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MORPHOLOGICAL DESCRIPTION OF *COSMOCERCA VRCIBRADICI* (COSMOCERCIDAE RAILLIET, 1916) AND *PHYSALOPTERA BAINAE* (PHYSALOPTERIDAE, LEIPER 1908) IN *CHALCIDES OCELLATUS* FROM EGYPT: A LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY

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Abstract

Cosmocerca vrcibradici (Cosmocercidae) and *Physaloptera bainae* (Physalopteridae) are two intestinal nematodes obtained from a scincid lizard, *Chalcides ocellatus* (Scincidae). The study gave the first morphological description of *C. vrcibradici* and *P. bainae* using light and scanning electron microscopy. A cosmocercid nematode *C. vrcibradici* assigned to genus *Cosmocera*, possessing small mouth surrounded by three lips provided with four sub-median cephalic papillae and each one has a pair of lateral amphids. Male was 2.4-2.7 (2.6±0.1) mm long; maximum width 0.2-0.23 (0.21±0.01) can be easily distinguished from its congeners by the presence of lateral alae and small well sclerotized gubernaculum and ended conically with a tapered tail. Females measured 1.9–3.7 (2.8±0.1) mm in length & 0.24–0.42 (0.38±0.01) mm in width ended with a tapered tail. Besides, all recovered females were gravid filled with large number of embryonated eggs. *Physaloptera bainae* assigned to genus *Physaloptera* characterized by long robust body measured 3.21-5.47 (4.35±0.10) long and 0.58-0.65 (0.62±0.01) in maximum width, oral openings with two semicircular pseudolabia, cuticle dilatation at anterior extremity, reflected over base of lips, forming cephalic collar and ended posteriorly with tapered tail. Only recovered parasites were females with didelphic uterus the main characteristics feature among between species. These observations have allowed a more detailed description of both nematode taxonomy and classification.

Key words: *Chalcides ocellatus, Cosmocerca, Physaloptera,* Morphological description.

Introduction

Species of the genus *Cosmocerca* Diesing (1861) are found among the parasitofauna in the stomach of amphibians (Martinez and Maggenti, 1989; Moravec and Baruš 1990; Bursey *et al,* 2015; Sou and Nandi 2015) and occasionally reptiles (Baker, 1987; González and Hamann, 2008; Hassan, 2016). Also, genus *Physaloptera* Rudolphi (1819) is parasite in the stomach of reptiles, birds, mammals and occasionally amphibians (Yamaguti, 1961). They infect both wild animals and pets, such as dogs and cats (Ortlepp, 1922; Chabaud, 1975) and require an insect as the intermediate host (Anderson, 2000). However, these parasites are scarcely studied in lizards (Brooks and Hoberg, 2000; Poulin and Leung, 2010), resulting in taxonomic confusion (Pereira *et al,* 2017). Detailed morphological data on these nematodes is important for improving the database and resolving current taxonomic issues.

The study aimed to give full description of the gastrointestinal helminths, *Cosmocerca vrcibradici* and *Physaloptera bainae* from *Chalcides ocellatus* in Egypt using light and scanning electron microscopy.

Materials and Methods

Worm collection and examination: A total of 30 ocellated skink, *Chalcides ocellatus* (Family: Scincidae) were randomly collected from Abu Rawash City, Giza Governorate, Egypt during the period from March to July 2020. All collected lizards were transported immediately to Laboratory of Parasitology Research at Department of Zoology, Cairo Faculty of Science. Each lizard was euthanized with a dose of sodium pentobarbital, subsequently necropsied, body cavities as well as internal organs were removed and examined for parasites infection under a stereomicroscope (Olympus, SZ51). Recovered parasites were counted, preserved in 70% ethanol, and cleared in 5% glycerin for micr-

oscopic species identification. Photomicrographs were taken by a light microscope supplied with a Leica ICC 50 HD Camera. For SEM, worms were fixed in 3% glutaraldehyde solution, washed in 0.1 M sodium cacodylate buffer (pH 7.4), dehydrated through a graded ethanol series (50%, 60%, 70%, 80%, 90% & 100%), and dried at 30°C for 30min using critical point drier (Leica, EM CPD300). After complete drying, nematodes were mounted on SEM stubs, coated with gold and examined with JEOL JSM-5200 SEM (Tokyo, Japan) at accelerating voltage 25 kV. Prevalence of infection was calculated (Bush *et al*, 1997). All body measurements (mean ±SD, followed by a range in parentheses) in millimeters (mm).

Ethics approval: All procedures contributed in this work comply with the ethical standards authorized by the Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Cairo University with Number CU/I/S/54/17.

Results

Twenty-one lizards (70%) had a mixed infection with two nematodes of family: Cosmocercidae: *Cosmocerca vrcibradici* (male & female) and family Physalopteridae: *Physaloptera bainae* (female only) with *C. vrcibradici* (4-9worms/host) with a higher infection rate than *Physaloptera baiane* (1-2worms/host).

Cosmocerca vrcibradici: Body of adult worm whitish in color and filiform in shape, small-sized, with maximum width at mid-body, Cuticle with fine transverse striations, Excretory pore situated slightly anterior to esophageal bulb, Deirids not seen. Mouth with 3 prominent lips, each one with an anteriorly directed V-shaped cuticular flanges provided with a pair of lateral amphids. Esophagus divided into anterior indistinct pharynx, cylindrical corpus and terminal posterior bulb with short trivulvated part followed by a long intestine. Nerve ring located at about 1/2 of esophageal length. Tail of both sexes was conical with pointed tip; Lateral alae well developed extending from halfway between anterior tip and nerve ring to beginning of caudal spike in both sexes, with prominent sexual dimorphism.

Male (3 mature ones): Body measured 2.4-2.7x0.2-0.23 (2.6±0.1x0.21±0.01). Esophagus 0.52-0.65 (0.6±0.02) in length including bulb, represented 23.8% of body length. Nerve ring, and excretory pore located at 0.16-0.23x0.21-0.37 (0.18±0.01x0.33±0.01) from anterior end, respectively. Posterior body end distinct curved ventrally, Intestine long and opened at body posterior end in crescent shape cloacal aperture at 0.38 from posterior body extremity. Spicules alate, equal in length, 0.037-0.052 (0.046±0.04) bent at midpoint with distal pointed end. Spoon shaped gubernaculum with well sclerotized margins. Body ended conically with a tapered tail measured 0.17-0.21 (0.18±0.01).

Female (8 mature ones): Body larger measured 3.1-3.4x0.29-0.38 (3.23±0.22x 0.36± 0.02). Esophagus 0.76-0.85 (0.81±0.01) long including bulb, represented 25% of body. Nerve ring and excretory pore located at 0.14-0.18 (0.162), 0.255-0.326 (0.281) from anterior extremity, respectively, Anus situated at 0.39-0.46 (0.43±0.22) from tail end. Vulva near mid-body with transverse slit opening, vulval lips not protruded. Tail straight, 0.06-0.09 (0.07±0.001), including terminal awl-shaped process. All females were gravid filled with large number of embryonated eggs, oval, thin-walled with smooth surface, surrounded with a delicate membrane, and measured 0.061-0.082×0.052-0.071 (0.093±0.01 x 0.061±0.01).

Taxonomic position: *Cosmocerca vrcibradici* Bursey and Goldberg (2004)
Family: Cosmocercidae Railliet (1916)
Host Type: Ocellated skink, *Chalcides ocellatus* Forskal (1775) (Family: Scincidae).
Locality: El-Giza Governorate, Egypt.
Site of infection: Large intestine.

Physaloptera bainae (3 mature females): Robust elongated body with posterior tapered end, white in color, measured 3.21-5.47x 0.58-0.65 (4.35±0.10x0.62±0.01) in maximum width, Distinct thick cuticle with trans-

verse striations more evident at body extremeities forming well-marked annulations at posterior end. Cephalic end dome-shaped composed of 2 semicircular, convex, lateral pseudo lips surrounding oral opening. Internal margins of each pseudolip with 2 cuticular folds forming large cephalic collaret; each lip armed with a variable number of teeth and two external papillae. Buccal capsule absent. Total esophagus length of 0.71-0.79 (0.74±0.10) divided into anterior muscular portion 0.41-0.64 (0.53±0.10) in length and posterior glandular one measured 0.14-0.19 (0.17±0.01) long and 0.24-0.29 (0.27 ±0.01) wide. Esophagus represented 17.01% of total body length. Nerve ring encircles muscular esophagus on posterior quarter. Vulvar opening without prominent lips. Uterus with two uterine branches (didelphic), eggs with thick shell. Anal opening located at 0.26-0.0.31 (0.28±0.10) from posterior extremity.

Taxonomic position: *Physaloptera bainae* Pereira *et al.* (2014)
Family: Physalopteridae Leiper (1908)
Host Type: Ocellated skink, *Chalcides ocellatus* Forskal (1775) (Family: Scincidae).
Locality: El-Giza province, Egypt.
Site of infection: Large intestine.

Details were given in figures (1, 2, & 3).

Discussion

Egypt has a relatively long history of reptile Nematology (Moravec *et al,* 1987). However, little attention was paid to the helminthes community of reptiles and few records existed in the literature (Sharpilo *et al*, 2001; Martin and Roca, 2004).

Cosmocerca were common nematode parasites infecting in the digestive tract of various amphibians (Yamaguti, 1938; Skrjabin *et al,* 1961; Moravec and Sey, 1985; Moravec and Baruš, 1990; Bursey *et al,* 2015; Sou and Nandi, 2015). Till now, about 30 species of *Cosmocerca* were reported worldwide (Rizvi *et al*, 2011; Bursey *et al,* 2015; Sou *et al*, 2018, 2019).The present species belongs to genus *Cosmocerca* with small mouth opening surrounded by 3 lips provided with four sub-median cephalic papillae and one pair of lateral amphids, long muscular esophagus divided into corpus and bulb lead to long intestine opened exteriorly with an anal opening in females, and cloaca in males, presence of lateral alae, gubernaculum, and somatic papillae along body (Kung and Wu, 1945). Also, the present parasite resembles *C. panamaensis* Martinez and Maggenti (1989); *C. vrcibradici* Bursey and Goldberg (2004); *C. oroensis* Bursey *et al.* (2013); *C. leytensis* Bursey *et al.* (2015); *C. multipapillate* Ni *et al.* (2020), and *C. smilie* Chen *et al.* (2020) in the presence of small mouth surrounded by 3 lips, lateral alae and gubernaculum in contrast to *C. bengalensis* Sou *et al.* (2018) that lacked a gubernaculum. Also, females of *C. panamaensis* Martinez and Maggenti (1989); *C. leytensis* Bursey *et al.* (2015); *C. bengalensis* Sou *et al.* (2018); *C. ornate* Sou *et al.* (2019) and *C. smilie* Chen *et al* (2020) differed from the present nematode by having round ended body with a long filamentous tail but resembles *C. vrcibradici* Bursey and Goldberg (2004), and in having straight tail with terminal awl-shaped process. *C. parva* Travassos (1925) isolated from large intestine of the Schneider's toad *Rhinella schneideri* in Argentina; *C. ornate* Moravec *et al.* (1987) in Egypt isolated from large intestine and rectum of marsh frog *Rana ridibunda*; *C. panamaensis* Martinez and Maggenti (1989) isolated from small intestine of strawberry poison frog *Dendrobates pumilio* in Panama; *C. oroensis* Bursey *et al.* (2013) isolated from large intestine of the Gunther's papua frog *Barygenys atra* in Papua New Guinea; *C. bengalensis* Sou *et al.* (2018) in *Hoplobatrachus tigerinus* in India; *C. smilie* Chen *et al.* (2020) from Asiatic toad *Bufo gargarizans* in China; *C. multipapillate* Ni *et al.* (2020) from the marine toad *Rhinella marina* in Australia. All were from anuran hosts. However, *C. vrcibradici* was the first *Cosmocerca* species to have a reptilian definitive host, reported in *Prionodactylus eigenmanni* and *P. oshaughnessy* from Brazil (Bursey and Goldberg, 2004). Also, others

reported some species from reptilian hosts, *C. zugi* Bursey *et al.* (2005) isolated from large intestine of ring-tailed gecko *Cyrtodactylus louisiadensis* in Papua, New Guinea; *C. leytensis* Bursey *et al.* (2015) from large intestine of bent-toed gecko *Cyrtodactylus gubaot* in Philippines. Hassan (2016) in Egypt detected *C. vrcibradici* from *Chalicides ocellatus* that was similar to the present nematode in host type and infection site.

By comparing the present parasite with other species of same genus, but from different hosts, the only morphologically related species was *C. vrcibradici* (Bursey and Goldberg, 2004). Also, this genus was closely related to *C. vrcibradici* (Hassan, 2016) as sharing the same host and geographic area.

Genus *Physaloptera* was established by Rudolphi (1819) as *Physaloptera clausa* type species parasitizing *Erinaceus dealbatus* Swinhoe (1870) that was placed in family Physalopteridae. Dujardin (1845) suppressed the genus *Physaloptera*, relocated its species to genus *Spiroptera*. Diesing (1861) reintroduced *Physaloptera* genus and added two additional species. Nowadays, 100 species of *Physaloptera* were known (Pereira *et al*, 2012) commonly parasitizing stomach of reptiles, birds, mammals, and sometimes amphibians (Yamaguti, 1961; Pereira *et al*, 2014), which larvae parasitized amphibians (Anderson, 2000). The main genus characters of *Physaloptera* were presence of a cephalic collar at anterior end, 2 lateral pseudolabia surrounding oral aperture armed with teeth, thick cuticle with transverse striations and 2-4 uterine branches in females (Ortlepp, 1922; Skrjabin and Sobolev, 1964; Chabaud, 1975; Pereira *et al*, 2012, 2014).

Body size, shape, and spicule length to total body length, pre and postcloacal papillae in males and uterus branches in females were the most important characteristics for *Phys-aloptera* species identification (São Luiz *et al,* 2015). Within genus *Physaloptera*, Seurat (1914) highlighted taxonomicimportance of uterus number that reorganiized species based on having 2 or 4 uteri.

Also, authors separated *Physaloptera* species into groups according to females' number of uterine branches (Ortlepp, 1922; Morgan, 1943; Skrjabin and Sobolev, 1964; Chabaud, 1975), who considered it an important genetic feature. Besides, Anderson *et al* (2009) reported the presence of two to four uterus branches as generic character of *Physaloptera*. Pereira *et al* (2012) reorganized *Physaloptera* species by number of uterus, and divided them into 58 didelphy species, two tridelphys and seven tetradelphy. In addition to uterus, other important diagnostic characters in *Physaloptera* were the apical anterior extremity morphology, position of excretory pore and vulvar position (Ortlepp, 1922; Morgan, 1943; Skrjabin and Sobolev, 1964; Steban *et al,* 1995; Torres *et al,* 2009). But, cervical or lateral alae were not used as species differentiation, although these were reported for *P. getula* by Seurat (1917), and Vaz and Pereira (1935), they described *P. bispiculata* with symmetrical lateral cephalic alae. As to host, the present species differed from *P. bispiculata* Vaz and Pereira (1935); *P. murisbrasilliensis* Diesing (1861); *P. hispida* Schell (1950); *P. longispicula* Quentin (1968); *P. galvao*i São Luiz *et al* (2015) and *P. goytaca* Ederli *et al.* (2018). Also, it differed from *P. galvao*i São Luiz *et al.* (2015) and *P. goytaca* Ederli *et al.* (2018) by uterine branches pattern and resemble *P. murisbrasilliensis* Diesing (1861); *P. bispicula*taVaz and Pereira (1935); *P. hispida* Schell (1950); *P. longispicula* Quentin (1968); *P. lutzi* Ramallo and Diaz (1998); *P. tupinambae* Pereira *et al* (2012); and *P. bainae* Pereira *et al.* (2014), all have didelphic uterine and shared same host type as the last 3 ones.

In the present study, didelphic uterus and host type, genus resembles *P. lutzi, P. tupinambae* and *P. bainae*. Also, the nematode was closely related to *P. bainae* with thick cuticle, transversal striations, and well-marked annulations.

Conclusion

The current study may represent the first comprehensive morphology of *C. vrcibradi*

and *P. bainae* collected from Egyptian *C. ocellatus* using light microscopy and SCM.

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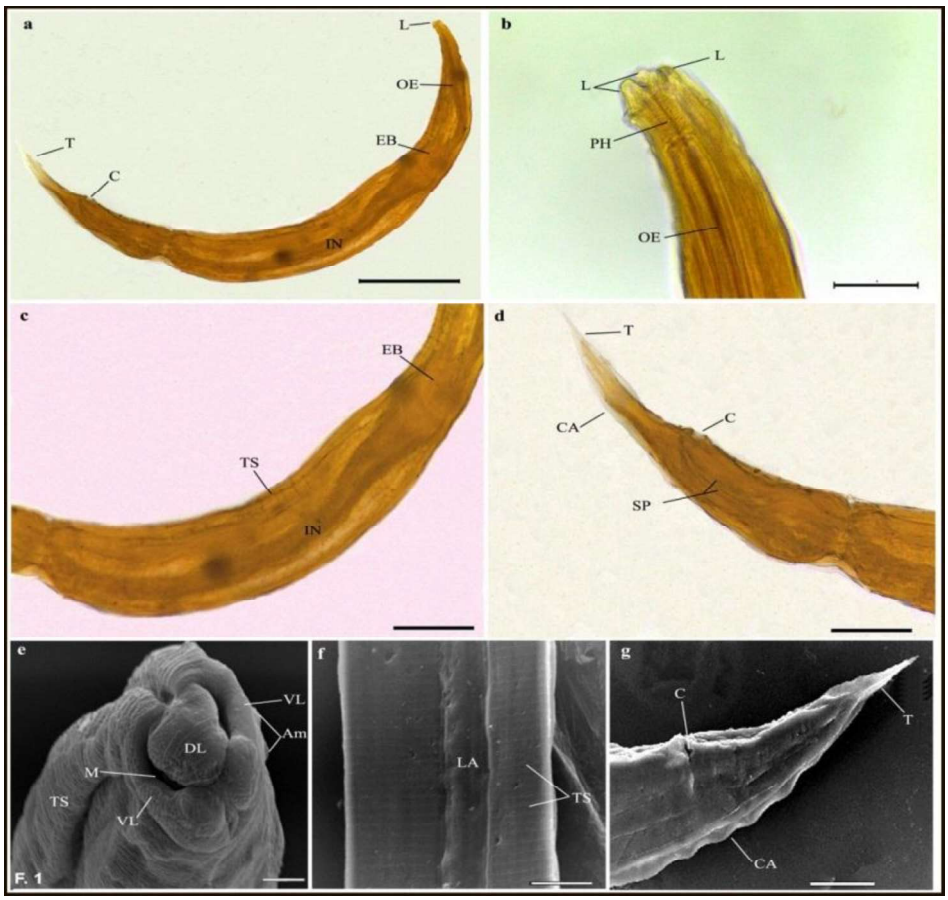
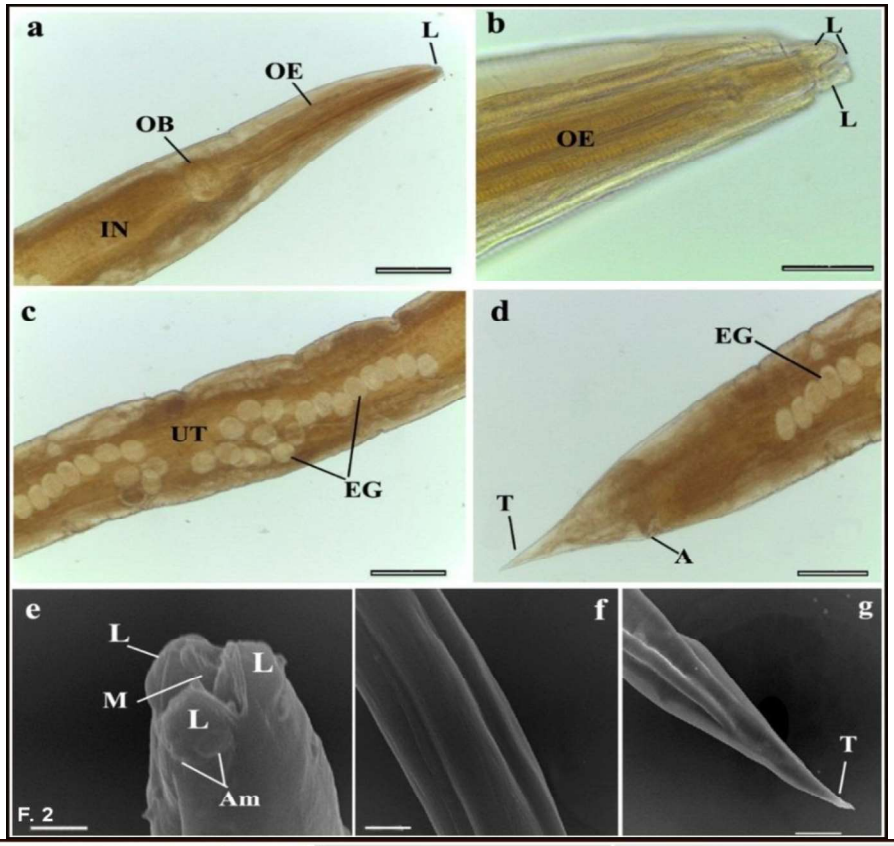
Explanation of figures

Fig. 1: Male *Cosmocerca vrcibradici* (a) Whole body showed mouth (M) surrounded by 3 lips (L), followed by esophagus (OE) ended by bulb (EB), led to long intestine (IN), transverse striations (TS), scale bar = 200µm. (b) Anterior end surrounded by three lips (L), followed by short pharynx (PH) and muscular esophagus (OE), scale bar = 50µm. (c) Mid-body showed esophageal bulb (EB) lead to long intestine (IN) and transverse striations (TS), scale bar = 200µm. (d) Posterior part terminated with pointed tail, caudal alae (CA), spicules (SP) and opened outside by cloaca (C), scale bar = 200µm. (e-g) SEM of male *C. vrcibradici* (e) Anterior part of the body showing mouth (M) with 3 lips (L) and a pair of lateral amphids (Am), scale bar = 10µm. (f) Body cuticle with transverse striations (TS) and lateral alae (LA), scale bar = 50µm. (g) Posterior part end with terminal tail (T) with caudal alae (CA) and opened outside by cloaca (C), scale bar = 100µm.
Fig. 2: Gravid female *Cosmocerca vrcibradici* (a) Anterior extremity surrounded by 3 lips (L), followed by esophagus (OE) ended by bulb (EB), scale bar = 200µm. (b) High magnifications of cephalic region surrounded by 3 lips (L) and esophagus (OE), scale bar = 50µm. (c) Midbody showed uterus (UT) filled with eggs (EG), scale bar = 200µm. (d) Posterior extremity end with pointed tail (T), anus (A), scale bar = 20µm. (e-g) SEM of female *C. vrcibradici* (e) Anterior part of body showed mouth (M) with 3 small V-shaped lips (L) and a pair of lateral amphids (Am), scale bar = 10µm. (f) Body cuticle, scale bar = 50 µm. (g) Posterior part end with terminal tail (T), scale bar = 50µm.
Fig. 3: Female *Physaloptera bainae* (a) Whole body showed mouth (M) surrounded by 2 pseudolips (L), cephalic collaret (CC), followed by esophagus (OE) end by bulb (EB) led to long intestine (IN), tubular uterus (UT) and caudal striations (CS), scale bar = 500µm. (b) Anterior extremity showed mouth (M) surrounded by 2 pseudolips (L), cephalic collaret (CC), followed by esophagus (OE) end by bulb (EB), scale bar = 200µm. (c) High magnifications of cephalic region showed cephalic collaret (CC), scale bar = 50µm. (d) Mid-body showed tubular uterus (UT) filled with eggs (EG) and cuticle with fine transverse striations (TS), scale bar = 200µm. (e, f) Posterior extremity with tapered tail (T), anus (A), scale bar = 200µm, 50µm respectively. (g-j) SEM of female *P. baiane,* (g, h) Anterior end showed mouth (M) with 2 pseudolips (L) surrounded by cephalic collarate (CC), scale bar = 10µm, i) Mid-body cuticle, scale bar = 10µm, (j) Posterior part end with terminal tail (T), anus (A), scale bar = 100µm.

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