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ARTICLE

Morphological redescription and morphometry of *Aniara sepulchralis* (Coleoptera: Cicindelidae) from Northeast Para, Brazil

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Abstract. *Aniara sepulchralis* specimens sampled at different locations (Belém and Bragantina microregions) show differences in size of some morphological structures such as head width, pronotum width, distance between eyes, pronotum base width, labral length, labral width, and spur of the third pair of legs. These changes may be due to biotic, abiotic, and structural factors peculiar to each environment. Differences in structures and morphological measurements of males and females of *Aniara sepulchralis* were observed, characterized as sexual dimorphism in relation to their teeth and labral-clypeal suture. Females present apical teeth and rectilinear suture, whereas males present a more rounded shape in both teeth and the labral-clypeal suture. Regarding measurements, females have greater body length, head width, eye distance, pronotum length, pronotum width, labral length, and pronotum base width than males.

Keywords. Coleoptera; Morphometry; Neotropical region; Sexual dimorphism.

INTRODUCTION

Formerly considered a subgroup within the family Carabidae (Ball & Bousquet, 2001, Arndt *et al.,* 2005), Tiger Beetles have recently been elevated to a distinct family, Cicindelidae (Duran & Gough, 2020). It is a diverse taxon with approximately 2,900 described species distributed worldwide (Pearson & Wiesner, 2023). They have an elongated body, coloration ranging from dark to metallic, long running legs, body length ranging from 5 to 40 mm, head without the presence of antenna grooves, and imbricate antennae, composed of 11 to 12 narrow and elongated segments (Gilbert, 1997; Pearson & Vogler, 2001; Ball & Bousquet, 2001).

Various tiger beetle species occur in forest floor leaf litter, grasslands, along rivers and water courses, sandy environments such as dunes and beaches, boulders, and on termite mounds (Knisley & Schultz, 1997; Lövei & Sunderland, 1996; Pearson & Vogler, 2001; Ball & Bousquet, 2001).

One of the least studied species of tiger beetles is *Aniara sepulchralis* (Fabricius, 1801), which is the only species within the genus *Aniara* Hope. Adults are black in color, with a convex body shape, elytra slightly more expanded than the thorax, sub sinuated and sub acuminate near the apical extremity, head and antennae proportionally shorter compared to other species. Running actively during the day, in grassy clearings and open terrain, it has functional flying wings, but rarely flies. Its geographic distribution is restricted to the northern region of South America, including Trinidad, Colombia, Ecuador, Venezuela, French Guiana, Guyana, Surinam, Brazil (Amapá, Pará, Amazonas, Rio Negro, and Mato Grosso) (Pearson & Vogler, 2001, Wiesner, 2020).

As in many cicindelid species, males of *A. sepulchralis* are smaller and less robust than females. Antennae and mouthparts also exhibit dimorphism in both size and shape. Generally, the antennae are larger and the mouthparts more

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conspicuous in male specimens. Legs, elytra and wings may also have characters peculiar to both sexes (Pearson & Vogler, 2001; Martins *et al.,* 2010; Casari & Ide, 2012). Some sexual differences in morphology, as well as between populations, may be directly related to the species' way of life and their habitat, as well as may be influenced by anthropogenic factors, such as urbanization (Talarico *et al.,* 2007; Papp *et al.,* 2020).

The aim of this study was to compare morphologically the species *Aniara sepulchralis* (Carabidae: Cicindelinae) collected in two locations (Belém microregion and Bragantina microregion) of the northeastern Pará. We wanted to verify parameters of sexual dimorphism and to measure variations of the morphological structures of the specimens from different localities. Any significant differences could be used to help discern the important biotic and abiotic influences on the populations of this species.

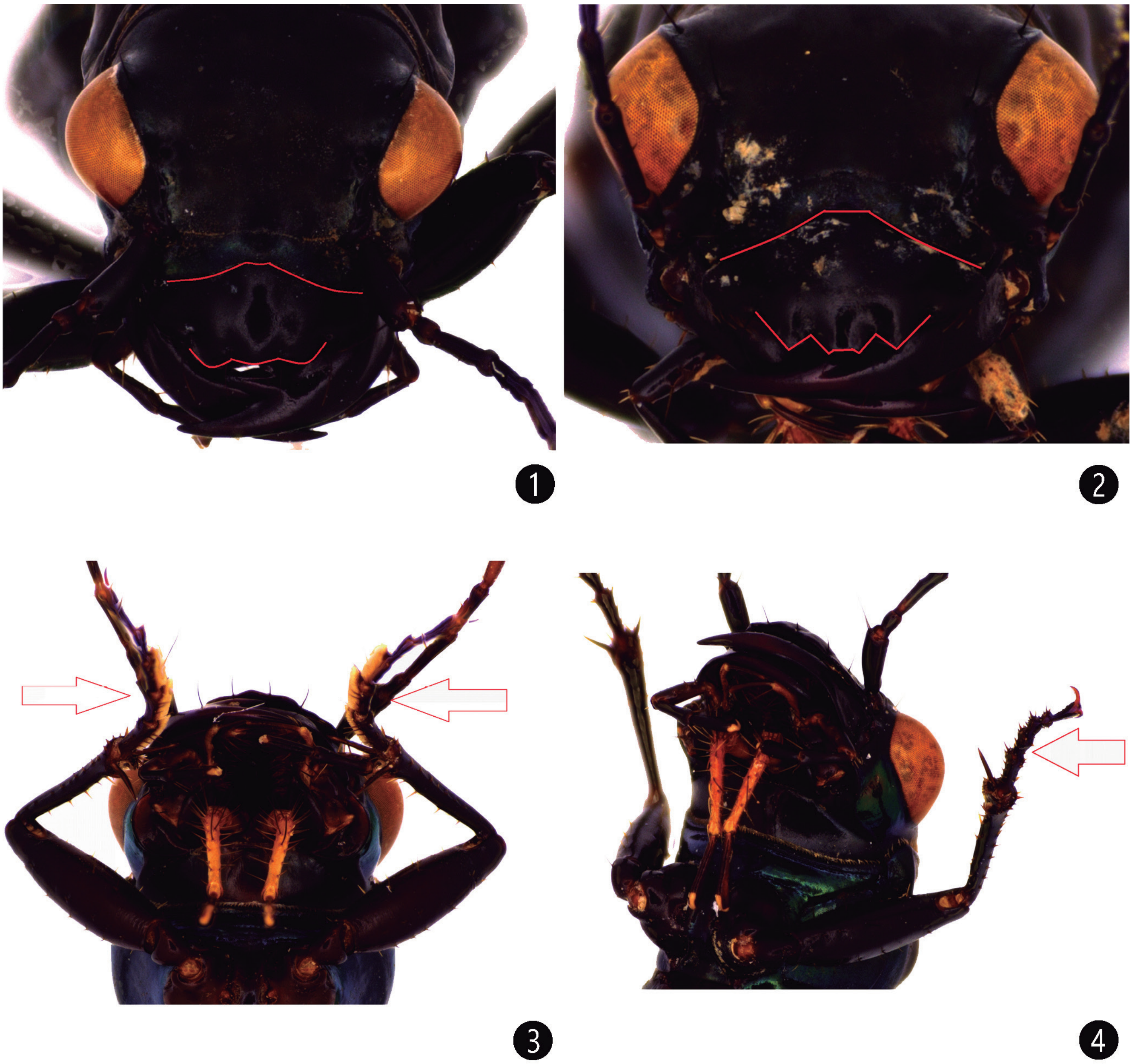
MATERIAL AND METHODS

The specimens analyzed in this study were collected in the municipality of Belém and Igarapé-Açu, Pará, Brazil. A total of 10 specimens was analyzed, five from Belém, PA and five from Igarapé-Açu, PA, two females and three males from each location.

The individuals used in the study were collected from agricultural areas, in the experimental farm of Igarapé-Açu – FEIGA (Fazenda Escola de Igarapé-Açu), and in the Brazilian Agricultural Research Corporation – Embrapa Eastern Amazon (located within the Belém metropolis).

The morphology of *A. sepulchralis* was described using standard external body characteristics (antennae, head, mouthparts, pronotum, legs, elytra and body length) (Ide, 2007, 2012).

For description and measurement, we used the ruler tool from the Leica LAS package. Measurements



Figures 1-4. Sexual dimorphism of the labrum and tarsus of *Aniara sepulchralis.* (1-2) labrum. (1) male; (2) female. (3-4) tarsus. (3) male; (4) female.

were based on total length: from the apex of the head to the last abdominal segment (dorsal view); head length: along the longitudinal line; head width: at eye level; interocular distance: between the nearest points between the eyes; antenna length: from the base of the pedicel to the apex of the last antennomers; pedicel length: from base to apex; pronotum length: at central level of the plaque region; pronotum width: at apex and base level; elytra length: at the level of the elytra/pronotum junction to the end point of the structure; elytra width: at the widest region; leg length I and III: from trochanter to last tarsomere; length of the trochanter femur, tibia, tarsus and spurs: both from base to apex level. All measurements are given in millimeters (mm).

The photographs were taken at the Invertebrate Laboratory of Universidade Federal do Pará, UFPA, Belém, using the camera-attached stereomicroscope. For all the photos taken, the self-assembly technique was used, which consists of capturing images in different focus planes for later mounting via software. The camera used was the Leica DFC 450 coupled to the Leica M205A stereomicroscope (Leica Microsystems GmbH, Wetzlar/Munich, Germany). The self-assembly was done using the Leica LAS package application.

For statistical analysis we used free software R, basic package, which can obtain the results of simple variance, as well as multivariate analysis using principal components (PCA), verifying which variables contributed to the comparison.

RESULTS AND DISCUSSION

General description

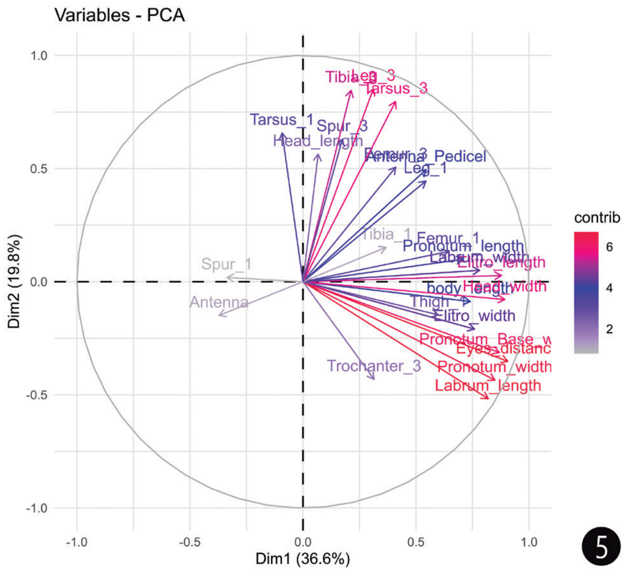
The species is entirely black in color with a body length ranging from 11.3 to 12.8 mm, and an overall fusiform shape and body proportion approximately three times longer than broad. The cuticle is heavily sclerotized with a rough elytral surface (Figs. 14-18).

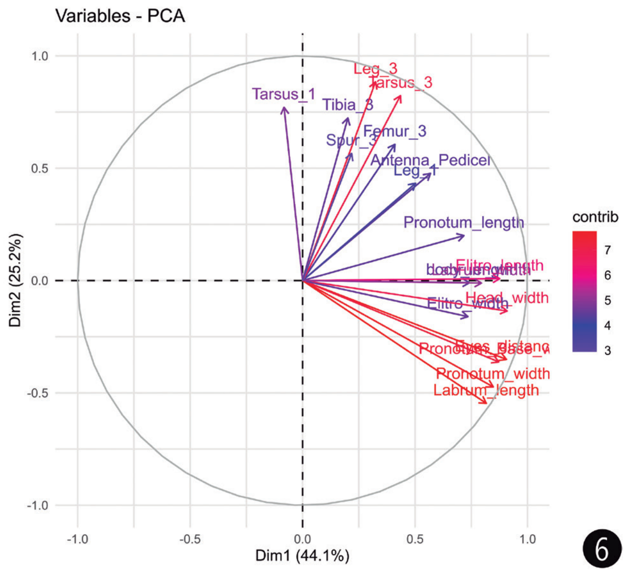
Head

Rectangular head, broader than long, forehead occupying almost all head space and with two frontal grooves protruding near the orbits and reaching the labral suture (Fig. 9). Two supraorbital bristles on each side with distinct punctuation, the bristle being at the apex longer than the other. Flat clypeus, partially fused to the front, separated only by a weak suture, compound eyes, laterally fitted to the head (Figs. 1-2).

Sub-rectangular labrum, broader than long with distal teeth with either rounded or triangular shapes and 4 bristles between the teeth (Figs. 1-2).

Robust mandibles, longer than broad and concave ventrally; right mandible with molar region consisting of three sinuous teeth at the apex of the inner region, left mandible with two robust and larger teeth than the right





Figures 5-6. Principal component analysis (PCA) to verify variables that contributed to the simple variance analysis.

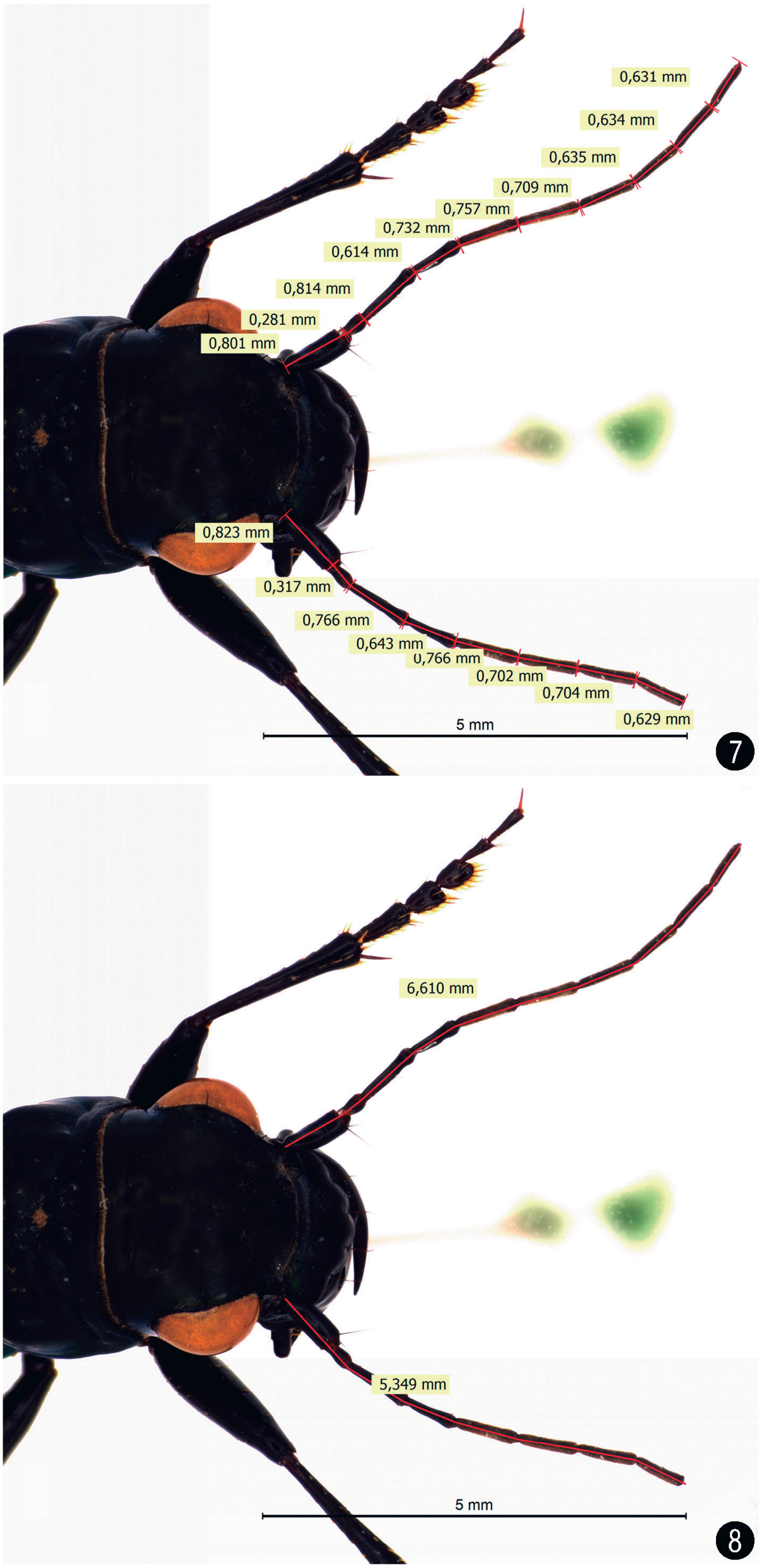
at the apex, and two teeth at the base, one larger and another smaller than at the apex (Figs. 1-2).

Unsegmented galea, with rounded apex composed of palpi and with licinia composed of thick bristles on the inner margin.

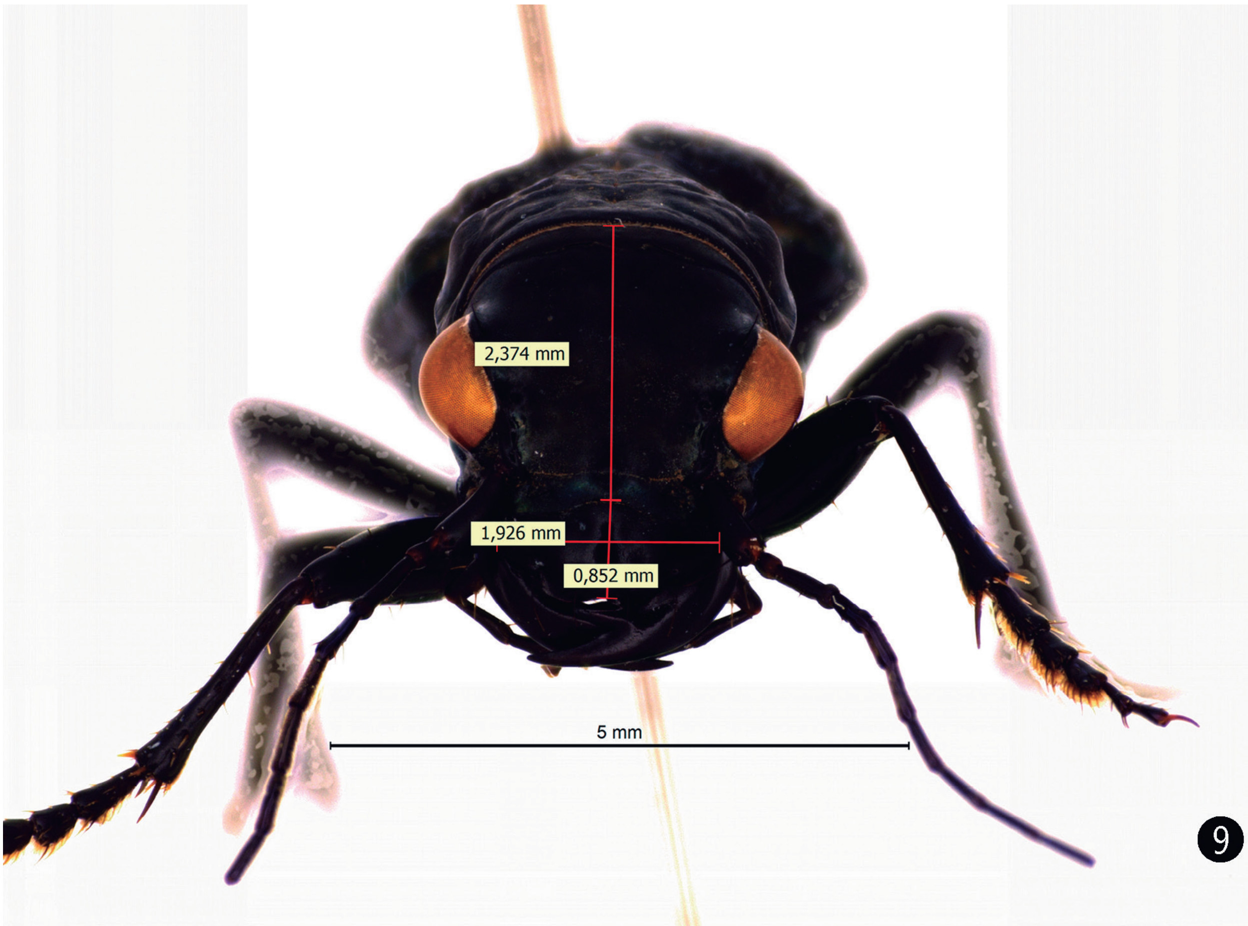
Segmented antennae with 11 antennomers; scape longer than the other segments, and with two bristles at the apex; short pedicel, rounded and without any bristles; 3-4 antennomers larger than pedicel, with antennomere 3 greater than 4, both have two bristles; 5-11 antennomers steadily smaller in size as they approach the apex, the 5th largest of these antennomers, and all have a large number of small bristles (Figs. 7-8).

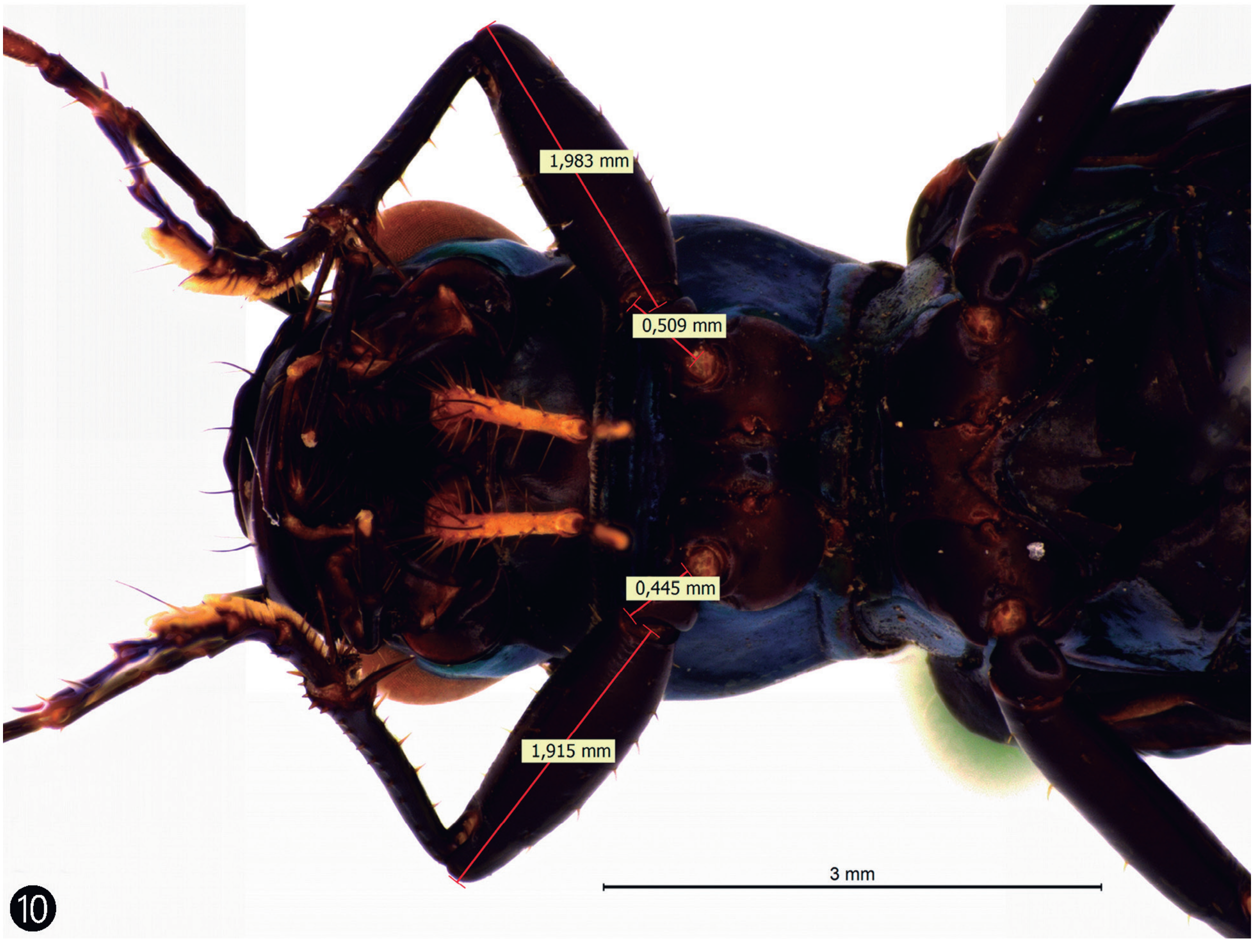
Thorax

Sub-square pronotum, slightly convex, corrugated microsculpture, wider anteriorly and posteriorly, elevations on the lateral side, slightly globular slightly curved

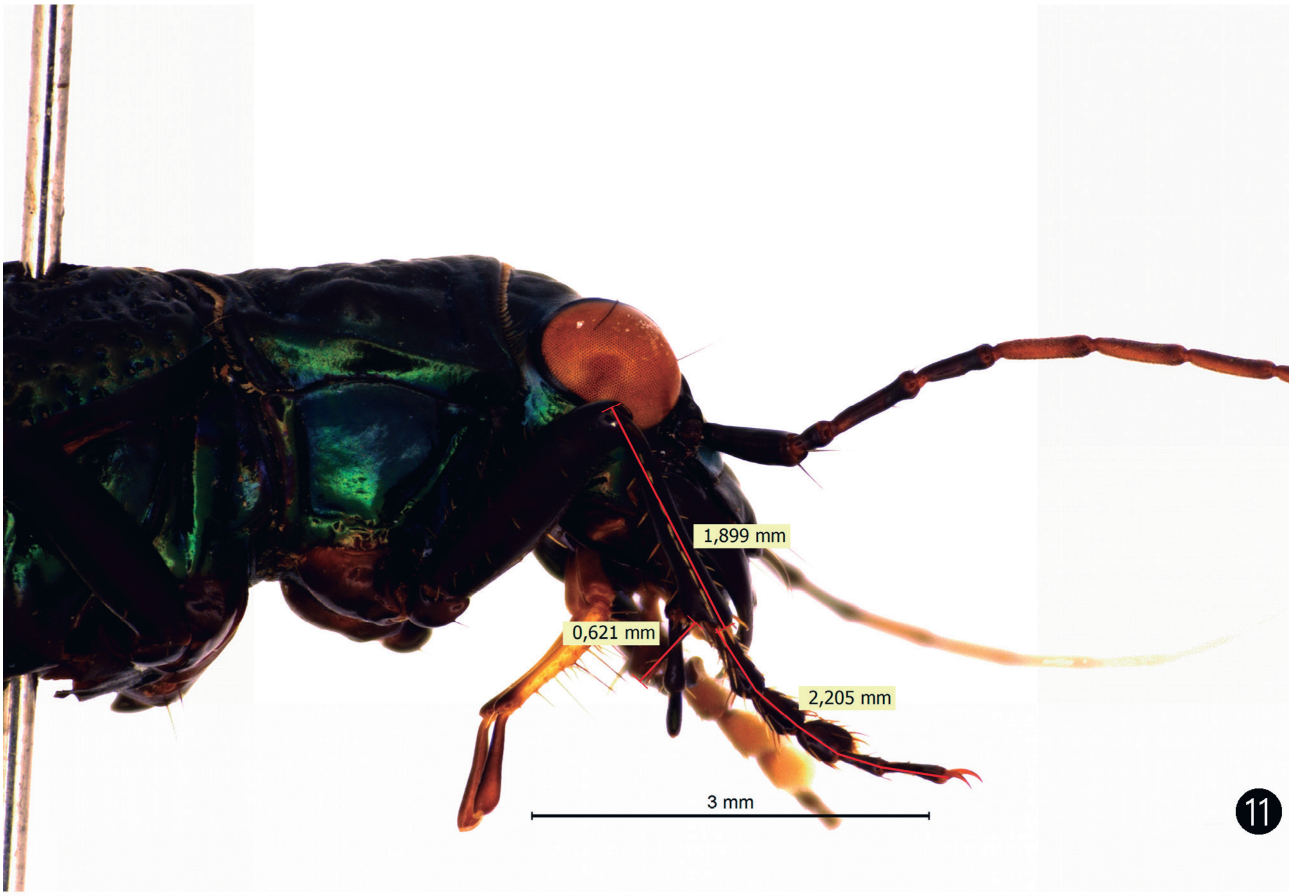


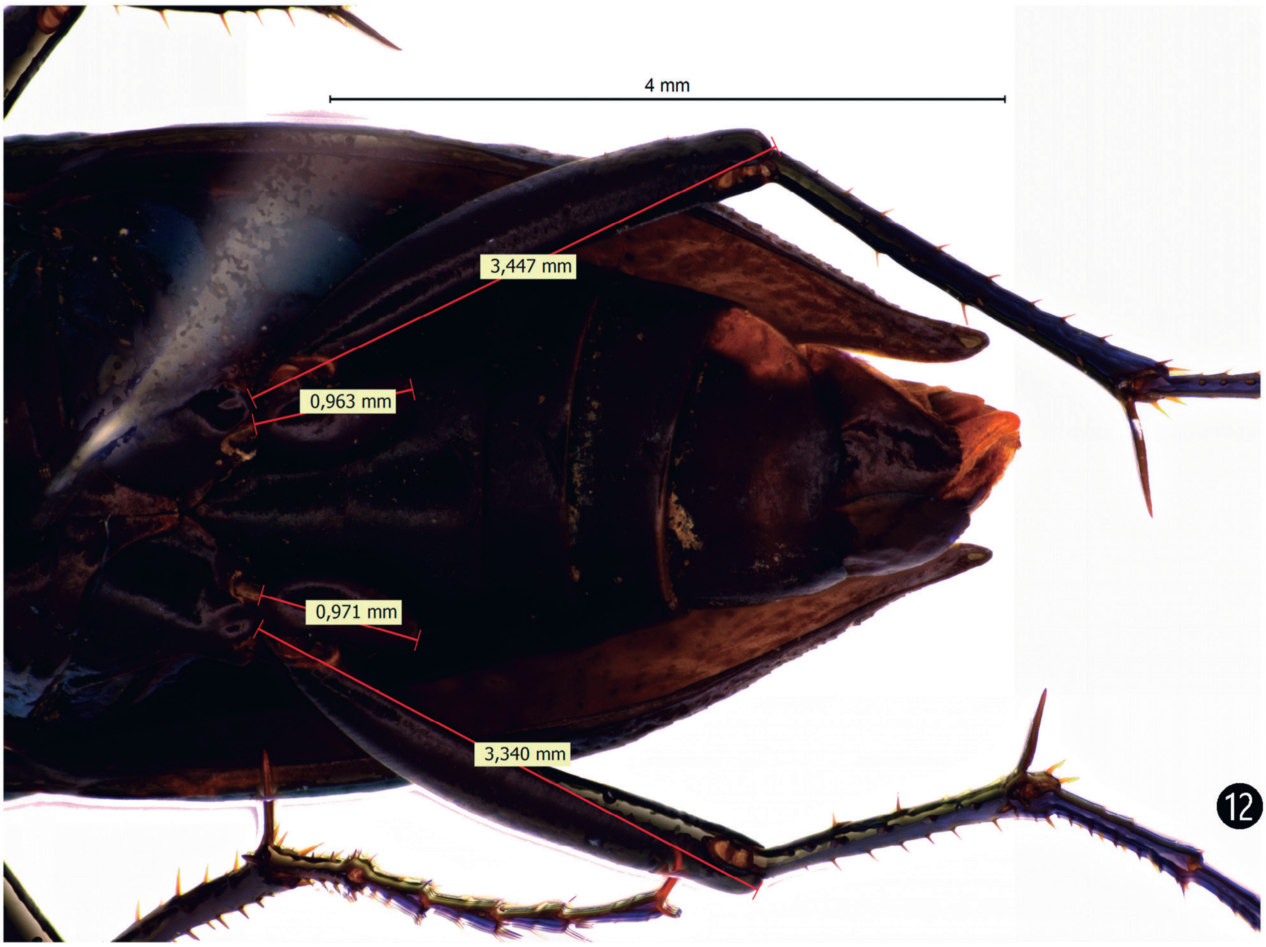
Figures 7-8. Antennas. (7) Pedicel length from base to apex; (8) Length of the antennas, from the base of the pedicel to the apex of the last antennomer.



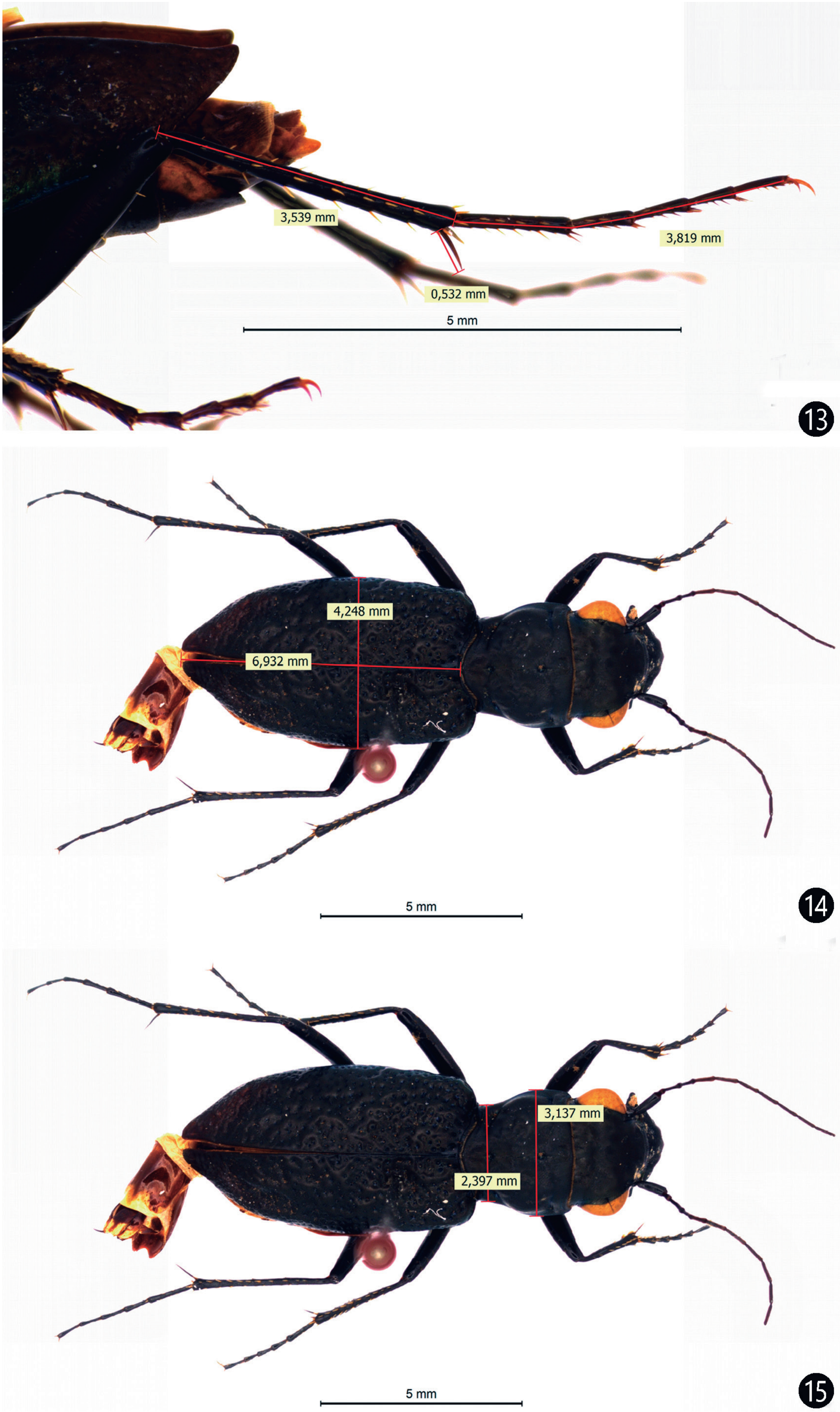


Figures 9-10. Head and first pair of legs. (9) Length of the head along the longitudinal line; (10) Length of the trochanter and femur of the first pair of leg.





Figures 11-12. First and third pair of legs. (11) Length of tibia, tarsus and spur of first pair of legs; (12) Length of the trochanter and femur of the third pair of leg.



Figures 13-15. Habitus of *Aniara sepulchralis.* (13) Length of tibia, tarsus and spur of third leg pair; (14) Elytra length at the level of the elytra; pronotum junction to the elytra endpoint; elytra width at the level of the widest region of the structure; (15) Width of pronotum at apex and base level.

Figures 16-18. Habitus of *Aniara sepulchralis.* (16) Head width at eye level; interocular distance between the nearest points between the eyes; (17) Total length from the apex of the head to the last abdominal segment in dorsal view; (18) Length of the pronotum at the central level of the plate region.

lateral margins, slightly rounded and short-bristled anterior and posterior angles, small and barely visible scutellum (Figs. 15-18).

Elytra convex, with parallel lateral margins, completely rough surface, distinct punctuations on entire surface, higher concentration near pronotum, completely short and rounded humeral angle and no bristles (Figs. 15-18).

Prothoracic legs large and thin, small trochanter, globose and without bristles. Large femur, largest leg joint, broader in the mid region and tapered towards the apex; longitudinally spaced bristles and in a line toward the apex. Tibia smaller than femur, homogeneously thin from base to apex, longitudinally spaced bristles and lined toward apex where they cluster; two symmetrical spurs at the apex (Figs. 10-13). Proximal tarsus, with first tarsomere larger than the others, 2-3 more globous, 4 smaller than the others, 5 larger than 2-4 with dilated apex, bearing two simple claws; bristles throughout the tarsus, and tarsomeres 1-3 with agglomerated bristles on the inside.

Metathoracic and mesothoracic legs large and thicker than prothoracic legs. Subtriangular femora without bristles. Trochanter a bean-like shape with a slight depression in the convex part. Large femur, wider in the middle region; spaced bristles, fewer in number than other parts, lined vertically toward the apex. Narrow tibia, larger than femur; longitudinally spaced bristles, in four rows, towards the apex, and with two apical spurs. Proximal tarsus with first tarsomere larger than the others and with the presence of bristles; tarsomeres 2-5 practically the same size and with the presence of bristles, tarsomere 5 with dilated apex, carrying two simple claws (Figs. 10-13).

Dimorphism

Sexual dimorphism within Cicindelidae and closely related Carabidae species has been widely studied. Such structures as legs, mouth and wings, distinguish the sexing of animals (Ide, 2007; Martins *et al.,* 2010; Moravec, 2015).

In *A. sepulchralis* there was no significant difference in the dorsal aspect (body shape and length) between male and female. However, some specific structures, such as the labrum and tarsi, exhibited differences (Figs. 1-4).

The labrum in both sexes has dark coloration, 4 bristles and 5 teeth. However, females have have triangular-shaped teeth and rectilinear labral suture. Males, on the other hand, have a more sinuous shape in both the teeth and the labral suture (Figs. 1-2). Such sexual differences have been observed in several species of Cicindelidae (Martins *et al.,* 2010).

Males and females also present differences in the bristles present in tarsomeres I, II and III. In males there are adhesive bristles on the inside, which are absent in females (Figs. 3-4). This dimorphism is observed in many species of Cicindelidae (Martins *et al.,* 2010). It is thought that these bristles help males grip the females in copulation and subsequent mate guarding.

Morphometry

Some variables that contributed little to variance within the principal component analysis (PCA) were excluded. These non-relevant variables were head length, antenna, tibia of leg 1, thigh of leg 1, femur of leg 1, spur of leg 1 and trochanter of leg 3 (Fig. 5).

After removal of non-relevant variables, the explanation of total variance was near 70% (Fig. 6). The graph shows how relevant each structure is for the analysis, where the red components have the highest contribution. Each component is consistent for the high or low values. The smaller the angle between the variables, the greater the relationship between them.

Although the sample sizes were small, the p values were so significant, we consider them appropriate for cautious biological interpretation. Regarding morphological differences based on the site from which they were collected, the moist significant variables included: head width ($p = 0.0014$), pronotum width ($p = 0.0088$), distance between eyes ($p = 0.0130$), width from the base of the pronotum ($p = 0.0176$), labral length ($p = 0.0214$), labral width ($p = 0.0273$) and spur ($p = 0.0858$). The specimens from Igarapé-Açu showed larger sizes than those from Belém (Table 1).

Behavior and microhabitat have been associated with variation within some morphological structures of several species of beetle. Species of the genus *Siagona* and *Carabus* showed variation in the body length and in the morphology of the compound eyes (Talarico *et al.,* 2007; Talarico *et al.,* 2011), and *Scaphinotus petersi* showed a difference in the head and pronotum (Ober & Connolly, 2015) that were correlated with anthropogenic interferences, such as proximity to urbanized environments

Table 1. Means and analysis of variance of body measurements of *Aniara sepulchralis* (Carabidae: Cicindelinae) in relation to locality, Belém and Igarapé-Açu, Pará, Brazil.

Measured variable	Means		*F*	p	
	Belém	Igarapé-Açu			
Body length	11,904	12,041	0,4335	0,5313	
Head width	3,128	3,364	25,9530	0,0014	***
Eyes Distance	2,387	2,557	10,9390	0,0130	**
Pronotum Length	2,738	2,792	0,5918	0,4669	
Pronotum width	3,027	3,202	12,9280	0,0088	***
Pronotum Base	2,422	2,558	9,5416	0,0176	**
Elytra Length	7,385	7,731	2,7954	0,1385	
Elytra Width	4,336	4,625	1,2291	0,3042	
Antenna pedicel	0,762	0,806	2,4823	0,1591	
Labrum Length	0,837	0,936	8,7108	0,0214	**
Labrum Width	1,823	1,949	7,7358	0,0273	**
Leg I	7,047	7,051	0,0005	0,9830	
Tarsus I	2,304	2,282	0,0509	0,8279	
Leg III	12,614	12,547	0,0290	0,8696	
Femur III	3,626	3,548	0,2580	0,6271	
Tibia III	3,896	3,899	0,0002	0,9905	
Tarsus III	4,007	4,105	0,3124	0,5936	
Spur III	0,574	0,708	3,9942	0,0858	*

Significant variables for p = '***' 0.01 '**' 0.05 '*' 0.1

Table 2. Means and analysis of variance of body measurements of *Aniara sepulchralis* (Carabidae: Cicindelinae) in relation to sex (male and female) sampled in Belém and Igarapé-Açu, Pará, Brazil.

Measured variable	Mean		*F*	p	
	Male	Female			
Body length	11,611	12,560	21,3011	0,0024	***
Head width	3,187	3,342	11,4260	0,0118	**
Eyes Distance	2,407	2,574	10,5020	0,0142	**
Pronotum Length	2,688	2,872	6,2074	0,0415	**
Pronotum width	3,064	3,216	11,5430	0,0115	**
Pronotum Base	2,443	2,546	4,2374	0,0785	*
Elytra Length	7,353	7,671	0,7881	0,4042	
Elytra Width	4,259	4,778	3,4638	0,1050	
Antenna pedicel	0,766	0,776	0,2320	0,6447	
Labrum Length	0,854	0,944	8,0190	0,0253	**
Labrum Width	1,856	1,905	0,4500	0,5239	
Leg I	6,924	7,056	0,0027	0,9603	
Tarsus I	2,289	2,217	1,6227	0,2434	
Leg III	12,360	12,624	0,0316	0,8639	
Femur III	3,469	3,721	2,0307	0,1972	
Tibia III	3,859	3,866	0,0832	0,7814	
Tarsus III	3,967	4,033	0,0463	0,8358	
Spur III	0,654	0,605	0,7834	0,4055	

Significant variables for p = '***' 0.01 '**' 0.05 '*' 0.1

(Papp *et al.,* 2020). However, both biotic and abiotic factors need to be tested in the future to determine if individuals of *A. sepulchralis* from the two study areas are associated with these human influences and, if so, which ones.

The female specimens presented larger body length and morphological structures (Table 2). Significant variables for sexual dimorphism were: Body length (p = 0.0024); head width (p = 0.0118), distance between eyes (p = 0.0142), pronotum length (p = 0.0415), pronotum width (p = 0.0115), labral length (p = 0.0253); and pronotum base width (p = 0.0785).

As observed in other studies, cicindelids/carabids commonly exhibit sexual dimorphism, as in spurs of the first segment of the tarsus (Fuente *et al.,* 2010), antenomers (Benitez, 2013; Fuente *et al.,* 2010) and abdominal segments (Alibert *et al.,* 2001). The female of *A. sepulchralis* is significantly larger, as in many invertebrates (Gould, 1996). This may be due to factors such as the ability to support the male's weight during copulation or the need to support more and heavier eggs for increased fecundity (Adams & Funk, 1997; Tammaru *et al.,* 2002). Possible cause(s) of this size dimorphism include a combination of environmental factors, such as humid or dry weather, and anthropogenic factors, such as soil or forest management and increased urbanization near these sites (Benitez, 2013; Papp *et al.,* 2020).

CONCLUSION

Aniara sepulchralis showed a significant difference in the size of the morphological structures, between populations and between male and female. Likely evolutionary influences include biotic and abiotic factors, such as climate and urbanization. In addition to sexual dimorphism in the size of certain structures, dimorphism in the form of the labrum and in the presence of adhesive bristles in male tarsi was evident. These indicate increasing differences in reproductive behavior as a possible factor between the two sites.

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