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Two new records of *Dactylogyrus* Diesing, 1850 (Monogenea: Dactylogyridae) from cyprinid fishes farmed in Cuba

Dos nuevos registros de *Dactylogyrus Diesing*, 1850 (Monogenea: Dactylogyridae) en peces Ciprínidos de cultivo para Cuba

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ABSTRACT | Two monogenean species were collected from gills of Labeo, *Labeo rohita* (Hamilton, 1822), and silver carp, *Hypophthalmichthys molitrix* Valenciennes, 1844 from four localities in Cuba: "La Sierpe" Fish hatchery and Zaza reservoir (Sancti Spíritus province); "Carlos Manuel de Céspedes" Fish hatchery (Santiago de Cuba province); and in the Aquaculture Technologies Company, EDTA (Havana province). Present specimens were identified by comparing them with the original morphometric characteristics of *Dactylogyrus labei* Musselius and Gussev, 1976 (Gussev, 1976) from *L. rohita*; and *Dactylogyrus hypophthalmichthys* Achmerow, 1952 from *H. molitrix*. The collected parasites were set in ammonium-picrate and glycerin solution and mounted in gelatin glycerin. Drawings, digital images, and morphometric data were presented for each species. These new monogenean records for Cuba also represent a new world geographical location.

Palabras clave
Monogeneos
Labeo rohita
Hypophthalmichthys molitrix
Dactylogyrus labei
Dactylogyrus hypophthalmichthys

RESUMEN | Dos especies de monogeneos fueron colectadas en filamentos branquiales de Labeo, *Labeo rohita* (Hamilton, 1822) y Tenca plateada, *Hypophthalmichthys molitrix* Valenciennes, 1844 en cuatro localidades del país: Centro de alevinaje "La Sierpe" y embalse Zaza (provincia Sancti Spíritus); Centro de alevinaje "Carlos Manuel de Céspedes" (provincia Santiago de Cuba); y en la Empresa de Desarrollo de Tecnologías Acuícolas, EDTA (provincia La Habana). Las dos especies fueron identificadas acorde a sus características morfológicas y medidas morfométricas como *Dactylogyrus labei* Musselius y Gussev, 1976 (Gussev, 1976) de *L. rohita*; y *Dactylogyrus hypophthalmichthys* Achmerow, 1952 de *H. molitrix*. Los parásitos colectados fueron fijados en solución de Amonio-picrato y glicerina, y montados en glicerina gelatina. Se presentaron dibujos, imágenes digitales y datos morfométricos para cada especie. Estos nuevos registros de monogeneos para Cuba también representan una nueva localidad geográfica mundial.

INTRODUCTION

The class Monogenea Van Beneden, 1858, includes several fish parasites with a worldwide distribution and are characterized by having a direct life cycle. Many monogenean species have been transferred together with their hosts, due to the international movement, causing its large dispersion throughout the world (Prieto *et al.*, 1985; Jiménez-García *et al.*, 2001; Vidal-Martínez *et al.*, 2002; Flores-Crespo & Flores, 2003).

The genus *Dactylogyrus* Diesing, 1850, comprises more than 900 described species (Gibson *et al.*, 1996). They are ectoparasites of the gills of several fish species with high commercial value. The high species diversity of dactylogyrids in cyprinids could be explained by the great diversity of existing host fishes of this family (Cyprinidae), which represents the most diverse of freshwater fishes in the world with around 1700 species and 200 genera (Helfman *et al.*, 1997).

Various species of *Dactylogyrus* are the reason for large economic losses in fish production systems in different countries, especially in the first life stages of fish. The observed damages depend on the intensity of the infestation (Flores-Crespo & Flores, 2003). When the infestations are massive, marked weight losses and high mortality rates are presented. Generally, the monogeneans show a marked specificity in their host-parasite relationship. According to Šimková *et al.* (2006), they can show a strict specificity to a single kind of host, to host species of the same genus, or to host species of different genera that are phylogenetically related.

In Cuba, previous studies about species of *Dactylogyrus* (Prieto *et al.*, 1993; Prats *et al.*, 2019) refer to the establishment of *D. aristichthys* Long & Yu, 1958 and *D. nobilis* Long & Yu, 1958 in *Hypophthalmichthys nobilis* (bighead carp); *D. lamellatus* Achmerow, 1952 in *Ctenopharyngodon idella* (grass carp); *D. vastator* Nybelin, 1924, *D. minutus* Kulwiec, 1927 and *D. extensus* Mueller and Van Cleave, 1932 in *Cyprinus carpio* (common carp); and *D. dulkeiti* Bychowsky, 1936, *D. baueri* Gussev, 1955, *D. formosus* Kulwiec, 1927, and *D. intermedius* Weger, 1910 in *Carassius auratus* (Goldfish).

In order to contribute to the knowledge of the helminthological fauna that affects aquaculture, we add two new species of dactylogyrids for Cuba with *D. labei* and *D. hypophthalmichthys*, as well as a new geographical location for these species.

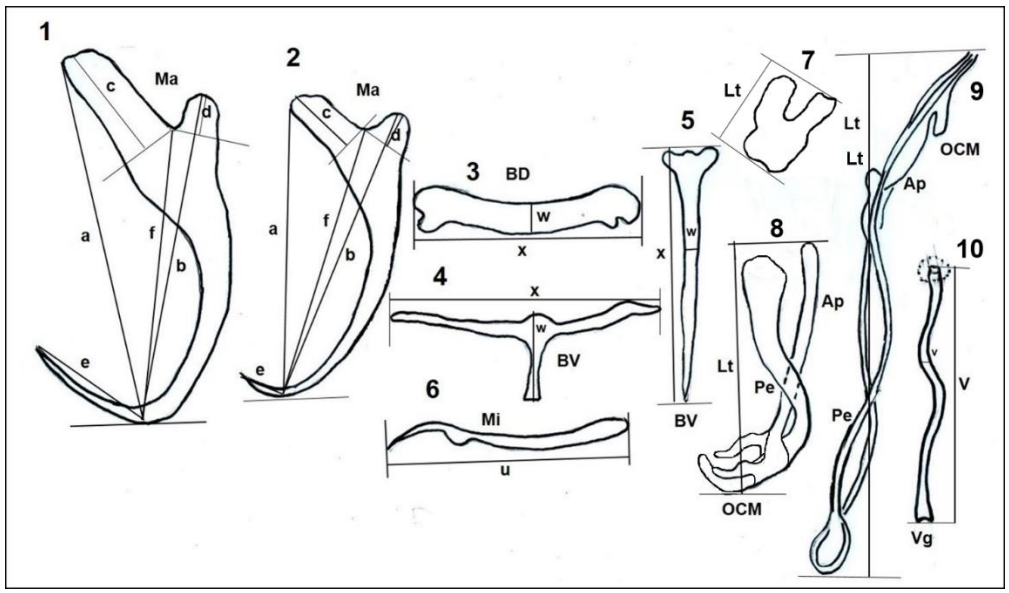
MATERIALS AND METHODS

The studied fishes were collected in 2019, in four locations in the country. The *L. rohita* specimens were collected in the localities: Zaza reservoir; "La Sierpe" Fish hatchery (Sancti Spíritus province) and "Carlos Manuel de Céspedes" Fish hatchery (Santiago de Cuba province). The *H. molitrix* specimens were collected in the Aquaculture Technologies Company, EDTA (Havana province). Ten specimens per locality were sampled. All fishes were measured and weighed (see Table 1).

Living fishes were transported to the laboratory, where they were sacrificed by severing the spinal cord and examined for parasites. Opercular dissections were conducted to expose and extract the gills. The samples were placed in a Petri dish containing water, and each branchial arch was carefully scraped to obtain the largest number of parasites.

The collected monogeneans were fixed in ammonium picrate and glycerin following the procedure described by Malmberg (1957) to examine the sclerotized structures. These semi-permanent preparations were observed with the aid of a Motic BA 210 digital microscope at 1000X magnification with an oil immersion lens. Digital images were taken and processed by Motic Images Plus (version 2). Once the material was analyzed, some samples were disassembled and re-mounted in Canada Balsam for permanent preservation. Measurements are presented in micrometers (µm), as follows: mean value ± standard deviation (min−max value) (Gussev, 1979; 1985). Prevalence and mean intensity of parasites were calculated according to Bush et al. (1997).

The studied reference specimens (slides LPCIP2019 [01–10]) were deposited in the collection of the Parasitology Laboratory, Fisheries Research Center, Havana, Cuba.



Figures 1–10. Schematic representation of the measurements taken of the sclerotized structures used in this study to identify two species of *Dactylogyrus*. **1–2**. Ventral view of the anchor, where a = anchor internal length, b = anchor external length, c = anchor inner root, d = anchor outer root, e = point length, and f = main part length. **3**. Ventral view of dorsal bar, where x = length and w = width. **4–5.** Ventral view of ventral bars, where x = length and w = width. **6.** Ventral view of the marginal hook, where u = the marginal hook shaft length. **7.** Ventral view of free complementary piece of male copulatory complex of *D. labei*, where Lt = piece length. **8–9.** Ventral view of the male copulatory organ of *D. hypophthalmichthys* and *D. labei:* Lt = total length of male copulatory organ. **10.** Ventral view of the vagina where V = length and v = width. Abbreviations: AP, accessory piece; BD, dorsal bar; Ma, anchor; Mi, marginal hook shaft; OCM, male copulatory organ; Pe, penis, Vg = vagina.

RESULTS

Two species of *Dactylogyrus* were identified from *L. rohita* (Hamilton, 1822) (Labeo) and *H. molitrix* Valenciennes, 1844 (Silver carp): *D. labei* Musselius & Gussev, 1976; and *D. hypophthalmichthys* Achmerow, 1952. Prevalence and mean intensity of parasites of the two species of *Dactylogyrus* are presented in Table 1; their morphometric variations in Tables 2-3 and their images in Figures 11-18.

Table 1. Prevalence and mean intensity of infestation for *Dactylogyrus* spp. of the cyprinids examined.

Host	***L. rohita* (n = 10)**			***H. molitrix* (n = 10)**
Locality*	1	2	3	4
LT (cm)	43,2	33,4	51,2	40,6
P (g)	913,3	343,7	1665	625
Parasites	*D. labei*			*D. hypophthalmichthys*
Prevalence (%)	100	100	100	50
Mean Intensity	11,3	4,2	9,5	2

***** Locations: 1 = Zaza reservoir (Sancti Spíritus province), 2 = "La Sierpe" Fish hatchery (Sancti Spíritus province), 3 = "Carlos Manuel de Céspedes" Fish hatchery (Santiago de Cuba province), and 4 = Aquaculture Technologies Company, EDTA (Havana province); P % = prevalence; I = mean intensity (parasites per infested fish); LT = average total length; P = average weight.

D. labei Musselius & Gussev, 1976 (Figures 11-15).

Host: *L. rohita* (Hamilton, 1822).

Site on host: gills.

Locations: Zaza reservoir (Sancti Spíritus province), "La Sierpe" Fish hatchery (Sancti Spíritus province), and "Carlos Manuel de Céspedes" Fish hatchery (Santiago de Cuba province).

Morphometric data (All measurements are given in micrometers, µm**):** (Based on 10 specimens; see also Table 2.). Medium worms, body 478.6 ± 66.1 (412.4 – 544.7) length and 90.6 ± 18.4 (77.6 – 103.6) width at

midbody. Two pairs of eye spots present anterodorsal to the pharynx. Spherical pharynx, diameter 16.7 ± 1.4 (14.8 - 18.0). Opisthaptor 86.2 ± 2.3 (84.4 - 90.1) long and 66.5 ± 14.8 (58.4 - 88.7) wide. Single pair of anchors (dorsal), inner length 46.2 ± 1.1 (44.1 – 48.1), outer length 35.9 ± 1.2 (34.3 – 38.3), with base length or main part 40.1 ± 1.3 (38.0 – 42.7) and point length 16.4 ± 0.9 (14.8 – 17.7); anchor outer root shorter than inner root, 4.5 ± 0.7 (3.5 – 5.7) in length, inner root elongated wide, 16.5 ± 1.3 (14.2 – 18.8) in length. Anchor filaments prominent. Two connecting bars: dorsal bar slightly bowed with laterally expanded ends, 26.0 ± 1.4 (23.8 – 28.4) length and 4.8 ± 0.4 (4.0 – 5.3) width; ventral bar "T"- shaped with pointed long end, 29.3 ± 2.2 (23.9 – 31.3) length and 2.4 ± 0.2 (1.9 – 2.8) width. Marginal hooks 7 pairs, equal in shape and unequal in size, hook lengths: I = 17.5 ± 0.6 (16.6 – 18.8), II = 15.5 ± 0.9 (14.3 – 16.9), III = 17.7 ± 1.1 (15.6 – 19.1), IV = 18.3 ± 0.6 (17.4 – 19.3), V = 18.5 ± 0.9 (17.3 – 19.8), VI = 18.0 ± 0.7 (16.6 – 19.4), VII = 18.8 ± 0.9 (17.1 – 20.1). Male copulatory complex 76.3 ± 3.0 (69.1−81.0) total length, composed of a tube (penis) "S"-shaped curved , 74.2 ± 1.5 (72.1 – 75.7) length, with bubble like inflated initial part of diameter 4.9 ± 0.4 (4.3 – 5.4); and accessory piece, 75.9 ± 1.9 (73.4 – 77.6) length. Complementary free piece of male copulatory complex, 13.5 ± 0.8 (12.2 - 14.9) in length. Testis and vessels deferens were not observed. Tubular vagina, 35.3 total length and 1.3 ± 0.3 (1.0 - 1.6) width. Ovary and oviduct not observed.

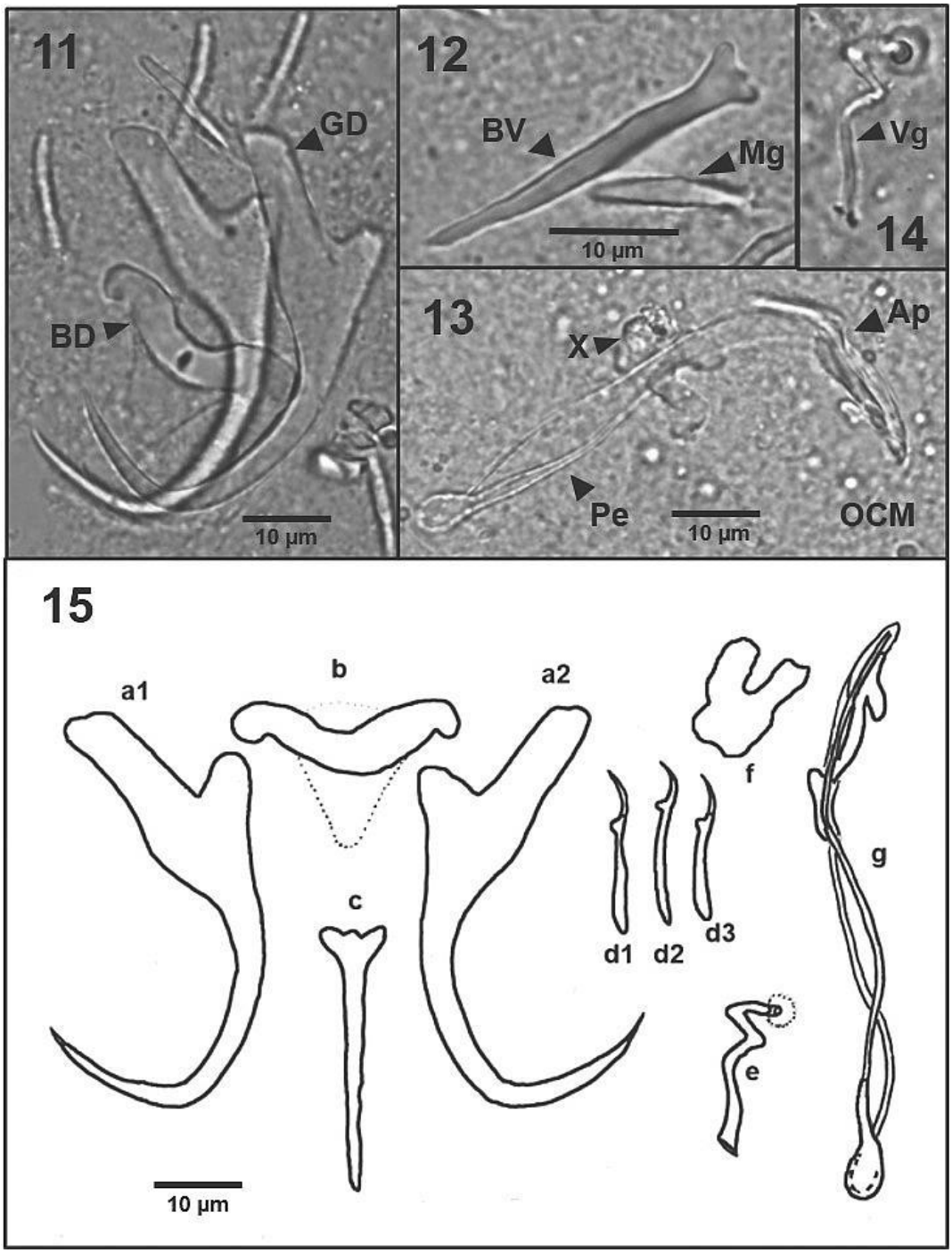
D. hypophthalmichthys Achmerow, 1952 (Figures 16 – 18)

Host: *H. molitrix* Valenciennes, 1844.

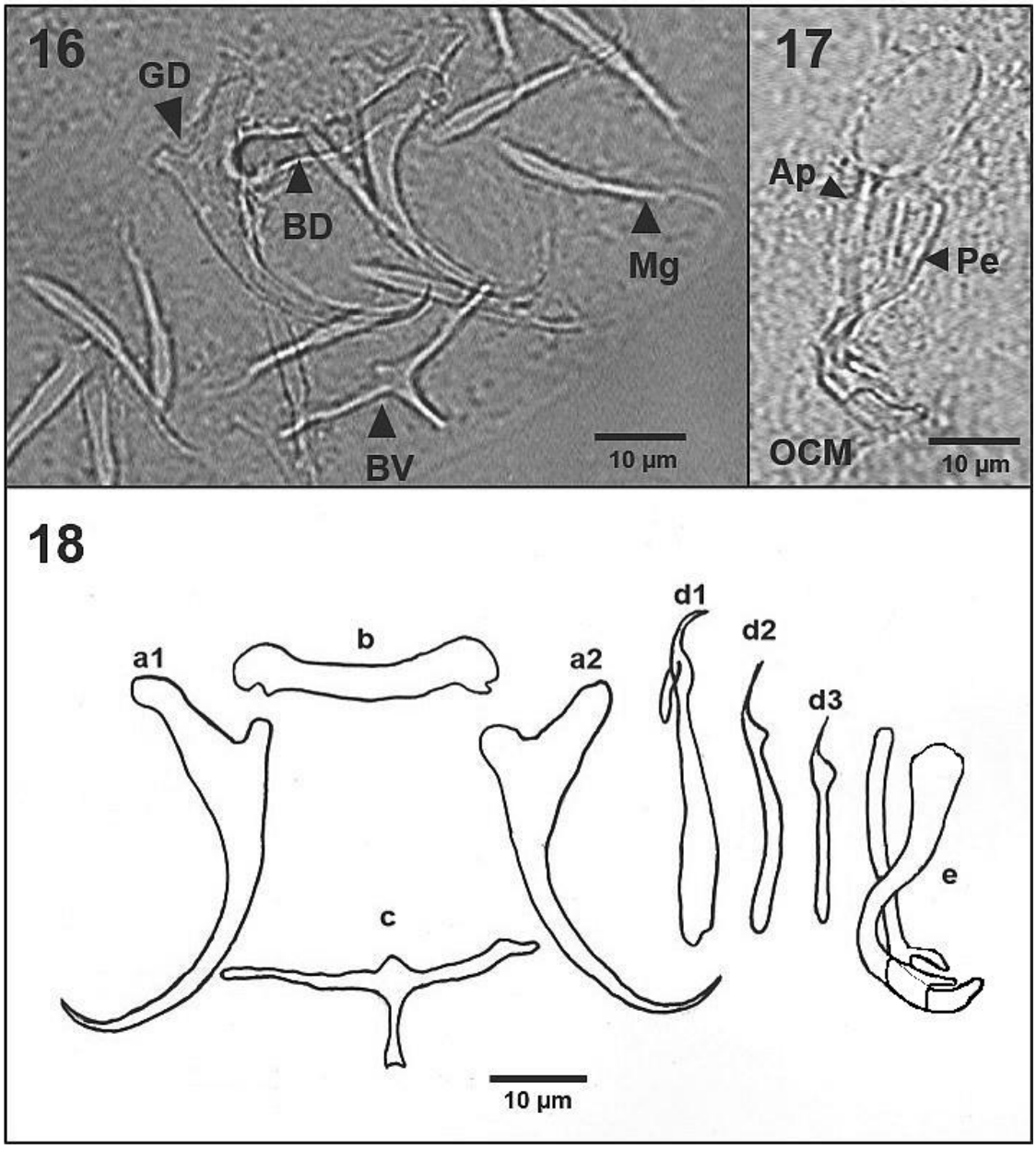
Site on host: gills.

Locality: Aquaculture Technologies Company, EDTA (Havana province).

Morphometric data (All measurements are given in micrometers, μm)**:** (Based on 2 specimens; see also Table 3.). Medium worms, body length 406.4 and width 90.8 ± 2.0 (89.4−92.2) at midbody. Two pairs of eye spots present anterodorsal to the pharynx. Pharynx diameter 33.5 measured by its wider portion. Opisthaptor 67.6 ± 1.1 (66.5 – 68.6) long and 110.5 ± 5.4 (106.7 – 114.3) wide. Single pair of anchors (dorsal), inner length 42.8 ± 1.3 (41.0 – 44.5), outer length 34.8 ± 0.3 (34.4 – 35.0), with base length or main part 37.3 ± 2.9 (32.5 – 39.7) and point length 9.6 ± 0.8 (9.0 – 10.2); anchor inner root, 13.0 ± 1.4 (11.0 – 14.0) long, outer root 4.1 ± 0.6 (3.6 – 4.8) long. Prominent hook filaments. Two connecting bars: dorsal bar slightly arched with expanded lateral endings, 23.8 ± 1.8 (22.7 – 25.8) length and 4.7 ± 0.1 (4.6 – 4.8) width, ventral bar "T"-shaped, 31.4 ± 0.1 (31.3 – 31.5) in length and 12.2 ± 0.2 (12.0 – 12.3) in width. Marginal hooks shaft 7 pairs, equal in shape, 33.1 ± 6.0 (22.8 – 42.8) long. Male copulatory organ, 33.5 total length, with a thick penis in tubular and slightly arched, 34.2 length and accessory piece, 31.7 length. Testis and vessels deferens were not observed. Vagina, ovary, and oviduct were not observed.



Figures 11–14. Photomicrographs of *D. labei* from gills of *L. rohita*. **11.** Ventral view of opisthaptor. **12.** Ventral view of the ventral bar and marginal hook. **13.** Male copulatory organ. **14.** Ventral view of the vagina. Abbreviations: BD = dorsal bar; GD = dorsal anchor; BV = ventral bar Mi = marginal hook; OCM = male copulatory organ; Vg = vagina; Pe = penis; Ap = accessory piece; B = base of the part initial of OCM; X = free complementary piece of male copulatory complex. Bar of scale 10 µm. **Figure 15.** Drawing of *D. labei* Musselius & Gussev, 1976 (atypical) of *L. rohita*. Ventral view of the sclerotized structures: opisthaptor; male copulatory organ and vagina. Abbreviations: b = dorsal bar; a1 y a2 = dorsal anchor; c = ventral bar; d1, d2 y d3 = marginal hook shaft; e = vagina; f = free complementary piece of male copulatory complex; g = male copulatory organ. Bar of scale 10 µm.



Figures 16–17. Photomicrographs of *D. hypophthalmichthys* from gills of *H. molitrix*. **16**. Ventral view of opisthaptor. **17.** Ventral view of male copulatory organ. Abbreviations: BD = dorsal bar; GD = dorsal anchor; BV = ventral bar Mi = marginal hook shaft; OCM = male copulatory organ; Pe = penis; Ap = accessory piece. Bar of scale 10 µm. **Figure 18.** Drawing of *D. hypophthalmichthys* Achmerow, 1952. Ventral view of the sclerotized structures: opisthaptor and male copulatory organ. Abbreviations: b = dorsal bar; a1 y a2 = dorsal anchor; c = ventral bar; d1, d2 y d3 = marginal hook; e = male copulatory organ. Bar of scale 10 µm.

Table 2. Comparison between the morphometric data variability of *D. labei* from *L. rohita* shown by Musselius & Gussev (1976); Chandra & Jannat (2002); Chiary *et al*. (2014); and Sujana & Shameem (2015) with the present study. All measurements are given in micrometers (µm).

Species	*D. labei* Musselius & Gussev, 1976				
Host	*L. rohita*	*L. rohita, L. gonius, Catla catla*	*C. catla*	*L. rohita, C. catla, Cirrhinus mrigala, C. idella, H. molitrix*	*L. rohita*
Country	India	Bangladesh	India	India	Cuba
Site on host	gills	gills	gills	gills	gills
Source	Musselius y Gussev, 1976	Chandra y Jannat, 2002	Chiary *et al*., 2014	Sujana y Shameem, 2015	Present study, 2019
Form	atypical	atypical	atypical	atypical	atypical
Body length	-	380 - 480	435 - 445	212 - 320	412 - 545
Body width	-	70 - 120	92 - 96	64 - 100	78 - 104
Diameter pharynx	-	-	56 - 64	16 - 24	15 - 18
Haptor length	-		76 - 80	54 - 66	84 - 90
Haptor width	-		60 - 66	72 - 90	58 - 89
Inner length of anchor	33 - 38	36 - 38	52 - 60	30 - 35	44 - 48
Outer length of anchor	-	-	-	-	34 - 38
Inner root	10 - 13	10 - 17	13 - 15	11 - 18	14 - 19
Outer root	4 - 7	2 - 7	7 - 9	3 - 6	3 - 6
Anchor point length	13 - 14	12 - 13	10 - 16	13 - 16	15 - 18
Anchor main part length	26 - 31	27 - 32	30 - 34	10 - 16	38 - 43
Dorsal bar total length	22 - 24	21 - 25	23 - 25	16 - 22	24 - 28
Dorsal bar width	3 - 4	4 - 6	4 - 6	3 - 6	4 - 5
Ventral bar total length	26 - 28	22 - 23	25 - 27	19 - 29	24 - 31
Ventral bar width	3	3 - 5	-	3	2 - 3
Copulatory organ total length	-	40 - 64	73 - 77	24 - 40	69 - 81
Diameter base penis	-	-	4 - 6	-	4 - 5
Vagina length	-	21 - 33	-	-	35
Vagina width	-	-	-	-	1 - 2
Marginal hook total length	-	12 - 17	11 - 19	14 - 21	14 - 20

Table 3. Comparison between the morphometric data variability of *D. hypophthalmichthys* from *H. molitrix* shown by Achmerow (1952); Gussev (1985) and Nitta & Nagasawa (2020) with the present study. All measurements are given in micrometers (µm).

Country	Russia	Russia	Japan	Cuba
Site on host	gills	gills	gills	gills
Source	Achmerow, 1952	Gussev, 1985	Nitta & Nagasawa, 2020	Present study, 2019
Body length	650 - 700	520	384 - 554	406
Body width	110 - 130	120	73 -117	89 - 92
Diameter pharynx	-	-	14 - 24	33
Haptor length	70 - 80	-	55 - 77	66 - 69
Haptor width	70	--	88 - 125	107 -114
Inner length of anchor	35	25 - 35	33 - 40	41 - 44
Outer length of anchor	-	-	-	34 - 35
Inner root	12	10 - 13	11 – 18	11 - 14
Outer root	5	4 - 6	4 - 6	4 - 5
Anchor point length	6	2 - 5	9 - 11	9 - 10
Anchor main part length	29	22 - 31	29 - 34	32 - 40
Dorsal bar total length	24	23 - 31	24 - 27	23 - 26
Dorsal bar width	4	3 - 6	3 - 5	5
Ventral bar total length	34	29 - 40	-	31
Ventral bar width	8	8 – 10	5 - 11	12
Copulatory organ total length	42	29 - 37	32 - 34	33
Marginal hook total length	29	19 - 46	26 - 44	23 - 43

DISCUSSION

The morphological and biometric characteristics of the collected specimens coincide with the genus *Dactylogyrus* Diesing, 1850 and correspond to the species described by Musselius & Gussev, (1976) for *L. rohita* as *D. labei*; and by Achmerow (1952) for *H. molitrix* as *D. hypophthalmichthys*.

For *D. labei*, Musselius and Gussev (Gussev, 1976) define two forms (typical and atypical) based on the morphology of the haptoral apparatus (opisthaptor) and the male copulatory complex. In the atypical form there is a free piece towards the front side of the male copulatory organ, which is not observed in the typical form. In addition, there are some differences between the dorsal and ventral bars of both forms. In the typical form, dorsal bar shows a posterior tongue-shaped process which is not observed in the typical form. The ventral bar of the typical form presents 5 rayes that are absent in the atypical form. The atypical form described in this work is similar to that presented by Musselius and Gussev (Gussev, 1976) for *D. labei*.

Comparing the morphometric data presented for *D. labei* in this material with those referred to in other studies (see Table 2), we observe slight variations. The range of values for the internal length of the hook and the total length of the male copulatory complex, presented by Chiary *et al.* (2014) and the present study, were greater than those shown by Musselius & Gussev (1976), Chandra & Jannat (2002), and Sujana & Shameem (2015) in their studies. This fact seems to be related to the size of the specimens collected by Chiary *et al.* (2014) and the present study, which presented major ranges. Another remark is presented to the measure referred to by Chiary *et al.* (2014) for the diameter of the pharynx, which is far superior to those shown by the rest of the researchers. Towards the back of the dorsal bar of *D. labei* specimens, there is a non-chitinoid tongue-shaped process observed at the photomicrographs that does not coincide with the morphology of the dorsal bar described by Musselius & Gussev, 1976 (Gussev, 1976) for the typical form of the species.

The presence of *D. labei* in polycultures in the country currently shows a high specificity to *L. rohita* species, despite living together with other cyprinids species in the ponds (*H. molitrix, H. nobilis*, *C. carpio*, *C. idella*). However, studies in India by Chandra & Jannat (2002), Chiary *et al.* (2014), and Sujana & Shameem (2015), show strict pattern host specificity, reporting other host species for *D. labei* (*L. gonius*, *C. catla*, *C. idella*, *H. molitrix*, *C. mrigala*). This specificity presented by *D. labei* to other host cyprinids species could be related to the close phylogenetic relationship between the species of this fish family and their ecological adaptation to new environmental conditions.

On the other hand, *D. hypophthalmichthys* was originally described by Achmerow (1952) in branchial filaments of *H. molitrix* collected in the lakes Petropavlovsk, Bolon and Udyl, at eastern Russia. In 1963, Yamaguti changed the species to genus *Neodactylogyrus* Price, 1938, but it had already been previously synonymized as *Dactylogyrus* by Mizelle & Donahue (1944). The presence of *D. hypophthalmichthys* in Cuba was recorded during the sanitary checkup of *H. molitrix* importation quarantine from Vietnam in the Aquaculture Technologies Company (EDTA) (Havana province) to improve the genetic bank of spawners. The structure of male copulatory complex, where the tip of the accessory piece holds half of the sigmoid penis and the ventral bar shape, made possible the identification of *D. hypophthalmichthys*. The examined specimens in this study coincide with descriptions and illustrations of *D. hypophthalmichthys* of Achmerow (1952), Gussev (1985) and Nitta & Nagasawa (2020).

Prevalence and mean intensity of parasites for both species of monogeneans were low (see Table 1). However, danger of epizootics must be considered because pathogenicity can be increased due to the new habitat conditions and management of their hosts in culture. This work adds two new records of *Dactylogyrus* for Cuba and provides a new geographical distribution for the species *D. labei* and *D. hypophthalmichthys*. Surveillance for both monogeneans parasites is important for the aquaculture industry.

Aquaculture research on the use of bioactive extracts of plants has focused on specific characteristics or benefits of GRAS plants or their compounds, mostly in their microbicidal characteristics at lethal doses (Kakoolaki *et al.*, 2016; Aftabuddin *et al.*, 2017; Marisa Halim *et al.*, 2017; Palanikumar *et al.*, 2020; Reverter *et al.*, 2021). These plants can be used as part of a holistic approach to treat vibriosis and the imbalances it causes on shrimp health (oxidative stress and immune suppression). In this study, we have

determined that sublethal doses for pathogenic *Vibrio* spp. of shrimp (for a comprehensive anti-virulence strategy) of *A. sativum*, *O. vulgare*, *C. sinensis* and *M. citrifolia*, allow taking advantage of their anti-QS, antioxidant, and immunomodulatory characteristics, as part of a holistic approach for shrimp health management.

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