

HELMINTHOLOGIA, 55, 1: 70 - 76, 2018

## Morphology of *Emoleptalea nwanedi* n. sp. from *Schilbe intermedius* from Nwanedi-Luphephe Dam, Limpopo Province, South Africa

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### Article info

Received June 7, 2017  
Accepted October 5, 2017

### Summary

A new species, *Emoleptalea nwanedi* n. sp. is described from the intestine of *Schilbe intermedius*, the silver catfish or butter barbel, from the Nwanedi-Luphephe Dam in the Limpopo Province of South Africa. Fish were collected using gill nets where after they were euthanised and dissected. The parasites were sampled, fixed in 70 % EtOH and stained with Van Cleave's haematoxylin. This species represents an addition to the African cluster of *Emoleptalea* species previously described and differs from the known species due to its unique size, equal size of oral and ventral suckers, position of ovary and seminal receptacle, number of vitelline follicles and their size, as well as the unique ciliated receptors on the wall of the acetabulum. This is the first record of this parasite from the silver catfish and from southern Africa.

**Keywords:** trematode; *Emoleptalea*; *Schilbe*; southern Africa

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### Introduction

According to Jones and Bray (2008), the family Cephalogonimidae Looss, 1899 is a small family of freshwater fish parasites from Africa and Asia consisting of small, spinous digeneans in the gastro-intestinal tract of fishes, amphibians and reptiles. According to these authors its major differential character is the position of the genital pore opening at the anterior extremity of the body and the presence or absence of circumoral spines at the anterior end of the body.

The first species within this family was described by Looss in 1899 under the genus *Leptalea* Looss, 1899 which he later renamed *Emoleptalea* Looss, 1900. The parasite was described as *Emoleptalea exilis* (Looss, 1899) Looss, 1900 from the midgut of *Bagrus bayad* (Forsskål, 1775) from the Nile River in Egypt. Dollfus (1950) described *Emoleptalea synodontis* Dollfus, 1950 from the intestine of *Synodontis notatus* Vaillant, 1893 from Lake Maka, Belgian Congo, presently known as the Democratic Republic of the Congo. Thomas (1958) followed with a description of *Emoleptalea*

*proteropora* Thomas, 1958 from the intestine of *Clarias senegalensis* Valenciennes, 1840, presently known as *Clarias anguillaris* (Linnaeus, 1758), from the Black Volta River, Gold Coast, Ghana, West Africa. Two species were described by Shrivastava (1960) from India namely *Emoleptalea loossi* Shrivastava, 1960 and *Emoleptalea dollfusi* Shrivastava, 1960 which were both described from the intestine of *Saccobranchus fossilis* (Bloch, 1794), currently known as *Heteropneustes fossilis* (Bloch, 1794) sampled from Raipur in India.

Ramadan *et al.* (1987) described *Paramasenia rifaati* Ramadan, Saoud & Taha, 1987 from *Synodonis schall* (Bloch & Schneider, 1801) and *Synodontis serratus* Rüppell, 1829 from the Sharkiya Governorate in Egypt. This genus was later synonymised with the genus *Emoleptalea* by Jones and Bray (2008) and is now known as *Emoleptalea rifaati* (Ramadan, Saoud & Taha, 1987) n. comb. Three species previously described under the genus *Oudhia* were synonymised with the genus *Emoleptalea* by Jones and Bray (2008). These are *Oudhia horai* Gupta, 1955 described by Gupta

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(1955) from *H. fossilis* at Imphai (Manipur State), India, now known as *Emoleptalea horai* (Gupta, 1955) n. comb., *Oudhia hardayali* Kumar & Agarwal (1980) described by Kumar and Agarwal (1980) from the intestine of *Mystus vittatus* (Bloch, 1794) from Soraha Tal, Ballia, India, now known as *Emoleptalea hardayali* (Kumar & Agarwal, 1980) n. comb. and *Oudhia kanungoi* Agarwal & Agarwal, 1985 described from the intestine of *Rita rita* (Hamilton, 1822) by Agrawal and Agarwal (1985) from Uttar Pradesh and Madhya Pradesh, India, now known as *Emoleptalea kanungoi* Agarwal & Agarwal, 1985) n. comb.

In the present study, specimens of the genus *Emoleptalea* were sampled from an isolated water body, the Nwanedi-Luphephe Dam situated in the northern part of the Limpopo Province in South Africa. These specimens were sampled from the silver catfish, *Schilbe intermedius* Rüppell, 1832, commonly also known as the butter barbel.

### Material and Methods

Fish was collected with gill nets from the Nwanedi-Luphephe Dam. They were euthanised with clove oil where after the intestines were examined for parasites using a Nikon Model C-Leds stereo microscope. Specimens for light microscopy were fixed in 70 % EtOH and later stained in Van Cleave's haematoxylin, routinely prepared and mounted on slides using DPX as mounting medium. All measurements were made using the NIS-Elements AR imaging (Nikon, Japan) software programme. Morphological measurements are presented in the text as follows: minimum to maximum values, followed by the average value and standard deviation in parentheses. For scanning electron microscopy specimens were fixed in 2.5 % gluteraldehyde, washed in Millonig's phosphate buffer (pH = 7.2) and dehydrated through a graded series of ethanol for one minute in each concentration. They were critically point dried (Polaron, Watford, UK), mounted on stubs, sputter coated with carbon (QT150ES, Quorum Technologies, USA) and examined with a Zeiss Supra 55 Variable Pressure Field Emission Scanning Electron Microscope (VP FE-SEM) (Carl Zeiss, Germany) at 1-2 kV.

### Results

Family Cephalogonimidae  
*Emoleptalea nwanedi* n. sp.  
 (Figs. 1, 2A – H, 3A – H)

Description: Diagnosis based on whole mounts of 25 mature worms and 12 worms observed with SEM: Body (Figs. 1, 2A) small, dorsoventrally flattened, measuring 582 – 722 (653 ± 55; 25) long and 320 – 407 (364 ± 33; 25) wide. Oral sucker situated subterminally at distance of 2.5 – 8.8 (5.7 ± 1.9; 25) from anterior extremity of body. Sucker slightly longer than wide, measuring 91 – 123 (110 ± 11; 25) x 79 – 115 (93 ± 8; 25). Oral sucker well-developed (Fig. 2E), muscular, very stretchable, not covered with

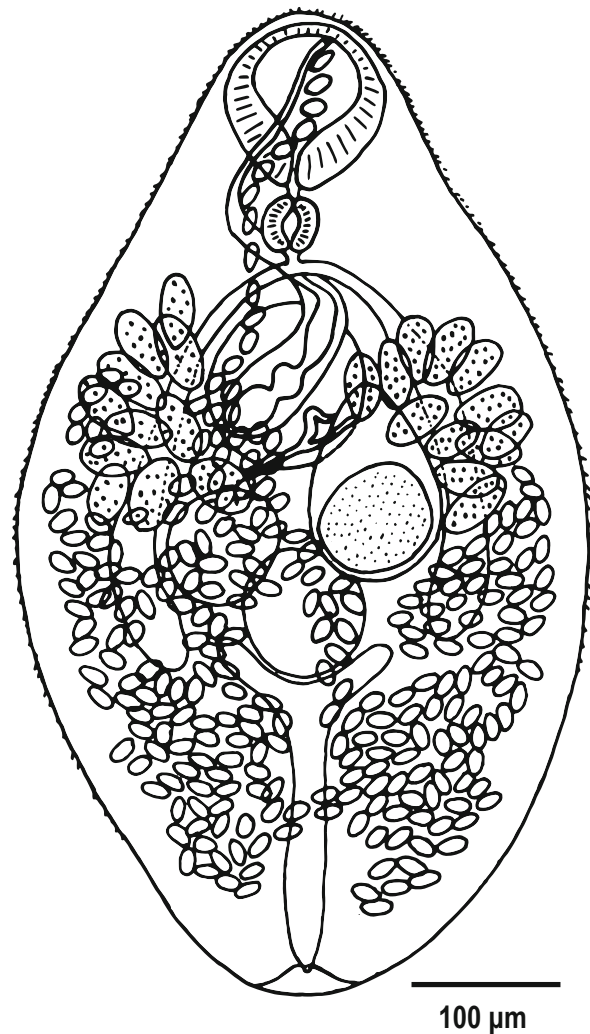


Fig. 1. Microscopic projection drawing of the adult of *Emoleptalea nwanedi* n. sp. from the intestine of *Schilbe intermedius*.

spines (Fig. 3C). Sensory receptors on wall of oral sucker consist mainly of large non-ciliated receptors (Fig. 3C). Only a few of these receptors were observed on and around oral sucker.

Pre-pharynx present but very short (Fig. 2E), only visible in a few specimens, measuring 5.2 – 14 (10 ± 2.4; 25) x 5.6 – 13 (10 ± 2.4; 25). It leads to small but well-developed pharynx (Fig. 2E), 30 – 43 (38 ± 4; 25) x 30 – 46 (38 ± 3.7; 25), followed by short oesophagus measuring 4.5 – 16 (10 ± 4; 25) long and 10 – 17 (13 ± 2; 25) wide. Intestinal bifurcation in forebody, caeca simple, slender and extends to end of posterior testis where they end blind, measuring 279 – 356 (319 ± 17; 25) long x 25 – 39 (31 ± 4; 25) wide.

Acetabulum (Figs. 3B,D) situated 157 – 223 (188 ± 22; 25) from anterior end of body and 59 – 99 (77 ± 16; 25) posterior to oral sucker. Almost equal in size compared to oral sucker, situated pre-equatorial and measures 90 – 123 (108 ± 11; 25) long x 95 – 125 (109 ± 9; 25) wide. Acetabulum muscular, well-formed

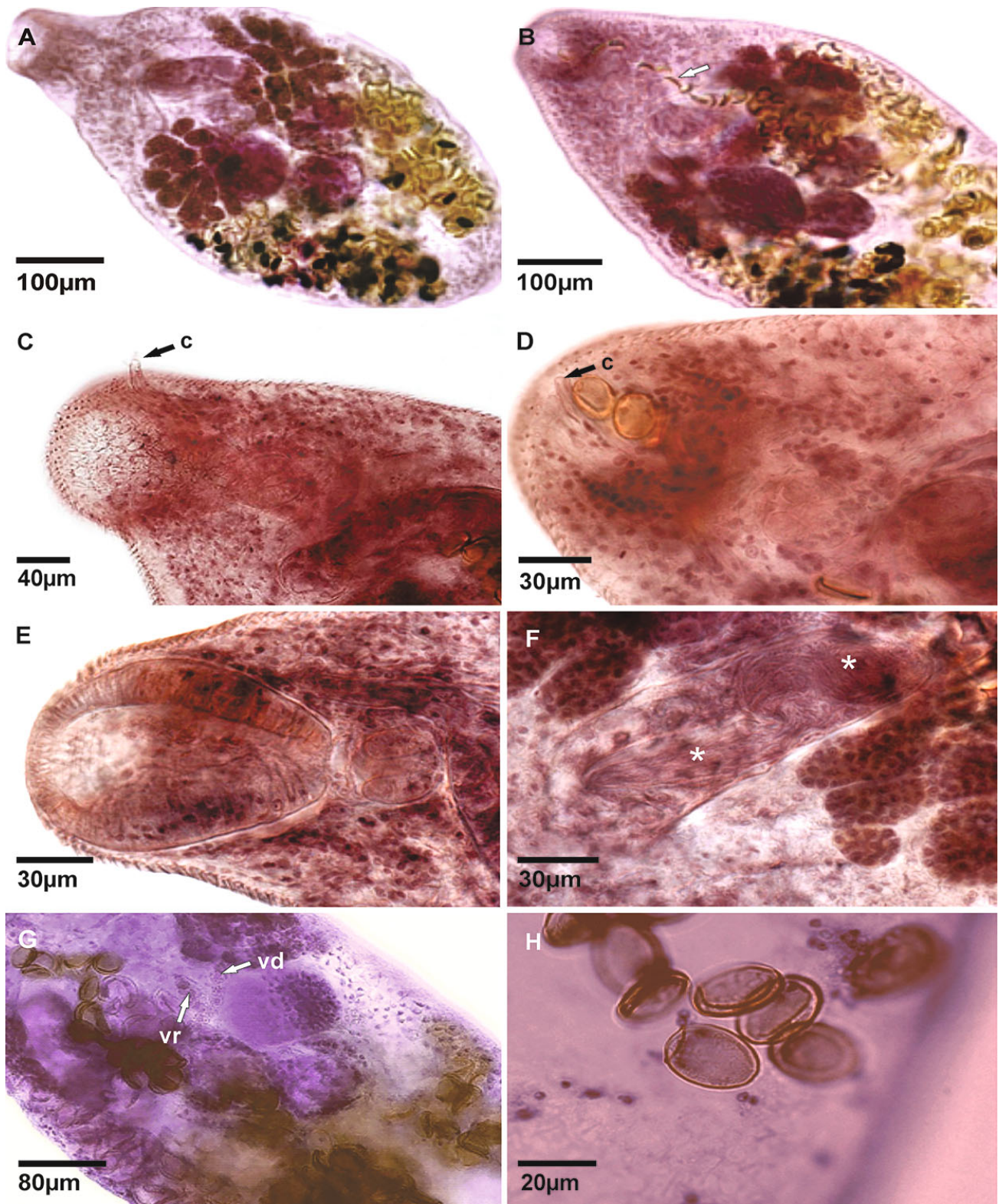


Fig. 2. Light micrographs of the adult of *Emoleptalea nwanedi* n. sp. from the intestine of *Schilbe intermedius*. 2A – whole mount; 2B – anterior half showing anteriorly directed uterus with eggs (arrow); 2C,D – cirrus opening (c) medially to oral sucker; 2E – oral sucker and pharynx separated by pre-pharynx; 2F – bipartite seminal vesicle (\*); 2G – vitelline duct (vd) and reservoir (vr); 2H – operculated eggs with collar.

(Fig. 3D) and very protractible. Two types of sensory receptors observed on wall of acetabulum. First type represents bulbous receptors from which a short cilium protrudes. Second type, only found on wall of acetabulum, consists of broad plate-like base with short cilium protruding from centre of plate (Fig. 3G).

Male reproductive system consists of two testes lying oblique and intercaecal in anterior part of hindbody (Figs. 1, 2A,B). Anterior testis almost round in shape, situated 281 – 369 ( $317 \pm 33$ ; 25) from anterior end of body and measures 72 – 98 ( $85 \pm 9.9$ ; 25) in length x 75 – 101 ( $87 \pm 9.5$ ; 25) wide. Round posterior testis situated 310 – 379 ( $336 \pm 27$ ; 25) from anterior extremity of body measures 72 – 99 ( $84 \pm 8.8$ ; 25) long x 72 – 97 ( $86 \pm 9.7$ ; 25) wide. Cirrus sac elongated, claviform, sigmoid, occupying most of space between pharynx and acetabulum, sometimes overlaying acetabulum, measuring 130 – 174 ( $151 \pm 14$ ; 25) long x 42 – 60 ( $51 \pm 5$ ; 25) wide. Within it bipartite internal seminal vesicles present (Fig. 2F), consisting of posterior part measuring 49 – 89 ( $67 \pm 13$ ; 25) x 21 – 35 ( $29 \pm 3.9$ ; 25) and anterior part measuring 51 – 82 ( $70 \pm 9.6$ ; 25) x 16 – 24 ( $19 \pm 2.3$ ; 25). From here it extends into elongated pars prostatica measuring 58–94 ( $75 \pm 11$ ; 25) x 17 – 35 ( $25 \pm 5$ ; 25), followed by long ejaculatory duct, 124 – 149 ( $138 \pm 7$ ; 25) x 9.8 – 12.8 ( $10.8 \pm 0.8$ ; 25), that extends to lateral edge of oral sucker where it opens as a genital pore (Figs. 2C,D). Extending cirrus is not covered with spines.

Female reproductive system consists of round to slightly oval ovary, measuring 96 – 123 ( $111 \pm 10$ ; 25) in length and 79 – 101 ( $90 \pm 7$ ; 25) wide. Ovary situated almost in middle of body, submedian, but overlays edge of anterior testis (Figs. 1, 2B). Ovary situated 219 – 322 ( $263 \pm 38$ ; 25) from anterior end of body. Seminal receptacle large, sometimes overlaying ovary, situated 253 – 302 ( $274$

Excretory vesicle “Y”-shaped consisting of elongated stem measuring 131 – 172 ( $155 \pm 13$ ; 25) long x 26 – 45 ( $35 \pm 5.9$ ; 25) wide, followed by anterior parts measuring 63 – 104 ( $85 \pm 14$ ; 25) long x 15 – 27 ( $19 \pm 3$ ; 25) wide. Pore opens terminally at posterior end of body (Fig. 3A).

Tegument of anterior part of forebody covered by scale-like spines (Fig. 3E) which are embedded in tegument all around oral and ventral suckers (Fig. 3B). These spines are densely packed, posteriorly directed and can retract into body. Posterior to acetabulum these spines are more sharply pointed and seemingly arranged in rows (Fig. 3F). From anterior part of hindbody, these spines decline in number and concentration towards the posterior end where body is only covered by a rough spine-less tegument (Fig. 3A). Enlarged circumoral spines absent. Very few sensory receptors present elsewhere on body of the worm. A few large non-ciliated receptors were observed randomly on body (Fig. 3C), whereas a few small bulbous ciliated receptors and longer ciliated receptors (Fig. 3H) were observed in the area between the two suckers.

Seventy-one specimens of *S. intermedius* were sampled and examined for parasites. Only 29 of these were found to harbour *Emoleptalea* parasites, resulting in a prevalence infection of 40.8 %. A total number of 1468 parasites were collected. The mean intensity was calculated as 50.6 and the mean abundance as 20.7.

It also became apparent that the size of *S. intermedius* specimens collected had an effect on the number of parasites that possibly could be sampled. Small fish, less than 16 cm, were seldom infected, while sizes ranging between 16 to 25 cm yielded more parasites, but specimens larger than 25 cm were infected with the most parasites. This data is summarised in Table 1.

Table 1. Infection statistics of *Schilbe intermedius* with *Emoleptalea nwanedi* n. sp. regarding the size of the host vs. the number of parasites found.

Length of sampled fish	Prevalence (%)	Abundance	Intensity	Intensity range
< 16 cm	14.2	1.6	11.5	2 – 14
16 – 25 cm	48	8.9	18.5	2 – 147
> 25 cm	56	39.8	70.1	1 – 327

$\pm 19$ ; 25) from anterior end of body. This sperm filled structure measures 61 – 91 ( $76 \pm 11$ ; 25) in length and 46 – 88 ( $58 \pm 9.7$ ; 25) wide. Uterus coils mainly in post-testicular region, descends on the right to close to posterior end of body where after it ascends on the left again, occupying most of hindbody. Eggs are numerous and small, measuring 21 – 24 ( $23 \pm 0.9$ ; 25) long x 13 – 16 ( $14 \pm 0.9$ ; 25) wide. Eggs are operculated forming a slight shoulder edge at opposite ends of opercule line (Fig. 2H).

Vitelline follicles in lateral fields are grouped together forming a cluster of 10 – 14 ( $12 \pm 1.1$ ; 25) follicles (Fig. 2A) on both sides of body on either side of acetabulum to half way of anterior testes flowing into a vitelline reservoir (Fig. 2G) before discharging into ootype. Individual follicles measure 42 – 61 ( $51 \pm 6.5$ ; 25) long x 30 – 42 ( $36 \pm 3.9$ ; 25) wide.

### Taxonomic summary

Type and only known host: *Schilbe intermedius* Rüppell, 1832.

Type and only locality: Nwanedi-Luphephe Dam, Limpopo Province, South Africa ( $22^{\circ}38'36''S$   $30^{\circ}24'29.5''E$ ).

Site of infection: Duodenum.

Prevalence of infection: 40.8 %.

Specimens deposited: Deposited in the Aquatic Parasitology Research Group, Department of Biology, Sefako Makgatho Health Sciences University, Pretoria, South Africa. Adult specimens: holotype: 2011/03/15/01; voucher specimens: 2012/02/29/01-16.

Etymology: The species epithet, *nwanedi*, derived from the type locality, an ecological jewel in the Limpopo River System.

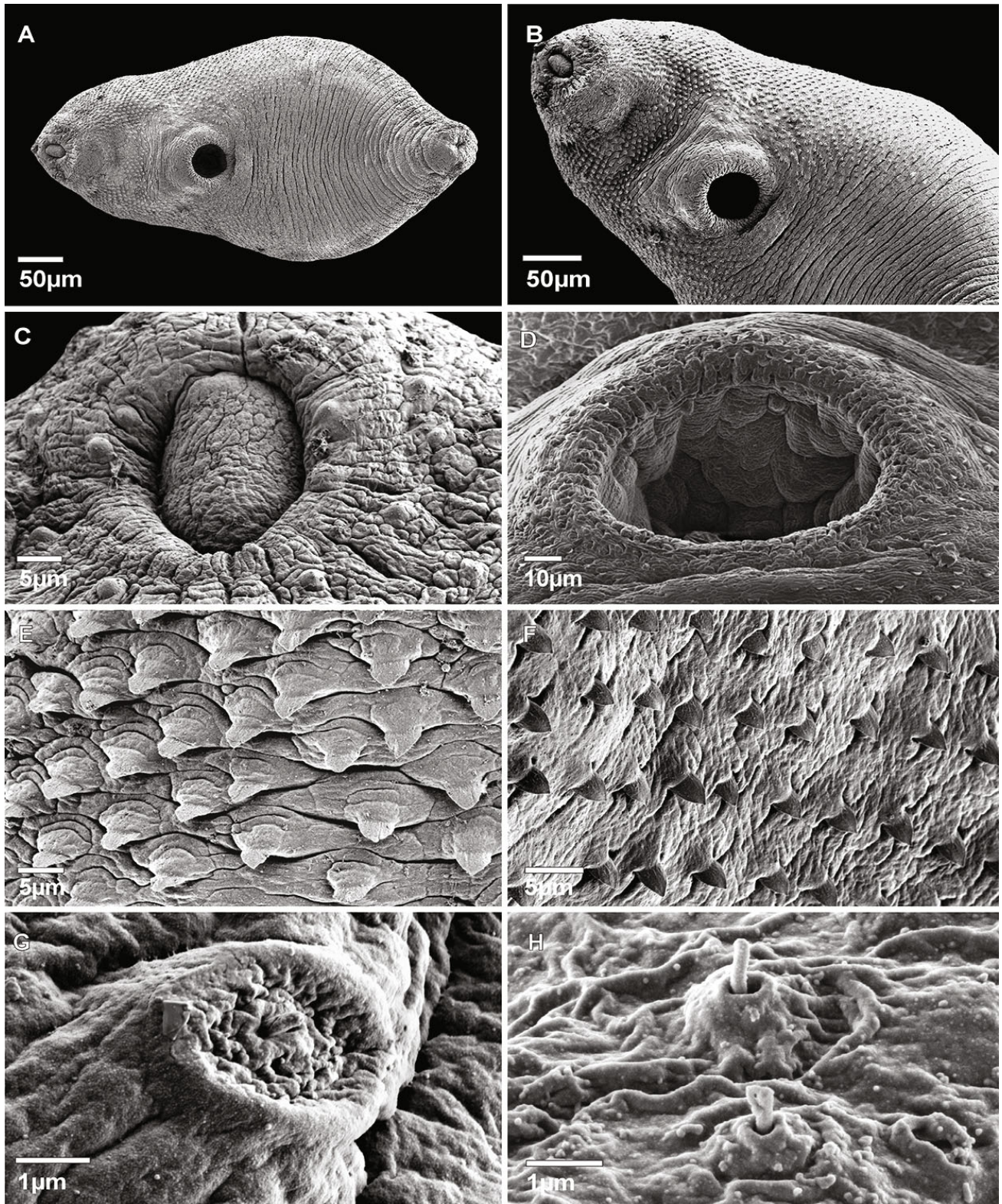


Fig. 3. Scanning electron micrographs of the adult of *Emoleptalea nwanedi* n. sp. from the intestine of *Schilbe intermedius*. 3A – whole mount; 3B – anterior half showing body spines; 3C – oral sucker showing non-ciliated receptors; 3D – acetabulum showing receptors; 3E – tegumental features between oral sucker and acetabulum; 3F – body covering posterior to acetabulum; 3G – enlarged plate-like receptors on acetabulum edge; 3H – longer ciliated receptors on body and around suckers.

## Discussion

From the introduction it is clear that species within the genus *Emoleptalea* can be clustered into two groups, those described from Africa and those from India. The Indian group now includes five species namely *E. horai*, *E. loossi*, *E. dollfusi*, *E. hardayali* and *E. kangungoi*. All five of these species make use of catfish as their definitive host. *Emoleptalea loossi*, *E. dollfusi* and *E. horai* were found in the Asian stinging catfish (*Heteropneustes fossilis*) (Shrivastava, 1960; Gupta, 1955), *E. hardayali* in the striped dwarf catfish (*Mystus vittatus*) (Kumar & Agarwal, 1980) and *E. kangungoi* in the striped catfish (*Rita rita*) (Agarwal & Agarwal, 1985).

The African group also includes five species namely *E. exilis*, *E. synodontis*, *E. proteropora*, *E. rifaati* and *E. nwanedi* n. sp. Of these only *E. proteropora* is found in a *Clarias* species, namely *Clarias anguillaris* (Thomas, 1958). *Emoleptalea exilis* was described from the black Nile catfish, *Bagrus bayad* (Looss, 1899) Looss, 1900. The remaining three species were all described from squeakers: *E. synodontis* from the one-spot squeaker, *Synodontis notate* (Dollfus, 1950), *E. rifaati* on the other hand, was described from two squeaker species, namely the Nile squeaker, *Synodontis schall* and the shield-head squeaker, *Synodontis serratus* (Ramadan, Saoud & Taha, 1987). The newly described *E. nwanedi* n. sp. however, was described from a siluriforme fish, the silver catfish *S. intermedius*.

The species material was collected over a period of three years during three different field trips resulting in the size ranges as indicated in the results. The present species show minor morphological differences compared to the four *Emoleptalea* spp. described from Africa and the five species described from India. The following five differences indicate the present species to differ from the nine known species: the present species is the smallest of all the *Emoleptalea* species described from Africa and India to date. The oral sucker of the present species is more or less of equal size to the acetabulum, compared to the already described species that indicates that the suckers are not uniform in size. The ovary in the present species most often overlays the anterior testis, compared to other species that indicate the ovary to be a distance away. The large seminal receptacle most often overlays the ovary not as indicated for other described species. The vitelline follicles are of greater numbers and size compared to the rest of the African species, and even more so for the Indian species.

This study also represents the first description of an *Emoleptalea* species using scanning electron microscopy. These results clearly show the body to be covered with triangular spines that are arranged anteriorly in rows. Another unique feature is the ciliated receptors on the acetabulum that protrude from a plate-like base. Electron microscopy also shows that the cirrus is not covered with spines.

The only related genus of digeneans from African and Asian freshwater fishes that appears morphologically similar to *Emoleptalea* in the Cephalogonimidae, is *Masenia* Chatterji, 1933. Two species

have previously been described from Africa, i.e. (i) *Eumasia bangweulensis* Beverley-Burton 1962 described from *Clarias melandi* (synonymised with *Clarias ngamensis*) from the Mangweula Swamps in northern Rhodesia (now Zimbabwe) (Beverley-Burton, 1962) and characterised by 48 circumoral spines and vitelline follicles that occur in two groups on either side of the ventral sucker; (ii) *Eumasia synodontis* Khalil & Thurston 1973 described from *Synodontis victoriae* from Jinja on Lake Victoria, Uganda (Khalil & Thurston, 1973), with 36 – 40 circumoral spines and vitelline follicles that extend from the level of the ventral sucker to the level of the margin of the posterior testis. Jones and Bray (2008) synonymised *Eumasia* with *Masenia*, but these species differs from *Emoleptalea* species by a crown of circumoral spines at the anterior end of the body and vitelline follicles that are mainly located between the ventral sucker and the level of the testes.

This is the first description of an *Emoleptalea* species from southern Africa and from *S. intermedius*. Follow-up studies were also attempted in order to try and find the intermediate hosts. Two freshwater snail species were collected. *Lymnaea natalensis* Krauss, 1848 was found to secrete 27-spined echinostome cercariae, and *Biomphalaria pfeifferi* (Krauss, 1848) was found to secrete strigeid cercariae, categorised as the pharyngeal, distome, longifurcate type. The metacercarial stage also remains unknown, but it is suspected that the larval stage of the parasite utilises smaller fish species as second intermediate hosts.

## Acknowledgements

This work is based on the research supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa (Grant No 101054) and the University of Limpopo. Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the NRF does not accept any liability in this regard. We wish to acknowledge the Department of Science and Technology of South Africa in partnership with the National Research Foundation for grants received to purchase the Field Emission Scanning Electron Microscope and ancillary equipment used in this investigation.

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