

## *Mormyrogyrodactylus gemini* gen. et sp. n. (Monogenea: Gyrodactylidae), a new gyrodactylid from *Marcusenius macrolepidotus* (Mormyridae) from South Africa

Wilmien J. Luus-Powell<sup>1</sup>, Sevid N. Mashego<sup>2</sup> and Lotfi F. Khalil<sup>1</sup>

<sup>1</sup>School of Molecular and Life Sciences, University of the North, P/Bag X1106, Sovenga, 0727, South Africa;

<sup>2</sup>Department of Zoology, P.O. Box 524, Rand Afrikaans University, South Africa

Key words: Monogenea, Gyrodactylidae, *Mormyrogyrodactylus*, Mormyridae, *Marcusenius macrolepidotus*, South Africa

**Abstract.** *Mormyrogyrodactylus gemini* gen. et sp. n. (Gyrodactylidae: Gyrodactylidae), a viviparous monogenean is described from the skin and fins of *Marcusenius macrolepidotus* (Peters) from South Africa. This new genus is unique in having a large cup-shaped cirrus armed with one large needle-like spine and numerous small hair-like spinelets. A peduncular bar is present. The haptor is clearly demarcated from the body and situated on a small pedicle. The haptor has one pair of large anchors, a ventral bar complex, a small dorsal bar and 16 evenly spaced marginal hooks. The ventral bar complex consists of three parts: an inverted U-shaped piece with two semi-attached bars, each bar consisting of a base and an extension. An accessory inverted T-shaped sclerite is present on the posterior rim of the haptor. *Mormyrogyrodactylus* is the fifth genus of the Gyrodactylidae to be described from Africa.

The Gyrodactylidae comprises 28 genera, the species of which parasitize mostly marine and freshwater fishes. Three genera were recorded from other hosts, i.e. *Gyrdicotylus* Vercammen-Grandjean, 1960 from the African clawed toad, *Xenopus laevis* (Harris and Tinsley 1987), *Neogyrodactylus* Baugh, 1957 from a parasitic crustacean, *Argulus indicus* (Baugh 1957) and *Isancistrum* de Beauchamp, 1912 from a cephalopod (De Beauchamp 1912). Gyrodactylids have a worldwide distribution (Cone 1995) with four genera recorded from Africa, i.e. the cosmopolitan *Gyrodactylus* Nordmann, 1832; *Macrogyrodactylus* Malmberg, 1957 and *Afrogyrodactylus* Paperna, 1968 described from freshwater fishes endemic to Africa; and *Gyrdicotylus* described from the African clawed toad.

During a parasitological survey of mormyrid fishes in the Northern Province and Mpumalanga, South Africa, a previously undescribed viviparous monogenean from the external surface of *Marcusenius macrolepidotus* (Peters) was found. This monogenean could not be assigned to any known genus within the Gyrodactylidae and therefore is described as a new species of a new genus.

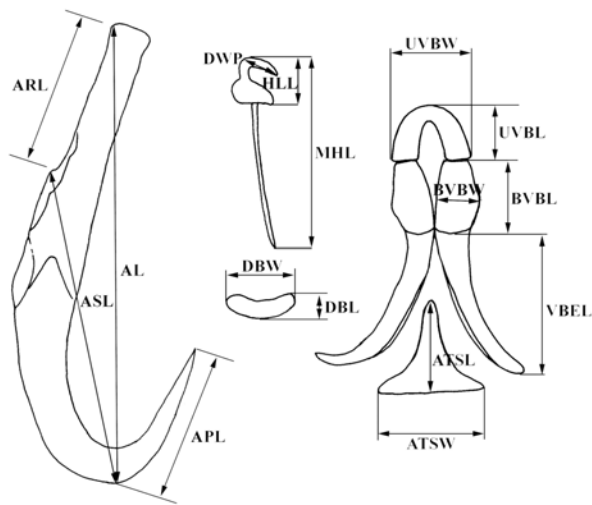
### MATERIALS AND METHODS

Seasonal surveys were undertaken during 1998 and 1999 at Nwanedi-Luphephe Dams and additional sampling of fish was carried out to include sampling localities representative of selected river systems where mormyrid fishes occur naturally in South Africa.

Fishes were collected using different standard collecting methods as dictated by the prevailing conditions at the

particular sampling locality. This was mostly selective gill netting (mesh sizes: 30, 50, 70, 90 mm), cast netting and electrofishing.

Hosts that could not be examined immediately were kept alive in holder tanks with well aerated water and water temperature similar to the temperature of the dam or river. A total of 178 *M. macrolepidotus*, ranging in size from 7.5 cm to 32.3 cm total length (TL), and 78 *Petrocephalus wesselsi* Kramer et Van der Bank, ranging in size from 4.9 cm to 13.3 cm TL, were examined for parasites. Both sexes were approximately equally represented in the sample. A skin smear was made by holding the fish firmly on the head, covered with a damp cloth and scraping the skin on both sides with a glass slide. Each fish was measured, weighed and placed in a separate container for further examination. Monogenetic parasites collected from the skin smear of *M. macrolepidotus* were placed individually, with a fine brush, in a petri dish with filtered dam water. They were fixed by adding hot ( $\pm 70^{\circ}\text{C}$ ) alcohol-formalin-acetic acid (AFA) fixative and stored in 70% ethanol. Unstained specimens used for measurement of the anchors and marginal hooks were mounted in glycerin jelly under slight coverslip pressure and sealed with clear nail varnish. Specimens used for body measurements and detailed anatomical studies were stained in Horen's trichrome, counterstained in acetocarmine and mounted in Canada balsam or Entellan. The haptoral sclerites were measured following the scheme of Malmberg (1970) (Fig. 1). Body measurements include total body length, i.e. anterior extremity to posterior extremity of anchors, and maximum width. All measurements are in micrometres ( $\mu\text{m}$ ) and based on ten specimens. The average is given with the range following in parentheses. Terminology of haptoral armament was modified from Mizelle and Kritsky (1967) and Ernst et al. (2000).



**Fig. 1.** Anchors, marginal hook, dorsal bar and ventral bar complex characters and measurements of *Mormyrogyrodactylus gemini* sp. n. Abbreviations: ARL – anchor superficial root length; ASL – anchor shaft length; AL – anchor total length; APL – anchor point length; DWP – distal width of point of hooklet; HLL – length of hooklet; MHL – total length of marginal hook; DBW – dorsal bar width; DBL – dorsal bar length; UVBW – U-shaped piece of ventral bar complex width; UVBL – U-shaped piece of ventral bar complex length; BVBW – base of ventral bar extension width; BVBL – base of ventral bar extension length; VBEL – ventral bar extension length; ATSL – accessory T-shaped sclerite length; ATSW – accessory T-shaped sclerite width.

For scanning electron microscopy, specimens were ultrasonically cleaned for 10–12 seconds to remove mucus and debris. Due to their small size, specimens were placed in a small plastic microtissue capsule assembly with copper grids (G200Ht) at both ends. Monogeneans were dehydrated through graded ethanol series, critical point dried, mounted on aluminium stubs and sputter coated with gold-palladium. Another method was used to replace the drying technique: some specimens were immersed in a small volume of hexamethyldisilazane (30 min) and dried by placing them under a slight vacuum to remove the hexamethyldisilazane. The latter method is commonly used for copepods but was found, however, to be less suitable for monogeneans. Specimens were viewed and photographed in a JEOL 6100 scanning electron microscope between 5 and 7 kV.

## RESULTS

### *Mormyrogyrodactylus* gen. n.

#### Generic diagnosis

Gyrodactylidae: body divisible into cephalic region, trunk, peduncle and haptor. Tegument thin, smooth. Cephalic region bilobed, cephalic glands present. Pharynx compound, posterior portion composed of two hemispherical parts, anterior portion with pharyngeal papillae. Eyes absent. Gut bifurcate, terminating blindly in posterior part of trunk. Cirrus large, consisting of

cirrus pouch with numerous small hair-like spinelets, one needle-like spine medially. Peduncular bar present. Haptor clearly demarcated from trunk, situated on a small pedicle. Haptor with one pair of anchors, ventral bar complex, dorsal bar and 16 marginal hooks. Marginal hooks of equal size, evenly spaced on dorsal margin of haptor. Haptor with additional suction discs at anterior extremity. Ventral bar complex consisting of inverted U-shaped piece with two semi-attached bars; each bar consisting of base and extension. Haptor with additional inverted T-shaped sclerite supporting haptor posteriorly. Type and only species: *Mormyrogyrodactylus gemini* sp. n.

**E t y m o l o g y :** The generic name derives from the mormyrid host.

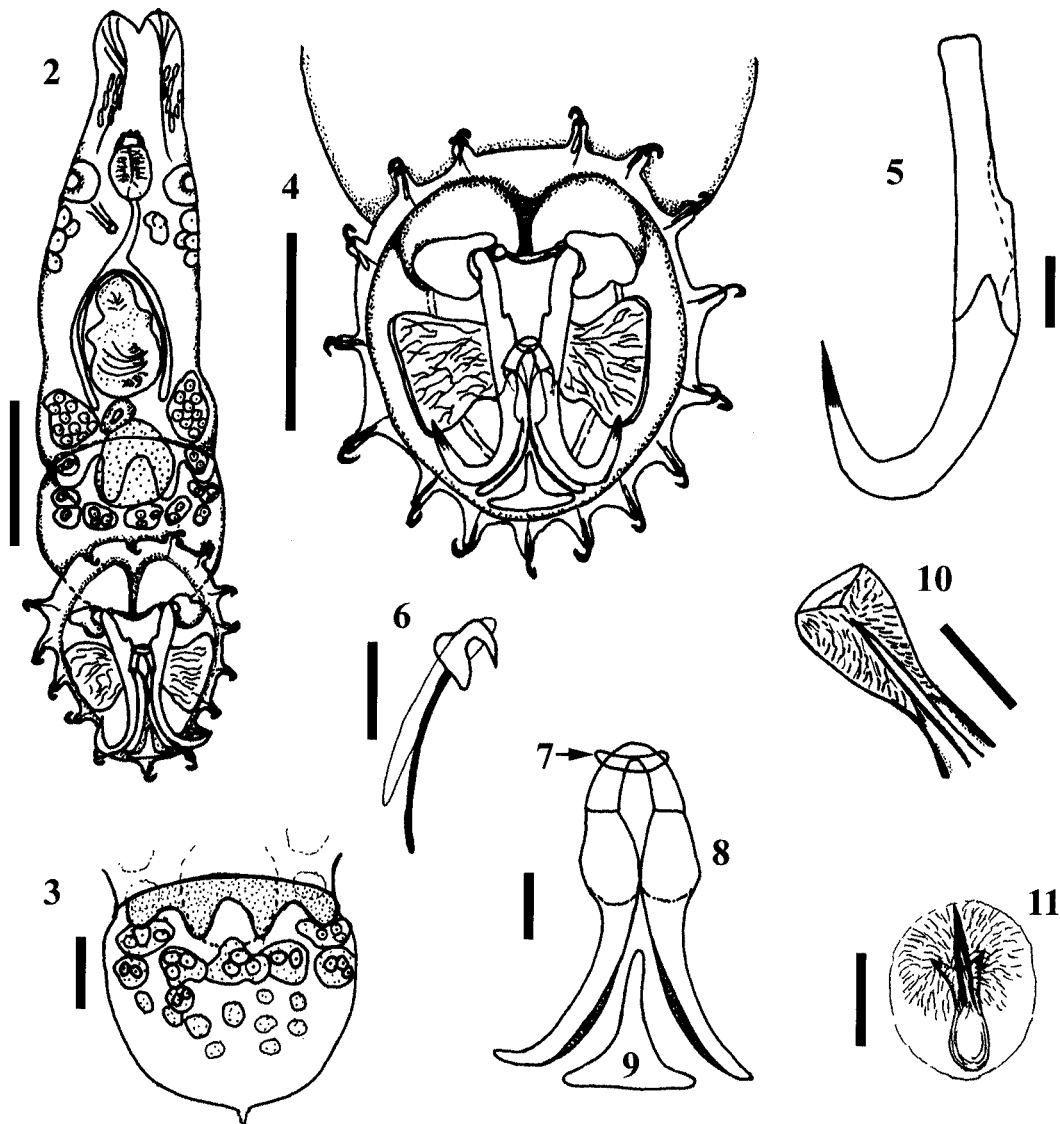
#### Remarks

This genus appears to be most similar to *Swingleus* Rogers, 1969 (Rogers 1969) and in general body shape resembles *Polyclithrum* Rogers, 1967 (Rogers 1967a) but differs from the above mentioned genera by the arrangement of marginal hooks. It differs from all genera of the Gyrodactylidae in the following way: a ventral bar complex, consisting of three structures; additional suction discs at anterior extremity of haptor; the haptor situated on a pedicle; and the cup-shaped cirrus with numerous hair-like spinelets and one large spine medially.

### *Mormyrogyrodactylus gemini* sp. n. Figs. 2–19

**Description:** Medium-sized gyrodactylid with fusiform body (Figs. 2, 12). Body length including haptor 570 (477–685), width 163 (119–221). Body narrow in centre, wider in regions of pharynx and gonads (Fig. 14). Cephalic region with two prominent lobes, each containing adhesive area terminating in single spine (Figs. 2, 13). Cephalic glands prominent, extending laterally to anterior region of uterus (Fig. 2). Posterior section of pharynx bilobed, lobes of equal size (Fig. 2). Small anterior section of pharynx terminating into mouth with pharyngeal papillae (Fig. 13). Mouth ventral with lateral elevations (Fig. 13). Gut bifurcated with crura ending blindly at gonads.

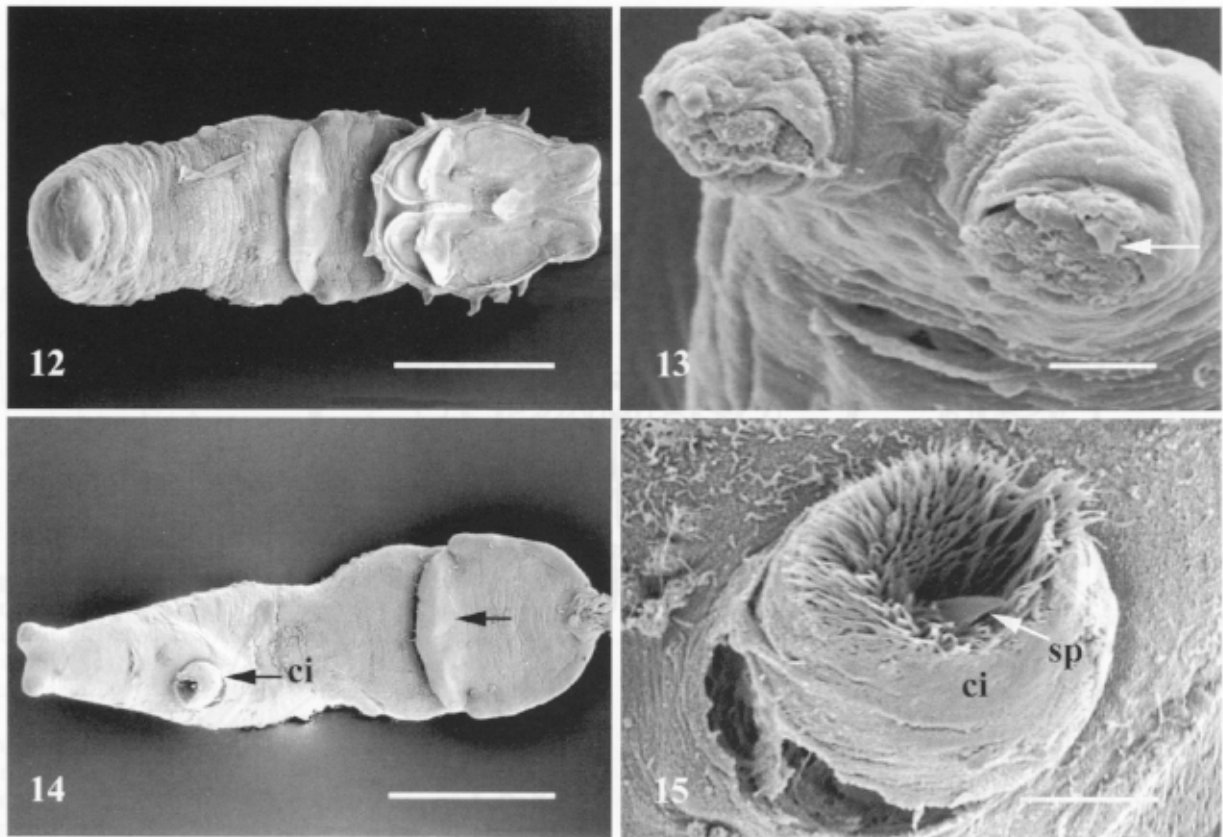
Cirrus pouch large, 45 (41–47) in diameter, with numerous small hair-like spinelets (Figs. 10, 11, 14, 15), located ventrally to right of and posterior to pharynx, with large needle-like spine medially (Fig. 15, arrowed). Uterus median, usually containing developing embryos (Fig. 2). Ootype, usually containing large oocyte, located immediately posterior to uterus. Ovary, containing many maturing oocytes, globular, partially surrounding testis. Single testis large and round. A glandular peduncular bar present on ventral surface of peduncle, anterior to haptor, with three indentions (Figs. 2, 3). Peduncular bar covered by smooth tegument protruding antero-ventrally beyond the surrounding tissue (Figs. 12, 14, 16).



**Figs. 2–11.** *Mormyrogyrodactylus gemini* sp. n., microscope projection drawings. **Fig. 2.** Whole worm, ventral view. **Fig. 3.** Peduncle with peduncular bar anteriorly, ventral view. **Fig. 4.** Haptor, ventral view. **Fig. 5.** Anchor, ventral view. **Fig. 6.** Marginal hook. **Fig. 7.** Dorsal bar, ventral view. **Fig. 8.** Ventral bar complex, ventral view. **Fig. 9.** Accessory T-shaped sclerite, ventral view. **Fig. 10.** Inverted cirrus. **Fig. 11.** Everted cirrus. Scale bars: Fig. 2 = 100  $\mu$ m; Figs. 3, 5, 7–11 = 25  $\mu$ m; Fig. 4 = 50  $\mu$ m; Fig. 6 = 10  $\mu$ m.

Haptor obovate in outline and ventrally concave (Figs. 4, 17), clearly demarcated from body and situated on pedicle (Fig. 18). Haptor consisting of dorsal flap (Figs. 18, 19) bearing 16 marginal hooks of equal size and inner anchor membrane bearing one pair of anchors supported by a small dorsal bar and a ventral bar complex (Figs. 4, 17). Two distinct regions with fine branching striations on inner anchor membrane laterally (Fig. 4). Additional suction discs present at anterior extremity of haptor (Fig. 17, indicated by arrow). Haptor length 171 (142–220), width 137 (136–139). Anchors large, each composed of base with elongated superficial root, elongated shaft and recurved point (Fig.

5); total length 123 (101–134), length of shaft 88 (76–96), length of point 44 (36–49), length of root 38 (29–45). Superficial roots of anchors with lateral membranous extensions and connected by transverse muscle band (Fig. 4). Tip of anchor point with fine longitudinal striations. Dorsal bar small (Fig. 7), articulated between anchors without attachment; length 15 (14–16), width 5.6 (5.2–6.7). Ventral bar complex prominent, consisting of three structures (Fig. 8): an elevated inverted U-shaped piece, length 20.3 (18–22), width 21.4 (19–23); and two semi-attached bars, each composed of base and extension, extending to rim of haptor (Fig. 17); length of base 19 (13–22), width 15 (10–16) length of bar



**Figs. 12–15.** *Mormyrogyrodactylus gemini* sp. n., scanning electron micrographs. **Fig. 12.** Whole worm, ventral view. **Fig. 13.** Cephalic region with mouth and prominent lobes terminating in a single spine (arrowed). **Fig. 14.** Cephalic region, trunk, cirrus (ci) and peduncle (haptor removed) with peduncular bar (arrowed), ventral view. **Fig. 15.** Cirrus (ci) with hair-like spinelets and needle-like spine (sp), ventral view. Scale bars: Figs. 12, 14 = 100  $\mu$ m; Figs. 13, 15 = 10  $\mu$ m.

extension 45 (41–46). An accessory inverted T-shaped sclerite (Figs. 9, 17), length 32 (31–33), width 51 (49–52) present at posterior rim of haptor. Elevated U-shaped piece of ventral bar complex articulating in folds of anchors with prominent markings on anchor base (Fig. 5).

Dorsal flap of haptor extends to form 16 radially arranged marginal hooks of similar size and shape. Shaft of each hooklet (Fig. 6) with slight enlargement proximally, total length of marginal hook 26 (24–28), length of hooklet 7.5 (6–8), distal width of point 5.1 (3–6).

**Type host:** *Marcusenius macrolepidotus* (Peters, 1852) (Osteoglossiformes: Mormyridae).

**Site of infection:** Fins and ventral side of body.

**Type locality:** Nwanedi-Luphephe Dams (22°39'S, 30°25'E), Northern Province, South Africa (12/02/1998).

**Other localities:** Tzaneen Dam (23°48'S, 30°10'E), Northern Province; Arabie Dam (24°46'S, 29°25'E), Loskop Dam (25°22'S, 29°10'E), Blyde Canyon Dam (24°32'S, 30°48'E), Mpumalanga, South Africa.

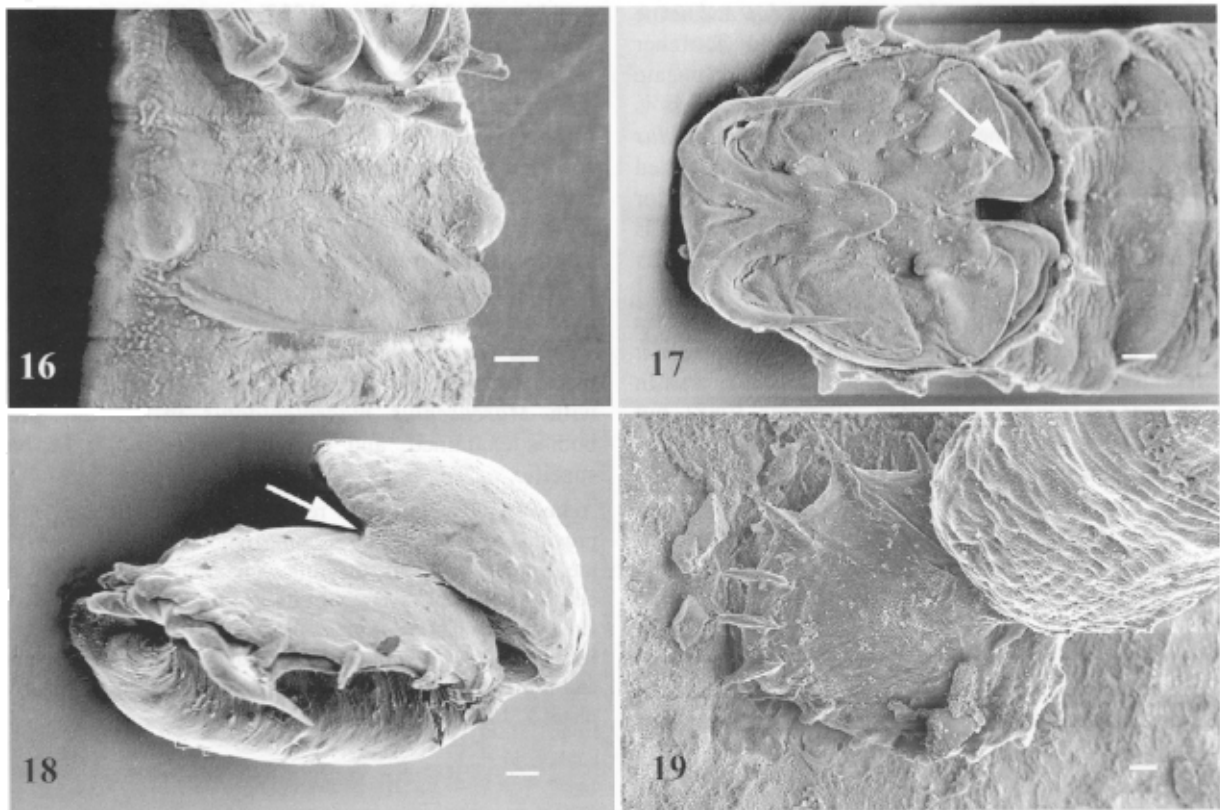
**Prevalence:** 55% of examined hosts at Nwanedi-Luphephe Dams were infested.

**Deposition of types:** Holotype, South Africa Museum, Cape Town, SAM A29436; Paratypes, SAM A29437; M-348 Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice.

**Ety m o l o g y:** The specific name derives from the twin dams, Nwanedi-Luphephe, the type locality.

## DISCUSSION

*Fundulotrema* was established by Kritsky and Thatcher (1977) to accommodate gyrodactylids with a peduncular bar and 16 evenly spaced marginal hooks. Cone and Odense (1988) rediagnosed this genus and revealed that, in all known species of the genus, marginal hooks i–iii are grouped anteriorly on the haptor, similar to the arrangement recorded for *Swingleus* and *Accessorius* Jara, An et Cone, 1991. It differs, however, from *Polyclithrum* which has hooks i–iv grouped anteriorly. However, the present species of *Mormyrogyrodactylus* has an even distribution of marginal hooks. *Mormyrogyrodactylus* has some characteristics of the Polyclithrinae but the distribution of marginal hooks makes the affinities with this subfamily unclear.



**Figs. 16–19.** *Mormyrogyrodactylus gemini* sp. n., scanning electron micrographs. **Fig. 16.** Peduncle with peduncular bar, ventral view. **Fig. 17.** Haptor with suction discs (arrowed), ventral view. **Fig. 18.** Whole worm, showing the pedicle (arrowed), dorso-lateral view. **Fig. 19.** Haptor with marginal hooks loosely attached to the host's skin, dorsal view. Scale bars = 10  $\mu$ m.

Presently there are seven gyrodactylids which possess a peduncular bar. These include the five species of *Fundulotrema*, which were all previously included in *Gyrodactylus*, i.e. *F. prolongis* (Hargis, 1955), *F. megacanthus* (Wellborn et Rogers, 1967), *F. trematoclitrus* (Rogers, 1967), *F. foxi* (Rawson, 1973), and *F. stableri* (Hathaway et Herlevich, 1973) (see Kritsky and Thatcher 1977), and the two species described within *Swingleus*, i.e. *Swingleus polyclithroides* Rogers, 1969 and *S. ancistrus* Billeter, Klink et Mangel, 2000. The peduncular bar of *Mormyrogyrodactylus* is covered by tegument and protrudes from the ventral surface showing a distinct ridge when studied with the aid of scanning electron microscopy. *Mormyrogyrodactylus* does not use the peduncular bar to assist in attachment on the host, therefore the glandular appearance cannot be explained at this stage. No spines or oblong pits were observed as recorded for the peduncular bar of *F. prolongis*, *F. trematoclitrus*, *S. polyclithroides* and *S. ancistrus* (see Hargis 1955, Rogers 1967b, Rogers 1969, Billeter et al. 2000). The peduncular bar of *Mormyrogyrodactylus* differs from that of *F. megacanthus*, which has been described as a sclerotised structure with an inverted V-shape with tips bending at right angles to the bar (Wellborn and Rogers 1967). The possible function of the peduncular bar has been discussed by Cone and

Odense (1988). We obtained similar results studying live specimens. The parasite uses the bar to stabilise the pedunculate haptor (as defined by Cone and Cusack 1988), which is very flexible. The haptor is situated on a small pedicle and can rotate more than 180° in both directions. When placing the parasite on a smooth glass surface, it pushes the haptor down and attaches itself, presumably with the aid of the “suction discs” at the anterior extremity of the inner anchor membrane of the haptor. While attached in this manner, the cephalic lobes and the marginal hooks move constantly until the parasite finds a more suitable substratum to attach. On the host's surface, the parasite attaches itself loosely with the aid of the marginal hooks and anchor tips (Fig. 19). The marginal hooks do not penetrate the host's skin as in the case of *Gyrodactylus salmonis* (see Cone and Odense 1984). No depressions or definite markings were observed on the host's skin surface.

The ventral bar differs from that in all known gyrodactylids in being an inverted U with two semi-attached bars. The term ventral bar complex is proposed for these structures. *Fundulotrema prolongis*, *F. trematoclitrus*, *F. megacanthus*, *Accessorius peruensis* and *Swingleus polyclithroides* possess a ventral bar with a shield or membrane, but in some genera the ventral bar shield or membrane is lacking, e.g. in *Phanero-*

*thecium*, *Afrogyrodactylus*, *Metagyrodactylus* and some species of *Gyrodactylus* (see Kritsky and Thatcher 1977). *Polyclithrum* differs in having an elongate posteriorly directed process (Rogers 1967a) which is similar as seen in some species of *Macrogyrodactylus* (see Khalil and Mashego 1998). The additional inverted T-shaped accessory sclerite found on the posterior extremity of the haptor of *Mormyrogyrodactylus* is similar to the sclerites described for *Macrogyrodactylus* (see Khalil and Mashego 1998).

The cirrus of *Mormyrogyrodactylus* is similar in structure as described for *Afrogyrodactylus characinis*: "...it consists of a muscular pouch with an inner wall in which rods or spines are inserted" (Paperna 1968), but differs in having an additional large needle-like spine. The "muscular pouch" of *Mormyrogyrodactylus* differs from the true cirrus sac as reported for *Phanerothecium* where only the muscular tube protrudes (Kritsky and Thatcher 1977), whereas in *Mormyrogyrodactylus* the entire pouch with the spinelets protrudes to push the large spine to the exterior. This cirrus pouch differs from the condition in other genera where the cirrus consists of a spine and one to several spinelets situated on a muscular bulb.

Although Malmberg (1998) stated that "similarities in the shape of the haptor cannot be used for expressing relationships in the Gyrodactylidae" because "the different types of haptors do not correlate with the types of protonephridial systems", it was still necessary to compare the morphology of the different genera. To establish relationship with the other genera within the Gyrodactylidae, further studies on the protonephridial system of *Mormyrogyrodactylus* is recommended.

At Tzaneen Dam, Northern Province, *M. gemini* was in association with an undescribed, much smaller *Gyrodactylus* sp. It was never recorded from the other mormyrid host, *Petrocephalus wesselsi*, which co-exists with *Marcusenius macrolepidotus* in all the water bodies examined, and appears, therefore, to be host specific.

**Acknowledgements.** This research was supported by funds provided by the Research Administration Office, University of the North. We thank the following units, departments and people from the University of the North: Electron Microscope Unit and Department of Zoology and Biology for use of facilities, Mrs. A. Moller for assistance in SEM-preparations and Mr. R. Sandrock for his help and patience with the photo plates.

## REFERENCES

- BAUGH S.C. 1957: On the morphology of *Neogyrodactylus indicus* n.g., n.sp., a viviparous monogenetic trematode (Fam. Gyrodactylidae) from *Argulus indicus* Weber. Parasitology 47: 40–45.
- BILLETTER P.A., KLINK M.M., MAUGEL T.K. 2000: A new species of *Swingleus* (Monogenea: Gyrodactylidae) from the mummichog *Fundulus heteroclitus*, in the Delaware Bay. J. Parasitol. 86: 1219–1222.
- CONE D.K. 1995: Monogenea (Phylum Platyhelminthes). In: P.T.K. Woo (Ed.), Fish Diseases and Disorders. Volume 1. Protozoan and Metazoan Infections. CAB International, Wallingford, pp. 289–327.
- CONE D.K., CUSACK R. 1988: A study of *Gyrodactylus colemanensis* Mizelle and Kritsky, 1967 and *Gyrodactylus salmonis* (Yin and Sproston, 1948) (Monogenea) parasitizing captive salmonids in Nova Scotia. Can. J. Zool. 66: 409–415.
- CONE D.K., ODENSE P.H. 1984: Pathology of five species of *Gyrodactylus* Nordmann, 1832 (Monogenea). Can. J. Zool. 62: 1084–1088.
- CONE D.K., ODENSE P.H. 1988: Light and scanning electron microscope studies of *Fundulotrema prolongis* (Monogenea; Gyrodactylidae) parasitizing *Fundulus diaphanus* (Cyprinodontidae) in Nova Scotia, Canada, with an emended diagnosis of *Fundulotrema*. Proc. Helminthol. Soc. Wash. 55: 224–228.
- de BEAUCHAMP P. 1912: *Isancistrum loliginis* n. g. n. sp. Trematode parasite du Calmar et l'inexistence de *Solenocotyle chiajei* Diesing. Bull. Soc. Zool. Fr. 37: 96–99.
- ERNST I., WHITTINGTON I.D., JONES K.K. 2000: Three new species of *Polyclithrum* Rogers, 1967 (Gyrodactylidae: Monogenea) from mugilid fishes from Australia and Brazil, with a redescription of *P. mugilini* Rogers, 1967. Syst. Parasitol. 45: 61–73.
- HARGIS W.J. 1955: Monogenetic trematodes of Gulf of Mexico fishes. Part 1, The superfamily Gyrodactyloidea. Biol. Bull. (Woods Hole) 108: 125–137.
- HARRIS P.D., TINSLEY R.C. 1987: The biology of *Gyrdicotylus gallieni* (Gyrodactylidae), an unusual viviparous monogenean from the African clawed toad, *Xenopus laevis*. J. Zool. Lond. 212: 325–346.
- KHALIL L.F., MASHEGO S.N. 1998: The African monogenean gyrodactylid genus *Macrogyrodactylus* Malmberg, 1957, and the reporting of three species of the genus on *Clarias gariepinus* in South Africa. Onderstepoort J. Vet. Res. 65: 223–231.
- KRITSKY D.C., THATCHER V.E. 1977: *Phanerothecium* gen. nov. and *Fundulotrema* gen. nov. Two new genera of viviparous Monogenoidea (Gyrodactylidae), with a description of *P. caballeroi* sp. nov. and a key to the subfamilies and the genera of the family. Excerta Parasitológica en Memoria del Doctor Eduardo Caballero y Caballero. Instituto de Biología, Universidad Nacional Autónoma de México, Publicaciones Especiales 4: 53–60.
- MALMBERG G. 1970: The excretory system and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). Ark. Zool. 23: 1–235.
- MALMBERG G. 1998: On the evolution within the family Gyrodactylidae (Monogenea). Int. J. Parasitol. 28: 1625–1635.
- MIZELLE J.D., KRITSKY D.C. 1967: Studies on monogenetic trematodes. XXX. Five new species of *Gyrodactylus* from the Pacific Tomcod, *Microgadus proximus* (Girard). J. Parasitol. 53: 263–269.

- PAPERNA I. 1968: Monogenetic trematodes collected from freshwater fish in Ghana. Second report. *Bamidgeh* 20: 88–100.
- ROGERS W.A. 1967a: *Polyclithrum mugilini* gen. et sp. n. (Gyrodactylidae: Polyclithrinae subfam. n.) from *Mugil cephalus* L. *J. Parasitol.* 53: 274–276.
- ROGERS W.A. 1967b: Six new species of *Gyrodactylus* (Monogenea) from the southeastern U.S. *J. Parasitol.* 53: 747–751.
- ROGERS W.A. 1969: *Swingleus polyclithroides* gen. et sp. n. (Monogenea: Gyrodactylidae) from *Fundulus grandis* Baird and Girard. *Tulane Stud. Zool. Bot.* 16: 22–25.
- WELLBORN T.L. Jr., ROGERS W.A. 1967: Five new species of *Gyrodactylus* (Trematoda: Monogenea) from the southeastern U.S. *J. Parasitol.* 53: 10–14.

Received 22 November 2001

Accepted 12 July 2002