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Phenotypic description of Egyptian endemic centipedes, genus *Scolopendra* Linnaeus, 1758 with a histological study of its venom glands

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Abstract

Centipedes are widely distributed over all continents. They have significant medicinal value and high toxicity, making them an intriguing subject for evolutionary research. The current study aims to provide the first comprehensive analysis of the morphology, description, and distribution preferences of the Egyptian Scolopendromorpha centipede fauna. According to surveillance research in Egypt, two *Scolopendra* species have been re-described. *Scolopendra canidens*Newport.1844 were discovered in only one location, whereas *Scolopendra morsitans s* Linnaeus,1758 was more abundant. Both venomous glands were histochemically investigated. This study is the first comparative report on the histology and histochemistry of the venom glands of the Egyptian centipede species. Both species' glands are primarily composed of columnar secretory cells radially arranged side by side and open through pores in a central chitinous duct. Each secretory cell is enclosed in striated muscle fibers. The secretion of both glands takes the shape of small PAS-positive granules, suggesting the presence of neutral polysaccharides. According to this surveillance study, the most abundant species was *Scolopendra morsitans s* Linnaeus,1758. *Scolopendra canidens* canidensNewport.1844 were found only on the Northwest coast of Egypt. The findings also revealed that the analyzed species are comparable in terms of their venom morphology and fundamental chemical composition.

Keywords Centipedes · Scolopendra · Egypt · Morphology · Poisonous gland

Abbreviations

PAS	Periodic acid–Schiff
S.	Scolopendra
pН	Potential of hydrogen
EDTA	Ethylenediaminetetraacetic acid
SC	Secretory cells
L	Lumen
Μ	Muscle sheath
С	Chitinous envelope
H&E	Hematoxylin and eosin

Background

Typically, morphological variation is responsible for differential adaptations to diverse ecosystems. Consequently, the study of intraspecific morphological variation is essential for comprehending the diversity of life and determining whether morphological variability is due to ecological and environmental causes or palaeogeographic history (Adams et al. 2004; Kamilari and Sfenthourakis 2009). *Scolopendra* has become an important research material for historical biogeography. Due to its limited mobility, it has also become a significant research material for historical biogeography (Kang et al. 2017).

Centipedes are spread across all continents except Antarctica. They are an economically significant group of arthropods that play a key role in managing the terrestrial ecosystem's toxic pests (Yadav 1994). Because of their toxic nature and painful bite, they are often regarded as significant creatures, particularly the Scolopendridae family members. They are elusive animals found in stones, potted plants, bushes, the bark of trees, gardens, rotting wood, damp soil, woodland, and cultivated and semi-cultivated soil (Sureshan

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et al. 2003). Taxonomic studies of Scolopendrid centipedes have frequently documented diversity at the level of individuals and populations and have applied these data to species separation challenges (Joshi and Edgecombe 2017). The common and complex centipede genus is *Scolopendra* Linnaeus, 1758. In recent years, the systematics and taxonomy of Old World *Scolopendra* species have been investigated. Diagnosis, classification key, and an illustrated list of Old World species of *Scolopendra* were established by Lewis (2010). Additionally, previous research has focused on the systematics and distribution of several species of *Scolopendra* (Shelley 2002, 2006; Shelley et al. 2005). Several studies (Kraepelin 1903; Attems 1930; Lewis 1985, 1996, 2001; Lewis and Gallagher 1993; Zapparoli 2002; Simaiakis and Mylonas 2008) have demonstrated that there are nine species widespread in the Mediterranean region, including the most widely distributed species, *Scolopendra cingulate* (Latreille 1829), which also exists in western Asia. Recent studies on the systematics and taxonomy of New World *Scolopendra* species are rare.

Scolopendra is typically composed of 42 nominal species in the Old World, as described by Lewis (2010). However, the precise detail of the distributional records is hampered by its dispersed distributional records. The ranges of some species are yet unknown and need to be updated. Certain *Scolopendra* species, such as *S. morsitans s* Linnaeus, 1758,

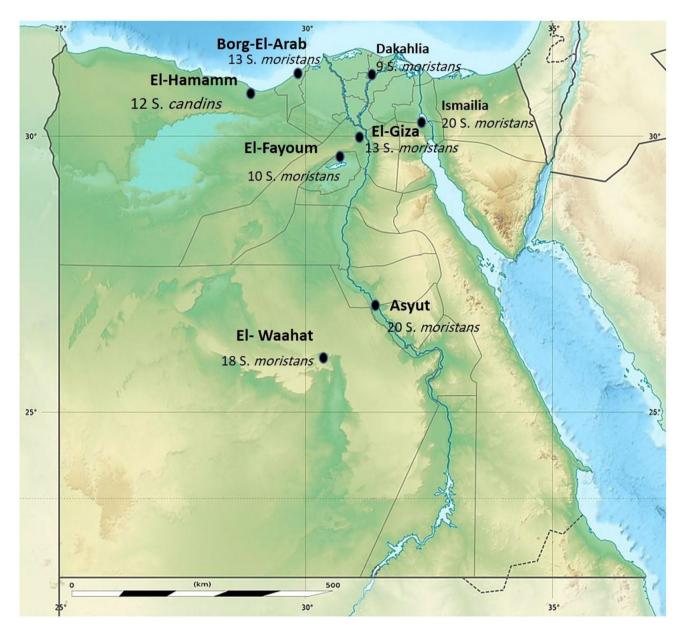


Fig. 1 Egypt map showing the samples collection sites. https://upload.wikimedia.org/wikipedia/commons/thumb/9/9e/Egypt_relief_location_map.jpg/650px-Egypt_relief_location_map.jpg?20170818140153

and *S. subspinipes* Leach, 1814, have been identified as widespread and recognized as introduced by human transport due to their ecological preferences and, in some cases, economic value (Shelley et al. 2005).

According to Guizze et al. (2016), species of the *Scolopendra* genus can reach up to 300 mm in length, and certain species are responsible for the vast majority of centipede-related human accidents worldwide. They frequently cause human accidents by injecting venom from glands within their maxillipeds, because they are well adapted to urban environments (Antoniazzi et al 2009). This gland secretion is used to kill or congeal prey by injection through horizontal movements of its maxillipeds (Stankiewicz et al. 1999).

This research aims to provide the first comprehensive examination of the morphology, description, and distribution preferences of the Egyptian Scolopendromorpha centipede fauna. In addition, the histology and histochemistry of its venom glands were studied.

Methods

Surveillance studies

Sites of collection

Scolopendra was collected and maintained in captivity from different areas along most of the governorates of Egypt (Fig. 1) from July 2019 to August 2020, as follows: 13 Samples from El-Giza (El-Mansouriya and Osim), 18 Samples from Baharia Oasis desert, 10 Samples from El-Faiyoum (Tamiya Center), 20 Samples from Ismailia (El-Qantra), Assiut: (3 Samples from (Borg El-Arab). To measure the length and conduct morphological analysis, 8–20 living animals were collected from each location of the study.

Identification

Scolopendra from each area was cursorily examined after anesthesia with 70% ethyl alcohol for the following characteristics: color, head (eyes, antenna, maxilliped), stigmata, legs, and anal cirri. The identification of samples was according to Lewis's (2010) identification key of oldworld *Scolopendra* species.

Histological studies

The venom apparatus captured from different areas was utilized. The venom apparatuses of ten adult specimens of each *Scolopendra canidens* (El-Hammam location) and *Scolopendra morsitans* (Assiut and El-Giza locations) captured were utilized in the present study. The maxillipeds were removed from each specimen and quickly immersed in a 10% neutral buffered formalin fixative (pH 7.4), then decalcified by EDTA. The fixed samples were thoroughly washed in running water, dehydrated, cleaned, impregnated, embedded in paraffin wax, sectioned at $4-5 \mu m$ thickness, and stained with hematoxylin–eosin for histological examination. For the identification of neutral and acidic mucosubstances, the sections were subjected to the periodic-acid Schiff (PAS) and Alcian blue, pH 2.5, techniques, as well as bromophenol blue for protein identification (Pearse 1985).

Using Image-Pro Express software (Media Cybernetics) and a digital camera attached to a ZEISS stereomicroscope and an Olympus (E330-ADU1.2X) microscope, photomicrographs were captured.

Results

The collected specimens were identified and displayed in Table 1.

Scolopendra canidens Newport.1844.

Figure 2

S. canidens Newport 1844 Ann. Mag. nat. Hist. 13: 98.

S. canidens cyrenaica Verhoeff 1908 Zool. Jahrb., Syst. 26: 274.

S. canidens canidens: Attems 1930 Das Tierreich 54: 36. S. canidens: Würmli 1980 Sber. Österr. Akad. Wiss. 189: 339 & 346.

Distribution: Italy, Morocco, Algeria, Libya, Egypt, Palestine, Turkey, Syria, Israel, Jordan, Tunisia, Iraq, Iran, Uzbekistan, Saudi Arabia, Yemen, Tajikistan, Armenia, Azerbaijan, Turkmenistan, Afghanistan.

Distribution in Egypt: (Table 1) The distribution of this species is less widespread than S. *morsitans*, which is restricted to the northwestern coast.

Description: Fig. 2 Tergite without curved anterior transverse sulcus, tarsus of the ultimate leg without spur, Coxopleural process long or moderate length and with six or more spines and 1 or 2 side spines. Tergite with a median longitudinal suture, basal 5, 6 or more antennomeres glabrous. First pair of legs with two tarsal spurs, rarely with only one. Each forcipular coxosternal tooth plate with four teeth, the lateral large and well separated, the medial three small and more or less fused. Forcipules trochanteric-prefemoral process without teeth. Tergite 1 lacks longitudinal sutures, while

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	Site	Altitude	No. of sam- ples	Color	Average length	Species
1.	Northern Giza: Aosem and Mansoura region	29.9870° N 31.2118° E	13	Olive green with reddish ultimate legs	7.2 ± 0.68	Scolopendra moristans
	Southern Giza: El Wahat El Bahariya	28° 21′ 5.36″ N 28° 51′ 44.6″ E	18			
2.	El-Fayoum (Tamya region)	29.308374° N 30.844105° E	10	Yellowish green with red- dish ultimate legs	6.9 ± 0.36	Scolopendra moristans
3.	Dakahlia Governorate (Delta) Belkas center	31° 14' N 31° 22' E	9	Yellowish green with red- dish ultimate legs	9.6 ± 0.32	Scolopendra moristans
4.	Ismailia Governorate) El Qantara city)	30.85° N 32.31° E	20	Yellowish green with red- dish ultimate legs	5.9 ± 0.56	Scolopendra moristans
5.	Asyut Governorate Dairut—Manfalut—Man- qabad	27° 11′ N 31° 10′ E	20	Deep green with reddish ultimate legs	7.6±1.1	Scolopendra moristans
6.	Alexandria Borg El-Arab region	30° 50′ 56″ N 29° 36′ 42″ E	13	Yellowish green with red- dish ultimate legs	4.7 ± 0.69	Scolopendra moristans
7.	Marsa Matrouh	30.841852° N 29.394043°	12	Yellowish-brown	8.1 ± 1.3	Scolopendra canidens
	El Hammam city	Е	10	Deep green	5 ± 0.5	Scolopendra moristans

Table 1 The distribution and morphometric comparison of the two collected samples

tergite 21 median suture is complete or nearly so. Antennal segments have 18 antennomeres and 10–12 glabrous ones at the base. The setae gradually appeared on the prefemoral process of most terminal legs, which had two or three spines. The prefemoral process of the ultimate leg typically has more than two spines, and both male and female ultimate legs are glabrous.

Scolopendra morsitans Linnaeus, 1758.

Figure 3.

S. morsitans s L. 1758 Systema Naturae 1: 637.

S. morsitans s scopoliana C. L. Koch 1841 M. Wagner, Reis Alger. 3: 222.

S. morsitans s: Attems 1930 Das Tierreich 54: 23.

S. m. scopoliana: Attems 1930 Das Tierreich 54: 23.

S. morsitans s amazonica Bücherl 1946 Mem. Inst. Butatan 19:135.

S. amazonica: Jangi 1959 Ent. News 70: 253.

S. jodhpurensis Khanna 1977 Oriental Insects 11: 154.

Distribution: Mexico, the Caribbean, Central and South America, Africa, Australia, Asia, the islands of the Atlantic and Indian, as well as the western and central Pacific Oceans [7,10].

Distribution in Egypt: (Table 1) This species has a broader distribution in Egypt.

Description: Fig. 3 Some tergites anterior to tergites 20 and 21 marginate. Prefemora of ultimate legs with spines not on elongated processes but sometimes on a swollen base

mostly with sternite paramedian sutures although these may be weak and incomplete, Prefemora of ultimate legs with 5 to many spines ventrally. Tergite 21 with a median longitudinal suture, Head plate without anterior diverging paramedian sutures, and basal plates. Forcipular coxosternum without ramifying sutures. Head plate with incomplete posterior lateral sutures and head plate lacking a median suture. Antennal segments with 19 antennomeres, basal 5-10 antennomeres glabrous. Leg 1 with a single tarsal spur. Coxopleuron with a side spine and coxopleural process with 3-5(7), mainly four apical spines. The pretarsus (claw) of the ultimate leg is shorter than the second tarsus and does not serrate ventrally. Ultimate leg prefemur with 7-10 ventral spines, typically in 3 rows of 3. Ultimate leg prefemur with 4 to 6 dorsomedial spines in two rows. The porose area extends to the posterior edge of the coxopleuron. Prefemur, tibia, and often tarsus of the ultimate leg of male dorsally flattened with swollen edges.

Histochemical

Figures 2 and 3D demonstrate the venom apparatus of both collected species, which consists of a pair of equal-sized maxillipeds found within a chitinous covering and the venom glands situated anteriorly in the prosoma on either side of the first segment of the body. The glandular epithelium is folded and composed of secretory epithelium surrounded by a striated muscle sheath. The secretory epithelium contains numerous cytoplasmic venom granules and high columnar venom-producing cells. The glandular canal is surrounded

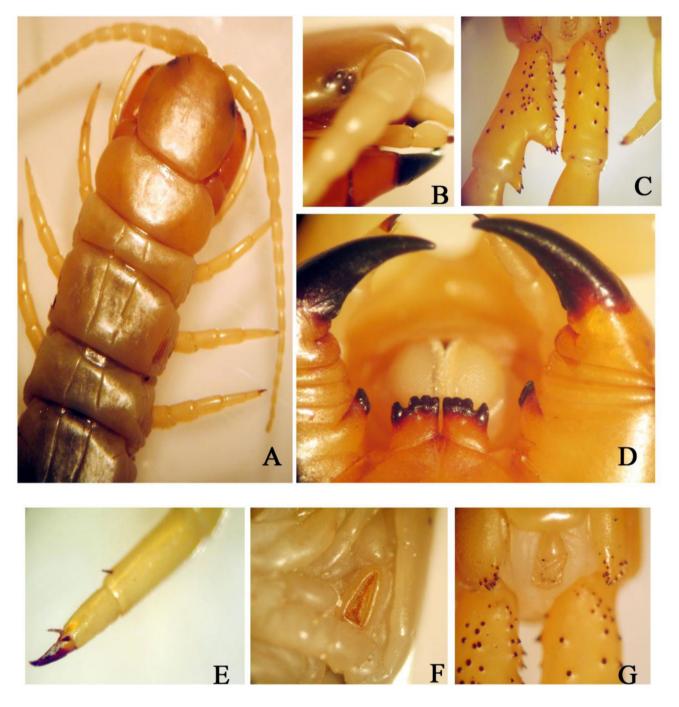


Fig. 2 Scolopendra canidens A Head plate with antenna and trunk segments, B 4 eyes in lateral sides, C Prefemure of the terminal leg, D forcipular coxosternal teeth plate, trochant-prefemoral process, and

by a chitinous interior layer and simple cuboidal epithelium and lacks muscles. (Figs. 4, 5A, B).

The venom-producing cells are filled with granules with small nuclei situated at their bases. (Figs. 4, 5A).

2 poisonous claw in the ventral side, \mathbf{E} leg with 3 lateral spines, \mathbf{F} spiracle, \mathbf{G} prefemure of the ultimate leg with coxsoternal process with spines in ventral view

Due to the presence of carbohydrate excretion, the venom-producing cells exhibited a positive Alcian-PAS reaction (Figs. 4B, 5C). In addition, they reacted positively with bromophenol blue techniques due to the presence of protein inside the cells (Figs. 4C, 5B).

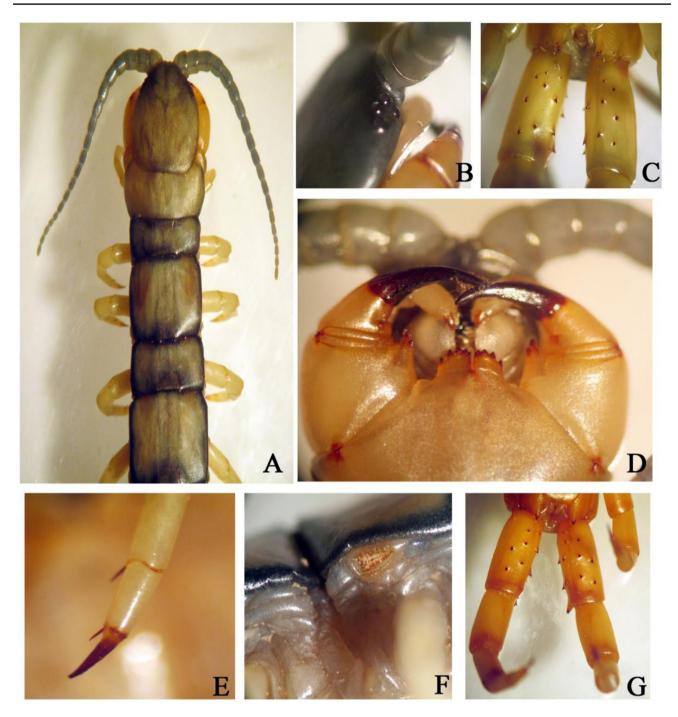


Fig. 3 *Scolopendra moristans*; A Head plate with antenna and trunk tergites, **B** 4 eyes in lateral sides, **C** Prefemure of the terminal leg, **D** forcipular coxosternal teethplate, trochantero-prefemoral process, and

2 poisonous claw in ventral side, E leg with 2 lateral spines, F spiracle, G prefemure of the ultimate leg with coxsoternal process with spines in ventral view

Discussion

All the scolopendrid species studied in the current research are prevalent in the Middle East, although there are few studies in Egypt. *Scolopendra cingulata, Scolopendra Canidens,* and *Scolopendra morsitans* have been historically found in Egypt; however, we could not find S. *cingulata.*

S. *cingulata* has been recorded in North Africa by Lewis (1985) and Minelli (2006), but we were unable to locate this species during our inquiry. S. *canidens* have a limited distribution on the northeastern shore of Egypt, whereas S.

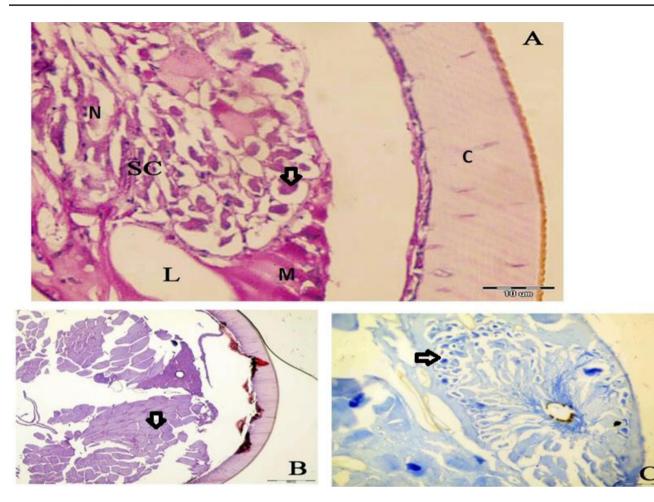


Fig. 4 A Photomicrograph showing a section of *S. canidens* through the venom glands. The secretory cells (SC) with basal nuclei; lateral lumen (L) and glandular muscle sheath (M); surrounded by ca chitinous envelope (C). H&E. **B** Photomicrograph an f section through *S.*

canidens stained with Alcian-PAS for carbohydrates (arrow). **C** Photomicrograph of a section of *S. canidens* through the venom glands stained with bromophenol blue for proteins (arrow)

morsitans s is more widespread. S. *canidens* appears to be generally restricted to more humid parts, the former to the rest of the country.

As the two centipede species identified belong to the same Scolopendrinae subfamily, they have comparable characteristics. In general, the histological and histochemical results did not show significant differences between the two studied species. The venom glands of collected species are located inside each maxilliped of the first pair behind the head. Each one is covered with external striated muscles. Each gland ends with its duct at the venom subterminal pore located on claw's outer curvature. The glandular epithelium is a folded mass of secretory epithelium surrounded by a striated muscle sheath and chitinous membrane. Despite the study of Dugon and Arthur (2012) that each secretory unit in the venom glands of all centipede species is composed of identical cells, the glands units of the two species have nearly identical structure of these units. Consequently, the glands of S. canidens and S. morsitans s consists of many small secretory units extending almost perpendicularly from the calyx to the basal lamina. Between the calyx and the basal lamina, which is also enveloped in striated muscle, secretory units are alternated by striated muscle.

Both collected Egyptian species had PAS-positive, indicating that the venom of this centipede species contained neutral mucosubstances and polysaccharides. According to Taib and Jarrar (1993) and Jarrar and AL-Rowaily (2008), scorpion species' venom has been shown to contain neutral mucosubstances and are believed to contain the most toxic species that produce large amounts of these chemicals. Because neutral mucosubstances are a glucosamine polymer, they may play a function in osmoregulation and transport the venom protein fragment into the victim's tissue (Jarrar and AL-Rowaily 2008). The PAS status of the *Scolopendra morsitans* and *Scolopendra canidens* glands indicates the presence of venom-containing acid mucosubstances, which may facilitate venom absorption after injection into the victim.

Fig. 5 A Photomicrograph showing a section of *S. morsitans* through the ven glands. The secretory cells(SC) with basal nuclei; lateral lumen (L) and glandular muscle sheath (M); surrounded by a chitinous envelope (C). H&E.**B** Photomicrograph of a section through *S.*

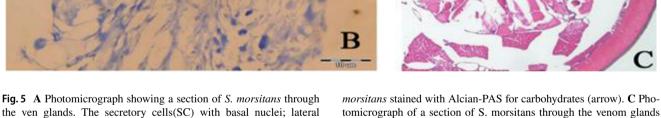
Conclusion

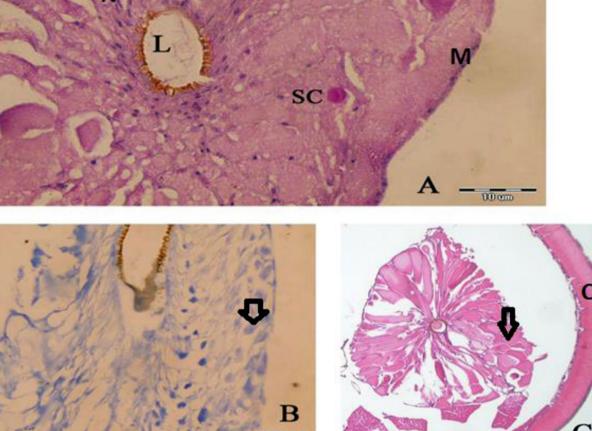
This study surveyed the endemic Egyptian *Scolopendra*, two Egyptian *Scolopendra* species were re-described, and *Scolopendra canidens* Newport.1844 was found in only one location. *Scolopendra morsitans* Linnaeus,1758 was more abundant. In this study, the histology and histochemistry of the venom glands of Egyptian centipedes were compared, which is also regarded as the first comprehensive investigation of newly collected Egyptian specimens. stained with bromophenol blue for proteins (arrow)

Author contributions Both authors gathered the data, analyzed the data, and wrote and discussed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.





Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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