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ORIGINAL

Morphology and molecular taxonomy of the tongue worm, genus *Raillietiella* (Pentastomida) from the lungs of berber skinks *Eumeces schneideri* (Scincidae): First report

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Light and scanning electron microscopy;
Molecular study

Abstract In the present study, pentastomids belonging to the order *Cephalobaenida* were isolated from the lungs of Berber skinks *Eumeces schneideri* (Famiy: Scincidae), which were morphologically described by light and scanning electron microscopy and taxonomically justified by 18s rDNA molecular analyses of the parasites. Seventeen host specimens were collected from well–vegetated wadis at high altitudes, Jizan, Saudi Arabia as new type locality; twelve specimens (70.59%) were infected. All of the recovered parasites were adults, possessed small broadly triangular cephalothorax flattened on the ventral surface and merged smoothly with a uniformly thick and squat abdomen and terminated in a pair of divergent lobes. The results obtained indicated that the parasites belong to the sharp–tipped posterior–hook *Raillietiella* spp. distinguished from other raillietiedids of the same group some important characteristic features including annulus number, shape and dimensions of the buccal cadre, copulatory spicules, and anterior and posterior hooks. The anterior hook of the female specimens (n = 5) had a blade length (AB) of 135 ± 5 (110–146) μm and shank length (BC) 158 ± 5 (150–169) μm while the posterior hook was much larger with AB measuring 221 ± 5 (200–236) μm and BC 286 ± 6 (280–289) μm. For the male specimens (n = 5), the anterior hook had an AB of 73 ± 3 (72–75) μm and a BC 102 ± 5 (100–103) μm. The posterior hook was much larger with AB 190.6 ± 5 (190–191) μm and BC 221 ± 5 (280–289) μm. The morphological characterization of the recovered parasites was closely similar to *R. aegypti* previously isolated from the same host. Sequence

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alignment by the maximum likelihood analysis for the data obtained from the 18S rDNA analysis of the parasites exhibits identities ranging between 88–95% with pentastomid genera recovered from the GenBank. The phylogenetic tree supported the inclusion of the parasites within the monophyletic Pentastomida clade with maximum identity to the raillietiellid species. The recovered sequences from the present study were deposited in GenBank under Accession number MK970649.1. The present molecular analysis was the first to confirm the taxonomic position of *R. aegypti* isolated from the host examined.

PALABRAS CLAVE
Pentastómido;
Raillietiella aegypti;
Cephalobaenida;
Eumeces schneideri;
Microscopía óptica y microscopía electrónica de barrido;
Estudio molecular

Morfología y taxonomía molecular de un pentastómido del género *Raillietiella* obtenido del pulmón del eslizón bereber *Eumeces schneideri*: Primer informe

Resumen En el presente estudio se aisló un pentastómido perteneciente al orden *Cephalobaenida* del pulmón de un eslizón bereber (*Eumeces schneideri*, Familia: Scincidae). Se efectuó su descripción morfológica basada en observación por microscopía óptica y de barrido y se justificó su ubicación taxonómica mediante análisis molecular del gen 18S del ADNr. Se recolectaron 17 especímenes del citado huésped en valles ubicados a elevadas altitudes, en la región de Jizan (Arabia Saudí); 12 de ellos (70,59%) estaban infectados. Todos los parásitos recuperados eran adultos y poseían un pequeño cefalotórax triangular, aplanado en la superficie ventral, que se fusionaba con un abdomen abultado y terminado en un par de lóbulos divergentes. Los resultados indicaron que este parásito pertenece a *Raillietiella* spp., que agrupa especies con gancho posterior puntiagudo; estas se distinguen de otros miembros de la Familia *Raillietiella* por algunos rasgos característicos, como el número de anillos y la forma y dimensiones del cuadro bucal, las espículas copulatorias y los ganchos anterior y posterior. La caracterización morfológica demostró que el parásito recuperado era muy similar a *R. aegypti*, previamente aislada del mismo huésped. El alineamiento de secuencias mediante el método de probabilidad máxima basado en el análisis del gen 18s del ADNr detectó identidades del 88–95% con los géneros de pentastómidos disponibles en GenBank. Dentro del árbol filogenético se pudo incluir este parásito dentro del clado monofilético pentastómido con máxima identidad con las especies de *Raillietiella*. Las secuencias obtenidas fueron depositadas en GenBank, con número de acceso MK970649.1. El presente análisis molecular confirma por primera vez la posición taxonómica de *Raillietiella aegypti*, anteriormente aislado del mismo huésped.

Introduction

Pentastomida[17] is a subclass of wormlike crustacean endoparasites with five anterior appendages one mouth, and two pairs of hooks for their attachment to the host. They have approximately 131 species distributed in two orders; Cephalobaenida and Porocephalida and seven families: Cephalobaenidae, Linguatulidae, Porocephalidae, Ralliietiellidae, Reighardiidae, Sebekidae and Subtriquetridae[2], in addition to three Middle Cambrian fossil groups[9]. The two orders can be distinguished mainly by differences in the disposition of the hooks relative to the mouth and by the form of the reproductive tract[1]. All of the species of these groups attain sexual maturity in the respiratory tract of vertebrates.

Parasites of the order Cephalobaenida are characterized by the presence of a subterminal or ventral mouth situated anterior to the hooks disposed in a trapezium[5,14]. Three genera of the order Cephalobaenida were recorded: (a) *Cephalobaena*[34], infecting the lung of American tree snakes; (b) *Reighardia* Ward, 1899 infecting the lung of marine birds as definitive hosts[12,20]; (c) *Raillietiella*[53], the largest pentastome, worm-like, blood sucking parasites inhabiting the upper respiratory tract of insectivorous small lizards[29,40,46], which have piercing mouthparts surrounded by two pairs of compound hooks that embed into the lung to facilitate feeding on the host blood[36,51].

The life cycle of pentastomids includes two hosts; the eggs are either coughed out by the host or leave the host body through the digestive system. The eggs are then ingested by an intermediate host, which is commonly either a fish or a small herbivorous mammal. The definitive host becomes infected by pentastomids after eating the intermediate host, the parasite crawls within the host body into the respiratory tract from the esophagus[2,37,52]. Humans can serve as an accidental definitive host after ingesting raw or poorly cooked viscera (i.e., liver, lungs, and trachea) of the intermediate hosts[22].

The pathologic effect of pentastomid infection may be very serious and/or lethal, causing lesions in lung tissues and obstruction of the trachea that can result in interstitial subacute pneumonia with congestion[36,38,49]. In Egypt, ralliettiellids were first recorded by Ali et al. [3], who described two pentastomids, *R. affinis*[54] and *R. aegypti* as a new species from Egyptian desert lizards, with much focus on *R. affinis*. Scant information with incomplete morphology and phylogeny is available worldwide for *R. aegypti*. The nuclear18S ribosomal DNA (18S rDNA) has proved to be a potential marker during the characterization of eukaryotes[23,41]. Its highly conserved nature and slow rate of evolutionary mutation make 18S rDNA suitable for interspecies distinction at higher taxonomic levels[57].

In the present study, a morphological description of a pentastomid infecting the lungs of Berber skinks *Eumeces schneideri* (Scincidae) captured in Jizan, Saudi Arabia was carried out by light and scanning electron microscopy. Furthermore, the 18S rDNA molecular analysis of the parasite was conducted to determine the exact phylogenetic position of these parasites.

Materials and methods

Sample collection and parasitological study

Seventeen specimens of the berber skink *Eumeces schneideri* (Reptilia: Scincidae), 10 males and 7 females, were collected by hand or noose from well-vegetated wadis at high altitudes, in Jizan, Saudi Arabia (17.6548° N, 42.8871°E) during the year 2017. Specimens were collected seasonally, during summer (5 males, 4 females) and winter (5 males, 3 females). Animals were kept alive in glass cages with sand and alluvium in the animal room at 25–30 °C and fed insect larvae. Identification of the examined species was carried out according to Arnold[11] and Al-Sadoon[10]. When examined, a cotton ball soaked with isoflurane was placed inside a plastic bag containing the animals and left inside to allow sufficient time for the anaesthetic gas to cause euthanasia. Alternatively, the animals were left in the sealed bag for sufficient time to achieve anaesthesia, at which time they were removed from the bag and injected intraperitoneally with an overdose of sodium pentobarbital. These were in agreement with the regulatory laws regarding experimental ethics of animal use and collecting permits, Institute of Animal Care and Use Committee (protocol number BSU/FS/2015/10).

Following dissection, pentastomids were recovered from lungs using a ZEISS Compact Greenough stereomicroscope (Model Stemi 305), heat fixed in 10% neutral buffered formalin for 15 min and then preserved in 70% ethanol in 5% glycerol solution to avoid sudden drying. Finally, samples were transferred to lactophenol for clearance. The prepared samples were examined using differential interference contrast (DIC) light microscopy with digital image analysis system (analysis auto 5.0) and photographed by an Olympus research photomicroscope supplied with a built-in camera (Model BX53F, Tokyo, Japan). Drawings were made with the aid of a drawing tube.

For scanning electron microscopy, samples were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, washed in the same buffer, and dehydrated in a graded alcohol series. Samples were then processed in a critical point drier ''Bomer-900'' with freon 13, sputter-coated with gold-palladium in a Technics Hummer V, and finally examined with a Jeol scanning electron microscope (Model JSM7610F, Jeol Ltd, Japan). Identification of the recovered pentastomids was based on the key published by Rego[48] and Ali et al.[4,6].

Molecular analyses

Genomic DNA was extracted from the ethanol preserved samples of the isolated parasites using QIAmp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions, and further stored at − 20 °C until used. PCR reactions were carried out using the following primers[25] (28Sa F: 5′-TGCTTGTCTCAAAGATTAAGCC-3′, 28Sb R: 5′-TGCTTGTCTCAAAGATTAAGCC-3′). To target the 18S rRNA gene in a final reaction, a total volume of 50 μl was prepared containing 1 μl of DNA templates (100 ng), 0.5 μl (50 pmol) of each primer, 2 μl of dNTPs (Advanced Bioenzymes, UK), 1 μl DNA polymerase (2 U) (Advanced Bioenzymes, UK), 5 μl of 10 × buffer (500 mM KCl, 100 mM Tris HCl pH 9.0, 1.5 mM $MgCl_2$), and 40 μl of dH_2O. The temperature profile was as follows: Initial denaturation at 95 °C for 5 min, followed by a denaturation step of 35 cycles at 94 °C for 45 sec, annealing at 49 °C for 45 sec, extension at 72 °C for 1 min, and a final extension at 72 °C for 1 min. The amplification reactions were carried out in a PCR Thermocycler (PTC 100, MJR Research, USA). Two microliters of bromophenol blue were added to the aliquots of PCR products and the corresponding amplicons were electrophoresed on 2.5% agarose gel, stained with ethidium bromide, visualized using a UV transilluminator.

Sequencing and phylogeny

The PCR product was purified using a kit (Roche Diagnostics, Germany), and sequencing templates were prepared using a plasmid preparation kit (Machery-Nagel) and aBIO Dye Terminator v 3.1 Ready Sequencing Kit (Applied Biosystems) and 310 Automated DNA Sequencer (Applied Biosystems, USA) using the same primers. To identify related sequences, a BLAST search was carried out on the NCBI database. A BLAST rectangular tree was created from genetic distances calculated using the Jukes-Cantor model for nucleotide comparisons[35] between the query sequence and arthropod sequences. The tree was built from the distance metrics using Fast Minimum Evolution (FastME) according to Desper and Gascuel[16]. Moreover, data of DNA sequences were aligned using CLUSTAL-X multiple sequence alignment[58] and compared with previously deposited sequences of pentastomids recovered from GenBank to analyze intra-specific differences. The alignment was corrected manually using the alignment editor on BIOEDIT software 4.8.9. A phylogenetic tree was constructed using the MEGA program version 7[56].

Results

Among the 17 specimens of *E. schneideri* examined, twelve animals (prevalence 70.59%), eight males and four females, had their lungs infected by adult pentastomids. The infection increased during summer (4 males, 4 females) with a percentage of 89% and decreased during winter (4 males, 0 males) with a percentage of 57%. Adults were identified by the presence of tapered hooks devoid of chitinous accessories. The examined male hosts were generally more heavily infected (mean intensity of 4.3) than females (2.2).

Morphology

Raillietiella aegypti Ali et al.[3] Fig. 1 (A-G), Fig. 2 (A-H), Fig. 3 (A-H)

Measurements and description based on the holotype female and male and paratype females.

Female (based on 5 specimens): body pyriform, annulated 25 ± 2 (24–28), widened anteriorly between 3–14 annuli and tapered posteriorly to a bilobed terminal segment. Body was 16.22 ± 4 (15.25–18.31) mm long and 4.16 ± 0.21 (3.6–5.9) mm wide. Cephalothorax trapezoidal, slightly wider and indistinct from abdomen, rostrum not prominent, with a sclerotized U– shaped buccal cadre and a thick–framed terminal mouth. Two pairs of hooks, a pair of sharp anterior hooks with AB 135 ± 5 (110–146) μm and BC 158 ± 5 (150–169) μm and a pair of sharp posterior hooks with AB 221 ± 5 (200–236) μm and BC 286 ± 6 (280–289) μm. Four dorsal and prominent apical papillae present, one adjacent to each hook. Abdomen with distinct annuli, parapodial lobes present, anus ventral; caudal papillae indistinct; uterus straight, tube–like, and opened anteriorly.

Male (based on 5 specimens): body morphology was similar to that of the female; with a pyriform body, annulated 19 ± 2 (16–21), widest anteriorly, 12.7 ± 3 (10.5–13.3) mm long and 3.45 ± 0.13 (3.2–5.8) mm wide, anterior hook with AB 73 ± 3 (72–75) μm and BC 102 ± 5 (100–103) μm; posterior hook larger with AB 190.6 ± 5 (190–191) μm and BC 221 ± 5 (280–289) μm. Dorsal papillae present; cephalothorax trapezoidal, widest at annuli 3–12, indistinct from abdomen. Two copulatory spicules curved with absolute uniform width; anus ventral; testis single, rounded, opened anteriorly.

Molecular study

Sequence alignment by the maximum likelihood analysis for the data obtained from 18S rDNA analysis of the parasite isolated from the host examined yielded 1405 bp, which exhibit identities ranging between 88–95% with pentastomid genera recovered from GenBank. BLAST computed a pairwise alignment between a query and the database sequences searched; a tree was constructed and constituted different clades from arthropod species (Figs. 4 and 5). It was observed that a separate monophyletic clade of Pentastomida was constructed including the present sequences and pentastomid species. Moreover, the results obtained strongly support that pentastomes are included within crustaceans and most closely related to branchiurans. The constructed phylogenetic tree by the maximum likelihood method based on the Tamura 3-parameter model among different species of the class Pentastomida arranged the aligned sequences and the present one into two main clades; the first clade (clade I) included species of the order Porocephalida[34] while the second clade (clade II) included species of the order Cephalobaenida[34] which was subdivided into two subclades: the first subclade (subclade A) arranged the species of family Reighardiida[7]: *Hispania vulturis* and *Reighardiasternae*[19] exhibited 94.90% and 94.76 identities respectively with the query sequences. The second subclade (subclade B) arranged the species of family Raillietiellida[7] to which the present sequences were included, the subclade B species and their identities being: *R. orientalis*[31], 95.33% and *Raillietiella* sp. [25], 95.89% showed maximum identities with the query sequences. Estimates of Evolutionary Divergence between Sequences were shown in Table 1. The recovered sequences from the present study were deposited in GenBank under Accession number MK970649.1.

DISCUSSION

Studying parasite diversity worldwide is important for at least two major reasons. First, parasites are now recognized as playing important roles in ecosystem fractions by influencing the populations and communities of their hosts[39]. Second, many parasite species are agriculturally and medically important[15,26,43]. Therefore, the identification of parasitic worms based on microscopic observations and PCR amplification/sequencing of 18S rDNA from isolated parasites is highly recommended for successful investigations and taxonomy[44]. There are many parasitic crustaceans, such as pentastomes, whose adult morphology is devoid of crustacean features.

Family Raillietiellidae represented by the single pentastomid genus *Raillietiella* is characterized by a quite wide diversity of hosts[7,8]. The taxonomy and systematics of the pentastomid genus *Raillietiella* were reviewed by Ali et al.[6] in the description of *R. cartagenensis* and in the redescription of *R. amphiboluri*[42], *R. kochi*[33], *R. shipleyi*[33], and *R. indica*[24]. Ali et al.[6] reorganized the known and valid species of *Raillietiella* into five groups (Table 2): small lizards, varanid lizards, amphisbaenians, snakes, and amphibians, which are mainly based on host characteristics (host type, ecology, and zoogeography)[50]. Two of these groups (groups I and II; after Ali et al.[6]) include species infecting small insectivorous lizards and are easily differentiated by the initial ideas of Self[55] into species with sharp-tipped posterior hooks (group I) and blunt-tip posterior hooks (group II). Species differentiation in these two groups is mainly based on a combination of characteristics, including body size, annulus number, posterior-hook dimensions, and size and shape of the male copulatory spicule[50]. The sharp-tipped posterior-hook of *Raillietiella* spp. includes six well-characterized species: *R. amphiboluri*[42] infecting the Australian bearded lizard *Amphibolurus barbatus*; *R. chamaeleonis*[28] in *Chamaeleo oustaleti* and *Chamaeleo verrucosus* from Madagascar; *R. mottae*[9] from *Tropidurus hispidus* from Northeastern Brazil; *R. morenoi*[1] from *Gallotia atlantica* in the Canary Islands, *R. aegypti*[3] in different small lizards from Egypt; and

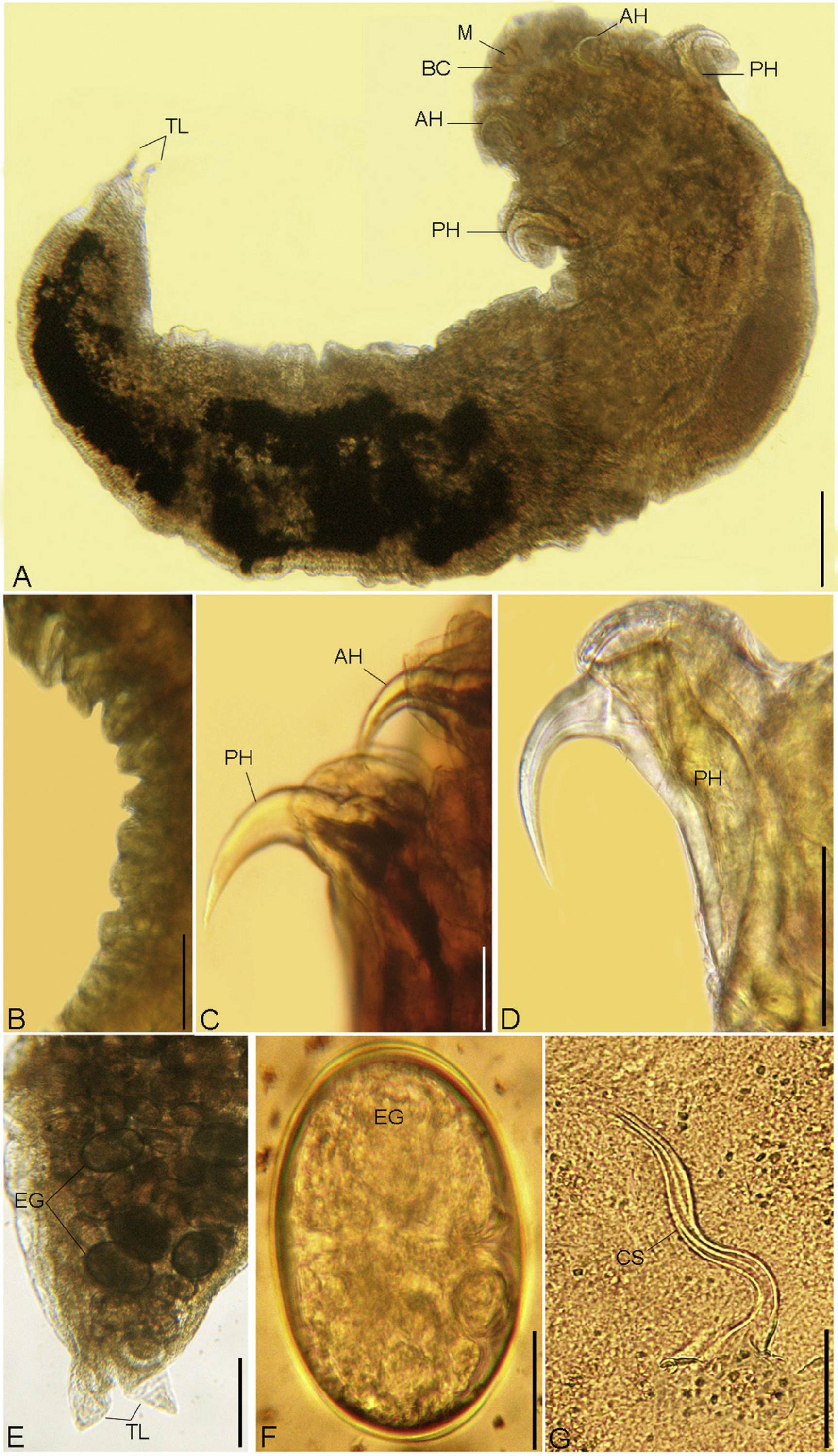

Figure 1 (A - G). Photomicrographs of *R. aegypti* showing: A Whole mount preparation of adult female with a broad cephalothorax that consists of a terminal mouth (M) at the apex supported by buccal cadre (BC), a pair of anterior hooks (AH), and a pair of sharp tipped posterior hooks (PH), abdomen terminated in divergent terminal lobes (TL), bar 50 μm. B Abdominal annuli, bar 100 μm. C, D Anterior (AH) and posterior (PH) hooks, bars 100 μm. E Posterior part of a female abdomen with terminal lobes (TL), note the presence of eggs (EG), bar 50 μm. F Eggs (EG), bar 10 μm. G Copulatory spicule of male (CS), bar 10 μm.

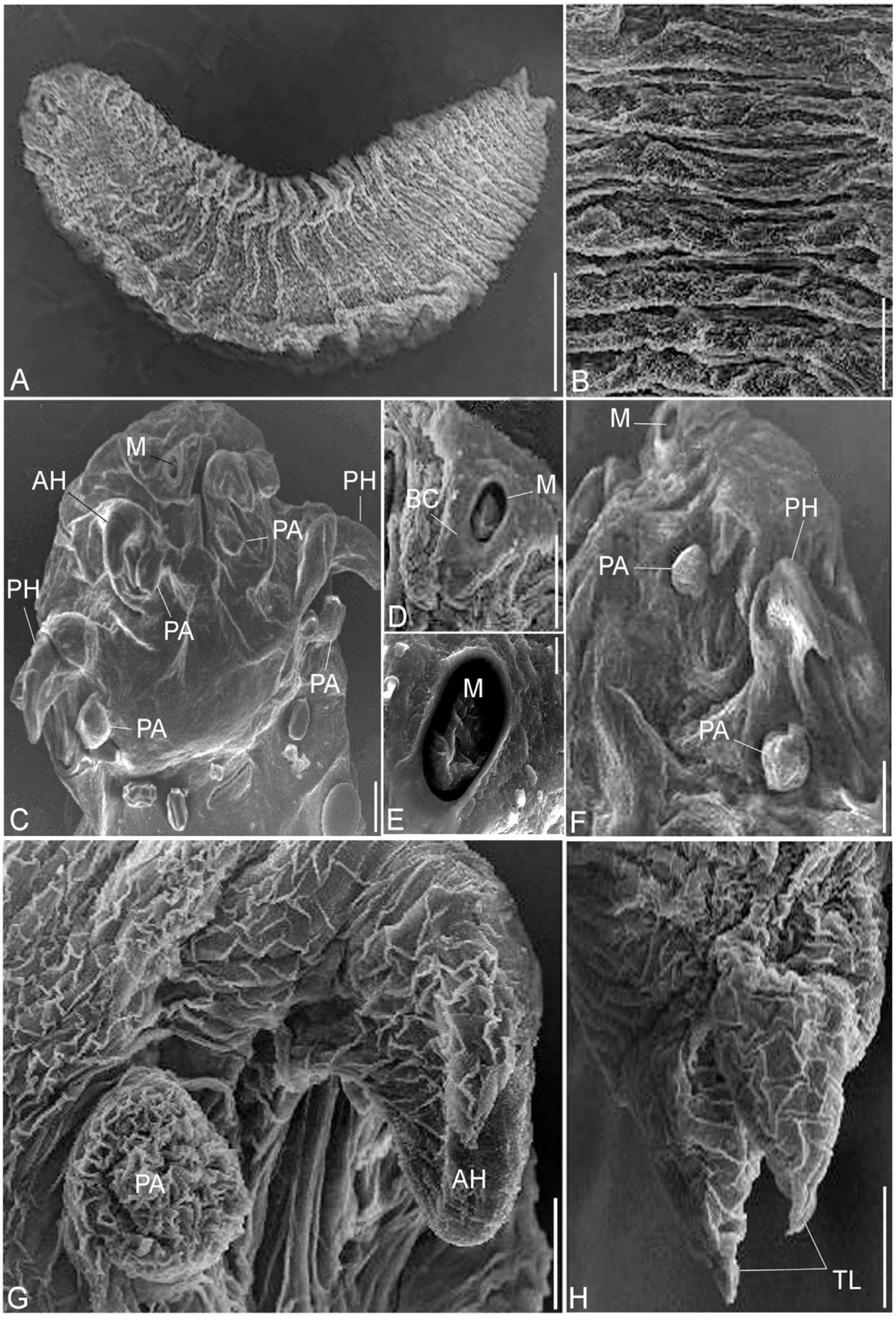

Figure 2 (A - H). Scanning electron micrographs of *R. aegypti* showing: A The adult parasite with an anterior cephalothorax and abdomen terminated in divergent terminal lobes, bar 500 μm. B Abdominal annuli, bar 100 μm. C Cephalothorax consists of a terminal mouth (M), two pairs of hooks, anterior (AH) and sharp posterior (PH), each with prominent cephalic papillae beside (PA), bar 100 μm. D, E The terminal mouth surrounded by a buccal cadre (BC), bars 100 μm; 10 μm. F Lateral view, posterior hook (PS) and papillae (PA), bar 100 μm. G Anterior hook (AH) and its papillae (PA), bar 50 μm. H Abdomen terminated in divergent lobes surrounding anal opening, bar 100 μm.

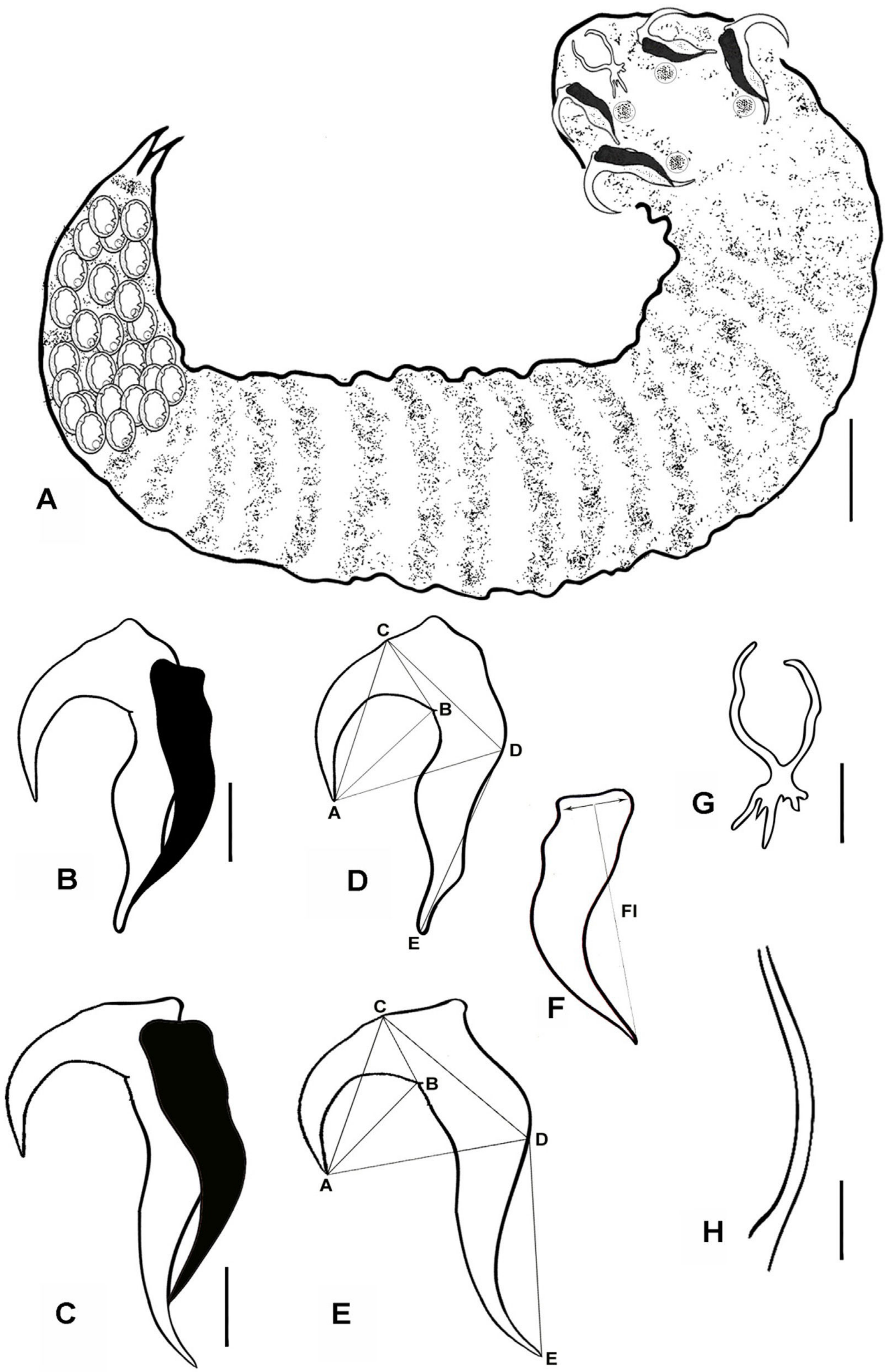

Figure 3 **(A - H).** Line diagrams showing: A Paratype female of *R. aegypti* with annuli, there are lobes and hooks in the area of cephalothorax, bar 50 μm. B. Anterior hook with supporting fulcrum; C. Posterior hook with supporting fulcrum, bar 10 μm. D, E, F. Hook measurement conventions. Anterior (D) and posterior (E) hooks and fulcrum (F) of an adult female, AC, Blade length; AD, Hook length; BC, Base length; CD, Plateau length; AB, Hook gape; DE Hook rest length; Fl, fulcrum length, measured from mid–point between anterior most lateral projections and posterior end, bar 10 μm. G. Buccal cadre, bar 10 μm. H. Copulatory spicule, bar 10 μm.

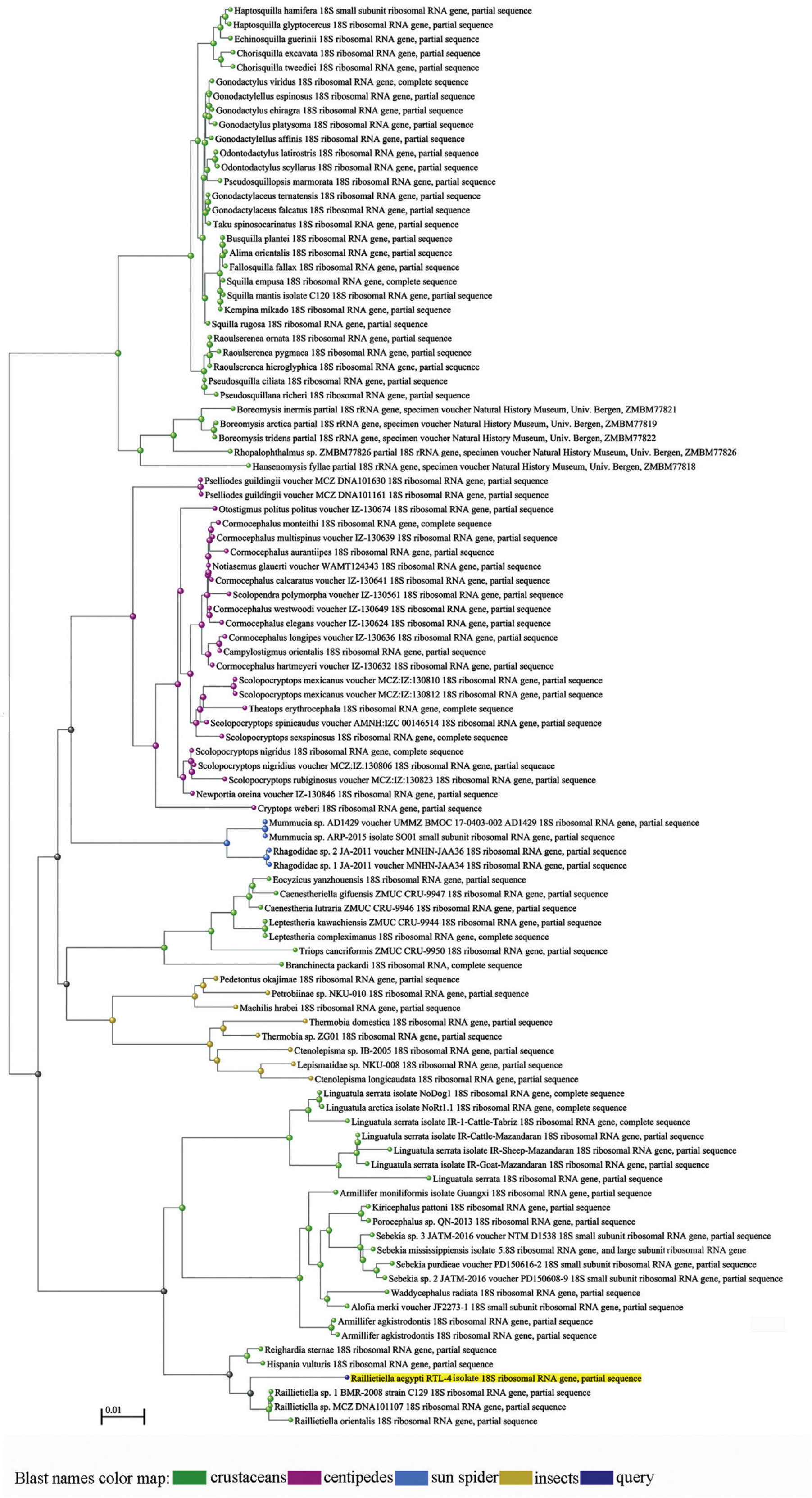

Figure 4 A rectangular tree drawn through the BLAST analysis. The terminal nodes are labeled by blast name to highlight taxonomic trends. The *R. aegypti* query sequence clusters with the arthropod sequences generated from the database sequences obtained from BLAST. The different blast names in the map distinguished by colors for crustaceans, centipedes, sun spider, insects and the query sequences.

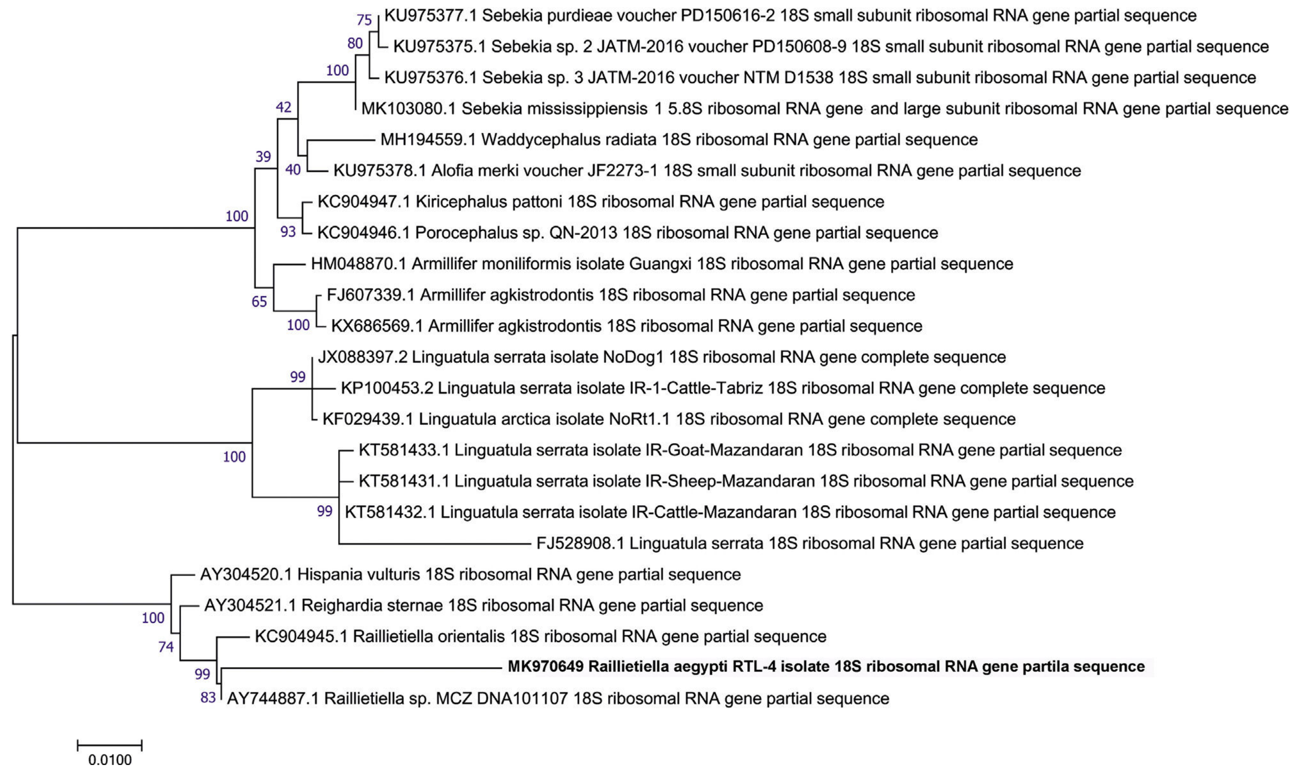

Figure 5 Molecular phylogenetic analysis by the Maximum Likelihood method: The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree (s) for the heuristic search was/were obtained automatically by applying the Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. There were a total of 1396 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

R. cartagenensis[6] in *Hemidactylus* sp. and *Gonatodes* sp. from Colombia. The reported presence of *R. affinis*[13] in *Lepidactylus lugutris* from the British Salomon Island still awaits confirmation and also that in *Lioheterodon modestus* from Madagascar due to the inclusion of *R. chamaeleonis* as a host[27]. The present *Raillietiella* sp. could be distinguished from *Raillietilla* sharp-hooked species of small lizards through different combinations of characteristics (Table 3), such as measurements of body length, annulus number, anterior-hook and posterior-hook size, and dimensions of the male copulatory spicule. Furthermore, it can be differentiated from similar species in group I. The taxonomic and systematic morphological characteristics stated by Ali et al.[6] and others referring to male hooks[50] are reliable in distinguishing and separating the present *Raillietiella* sp. from the rest. Based on light and scanning electron microscopy, the present parasite should be classified morphologically as *R. aegypti*[3] according to the following criteria: the same host with a new type locality; it is a group I type of *Raillietiella* spp. and the dimensions of anterior and posterior hook. This is the first report of a pentastomid in a reptile species from Saudi Arabia. The congruence between molecular and morphological data in the taxonomic justification of pentastomids was demonstrated by Mohanta and Itagaki[44].

Our analysis corroborates the monophyly of Pentastomida and the results thus provide strong support for Wingstrand's proposal that pentastomes are highly modified crustaceans most closely related to branchiurans. Data derived from the DNA analysis coincide with those from morphological studies in accordance with Wägele[61] and Nielsen[45]; the maximum identity obtained for the present sequences was 95.89% with *Raillietiella* sp. recovered from different hosts and localities with some morphological differences that exclude the suggestion to classify the present species as being the same. Moreover, since the present parasite is morphologically the same as *R. aegypti* described previously with no molecular confirmation, the present study represents the first one to confirm the taxonomy of *R. aegypti*. The results of molecular analyses vary according to the selected species, sample size, and particular methods of analysis used, where the sequences of 18S rRNA may contain consistent phylogenetic information for cladogenetic events as old as the Median Cambrian[9,47]. This morphology–based taxonomy is consistent with present molecular phylogenies, which are supported by previous observations. Consequently, revision on the morphology–based taxonomy or inclusion of molecular data from an extensive range of taxa should be necessary to elucidate the phylogenetic relationships of the Pentastomida.

Table 1 Estimates of Evolutionary Divergence between Sequences. The number of base substitutions per site from between sequences is shown. Standard error estimate (s) are shown above the diagonal. Analyses were conducted using the Maximum Composite Likelihood model. The analysis involved 23 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd. All positions containing gaps and missing data were eliminated. There were a total of 1396 positions in the final dataset. Evolutionary analyses were conducted in MEGA7

Species	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
MK970649 *Raillietiella aegypti*		0.009	0.010	0.010	0.010	0.019	0.019	0.019	0.019	0.019	0.020	0.020	0.020	0.019	0.019	0.020	0.020	0.020	0.020	0.018	0.021	0.018	0.019
AY744887.1 *Raillietiella sp.*	0.048		0.003	0.003	0.002	0.013	0.013	0.012	0.012	0.012	0.013	0.013	0.012	0.012	0.013	0.013	0.013	0.013	0.013	0.013	0.015	0.013	0.013
AY304521.1 *Reighardia sternae*	0.058	0.009		0.003	0.004	0.013	0.013	0.012	0.012	0.012	0.012	0.013	0.012	0.012	0.013	0.012	0.013	0.013	0.013	0.013	0.016	0.013	0.013
AY304520.1 *Hispania vulturis*	0.059	0.011	0.008		0.004	0.013	0.013	0.012	0.012	0.013	0.012	0.012	0.013	0.012	0.013	0.013	0.013	0.013	0.013	0.013	0.015	0.013	0.013
KC904945.1 *Raillietiella orientalis*	0.055	0.006	0.014	0.015		0.013	0.014	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.013	0.014	0.014	0.014	0.014	0.016	0.014	0.014
JX088397.2 *Linguatula serrata*	0.121	0.076	0.074	0.073	0.081		0.001	0.014	0.014	0.014	0.015	0.015	0.014	0.014	0.002	0.014	0.015	0.015	0.015	0.005	0.009	0.005	0.005
KF029439.1 *Linguatula arctica*	0.122	0.077	0.075	0.074	0.082	0.001		0.014	0.014	0.014	0.015	0.015	0.014	0.014	0.002	0.014	0.015	0.015	0.015	0.005	0.009	0.005	0.005
HM048870.1 *Armillifer moniliformis*	0.121	0.069	0.070	0.071	0.075	0.084	0.083		0.004	0.004	0.003	0.004	0.004	0.004	0.015	0.005	0.005	0.005	0.005	0.015	0.016	0.015	0.015
KC904947.1 *Kiricephalus pattoni*	0.121	0.070	0.070	0.071	0.075	0.084	0.083	0.016		0.001	0.004	0.004	0.004	0.003	0.015	0.004	0.004	0.004	0.004	0.015	0.017	0.015	0.015
KC904946.1 *Porocephalus sp.*	0.122	0.071	0.072	0.072	0.076	0.084	0.083	0.014	0.003		0.004	0.004	0.004	0.003	0.015	0.004	0.004	0.004	0.004	0.014	0.016	0.014	0.015
FJ607339.1 *Armillifer agkistrodontis*	0.124	0.072	0.072	0.071	0.076	0.090	0.089	0.012	0.014	0.015		0.001	0.005	0.004	0.016	0.005	0.005	0.005	0.005	0.015	0.017	0.016	0.016
KX686569.1 *Armillifer agkistrodontis*	0.125	0.073	0.073	0.072	0.077	0.091	0.090	0.013	0.015	0.016	0.002		0.005	0.004	0.016	0.005	0.005	0.005	0.006	0.016	0.017	0.016	0.016
MH194559.1 *Waddycephalus radiata*	0.125	0.072	0.072	0.072	0.076	0.088	0.087	0.019	0.019	0.019	0.024	0.025		0.004	0.015	0.005	0.005	0.005	0.005	0.015	0.017	0.015	0.015
KU975378.1 *Alofia merki*	0.122	0.069	0.069	0.069	0.074	0.086	0.085	0.014	0.013	0.013	0.017	0.017	0.014		0.015	0.003	0.004	0.004	0.004	0.015	0.017	0.015	0.015
KP100453.2 *Linguatula serrata*	0.125	0.080	0.078	0.077	0.085	0.004	0.004	0.088	0.088	0.088	0.094	0.095	0.092	0.090		0.015	0.015	0.015	0.015	0.005	0.010	0.005	0.005
MK103080.1 *Sebekia mississippiensis*	0.126	0.072	0.072	0.073	0.077	0.087	0.087	0.020	0.016	0.017	0.022	0.024	0.021	0.013	0.091		0.002	0.002	0.002	0.015	0.017	0.015	0.015
KU975376.1 *Sebekia sp.*[3]	0.128	0.074	0.073	0.075	0.079	0.089	0.088	0.020	0.017	0.018	0.023	0.025	0.024	0.015	0.093	0.004		0.001	0.002	0.015	0.017	0.016	0.016
KU975377.1 *Sebekia purdieae*	0.128	0.075	0.074	0.075	0.079	0.089	0.088	0.020	0.019	0.020	0.023	0.025	0.023	0.015	0.093	0.004	0.003		0.001	0.015	0.018	0.016	0.016
KU975375.1 *Sebekia sp.*[2]	0.128	0.074	0.073	0.074	0.079	0.088	0.088	0.022	0.019	0.020	0.024	0.026	0.022	0.017	0.092	0.004	0.004	0.001		0.015	0.017	0.015	0.016
KT581432.1 *Linguatula serrata*	0.119	0.079	0.078	0.077	0.084	0.023	0.023	0.089	0.090	0.088	0.096	0.096	0.093	0.092	0.025	0.093	0.095	0.095	0.094		0.006	0.001	0.001
FJ528908.1 *Linguatula serrata*	0.141	0.098	0.098	0.097	0.103	0.054	0.054	0.102	0.106	0.105	0.109	0.110	0.111	0.106	0.054	0.109	0.111	0.111	0.110	0.030		0.006	0.006
KT581431.1 *Linguatula serrata*	0.120	0.081	0.081	0.080	0.086	0.025	0.026	0.092	0.092	0.091	0.098	0.099	0.096	0.094	0.027	0.096	0.097	0.097	0.096	0.002	0.032		0.002
KT581433.1 *Linguatula serrata*	0.122	0.081	0.081	0.080	0.086	0.025	0.026	0.092	0.092	0.091	0.098	0.099	0.096	0.094	0.027	0.096	0.097	0.097	0.096	0.002	0.032	0.004	

Table 2 The five groups of *Raillietiellids* modified from Ali et al[6]

Group	Species	References	Body shape	Caudal lobes	Female	Hosts	Distribution
A	*R. geckonis* *R. affinis* *R. mabuiae* *R. gehyrae* *R. indica* *R. hemidactyli* *R. aegypti* *R. amphiboluri* *R. chamaeleon* *R. cartaginensis*	DIESING [18] BOVIEN [17] HEYMONS [33] BOVIEN [13] GEDOELST [24] HETT [30] ALI et al.[3] MAHON[42] GRETILLAT & BRYGOO [28] ALI et al., [6]	Generally small, cigar-shaped, hooks supported by prominent divergent podial lobes	Bifid or divergent posterior lobes, forward projecting	3.6-18 mm	Mainly small lizards, one species in a toad	Indonesia, Asia, Africa
B	*R. kochi* *R. shipleyi*	HEYMONS[33] HEYMONS[33]	Thick body,'fleshy', conspicuously annulated	Forward projecting or swollen	19-34 mm	Varanid lizards	Africa
C	*R. orientalis* *R. boulengeri* *R. agcoi*	HETT [31] VANEY & SAMBON[60] TUBANGUI & MASILUFIGAN [59]	Elongate species	Plump caudal lobes	34-61 mm	Snakes	Asia, Africa, Philippines
D	*R. meditteranea* *R. spiralis*	HETT [31] HETT [32]	Variable length	Rounded and swollen	20-35 mm	Snakes	Western Asia
E	*R. furcocerca* *R. bicaudata*	DIESING [17] HEYMONS [34]	Variable length	Rounded and swollen	20-35 mm 26-60 mm	Snakes and amphisbaenians	Western Asia
	R. gigliolii *R. ehamaeleonis* *R. schoutedeni* *R. congolensis*	HETT [32] GRETILLAT & BRYGOO [28] FAIN [21] FAIN [21]	Generally long and slender species	Pronounced, elongate and slender, or peg- like		Snakes, lizards and amphisbaenians	North and South America, Madagascar, Africa

Table 3 A summary of the principal characteristics of sharp-hooked *Raillietiella* species from insectivorous lizards

Species	Host	Female				Male			
		Length (mm)	Annulus number	Posterior hook (μm)		Length (mm)	Annulus number	Posterior hook (μm)	
				AB	BC			AB	BC
R. amphiboluri MAHON[42]	*Amphibolurus barbatus*	32 (20–44)	32.5 (30–34)	208 (200– 220)	370	8-12	27–29	—	—
R. chamaeleonis GRETILLAT & BRYGOO[28]	*Chamaeleo oustaleti*	15–18	17–22	—	277	6.5	17–22	—	—
R. aegypti ALI et al.,[3]	*Eumeces schneideri*, *Uromastyx aegyptia* *Agama mutabilis* *Agama stellio*	26	26 (25–27)	247 (237– 267)	384 (366–410)	< 13	22 (21–24)	135 (118–153)	212 (148–238)
R. cartaginensis ALI et al.,[3]	*Gonatodes sp.* *Hemidactylus sp.*	61–69	—	190–200	315–325	3.9–4.7	—	—	—
R. morenoi ABREU-ACOSTA et al.,[1]	*Gallotia atlantica*	25	36	309	401.7	5.7	30	192	267.8
R. mottae ALMEIDA et al.,[9]	*Tropidurus hispidus*	7.2-7.8	—	222.5 (224-226)	363.25 (360-365)	3.5-4.3			
R. kochi HEYMONS[33]	*Varanid lizards*	27 (23-32)	28-29	265	405	7	—	—	—
R. aegypti (present study)	*Eumeces schneideri*,	16.22 ± 4 (15.25–18.31)	25 ± 2 (24–28)	221 ± 5 (200–236)	286 ± 6 (280–289)	12.7 ± 3 (10.5–13.3)	19 ± 2 (16–21)	190.6 ± 5 (190–191)	221 ± 5 (280–289)

Animal Rights Statement

Authors declare that the experiments on animals were conducted in accordance with local Ethical Committee laws and regulations as regards care and use of laboratory animals.

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