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DESCRIPTION OF THE FEMALE OF *DIPLOTHYRUS SCHUBARTI* LEHTINEN, 1999
(HOLOTHYRIDA: NEOTHYRIDAE) AND NEW SPECIES OCCURRENCES IN BRAZIL

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ABSTRACT — The order Holothyrida includes one of the rarest groups of mites. For the moment, 28 species are known, but only one occurs in Brazil. This work aimed to describe the female of *Diplothyrus schubarti*, along with presenting the first occurrence of organisms of this order in caves. A total of 54 specimens were collected, in 17 caves in three municipal districts in the state of Pará, northern Brazil. The differences that stand out in the female of *D. schubarti*, in relation to the other species of the genus, are the absence of the membranous process in the palpus and the absence of the distinct cuticular strip running postero-ventral towards the edge of the dorsal shield.

KEYWORDS — Acari; Parasitiformes; Holothyrida; ceotropics; cave mites

INTRODUCTION

The order Holothyrida is characterized by the large body size (2 to 7 mm), by being strongly sclerotized and having, as habitat, the litter present on the soil. These mites are still very little known, but it is known that their species compose one of the most primitive orders within the Parasitiformes group (Krantz and Walter, 2009).

The first taxon, *Holothyrus coccinella*, was described by Gervais (1842). Currently, the order is composed by 3 families (Allothyridae, Holothyridae and Neothyridae), totaling 15 genera and 28 species (Kontschán and Mahunka, 2004; Gerlach *et al.*, 2010; Klompen 2010).

The geographical distribution of the Holothyrida order comprises several places; Costa Rica, Panama and Dominican Republic (Central America); Venezuelan, Peru, French Guiana and Brazil (South America); Seychelles and Mauritius Islands (Africa); Sri Lanka, Philippines and Luzon island (Asia); New Guinea, Louisiade Archipelago, New Caledonia, Australia, New Zealand and Lord Howe Island (Oceania) (Lehtinen, personal information 2011). Among the species described for the Neotropical Region, three were found in the northern portion of South America (Kontschán and Mahunka, 2004; Klompen, 2010).

The first described taxon was *Neothyrus ana*

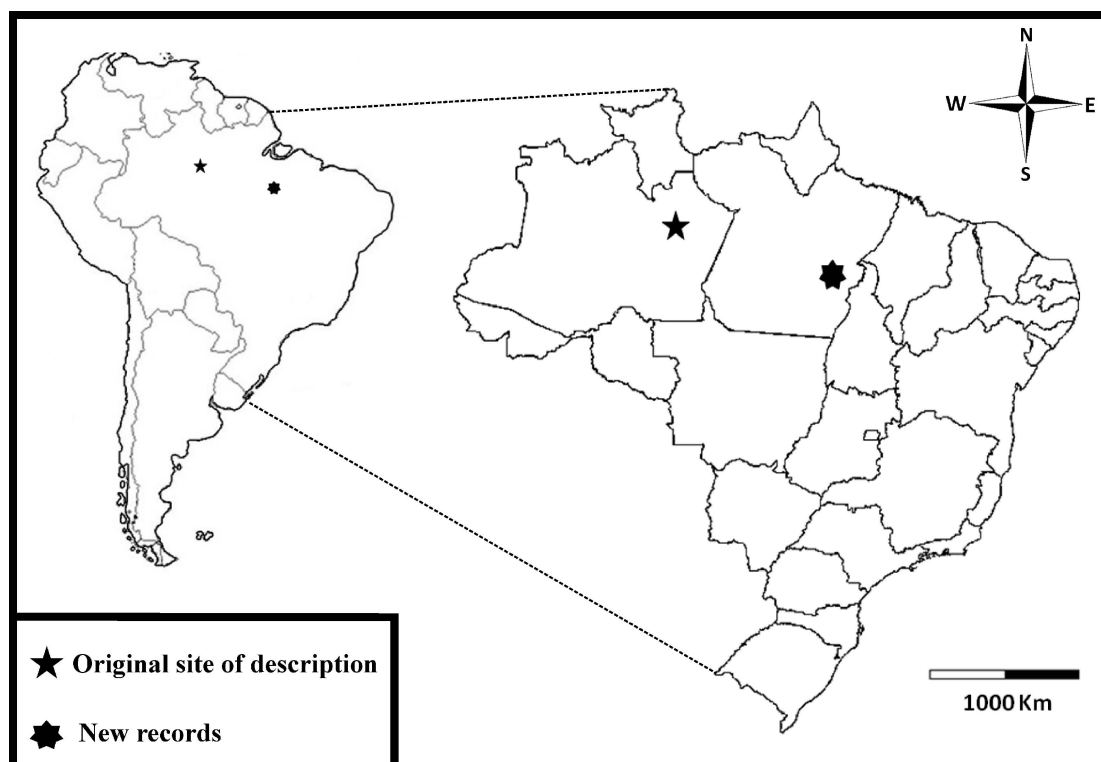


FIGURE 1: Occurrence sites of *Diplothyrsus schubarti* species in Brazil.

Lehtinen (1981), present in the Peruvian Amazon. The second species, *Diplothyrsus schubarti*, was described from specimens found in Brazil in a primary forest in the Adolpho Ducke Forest Reserve, located in the municipal district of Manaus, Amazon State, in the northern region of the country (Lehtinen, 1999). The third, recently described, is the species *D. lecorrei*, found in the French Guyana Amazon, north region of the South American continent (Klompen, 2010).

In the descriptive work of the Brazilian species, Lehtinen (1999) only used the male for the morphologic description and analysis. Thus, the objective of the present work is to conduct the description of the female of *D. schubarti*, which until now was not available for study, although present in collections of INPA. Furthermore, the present work presents new occurrences of the species in Brazil, all associated to ferruginous caves.

MATERIALS AND METHODS

Study area

Collections of mites were carried out in a total of 249 caves formed in iron ore, located in the municipal districts of Carajás, Parauapebas and Canaã dos Carajás, all located in the southeast portion of the state of Pará. The ferriferous formation of Carajás, where such caves are inserted, is totally under the domain of the Amazon Forest, located in the north area of Brazil. Some of the caves are found inserted within a National Forest (Flona de Carajás). This area, although comprising a protected area, presents multiple anthropic uses, such as iron ore exploration.

The ferriferous formation of Carajás is composed by plateaus whose topography is maintained by a ferruginous topmost breccia, named "canga", under which the lateritic ore occurs, with a thickness that can surpass, locally, 200 meters (Lindenmayer *et al.*, 2001). The entrances of the caves are in this lat-

TABLE 1: Morphological measurements of *Diplothyryus schubarti* males and females.

	Female	Male
Idiosoma (length)	1720 - 1760 μm	1680 - 1720 μm
Idiosoma (width)	1160 - 1200 μm	1120 - 1160 μm
Palpus	690 - 675 μm	660 - 705 μm
Subcapitulum	350 - 400 μm	350 - 375 μm
Chelicera	385 - 395 μm	375 - 385 μm
Leg I	1875 - 2070 μm	1875 - 2015 μm
Leg II	1705 - 1755 μm	1725 - 1885 μm
Leg III	1635 - 1835 μm	1685 - 1835 μm
Leg IV	2005 - 2220 μm	2035 - 2225 μm
Anal opening (length)	130 - 135 μm	130 μm
Anal opening (width)	170 - 172 μm	123 - 126 μm
Genital opening (length)	500 - 525 μm	110 - 115 μm
Genital opening (width)	518 - 525 μm	157 - 168 μm

eritic crust, and the development of conduits and galleries mainly occur below this thick layer of iron.

Methods

The collection was conducted through manual capture by using tweezers and brushes inside caves, all of the specimens being fixed in 70% alcohol and taken to the laboratory for identification.

The measurements and morphological observations were based on 3 females and 3 males clarified in Nesbitt's solution, dismembered and later mounted on slides and glass coverslips using Hoyer's solution (Evans 1992; Krantz and Walter 2009). The idiosoma and the genital opening measurements were taken based on 3 additional females and 3 males maintained whole and mounted on temporary slides with glycerin.

The ultrastructural analyses were conducted using the scanning electron microscope. The male and the female were placed on aluminum support stubs, placed over aluminum foil film with carbon tape, sputter-covered with gold (Baltec SCD 050), and observed in a LEO EVO 40 XVP scanning electron microscope (Leo Electron Microscopy).

RESULTS

Geographic distribution

A total of 54 specimens were collected, from which 21 were male, 10 were female and 23 were juveniles. The organisms were found in 9 caves in the municipal district of Carajás, 9 caves in the municipal district of Parauapebas and 1 caves in the municipal district of Canaã de Carajás, all located in the south of the state of Pará, northern Brazil (Figure 1).

According to the work of Lehtinen (1999), the distribution of *Diplothyryus schubarti* is restricted to the northeast area of the state of Amazonas, in the municipal district of Manaus. The new occurrences extend the distribution of the species by about 1100 km to the east, these also being the first occurrences of *D. schubarti* in the cave environment (Figure 1).

Material examined from Pará State, Brazil

One immature from Cave N4E-03 (6°02'24.3"S, 50°09'38.7"W), Carajás, coll. R. Andrade *et al.*, 20 April 2010; one male from Cave N4E-48 (6°02'15.3"S, 50°09'36.6"W), Carajás, coll. R. Andrade *et al.*, 19 April 2010; one immature from Cave N4E-79 (6°01'58.5"S, 50°09'05"W), Carajás, coll. R. Andrade *et al.*, 19 April 2010;

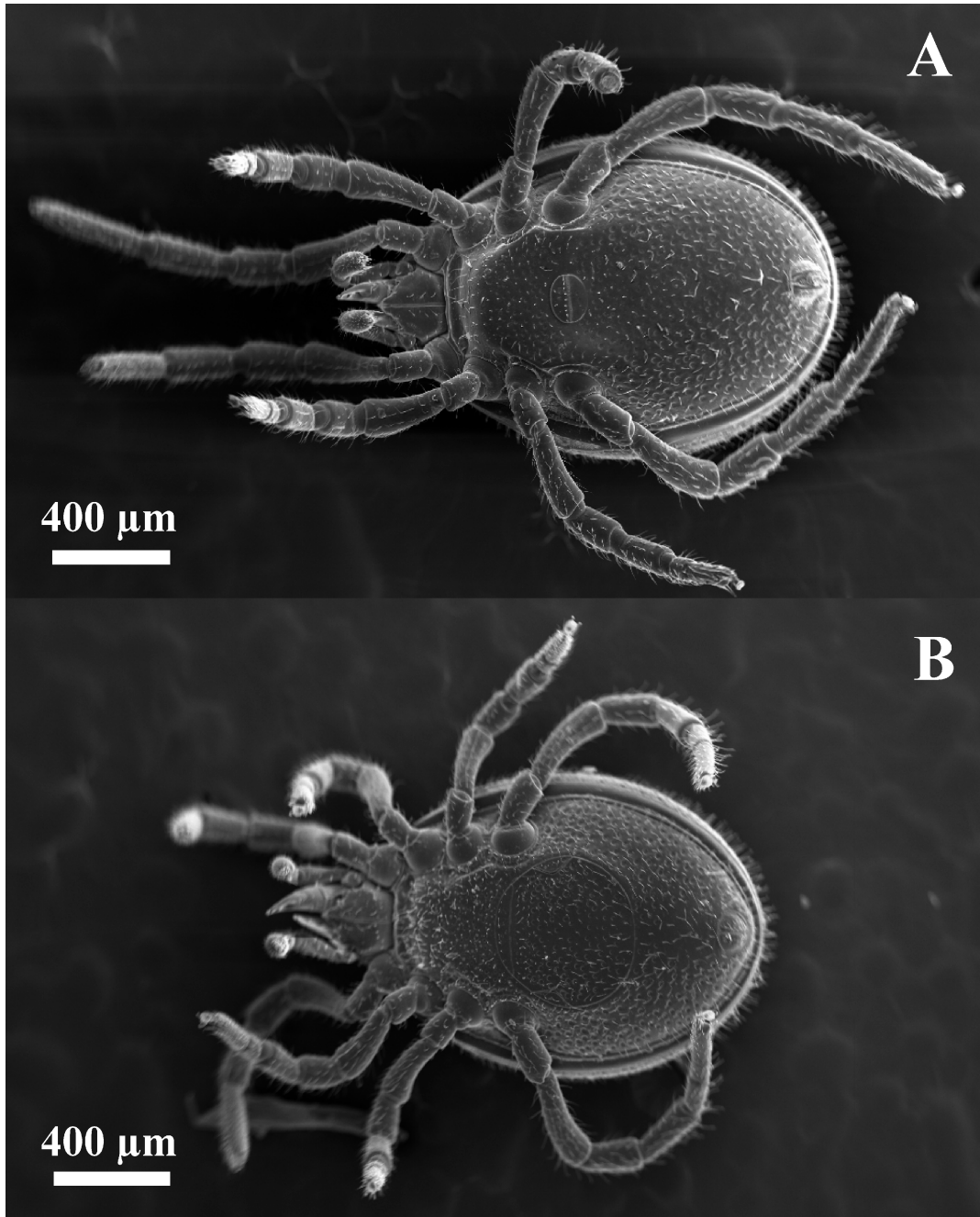


FIGURE 2: *Diplothyrsus schubarti*. Overview of the ventral idiosoma. A – male, B – female.

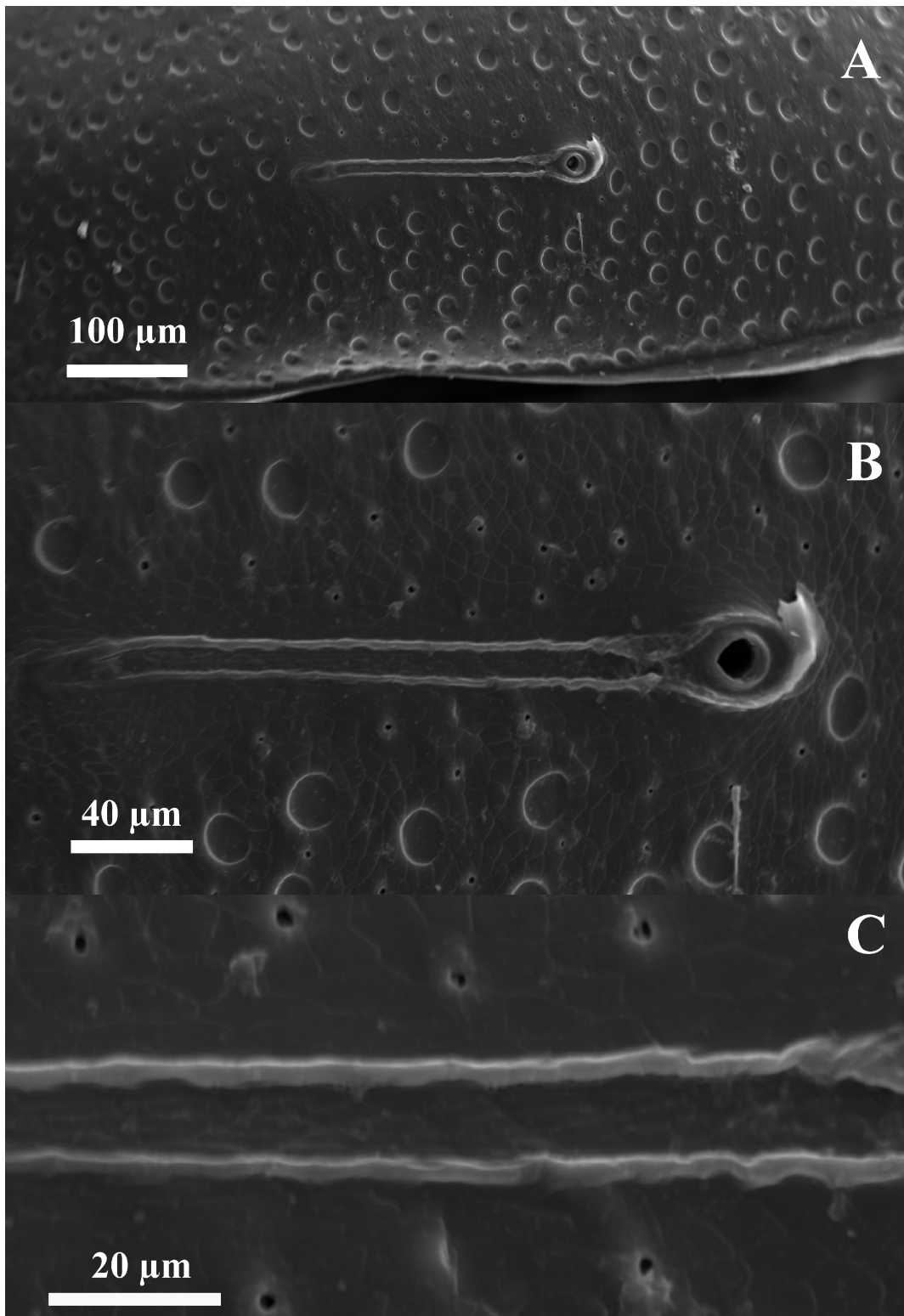


FIGURE 3: *Diplothyrys schubarti*. Detail of tegument, opening of Thon's organ of female.

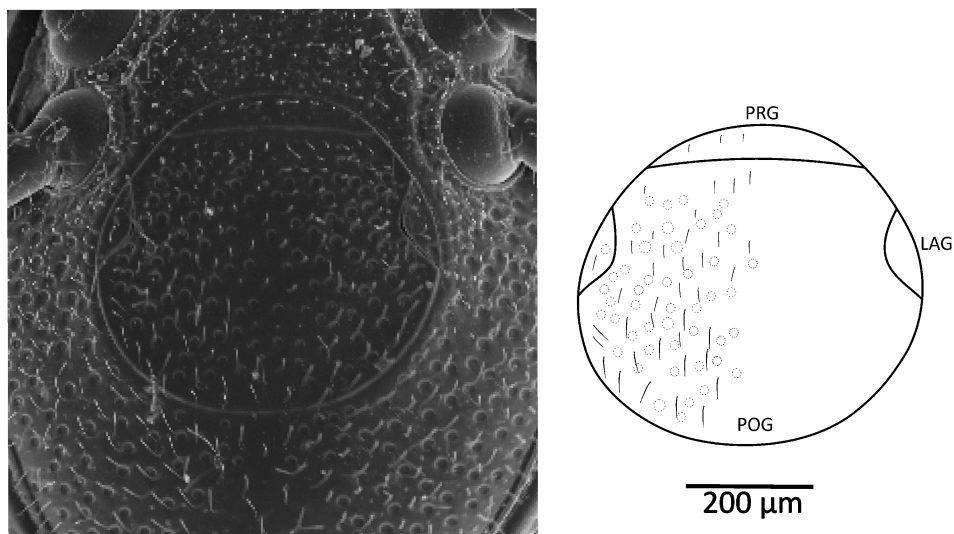


FIGURE 4: *Diplothyrys schubarti*. Genital opening of female. PRG: pregenital shield, LAG: lateroetinal shield, POG: posgenital shield.

five immatures from Cave N4E-85 (6°02'02.6"S, 50°09'25.6"W), Carajás, coll, R. Andrade *et al.* 19 April 2010; one female from Cave N4WS-14 (6°03'53.8"S, 50°11'21"W), Carajás, coll, R. Andrade *et al.* 20 April 2010; two males from Cave N4WS-15 (6°03'59"S, 50°11'21.9"W), Carajás, coll, R. Andrade *et al.* 20 April 2010; one immature and a male from Cave N5S-70 (6°03'59"S, 50°11'21.9"W), Carajás, coll, R. Andrade *et al.* 14 April 2010; three immatures from Cave N5S-73 (6°03'59"S, 50°11'21.9"W), Carajás, coll, R. Andrade *et al.* 19 April 2010; nine immatures, six males and six females from Cave N5S-74 (6°06'01.2"S, 50°08'04.7"W), Carajás, coll, R. Andrade *et al.* 14 April 2010;

One immature from Cave N4E-44 (6°01'55"S, 50°09'50.2"W), Parauapebas, coll, R. Andrade *et al.* 19 April 2010; two males and two females from Cave N4E-72 (6°01'56.6"S, 50°09'13.1"W), Parauapebas, coll, R. Andrade *et al.* 19 April 2010; one male from Cave N4E-76 (6°01'59"S, 50°09'03"W), Parauapebas, coll, R. Andrade *et al.* 19 April 2010; two immatures and one female from Cave N4E-89 (6°01'58.7"S, 50°09'06.4"W), Parauapebas, coll, R. Andrade *et al.* 19 April 2010; one male and one female from Cave N4E-92 (6°02'21.8"S, 50°09'31.2"W), Parauapebas, coll, R. Andrade *et al.* 19 April 2010; four males from Cave S11D-01

(6°23'53.9"S, 50°21'25.2"W), Parauapebas, coll, R. Andrade *et al.* April 2010; one female from Cave S11D-10 (6°23'54.8"S, 50°21'25.1"W), Parauapebas, coll, R. Andrade *et al.* April 2010; a female from Cave S11D-17 (6°23'55.4"S, 50°21'22.8"W), Parauapebas, coll, R. Andrade *et al.* April 2010; one female from Cave S11D-74 (6°23'32.1"S, 50°19'06.6"W), Parauapebas, coll, R. Andrade *et al.* April 2010; three males from Cave S11-27 (6°24'42.2"S, 50°21'09.4"W), Canaã dos Carajás, coll, R. Andrade *et al.* April 2010. For all geographical coordenadas were used Datum-SAD 69.

The specimens are deposited in the Invertebrate Collection of Lavras (ISLA) in the Zoologia Sector/Biology Department of the Federal University of Lavras (UFLA), Lavras, Minas Gerais state, Brazil (ISLA 1454-1463); The Mite Collection "Geraldo Calcagnolo" in the Instituto Biológico, Campinas, São Paulo state, Brazil (7463 e 7464); and at Museum of Zoology (MZLQ) in the Departamento de Entomologia e Acarologia, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo state, Brazil (MZLQ 12300-1305).

Description of the female of the species

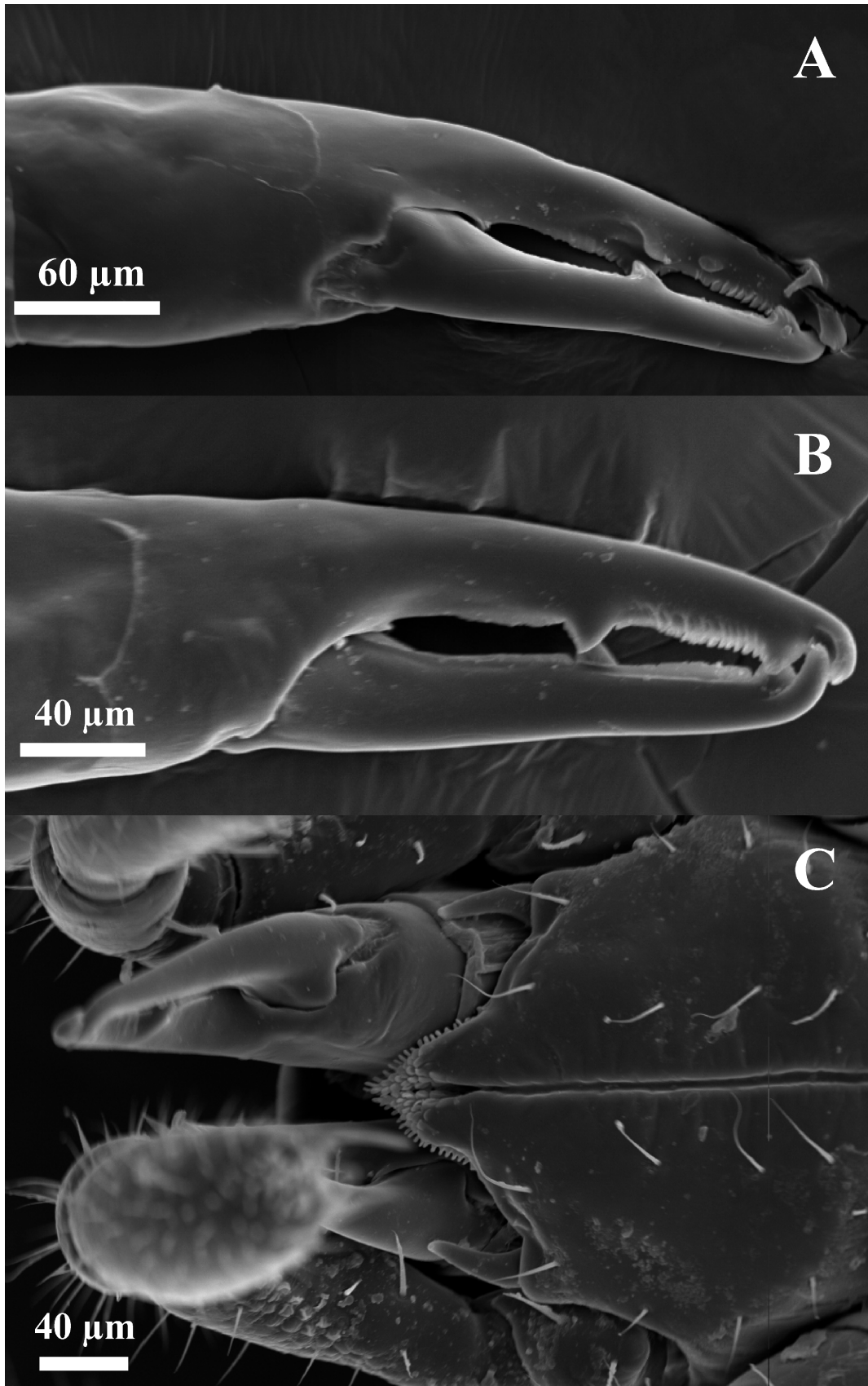


FIGURE 5: *Diplothyrys schubarti*. View of the Chelicerae (A and B) and gnathosoma (C).

Diplothyrus schubarti

Idiosoma — The female idiosoma well sclerotized, of light brown coloration, its length 1720 – 1760 μm and width 1160 – 1200 μm (Figure 2A and 2B).

Dorsal view — Strongly arched, hypertrichous, the entire dorsum being covered by concavities. On the lateral area of the dorsal disk, it is possible to see the stigma, a small opening located above the fourth coxa. The Thon's organ is visible externally, however it is short (235 – 240 μm), extending to the anterior region and located above the third and fourth coxae (Figure 3).

Ventral view — Presents a homogeneous distribution of setae on the ventral shield. Sternal lyrifissures (if present) not observed. The ventral shield is separate from the dorsal shield by a continuous fissure. The anal opening of the female is composed by two valves, with length of 130 – 135 μm and width 170 – 172 μm .

The genital area of the female has a length of 525 – 530 μm and width of 500 – 505 μm , with rounded shape. Similar to that observed for the remainder of the body, the female genital opening presents round concavities and hypertrichy. The central plate (post-genital shield) is notably larger than the other plates located on the sides (laterogenital shield) and on the anterior area (pregenital shield) (Figure 4).

Legs — Length of the legs I-IV (female): 1875 – 2070 μm , 1545 – 1705 μm , 1635 – 1835 μm and 2005 – 2220 μm , respectively.

Subcapitulum — Length of 350 – 400 μm . Possesses 3 pairs of setae on the central region, a pair of setae on the central portion of the anterior area, a pair of setae located below the corniculi and three pairs of setae on the region of the posterior lateral margin. Gnathotectum (Figure 5C).

Palpus — Total Length 675 – 690 μm .

Chelicerae — Length 385 – 395 μm . The fixed digit is covered by two robust teeth, one on the anterior portion and the other on the central area. There is a row of small denticles between the teeth a row of small denticles is observed. Posterior to the central digit fine serrated edge is observed that is on the

internal part of the fixed digit. The movable digit also possesses two robust teeth, one on the anterior portion and the other on the central area (Figure 5A and 5B).

The morphological measurements of males do not considerably differ from those of the females, with an exception for the genital plates (Table 1).

DISCUSSION

The main difference between *D. lecorrei* and *D. schubarti* is the presence of two dorsolateral openings that are connected in a distinct posteroventral cuticular strip towards the edge of the shield, which is only present in the species *D. lecorrei*. The females of *D. schubarti* present smaller lateral genital plates (LAG) that do not touch the pre-genital plate (PRG). *Diplothyrus lecorrei* has a membranous process on the fixed digit of the chelicera and 13 pairs of setae on the subcapitulum, *D. schubarti* does not have such a cheliceral process and carries only 8 pairs of setae on the subcapitulum.

The morphological measurements observed in the present study did not demonstrate the existence of sexual dimorphism in *D. schubarti*. This absence of differentiation in the size of the morphological structures also occurs in *D. lecorrei*, and in other species of other genera, such as *Australothyrus*. However, as it is common in this order, a differentiation between the sexes exists in the genital plates, with the females presenting a larger opening, composed by a group of four valves in the genital area, while in the male, only two small valves are observed (Van der Hammen, 1983; Klompen, 2010).

Little is known regarding the living habits of the Holothyrida species. Some specimens can be found in the soil, amid the litter and under stones. As for the eating habits, according to some authors, species of the families Allothyridae and Holothyridae are scavengers (Krantz and Walter 2009; Walter and Proctor 1998). However, the cave habit is not common among the species of Holothyrida, this being the first record of specimens belonging to the family Neothyridae in caves. The specimens found in the hypogean environment were collected associated with the organic matter in the entrance of the

caves, walking on the floor in areas of partial shade and in more interior aphotic portions.

Information on the biology and the habits of *D. schubarti* is still scarce. Its geographical distribution, although wider, still remains restricted to only two areas, making it necessary that the caves, where these specimens were found, become the target of preservation action. Such a measure is indeed necessary, mainly when considering that the area is under intense mineral exploration, and the caves (and their associated species), run the risk of disappearing.

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