The description of Mediorhynchus africanus *n. sp. (Acanthocephala: Gigantorhynchidae) from galliform birds in Africa*

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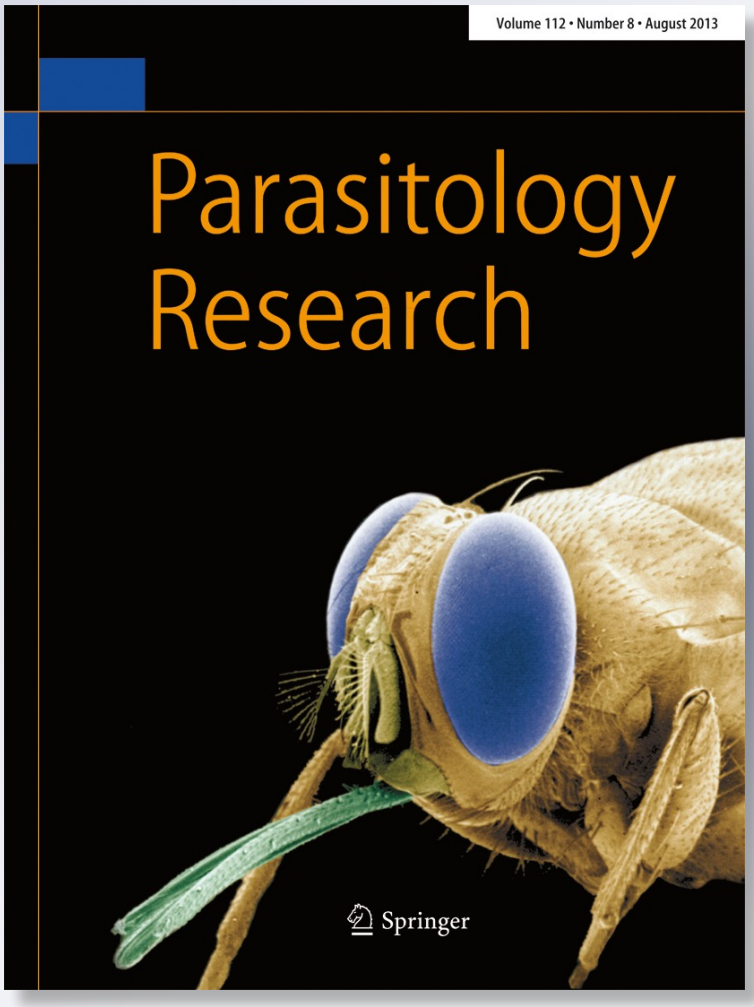

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ORIGINAL PAPER

The description of *Mediorhynchus africanus* n. sp. (Acanthocephala: Gigantorhynchidae) from galliform birds in Africa

Omar M. Amin · Paul Evans · Richard A. Heckmann · Atif M. El-Naggar

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Abstract *Mediorhynchus africanus* n. sp. is described from specimens collected from the helmeted guinea fowls, *Numida meliagris* Linn. 1758 in Kruger National Park and elsewhere in subSaharan Africa from the same and other galliform birds. These specimens were previously assigned to *Mediorhynchus gallinarum* Bhaleroa (Proc Zool Soc Lond Ser B Syst Morph 107:199–203, 1937) described from chickens, *Gallus gallus* L. in India and subsequently reported from other Asian countries. The identification of the African forms as *M. gallinarum* was based on similarities in the structure and measurements of the proboscis, proboscis armature and receptacle, lemnisci, and reproductive organs. A detailed study of specimens from South Africa and descriptions reported from elsewhere in Africa revealed marked differences that clearly distinguish the African material as new species. The African specimens are pseudo-segmented and flattened, the proboscis has two prominent apical pores, sensory pits are prevalent throughout the trunk, the posterior end of the female is broad with dorsoterminal dome-like extension opposite the subterminal gonopore, and the eggs are large. The Asian specimens from Indonesia and elsewhere are cylindrical and non-segmented, the proboscis lacks prominent apical pores, sensory pits are rare on the trunk, the posterior end of the female is pointed with a terminal gonopore, and the eggs are markedly smaller. We used DNA sequence from one mitochondrial gene (cytochrome oxidase subunit I) and one nuclear gene (18S ribosomal RNA) to infer the phylogenetic relationships of *M. africanus* and *M. gallinarum* and selected Acanthocephala. *Mediorhynchus* is monophyletic and *M. africanus* and *M. gallinarum* are allopatric sister species (9.7 % sequence divergence). All findings indicate that *M. africanus* should be ranked as a separate species.

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Introduction

The Asian distribution of *Mediorhynchus gallinarum* (Bhaleroa 1937) and the African distribution of *M. africanus* n. sp., which was previously confused with *M. gallinarum* are well documented. The Asian material included the original description from a single female as *Leiperacanthus gallinarum* by Bhaleroa (1937) from India. Tubangui and Masilungan (1946) described *M. gallinarum* from Manila also as *Leiperacanthus gallinarum*. Petrochenko (1958) placed *M. gallinarum* in *Empodius* Travassos, 1916, and based his description on Tubangui and Masilungan's (1946) account. Yamaguti (1954) described his specimens from Celebes (now Sulawesi, an Indonesian province) as *Empodius* sp. Nath and Pande (1963) described their specimens from India. Talbot (1971) reported but did not describe his specimens of *M. gallinarum* (except for Fig. 1 of a male) from Papua New Guinea. Schmidt and Kuntz (1977) reported, but did not describe, *M. gallinarum* from Terabanon Concepción and Palawan Island, revised the genus *Mediorhynchus* Van Cleave 1952, provided a key to the 29 species known then, and noted 17 other species "of uncertain

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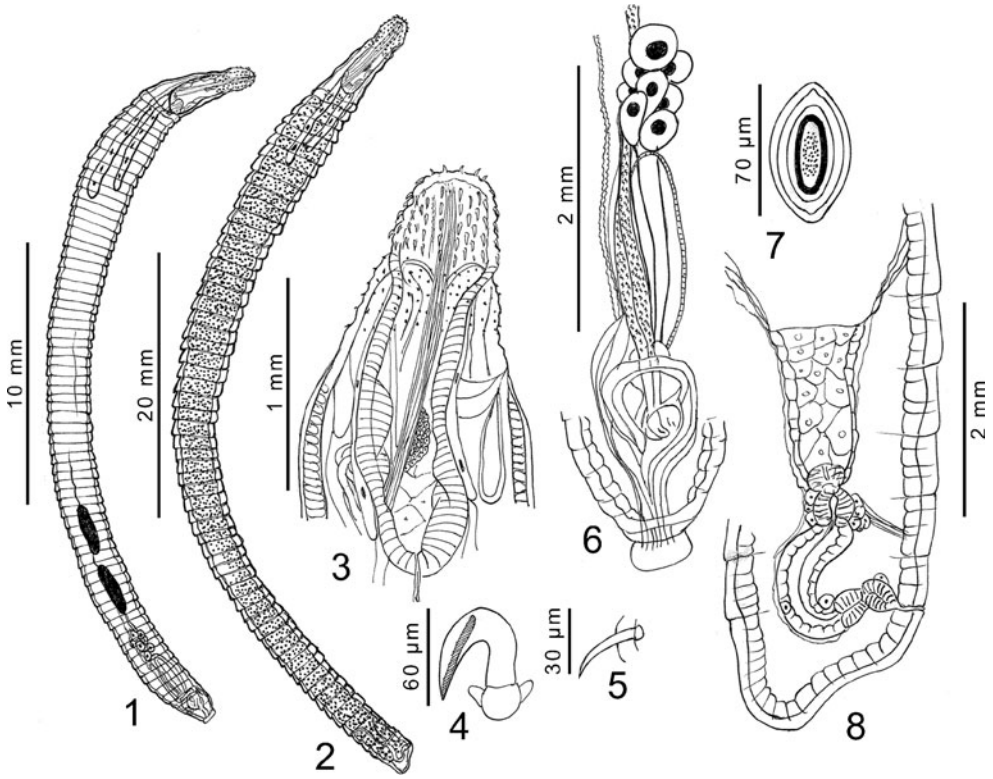

Figs. 1–8 Paratype specimens of *Mediorhynchus africanus* n. sp. from *Numida meliagris*. *1* An adult male paratype; note the unsegmented anterior part of the trunk. *2* A gravid Female; note the dorsal extension of the posterior end of the trunk. *3* The proboscis and receptacle of a paratype female. *4* A hook at the middle position of the proboscis. Note the lateral groove and the wings on the rounded posterior end of the root. *5* A spine from the posterior proboscis. *6* The posterior portion of the male reproductive system. Note the mononucleated cement glands, two cement gland ducts overlapping Saefftigen's pouch adjacent to two sperm ducts (with S pattern for sperms). *7* A ripe egg. *8* Female reproductive system. Note the sharply curved uterus with fibrillar connectors at its anterior end to the body wall and similar connectors at the anterior dorsal and ventral sides of the uterine bell

or no validity." Humphrey (1979) reported, but did not describe, *M. gallinarum* from Papua New Guinea. Amin et al. (2013) described a population of *M. gallinarum* from Indonesia and provided the first SEM images of that species.

The African reports of the new pseudo-segmented species previously confused with *M. gallinarum* included the three descriptive accounts of Harris (1973), Junker and Boomker (2006) and Southwell and MacFie (1925) from Kenya, South Africa, and Transvaal and Nyasaland (now Pretoria and Malawi, southeast Africa), respectively. Harris (1973) described *Mediorhynchus selengensis* as a new species from a galliform bird in Kenya, East Africa, that was considered a junior synonym of *M. gallinarum* (see Schmidt and Kuntz 1977). All African reports named *M. gallinarum* except for (1) those by Southwell and MacFie (1925) and Fabiyi (1972) who used the name *Empodius segmentatus* Marval (1902), (2) and by Vercruysse et al. (1985) who used the name *M. selengensis* as Harris (1973) did in his description. Fabiyi (1972) did not describe his specimens from Nigeria that were earlier described by Southwell and MacFie (1925) from southeast Africa.

Junker and Boomker (2006) provided a detailed description of specimens from guinea fowl in Kruger National Park, South Africa and Harris (1973) gave a reasonable description of the same species that he named *M. selengensis*. Fabiyi (1972) reported "*Empodius segmentatus* Marval, 1902" from Guinea fowl in Nigeria. This acanthocephalan appears to be the same as *E. segmentatus* (Marval, 1902) described by Southwell and Macfie (1925) which agrees with the description of *M. africanus*. *Empodius* (Skrjabin 1913) Travassos 1916 is a synonym of *Mediorhynchus*. All other reports from Africa were primarily ecological surveys dealing with prevalence rates and host–parasite relationships but not with morphology or taxonomy. These included reports from elsewhere in South Africa (Junker and Boomker 2007; Junker et al. 2008; Davies et al. 2008) and from Somalia, East Africa (Cancrini et al. 1988 and Terregino et al. 1999) as well as from the Central African state of Berkina Faso (formerly Upper Volta) (Vercruysse et al. 1985). Other African poultry from Kenyan villages that were examined (Irungo et al. 2004) and from West Africa in Nigeria (Fatihu et al. 1991) were not infected with *Mediorhynchus*.

The taxonomic status of *Mediorhynchus* species within the Acanthocephala class, Archiacanthocephala, is no less confused when evaluated from the perspective of gene and whole

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genome studies. Phylogenetic reconstructions using 18S ribosomal RNA genes (Garey et al. 1996; Near et al. 1998; Verweyen et al. 2011), 18S and 28S ribosomal RNA genes (Garcia-Varela and Nadler 2005), 18S, 28S ribosomal RNA genes and mitochondrial cytochrome c oxidase subunit 1 (cox 1) sequences (Garcia-Varela and Nadler 2006), whole mitochondria (Min and Park 2009; Weber et al. 2013), telomere sequence (Bombarova et al. 2009) or EST analysis (Witek et al. 2008) place *Mediorhynchus* and Archiacanthocephala in varying relationships. Regardless of the molecular character(s), the genus *Mediorhynchus* is unambiguously and consistently identified as a member of the monophyletic and basal Archiacanthocephala class. Our purpose in this study is to confirm the unique phylogenetic status of *M. africanus* and *M. gallinarum* using gene sequence analysis. These data and a much more robust taxon sampling will be required to further a clear understanding of the relationships of members of Archiacanthocephala, and the multiple genera in the other classes of Acanthocephala. Such studies are needed to ultimately resolve the observed paraphyly of Archiacanthocephala based on morphological characters (Monks 2001).

The present report supports the case that both the Asian and African species, previously described as *M. gallinarum* or its synonyms, are in reality two distinct species, based on morphological and gene sequence studies.

Table 1 Acanthocephalans and Rotifera sampled and GenBank accession numbers

Species	GenBank accession number	
	COI	18S
Acanthocephaus dirus	DQ089718	AY830151
Acanthocephaloides propinquus	DQ089713	AY830149
Centrorhynchus sp.	DQ089716	AY830155
Echinorhynchus truttae	DQ089710	AY830156
Filisoma bucerium	DQ089722	AF064814
Floridosentis mugilis	DQ089723	AF064811
Gorgorhynchoides bullocki	DQ089715	AY830154
Illiosentis sp.	DQ089705	AY830158
Koronacantha pectinaria	DQ089707	AF092433
Leptorhynchoides thecatus	DQ089706	AF001840
Macracanthorhynchus ingens	AF16997	AF001844
Mediorhynchus sp.	AF416996	AF001843
Mediorhynchus africanus	KC261351[a]	KC261353[a]
Mediorhynchus gallinarum	KC261352[a]	KC261354[a]
Moniliformis moniliformis	AF416998	Z19562
Neoechinorhynchus saginata	DQ089704	AY830150
Oligacanthorhychus tortuosa	AF416999	AF064817
Plagiorhynchus cylindraceus	DQ089714	AF001839
Polyacanthorhynchus caballeroi	DQ089724	AF388660
Polymorphus sp.	DQ089721	AF064815
Polymorphus brevis	DQ089717	AF064812
Pomphorhynchus bulbocolli	DQ089709	AF001841
Profilicollis altmani	DQ089720	AF001838
Rhadinorhynchus sp.	DQ089712	AY062333
Transvena annulospinosa	DQ089711	AY830153
Brachionus patulus	AF416995	AF154568

[a] Sequences obtained in this study

Materials and methods

Pseudo-segmented specimens of *M. africanus* collected from the helmeted Guinea fowl, *Numida meleagris* Linn., in South Africa that were identified as *M. gallinarum* and provided by Dr. K. Junker, University of Pretoria at Onderstepoort, South Africa. Specimens were collected in Kruger National Park in 1989 (Junker and Boomker 2006) and in Limpopo Province in 2010 (see Junker and Boomker 2007 and Junker et al. 2008). These specimens were used for microscopical, SEM, and gene frequency studies.

For microscopical studies, worms were punctured with a fine needle and subsequently stained in Mayer's acid carmine, destained in 4 % hydrochloric acid in 70 % ethanol, dehydrated in ascending concentrations of ethanol (24 h each), and cleared in graduated concentrations of terpineol in 100 % ethanol to 100 % terpineol, then 50 % terpineol in 50 % Canada balsam (24 h each). Whole worms were then mounted in Canada balsam. Measurements are in micrometers, unless otherwise noted; the range is followed by the mean values between parentheses. Width measurements represent maximum width. Trunk length does not include proboscis, neck, or bursa.

Type specimens were deposited at the Harold W. Manter Laboratory (HWML) collection, at the University of Lincoln, Nebraska. Many voucher specimens are in the collection of the University of Pretoria at Onderstepoort, South Africa (Dr. K. Junker).

For SEM studies, specimens previously fixed in 70 % ethanol were placed in critical-point drying baskets and dehydrated using ethanol series of 95 and 100 % for at least 10 min per soak followed by critical-point drying (Lee 1992). Samples were mounted on SEM sample mounts, gold coated and observed with a scanning electron microscope (XL30 ESEMFEG; FEI, Hillsboro, Oregon). Digital images of the structures were obtained using digital imaging software attached to a computer.

For gene sequence studies, DNA was extracted from specimens fixed and preserved in 70 % ethanol using a Qiagen DNAeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA, USA). A 1-cm portion of the specimen was soaked in 500 μl of ATL buffer for 10 min prior to DNA digestion. Samples were macerated by scissors and the protocol followed as outlined by the manufacturer. Specimens of *M. africanus*

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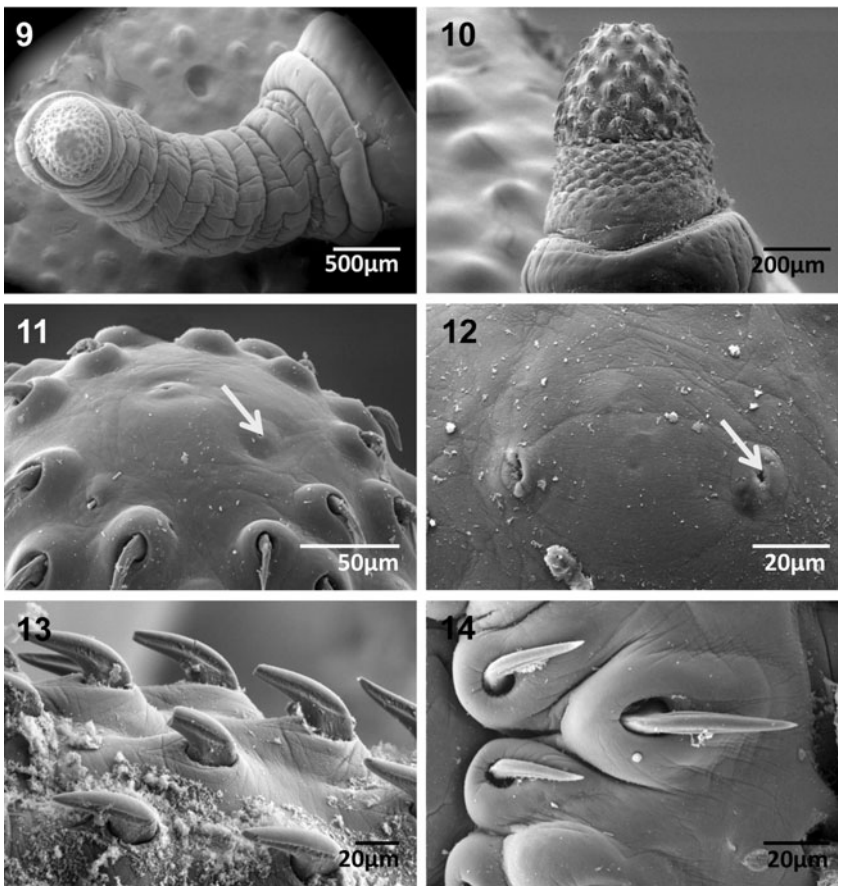
and *M. gallinarum* yielded 5–10 μg of DNA as determined by spectroscopy and staining after electrophoresis.

For PCR amplication of a 682-bp fragment of the mitochondrial cytochrome oxidase subunit 1 gene (CO1), we used the primers 5′-AGTTCTAATCATAA(R)GATAT(Y)GG-3′ and 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al. 1994). Primers used for the amplification of a 1,747-bp fragment of the nuclear 18S ribosomal RNA gene (18S) were 5′-AGATTAAGCCATGCATGCGTAAG-3′ and 5′-TGATCCTTCTGCAGGTTCACCTAC-3′ (Near et al. 1998). Reaction cocktails were 12.5 μl in volume and included the following reagents: DNA template (~150 ng), nuclease free water (2.25 μl), oligonucleotide primers (10 pmol each), and Promega GoTaq® Green Master Mix (6.25 μl). The thermal profile began with an initial denaturation step of 95 °C for 2 min to activate the enzyme, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s, and concluded by a rapid cool down to 4 °C. Successful amplifications were verified qualitatively by viewing PCR products under ultraviolet radiation following electrophoresis through 1.0 % agarose gels. Millipore MultiScreen$_{\mu 96}$ filter plates were used to purify PCR products following the manufacturer's recommended protocol.

Cycle sequencing reactions were performed using the ABI Big Dye Terminator protocol (Applied Biosystems, Foster City, CA). Reaction cocktails were 10.5 μl in volume, and were mixed using the following reagent amounts: purified PCR product (~150 ng), nuclease free water (2.75 μl), 5× Tris buffer (1.75 μl), primer (6 pmol), and dye terminator reaction mix (0.5 μl). Both DNA strands were sequenced using the same primers that were used to amplify the genes via PCR. The thermal profile for the sequencing reactions consisted of 25 cycles of 96 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min, followed by a rapid cool down to 4 °C. All sequencing was carried out on an ABI 3730xl automated sequencer in the DNA Sequencing Center at Brigham Young University. Sequence data for COI and 18S generated from this study are available from GenBank.

Sequences (Table 1) were initially aligned with Sequencher v. 4.8 (Gene Codes Corp.) and subsequently corrected by eye. Tree reconstructions were carried out using maximum parsimony (MP) maximum likelihood (ML) methods. Bootstrapping of MP, distance-based reconstructions entailed 1,000 replications, with random additions of taxa, on informative sites only in MP. The PAUP*4.2a program package (Swofford 2003) was used for MP reconstructions. The appropriate model of sequence evolution was selected using jModelTest 0.1.1 (Posada 2008) as implemented in PhyML v. 3.0 (Guindon and Gascuel 2003). ML phylogenies were generated using TreeFinder (version of October 2008; Jobb 2008), and nodal support was estimated by performing 1,000 bootstrap replicates. *Brachionus plicatus*, a rotiferan, was used as the outgroup.



Figs. 9–14 SEM of adult mature paratype specimens of *Mediorhynchus africanus* n. sp. *9* Anterior part of a specimen showing the proboscis, anterior somewhat contracted unsegmented portion, and the beginning of the pseudo-segmented trunk. *10* The anterior cone-shaped proboscis with hooks and the posterior proboscis with spines only anteriorly connected to the trunk with mild shoulders. *11* Apical end of the proboscis showing the two sensory pores. *12* Higher magnification of the apical pores shown in Fig. 3. *13* Lateral view of proboscis hooks showing the longitudinal lateral slits. *14* Dorsal view of anterior hooks deeply set in elevated dome-shaped grids

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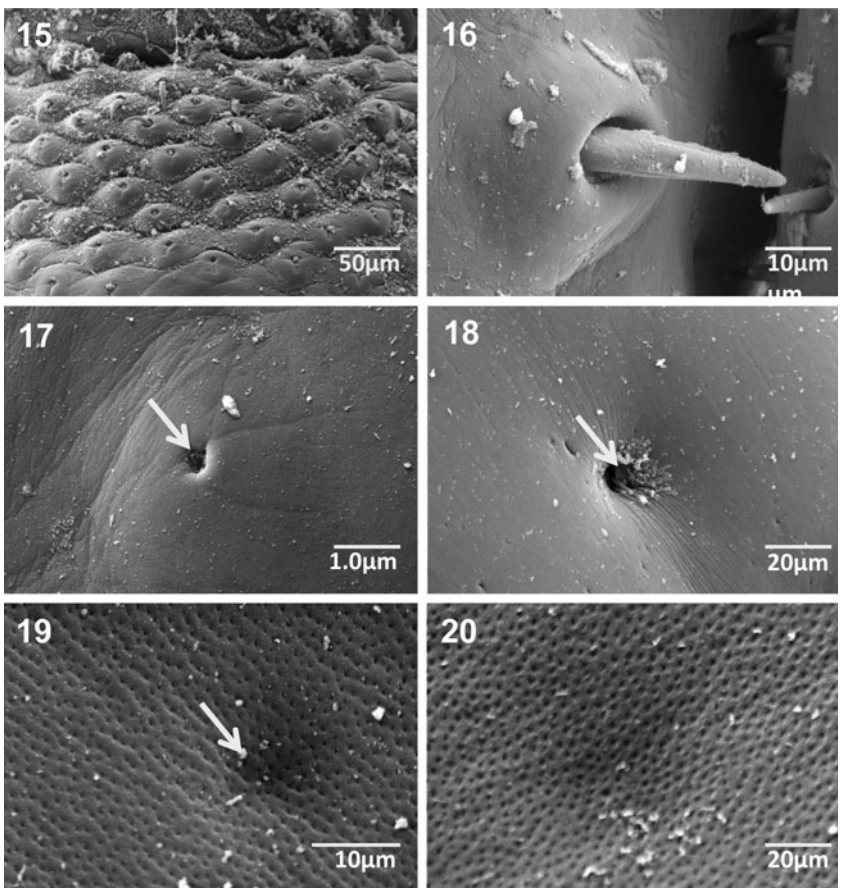
Results

Morphological observations and/or measurements of the new species are based on the study of our own South African specimens provided courtesy of Dr. K. Junker, Pretoria, South Africa from collections reported by Junker and Boomker (2006, 2007) and Junker et al. (2008), as well as on accounts by Junker and Boomker (2006) from *N. meliagris* in South Africa, by Harris (1973) from *Pternistes leucoscepus* in Kenya, and by Southwell and MacFie (1925) from *Numida ptilorhyncha* in Travsvaal and Nyasaland. The measurements from Junker and Boomker (2006), Harris (1973), and Southwell and MacFie (1925) fit within the range of measurements of our own South African specimens of *M. africanus*.

Description

Mediorhynchus africanus n. sp.

General With characters of the genus *Mediorhynchus*. Robust, intermediate in size, pseudo-segmented and laterally flattened throughout except for short somewhat narrower anterior cylindrical portion (Figs. 1, 2, 9, 22–25). Pseudo-segmentation less pronounced in younger worms. Up to 100 annuli in females, fewer in males. Shared structures larger in females than in males. Body wall aspinose, with many fragmented nuclei and electron-dense micropores at epidermal surface (Figs. 19–21, 32). Proboscis in 2 parts occasionally manifesting all armature variations in single proboscides. Ridge between anterior and posterior proboscis prominent. Spines on posterior proboscis easily mistaken for trunk spines in partially retracted proboscides (Figs. 3, 10). Anterior proboscis pear-shaped or apple-shaped with truncated bare apical end, two prominent apical pores (Figs. 11, 12, 17), and 18–22 longitudinal rows of 4–6 hooks each. Hooks 35–76 long; smallest anteriorly and longest at middle. Hooks invariably with longitudinal lateral slits (Figs. 4, 13) set in elevated hexagonal grids. Hook roots about as long as blades, simple, directed posteriorly. Posterior end of roots markedly rounded, each with1 pair of prominent accessory lateral ribbed wings (Fig. 4). Posterior proboscis conically shaped, broader posteriorly at junction with anterior trunk, with 26–40 longitudinal rows of 2–6 spines each measuring 14–43 long; longer anteriorly. Posterior most zone of posterior proboscis devoid of spines; easily confused with neck (Fig. 3). Spines very thin, curved posteriorly or undulating, with small knob-shaped roots (Fig. 5). Both hooks and spines partially embedded in raised dome-shaped cuticular swellings (Figs. 13–16). Borderline between posterior proboscis and neck salient. Neck



Figs. 15–20 SEM of adult mature paratype specimens of *Mediorhynchus africanus* n. sp. *15*. The surface of the spiny area of the posterior proboscis showing dome-shaped elevations in which spines embed; most spines are broken off. *16* Higher magnification of a spine deeply imbedded in elevated grid. *17* High magnification of a sensory pore on the apical proboscis. *18* High magnification of a sensory pore on the collar of the proboscis at the anterior margin of the trunk. Those pores are widely distributed throughout the trunk. *19* Micropores in the anterior third of trunk. *20*. Micropores in the middle third of trunk

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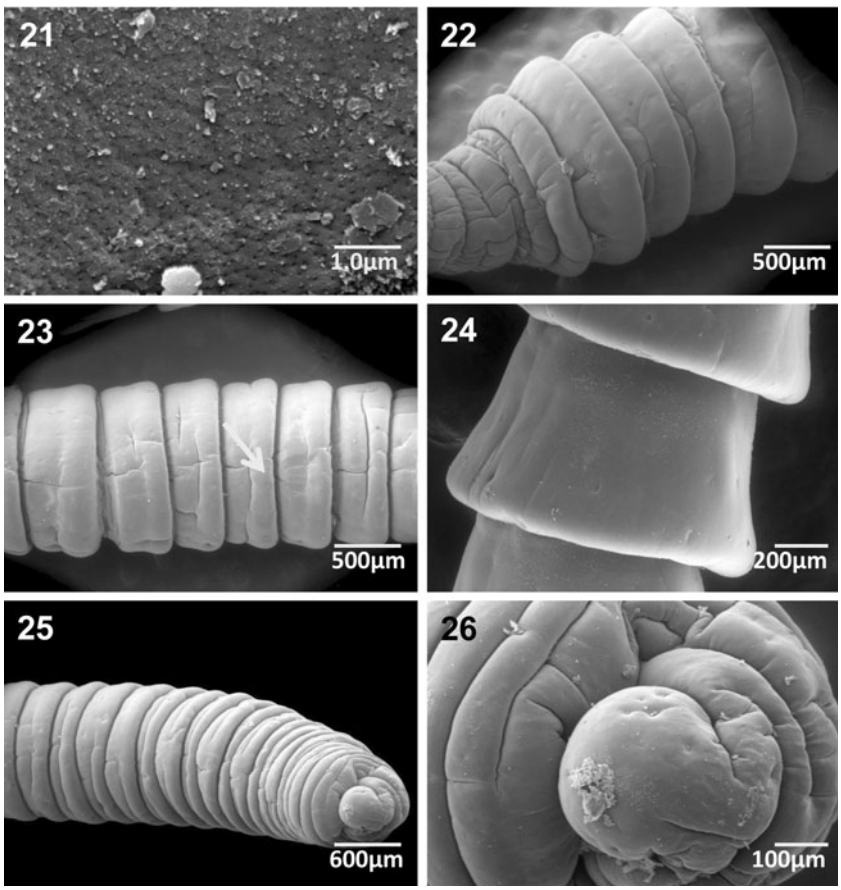
unremarkable. Sensory pits many throughout trunk of males and females (Figs. 18, 26, 29). Proboscis receptacle about twice as long as both proboscides, single-walled with anterior portion encased in jacket of adjacent retractor fibers. Proboscis retractor muscles prominent, attached to proboscis receptacle near its posterior end dorsally. Dorsal and ventral ligament strands nucleated. Cephalic ganglion near middle of receptacle adjacent to retractor muscles. Ventral and dorsal ligament strands prominent (Fig. 3). Lemnisci long, digitiform, unequal, unattached, usually with 6 giant nuclei each. Occasionally shorter lemniscus with 5 nuclei and longer lemniscus with 7 (Figs. 1, 2).

Males (based on 19 adults) Trunk 3.0–74.0 mm long by 0.5–2.80 mm wide. Anterior proboscis 250–339 long 278–478 wide. Posterior proboscis 291–332 long by 591–689 wide posteriorly at junction with anterior trunk. Proboscis receptacle 0.64–1.23 mm long by 0.11–0.44 mm wide. Shorter lemniscus 1.60–3.38 mm long by 0.21–0.31 mm wide, with 5 or 6 giant nuclei. Longer lemniscus 2.15–3.47 mm long by 0.21–0.33 mm wide with 6, occasionally 7, giant nuclei. Reproductive system in posterior third of trunk. Testes large, oblong, short distance apart, rarely contiguous or distant (Fig. 1). Anterior testis 0.37–4.10 mm long by 0.30–1.10 mm wide. Posterior testis 0.32–3.15 mm wide. Cement glands 8, clustered, larger anteriorly, each with 1 large central single giant nucleus and with independent cement ducts emptying at posterior end of Saefftigen's pouch, along with prominent common sperm duct (Fig. 6). Male gonopore terminal (Fig. 1).

Females (based on 14 mature specimens) Trunk 4.00–110.00 mm long by 0.60–4.00 mm wide. Anterior proboscis 300–438 long by 330–510 (480) wide. Posterior proboscis 323–365 long by 99–745 wide posteriorly at junction with anterior trunk. Proboscis receptacle 0.64–1.80 mm long by 0.11–0.55 mm wide. Shorter lemniscus 1.60–5.50 mm long by 0.19–0.33 mm wide, with 5 or 6 (usually 6) giant nuclei (Fig. 2). Longer lemniscus 2.12–7.31 mm long by 0.19–0.62 mm wide with 6 giant nuclei. Reproductive system rather short, with marked curvature of uterus into posterior loop. Junction between uterus and uterine bell connected to ventral body wall with filaments. Uterine bell with many large nucleated cells; its dorsal and ventral anterior ends connected to body wall with filaments (Fig. 8). Posterior end of trunk broad, flat, with large postero-dorsal knob (Figs. 27-30). Gonopore sub-ventral occasionally covered by looping posterior trunk expansion. Eggs ovoid (Figs. 7, 31) 65–87 long by 39–52 wide.



Figs. 21–26 SEM of an adult mature paratype female of *Mediorhynchus africanus* n. sp. *21* Micropores in the posterior third of trunk. *22* Segments in anterior trunk. *23* Segments in middle trunk. *24* Higher magnification of segments in middle trunk. *25* Segments in posterior trunk. Note the curling of the dorso-posterior trunk expansion over the sub-ventral gonopore. *26* Higher magnification of trunk expansion in Fig. 25 showing the presence of sensory pores

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Taxonomic summary

Type host Helmeted Guinea fowl, *Numida meliagris* Linn. 1758 (Galliformes: Numididae) (South Africa, Burkina Faso).

Other hosts Yellow-necked spurfowl, *P. leucoscepus* (Galliformes: Phasianidae) (Kenya); crested-billed Guinea hen, *N. ptilorhyncha* Licht. (Galliformes: Numididae) (Transvaal, Nigeria, Nyasaland).

Other hosts presumably infected with the same species Domestic chicken, *Gallus gallus domesticus* Linn., 1758 (Galliformes: Phasianidae) and vulturine Guinea fowl, *Acryllium vulturinum* Hardwicke, 1834 (Galliformes: Numididae) (Somalia).

Type locality Kruger National Park, Limpopo and Mpumalanga provinces (24° 50′S, 31° 35′E), South Africa (Junker and Boomker 2006).

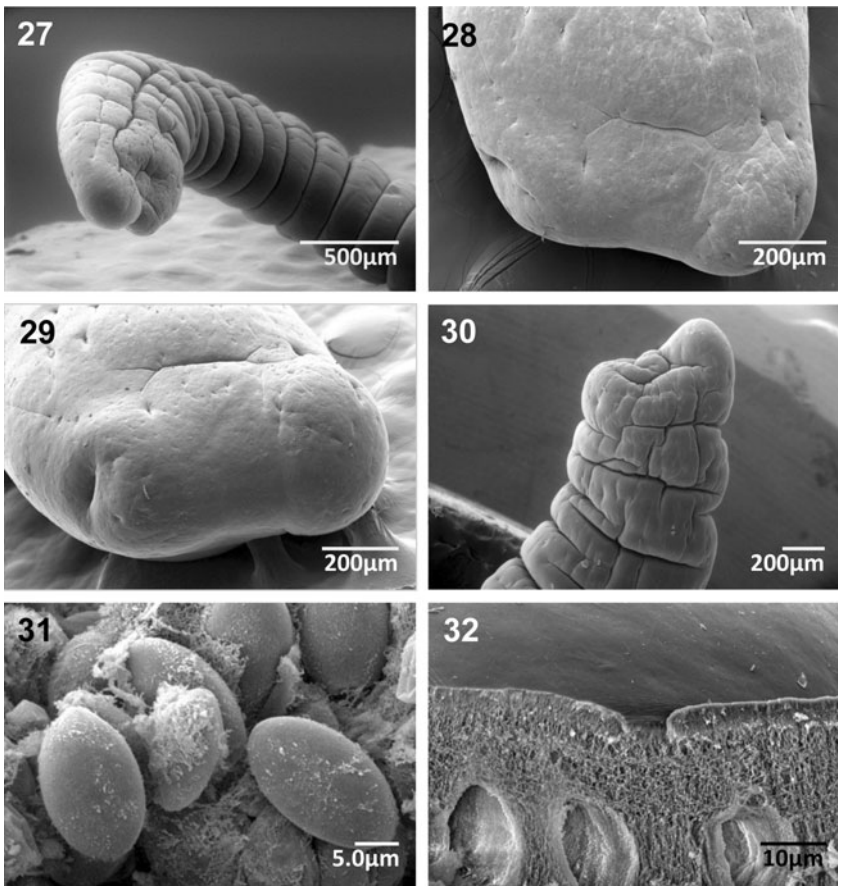
Other localities Kenya (Harris 1973), Burkina Faso (Vercruysse et al. 1985), Transvaal and Nyasaland (Southwell and MacFie 1925), Nigeria (Fabiyi 1972), Somalia (Cancrini et al. 1988, and Terregino et al. 1999).

Type specimens HWML collection no. 49748 (holotype male), no. 49749 (allotype female and paratypes). Many voucher specimens are in the collection of Dr. K. Junker, University of Pretoria at Onderstepoort, South Africa.

Etymology The new species is named for its distribution in subSaharan Africa.

Comparisons

Specimens of *M. africanus* are commonly found in galliform birds, especially Guinea fowls that have a restricted distribution in subSaharan Africa, except for a small population of helmeted Guinea fowls in Morocco (Del Hoyo et al. 1994). For ecological studies and host parasite relationships of the South African material, see Junker and Boomker (2007) and Junker et al. (2008). Specimens of *M. gallinarum* are mostly found in chickens from various Asian locations from India to Indonesia, the Philippines, Borneo, and Palawan (Amin et al. 2013). Morphologically, the two species have similar proboscis, proboscis armature and receptacle, lemnisci, and reproductive structures, but can be distinguished as follows. In *M. gallinarum*, the trunk is cylindrical, non-segmented, and with very few sensory pits, the proboscis has no visible apical pores,



Figs. 27–32 SEM of young paratype female of *Mediorhynchus africanus* n. sp. *27* The posterior part of a specimen showing the unremarkable segmentation in younger specimens and the relatively laterally flattened trunk. *28* Lateral view of the posterior end of another specimen showing the prominent dorso-posterior expansion of the trunk (*lower right*). *29* Terminal view of another young female showing the subterminal position of the gonopore (*left*). Note sensory pores. *30* Lateral view of the same specimen in Fig. 27 showing the dorso-posterior trunk expansion. *31* Eggs broken out of a gravid female. *32* Cross-section of the body wall from the anterior third of a trunk. Note the opening of a micropore (*top center*) and the tegument, muscle layers and the lacunar channels

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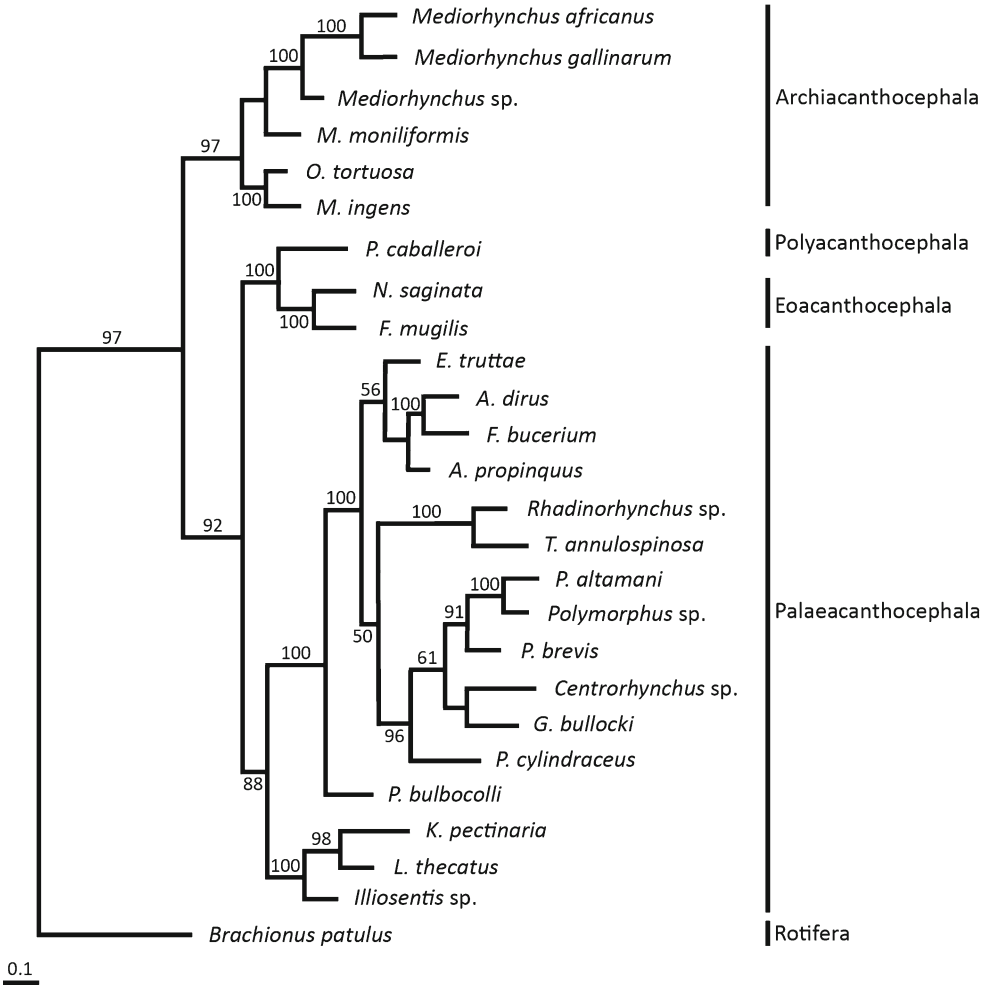

Fig. 33 Maximum likelihood tree inferred from the concatenated (COI+18S) dataset. *Numbers* near internal nodes show ML bootstrap clade frequencies. Branch lengths are scaled to the inferred amount of substitutions per site

the posterior end of the female is pointed with a terminal gonopore. The eggs measure 47–57×24–32 (Amin et al. 2013). It has a restricted distribution is Asia, usually in chickens. In *M. africanus*, the trunk is pseudo-segmented and flattened laterally and has many sensory pits, two prominent apical pores on the proboscis, the posterior end of the female is broad with a sub-ventral gonopore and a dome-shaped expansion of the postero-dorsal end. The eggs are larger, 65–86×39–52. The natural distribution of *M. africanus* is restricted to subSaharan Africa usually in Guinea fowls.

Phylogenetic analysis

The phylogenetic status of *M. africanus and M. gallinarum* was inferred from one mitochondrial (COI) and one nuclear gene sequence (18S) totaling 2429 nucleotides. Separate analyses of the two genes yielded congruent phylogenies. The protein coding COI gene sequence differed by 9.7 % between *M. africanus* and *M. gallinarum* and the transition/transversion ratio was 1.4. The results of the phylogenetic analyses were consistent between the different methods applied, maximum parsimony (MP) and maximum likelihood (ML). Identical nodal structure was observed with MP and ML analysis and the phylogeny inferred by ML is shown in Fig. 33. Absolute support for the separation of *M. africanus* and *M. gallinarum* as sister species was obtained with both MP and ML analysis.

The concatenated (COI+18S) dataset included 26 taxa with 1598 characters (Table 2) of which 784 were parsimony informative. Both MP (not shown) and ML (Fig. 1) analysis yielded the same topologies. The ML analysis yielded a single best tree with a likelihood score of 18,456.8680, a consistency

Table 2 Tree statistics for concatenated (COI+18S) data set

Total characters	Uninformative characters	Constant characters	Informative characters	CI[a]	Tree length[a]	−ln likelihood[a]
1598	161	1253	784	0.42	2,799	18456.8680

[a] Consistency Index (CI) and Tree Length from parsimony (MP) inference. −ln likelihood from maximum likelihood (ML) inference

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index (CI) of 0.42 and a length of 2,799 steps (Table 2). The −ln likelihood score for the first alternative topology was 18,388.4554. Separate analyses of the two genes yielded congruent phylogenies. The protein coding COI gene sequence differed by 9.7 % between *M. africanus* and *M. gallinarum* and the transition/transversion ratio was 1.4.

The MP and ML analyses of the combined mitochondrial and nuclear (COI+18S) dataset showed that *Mediorhynchus* is a monophyletic assemblage with absolute support for the separation of *M. africanus* and *M. gallinarum* as separate species. The intent of these analyses was to demonstrate the phylogenetic separation of *M. africanus* and *M. gallinarum*. Although samples were limited in this study, we note additional molecular support for the reciprocal monophyly of the Arciacanthocephala and Paleacanthocephala major groups of Acanthocephala as has been previously noted (Near et al. 1998; Van Cleave 1952). Analysis of additional samples including both molecular and morphological characters will provide a robust dataset to distinguish between the biogeographic hypotheses of Gondwanian, Tertiary, or Pleistocene allopatric origin of these and other species pairs in the acanthocephalans.

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