

# New record of *Thubunaea Pudica* Seurat, 1914 (Spirurida: Physalopteridae) in lizard *Trapelus mutabilis* from Egypt

Original  
Article

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## ABSTRACT

**Background:** Studies on nematode taxa remain poorly described in cold blooded animals, with rareness of data on the helminth community of Egyptian ones, especially lizards. The available literatures are mostly restricted to ecological studies rather than descriptive ones.

**Objective:** To identify and give full description for nematodes that inhabit the Desert *Agama*, *Trapelus mutabilis* (*T. mutabilis*) caught from El-Dabaa desert, Egypt.

**Material and Methods:** Nineteen *Agama* lizards having the characteristic morphological criteria of *T. mutabilis* were dissected and examined for parasitic infection. Gastrointestinal nematodes were collected, fixed and identified using light microscopy. Those subjected for scanning electron microscopy (SEM) were dried, coated and examined.

**Results:** Seven out of nineteen dissected lizards were found to be infected with the nematode *Thubunaea pudica* (*T. pudica*) (Family: Physalopteridae). They were collected from the stomach and small intestine of *T. mutabilis*. The main characteristics of adult *T. pudica* are: symmetrical anterior cephalic structure similar in both sexes, vulva is situated in the first tenth of the body, the tip of male tail ends beyond well-developed caudal alae with 32 true caudal papillae and two subequal stout spicules.

**Conclusion:** The study added the first fully described details for *T. pudica* using both light and SEM. Moreover, *T. mutabilis* lizard represents a new host record for *T. pudica* in a new geographic locality El-Dabaa desert as there are no reports of this species recorded from Egypt.

**Keywords:** Egypt, El-Dabaa deserts, light microscope, reptile, SEM, *Thubunaea pudica*, *Trapelus mutabilis*.

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## INTRODUCTION

Although more than 400 species of reptiles have been reported in Egypt<sup>[1]</sup> there is minimal data on their helminth assemblage. Worldwide studies on the helminth communities of reptiles are limited to information regarding their ecology and taxonomy rather than their detailed description<sup>[2]</sup>.

Lizards are good models for parasitological and ecological studies that describe their helminthic infections, different feeding habits, activities and flexibility of habitat location<sup>[3]</sup>. Previous studies demonstrated the high biodiverse capacity of parasitic nematodes that accompanies the varied predispositions of lizards<sup>[4]</sup>. However, this parasite fauna is rarely explored in lizards as a host, with respect to the taxonomic aspects, compared to other groups of vertebrates<sup>[5,6]</sup>.

The lizard family Agamidae Gray, 1827 is represented in Africa by five genera: *Agama* Daudin, 1802; *Trapelus*

Cuvier, 1817; *Uromastix* Merrem, 1820; *Acanthocercus* Fitzinger, 1843 and *PseudoTrapelus* Fitzinger, 1843. Many species of this family were classified as members of the genus *Agama*, until Moody<sup>[7]</sup> reviewed previously proposed generic names for species-groups.

The genus *Trapelus* Cuvier, 1817 which is distributed from northern Africa across the Middle East to Asia consists of 15 species. Five species occur in northern Africa: *T. mutabilis* Merrem, 1820 and *T. pallidus* Reuss, 1833 in Egypt; *T. schmitzi* Wagner and Böhme, 2007 in Algeria and Niger; *T. tournevillei* Lataste, 1880 in Algeria and Tunisia; and *T. savignii* Dumeri and Bibron, 1837 in Eastern Egypt. They are characterized by short thick heads, deeply sunken tympana and a few spiny scales above the ear opening<sup>[7]</sup>.

*T. mutabilis* lizard, is widespread, occurring across northern Africa from the western Sahara through Mauritania Mali, Morocco, Algeria, Tunisia, Libya, and the Sudan to Egypt<sup>[8]</sup>. It differs from its close relative species, *T. pallidus* within the genus by possessing

irregular dorsal rhomboidal and scattered enlarged scales; but it has similar scales on the hind limbs and base of the tail. Coloration of the live specimen is sandy gray with four to five transverse bands. Male specimen has violet blue throat<sup>[8,9]</sup>.

The nematode family Physalopteridae constitutes three subfamilies: Thubunaestinae Sobolev, 1949, occurs only in reptiles; Physalopterinae Railliet, 1893, is common in reptiles, birds, mammals, rare in amphibians and absent in fish<sup>[10]</sup>; and Proleptinae Schulz, 1927, occurs mainly in fish and rarely in reptiles. Two genera are included in Thubunaestinae; *Thubunaea* Seurat, 1914 and *Physalopteroides* Wu and Liu, 1940. They are parasites of reptiles and are characterized by absence of a cephalic ring, presence of many caudal papillae with an ornamented cuticle forming papillary plates on male caudal surface<sup>[11]</sup>. The two genera differ from each other by the symmetry of their cephalic structures which is symmetrical in *Thubunaea*, and asymmetrical in *Physalopteroides*<sup>[11]</sup>.

Pereira *et al.*<sup>[12]</sup> pointed to the fact that the taxonomy of several nematodes remain poorly described in these cold-blooded animals in Egypt. Hence, full morphological studies of these parasites are important to complete the database and help clarify the current taxonomic confusion. Accordingly, the present study was planned to give for the first time, a full detailed descriptive light and SEM picture of adult nematode species affecting *T. mutabilis* lizard caught from EL-Dabaa desert, Egypt.

## MATERIAL AND METHODS

Nineteen *Agama* lizards caught from EL-Dabaa desert, Matrouh (30°1'36"N, 28°26'9"E) during the period from September 2014 to August 2015, having the morphological and characteristic picture of *T. mutabilis* (Reptilia: Agamidae), were examined for parasitic infection. The collected hosts were anesthetized using chloroform. The gastrointestinal tracts were removed in 0.7% saline solution and each part was examined separately. Nematodes found in the stomach and anterior parts of small intestine were selected. Live specimens were washed in physiological saline and fixed relaxed in warm 70% ethanol with 10% glycerol. Specimens were cleared in lactophenol then mounted in glycerin jelly for light microscopical examination and identification.

Specimens were measured and examined using Olympus CX31 microscope, and Zeiss Stemi 2000-C microscope, and representative photomicrographs were taken with E-330DC 7.4V digital camera. Drawings were made with the aid of camera Lucida.

For SEM, specimens were isolated carefully and washed in isotonic saline solution, followed by

phosphate buffer saline and fixed overnight in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C. Specimens were post fixed in 1% osmium then dehydrated through graded series of ethanol. Dehydration was performed in two changes of absolute ethanol and critical point dried. Specimens were mounted on stubs, coated with gold and examined with a JEOL (5300 JSM) SEM at an accelerating voltage of 25 K.V.

Identification of the host, followed the key for the African taxa of the genus *Trapelus*<sup>[9]</sup>, and identification of the collected nematode species followed the key of Anderson *et al.*<sup>[13]</sup> and Gibbons<sup>[14]</sup>. All measurements were given in millimetre.

**Ethical Consideration:** Zoology Department's council of the Faculty of Science, Tanta University, Egypt, approved the experimental design and the plan of work.

## RESULTS

From seven out of the nineteen identified *T. mutabilis* lizard examined for gastrointestinal parasitic infection, twenty-nine nematode specimens (eighteen females and eleven males) of *T. pudica* Seurat, 1914 belonging to family Physalopteridae were identified and described using light and SEM.

**Family:** Physalopteridae Railliet, 1893

**Subfamily:** Thubunaestinae

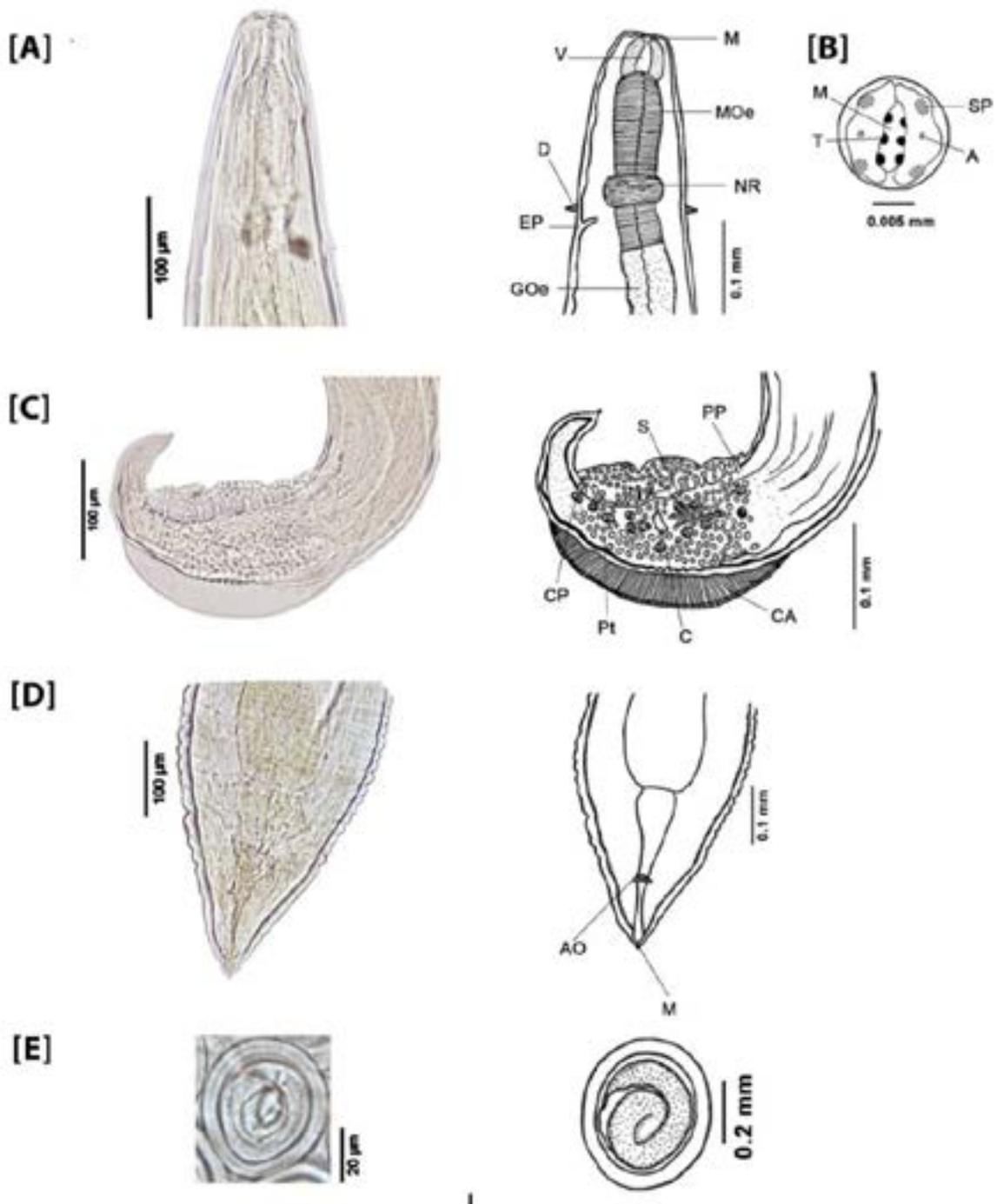
*T. pudica* Seurat, 1914 (Figures 1 and 2).

**General description:** Adults are medium sized with finely transverse striation of cuticle along their entire body; cephalic end is rounded and similar in both sexes. Mouth is dorsoventrally elongated and surrounded by two symmetrical lips. Each lip is provided with three rounded forwardly directed teeth on its inner side, with a pair of submedian papillae and a small lateral amphid between them (Figures 1B, and 2A). Deirids are small, simple, and situated in the lateral cervical region slightly below nerve ring level (Figures 1A and 2B). Mouth leads into short vestibule (Figure 1A). Oesophagus is composed of anterior muscular part, encircled by nerve ring and posterior glandular part. Excretory pore opens to the exterior, slightly anterior to the junction of both portions of oesophagus immediately behind the nerve ring (Figures 1A and 2C).

**Male** (seven adult specimens): Body length: 5.25-10.85; maximum width: 0.10-0.42. Vestibule length is 0.03-0.07. Entire oesophagus measures 0.83-2.06 in length, muscular portion is 0.1-0.2. Nerve ring is 0.03-0.07, excretory pore is 0.2-0.22, and deirids are 0.16-0.17 from anterior end of body, respectively. There are two stout subequal spicules; the left spicule is longer measuring 0.05, while the right one measures 0.04. Tip of tail is pointed distally with well-developed caudal alae (0.02-0.03 wide) bearing numerous stout papilliform

irregularly arranged cuticular ornamentation (protuberances) on the ventral side. Caudal papillae can be distinguished by their larger size and defined shape. There are about 32 papillae; 9 pairs precloacal and 14 postcloacal. Of the 14 postcloacal papillae 9 are

on one side and 5 on the other side. SEM revealed that papillae are rather similar in appearance, while light microscopy revealed them as pedunculated (Figures 1C, and 2E). Minute lateral phasmidal pores are located slightly anterior to the tail projection (Figure 2F).



**Fig 1. *Thubunaea pudica*.** Camera Lucida.

**A**, anterior end of female, lateral view showing mouth (M), vestibule (V), muscular (MOe) and glandular (GOe) parts of oesophagus, nerve ring (NR), deirid (D) and excretory pore (EP).

**B**, enface view of cephalic region showing mouth (M) surrounded by two symmetrical lips; each lip has three rounded teeth (T), a pair of submedian papillae (SP) and a small lateral amphid (A).

**C**, caudal end of male, subventral view showing cloaca (C), caudal alae (CA), caudal papillae (CP), pedunculated papillae, (PP) protuberances (Pt) and two spicules (S).

**D**, posterior end of female showing anal opening (AO) and small mucron (M).

**E**, larvated egg, lateral view.

**Female** (seven gravid specimens): Length of body is 17.45-22.10 and maximum width is 0.7-1.05. Length of vestibule is 0.04-0.042. Length of entire oesophagus is 2-3.35, of which the muscular portion measures 0.2-0.25. Deirids and nerve ring are 0.2-0.21 and 0.16-0.3 respectively from anterior end of the body (Figures 1A, and 2B). Vulva is situated 2.05-3.7 from anterior extremity as a transverse slit bound anteriorly and posteriorly by slightly swollen lips (Figure 2D). Vagina is long and muscular. Two uteri contain numerous eggs with larvae. Eggs are oval, thick-walled, embryonated, and measure 0.03X0.04- 0.05X0.04 (Figure 1E). Tail

is conical, rounded containing anal opening and terminating in a small mucron (Figures 1D, and 2G).

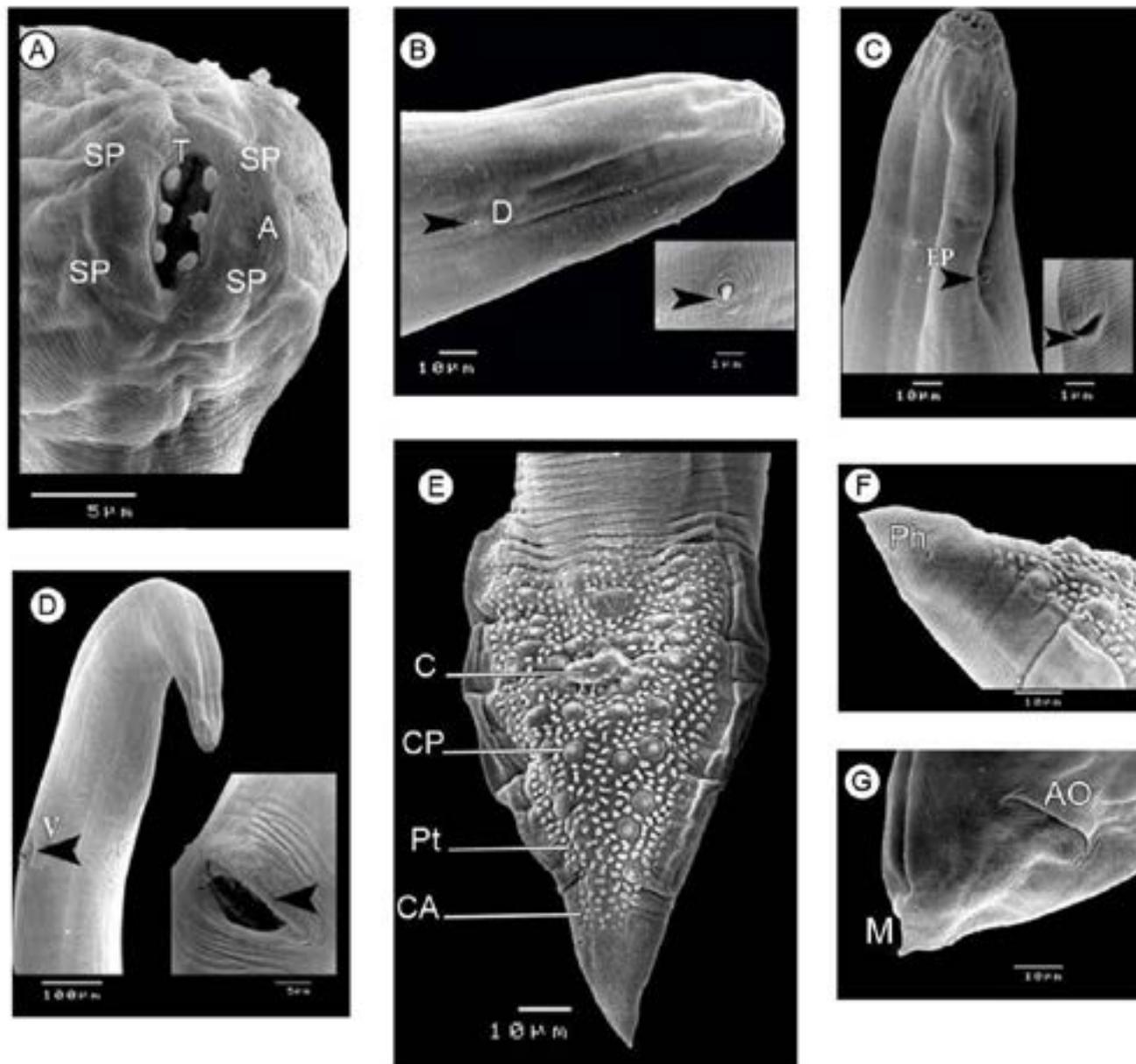
**Taxonomy summary:**

**Type of host:** Desert *Agama*, *T. mutabilis* Merrem, 1820. Collection site: El-Dabaa desert, Matrouh (30°1'36"N, 28°26'9"E).

**Collection time:** September 2014 to August 2015.

**Site of infection:** Stomach lumen and anterior part of small intestine.

**Prevalence:** 36.8% (7 out of 19 hosts examined), with mean intensity of 4.14 (1-17 worms/host).



**Fig 2.** *Thubunaea pudica*. SEM micrographs.

**A**, apical view of female, showing two symmetrical lips, 3 rounded teeth (T), a pair of submedian papillae (SP) and small lateral amphid (A) on each lip.

**B and C**, anterior end of female, lateral view showing deirids (D) and excretory pore (EP).

**D**, anterior end of female, ventrolateral view showing vulva (V).

**E**, caudal end of male, ventral view showing cloaca (C), caudal alae (CA), caudal papillae (CP) and protuberances (Pt).

**F**, caudal end of male, ventrolateral view showing phasmid (Ph).

**G**, posterior end of female, ventral view showing the anal opening (AO) and small mucron (M).

**DISCUSSION**

In our study, description of the discovered “*Thubunaea*” species is based on the generic characters given by Seurat<sup>[15]</sup> and Belle<sup>[16]</sup>. These characters are: mouth with two lateral simple lips, each lined with three small teeth in the internal surfaces and a pair of submedian papillae; no lateral flanges; cervical papillae immediately behind the nerve ring; short vestibule; male caudal alae are well developed with four pairs of pedunculated pre-anal papillae and four to five pairs of pedunculated post-anal papillae and numerous sessile papillae around the anus; two stout subequal spicules present; female posterior end terminating in a small conical point; vulva is in the anterior fifth of the body; two parallel uteri; oviparous eggs. Type species: *T. pudica* Seurat, 1914.

The morphological characterization and measurements of our nematode specimens are similar to the original description of *T. pudica* reported from several species of reptiles, including *Scincus scincus*, from North Africa<sup>[15]</sup>. In Egypt, *T. pudica* has been included in the checklist from the stomach of the snake *Psammodphis schokari* by Myers *et al.*<sup>[17]</sup> and from the stomach of *Scincus scincus* in Abu Rawash<sup>[17]</sup>.

In comparison with the previously reported *T. pudica*, our present specimen is identical to the description by Belle<sup>[16]</sup> except for the length of the male spicules, where the present male spicules are shorter; left spicule is 0.05 mm vs. 0.16 mm and right spicule is 0.04 mm vs. 0.10 mm.

The present description somewhat resembles the description of Moravec *et al.*<sup>[18]</sup>, except for some

biometric features, where the present female specimens are longer in length (17.45-22.1 mm vs. 15.64-19.17 mm) and larger in width (0.7-1.05 mm vs. 0.381-0.54 mm). The present specimens also have longer oesophagus compared to that described by Moravec *et al.*<sup>[18]</sup> (2-3.35 mm vs. 1.93-2 mm). These differences may be due to host variation and parasitological technique (type and concentration of fixative and relaxing agents), since all other general morphological features are the same.

The number of male caudal papillae in the present specimens is 32 which is in agreement with the type species of Seurat, 1914, but differs from that reported by Moravec *et al.*<sup>[18]</sup> who detected 35 papillae in one larger specimen. This difference may be because the most anterior pairs of preanal papillae are usually poorly observable than others or may be hidden in the wrinkles at this part. It is also concomitant with Sharpilo<sup>[19]</sup> who reported that there is a probable intraspecific variability in the number and distribution of caudal papillae in male *Thubunaea* members. The difference between SEM and light microscopy of sessile-pedunculated appearance in the caudal papillae observed in our study may be explained by their ability for retraction.

Although more than twenty three species of *Thubunaea* are currently distributed all over the world<sup>[20]</sup>, their ultrastructural-topography has rarely been studied using SEM, which provides detailed information to assess the validity of this genus. The difference in morphological and biometric measurements between the present *T. pudica* and the other reported species are illustrated in table (1). To the best of our knowledge, SEM studies are still scarce

**Table 1.** SEM characteristics of male *Thubunaea* species

	<i>T. ctenosaur</i> <sup>[21]</sup>	<i>T. mobedii</i> <sup>[22]</sup>	<i>Thubunaea</i> spp. <sup>[23]</sup>	<i>T. leonregagnonae</i> <sup>[24]</sup>	<i>T. eleodori</i> <sup>[25]</sup>	<i>T. pudica</i> (Present study)
<b>Cuticle</b>	Smooth	With fine cross striation	With transverse striation	With fine transverse striation	Finely annulated	With finely transverse striation
<b>Pseudo-labium</b>	With a pair of sub-median papillae and 1 amphid	With a pair of papillae on outer margin and 1 amphid	With heavily toothed outer margin and 2 amphids	With a pair of sub-median papillae and 1 amphid	With a pair of papillae and 1 amphid	With a pair of sub-median papillae and 1 amphid
<b>Excretory pore site*</b>	0.16	0.27	0.37	0.17	0.20	0.21
<b>Length</b>	8.1	13		6.19	10.25	6.9
<b>Oesophagus</b>	0.85	1.5		0.8	1.16	1.29
<b>No. of pedunculated papillae</b>	16	Absent	Data are not available for male	10-16	12	16
<b>No. of sessile papillae</b>	17	28		11-14	14	16
<b>Spicules</b>	Two equal spicules 0.066	Absent		Absent	Not observed	Two sub-equal spicules; left 0.05 and right 0.04

\* From the anterior end, All measurements in mm

for members of this genus, being available only for five species; *T. ctenosauri* from *Ctenosaura pectinata* in Mexico<sup>[21]</sup>; *T. mobedii* from *Laudakia nupta* in Iran<sup>[22]</sup>; *Thubunaea* sp. from *Paraechinus aethiopicus* in Central Saudi Arabia<sup>[23]</sup>; *T. leonregagnonae* from *Sceloporus pyrocephalus* in Mexico<sup>[24]</sup> and *T. eleodori* from *Liolaemus eleodori* in Argentina<sup>[25]</sup>.

The present study provides for the first time SEM morphological characterizations of both male and female *T. pudica* in Egypt. It shows the accurate symmetrical description of the anterior cephalic region, as well as location of amphids, full description of caudal region in males, including papillae distribution and the position of phasmids in both sexes. Moreover, this study records for the first time *T. pudica* in *T. mutibilis* lizard host in El-Dabaa desert as a new geographical locality in Egypt.

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**Author's contribution:** SF Harras shared in designing the study and research implementation, and in identifying the worms, reviewed the literatures, collected the data, and wrote the manuscript. RA Elmahy proposed the research idea, shared in collection and identification of the worms.

**Conflict of interest:** Authors confirm that there are no known conflicts of interest associated with this study.

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