Journal of the Egyptian Society of Parasitology, Vol.52, No.1, April 2022
J. Egypt. Soc. Parasitol. (JESP), 52(1), 2022: 99 - 106
(Online: 2090-2549)

MORPHOLOGICAL IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF *LECITHODENDRIUM ASWANI N. SP.* (DIGENEA: LECITHODENDRIDAE) FROM BATS IN ASWAN, EGYPT

By
NAGLA MUSTAFA KAMEL SALEH, ATEF IBRAHIM SAAD, ASMAA AHMED ABUELMAGD AND ABUELHASSAN ELSHAZLY YOUNIS*
Department of Zoology, Faculty of Science, Aswan University, 81528 Aswan, Egypt
(*Correspondence: abssan2000@aswu.edu.eg, ORCID: AE Younis: 0000-0001-7476-9320)

Abstract

Bats attract zoologists' attention due to their well-documented vulnerability to a wide array of animal and human diseases. During the current parasitological survey of bat parasites from October 2016 to March 2020, *Lecithodendrium aswani* n. sp. was detected and described based on morphological and molecular characters from the liver tissue of *Rhinopoma hardwickii cystops*, establishing a new infection site and locality record in Aswan Governorate. Light and scanning electron microscopy studied the morphology and surface topography, respectively. *L. aswani* n. sp. was detected in 1% (5/500) bats. Also, molecular analyses showed that the partial ribosomal ITS1-5.8S-ITS2 region sequence deposited to GenBank database under the accession number MW598491 as distinct species related to other *Lecithodendrium* sequences. Phylogenetic study proved a strong connection between the new species' DNA and family Lecithodendriidae.

Keywords: Bats, *Lecithodrendrium*, Descriptive morphology, SEM, Molecular characterization.

Introduction

Order Chiroptera is the second most diverse among mammalian orders and exhibits great physiological and ecological diversity, with over 1440 species. Bats are excellent ecological indicators of habitat quality, play an important ecological role in soil fertility, forest regeneration, pollination of plants and seed dispersal, and provide an ecological role by controlling some arthropods (Tremlett *et al,* 2021). Because of its high concentrations of limiting nutrients like nitrogen and phosphorous, bat guano has long been mined from caves for use as fertilizer on agricultural crops (Misra *et al,* 2019). Bats are reservoirs of many infectious zoonotic diseases worldwide (Calisher *et al,* 2006) including Egypt (Morsy *et al,* 1987) as well as poultries (Hafez and Attia, 2020) They sheltered close to humans with a chance to transmission of some zoonotic parasitosis (Saoud and Ramadan, 1976), either direct or indirect way (Edungbola, 1981). They are a potential epidemiologic source of many risk diseases, including rabies, Ebola, leptospirosis, histoplasmosis, and pseudotubercuosis *Bartonella* spp. and *Borrelia* spp. (Dobson, 2005; Luis *et al,* 2013; Urushadze *et al,* 2017; Szentiványi *et al,* 2019), and trypanosomes (Morsy *et al,* 1986) as well as coronaviruses (Banerjee *et al,* 2019). Also, they are infested with many zoonotic ectoparasites (Farina and Lankton, 2018)

Family *Lecithodendriidae* have 12 genera, including *Lecithodendrium* Looss, 1896. Literature on bats' parasites in Egypt is fairly comprehensive. Though, bats attracted the attention of many scientists in Egypt as Looss (1896, 1899); Odhner (1911); Abdel-Azim (1936); Macy *et al* (1961); Heyneman and Macy (1962); Saoud (2009); Saoud and Ramadan (1977, 1980); El-Naffar (1978); Fahmy *et al* (1984) and EI-Damarany *et al.* (1997).

The present work has been done to survey the *Lecithodendriid* parasites infecting bats in Aswan Governorate and described as new species *aswani*

Material and Methods

Study site and location: Over a three-year from October 2016 to March 2020. This study was conducted in three cities (Aswan, Kum Umbo and Edfu) Aswan Governorate.

A total of 500 bats were trapped alive

from caves, abandoned buildings, and fruit-trees in Aswan, including well-known cave systems like Abu Sibira and Elsil. Edfu is located on the west bank of the Nile River between Esna and Aswan as home to cities of Hilal, Mahamid, Eshahid, Al-Atwany, Tunab, and Al West Sibaia, but Silwa, Kalabasha, Fares, and Gebel Elsilsila (mountain) were located in Kum Umbo. Despite the challenges of hunting bats, hunters were able to capture bats using mist nets in early morning, put them in labeled soft cloth bags, and transport them immediately to experimental laboratory. They were identified by species & sex using standardized keys (Dietz, 2005). They were anesthetized and dissected out for liver (Jonsson *et al*, 2004), and trematode samples were stored at -20°C in 70% ethanol for molecular studies. The remaining parasites were washed in 0.9% saline solution, flattened, fixed in 70% ethanol, stained in alum carmine, dehydrated in ascending ethanol, cleared in xylene, and mounted in DPX.

Ethical approval: The study was approved by Aswan University's Research Council. It followed a standard operating procedure approved by Aswan University's Animal Use and Care Committee, which went with the experimental animals dealt with according the rules of Helsinki (2000).

Light microscopy*:* Parasites were counted per infected bat, examined and photographed using light microscope (Olympus BX43, Japan) with a digital camera. Measurements were taken throughout the process of drawing with a drawing tube (Shaheen, 2007)

Scanning electron microscopy: Worms were fixed in 0.1M sodium cacodylate buffer with a PH of 4.7 and fixed in glutaraldehyde for six hours at 4°C, followed by several washes in the same cooled cacodylate buffer. After 2 hours at 4°C, the worms were post-fixed in 1% osmium tetroxide (OSO_4), rinsed twice in cacodylate buffer, dehydrated in an ascending ethanol, and then were immersed in pure acetone. Specimens were treated with Feron13 in a Bomer-900 critical point dryer, and were sputtered with gold in a technics Hummer V (Lee, 1993) and analyzed using a Joel JSM-5400L V SEM operating at 15 KV at Assiut University's EM Unit.

Molecular analysis: Individual genomic DNA (gDNA) samples from *L. aswani* n. sp. were preserved in ethanol extracted using a slightly modified phenol/chloroform standard technique (Saad *et al,* 2018). Materials were digested with proteinase K in ALT buffer (Dneasy Kit, Qiagen) for an overnight at 56°C and precipitated with 5.2M ammonium acetate. Pellets were diluted in 20-50µL of deionized water, depended on size. Using a Nanodrop (Implen NP80, Germany), integrations and concentrations of gDNA samples were determined spectrophotometric.

Forward D1 (5′-AGGAATTCCTGGTAA GTGCAAG-3′) & reverse D2 (5'GCTATC CTGA GRGAAACTTCG3'), primers were used to amplify ITS-1, 5.8S, ITS2 (Galazzo *et al,* 2002). For PCR amplifications, a total volume of 50µL was used, which included 1x thermal buffer (Bioline, UK, My Taq Red Reaction buffer), 10-20pmol forward and reverse primers, a 10mM dNTP mix (Alliance Bio, USA), 5µL Taq polymerase (Bioline, UK), and 0.1-0.2µg of gDNA. The amplifications were performed in a thermocycler (Sensoquest Lab cycler, SensoQuest GmbH, Germany) with the following cycling conditions: initial denaturation at 95°C for 5 minutes, 35 cycles at 94°C for 30 seconds, annealing at 56°C for 30 seconds, elongation at 72°C for 30 seconds, and final extension at 72°C for 5 minutes. PCR products were examined and stained with ethidium bromide on a 1% agarose gel. UV gel documentation system (UVP Bio-Doc IT-220 Imaging system, BioExpress, USA) was used for photographing the DNA bands.

Sequencing and phylogenetic analysis: Purification PCR products followed the manufacturer's instructions using either the Zymo Research DNA clean and concentrator Kit (USA) or QIAquick® PCR Kit (Qiagen, Hilden, Germany). Purified PCR products were sequenced in both directions (forward & reverse) using the same primers as in initial

PCR and an Applied BioSystems (Model 3730XL) automated DNA sequencing instrument (USA). Each sequence was homologized by using NCBI Blast tool (http://www.ncbi.nlm.nih.gov). Following manual comparison of each forward sequence to its reverse complement, the CAP3 algorithm combined them. Phylogenetic analysis was done using Phylogeny.fr web tool (Dereeper *et al,* 2010).

Results

Three different species: *A. tridens tridens, R. aegyptiacus aegyptiacus*, and *R. hardwickii cystops* of families: Rhinopomatidae, Pteropodidae, and Hipposideridae, respectively. Only *R. hardwickii cystops* harbored *Lecithodendrium* species in liver of *5/*500 (1%), captured from Edfu and Aswan Cities.

Morphology (Based on 5mature samples): Body was small, pyriform or oval in shape, with rounded posterior end measuring 430-1080x340-830µm (average 750-550). Tegument provided with spines, length /width ratio (1:1.26-1.30); Oral sucker small, oval or round in shape, and terminal, measured 95-170x85-190µm (av. 105.8x125.2) but slightly larger than ventral one. Ventral sucker oval, or round in shape, measured 75-130x 85-150µm (av. 111x101), located pre-equatorial or equatorial, parallel to two testes. Oral sucker/ventral sucker ratio (I1:0.95-1.24), Pharynx small, oval or round in shape and measures 31-48x29-45µm (av.35x29.8). Oesophagus short, measures 19-48x21-35µm (av.33x25) and bifurcates into two laterally divergent intestinal caeca that end blindly a little in front of testes. Testes oval or irregularly round in shape smooth in outline, located in para-acetabular region. Right testis 62-184x92-193µm (av.101x123) and left one 78-186x88-174µm (av.103x95). Vasa differentia arise from testes inner upper borders uniting anteriorly and immediately entering pseudo-cirrus sac measures 46-75x 35-65µm (av.57x48.6) to form vesicula seminalis in between ventral sucker and intestinal bifurcation; Genital pore locates anterior to ventral sucker. Ovary rounded or slightly oval structure posterior to ventral sucker, measures 65-167x58-144µm (av.115x88.7). Vitellaria post testicular forming 2 follicular sets. Follicles on right side 9-18, on left one 7-19. Uterus occupies posterior body half. Eggs oval, small, and yellow in color, measure 10-25x10-20µm. Excretory bladder Y or V-shaped extended to body half opens into an excretory pore at body posterior end.

SEM: adult worms showed concaved body, entirely covered with numerous claw-shaped spines. Oral sucker almost oval or round in shape, its lip lacked tegumental spines. Oral sucker and mouth opening, anteroventral in position. Ventral sucker oval, pre-equatorial or equatorial, with spines covering lumen. Genital pore locates on a protruded portion in front of the ventral sucker.

Taxonomic summary:

Family*:* Lecithodendriidae Odhner, 1910

Genus*:* *Lecithodendrium* Odhner, 1910

Species*:* *aswani* new species (after Aswan City)

Deposit: Public Museum, Faculty of Science, Aswan University, Egypt,

Deposition number (Zoo-Inv-Trem.La.01/ 2021)

Molecular analysis: By agarose gel analysis, sequence length of ITS1, 5.8S, & ITS2 ampilicons in *L. aswani* n. sp. was estimated to be between 1400 &1500bp. Resulted sequence was deposited in Genbank with an accession number (MW598491). Blast analysis revealed that the sequence generated in this study was distinct from all other sequences in the GenBank databases. A similarity score was 95.75% to a sequence from an unspecified *Lecithodendrium* sp. (MW414609),over 86% to 2 *L. linstowi* sequences (MW001039 and JF784190), and 87.04% to a sequence (JF784192) from *L. spathulatum*. Also, *Lecithodendrium*'s phylogenetic analysis included the relevant 6, ITS-1, 5.8S, ITS2 sequences, with the novel one. Both *L. aswani* n. sp. and an unnamed species from Brazil were closely related, to genus *Lecithodendrium*. Details were given in tables (1, 2, & 3) and figures (1, 2, 3, 4, & 5)

Table 1: Mean intensities, prevalence and mean abundance of *L. aswani n. sp.*

Parasite	Frequency	Prevalence %	Parasites burden	Mean Intensity
L. aswani n.sp.	5	1	1-3	0.33 -1

Table2. Infection distribution in 2 locations

Distribution	Aswan Host	Percentage %	Edfu Host	Percentage
Examined bat	150	30	200	40%
Infected by *L. aswani n.sp.*	*R. hardwickii* 2	40	*R. hardwickii* 3	60%

Table 3: Comparison of measurements, morphometric percentages and morphometric ratios between different spinose described species of *L. aegyptiacus* and present material (Measurements in micron unless otherwise mention).

Character	*L. aegyptiacus* (Saoud and Ramadan,1976b)	Present study
Cuticle	Spinose	Spinose
Length	0.57-1.07	0.43-1.08mm
Width	0.42-0.73	0.34-0.83mm
Ratio	1:1.3-1.46	1:1.26-1.30
Oral sucker	0.08-0-11 x 0.08-0.13	95-170x85-190 µm
Ventral sucker	0.075-0.11x 0.75-0.113	75-130x 85-150 µm
Suckers Ratio	0-93-1.9: 1	1:0.95-1.24
Pharynx	0.037-0.058 x 0.034-0.060	31-48 x29-45 µm
Oesophagus	0.026-0.132	19 -48 x 21-35 µm
Right testis	0.075-0.160 x 0.094-0.175	62-184x92-193 µm
Left testis	0.079-0.185 x 0.094-0.177	78-186x 88-174 µm
Cirrus pouch	0.053- 0.98 x 0.075-0.143	46-75x 35-65 µm
Ovary	0.072-0.147 x 0.055-0.132	65-167 x58-144 µm
Ovum	19-25 x 9-13 um	10-25x10-20µm
No. vitelline	Rg (9-22) , Lg (11-25)	Rg (9-18),Lg (7-19)
Host	*A. tridens* and *R. hardwickei cystops*	*R. hardwickii cystops*
Site of infection	Small intestine	Tissue of liver
Location	Qena, Luxor, Giza and Cairo, Egypt	Edfou and Aswan Governorate, Egypt

Discussion

Helminthes infecting *Pipistrellus kuhli*, *Rousettus aegyptiacus aegyptiacus* and *Rhinopoma hardwickei cystops* were studied (Saoud and Ramadan, 1977; Ramadan *et al,* 1988). *L. granulosum* was first reported in Egypt (Macy *et al,* 1961). Matskási (1971) reported *L. linstowi* as a harvest mouse parasite. Matskási (1973) reported *L. linstowi* in India. Saoud and Ramadan (1976b) gave an overview of genus *Lecithodendrium* Looss, 1896 infecting some Egyptian b-ts. They recorded *L. linstowi* and identified *L. aegyptiacus* and *L. duboisi* as new species from small intestines of *A. tridens tridens*, *R. hardwickei cystops*, and *T. nudiventris nudiventris*, respectively. Matskási (1980) in Iraq reported *L. linstowi*. The nine species were: *L. linstowi* Dollfus, 1931; *L. scotophilt* Kumari and Gupta, 1984; *L. abdussalami* Kumari and Gupta, 1984; *L. duboisi* Saoud and Ramadan, 1976b; *L. mystacini* Zdzitowiecki, 1969; *L. gurdaspurense* Kumari and Gupta, 1984; *L. minutum nabhaensis* Kumari and Gupta, 1984; *L. bezubiki* Kumari and Gupta, 1984; and *L. aegyptiacus* Saoud and Ramadan, 1976b. Also, Botella *et al.* (1993) in Spain identified *L. mystacini* and *L. linstowi*. El-Damarany *et al.* (1997) in Sohag reported a *Lecithodendrium* sp. Esteban *et al.* (2001) in Spain reported *Lecithodendrium* spp. Kirillov *et al.* (2012) reported *L. Rysavyi*, *L. Linstowi*, and *L. Skrjabini.* Lord *et al.* (2012) in South Lancashire and Greater Manchester reported *L. linstowi* and *L. spathulatum*. Hovat *et al.* (2017) in Serbia reported *L. linstowi*, which was reported by Sümer and Yildirimhan (2018) in Turkey.

The present is the first *Lecithodendrium* species parasitizing bat liver tissue, With the exception of spine-covered tegument *L. aegyptiacus*, but other species lacked tegumental spines. Besides, the present species differed from tegumental spines *L. aegyptiacus* by: 1- Smaller length/width ratio, 2- Larger oral & ventral suckers, 3- Oval ovary lobul-

ated in former one, 4- Vitelline follicles few in numbers, 5- Eggs large sized, moreover liver was the infected site instead of the small intestine in *L. aegyptiacus*.

L. aswani n. sp. molecular sequence was unique with great similarity (95.75%) to the sequence recently deposited to GenBank (MW414609) from an undefined cercaria recovered from a *Melanoides tuberculata* in Brazil (Lopes *et al,* 2021). This unspecified larval trematode was a novel parasite in Americas and categorized as a member of family Lecithodendriidae. *Melanoides tuberculata* was encountered in Egypt (Yousif *et al,* 2009). Again, the present one was comparable (86.98%) to the sequence of *L. linstowi* derived from cercariae of *Lymnaea stagnalis*, *Stagnicola palustris*, and *Bithynia tentaculata* in Danish freshwater lakes (Duan *et al,* 2021). It was similar (87.04%) to *L. spathulatum* sequence (JF784192) obtained from pipistrelle bats (*Pipistrellus pipistrellus* & *P. pygmaeus*) in Greater Manchester and Lancashire, England (Lord *et al,* 2012). The present species showed 86.67% great similar to a sequence of *L. linstowi* (JF784190) (unpublished data).

Saoud and Ramadan (1976b) reported *Lecithodendrium* in *R. hardwickei cystops, T. nudiventris nudiventris*, and *A. tridens tridens* with prevalence of 1.3, 12.8, & 14.8%, respectively, whereas the current one was 1% in *R. hardwickei cystops*. Also, a 5.88% prevalence of *L*. sp. was in Sohag (El-Damarany *et al,* 1997), 93% in Spain (Esteban *et al,* 2001), 84.4% infected *L. linstowi* and a 19.6% infection *L. spathulatum* in England infected (Lord *et al,* 2012), 19.5% in Serbia (Horvat *et al,* 2017), 80% in Turkey (Sümer and Yildirimhan (2018).These variations could be attributed to host-parasite system, host specificity, diet, sex, or ecological factors (Saoud and Ramadan, 1976a; Tinsley *et al,* 2020).

Consequently, based on the diagnostic differences between the present one and *L. aegyptiacus,* and molecular analysis, and hence the name *L. aswani* new species.

Conclusion

No doubt, bats are hosts for many ectoparasites and act as reservoirs for several infectious agents exhibiting zoonotic potential.

Lecithodendrium aswani n. sp, as the first species parasitized *R. hardwickei cystops* liver tissue trapped in Aswan Governorate. This was proved by descriptive morphology from other species encountered, molecular and phylogenetic analyses**.**

References

Abdel-Azim, M, 1936: On the life history of *Lecithodendrium pyramidum* Looss, 1896 and its development from xiphidocercaria *C. pyramidum* sp. nov. from *Melania tuberculata.* Ann. Trap. Med. Parasitol. 30: 351-156.

Banerjee, A, Kulcsar, K, Misra, V, Frieman, M, Mossman, K, 2019: Bats and coronaviruses. Viruses 11, 1:41-8.

Botella, P, Sanchez, L, Esteban, JG, 1993: Helminth fauna of bats in Spain. III. Parasites of *Pipistrellus pipistrellus* (Schreber, 1774) (Chiroptera: Vespertilionidae). Res. Rev. Parasitol. 53, 1/2:63-70.

Calisher, CH, Childs, JE, Field, HE, Holmes, KV, Schountz, T, 2006: Bats: important reservoir hosts of emerging viruses. Clin. Microbiol. Rev. 19:531-45

Dereeper, A, Audic, S, Claverie, JM, Blanc, G, 2010: Blast-Explorer helps you building datasets for phylogenetic analysis. BMC Evol. Biol. 10:1-6.

Dietz, C, 2005: Illustrated identification key to the bats of Egypt. Electronic Publication, Version, 1.

Dobson, AP, 2005: What links bats to emerging infectious diseases? Science310:628-9.

Duan, Y, Al-Jubury, A, Kania, PW, Buchmann, K, 2021: Trematode diversity reflecting the community structure of Danish freshwater systems: molecular clues. Parasit. Vectors14, 1:1-15.

Edungbola, LD, 1981: Parasites of house-dwelling, insectivorous bats from Alabe, Kwara State, Nigeria. J. Parasitol. 67, 2:287-8.

El-Damarany, M, EL Nazer, M, Mazen, N, 1997: On some trematodes recovered from bats (Chiroptera) in Sohag, southern Egypt. J. Egypt. German Soc. Zool. 22:43-58.

El-Naffar, MK, OMran, L, Mandour, AM, 1978: *Acanthatrium* (A) *assiuti* sp. n. and other trematodes recovered from the bat (*Vespertilioinesi)* collected from Assiut. J. Egypt. Soc. Para-

rasitol. 32, 1:107-12.
Esteban, JG, Amengual, B, Cobo, JS, 2001: Composition and structure of helminth communities in two populations of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) from Spain. Folia Parasitol. 48, 2: 143-8.
Fahmy, MAM, Arafa, MS, Khalifa, R, Abdel Rahman, AM, Mounib, ME, 1984: Studies on some helminthes parasites in some mammals in Assuit Governorate, II Trematode parasites of the desert bat *Rhinopoma hardwickei cystops.* Assiut Vet. Med. J. 11: 54-57.
Farina, LL, Lankton, JS, 2018: Chiroptera: Pathology of Wildlife and Zoo Animals: 607-33.
Galazzo, DE, Dayanandan, S, Marcogliese, D J, McLaughlin, JD, 2002: Molecular systematics of some North American species of *Diplostomum* (Digenea) based on rDNA-sequence data and comparisons with European congeners. Can. J. Zool. 80, 12: 2207-17.
Hafez, **MH, Attia, YA, 2020:** Challenges to the poultry industry: Current perspectives and strategic future after COVID-19 Outbreak Front Vet Sci. 2020; 7: 516: Online 2020 Aug 26.
Heyneman, D, Macy, RW, 1962: Helminths reported from bats (Chiroptera) in Egypt with an illustrated key to the common flukes. J. Egypt. Publ. Hlth. Assoc. 25, 109-142.
Horvat, Ž, Čabrilo, B, Paunović, M, Karapandža, B, Jovanović, J, Budinski, I, Čabrilo, O B, 2017: Gastrointestinal digeneans (Platyhelminthes: Trematoda) of horseshoe and vesper bats (Chiroptera: Rhinolophidae and Vespertilionidae) in Serbia. Helminthologia. 54, 1:17-25.
Jonsson, NN, Johnston, SD, Field, H, de Jong, C, Smith, C, 2004: Field anesthesia of three Australian species of flying fox. Vet. Rec. 154, 21:664 doi: 10.1136/vr.154.21.664.
Kirillov, AA, Kirillov, NYu, Vehkink, VP, 2012: Trematodes of bats (Chiroptera) from the middle volage region.46, 5:384-413.
Kumari, S, Gupta, NK, 1984: On two new species of trematodes from bats in India. II. Revista iberica de Parasitologia. ISSN: 0034-9623
Lee, MR, 1993: The petrography, mineralogy and origins of calcium sulphate within the Cold Bokkeveld CM carbonaceous chondrite. Meteoritics28, 1: 53-62.
Looss, A, 1896: Recherches sur la fauna parasitaire de l'Égypte. Premier Partie. Mem. Inst. Egypt. 3:1-252.
Looss, A, 1899: Weitere Beiträgc zur kenntnis der Trematoden: Fauna Aegyptens, zugleich Versuch einer natiirlichen Gleiderung des Genus *Distomum* Retzuis. Zool. Jber. S. 12:52-784.
Lopes, AS, Pulido-Murillo, EA, López-Hernández, D, de Melo, AL, Pinto, HA, 2021: First report of *Melanoides tuberculata* (Mollusca: Thiaridae) harboring a xiphidiocercaria in Brazil: A new parasite introduced in the Americas? Parasitol. Inter. 82:102284.
Lord, JS, Parker, S, Parker, F, Brooks, DR, 2012: Gastrointestinal helminths of pipistrelle bats (*Pipistrellus pipistrellus/Pipistrellus pygmaeus*; Chiroptera: Vespertilionidae) of England. Parasitology 139, 3:366-74.
Macy, RW, Heyneman, DO, Kuntz, RE, 1961: Records of trematodes of the families Lecithodendriidae, Dicrocoeliidae, & Heterophyidae from Chiroptera collected in Egypt and Yemen, SW Arabia. Proc. Helminthol. Soc. Wash. 28:13-7.
Matskási, I, 1971: The Hungarian harvest mouse (*Micromys minutus pratensis*), a new host of the bat fluke *Lecithodendrium linstowi* (Trematodes). Parasit. Hung. 4:137-144.
Matskási, I, 1973: Trematodes of bats in India. Parasit. Hung. 6: 77-97.
Matskási, I, 1980: Trematodes of bats in Iraq. Parasit. Hung. 13: 7-12.
Misra, PK, Gautam, NK, Elangovan, VA, 2019: Bat guano: A rich source of macro and micro-elements essential for plant growth. Ann. Plant Soil Res. 21, 1:82-6.
Morsy, TA, Khalid, MLM, Bebars, MA, Abdel Hamid, MY, 1986: *Trypanosoma vespertilionis* (Battaglia, 1904) from Egyptian Sars. J. Egypt. Soc. Parasitol. 16, 2:373-8.
Morsy, TA, Salama, MMI, Abdel Hamid, MY, 1987: Detection of *Leishmania* antibodies in bats. J. Egypt. Soc. Parasitol.17, 2:797-8.
Odhner, T, 1911: Nordafricanische Trematoden Grisstenteils vom Weissen Nil (von der Schwedischen zoologischen Expedition gesammelt). I. Fascioliden. Jäigerskiold, LA Zool. Exped. Egypt.78, 23: 4.
Ramadan, MM, Saoud, MFA, Aly, AH, 1988: *Caprimolgorchis jhai* sp. nov. (Trematoda: Lecithodendriidae), an intestinal parasite of Egyptian house sparrow. Parasitol. Res**.** 75:28-30.
Saad, AI, Younis, AE. Rabei, JM, 2018: Experimental life cycle of *Contracaecum quadripapillatum* n. Sp. In white pelican (*Pelecanus erythrorhynchus*) at Lake Nasser, Egypt: Morphological and genetic evidences. J. Egypt. Soc. Parasitol. 48, 3:587-98.
Saoud, MFA, 2009: On a new trematode, *Trem-*

ajoannes buckleyi gen et sp. nov. (Lecithodendriidae) from Central American bats with some notes on *Phaneropsolus orbicularis* (Diesing, 1850) Braun, 1901. J. Helminthol. 38:97-108.

Saoud, MFA, Ramadan, MM, 1976a: Studies on the helminth parasites of bats in Egypt and the factors influencing their occurrence with particular reference to digenetic trematodes. Zeitschrift für Parasitenk.51, 1:37-47.

Saoud, MFA, Ramadan, MM, 1976b: Studies on trematodes of the genus *Lecithodendrium* Looss, 1896 from some Egyptian bats, with a description of two new species. J. Helminthol. 50, 4:281-5.

Saoud, MFA, Ramadan, MM, 1977: A review of trematodes genus *Anchitrema* Looss, 1899 *(Dicroceliidae)*, with a redescription of *Anchitrema sanguineum* (Soncino, 1894) and *Anchitrema longiformi* n. sp. from some Egyptian bats. Z. Parasitked. 54: 61-67.

Saoud, MFA, Ramadan, MM, 1980: A review of genus *Acantbatrium* Faust, 1919 (Trematoda: Lecithodendriidae) with a description of two new species. J. Egypt. Soc. Prasitol. 10, 2:387-92.

Shaheen, L, 2007: Tube drawing principles: Understanding processes, parameters key to quality, retrieved 2008-10-.

Sümer, N, Yildirimhan, HS, 2018: Helminth parasites of the whiskered brown bat, *Myotis aurescens*, Kuzyakin, 1935 (Chiroptera: Vespertilionidae) from Turkey. Acta Zool. Bul. 70:113-6.

Szentiványi, T, Christe, P, Glaizot, O, 2019: Bat flies and their micro-parasites: Current knowledge and distribution. Front. Vet. Sci. 6:115.

Tinsley, RC, Vineer, HR, Grainger-Wood, R, Morgan, ER, 2020: Heterogeneity in helminth infections: Factors influencing aggregation in a simple host-parasite system. Parasitology 147: 65-77.

Tremlett, CJ, Peh, KSH, Zamora-Gutierrez, V, Schaafsma, M, 2021: Value and benefit distribut-ion of pollination services provided by bats in the production of cactus fruits in central Mexico. Ecosystem Services 47:101197.

Urushadze, L, Bai, Y, Osikowicz, L, McKee, C, Sidamonidze, K, Putkaradze, D, 2017: Prevalence, diversity, and host associations of *Bartonella* strains in bats from Georgia (Caucasus). PLoS Negl. Trop. Dis. 11:1-19.

Yousif, F, Ibrahim, A, Sleem, S, El-Bardicy, S, Ayoub, M, 2009: Morphological and genetic analyses of Melanoides tuberculata populations in Egypt. Glob. J. Mol. Sci. 4: 112-7

Explanation of figures

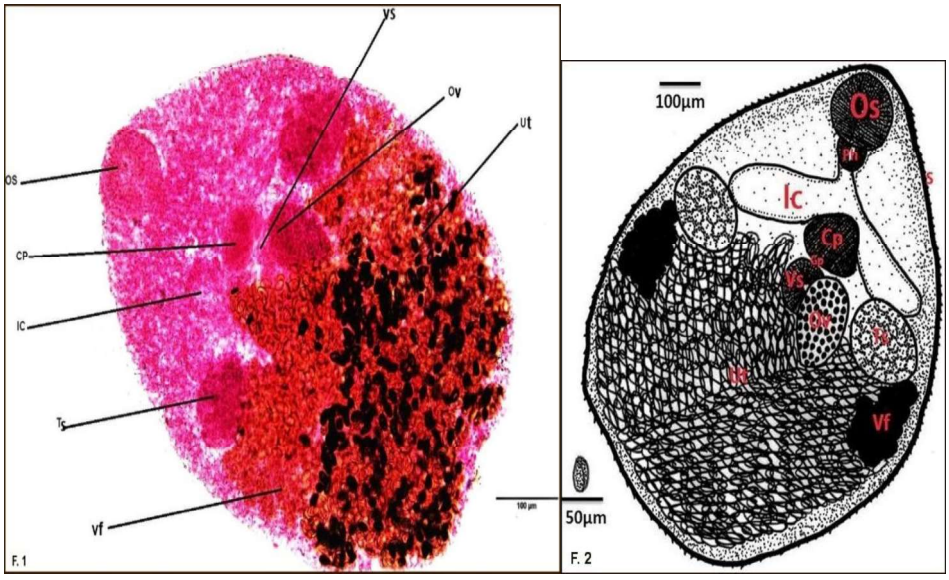
Fig. 1: *L. aswani* n. sp.: Ventral view of whole mounted parasite. OS, oral sucker; VS, ventral sucker; Gp, genital pore; Ph, pharynx; VF, Vitelline follicle; CP, cirrus pouch; Ov, ovary; Ts, testes; Ut, uterus; IC, intestinal caecum.

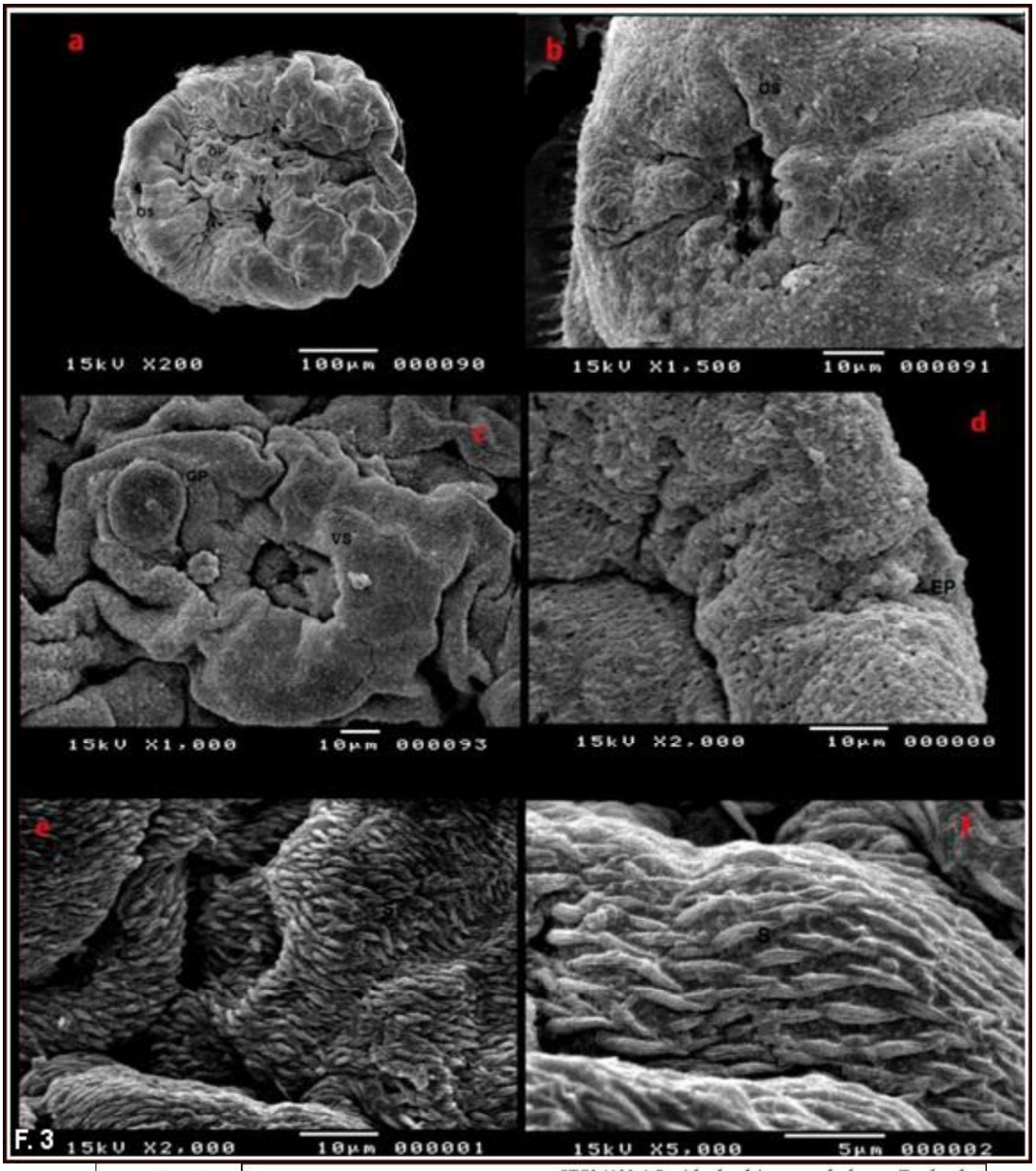
Fig. 2: *L. aswani* n. sp.: Camera Lucida drawing of whole mounted parasite.

Fig. 3:SEM of *L. aswani n. sp.* a, whole ventral view of adult worm; b, high magnification oral sucker; c, high magnification of ventral sucker & genital pore; d, high magnification of excretory pore; e, body wall with spines; f, high magnification of spines in body wall.

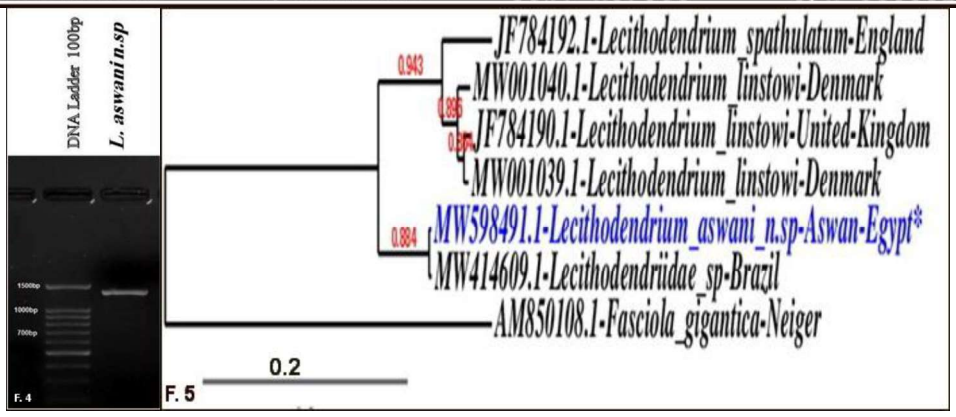
Fig. 4: Ampilicons of ITS1, 5.8S, ITS2 region of *L. aswani n. sp.*

Fig. 5: Phylogenetic tree of ITS1, 5.8S, ITS2 region of *Lecithodendrium.*




a
b
OS
15kV X200 100µm 000090
15kV X1,500 10µm 000091
GP
VS
d
EP
15kV X1,000 10µm 000093
15kV X2,000 10µm 000000
e
S
15kV X2,000 10µm 000001
15kV X5,000 5µm 000002

F. 3


DNA Ladder 100bp
L. aswani n.sp
1500bp
1000bp
700bp
JF784192.1-Lecithodendrium_spathulatum-England
MW001040.1-Lecithodendrium_linstowi-Denmark
JF784190.1-Lecithodendrium_linstowi-United-Kingdom
MW001039.1-Lecithodendrium_linstowi-Denmark
MW598491.1-Lecithodendrium_aswani_n.sp-Aswan-Egypt*
MW414609.1-Lecithodendriidae_sp-Brazil
AM850108.1-Fasciola_gigantica-Neiger
0.943
0.884
0.2

F. 4 F. 5