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Ultrastructural analysis and polymorphisms in *Coarazuphium caatinga* (Coleoptera: Carabidae: Zuphiini), a new Brazilian troglobitic beetle

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Abstract

Coarazuphium caatinga sp. n. occurs in limestone caves located in Campo Formoso municipality, in the Brazilian Caatinga (Bahia, Brazil). The new species is close to *C. formoso* although they are morphologically distinct by the elytra sinuosity, which is more pronounced in *C. caatinga*; the aedeagus is more tapered at the tip in this last species. Important traits found in *C. caatinga* are the variable size presented by the eyes, and the remarkable variability of body pigmentation among specimens; both traits do not seem to be correlated. *Coarazuphium* Gnaspini, P., Vanin, S.A. & Godoy, N.M., 1998, species exhibit advanced troglomorphic characters in comparison to other Brazilian cave beetles, as are increased extra-optic sensory structures, presence of particular sensilla, and sensory and gustatory receptors. These characters are not detected under routine microscopy and thus require ultrastructural methods for their study.

Key words: eyes, coloration variability, ground beetle, caves, hypogeous, sensilla

Introduction

Obligatory cave-dwelling invertebrates usually possess singular morphological traits, such as the elongation of appendages (including sensorial structures), reduction of eyes, and wings. Those species are called troglobionts, by the Schinner-Racovitza classification system (modified by Holsinger & Culver 1988).

To date, all members of the genus *Coarazuphium* Gnaspini, P., Vanin, S.A. & Godoy, N.M., 1998, found in Brazilian caves are troglobitic species. According to Gnaspini & Trajano (1994), *Coarazuphium* species are considered those that have the most advanced troglomorphic traits among all Brazilian cave beetles. Some singular features of these species are not macroscopic and thus require of ultrastructural analyses for determining them. Recently, a new species *Coarazuphium whiteheadi* Ball and Shpeley 2013, was found in Mexico, probably a hypogaeic (troglophilic) specie (Ball & Shpeley, 2013), the only species of this genus not strictly troglobiont.

Coarazuphium species are closely related to two other Zuphiini genera: *Zuphium* Latreille, 1806 and *Parazuphium* Jeannel, 1942 (Godoy & Vanin 1990). Recently, Andújar *et al.* 2011 have described a new blind species of *Parazuphium* from Morocco. The authors stated that this new species has traits in common with *Ildobates* Español, 1966, and that this is probably a closely related genus (Ortuño *et al.* 2004; Ribera *et al.* 2006). Ball and Shpeley (2013) proposed that shared features of *Zuphioides* and *Coarazuphium* suggest that they are sister taxa, and noted that *Coarazuphium* posses the mostly derivate features, considering life habits. However, more detailed phylogenetic analysis would be needed to establish the position of those four genera within Zuphiini.

In this work, we describe the eighth species of the genus, *C. caatinga* found in limestone caves from Brazil. This description also focuses on the ultrastructural analysis of antennae, mouthparts, and legs.

Material and methods

Seven specimens were collected at Toca do Gonçalves Cave (10°30'41"S 40°53'39.8"W, 546 m a.s.l.), and also at Toca de São Tomé Cave (10°36'06"S 40°55'57"W, 532 m a.s.l.), both located in Campo Formoso municipality, Bahia, Brazil (Fig. 1). Specimens were visually searched for throughout the base and walls of the caves and were collected between January of 2008 and January of 2013. Specimens were captured with a fine brush and placed in vials containing 70% ethanol.

Measurements and drawings were made under a stereomicroscope and a *camera lucida* microscope. To dissecting male and female genitalia, fine entomological pins were used. Male genitalia were prepared in Kayser glycerol gelatin. Female genitalia were cleared in KOH water solution. Genitalia drawings were made under a Leica MDLS phase contrast microscope. Micrographic images were obtained using the AxioCam ERc 5s program connected to a Primo Star microscope (ZEISS). The stereoscope images were obtained using the Leica M205 A, with the program Leica Application Suite auto-handling to combine the images. Ultrastructural analyses were conducted through use of a scanning electron microscope. Parts from Paratype 1 were attached on an aluminum support stub, placed over a film of aluminum foil with carbon tape, sputter-covered with gold (Baltec SCD 050), and examined in a LEO EVO 40 XVP scanning electron microscope (Leo Electron Microscopy). All images were prepared and grouped on plates using *Photoshop CS6* program.

For naming male and female genitalia we followed Ball & Shpeley (2013) and Liebherr & Will (1998) respectively. We followed the criteria of Schneider (1964), McIver (1975), Zacharuk (1980), Kim & Yamasak (1996) and Merivee *et al.* (2000) for naming ultrastructural morphological parts and assessing their function. Minimum and maximum morphometric data from paratypes are given in parenthesis (in mm).

Type specimens are deposited in the Zoology Collection (Coleção de Invertebrados Subterrâneos de Lavras), Seção de Invertebrados Subterrâneos (ISLA 2282—Male holotype ISLA 2280—Female paratype 1; ISLA 2281—Female paratype 2; ISLA 2283—Male paratype 3 and ISLA 2284—Male paratype 4; all specimens from Toca do Gonçalves Cave; and ISLA 3974—Male paratype 5; ISLA 3975—Female paratype 6; ISLA 3976—Female paratype 7; from Toca São de Tomé Cave), at the Federal University of Lavras (UFLA), Minas Gerais, Brazil. Other species were also examined: *C. formoso* (ISLA 1057—Male holotype; ISLA 1058—Female paratype 1; ISLA 1059—Male paratype 2 and ISLA 1060—Male paratype 3), *C. tapiaguassu* (ISLA 1493—Male holotype; ISLA 1494—Female paratype 1; ISLA 1495—Male paratype 2 and ISLA 1496—Male paratype 3) and *C. cessaïma* (ISLA 2288—male).

Taxonomy

Family CARABIDAE Latreille, 1802

Tribe Zuphiini Bonelli, 1810

Genus *Coarazuphium* Gnaspini, Vanin & Godoy, 1998

Coarazuphium caatinga sp. n.

Description. Holotype male (Fig. 2A). Total length, from the apex of the mandible to the apex of the elytra: 6.43 mm (5.51–6.43), width, from at the widest region of the elytra: 1.98 mm (1.73–2.00). Body coloration varies from dark brown to yellowish (Figs. 1B, 3), dorsal integument of the elytra covered with short recumbent hairs.

Head. Subtrapezoidal (Fig. 2) with similar width and length, width/length ratio: 1.02 (0.94–1.11). Maximum width of head at its base, 1.30 (1.13–1.38). Head slightly narrower than pronotum. Dorsal surface with one pair of setae internal to the ocular area and one pair of lateral setae located immediately behind ocular area, one pair at the widest region of the head, and two pairs close to posterior margin of head (both more internally placed). Ventral surface with two pairs of posterior setae close to median line of head, another pair inside the median line of head, and an anterior pair, close to margin of gular region (Fig. 2B). Eyes reduced and depigmented, situated laterally at the end of antennal insertion of the head. Although all specimens analyzed had reduced and depigmented eyes, they showed a high variation in the ocular area size. The eyes varied from 0.084 to 0.143 times the length of head, indicating a polymorphism of this trait in the species (Fig. 4).

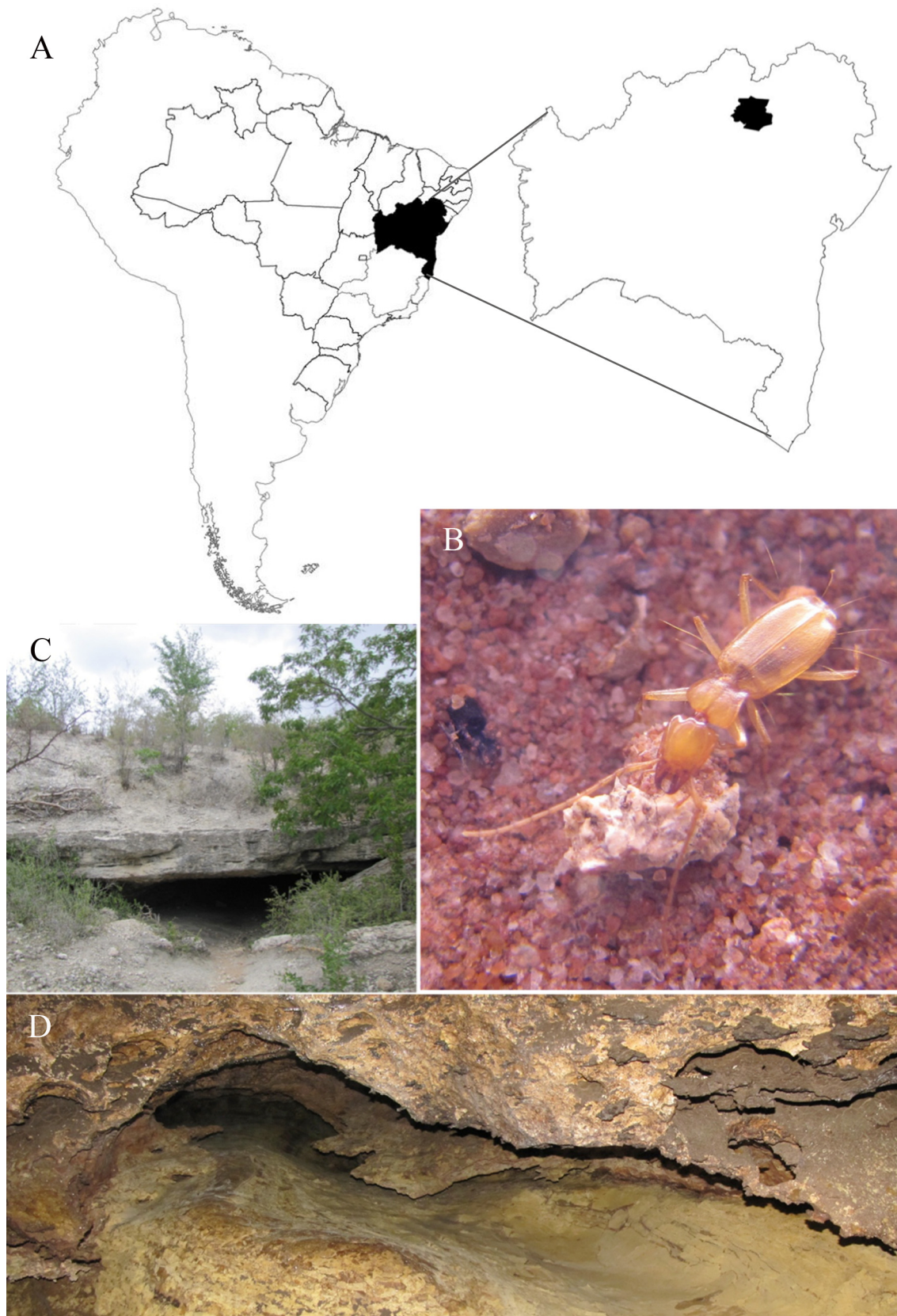


FIGURE 1. (A) Localization of Campo Formoso, type municipality at Bahia state, Brazil. (B) Live specimen of *Coarazuphium caatinga* sp. n. (C) Toca do Gonçalves Cave entrance. (D) Conduct of Toca do Gonçalves Cave.

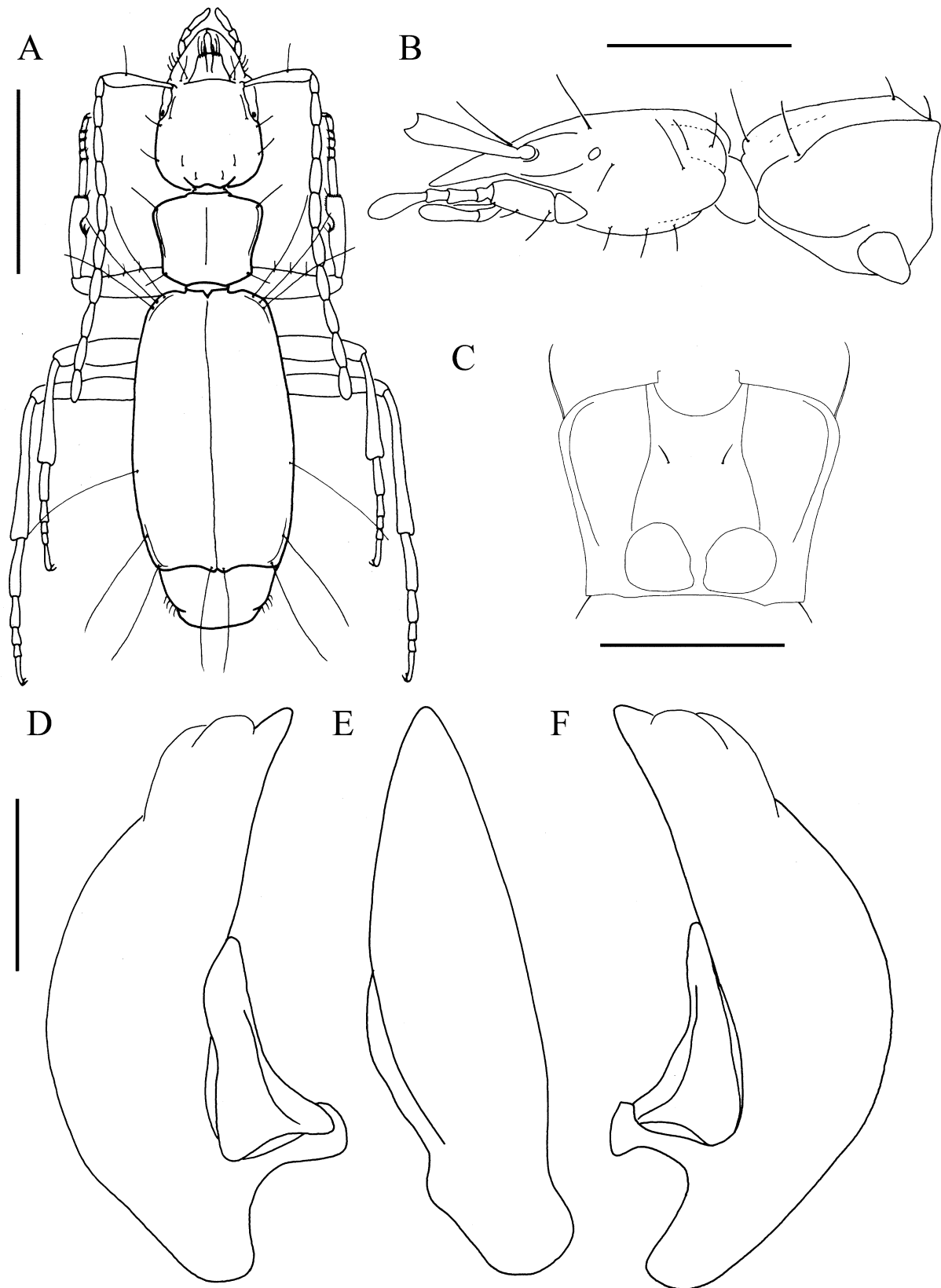


FIGURE 2. *Coarazuphium caatinga* sp. n. (A) Habitus from paratype 1. (B) Head and pronotum lateral view. (C) Prothorax, ventral view. (D) Aedeagus, left lateral view. (E) Aedeagus, dorsal view (F) Aedeagus, right lateral view. Scale bar (A = 2 mm; B = 1 mm; C = 0.857 mm and D, E, F = 0.25 mm).



FIGURE 3. Color variation among *Coarazuphium caatinga* sp. n. specimens.



FIGURE 4. Different *Coarazuphium caatinga* sp. n. specimens, showing differences in ocular area size. Dashed circles indicate each specimen eyes size. Eyes scale bar from left to right, from the top to the bottom: 126 μm ; 106 μm ; 97 μm ; 146 μm ; 103 μm ; 93 μm ; 141 μm ; 110 μm .

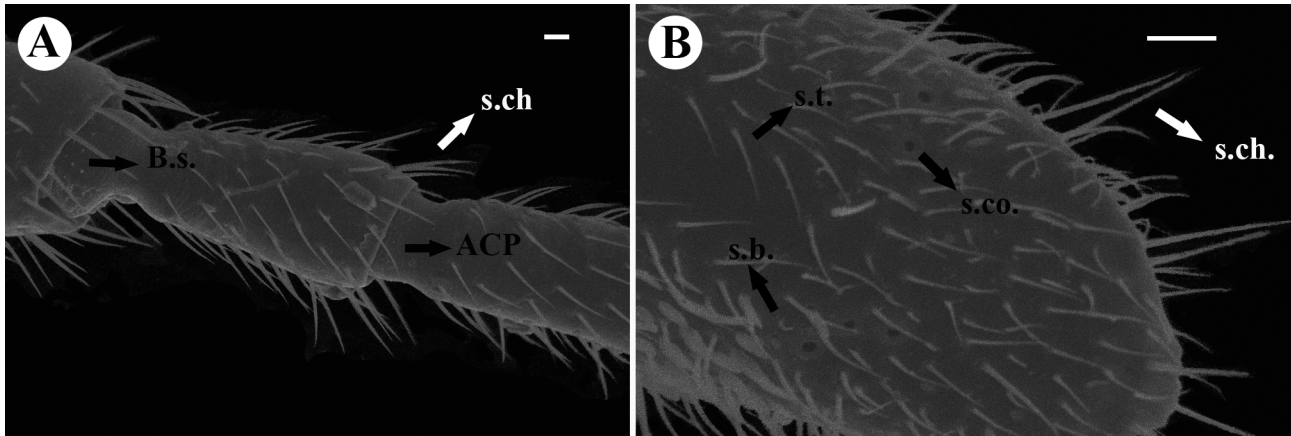


FIGURE 5. Scanning electron micrograph showing antennal segments and sensilla in *Coarazuphium caatinga* sp. n. s.ch. show sensilla chaetica, s.t. trichoid sensilla, s.b. sensilla basiconica, s.co. coeloconic sensilla, B.s. Böhm sensilla. ACP, appendages of cuticular plates. (A) Scape, pedicel and first flagellum. (B) Terminal antennomere. Scale bar (A, B = 20 μ m).

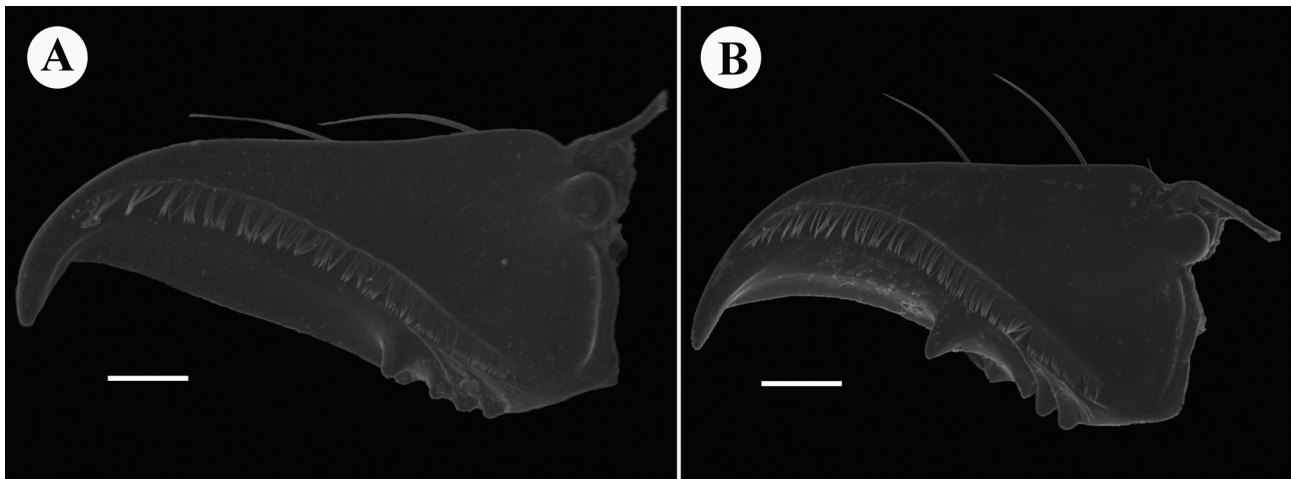


FIGURE 6. Scanning electron micrograph showing ventral view from left mandible. (A) *Coarazuphium caatinga* sp. n. (B) *C. formoso*. Scale bar (A, B = 100 μ m).

Antennae. Antennae filiform and flagellar, (Fig. 2A) 4.75 mm (4.27–4.86), 4.63 (3.83–4.72) times longer than pronotum; first antennomere elongate, shorter than 2–4 together. First antennomere with a long bristle close to the middle. Antennomeres are almost round in cross-section, except for the tip of the terminal, which is laterally flattened (Fig. 5B).

Sensilla on the antennae of one specimen were examined. The sensilla chaetoid (s.ch) (sensory bristles or spines) are present on all antennomeres. Appendages of cuticular plates (ACP) were abundant at the bases of all antennomeres, close to the intersegmental joints (Fig. 5A). Trichoid sensilla (s.t.) (sensory hairs), basiconic sensilla (s.b.) and coeloconic sensilla (s.co.) can all be found from the 4th to 11th antennomeres. Some Böhm sensilla (B.s.) (sensory pit-pegs) are also present in areas opposite the intersegmental membrane between head and scape, as well as between scape and pedicel on the scape and pedicel bases, respectively (Fig. 5).

Mouthparts. Sensilla on the mandible, maxilla, labial palpus, labrum, and clypeus of the Paratype 1 were examined. On the dorsal surface, a series of hair sensilla projects from the submolar region to near the cuticular processes (Fig. 6A). The mandible is acutely bent inwardly at its tip. On the ventral side, longitudinal rows of setae are present.

The maxilla basically consists of the lacinia, galea and maxillary palp (Figs. 7A–D). The lacinia is shorter than the galea, with an acute and curved end, with rows of long setae and cuticular processes. The galea is biarticulated, composed of two segments, with different types of basiconic sensilla. The four-palpomere from maxillary palp is long and filiform with spaced basiconic sensilla present on the surfaces of the segments. Trichoid sensilla are

distributed along the maxillary palp, and they become more abundant and smaller on the last segment. There are also grooves in this segment that may indicate a sensory organ or gustatory receptors (Figs. 7C–D).

The labium is composed of three segments; Labial palpomere 1 is glabrous; labial palpomere 2 has one pair of medium setae and two pairs of long setae, which has some sensilla chaetoid within, and Labial palpomere 3 also has chaetoid sensilla covering it (Fig. 8A). The labrum is quadrangular and presents tree pairs of setae in sequential sizes on dorsal margin (Figs. 8B).

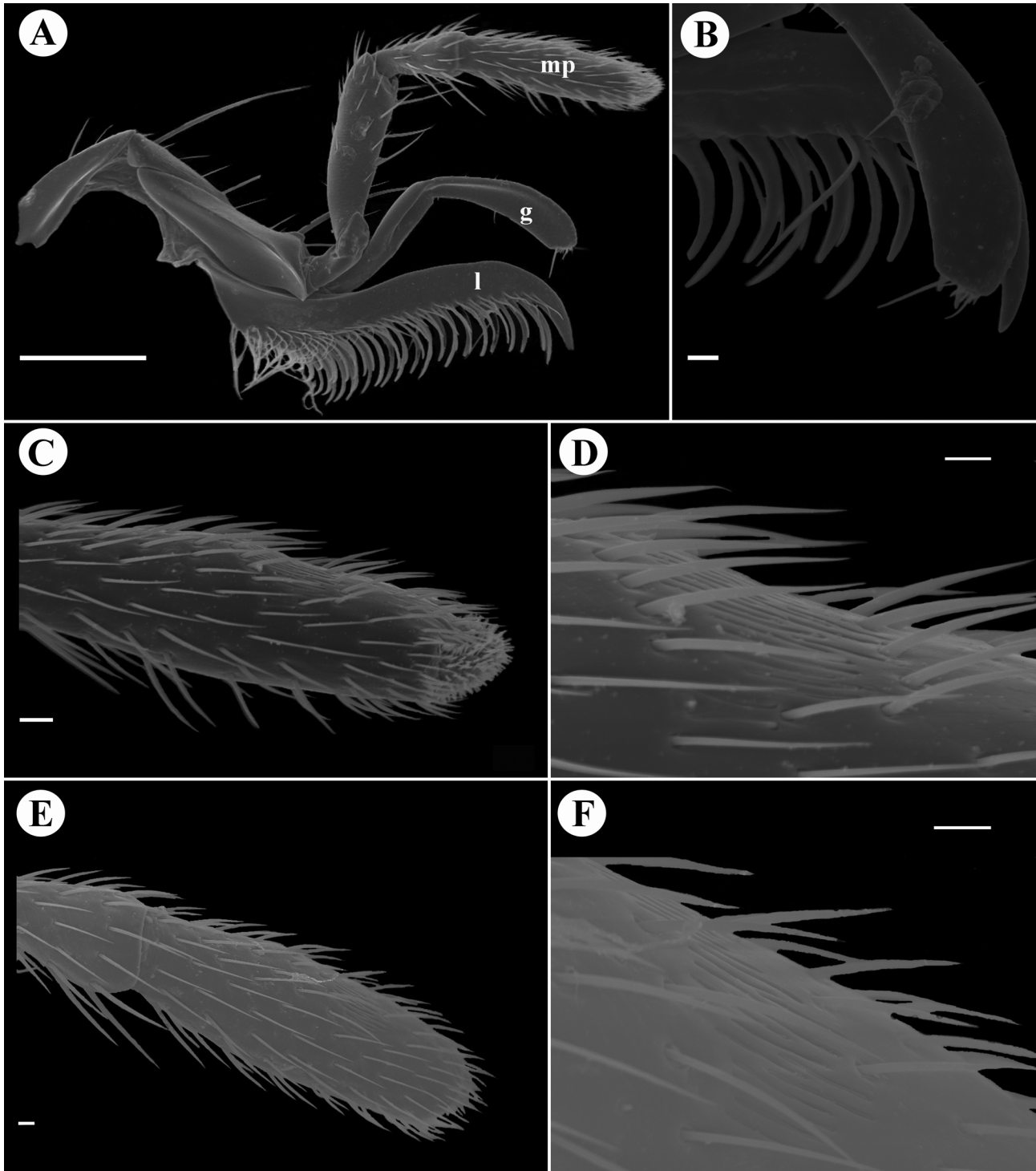


FIGURE 7. Scanning electron micrograph showing maxilla. m.p. = maxillary palp; g = galea; l = lacinia. (A) Right maxilla, dorsal view from *Coarazuphium caatinga* sp. n. (B) Right galea apices, dorsal view from *C. caatinga* sp. n. (C) Right maxillary palp apices from *C. caatinga* sp. n. (D) Close-up on maxillary palp apices, view of a probable sensilla organ from *C. caatinga* sp. n. (E) Right maxillary palp apices from *Coarazuphium formoso*. (F) Close-up on maxillary palp apices, view of a probable sensilla organ from *C. formoso*. Scale bar (A = 200 μ m; B, C = 20 μ m; D, E = 10 μ m; F = 100 μ m).

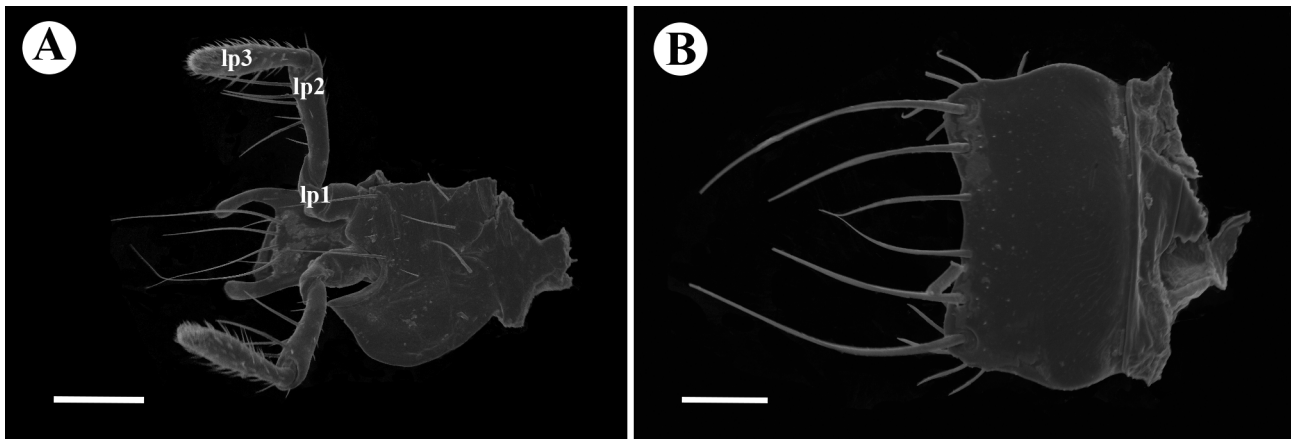


FIGURE 8. Scanning electron micrograph showing mouth parts (A) Labium with labial palpus from *Coarazuphium caatinga* sp. n., lp1 = first labial palp, lp2 = second labial palp and lp3 = third labial palp. (B) Dorsal view of the labrum from *Coarazuphium caatinga* sp. n. Scale bar (A = 200 μ m; B = 100 μ m).

Pronotum. Shape trapezoidal, 1.32 (1.19–1.32) times wider than long (Figs. 2A–B). Maximum width closes to anterior angle as wide as head. Posterior angle is acute. Dorsal surface (Fig. 2A) with two pairs of erect setae: one close to the anterior angle of the pronotum and the other, shorter, close to the posterior angle. Ventral surface with one pair of anterior setae medially located (Fig. 2C).

Elytra. Elytra are free (Fig. 2A), together 1.72 (1.68–1.76) times longer than wide. Maximum width nearly one third the distance from the apex and 1.44 (1.28–1.48) times wider than pronotum. Apex of elytra is very sinuous. Seven large setae on each elytron: 3 close to the anterior angle, 2 marginal in posterior half, and 2 on posterior margin. Hind wings absent. Abdominal sterna 1–5, glabrous, sixth sternum with a small pair of setae close to its posterior margin.

Legs. Procoxa glabrous (Fig. 2B); mesocoxa with two, and metacoxa with one pair of setae close to the anterior margin. Pro-, meso- and metatrochanter bear one medial setae. Profemur with long and short setae. Profemur 1.16(1.04–1.13), as long as the mesofemur and 0.91(0.67–0.86) times the length of metafemur. Protibia 0.84 (1.0–1.15) as long as the mesotibia and 0.62(0.67–0.76) times the length of metatibia. Protibia 1.02(1.15–1.30) times longer than protarsus. Mesotibia 1.04(0.8–0.95) times the length of mesotarsus and metatibia 1.02(0.88–1.06) times longer than the metatarsus. First tarsomere almost equal to tarsomeres 2–4 together. Length of protibia and tarsus together 2.29 (2.14–2.38) times the length of the pronotum. Mesotibia and tarsus length 2.68 (2.28–2.52) times, and metatibia and tarsus length 3.63 (3.19–3.60) times the length of pronotum.

The ultrastructural analysis showed that on the femur, trichoid sensilla are regularly distributed on the whole surface. ACP are abundant at the base of the protibia, close to the intersegmental joint, where there is also an aggregate of basiconic sensilla. The protibia also has a row of trichoid sensilla, which become more abundant at the apex, and spaced basiconic sensilla occur at its border (Fig. 9A). The tarsus has abundant trichoid sensilla (Fig. 9B); and it also has some articulo seta on the ventral side of tarsus, which occurred only in males (Fig. 9C).

Aedeagus. Dorsally curved and elongate (PW/PL 0.287) (Figs. 2D and 10A–C), narrowed apically, apical margin rounded, ostium membrane extensive (OM/PL 0.376). Left paramere (**lp**) about two times longer than wide with an irregular border, right paramere (**rp**) slighter curved and elongate (rp/lp 0.472).

Female genitalia. *Ovipositor* (Figs 11A–C): Gonocoxite 1 (**gc1**) subtriangular with patch of six long trichoid setae distally on ventral surface. Gonocoxite 2 (**gc2**) short, with apex pointed and turned out, in lateral aspect falciform; ventral surface toward margins with row of marginal pit pegs (**mpp**).

Female genital tract (Figs 11A–C): Bursa copulatrix (**bc**) inserted at base of common oviduct (**co**), bursal sacculus (**bs**) is broken in the specimen analyzed. Spermatheca (**sp**) is large and elongate, inserted laterally to the genital tract. Secondary spermatheca gland (**ssg**) markedly elongate and slender, located just above bursal sacculus.

Etymology. The epithet is given in apposition as a toponymic for the name of the biome (Caatinga) where both, Toca do Gonçalo Cave and Toca de São Tomé Cave are situated. The name Caatinga is a Tupi-Guaraní word meaning white forest (Kaa = forest, tínga = white), this semi-arid biome is found in areas of small rainfall in northeastern Brazil.

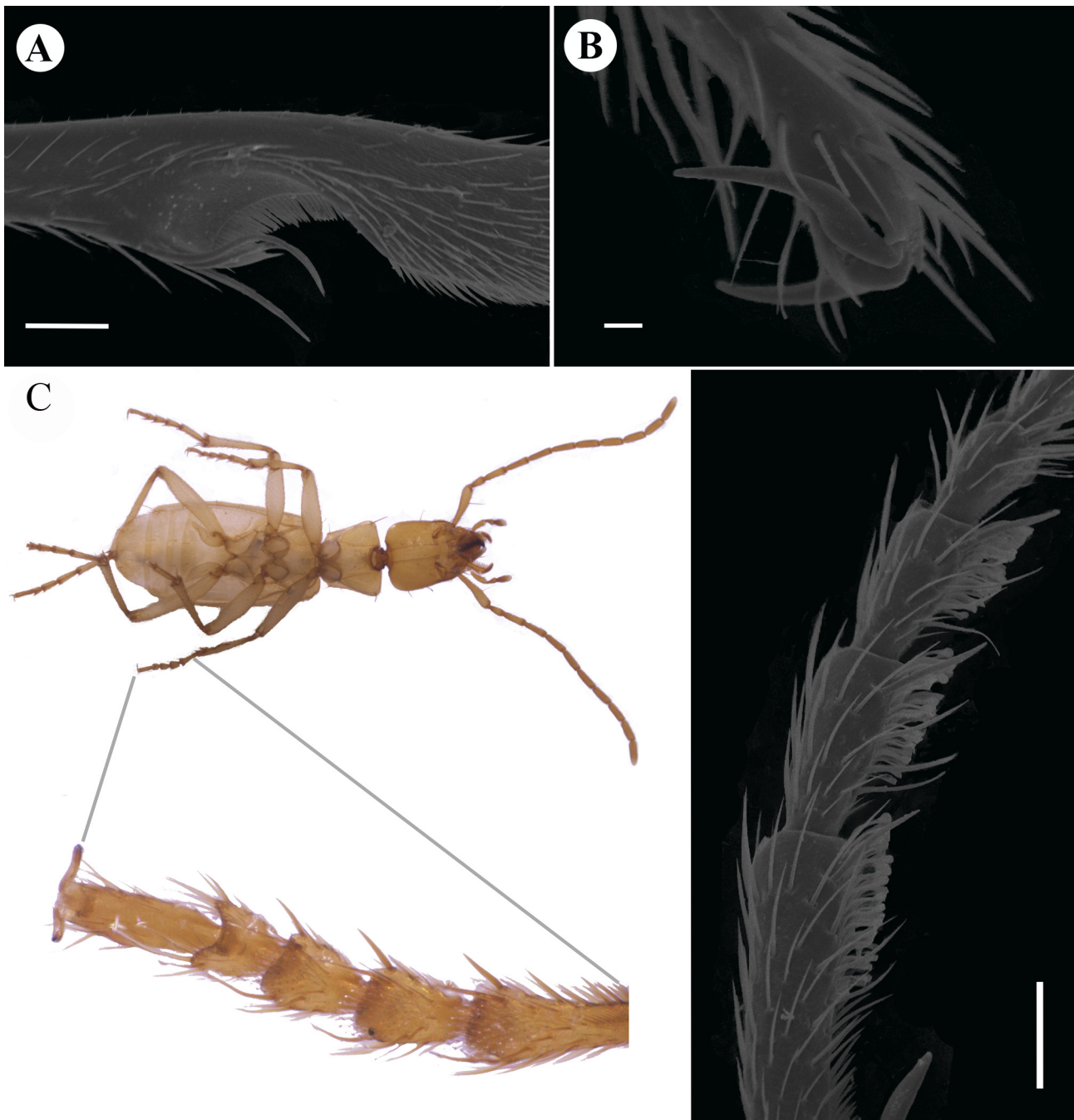


FIGURE 9. Proleg of *Coarazuphium caatinga* sp. n. (A) Scanning electron micrograph showing lateral view of profemur. (B) Scanning electron micrograph showing a close-up on apical portion of fifth tarsomere. (C) Position of articulo seta, on protarsomeres and a scanning electron micrograph showing a close-up on articulo seta. Scale bar (A = 100 μ m. and B, C = 20 μ m).

Differential diagnosis. All characteristics of *C. caatinga* are consistent with the description of the genus *Coarazuphium*. This species differs from all others of the genus by the following combination of characters: reduced and depigmented eyes, head slightly narrower than elytra, two pairs of dorsal setae at the posterior border of head, pronounced apical sinuosity of elytron. Furthermore, ultrastructural analyses show the lengthening of the mandible of *C. caatinga* (which has also a different disposition on the teeth) when compared with *C. formoso* (Fig. 6).

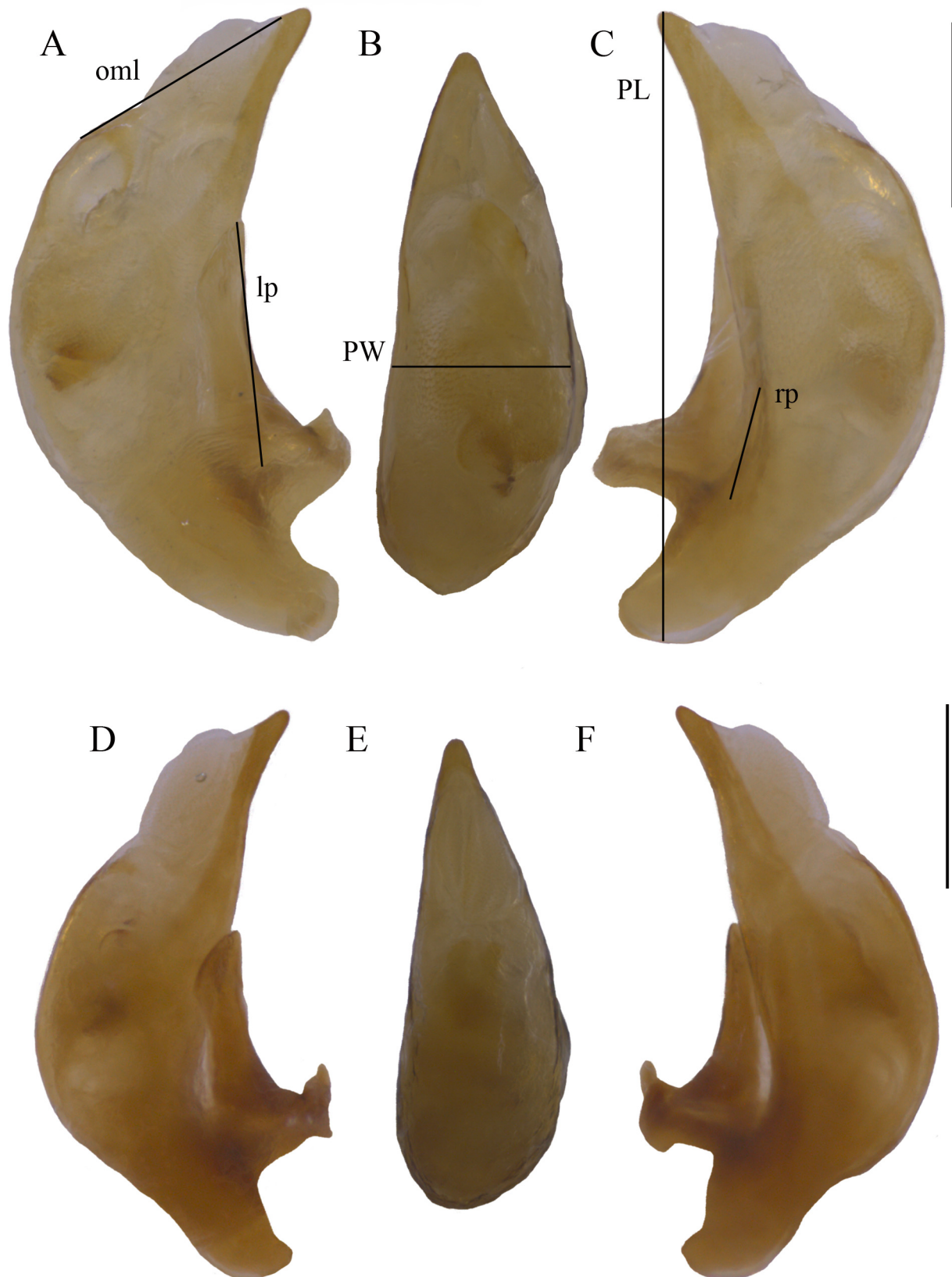


FIGURE 10. Picture showing differences among *Coarazuphium caatinga* sp. n. and *C. formoso* Aedeagus. (A), (B) and (C) Left lateral, dorsal and right lateral view, respectively, of *C. caatinga*. (D), (E) and (F) Left lateral, dorsal and right lateral view, respectively, of *C. formoso*. Scale bar (A, B, C, D, E and F = 200 μ m).

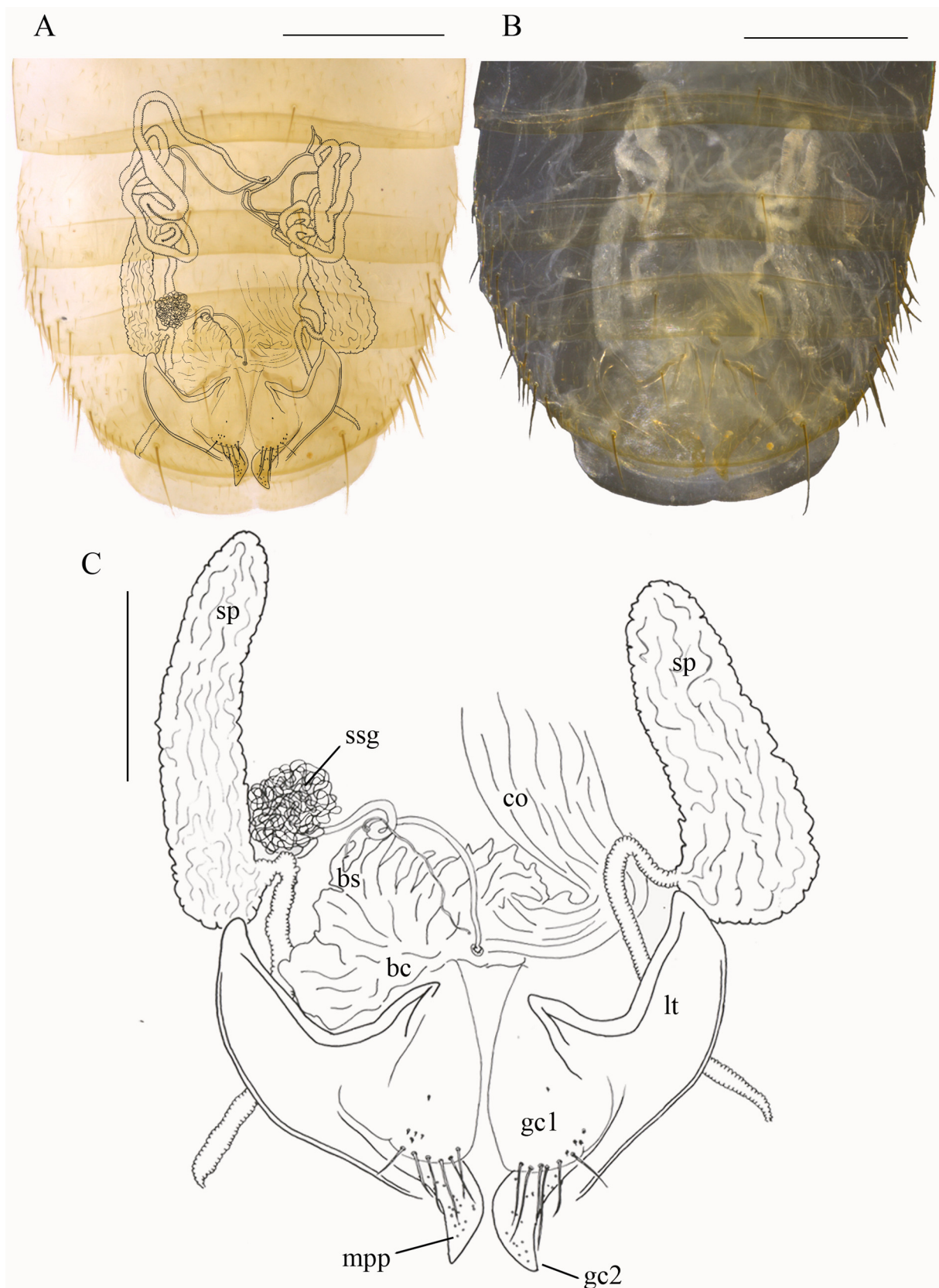


FIGURE 11. *Coarazuphium caatinga* sp. n. female genitalia and pygidial glands. (A) Female abdomen picture and genitalia drawings. (B) Female abdomen picture with contrast showing genitalia. (C) Schematic drawing on female genitalia, gc1 = gonocoxite 1; gc2 = gonocoxite 2; lt = Laterotergite; bc = bursa copulatrix; bs = bursal sacculus; co = common oviduct; ssg = secondary spermatheca gland; sp = spermatheca. Scale bar (A,B = 200 μ m; C = 250 μ m).

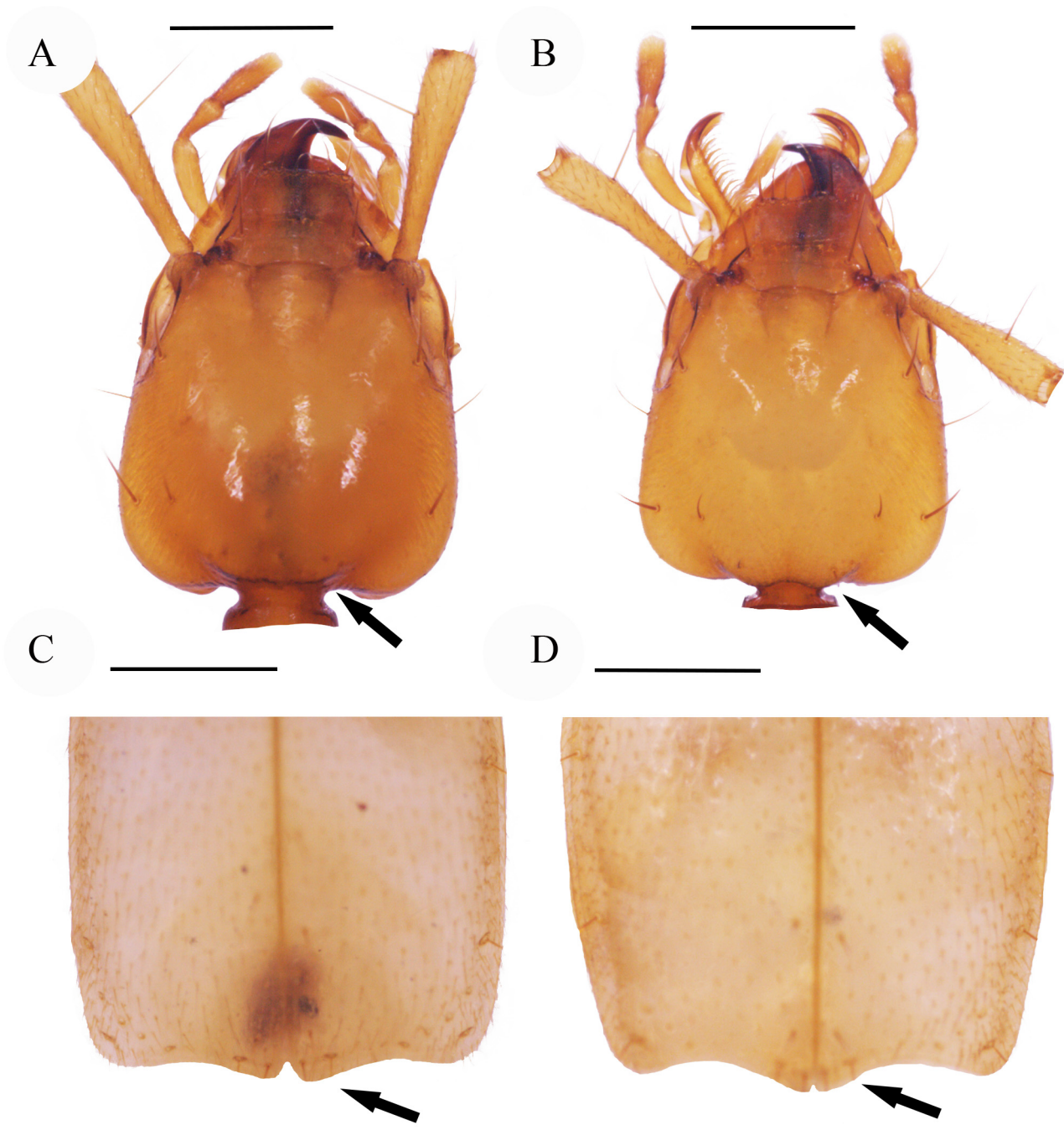


FIGURE 12. Picture showing differences among *Coarazuphium formoso* and *C. caatinga* sp. n. (A) Head of *C. formoso*. (B) Head of *C. caatinga* sp. n. (C) Elytra's sinuosity of *C. formoso*. (D) Elytra's sinuosity of *C. caatinga* sp. n. Scale bar (A–D = 500 μ m).

Key to species of the genus *Coarazuphium* (modify from Ball & Shpeley 2013)

1. Anophthalmous. Maximum width of elytra near middle. Male genitalia: right paramere styli- form, about as long as left paramere *C. cessaima* Gnaspini, Vanin & Godoy, 1998
- Microphthalmous. Maximum width of elytra near middle, or posteriad middle. Male genitalia: right paramere styli- form or not, distinctly shorter than left paramere 2
2. Elytron with apical margin truncate, not sinuate. Male right paramere styli- form or broad 3
- Elytron with apical margin sinuate. Male right paramere broad, not styli- form, distinctly shorter than left paramere 5
3. Head dorsally without setae posteriad the anterior supraorbital setae *C. tapiaguassu* Pellegrini & Ferreira, 2011
- Head dorsally with one to three pairs of setae posteriad the anterior supraorbital setae 4

4. Labrum with anterior margin broadly concave. Prosternal setae two pair. Maximum width (of elytra) posteriad transverse mid-line. Male right paramere broad, not styliform, distinctly shorter than left paramere. Brazil *C. pains* Álvares & Ferreira, 2002
- Labrum with anterior margin irregularly convex. Prosternal setae one pair. Male right paramere styliform, more than half length of left paramere. Mexico *C. whiteheadi* Ball & Shpeley, 2013
5. Head dorsally with a single pair of setae posteriad the anterior supraorbital setae. Male left paramere styliform *C. tessai* (Godoy & Vanin, 1990)
- Head dorsally with two pairs of setae posteriad the anterior supraorbital setae 6
6. Head dorsally with two pairs of setae (posterior supraorbitals and occipitals,) at posterior border of head capsule. Male left paramere broad, conchoids *C. bezerra* Gnaspini, Vanin & Godoy, 1998
- Without the above combination of characters. 7
7. Slight apical sinuosity of elytron (Figs. 12A–C). *C. formoso* Pellegrini & Ferreira 2011
- Pronounced apical sinuosity of elytron (Figs. 12B–D). *C. caatinga* **sp. n.**

Habitat, ecological considerations and threats

Toca do Gonçalves cave is one of the richest caves considering troglobitic species in Brazil. However, it is also one of the most altered caves in the Brazilian Caatinga formation, due to its human utilization (Prevorcnik *et al.* 2012). The historical human impacts in the cave start with the weekly cave-extracted water from the cave using a diesel-water pump, which polluted the cave atmosphere and phreatic waters. More recently (in 2010) an additional electric water pump was installed inside the cave for irrigation. The pump was consuming a huge amount of water, resulting in a severe decrease in the phreatic level. After that, the Brazilian Agency of the Environment, prohibited the cave-extracted water, and both water pumps were removed in 2012. The cave has two main levels; the upper level is drier, while the lower level is very moist, with many contacts with the phreatic level. Most *C. caatinga* specimens were found in deepest areas of the lower level of the Toca do Gonçalves cave. In a visit to the cave in January 2013, no individuals were observed in the cave. The lower level was extremely dry, probably due to a severe drought occurring in the area. Specimens probably migrate to inaccessible areas accompanying the moist areas.

Three specimens were found in Toca de São de Tomé Cave, located around 11 km from the Toca do Gonçalves cave. Those specimens were found in an old guano pile, located in a deep portion of the cave, which was considerably moist. This fact indicated that the species is distributed in a wider area, which comprises voids that interconnect some large caves in the region.

Discussion

The new *Coarazuphium* species is morphologically similar to *C. formoso* Pellegrini & Ferreira, 2011a. Among these characteristics are the general shape of the body, the number of setae along the body and the colour variation, among others. Both species were found in caves located in the municipality of Campo Formoso, Bahia State, which, together with *C. cessaima*, are the only records of the genus in the UNA carbonate formation. The other species of the genus occur in carbonates of the Bambuí group, with the exception of *C. tapiaguassu*, which occurs in ferruginous caves in the Brazilian Amazon, Pará state (Pellegrini & Ferreira 2011b).

Although both species are very similar, *C. caatinga* presents a more pronounced sinuosity in the posterior margin of the elytra and grooves in the back portion of the head (Fig. 12). Furthermore, when compared to *C. formoso*, the aedeagus is somewhat larger, less tapered at apical portion in lateral view and smaller ostial membrane (Figs. 10).

Studies involving the comparison of mouthparts of different species of Cholevinae (Leiodidae) revealed that troglobite organisms exhibit different degrees of modification of these structures as specializations to subterranean life (Moldovan *et al.* 2004). According to these authors, the permanent absence of light is compensated by elongation of not only locomotor appendages and antenna, but also the oral structures. In Cholevinae species, there occurs a lengthening of the maxillary and labial palpi. In *C. caatinga*, the mandible was bigger and more elongated, and the teeth at the base portion show differential size and disposition when compared to *C. formoso*. When comparing the maxillae of these species, it is possible to observe a peculiarity in the galea. Although both exhibit a structure that is likely to have a sensory function (Fig. 6–7), this is much more evident and has a larger diameter in *C. caatinga* compared to the same organ present in *C. formoso*.

Although antennae sensilla presents exactly the same disposition when compared with *C. formoso*, some differences in the distribution patter of the coeloconic sensilla were recognized when compared with *C. tapiaguassu*. In *C. caatinga*, those sensilla occur from the fourth antennomere to the tip of the antennae, while in *C. tapiaguassu* they are present from the fifth antennomere to the tip of the antennae. According to Pellegrini & Ferreira (2011b), the presence of coeloconic sensilla in a lesser number of antennomers, as occurs in *C. formoso* and *C. caatinga*, indicates that those species are more specialized in searching for water (or moist areas). It is worth noting that those two species are found in drier caves than those ones inhabited by *C. tapiaguassu*.

Until now, the only species of *Coarazuphium* believed to be totally anophthalmic is *C. cessaïma* (Gnaspini *et al.* 1998). However, representatives of the species analyzed in this study (collected in the type locality) presented an ocular area of extremely reduced size. Even though the presence of pigmented eyes was not noticed, the ocular area is present in all specimens of *C. caatinga*, and this structure shows an evident size polymorphism. Although this polymorphism has not been reported in other species of *Coarazuphium* it should be noted that that most species of this genus have been described based on one or a few individuals, what may have made it difficult to observe. We also observed an evident colour variation among individuals of *C. caatinga*. Again, it is difficult to say that this feature is common to all species of this genus based on the small series used in previous descriptions. The variations in the degree of troglomorphism of organisms of the same population have already been reported in previous studies for different groups. The troglobite fish of the *Poecilia mexicana* Steindachner, 1863, species, for example, show a wide variation not only in the size of the eyes, but also in head size. These variations were related to the distance from the entrance of the cave in which each specimen was found (Fontanier & Tobler, 2009).

The isopod *Asellus aquaticus* Linnaeus, 1758, also features morphological variations within a population as to the depigmentation of the body and eyes, length of sensory appendages and body proportions (Prevorcnik *et al.* 2004). There are three main hypotheses for the presence of such polymorphisms in *A. aquaticus*. The first was proposed by Kossing & Kossing (1940), attributing the morphological variability to "relaxed selection" that occurs in caves. However, Culver (1982) and Sket (1994) consider the migration from the surface as the more likely explanation. Finally, Veronik *et al.* (2003) suggest fragmentation events and gene flow as the main factors for the emergence of such variations.

In case of *Coarazuphium* apparently there are no epigeal populations that could contribute to the gene flow of cave individuals. Thus, two hypotheses were formulated for the polymorphisms observed in this species: *i*) the pigmentation and eyes are undergoing selection due to pressures of the subterranean environment, but their state of complete "regression" are not yet fully established in populations, *ii*) eyes and pigmentation make up "neutral" characters for these organisms, not undergoing any selection. However, those hypotheses are speculative, deserving to be the focus of future studies.

Both sexes also differ in the appearance of Laboulbeniales fungi (Ascomycetes) (Gnaspini *et al.* 1998). Females showed infection on the elytra, whereas males have such fungi on the legs and abdominal segments.

Gnaspini & Trajano (1994) suggested that the genus *Coarazuphium* is the most specialized to subterranean life in Brazil. These authors based this solely on visible features in stereomicroscopes, such as reduced eyes and pigmentation, and elongation of locomotor and sensory appendages. Pellegrini and Ferreira (2011a,b) pointed out the importance of using ultrastructural analysis for comparison of different degrees of adaptation to caves. This is the second study that compares the ultra-structures of species of the genus *Coarazuphium* in which dissimilarities between species become more evident. However, it would be interesting to conduct studies comparing troglobitic organisms of this genus with epigeal species phylogenetically close to *Coarazuphium*. Such a comparison would reveal, precisely, which ultrastructural modifications really make up specializations to subterranean life.

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