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Source: Comparative Parasitology, 80(1):39-46. 2013.

Published By: The Helminthological Society of Washington

DOI: <http://dx.doi.org/10.1654/4562.1>

URL: <http://www.bioone.org/doi/full/10.1654/4562.1>

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The Finding of *Mediorhynchus gallinarum* (Acanthocephala: Gigantorhynchidae) in Chickens from Indonesia, with Expanded Description Using SEM

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ABSTRACT: The original description of *Mediorhynchus gallinarum* (Bhalerao, 1937) and subsequent descriptions by other observers were riddled with errors and misinterpretations. The present collection of many specimens of *M. gallinarum* from chickens, *Gallus gallus* L., in Indonesia provided the opportunity to describe the Indonesian population, report the full range of variation in morphometric characteristics, especially proboscis armature, correct a few misconceptions, and obtain scanning electron microscopy documentation of previously unreported structures including features of the proboscis and hooks, the epidermis, sensory pores, bursa, and egg topography. Additionally, Indonesia is a new locality record for *M. gallinarum*.

KEY WORDS: *Mediorhynchus gallinarum*, Acanthocephala, chickens, Indonesia, description, Asian-African populations, SEM.

The Asian and African distribution of *Mediorhynchus gallinarum* (Bhalerao, 1937) is well documented. The Asian material included the original description from a single female as *Leiperacanthus gallinarum* by Bhalerao (1937) from India. That description was marred by serious errors reviewed, in part, by Van Cleave (1947). Bhalerao (1937) assigned the genus to Palaeacanthocephala instead of Archiacanthocephala, regarded the longitudinal canals of the lacunar system as lateral instead of dorsal and ventral, interpreted proboscis hooks as in “eight horizontal rows ... each row containing 10 hooks,” thought that the proboscis receptacle was inserted at the base and not at the middle of the proboscis, misconstrued his “para-proboscideal sacs” as unique structures of taxonomic importance that prompted him to place his *Leiperacanthus* in a new family, Leiperacanthidae, and interpreted the proboscis receptacle as double-walled anteriorly. The “outer wall” of the anterior “double-walled” proboscis receptacle is actually a separate envelop of fibers distinct from the single-walled receptacle but adjacent to it, for the retraction of the proboscis. It was properly interpreted by Lundström (1942) as an “outer cylinder” of longitudinal fibers. Tubangui and Masilungan (1946) described *M. gallinarum* from

Manila also as *L. gallinarum* with “spines in the anterior region of the body,” presumably referring to the posterior proboscis, which he interpreted as “circular depression forming a sort of (spiny) collar... separating anterior region from rest of body,” referred to “four submedian proboscideal sacs,” and mistook the anterior part of the proboscis receptacle as double-walled. Petrochenko (1958) placed *M. gallinarum* in *Empodius* Travassos, 1916, also mistook the anterior part of the proboscis receptacle as double-walled, and based his description on the account of Tubangui and Masilungan (1946). Yamaguti (1954) described his specimens from Celebes (now Sulawesi, an Indonesian province) as *Empodius* sp., also mistook the anterior proboscis receptacle as double-walled, and further interpreted the posterior proboscis spines as emerging from the neck. Nath and Pande (1963) described their specimens from India and, like Bhalerao (1937), also referred to “four para-proboscideal sacs,” and erroneously showed the posterior proboscis with 20 rows of spines on one side each with 9 spines per row. Talbot (1971) did not describe his specimens of *M. gallinarum* (except for figure 1 of a male) from Papua and New Guinea, indicated that the “structure of *M. gallinarum* has (already) been adequately described from Indian specimens (Nath and Pande, 1963)” (implication of similarity), and predicted its presence in Indonesia on the basis of its high

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prevalence in villages on the West Irian border and on “the considerable interchange of people and livestock which occurs between these border areas.” Schmidt and Kuntz (1977) reported, but did not describe, *M. gallinarum* from Terabanon Concepción and Palawan Island, revised the genus *Mediorhynchus* Van Cleave, 1916, provided a key to the 29 species known then, and noted 17 other species “of uncertain or no validity.” Humphrey (1979) reported, but did not describe, *M. gallinarum* from Papua New Guinea and showed higher prevalence of worms from chickens raised in “extensive” terrain and low lands with greater distribution of intermediate hosts.

The African reports included the only 2 descriptive accounts of Harris (1973) and Junker and Boomker (2006) from the coastal states of Kenya and South Africa, respectively. Harris (1973) described *Mediorhynchus selengensis* as a new species from a galliform bird in Kenya that proved to be a junior synonym of *M. gallinarum* (see Schmidt and Kuntz, 1977). In his description, Harris (1973) confused the “outer cylinder” of longitudinal fibers adjacent to the proboscis receptacle as “a thick outer wall of circular muscles.” Junker and Boomker (2006) provided a detailed description of specimens from guinea fowl in Kruger National Park, South Africa that, however, included some inaccuracies such as the measurements of hook length that included “their roots.” 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; Davies et al., 2008; Junker et al., 2008) and from the coastal state of Somalia (Cancrini et al., 1988; Terregino et al., 1999) as well as from the Central African state of Burkina Faso (formerly Upper Volta) (Verduysee et al., 1985). Fabiyi (1972) reported “*Empodius segmentatus* Marvel, 1902” from Guinea fowl in Nigeria. This acanthocephalan is of questionable identity and may be *Empodisma segmentatus* Southwell and Macfie, 1925, which is probably a *Mediorhynchus* different from *Echinorhynchus segmentatus* de Marvel, 1902, which may be *M. gallinarum*. Other African poultry examined from Kenyan villages (Irungu et al., 2004) and from West Africa in Nigeria (Fatihu et al., 1991) were negative for *M. gallinarum* infections.

Although morphometric measurements and correctly interpreted morphological features in above descriptive reports fell within the range of our

observations, some reports showed discrepancies that will be noted in the following sections.

MATERIALS AND METHODS

Forty-six Isa Brown laying hens ages 50–52 wk (Fig. 1) were examined for acanthocephalans from 2 different sources in Sleman district in Daerah Istimewa Yogyakarta, Indonesia from October 2010 to February 2011. The “Special Region” (Province) of Daerah Istimewa Yogyakarta comprises 4 districts and 1 city: Kulon progo District, Gunung kidul District, Bantul District, Sleman District, and Yogyakarta City (Fig. 2). Twenty-six chickens were examined from a local wet market and 20 other chickens were examined from a poultry farm in Kaliurang Sleman (107°15'03" and 107°29'30"E; 7°34'51" and 7°47'30"S). The poultry farm was situated in an open rural environment (Fig. 3) and the chickens were maintained in spacious well-managed settings (Fig. 4), unlike crowded native chickens in unstructured settings (Fig. 5) that were not used in this study.

Collected specimens were refrigerated in water for 2 d until the proboscis was evaginated. 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 hr each), and cleared in graduated concentrations of terpineol in 100% ethanol to 100% terpineol, then 50% terpineol in 50% Canada balsam (24 hr each). Whole worms were then mounted in Canada balsam. Measurements are presented in micrometers, unless otherwise stated as range values followed by the mean in parentheses. Width measurements represent maximum width. Trunk length does not include proboscis, neck, or bursa. Voucher specimens were deposited in the University of Nebraska's State Museum's Harold W. Manter Laboratory (HWML) collection no. HWML-49729 in Lincoln, Nebraska, USA.

For scanning electron microscopy (SEM) studies, 12 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 ESEM-FEG; FEI, Hillsboro, Oregon). Digital images of the structures were obtained using digital imaging software attached to a computer.

Type or voucher cylindrical nonpseudosegmented Asian specimens of *M. gallinarum* and 14 other species of *Mediorhynchus* from the HWML at Lincoln and the U.S. National Parasite Collection (USNPC) at Beltsville, Maryland were examined for verification of the identity of our specimens and for comparative purposes. These specimens included *Mediorhynchus conirostris* Ward, 1966 (HWML 34878); *Mediorhynchus corcoracis* Johnston and Edmonds, 1950 (HWML 34649); *Mediorhynchus edmondsi* Schmidt and Kuntz, 1977 (USNPC 74356, 74358); *Mediorhynchus emberizae* (Rudolphi, 1819) (HWML 34507, 34508); *M. gallinarum* (USNPC 74360, HWML 34913, 34924, 34925); *Mediorhynchus grandis* Van Cleave, 1916 (HWML 30671, 30676, 30695–30697); *Mediorhynchus kuntzi* Ward, 1960 (HWML 34879); *Mediorhynchus leptis* Ward, 1966 (HWML 34521); *Mediorhynchus muritensis* Lündstrom, 1942 (HWML 34500); *Mediorhynchus orientalis* Belopolskaya, 1953 (USNPC 74366, 74368, 74369, HWML 34748, 34906); *Mediorhynchus papillosus* Van Cleave, 1916 (USNPC 74359,



Figures 1–6. Collecting *Mediorhynchus gallinarum* from chickens in Indonesia. **1.** Isa Brown laying chicken, the primary host of these worms. **2.** Collecting localities in the Yogyakarta region. **3.** Poultry farm in an open rural environment where collections were made. **4.** Interior of a laying house; chickens were maintained in spacious well-managed settings. **5.** Crowded native chickens in unstructured setting. **6.** Freshly collected worms, which are olive-green in color.

HWML 34477, 34915, 34916, 34920); *Mediorhynchus robustus* Van Cleave, 1916 (HWML 20798, 20799); *Mediorhynchus taeniatus* (Linstow, 1901) (HWML 34877); *Mediorhynchus tenuis* Meyer, 1931 (HWML 34649); *Mediorhynchus turnixena* (Tubangui, 1931) (USNPC 74361).

Additionally, 11 pseudosegmented specimens of *M. gallinarum* collected from the helmeted Guinea fowl, *Numida meleagris* Linn., in South Africa were identified by and provided courtesy of Dr. K. Junker, University of Pretoria at Onderstepoort, South Africa. Eight specimens

were collected in Kruger National Park in 1989 (Junker and Boomker, 2006) and 3 specimens 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.

RESULTS AND DISCUSSION

Forty-six Isa Brown laying hens were examined for acanthocephalans from 2 different sources in

Yogyakarta, Indonesia from October 2010 to February 2011. Twenty-six chickens were examined from a local wet market of which 3 chickens (11%) were infected with 40, 15, and 65 worms (total 120 worms; mean of 4.6). Of the 20 other chickens examined from a poultry farm (Figs. 3, 4), 2 chickens (10%) were infected with 25 and 200 worms (total 225 worms; mean of 11.2), respectively. These prevalence figures of 10–11% would have probably been greater if native local chickens raised under crowded unsanitary conditions (Fig. 5) were examined. Similarly, Terregino et al. (1999) noted that 79% of rural free-ranging chickens and 40% of chickens from a modern indoor intensive-rearing farm were infected with *M. gallinarum* and other parasites. The prevalence noted in other reports varied between 2.2 and 42.2% depending on habitat (Humphrey, 1979) and 24% in 7 localities (Talbot, 1971) in Papua New Guinea. Upon dissection of the intestinal tract, some worms were still attached to intestinal lining and caused bleeding (petechie). The infected laying hens presented with clinical symptoms including lack of appetite, loss of weight, diarrhea, and inability to walk. The possibility that other, unaccounted for, factors may have been involved in this clinical picture is not discounted.

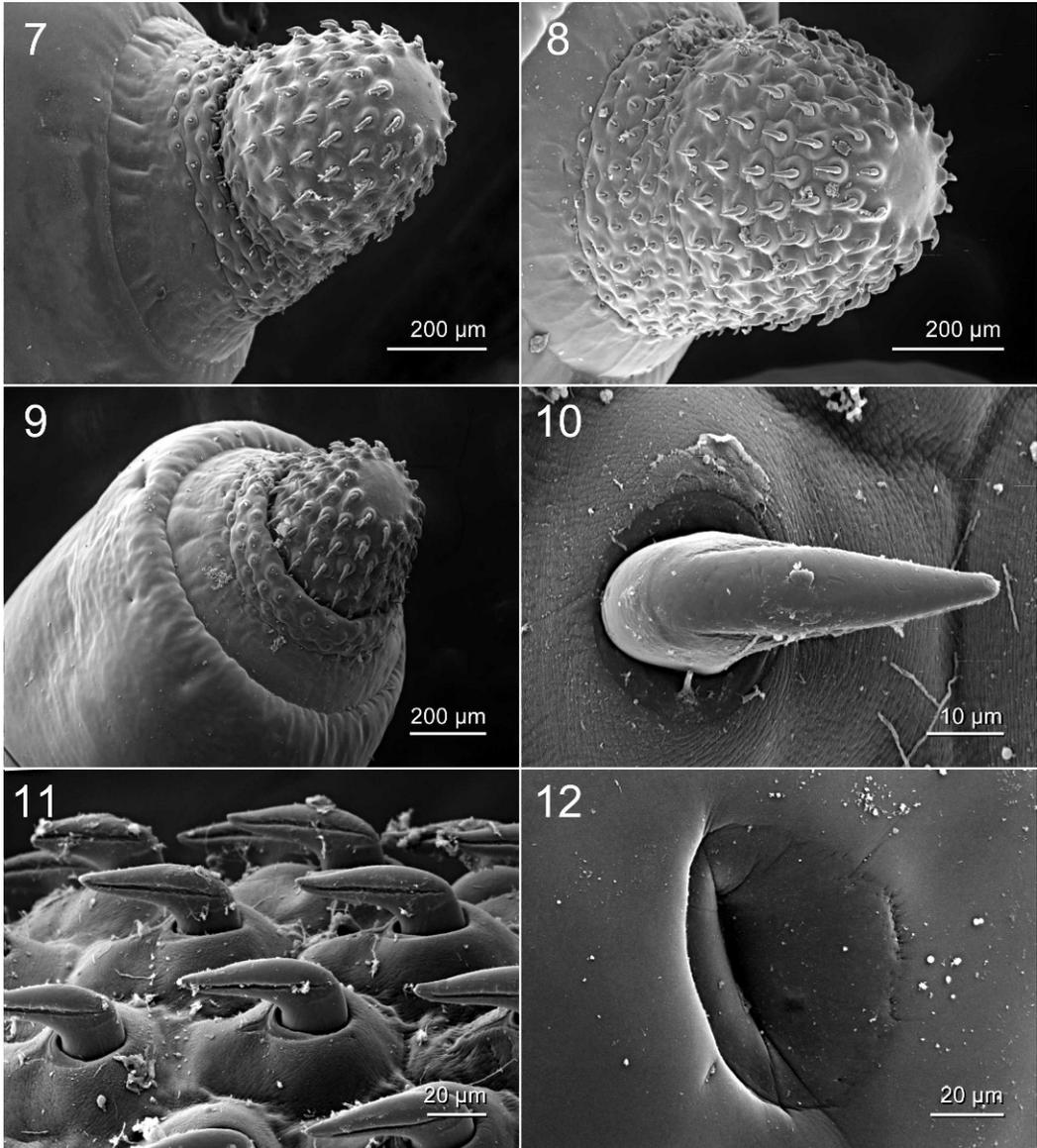
Description of the Indonesian population of *Mediorhynchus gallinarum*

General: With characters of the genus. Robust, olive-green worms with no pseudosegmentation (Fig. 6); creamy white upon recovery. Shared structures larger in females than in males. Trunk long, uniformly cylindrical, tapering at both ends. Body wall aspinose, with many fragmented nuclei and electron-dense micropores at epidermal surface. Proboscis in 2 parts (Fig. 7) occasionally manifesting all armature variations in single proboscides (Fig. 8). Spines on posterior proboscis often mistaken for trunk spines in partially retracted proboscides (Fig. 9). Anterior proboscis pear-shaped or apple-shaped with truncated bare apical end without pores and with 18–22 longitudinal rows of 5–6 hooks each. Hooks set in elevated hexagonal grids, longest at middle (Fig. 10), invariably with lateral slits (Fig. 11). Hook roots somewhat longer than blades, simple, directed posteriorly. Posterior end of roots markedly rounded, each with 1 pair of prominent accessory lateral ribbed wings. Posterior proboscis conically shaped, broader posteriorly at junction with anterior trunk, with 30–34 longitudinal rows of 2–6 spines each anteriorly; posterior zone devoid of spines. Spines very thin, curved posteriorly or

undulating, with small knob-shaped roots. Both hooks and spines partially embedded in raised dome-shaped cuticular swelling (Fig. 10, in part). No neck. Sensory pits at least at anterior trunk (Figs. 9, 12) and posterior trunk in females (Figs. 13, 14) and males (Fig. 18). Proboscis receptacle about twice as long as both proboscides, single-walled with anterior portion encased in jacket of adjacent retractor fibers. Cephalic ganglion near middle of receptacle. Lemnisci long, digitiform, unequal, unattached, usually with 6 giant nuclei each. Occasionally shorter lemniscus with 5 nuclei and longer lemniscus with 7. Reproductive opening terminal in both sexes.

Male (on the basis of 17 adults specimens with sperm): Trunk 8.87–46.25 (22.10) mm long by 0.87–1.80 (1.36) mm wide. Anterior proboscis 354–439 (412) long by 385–478 (419) wide with 18–20 (18.8) longitudinal rows of 5 hooks each. Hooks smallest anteriorly and longest at middle, 32–42 (39), 37–50 (46), 42–50 (46), 40–47 (46), 37–42 (41) long from anterior. Posterior proboscis 208–364 (271) long by 468–562 (502) wide posteriorly at junction with anterior trunk with 30–32 (31.5) longitudinal rows of 3–6 (4.6) spines each. Length of spines 25–35 (29), 25–32 (30), 25–32 (30), 30–32 (31), 27–30 (28) from anterior. Proboscis receptacle 1.04–1.54 (1.22) mm long by 0.32–0.46 (0.38) mm wide. Shorter lemniscus 2.12–5.12 (3.87) mm long by 0.21–0.33 (0.28) mm wide, with 5 or 6 giant nuclei. Longer lemniscus 3.30–5.87 (4.79) mm long by 0.21–0.32 (0.28) 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. Anterior testis 0.56–2.50 (1.51) mm long by 0.17–0.60 (0.42) mm wide. Posterior testis 0.60–2.56 (1.52) mm long by 0.20–0.62 (0.44) mm wide. Cement glands 8, clustered, larger anteriorly, 0.19–1.05 (0.48) mm long by 0.12–0.55 (0.34) mm wide, each with 1 large central single giant nucleus and with independent cement ducts emptying at posterior end of Saeffigen's pouch, 0.67–1.62 (1.17) mm long by 0.20–0.55 (0.36) mm wide, along with prominent common sperm duct. Bursa tilted ventrad (Fig. 17) bland without sensory or accessory structures (Fig. 18) but with elevated genital orifice (Fig. 18), 900–950 (925) long by 750–875 (812) wide.

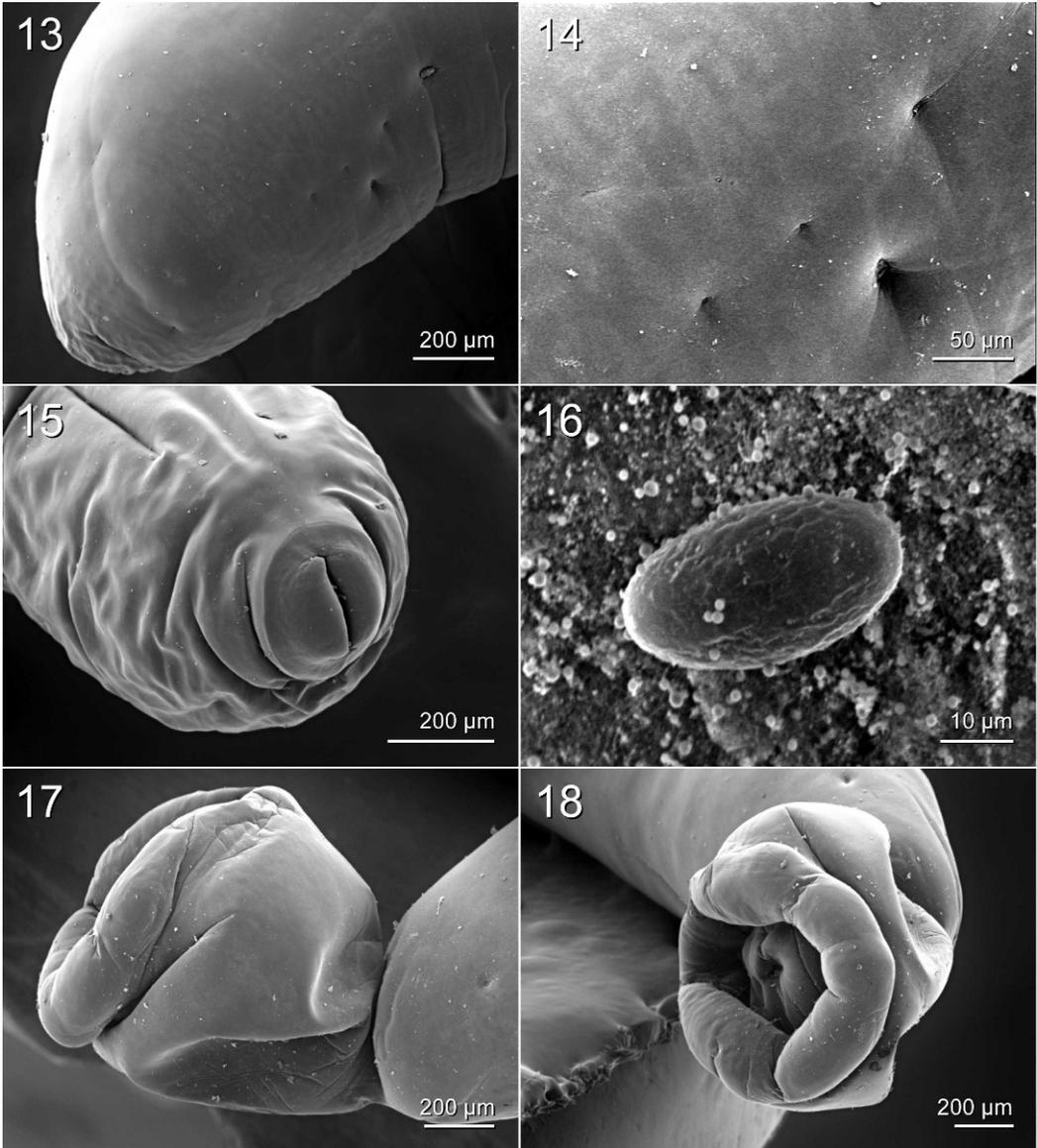
Female (on the basis of 25 mostly gravid specimens): Trunk 8.00–91.25 (30.05) mm long by 0.95–2.95 (1.57) mm wide. Anterior proboscis 385–478 (445) long by 426–520 (480) wide with 18–22 (20.7) longitudinal rows of 4–6 hooks each. Hooks



Figures 7–12. Proboscis, hooks, and sensory pits of *Mediorhynchus gallinarum*. **7.** The proboscis of a female worm showing its division into anterior region and conically shaped posterior region; only the anterior part of the posterior proboscis is armed with spines; the posterior unspiny part merges with the anterior trunk and is often confused with it. **8.** The proboscis of female worm showing the occasional presence of the full range of variation in the number of proboscis hooks of 4–6 per row and of spines of 2–6 per row in the anterior and posterior proboscis, respectively, in individual worms. **9.** The partial retraction of the proboscis in such worms led to the misinterpretation of spines of the posterior proboscis as trunk spines in some of the early descriptions. Note the sensory pit at the anterior trunk (upper left). **10.** A middle hook set in a raised hexagonal division of the proboscis. **11.** A number of proboscis hooks showing the lateral grooves characteristic of that species. **12.** Enlargement of the sensory pit shown in the anterior trunk of the specimen in Fig. 9.

smallest anteriorly and longest at middle, 32–50 (42), 45–52 (50), 42–50 (49), 42–50 (47), 25–47 (39) long from anterior. Posterior proboscis 177–385 (276) long by 499–645 (579) wide posteriorly at junction

with anterior trunk with 30–34 (32) longitudinal rows of 4–6 (4.8) spines each. Length of spines 27–35 (31), 27–37 (32), 22–42 (33), 25–37 (28), 22–32 (27) from anterior. Proboscis receptacle 1.00–1.72



Figures 13–18. Male and female structures. **13.** The posterior end of a female specimen showing 3 sensory pits. **14.** Enlargement of the sensory pit area shown in Figure 13. **15.** En face view of the terminal gonopore of a female showing its slit opening. **16.** An egg. **17.** Lateral view of a bursa showing its angle of articulation against posterior trunk. **18.** A near-face view of a bursa showing its plain structure, muscular rim, and elevated genital orifice. Note sensory pore on top.

(1.28) mm long by 0.34–0.54 mm wide. Shorter lemniscus 2.60–6.50 (4.29) mm long by 0.19–0.29 (0.24) mm wide, with 5 or 6 (usually 6) giant nuclei. Longer lemniscus 3.12–6.75 (4.76) mm long by 0.19–0.33 (0.25) mm wide with 6 giant nuclei. Reproductive system short, in posterior 5% of trunk, with prominent curvature of uterus and termina 1

slit-shaped gonopore (Fig. 15). Eggs ovoid (Fig. 16), 47–57 (54) long by 24–32 (29) wide.

Morphological comparisons

Complete morphometric comparisons were not possible because most reports, except for Junker and Boomker (2006), lack a complete set of measurements.

Only Bhalerao (1937) described his specimen's color as olive-green like ours but were creamy white upon recovery like Talbot's (1971) specimens. The size and morphology of the trunk, anterior and posterior proboscis, when properly interpreted, the proboscis receptacle, lemnisci, uterus, and testes was comparable in all collections, including ours, but markedly smaller in the apparently younger specimens (males 8–11 mm long, females 16–40 mm long) reported by Nath and Pande (1963) from India. Nath and Pande (1963) surprisingly reported and illustrated (Fig. 2) an elaborate posterior proboscis with 20 rows of spines on one side each with 9 spines and with no spineless posterior zone, a gross exaggeration of the total of 30–34 rows of 3–6 spines each in our Indonesian specimens that exhibited the widest range of variation in 1 locality.

Important characters of taxonomic significance include the proboscis armature and egg size and morphology. Our Indonesian specimens exhibited the widest range of variation from 1 locality in proboscis armature of 18–22 longitudinal rows of 5–6 hooks each on the anterior proboscis and 30–34 longitudinal rows of 2–6 spines each on the posterior proboscis. The usual armature reported was 20 rows of 5 hooks each and 30 rows of 5–6 spines each but varied between 18–22 rows of 4–5 hooks each and 25–32 rows of 2–6 spines each from different geographical locations.

Our specimens from Indonesia, in addition, are distinguished from others in all other locations by having the smallest hooks (25–52 long) and egg size ($47\text{--}57 \times 24\text{--}32$), only comparable with the specimens of Schmidt and Kuntz (1977) from Terabanon Concepción and Palawan Island that were not described but examined and measured by us (USNPC 74360, HWML 34913, 34924, 34925) (hooks longest at middle: 40–50 long, eggs $50\text{--}62 \times 25\text{--}30$). Hook length reached 70 (Harris, 1973) and 76 (Junker and Boomker, 2006) in African specimens, 66 (Bhalerao, 1931) in an Indian female specimen, 68 (Tubangui and Masilungan, 1946) in specimens from the Philippines, and 70 (Yamaguti, 1954) in specimens from Celebes. Comparative measurements of the markedly larger eggs were available for specimens from Africa: $65\text{--}75 \times 38\text{--}48$ (Harris, 1973) and $70\text{--}86 \times 43\text{--}52$ (Junker and Boomker, 2006) and from the Philippines: $64\text{--}68 \times 40\text{--}43$ (Tubangui and Masilungan, 1946 and Yamaguti, 1963, respectively) and Japan. On the basis of the taxonomically important characters of hook and egg size alone, our specimens and those of Schmidt

and Kuntz (1977) appear to be more similar to one another than either one to other specimens from other Asian and African locations.

Distribution

The largest assemblage of Asian *M. gallinarum* populations was reported from a group of islands between the Indian and the Pacific oceans in the Philippines (Tubangui and Masilungan, 1946), Terabanon Concepción and Palawan Island (Schmidt and Kuntz, 1977), Papua New Guinea (Talbot, 1971; Humphrey, 1997), Celebes (Yamaguti, 1954), and Indonesia (this paper) where the parasite appears to be endemic in the domestic chicken and related birds. The Indian collections from Muktesar and Mathura by Bhalerao (1937) and Nath and Pande (1963), respectively, in the landlocked northeastern state of Uttar Pradesh suggest dispersal from Southeast Asia through the Indo-Gangetic plain that spans most of the state with movement of people and domestic animals as Talbot (1971) proposed for the dispersal of *M. gallinarum* from Papua and New Guinea to Indonesia.

In Africa, *M. gallinarum* was also reported and described from coastal Kenya in East Africa (Harris, 1973) and in South Africa (Junker and Boomker, 2006), which have no direct human–animal traffic with the Asian-Pacific oceans' islands. Present or past routes of dispersal in this case are not known. The ecology and host–parasite relationships of *M. gallinarum* were also reported from elsewhere in Africa (Vercruyse et al., 1985; Cancrini et al., 1988, Terregino et al., 1999; Junker and Boomker, 2007; Davies, 2008; Junker et al., 2008) but the parasite was absent in poultry examined in other Kenya locations (Irungu et al., 2004) and in Nigeria, West Africa (Fatihu et al., 1991).

Population differences

The possibility that the Asian and the African populations of *M. gallinarum* represent 2 endemic centers that may have evolved independently from some hypothetical common ancestor may be supported on the basis of available morphological evidence including the pseudosegmentation and the presence of apical pores on the proboscis of the African specimens. This is being explored in a project using comparative gene sequence studies. The only descriptive accounts from Africa are those of Harris (1973) in Kenya and Junker and Boomker (2006) in South Africa, who reported pseudosegmented

specimens. The African specimens from Burkina Faso reported by Vercruyse et al. (1985) and currently deposited at the British Museum of Natural History were recently examined and reported to be pseudosegmented (David Gibson, personal communication). The Asian specimens, like ours from Indonesia, were not pseudosegmented. We examined specimens from the Junker and Boomker (2006) material for verification and future studies. Yamaguti's (1954) specimens from Celebes were reported to be "corrugated transversely" but his figures 6 and 7 of the anterior and posterior portions of worms show no segmentation. The "corrugated" state may have been a state of contraction of the middle portion of the trunk.

ACKNOWLEDGMENTS

We are grateful to Dr. Atif Naggar of Ain Shams University, Cairo, Egypt, currently at Brigham Young University, Provo, Utah, for his artful preparation of the plates (Figs. 1–12). This project was supported by an institutional grant from the Institute of Parasitic Diseases to O.M.A.

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