 (42.5-54.8)^{9}
Hamulus aperture distance	27.5 (21.1-30.2)^{9}	22.6	26.7 (21.1-30.2)^{10}
Hamulus point length	36.9 (31.7-41.3)^{9}	36.3	36.2 (31.7-41.3)^{10}
Hamulus inner curve length	1.7 (1.4-2.7)^{6}	4	2.1 (1.4-4)^{7}
Hamulus distal shaft width	5.5 (4.6-6.7)^{10}	6	5.7 (4.6-7.3)^{11}
Hamulus root length	26.7 (24.2-28.3)^{7}	-	26.7 (24.2-28.3)^{7}
Hamulus aperture angle (in degrees)	36.9 (31.5-45.4)^{7}	32.4	36.4 (31.5-45.4)^{8}
Hamulus point curve angle (in degrees)	4.4 (3.4-5.4)^{4}	-	4.4 (3.4-5.4)^{4}
Hamulus inner angle (in degrees)	40.4 (36-45.4)^{7}	37.2	40 (36-45.4)^{8}
Hamulus proximal shaft width	10.4 (8.2-12.1)^{10}	10.3	10.2 (8.2-12.1)^{11}
Marginal hook total length	34.4 (31.7-42.1)^{8}	35.2	34.8 (31.7-42.1)^{9}
Marginal hook shaft length	28.6 (26.1-33.4)^{9}	29.2	28.7 (26.1-33.4)^{10}
Marginal hook sickle length	6.2 (5.5-6.5)^{9}	6.6	6.3 (5.5-6.6)^{10}
Marginal hook sickle proximal width	4.6 (3.9-5)^{9}	5.5	4.7 (3.9-5.5)^{10}
Marginal hook sickle distal width	4.5 (3.9-5.1)^{9}	5	4.5 (3.9-5.1)^{10}
Marginal hook sickle toe length	1.9 (1.8-2.1)^{9}	2.1	2 (1.8-2.1)^{10}
Marginal hook aperture distance	5.5 (5-5.9)^{8}	5.3	5.4 (5-5.9)^{9}
Marginal hook in-step height	0.6 (0.5-0.9)^{8}	0.7	0.6 (0.5-0.9)^{9}
Ventral bar total length	19.6 (18.6-20.5)^{2}	-	19.6 (18.6-20.5)^{2}
Ventral bar total width	25.1 (24.8-25.4)^{2}	-	25.1 (24.8-25.4)^{2}
Ventral bar median length	6.1 (5.5-6.8)^{3}	-	6.1 (5.5-6.8)^{3}
Ventral bar membrane length	13.6 (12.7-14.5)^{3}	-	13.6 (12.7-14.5)^{3}
Ventral bar process length	3.7 (3.6-3.8)^{2}	-	3.7 (3.6-3.8)^{2}
Male copulatory organ diameter	18.4 (16.5-19.5)^{4}	21.2	18.9 (16.5-21.2)^{5}
Principal spine length	6.5 (6.3-6.6)^{3}	6.5	6.5 (6.3-6.6)^{4}
Small spine length	3.3 (3.1-3.5)^{3}	5.4	4.4 (3.1-5.4)^{4}
Dorsal bar length	11.9 (9.9-13.4)^{3}	-	11.9 (9.9-13.4)^{3}
Dorsal bar width	1.6 (1.2-1.9)^{3}	-	1.6 (1.2-1.9)^{3}

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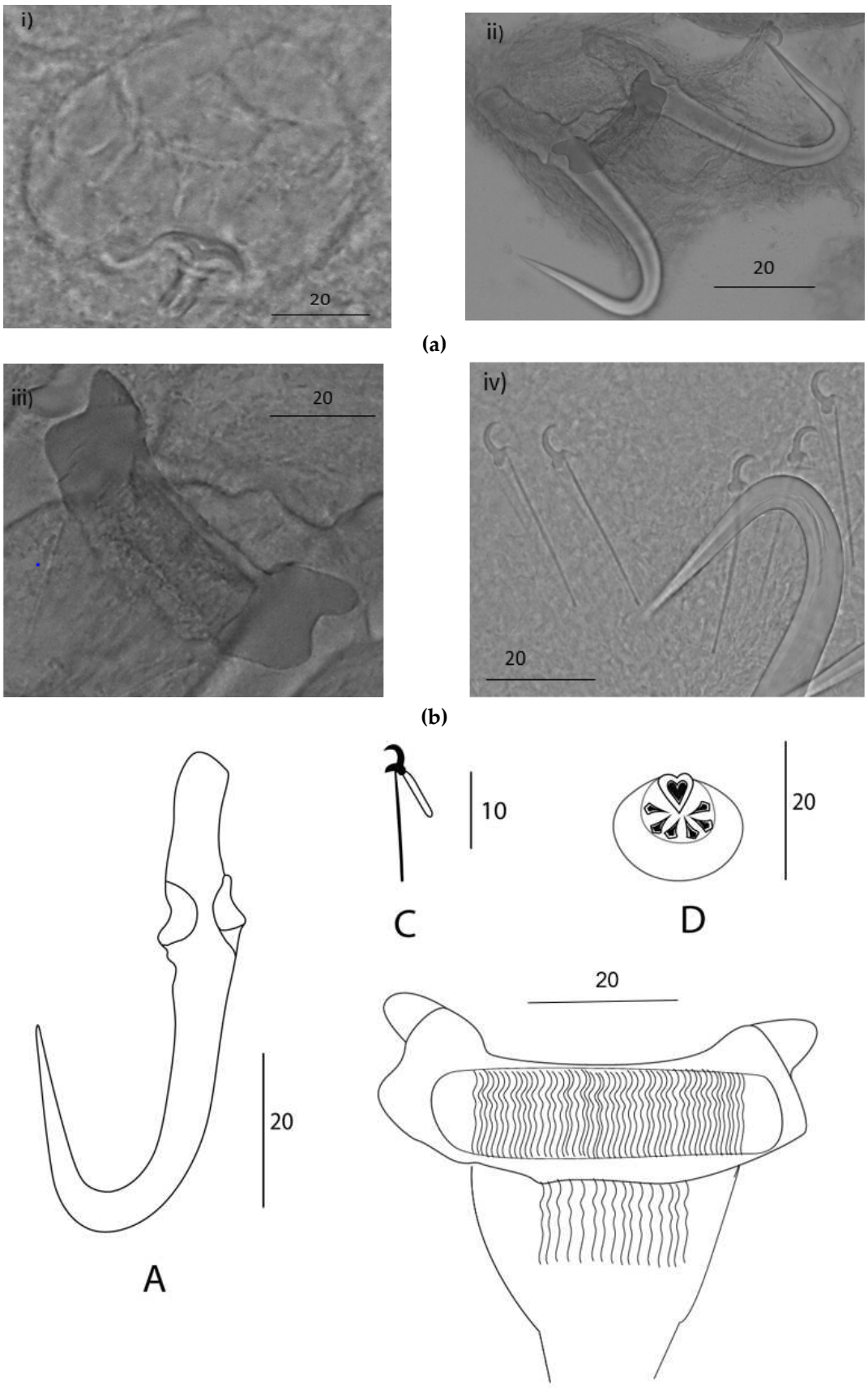

Figure 2. *Gyrodactylus nyingiae* n. sp. isolated from *Luciobarbus pallaryi*. (a) Micrograph of hamuli, male copulatory organs (MCO), ventral bar and marginal hooks. (b) Drawings of sclerotized structures of the haptor with (A); hamuli, (B); ventral bar, (C); marginal hook, and of the (D); male copulatory organ. Scale bars in µm.

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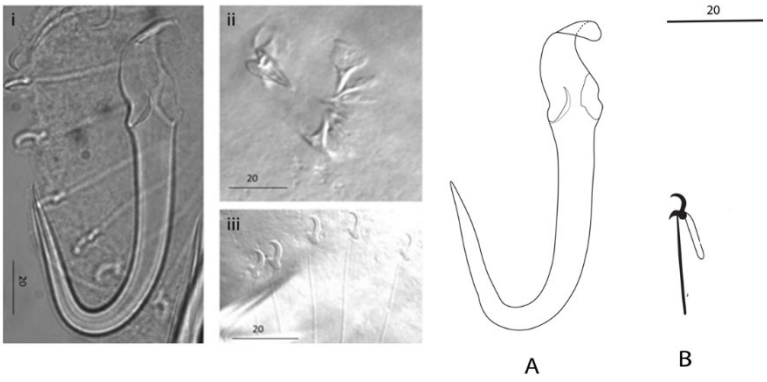

Figure 3. Micrograph and drawings of sclerotized structures of *Gyrodactylus nyingiae* n. sp. isolated from *Luciobarbus ksibi.*

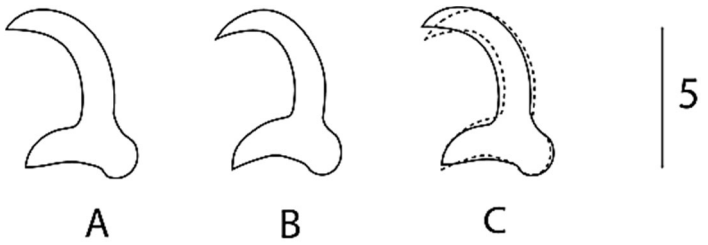

Figure 4. Overlay of marginal hook sickles (4C) of *G. nyingiae* n. sp. from *L. ksibi* (4A) and *L. pallaryi* (4B) (dotted outline). Scale bar represents 5µm.

Remarks:

The comparison with other gyrodactylid species is based on the phenotypic similarities with known parasite species and their occurrence from related hosts. From the three species of *Gyrodactylus* recorded from cyprinids in Africa, the here newly described species of *Gyrodactylus* can be differentiated by the longer hamuli: *G. nyingiae* n. sp. 76.5 (65.9-88.2) compared to a hamulus total length in *G. ivindoensis* of 55 (52-58), 32.1 (23-33) in *G. kyogae* and 43.3 (35.1-51.5) in *G. paludinosus*. Like *G. paludinosus*, *G. kyogae* has an upward projecting toe as opposed to that of *G. nyingiae* n. sp. whose toe points downwards. Also, the MCO of *G. nyingiae* n. sp. has one principal spine and five to six smaller spines arranged in a single row (Figures 2 and 3) as opposed to *G. kyogae* which has an unarmed MCO [29]. *Gyrodactylus kyogae*, in contrast to the other three species, lacks a ventral bar membrane. *Gyrodactylus ivindoensis* has shorter marginal hooks and a marginal hook total length of 22 (21-24) when compared to *G. nyingiae* n. sp. 34.8 (31.7-42.1). When comparing the relative length of the root to hamulus total length respectively, *G. nyingiae* n. sp. (26.7 vs 76.4), *G. ivindoensis* (19.4 vs 55) and *G. paludinosus* (15.4 vs 43.3) have similar ratios of the root length and total hamulus length (ca. 1:2.8). *Gyrodactylus kyogae* (9.2 vs 33.1) on the other hand has a different ratio of the relative root length to hamulus total length (1:3.5).

Due to the important biogeographical connections between the Middle East and Maghreb region during the Cenozoic period in the dispersal of freshwater fish fauna, it is interesting to compare the *Gyrodactylus* fauna of the Iranian region with the North African ones [3,30]. The freshwater species of *Gyrodactylus* mentioned by [31] and [32] were either known from Europe or Central Asia, or undescribed. It is therefore instructive to compare *G. nyingiae* n. sp. with widespread Palearctic species of *Gyrodactylus* infecting cyprinids, several of which are reminiscent of *G. nyingiae* n. sp. in marginal hook morphology. This includes *Gyrodactylus mutabilitas* Bychowsky, 1957 and *Gyrodactylus scardiniensis* Glaeser, 1974, which can both be distinguished from *G. nyingiae* n. sp. by virtue of their shorter hamulus root (max. 20 in *G. mutabilitas* and max. 23 in *G. scardiniensis* versus min. 24 in *G. nyingiae* n. sp.), and *Gyrodactylus schulmani* Ling, 1962 which has a hamulus of a total length of max. 44, shorter than the minimally 66 of *G. nyingiae* n. sp. A species described from a fish species endemic

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to Iran is *Gyrodactylus jalalii* Vanhove, Boeger, Muterezi Bukinga, Volckaert, Huyse & Pariselle, 2012, parasite of the cichlid host *Iranocichla hormuzensis* Coad, 1982. It can easily be distinguished from *G. nyingiae* n. sp. by its more pronounced ventral bar auricles and the sub rectangular ventral bar membrane as opposed to *G. nyingiae* n. sp. having small rounded anterolateral processes and a trapezium shaped ventral bar membrane. Following [33] *Gyrodactylus molnari* Ergens, 1978 infecting *Cyprinus carpio* Linnaeus, 1758 in Iraq is smaller in hamuli length (55-65) versus *G. nyingiae* n. sp. (65.9-88.2). Also, *G. molnari* has a longer dorsal bar (15-18), compared to *G. nyingiae* n. sp. (9.9-13.4), and an entirely different shape of marginal hook sickle. Therefore, *G. nyingiae* n. sp. can be distinguished from the aforementioned *Gyrodactylus* species by virtue of its longer hamulus total length, longer hamulus root, small rounded anterolateral process and trapezium shaped ventral bar membrane.

3.4. Multivariate Statistics

The morphological variation of the 12 specimens of *Gyrodactylus* was visualized based on a PCA performed on 19 standardized haptoral morphometric characters. The first two principal component axes contributed 25.9% and 19.6% of the variation respectively (Figure 5).

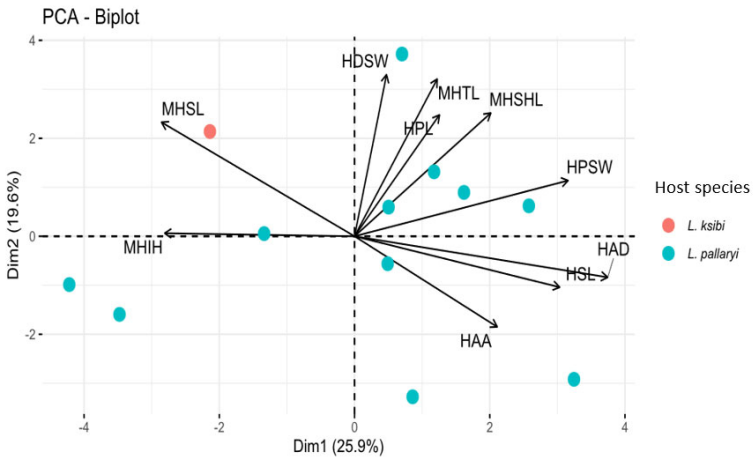

Figure 5. Biplot of PCA (first two axes) of all the 12 specimens of *Gyrodactylus* sp.

The biplot shows a single cloud which includes all the 12 specimens belonging to *Gyrodactylus*. From the PCA biplot we can confirm that we are dealing with a single species described herein as *Gyrodactylus nyingiae* n. sp.

4. Discussion

Gyrodactylus nyingiae n. sp. is the first described species of *Gyrodactylus* in Morocco. It is also the first gyrodactylid to be described from *Luciobarbus* in Africa, as previous studies on gyrodactylids infecting cyprinids in Africa have focused on the small barbs belonging to *Enteromius*, with only three *Gyrodactylus* species described so far [12].

In addition to the low prevalence and possible seasonality of members of *Gyrodactylus*, less research, a lack of reports on infections, and a lack of understanding of relationships between these monogeneans and cyprinid hosts [12], the current study's low number of gyrodactylids isolated from cyprinid hosts could be due to the fact that only the gills were examined for parasites. However, on native cichlid fishes in Morocco, [34] also found a species depauperate fauna and low abundances of gill-infecting dactylogyrids: hence, we cannot exclude that environmental conditions in Morocco's freshwater ecosystems limit the species richness and abundance of certain monogenean taxa. On the other hand, *Dactylogyrus* reaches higher species richness and higher infection intensities on Moroccan cyprinid-monogenean systems [4,35]

More research is needed in the African continent to understand the relationship, evolutionary history, and development of gyrodactylids and their hosts, as it is endowed with a diverse endemic fish fauna that undoubtedly harbors undiscovered parasite diversity [36,37].

5. Conclusions

Based on the morphoanatomical observation of opisthaptoral parts of 12 individuals of *Gyrodactylus* in the current study, we described a new species infecting two cyprinid hosts for the first time in Morocco. The new species is different from previously described gyrodactylids infecting cyprinid hosts because it has a longer hamulus total length, a longer hamulus root, a downward projecting toe, trapezium shaped ventral bar membrane with slightly striated median portion and small rounded anterolateral processes.

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