Redescription of Terrestrial Isopod *Porcellio laevis* Collected From Sohag Governorate, Egypt with an Overview of Epicuticular Structures Using Scanning Electron Microscopy

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Abstract: The terrestrial isopod *Porcellio laevis* belongs to the genus *Porcellio* in the family Porcellionidae. It is a cosmopolitan species and was collected previously in Egypt. In the present study, this species was collected from two sites, guava and citrus plantations in Sohag Governorate, Egypt. This species can be identified visually from many other species of the *Porcellio* genus by the smooth, glossy dorsal surface and long, slender uropods of males. *Porcellio laevis* was first described by Latreille in 1804, and since that time, all articles have depended on this description and were supported with drawings. The present work uses a light and scanning electron microscope to study the external morphology of this species to enhance our knowledge about its taxonomic status. Also, surface epicuticular microstructures were investigated in this species using scanning electron microscopy, which reflects the large diversity of these microstructures, including microscales, plaques, polygonal micro-ridges, and tricorn sensilla. **Keywords:** Swift woodlouse, Oniscidea, Porcellionidae, Microstructures, Upper Egypt.

1. Introduction

Crustacean isopods inhabit a great variety of environments, from marine to land [1-3]. Terrestrial isopods (Oniscidea), commonly known as woodlice or sowbugs, are the most successful crustacean group [4,5]. The family Porcellionidae and genus *Porcellio* Latreille, 1804, are most diverse within the Oniscidea and are distributed throughout Europe, Asia, Africa, America, and Australia [6,7]. Porcellionidae is characterized by antennae with two distinct flagellar articles and spear-shaped uropods that project beyond the body outline. Thier body cannot roll into a ball [8].

Genus *Porcellio* is characterized by a cephalon with pronounced side lobes, eyes of adults with more than 20 ommatidia, 1st pereon epimera with a concave hind margin, pleon epimera enlarged to form a continuous outline with the pereon, two pairs of pleopodal lungs, and telson with concave sides [6,7,9]. *Porcellio laevis* (Latreille, 1804) is a large and distinctive woodlouse, up to 20mm long, with a smooth dorsal surface and long uropods in the males. Vandel and Achouri et al. [10,11] place *P. laevis* as a characteristic representative of a distinctive North African group of *Porcellio* species.

According to the present authors' knowledge, there are no studies that revise the taxonomy of the terrestrial isopod *Porcellio laevis* in Egypt. However, this species attracted the attention of some authors to study the seasonal variation of heavy metal accumulation [12-14] and its phylogeographic and genetic diversity by using the mitochondrial cytochrome C oxidase subunit sequence [15]. This work revised the description of *Porcellio laevis* using illustrations, light

microscopy and scanning electron microscopy, as well as investigating surface epicuticular microstructures using SEM, which reflects the large diversity of these microstructures, including microscales, plaques, polygonal micro-ridges, and tricorn sensilla.

2. Materials and methods

A field collection of *Porcellio laevis* specimens was made from two different orchards at the Agricultural Research Center in Jazirat Shandaweel District in 2022. The research center is located approximately 12 kilometers north of Sohag City (between 26° 37' 41.65" N and 26° 38' 7.59" N and 31° 38' 47.44" E and 31° 39' 37.95" E). The first orchard contains guava trees and is surrounded by three types of orchards (Fig, olive, and pomegranate orchards) and field crops (Pl. 1A). The second is a part of a large citrus orchard located near a car road (Pl. 1B).

In both orchards, a systematic sampling technique using quadrate was performed. In the laboratory, adult specimens were selected and then separated into males and females according to their sexual characteristics. These specimens were preserved in a mixture of 70% ethanol and 1% glycerol. Only undamaged and complete adult specimens were chosen and designated as paratypes. Representative specimens of females and males were dissected, dehydrated, and mounted for light microscopy study, while others were prepared for scanning electron microscopy by washing them in buffer solution and then fixed in a mixture of 3 volumes of 4% glutaraldehyde and one volume of 1% osmium tetraoxide for 4 hours. They were dehydrated in a graded series of ethanol, critical point dried, gold coated, and viewed under a JEOL 5300 scanning electron microscope at an operating voltage range from 10-30 V.



Plate 1: A satellite map for the two collecting orchards and the adjacent areas, **A)**. Guava and **B)**. Citrus Orchards.

The examined material is preserved in the Zoology Department, Faculty of Science, Sohag University.

2.1. Identification

Identification of the present species, *Porcellio laevis*, depends on the keys of [16, 17, 18, 19, 20, 21].

3. Results

3.1. Examined Material

Paratypes: **5** $\stackrel{\circ}{\rightarrow}$ **and 4** $\stackrel{\circ}{\rightarrow}$, Guava orchard - Agricultural Research Center - Jazirat Shandaweel District- Sohag. **6** $\stackrel{\circ}{\rightarrow}$ **and 3** $\stackrel{\circ}{\rightarrow}$, Citrus orchard - Agricultural Research Center - Jazirat Shandaweel District - Sohag.

3.2. Description of adult male

The adult male has an ovate body with a convex shape and a length that is about twice the width of the body, measuring 9.8 mm and 5.94 mm in length and maximum width, respectively. The dorsal surface of the pereon (pereonites 1-7) is decorated with two longitudinal rows of painty-shaped chromatic patterns, while the pereon epimerae (1-7) lack such patterns and instead carry two rows of noduli laterales and two rows of glandular fields (pores). The first pereonite is concave, its epimera directed anteriorly to surround the cephalon up to the base of the lateral lobes. The epimerae of pereonites (2–7) are directed inwardly towards the posterior position of the body (uropods and telson) (Pl. **2A**). The pleon consists of six segments, five free pleonites, while the sixth one is fused with the telson, forming a pleotelson. Pleonites (1-2) with no epimera and

SOHAG JOURNAL OF SCIENCES

covered laterally by pereonite 7. Pleonites epimerae (3-5) are directed inward towards the telson. The lateral margins of epimera of pereonites (2-7) and pleonites (3-5) form a continuous line. Telson has a triangular shape with concave lateral margins, a straight anterior margin, and a narrowly rounded apex that exceeds the protopodite of the uropod.

3.2.1. The Cephalon

The cephalon of the specimen exhibits a slightly rectangular shape, measuring approximately 1.04 mm in length and 2.1 mm in maximum width (Pl. **2A**). The frontal margin of the cephalon produces three lobes: a median lobe and two lateral lobes (Pl. **2C**). The median lobe displays an arch-like structure, while the lateral lobes are more prominent than the central, with a rounded upper rim and a straight outer side. Conversely, the posterior margin of the cephalon appears straight. Each eye is characterized by an oval shape and is composed of (24-25) ommatidia in four rows (Pl. **2D**).

3.2.2. The mouthparts

As in all crustaceans, the cephalon has five pairs of appendages: the first and second antennae, the mandible, and the first and second maxillae. Additionally, the maxilliped is part of the first fused thoracic segment.

3.2.2.a. First antennae (Antennules): (Pl. 2E)

The first antennae, also known as antennules, are very small and positioned medially at the base of the antennae. Each antennule consists of two peduncles and a flagellum. The two peduncles are slightly cylindrical in form. The flagellum is triangular and has a sub-apical tuft of rod-like aesthetascs (34 in number).

3.2.2.b. Second antennae (Antennae): (Pl. 2F,G)

The second antennae are long, slender, and surpass the posterior margin of the first pereonite. Each antenna consists of five peduncles (p1-p5) and a flagellum with two articles (a1-a2). The peduncles gradually increase in length, from the smallest first peduncle to the longest fifth one.

Peduncle (1) is slightly triangular, peduncle (2) is trapezoidal, and Peduncles (3-5) are slender in shape. The fifth article is somewhat longer than the flagellum (about 1.3:1). Flagellum is composed of two jointed articles. The distal article of flagellum is barely longer than the proximal one (1.35:1) and ends with the terminal organ. The dorsal facet of the distal article possesses tricorn and aethetascs sensilla, as well as needle-like setae. Aethetascs are rod-shaped and arranged in five rows (with 4+4+2+4+2 rods in rows, respectively). The ventral facet of the distal article of the flagellum has a distinct bulbous region that incorporates densely packed elongated setae (hair-like in form) that are directed toward the flagellum apex.

3.2.2.c. Maxillulae (first maxillae): (Pl. 2H, J)

Each maxillula has two endites. The outer endite terminates with simple, strong, and inwardly curved teeth that are arranged in two rows (4+6). The free margin of the slender inner endite has two equal penicils and a lateral spine.

SOHAG JOURNAL OF SCIENCES



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Plate 2: Light (**A**-**C**), scanning electron (**D**-**I**) photomicrographs and camera lucida drawings (**J**, **K**) for *Porcellio laevis* showing: **A**). Dorsal surface of the male body, **B**). Dorsal surface of the female body, **C**). Lateral and median lobes of cephalon, **D**). Ommatidia of eye, **E**). Male right antennule, **F**). Male right antenna with the last flagellum article enlarged (arrow), **G**). Male second flagellum article showing aethetascs, **H**). Male outer lobe of maxillula, **I**). Male maxilla, with fine setae enlarged to illustrate biserration, **J**). Male maxillula lobes, and **K**). Male maxilla lobes.

3.2.2.d. Maxillae (second maxillae): (Pl. 2I, K)

The outer endite of the maxilla is distinctly wider than the inner one. The apical part of the outer endite is triangular and covered with fine setae. The apical part of the inner endite is slightly oval and also covered with fine biserrate setae. It is worth noting the presence of two rows of thick spine setae located in the furrow between the two endites.

3.2.2.e. Mandibles: (Pl. 3A, B)

The right and left mandibles have a similar shape, each consisting of pars incisive (incisors), lacinia mobilis, pilous (hairy) lobe, and pars molaris. Palp is absent in both mandibles. Pars incisive of the two mandibles has four blunt teeth. Lacinia mobilis is a strong, tooth-like structure, nearly trapezoidal in shape with a concave medial margin and two lateral cusps.

Pilous lobes of two mandibles carry a tuft of pilous-bristle setae and four spine-like simple setae.

Pars molaris of the two mandibles is a dichotomized molar penicil with a bundle of different-sized circumplumose setae. In both mandibles, there is a group of free penicil setae (11 in number and slightly clavate in shape) located between the pilous lobe and pars molaris.



Plate 3: Scanning electron photomicrographs (A, C, L, M) and camera lucida drawings (B, D, E-K) for male of *Porcellio laevis* showing: A, B). Ventro-lateral position of the left mandible, C). Ventral view of maxillipeds, with endite and palp enlarged at left corner, D). Ventral view of maxillipeds, E-K). Pereopods 1-7, respectively, L). First pereopod showing antennal grooming brush and hairy brush setae, and M). Microscales and needle-like setae on carpus of the second pereopod.

3.2.2.f. Maxilliped: (Pl. 3 C, D)

Each maxilliped consists of a large basis, a palp, and an inner endite. The basis is decorated with microscales and carries different-sized simple and spine-like setae. The palp of each maxilliped is composed of three articles. The first (proximal) article of the palp is rectangular in shape and has two strong simple setae carried on tubercles. The second article is trapezoidal in shape and has two groups of simple setae; each group arises from one socket that is carried on tubercles. The first group has two setae, while the second one has four different-sized setae. Additionally, a small seta is present on the

lateral margin of the second article. The distal article of the palp is slender, elongated, inwardly curved, carries a tiny seta, and terminates with a tuft of hair-like setae. The inner endite of the maxilliped is subrectangular, does not exceed the second palpal article, and bears six setae (three teeth-like setae on tubercles, one falcate seta on tubercles, one strong simple seta, and one tiny setae). A slender and elongated epipodite appears beside the lateral margin of the basis but does not exceed it.

3.2.3. The thoracic appendages (pereopods)

Each pereonite carries a pair of pereopods. Each pereopod consists of seven segments: a coxa, basis, ischium, merus, carpus, propodus, and dactylus (Pl. **3E-L**). The frontal and caudal surfaces of all pereopods (1-7) are similar in general shape, different in size, and equipped with microscales and sparsely needle-like setae (Pl. **3M**). The ventral surface of merus and carpus segments of pereopods (1-4) carry brushes of hairy setae, which are arranged in rows and have a clavate shape with striated shaft and terminate with two latero-distal spines (Pl. **4A**).

In addition, the carpus of pereopod 1 has a shallow depression on the frontal surface filled with transverse rows of antennal grooming brush setae. The setae in the middle resemble each other where each seta is marginally bi-serrated, medially furcated, and fused distally. On both sides of these setae, there are simple setae simple. (Pl. **4B**). The ventral surface of meri and carpi, and of pereopods (5-7), as well as propodi of pereopods (1-7) carry stout setae with quadrifid terminal part (Pl. **4C**). The dactylus of all pereopods is similar in shape and has two types of setae: the ungual seta and the dactylar (claw-like) seta, which are stout, simple, and not exceeding the claw (Pl. **4C**).

3.2.4. The abdominal appendages (pleopods and uropods)

The ventral surface of the free abdominal segments bears five pairs of appendages, or pleopods (1-5) (Pl. 4D). Each pleopod consists of two segments: the exopodite and the endopodite. Exopodites of pleopods (1-2) are subtriangular, with medial margin lacking setae, and both have a concave outer margin with a row of small setae (Pl. 4E, F). The proximal article of endopodite of pleopod 1 is subrectangular, while the distal article is styliform and both articles have no setae. The proximal article of endopodite of pleopod 2 is semicircular, while the distal one is flagelliform, and both articles lack setae. The endopodite of pleopod 1 is wider and shorter than the endopodite of pleopod 2. Respiratory fields of pleopods (1-2) without indentation Exopodites of pleopods (3-5) are slightly triangular in shape and carry spine-like setae on the outer margin (Pl. 4G-I). Endopodites of pleopods (3-5) are reduced. In addition, the genital papilla (apophysis) of males is spearheadshaped and shorter than pleopod 1 (Pl. 4E). The sixth abdominal segment carries one pair of appendages or uropods. Each uropod consists of protopodite and two rami: exopodite and endopodite. The protopodite is rectangular in shape. Exopodite is spearshaped, longer than endopodite, and extended posteriorly beyond the telson tip (Pl. 4J). The endopodite has a rod-like shape, extends beyond the telson tip, bears simple setae, and terminates with three pappose setae.

3.3. Thoracic noduli laterals: (Pl. 5A-F)

Pereonites (1-7) of *Porcellio laevis*, male and female, have one line of noduli laterales per each side. Distances between noduli position and both the lateral margin and the posterior margin of the pereonites, show little variations as shown in Plate (**5C-F**). Noduli were inserted more or less at the same distance from the posterior margin of the pereonites (Pl. **5C-F**). While both b/c and d/c ratios show similar trends in males and females. Noduli were positioned close to the lateral edge in pereonites (5-7) which give lower values for d/c ratio.



Plate 4: Scanning electron and light photomicrographs for male of *Porcellio laevis* showing: **A).** Hairy brush setae on carpus of the second pereopod, **B).** Antennal grooming brush setae (arrows) with enlarged seta on the left corner, **C).** Dactylus of pereopod 1, **D).** Ventral side of pleon showing pleopods (1-5), **E-I).** Pleopods from 1 to 5, respectively, showing exo- and endopodites and genital papilla, **J-K).** 1st and 2nd pleopods of female and **L).** Uropods of males.

3.4. Glandular fields: (Pl. 5A)

Each pereon epimera of *Porcellio laevis* males and females has a glandular field that is semi-ovoid. It is in the middle of the epimera, very close but separated from its outer edges.

3.5. Description of the adult females: (Pls 2B; 4J,K, 5G-M)

Generally, the morphological characteristics of the present female of *Porcellio laevis* are similar to those of its male, such as body shape, pereopods, pleopods, noduli laterales position, and cuticular scales setae. On the other hand, the morphological variations between both sexes of *Porcellio laevis* are summarized as follows:

- The number of aesthetascs in the flagellum of an antennule is 22 in females instead of 34 in males.
- The aethetascs of the antenna flagellum are arranged in 4 rows for females instead of 5 for males. The number of rod-like setae from the first to the fourth row of females is 4+3+5+2 on the dorsal side.
- The brush of hairy setae is absent on the ventral surface of all female percopods, while it was present on percopods (1-4) of males.
- Female pleopod-exopodites 1 and 2 are slightly rectangular and trapezoidal in shape, respectively, instead of triangular in the male.
- Endopodites of pleopods (1-5) are reduced in females, while in males, endopodites of pleopods (3-5) are reduced.

3.6. Cuticular scales

Male and female Porcellio laevis are equipped with varied cuticular microstructures. These microstructures include microscales, which cover various parts of the body, have curved outer margins and are arranged as shingles. They are found on antennules and antennae peduncles, the basis of maxilliped, epimerae of pereonites, and appear thick on the carpus and propodus of pereopods (Pls. 2E; 6A). Other microstructures are lifted, clavate-shaped scales that were found on pleonites (Pl. 6B), lifted wedge-shaped scales located on uropod exopodites (Pl. 6C), and Plaques that are found on pereonites, pleonites and uropods (Pl. 6D). All the previous microstructures (microscales, lifted scales and plaques) with a ridge facing caudal direction. A network of polygonal micro-ridges covers the pleopod exopodites and pleonites (1-5) (Pl. 6E). Additionally, tricorns sensilla are triangular structures with three pointed projections, two lateral and one middle. They are common exteroreceptors that are found in cephalon, on the tergites of pereon and pleaon as well as uropods. They are presented in three shapes, the first characterized by a broad, short middle projection and long, slender lateral projections. The second shape has a short, wide middle projection and two wider lateral projections. The third shape has a long, broad middle projection and very short lateral projections (Pl. 6D, F, G).

4. Remarks

The present species is cosmopolitan. Based on the morphology of the first male pleopod, it belonged to *laevis*-*hoffmannseggi* group, and it likely originated in North Africa [6, 22]. Previously to this work, *Porcellio laevis* was described from many regions around the world, and almost all of these works depend on morphological illustrations except work of [23] and [21] which was supported with SEM for some selected appendages, but they did not refer to epicuticular structure.

SOHAG JOURNAL OF SCIENCES



Plate 5: A). Light photomicrograph for the right side of male showing noduli laterales and glandular fields, **B).** Scanning electron photomicrograph showing a nodulus lateralis and plaques scales on third pereonite, **C-F).** The relative position of the noduli laterales on pereonites (1-7), for both sexes is defined by the ratios b/c and d/c where b=distance from the nodulus lateralis to the posterior margin of the pereonite, d= distance from the nodulus lateralis to the lateral margin of the pereonite and c= maximum length of the pereonal tergite (**C, D** for male, **E, F** for female), and **G-M).** Camera lucida drawings for female pereopods (1-7), respectively.

This species is similar to those reported from other parts of the world by possessing smooth terga, posterolateral angle of the first pereon tergite bluntly rounded, and uropods of male are elongated and spear-shaped [6, 22, 16, 18-21, 23, 24]. Also, lungs are restricted to pleopods I and II. Frontal margin with broad, convex median projection. Grooming antennae brush setae are associated with the first pereiopod of both sexes. In addition to that female pereopods lack brush of hairy setae, while it is present on male pereopods (1-4). The number of ommatidia shows some variations between individuals of the present species and those collected in other regions.

In the present species, ommatidia number ranged from (24-25), while they ranged from (8-31) ommatidia in individuals collected from Mexico [18], and 18 ommatidia in individuals collected from central Iraq [25]. Microstructures that are recorded in the present species have caudal directed rim which is adaptive to living in the soil. This enhances the movement abilities of the animal between cracks and beneath stones that

are needed to avoid predators, as well as environments that are either too wet or too dry [6].



Plate 6: Scanning electron photomicrographs for male showing epicuticular structures: **A).** Microscales arranged as shingles on the propodus of the third pereopod, **B).** Lifted, clavate-shaped scales (arrows) and third tricorn type on uropod exopodites, **C).** lifted wedge-shaped scales (arrows) and third tricorn type on uropod exopodites, **D).** Plaques and third tricorn type on tergite of the fourth pleonite, **E).** Network of polygonal micro-ridges on tergite of the third pleonite, **F).** The first tricorn type on cephalon and **G).** The second tricorn type on tergite of the second pereonite.

Tricorns sensilla, as surface structures, have mechanoreceptive functions and are widely distributed on terrestrial isopods cuticle and therefore have a role in the terrestrial adaptation of isopods [26]. These structures are found associated with plaques microstructures. Holdich and Lincoln [27] and Price and Holdich [28] reported that tricorns formed as a modification of the plaque, and this is why the two structures are spatially associated.

Network of polygonal micro-ridges assumed to be antiadhesive structures that prevent small wet particles from sticking to the cuticle or prevent animals from adhering to the substrate. These particles obstruct animal movement and lower the exchange of oxygen through cuticle [6]. The Network of polygonal micro-ridges of the present species lacks any tactile setae or tricorns inside them as reported in other terrestrial isopods [27, 28].

CRediT authorship contribution statement:

Ramadan S.A., and Ismail T.G.; write the original draft and

SOHAG JOURNAL OF SCIENCES

final manuscript, review and edit the final manuscript. Abd El-Rahman A.A.: collect specimens, SEM preparations and participate in writing the draft manuscript. All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

а	Aethetascs	ns	Needle-like setae
a2	Second antenna	0	Ommatidia
ag	Antennal grooming brush setae	ol	Outer lobe
ba	Basal article	p1	Peduncle article 1
c	Cephalon	p2	Peduncle article 2
cl	Claw	p3	Peduncle article 3
cs	Circumoplumose setae	p4	Peduncle article 4
ds	Dactylar seta	p5	Peduncle article 5
e	Eye	pi	Pars incisive
en	Endopodite	pl	Pilous lobe
ep	Epipodite	ple	Pleopods
ex	exopodite	pb	Pilous bristle setae
f	Flagellum	rs	Respiratory field
fp	Free penicil setae	SS	Simple setae
g	Glandular field	st	Stout seta
gp	Genital papilla	t	Teeth
h	Hairy brush setae	te	Telson
ie	Inner endite	ts1-ts3	Tricorn sensilla
			types
il	Inner lobe	tr	Terminal organ
11	Lateral lobe	u	Ungual seta
lm	Lacinia mobilis	uen	Uropod endopodite
ml	Median lobe	uex	Uropod exopodite
mp	Molar process	up	Uropod protopodite
mxp	Maxilliped palp	ur	Uropod
n	Noduli lateralis		

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