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Revealing male genital morphology in the giant ant genus *Dinoponera* with geometric morphometrics

Leonardo Tozetto*, John E. Lattke

Departamento de Zoologia, Universidade Federal do Paraná, Avenida Francisco Heráclito Dos Santos, S/n, Centro Politécnico, Curitiba, CEP: 81531-980, Brazil

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ABSTRACT

Genitalia include some of the most complex and morphologically diverse structures in insects, finding extensive use in taxonomy, but ant taxonomy is female biased and knowledge of the males is little explored, potentially depriving ant taxonomy of valuable information. We examine the male genital morphology of six species of *Dinoponera* and the variation among species and within species is described. We performed geometric morphometric analyses for the penisvalvae and lateropenite of the volsella. The results from the descriptions and statistical analyses show the genitalia offer valuable characters for species delimitation. What is presently known as *Dinoponera australis* can be differentiated into discrete populations, perhaps some representing cryptic species. The similarities between *D. australis* and *D. snellingi* suggest a close relation between them as well as between *D. gigantea* and *D. quadriceps*. We conclude that several genital characters, especially those of the penisvalvae, can be used to differentiate the species and might be useful to clarify the taxonomy of *Dinoponera*.

1. Introduction

The variation in insect male genital morphology may be so striking that even species with similar external morphology may often have very different genitalia, giving them great value in species delimitation (Shapiro and Porter, 1989; Hosken and Stockley, 2004). Some studies have even shown intraspecific variation in different insect groups (Arnqvist and Thornhill, 1998; House and Simmons, 2003; Mutanen et al., 2007), however, the breadth of this morphological variation in ants has been little explored.

Geometric morphometrics (GM) has proven a remarkable tool for investigating insect morphology. By analyzing the overall size and shape separately as components of the form, GM provides a sophisticated method to collect and analyze data to answer questions in anatomy and evolutionary biology (Adams et al., 2004; Lawing and Polly, 2009; Klingenberg, 2016). Although it is mostly applied to insect wings (see Tatsuta et al., 2018), genitalia have also been subjected to this analysis (e.g., Mutanen et al., 2007; Song, 2009; Jauset et al., 2017; García-Román et al., 2019).

Despite the remarkable success of GM analyses in Hymenoptera (Tatsuta et al., 2018), few studies have covered their genitalia (e.g., Querino et al., 2002; Prous et al., 2011; Özenirler and Aytekin, 2015; Vieira et al., 2015). GM analysis in ants has focused mostly on wing venation (e.g., Visicchio et al., 2004; Katzke et al., 2018) and other parts of the body (e.g., Seifert et al., 2014; Londe et al., 2015; Wagner et al., 2017; Friedman et al., 2019), but none on genitals so far.

Ant taxonomy is historically based on worker or gyne morphology and data on males are generally scarce, with comparatively far lesser resources for identifying them. Ant males are easily sampled with commonly used collecting techniques such as light or Malaise traps, but such specimens then lack concrete association with their morphologically very different female nestmates and are then usually relegated to a drawer or unit tray with anonymous males. An exception to this situation are the army ants, in which males are included as an essential part of alpha taxonomic treatment (Borgmeier, 1955), perhaps because of their striking external morphology and the ease of collecting them using light traps (Nascimento et al., 2011). This knowledge gap has received some attention in studies that revealed the value of male morphological characters, including their genitalia, for providing useful characters for taxonomy and systematics in other ant groups (LaPolla et al., 2012; Macgown et al., 2014; Wilson et al., 2016;

* Corresponding author.
E-mail address: leonardotozetto@gmail.com (L. Tozetto).

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Schmidt and Heinze, 2017). Some publications have included males for treatment at regional and generic levels (Yoshimura & Fisher, 2007, 2011; Boudinot, 2015, 2019; Borowiec, 2016). Despite these efforts, male sampling in ants is still meager as illustrated in the recent generic revision of the Ponerinae (Schmidt and Shattuck, 2014), which only considers workers for diagnosing each genus.

The ponerine ant genus *Dinoponera* Roger, 1861 has eight valid species (Lenhart et al., 2013) and is characterized by large, ground foraging workers that frequently surpass three centimeters in total body length, making them the largest ant in the world. Reproduction is through gamergates, a life history that potentially limits range expansion and burdens the males with a greater role for gene dispersal (Monnin and Peeters, 2008). They are broadly distributed throughout South America east of the Andes, found from southern Colombia south into the Amazon and Paraná river basins, reaching northern Argentina. While some species are frequently collected and can be locally abundant, others are relatively rare, both in the field and in collections. Their taxonomy is mostly based upon workers, with some species being quite difficult to recognize on account of an alpha taxonomy that uses subtle differences in hair color, petiolar shape, and luster of the integument (Kempf, 1971; Lenhart et al., 2013). *Dinoponera* males are particularly scarce in collections and are rarely collected, perhaps because only a few leave the nest to search for a mate (Paiva and Brandão, 1995; Monnin and Peeters, 1998). Mating in *Dinoponera quadriceps* is suicidal as males do not disengage from the female and the mated female must chew off the posterior part of the male's gaster and then clear her genital tract of the male's genitals (Monnin and Peeters, 1998). Males are known for six species (Lenhart et al., 2013; Escárraga et al., 2017) and some have been used in supporting alpha taxonomy (Borgmeier, 1937; Kempf, 1971) but most of these studies used one or two males, and a limited number of external characters. Lenhart et al. (2013) dissected and described the genitals of either one or two males for some species and described *Dinoponera snellingi* based only on the male, with emphasis on genital characters. Despite being a significant contribution, their reduced sampling of males, compared with the number of females, makes for a preliminary view of intraspecific morphological variation and does not allow studies of infraspecific variation.

The large size of *Dinoponera* facilitates dissections and the observation of characters, and their low diversity facilitates studies encompassing a single lineage distributed throughout a relatively large geographic area. The study of male genitalia in *Dinoponera* could provide information of interest for a better resolution of their alpha taxonomy and is also useful for defining morphological boundaries and intraspecific variation in a group with wingless females. Here we qualitatively and quantitatively study male genital morphology using geometric morphometric approaches. The use of different lines of evidence make for better supported taxonomic decisions, and it is our hope that this work will contribute with an ongoing revision of *Dinoponera* by A. Martins (unpublished ms).

2. Material and methods

We examined the genitalia from 25 males representing six species of *Dinoponera* borrowed from the following institutions (Table 1):

CPDC Centro de Pesquisas do Cacau, Itabuna, BA, Brazil.
DZUP Coleção Entomológica Padre Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, PR, Brazil.
MPEG Museu Paraense Emilio Goeldi, Belém, PA, Brazil.
MZSP Museu de Zoologia da Universidade de São Paulo, São Paulo, SP, Brazil.
UFES Universidade Federal do Espirito Santo, Vitória, ES, Brazil.
UFV Universidade Federal de Viçosa, Viçosa, MG, Brazil.

The males were identified using the keys in Lenhart et al. (2013) and compared with the descriptions (Borgmeier, 1937; Kempf, 1971; Lenhart et al., 2013; Escárraga et al., 2017). Additional information concerning worker taxonomy, morphology and species distribution was provided by Amanda Martins (unpub ms). One specimen (MZSP 62283) was identified as *D. snellingi*, but its ocelli are slightly larger and the ocellar promontory is more developed when compared with the description and illustration provided by Lenhart et al. (2013) of *D. snellingi*. Its volsella has a wider lateropenite that extends ventrally but, the penisvalva has no morphological differences from that of *D. snellingi*. One specimen (MZSP 62285) is morphologically unlike any other known male and is provisionally identified as *D.* sp. 1. It could represent either a male of *Dinoponera mutica* or of an undescribed species (A. Martins, pers. com.).

Specimens were softened in a water-filled relaxing container during one or more days at 37 °C. Genitalia dissections were carried out in 95% ethanol under a Zeiss Stemi SV6 microscope using fine forceps and entomology pins. To remove the genitalia, a forceps was used to tease out the genitalia and break muscular connections from the gaster. Once the genitalia are loosened, forceps were used to pull the genitalia out of the body. The genitalia were then soaked in 10% KOH for 24h at room temperature. Afterwards, the basal ring was easily dissociated from the valves with some careful wiggling. An entomology pin was then inserted between the bases of the penisvalvae to separate the two halves of the three pairs of valves. The three pairs of valves were then dissociated by gingerly grasping them with fine forceps.

To analyze shape variation, the genital valves were slide-mounted in lateral view and in a standard position across all specimens. High-resolution imagens of the genitals were obtained before and after dissections with an Axiocam 305 color camera coupled to a Zeiss Stereo Discovery v20, thereafter the brightness and contrast of the images have been corrected. The terminology herein follows Boudinot (2018) for genitalia and Harris (1979) for surface sculpturing.

2.1. Morphometric analysis

We describe the shape of the basal ring, volsella, and penisvalva. We analyze the shape of the lateropenite of volsella (digitus volsellaris) and the penisvalva in two dimensions. The shape was measured by landmark digitations using tpsDig 2.32 software (Rohlf, 2005). Given the fact that male genital shape shows curves and surfaces that are not amenable for defining landmarks; a combination of landmarks and semilandmarks was applied. Semilandmarks are appropriate for quantifying outline shapes that cannot be homologized across different individuals, and they can be analyzed together with landmarks (Bookstein, 1997; Gunz and Mitteroecker, 2012).

Because volsella structures can be flexible, we chose to set up landmarks only on the lateropenite. We digitized a total of two landmarks and 12 semilandmarks of the left lateropenite (Fig. 1A). The landmarks were delimited by the conjunction between the lateropenite and the cuspis volsellaris in the dorsal (Lv1) and in the anteroventral margin (Lv2). Semilandmarks started at Lv1 and were sequentially sampled by length to Lv2. We digitized a total of three landmarks and 16 semilandmarks of the left penisvalva (for *D. snellingi* from Itirapina we used the right penisvalva) (Fig. 1B). The landmarks corresponded to the anterodorsal margin of penisvalva,

Table 1
List of *Dinoponera* taxa with examined male genitalia.

Species	Locality	Institution	Unique Identifier
Dinoponera australis Emery, 1901	Brazil, PR Guarapuava, I 62, S. Sakagami	DZUP	DZUP 548845
	Brazil, PR, Laranjeira do Sul, 3–47, 4380, Coleção F. Justus Jr,	DZUP	DZUP 548844
	Brazil, GO, Campinas, 1935, R. Spitz col.	MZSP	MZSP 62281
	Brazil, GO, Jataí, xii.1972, F. M. Oliveira col.	MZSP	MZSP 62282
Dinoponera gigantea (Perty, 1833)	Brazil, PA, Benevides, 15.iii.1990, W. L. Overal col.	MPEG	MPEG.HHY 03045386
	Brazil, PA, Primavera, Quadripaum Fal. Feitoria, 27.xi.1992, armadilha de luz, J. Dias col.	MPEG	MPEG 03006727
	Brazil, PA, Paragominhas, −2.997500 -47.353611, i-vii.2011, 85m, R. Solar	UFV	UFV LABECOL 000449
Dinoponera lucida Emery, 1901	Brazil, ES, Alfredo Chaves, Matilde, RPPN Oiutrem, −20.550000−40.800000, 14−21.×.2009, Malaise, 600−800m, C. O. Azevedo & eq. col.	UFES	UFES 74864
	Brazil, ES, Alfredo Chaves, Matilde, RPPN Oiutrem, −20.550000−40.800000, 14−21.×.2009, Malaise, 600−800m, C. O. Azevedo & eq. col.	UFES	UFES 74864
	Brazil, ES, Domingos Martins, Mata do Pico do Eldorado, −20.371389−40.658056, 03 −10.xii.2004, Malaise T1, M. T. Tavares & eq. col.	UFES	UFES 106426
	Brazil, ES, Itaguaçu, Alto Laranjinha, Fazenda Binda, −19.800000−40.800000, 22−29.ix.2008, Malaise, M. T. Tavares & eq. col.	UFES	UFES 82143
	Brazil, ES, Laranja da Terra, Joatuba, Faz Betzet, −19.840278−40.827778, 05−12.×.2012, Malaise B-1, 280−430, M. T. Tavares & eq. col.	UFES	UFES 135693
	Brazil, ES, Santa Leopoldina, Suíça, −20.081833 −40.594139, 05−12.xi.2007, Malaise, C. O. Azevedo & eq. Col.	UFES	UFES 68910
	Brazil, ES, Pancas, Faz. Juliberto Stur - Área 1, −19.219583−40.773278, 24−31.i.2003, Malaise T4, Tavares, Azevedo & eq. col.	UFES	UFES 99293
	Brazil, BA, Barrolândia, 04.viii.2004, Santos, J. R. M col.	CEPLAC	DZUP 548849
Dinoponera quadriceps Santschi, 1921	Brazil, CE, Cráteus, Serra das Almas, 19−20.v.2014, Mini-luminosa, G. Melo & B. B. Rosa col.	DZUP	DZUP 548847
	Brazil, RN, Natal, Campus Central UFRN, vii.2012- i.2016, 4 ninhos, Dina col.	DZUP	DZUP 548850
	Brazil, RN, Natal, Campus Central UFRN, vii.2012- i.2016, 4 ninhos, Dina col.	DZUP	DZUP 548851
	Brazil, PB, João Pessoa, 09.ix.2002, A. Vasconcelos col.	CEPLAC	DZUP 548855
	Brazil, PB, Matureia, Pico do Jabre, −7.252500 −37.384722, 19.ii.2018, armadilha de luz, Ferreira col.	DZUP	DZUP 548852
	Brazil, PB, Matureia, Pico do Jabre, −7.252500 −37.384722, 19.ii.2018, armadilha de luz, Ferreira col.	DZUP	DZUP 548853
	Brazil, BA, Manoel Vitorino, 26−27.i.2006, Hora, R. R. Col.	CEPLAC	DZUP 548854
Dinoponera snellingi Lenhart et al. (2013)	Brazil, SP, Itirapina, 10.V.1990, R. Paiva col.	MZSP	MZSP 62283
	Brazil, MT, Itaum, iii-1974, M. Alvarenga col.	MZSP	MZSP 62284
Dinoponera sp. 1	Brazil, RO, Porto Velho, Rio Madeira, Área Mutum, Ahe Jirau, 07−20.ii.2013, F. Fernandes col.	MZSP	MZSP 62285

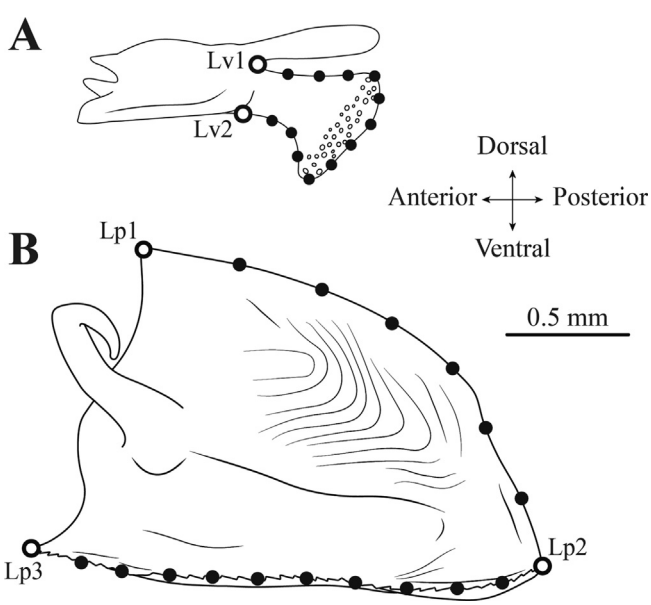


Fig. 1. Schematic drawing of *Dinoponera gigantea* genitals and landmarks. **A**: Lateropenite of volsella with the two landmarks (unfilled circles) and the twelve semilandmarks (filled circles). **B**: Penisvalva with the three landmarks (unfilled circles) and the sixteen semilandmarks (filled circles).

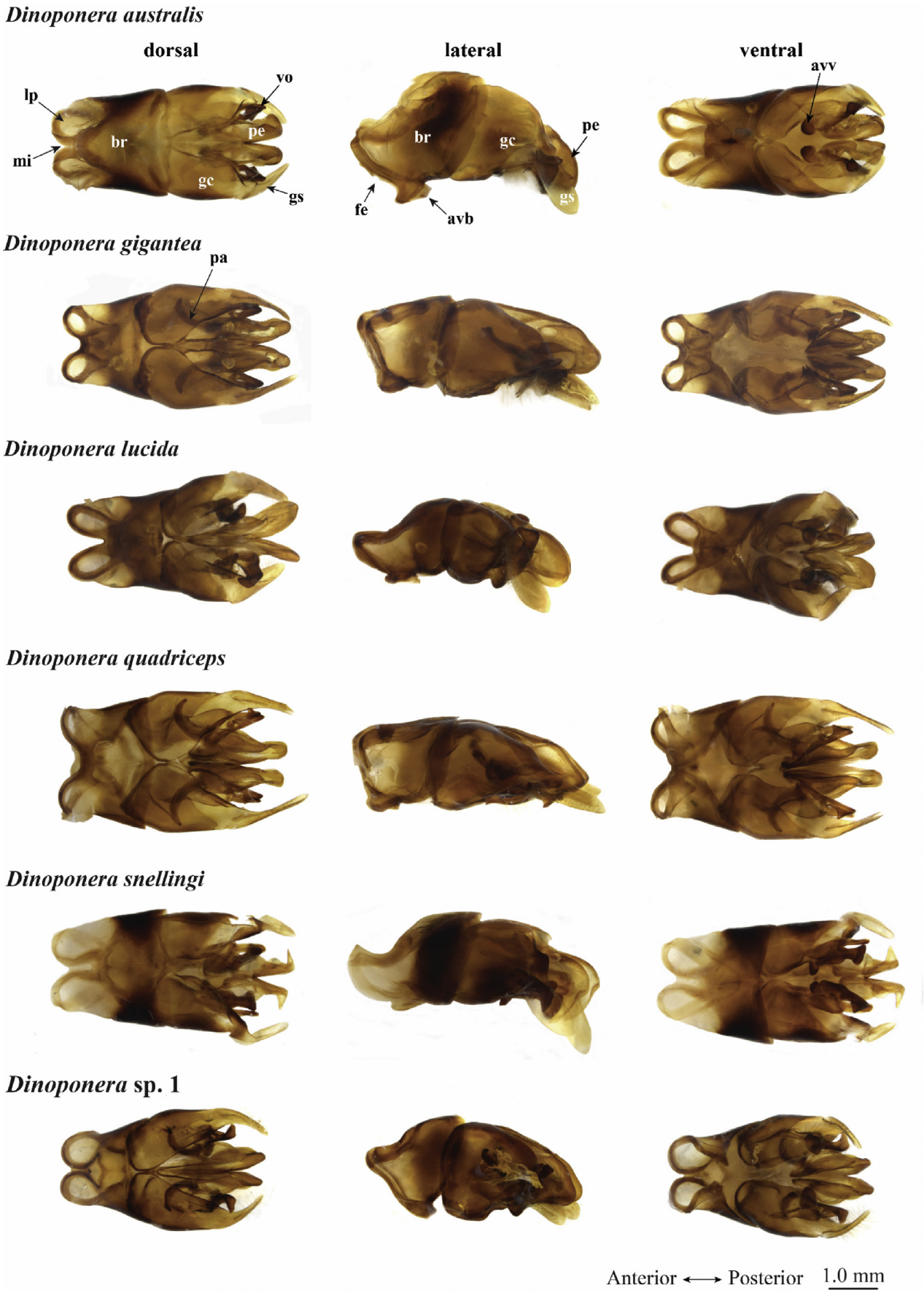
delimited by the penial apodeme (Lp1), the posterior point of the serrated ventral margin (Lp2) and the anterior point of the serrated ventral margin (Lp3). Semilandmarks started at Lp1 and were sequentially sampled by length to Lp2, then started at Lp2 to Lp3. We performed all geometric morphometric analyses in R (R Core Team, 2019) using GEOMORPH package v.3.0.5 (Adams et al., 2019). The landmarks were aligned using Generalized Procrustes Analysis. To test for allometry effects, we used a MANOVA on independent contrasts of size (centroid size) and a permutation test (999 permutations). To assess the differences between shape and species we also used MANOVA.

3. Results

The genitals are located at the posterior end of the abdomen, sometimes being half exposed. They consist of three pairs of valves surrounded anteriorly by a tubular basal ring. The outer valves are called gonopods, the middle valves volsellae, and the inner valves penisvalvae.

3.1. Basal ring

The basal ring, or cupula of Boudinot (2013), is a thin sclerite attached to abdominal tergum IX, which in either dorsal or ventral view has a thick anterolateral looped structure surrounding an internal fenestra, the loops separated anteriorly by a medial invagination (basal ring = br, lobular process = lp, fenestra = fe,


Dinoponera australis
dorsal
lateral
ventral
lp
vo
mi
br
pe
gc
gs
br
gc
pe
fe
avb
gs
avv
Dinoponera gigantea
pa
Dinoponera lucida
Dinoponera quadriceps
Dinoponera snellingi
Dinoponera sp. 1
Anterior ⟷ Posterior
1.0 mm

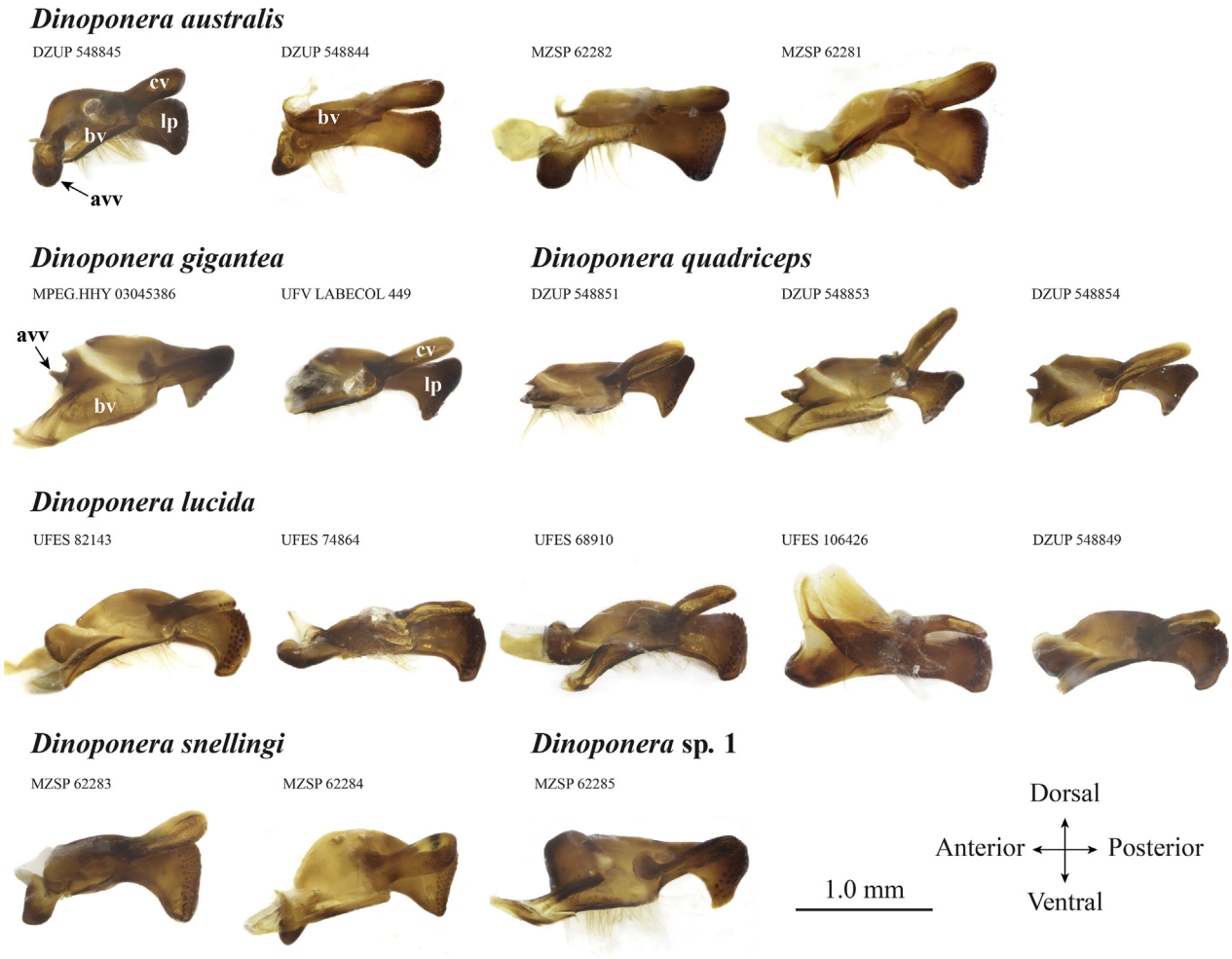


Fig. 3. *Dinoponera* left volsella. **avv**: anteroventral process of volsella, **bv**: basivolsella, **cv**: cuspis volsellaris, **lp**: lateropenite. Scale bar: 1.0 mm.

medial invagination = mi, Fig. 2). The ring's length varies from 38 to 50% of the genital capsule with a broadly convex posterior margin in lateral view.

Basal ring of *Dinoponera australis* in dorsal view is sub-rectangular, with semiparallel lateral margins. Fenestra in dorsal or ventral view teardrop-shaped with longitudinal axis semiparallel to longitudinal axis of genital capsule, medial invagination forms acute angle. Dorsal margin in lateral view anterodorsally concave, anteroventral process sub-quadrate to sub-triangular (anteroventral process of basal ring = avb, Fig. 2). Basal ring of *Dinoponera gigantea* in dorsal view trapezoid, anterior margin appreciably narrower than posterior margin. Fenestra ovoid, longitudinal axis of each forming obtuse angle, medial invagination convex. Anterior and dorsal margins meet at right angle in lateral view, dorsal margin straight, anteroventral process more or less rounded.

Basal ring of *Dinoponera lucida* in dorsal view oblong to trapezoid, anterior margin 20% narrower than posterior margin. Lobular process at least half as long as basal ring; fenestra teardrop-shaped, longitudinal axis of each forming acute angle, medial invagination in dorsal view acute. Anterior margin shaped as rounded lobe in lateral view, anterodorsal margin concave, posterodorsal margin convex, and anteroventral process sub-triangular to rounded. Basal ring of *D. quadriceps* in dorsal view sub-rectangular, lateral margins parallel. Fenestra shaped as narrow ellipse with longitudinal axis of each forming very open obtuse angle, medial invagination broadly concave. Basal ring in lateral view similar to that of *D. gigantea.*

Basal ring of *D. snellingi* in dorsal view trapezoid, anterior margin 70% narrower than posterior margin. Fenestra shaped as elongate teardrop with longitudinal axis of each semiparallel to longitudinal axis of capsule, similar to that of *D. australis*; medial invagination acute. Ring in lateral view anteriorly with upturned point, dorsoanteriorly with concavity and dorsoposteriorly with longer broad convexity. Basal ring of *D.* sp 1 in dorsal view shaped as short trapezoid, lateral margin concave, width of anterior margin 2/3 that of posterior margin; fenestra in dorsal view broadly oval with posterior angle, longitudinal axis of fenestra parallel to lateral margin of capsule, anteriorly converging with each other. Fenestra irregularly shaped in ventral view due to overlapping cuticular flange from lateral margin of ring, medial emargination acute. Dorsal margin in lateral view with anterior half broadly concave, posteriorly half weakly convex, almost straight. Ventral margin in lateral view reduced, anteroventral process sub-quadrate.

The rings of *D. australis* and *D. snellingi* are similar in general shape as seen dorsally and laterally, as well as in the shape and

Fig. 2. *Dinoponera* genital capsules. *D. australis* (MZSP 62282), *D. gigantea* (MPEG.HHY 03045386), *D. lucida* (DZUP548848), *D. quadriceps* (DZUP 548847), *D. snellingi* (MZSP 62283), *D.* sp 1 (MZSP 62285). **avb**: anteroventral process of basal ring, **avv**: anteroventral process of volsella, **br**: basal ring, **fe**: fenestra, **gc**: gonocoxite, **gs**: gonostylus, **lp**: lobular process, **mi**: medial invagination, **pa**: penial apodeme, **pe**: penisvalva, **vo**: volsella. Scale bar: 1.0 mm.

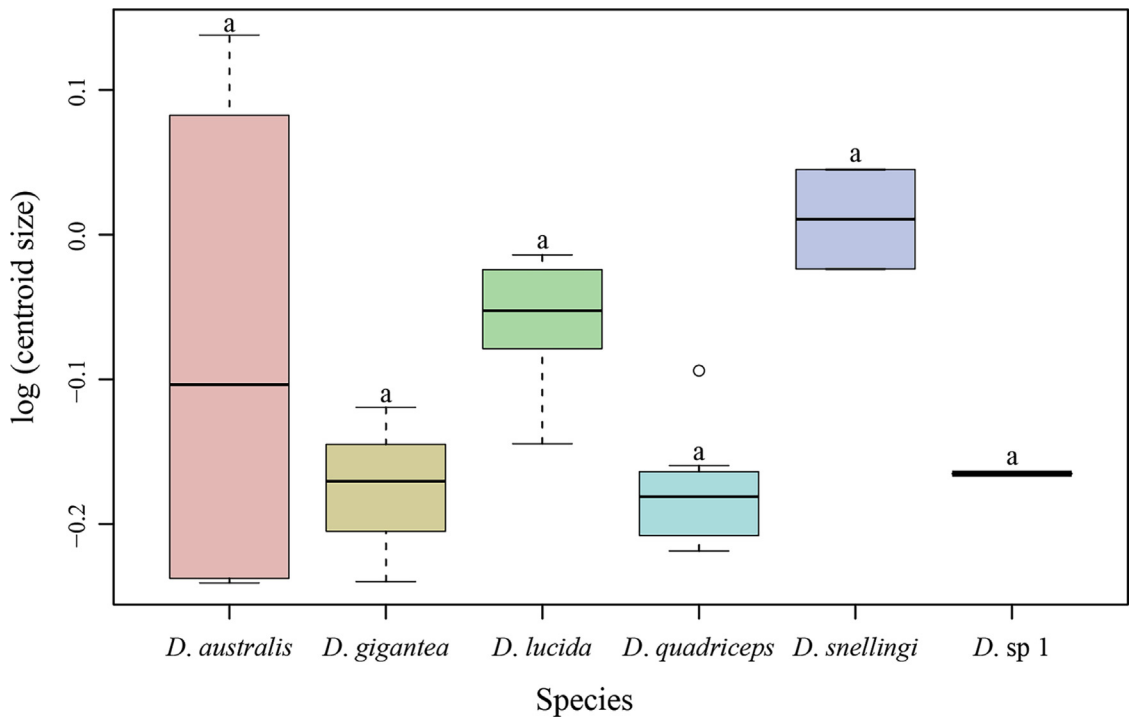


Fig. 4. Comparison between lateropenite centroid sizes (log) of six *Dinoponera* species, median (black line). Different letters represent significant differences.

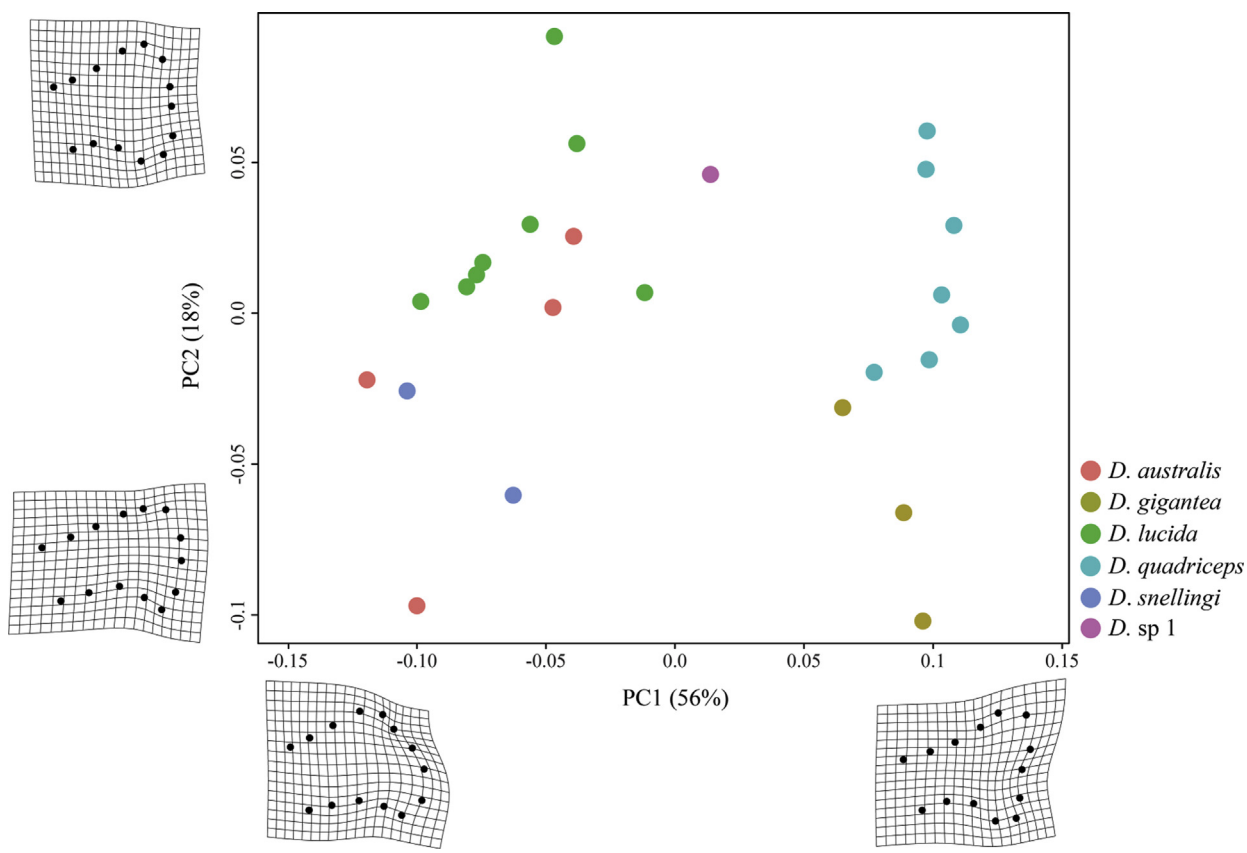


Fig. 5. Lateropenite morphospace of six *Dinoponera* species, based on discriminant analysis MANOVA with allometric effects.

orientation of the fenestra and shape of the medial emargination. The ring of *D. lucida* is also similar to these species, but the medial emargination is cleft deeper. The rings of *D. gigantea* and *D. quadriceps* are similar in lateral view with their anterior and dorsal margins forming a right angle with each other. The similarities in general shape seen dorsally are much less, both

Table 2
Permutation test for group differences in lateropenite and penisvalva shape. Here, we compare the distance pairs between *Dinoponera* species.

	D. australis	*D. gigantea*	*D. lucida*	*D. quadriceps*	*D. snellingi*	*D.* sp 1
D. australis	–	**0.026**	**0.019**	**0.002**	0.543	0.072
D. gigantea	**0.014**	–	**0.001**	**0.003**	0.089	0.166
D. lucida	**0.001**	**0.003**	–	**0.001**	**0.048**	**0.052**
D. quadriceps	**0.004**	**0.004**	**0.001**	–	**0.019**	0.095
D. snellingi	**0.031**	**0.058**	**0.002**	**0.018**	–	0.504
D. sp 1	0.111	0.554	**0.018**	0.081	0.169	–

Top diagonal, lateropenite *P*-values. Lower diagonal, penisvalva *P*-values. Bold values mean statistical significances lower than 0.05.

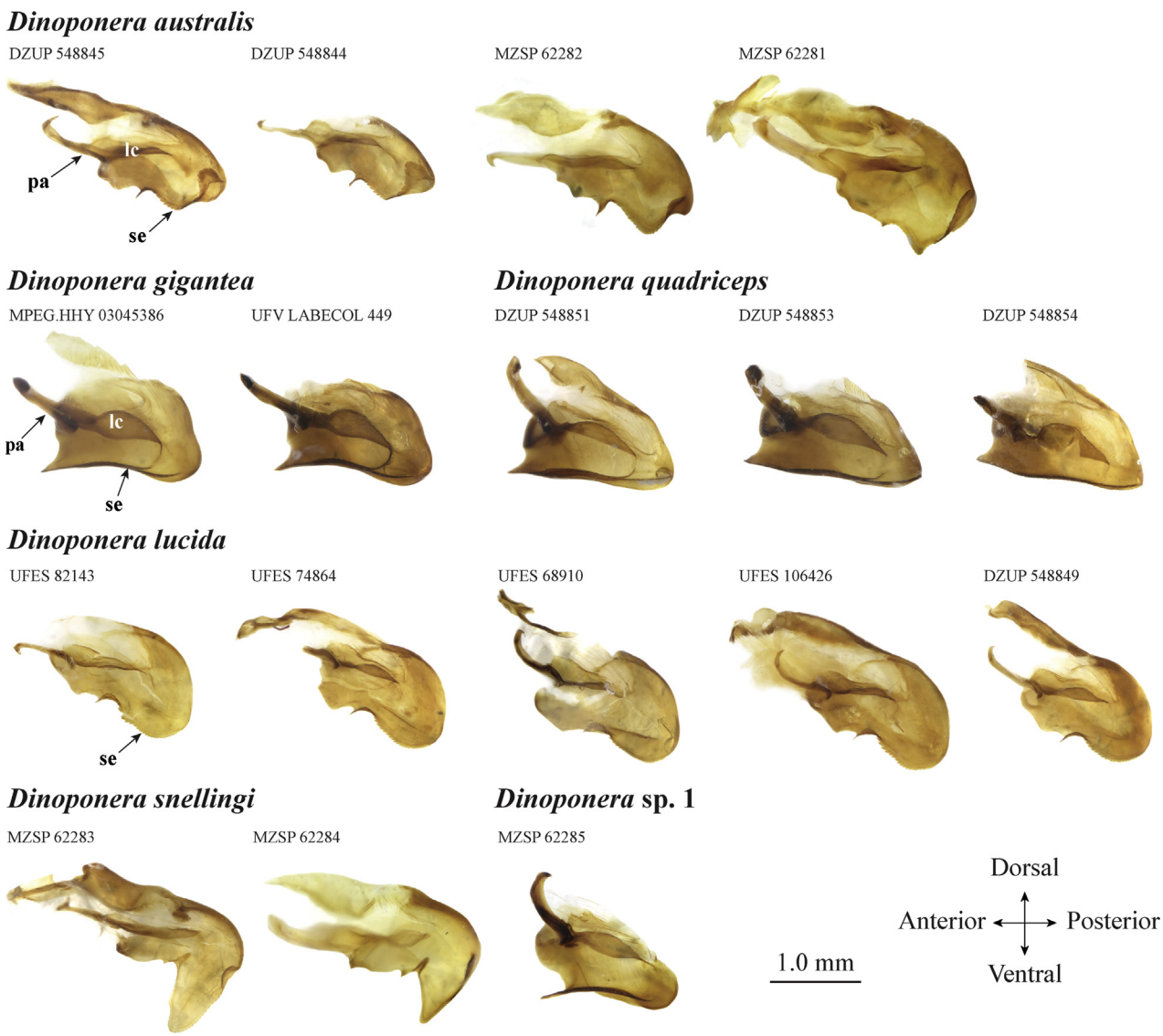


Fig. 6. *Dinoponera* left penisvalva. **lc**: lateral carena, **pa**: penial apodeme, **se**: serrated edge. Scale bar: 1.0 mm.

bearing in common a convex, and not acute, medial emargination. The ring of *D.* sp. 1 is distinctly different from all the others.

3.2. Gonopod (Parameres)

The gonopod is divided into a gonocoxite and a gonostylus (gonocoxite = gc, gonostylus = gs, Fig. 2). The gonostylus is distinctly differentiated from the gonocoxite by a ventral notch which forms a membrane and allows the gonostylus to fold. This movement exposes the penisvalva posteriorly and also affects the relative width of the gonopod by changing its longitudinal curvature. A gonopod that has been strongly curved will appear to be narrower.

Two basic gonostylus forms are easily recognized. In *D. quadriceps* and *D. gigantea* they are long and taper into a sharp apex, sometimes posteriorly inclined. In *D. australis*, *D. lucida,* and *D. snellingi* they are long and broad, slightly curved with a rounded

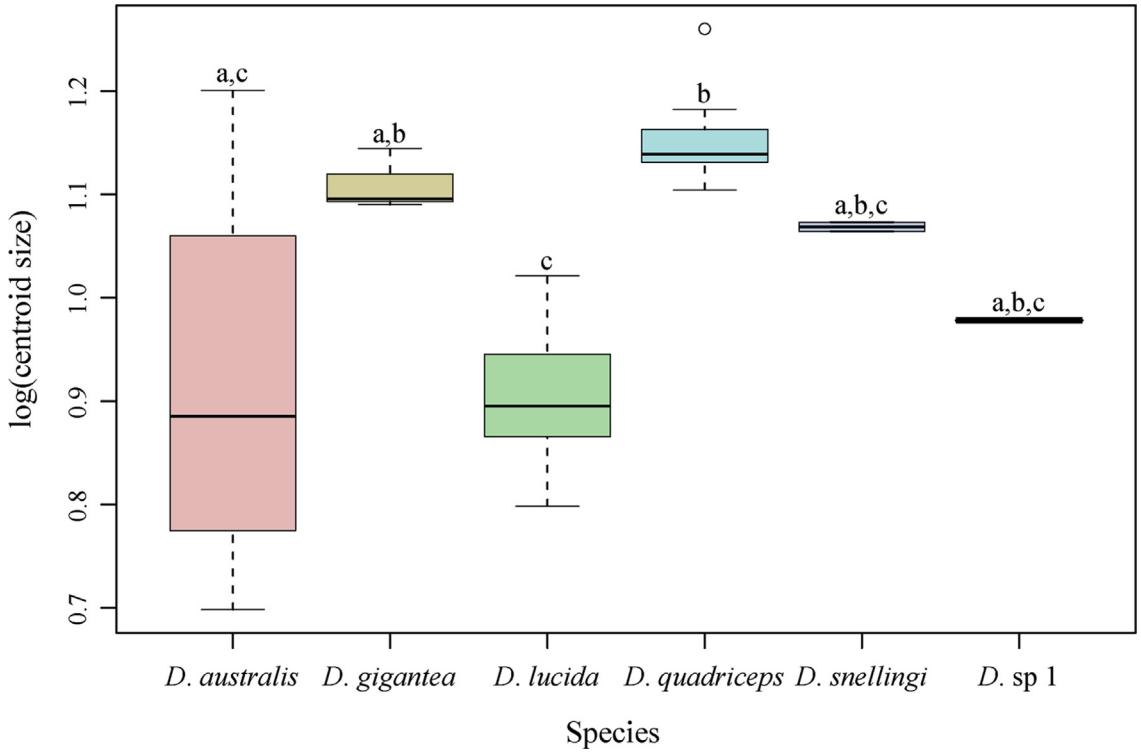


Fig. 7. Comparison between penisvalvae centroid sizes (log) of six *Dinoponera* species, median (black line). Different letters represent significant differences.

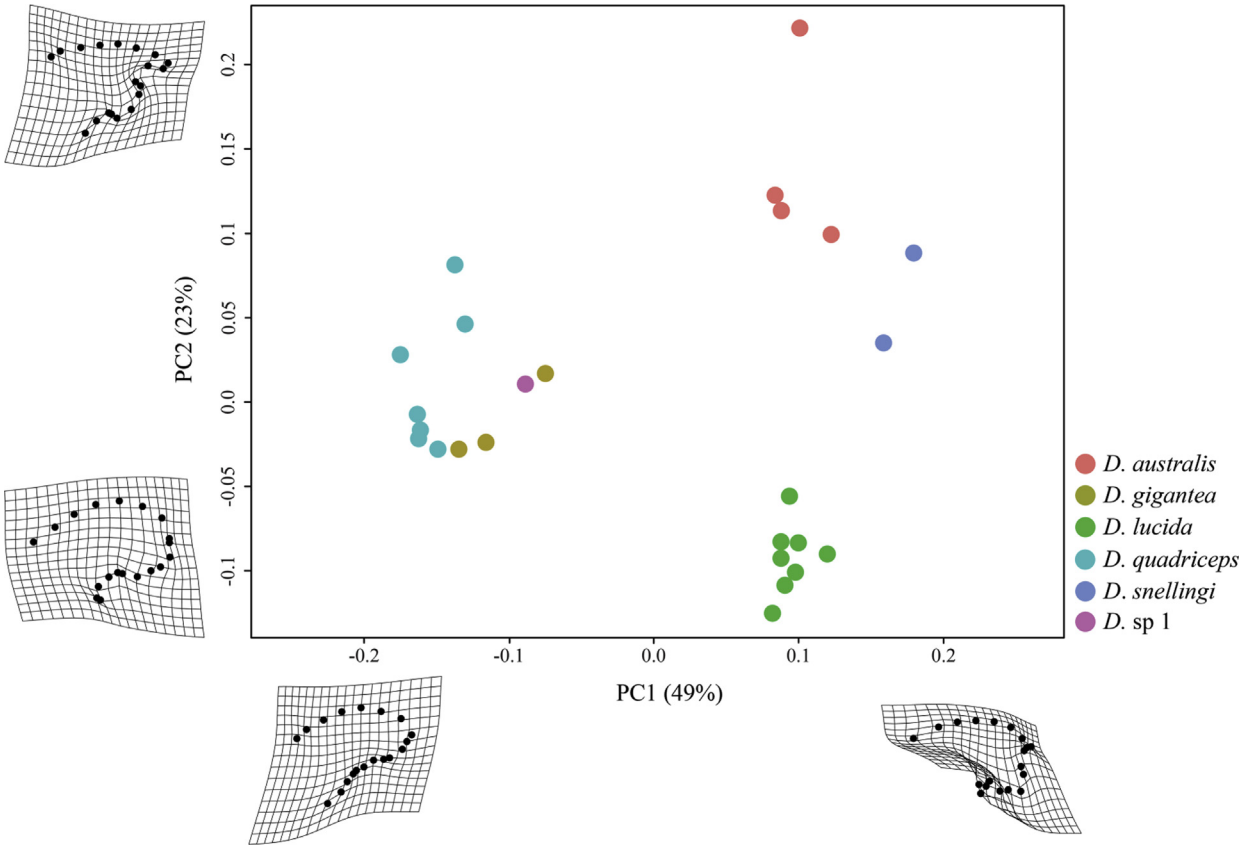


Fig. 8. Penisvalvae morphospace of six *Dinoponera* species, based on discriminant analysis MANOVA considering allometric effects.

apex. *D. lucida* can have a more or less broad gonostylus with some specimens showing it with a delicately convexity. In *D.* sp 1 it is short and broad, with a subquadrate apex.

3.3. Volsella

The volsella consists of the parossiculus (basivolsella and cuspis) and the lateropenite (digitus volsellaris) (volsella = vo, Fig. 2; basivolsella = bv, cuspis volsellaris = cv, lateropenite = lp, Fig. 3). It is articulated with the gonocoxite along its ventral edge and is housed by the penisvalva along the inner margin. It bears the lateral finger-like cuspis volsellaris with torulose sculpture on the medial-ventral margin. The lateropenite is laterally compressed, with posterior wide torulose sculpture. The anteroventral process varies in shape amongst the *Dinoponera* species and bears a minute echinulate sculpture (anteroventral process of volsella = avv, Fig. 3).

D. australis has a rectangular lateropenite in lateral view that widens modestly posterad with a vertical to weakly convex posterior margin and a broad cuspis volsellaris that reaches the lateropenite posterior margin. Lateropenite shape varies between specimens from Goiás State (MZSP 62282 and MZSP 62281) and Paraná State (DZUP 548845 and DZUP 548844) (Fig. 3). Specimens from Goiás are bigger and have a relatively wider lateropenite in lateral view with a more concave ventral margin. Specimens from Paraná have a subrectangular lateropenite and a broadly concave ventral margin, interestingly the lateropenite in these specimens are similar with *D. lucida* in the morphospace (Fig. 5). The anteroventral process projects ventrally and is either rounded (DZUP 548845 and MZSP 62282), sub-quadrate (DZUP 548844) or acute (MZSP 62281).

D. snellingi has an arched volsella in lateral view. The Itirapina specimen (MZSP 62283) has a very concave ventral margin with a pronounced posteroventral projection but the dorsal margin is broadly convex. In contrast, the other specimen (MZSP 62284) has a very convex dorsal margin and a more modestly concave ventral margin and posteroventral projection. The cuspis volsellaris reaches the lateropenite posterior margin in both specimens. The anteroventral process in lateral view projects ventrally and is subquadrate in one specimen (MZSP 62283) and triangular in the other (MZSP 62284), but both bear minute teeth.

D. lucida has a posteriorly widening lateropenite with a ventral edge that shows continuous variation in lateral view, mostly broadly concave with a posteroventrally hooked lobe, sometimes rounded. The posterior lateropenite margin is usually more convex than in *D. australis* or *D. snellingi.* The cuspis volsellaris is short, never reaches the lateropenite posterior margin, and the anteroventral process is sub-quadrate to rounded, never projecting ventrally as in *D. australis* or *D. snellingi*.

D. gigantea, D. quadriceps and *D. sp.* 1 show similarities in lacking a strongly ventrally projecting anterior process and having a strong posteroventral concavity with a ventrally pointed process. The lateropenite in *D. gigantea* and *D. quadriceps* has a posterodorsal projection that noticeably overhangs its sloped posterior margin, but in *D.* sp. 1 this projection is not pronounced. The cuspis volsellaris is variable in length but never exceeds the lateropenite posterior margin and the anteroventral process is anteriorly bidentate in *D. gigantea* and *D. quadriceps*, but rounded in *D. sp.* 1.

The geometric morphometrics analysis of the lateropenite evidenced that shape is significantly influenced by allometry (Procrustes Manova, $P < 0.005$). When analyzing the centroid size, no differences were found between species (Fig. 4). The discriminant analysis found that the lateropenite is different among *Dinoponera* species (Procrustes MANOVA, $P < 0.001$) (PC1 accounted for 56% and PC2 accounted for 18%, Fig. 5). Positive PC1 scores indicate a concave posterior margin, whereas negative scores correspond to a convex posterior margin. On the other hand, positive PC2 scores are related to a rounded ventroposterior edge, whereas negative scores indicate a ventrally protruding ventroposterior edge. Overall, the lateropenite shape is different amongst the species except for *D. snellingi* with *D. australis* ($p = 0.45$) and for *D. gigantea* ($p = 0.09$). Additionally, *D.* sp 1 is different only with *D. lucida* ($p = 0.95$) (Table 2).

3.4. Penisvalva

The penisvalva of *Dinoponera* species show considerable variation in general shape and size, ranging from rounded in *D. lucida*, rounded to subtriangular in *D. australis*, V-shaped in *D. snellingi*, and triangular in *D. gigantea*, *D. quadriceps,* and *D.* sp. 1 (penisvalva = pe, Fig. 2; Fig. 6).

The dorsoposterior profile of the penisvalva of *D. gigantea, D. quadriceps,* and *D.* sp 1 in lateral view is divided by a very blunt angle into a straight and posteriorly inclined dorsal margin, and a steeply descending posterior margin that is mostly broadly convex with a shallow median concavity. The dorsoposterior margin is continuously convex in *D. lucida*, but in *D. australis* and *D. snellingi* it is dorsally slightly convex.

Apex shape comes in four different forms: slightly angular in *D. quadriceps*, slightly rounded and directed more posteriorly in *D. gigantea* and *D.* sp 1, rounded and directed more ventrally in *D. lucida. D. australis* and *D. snellingi* share a ventroposterior, laterally projecting lobe with a serrated edge that folds anterad to a varying degree.

There are three general shapes for the ventral profile: (1) Weakly sinous in *D. quadriceps*, *D. gigantea,* and *D.* sp 1 with a ventral, laterally projecting serrated edge that forms a shelf with variable degrees of dorsal curvature (serrated edge = se, Fig. 6). In these species the anteroventral edge of the valve extends beyond the apodeme, ending in a projecting point (penial apodeme = pa, Fig. 6). (2) In *D. australis* and *D. snellingi* the ventral margin is irregularly sinuate in lateral view with a posteroventral projecting lobe with a serrate edge that in *D. australis* is convex to bluntly triangular and of varying length but relatively short, never longer than its basal width. The lobe in *D. snellingi* forms a conspicuous, ventrally triangular projection that is longer than its basal width. Just anterad of the posteroventral lobe these species present a narrow and short serrated lobe that may be folded or twisted, anterior to this the ventral edge lacks serration. (3) *D. lucida* is strongly sinuous with a posteroventral broadly rounded, serrate-edged lobe and a brief lateral serrate lobe. Posterior to this lobe the ventral edge lacks serration.

The lateral carina is normally straight to weakly curved in all species (lateral carina = lc, Fig. 6). In *D. quadriceps*, *D. gigantea*, and *D.* sp 1 the lateral carina forms a concavity with the ventral edge that houses the lateropenite and the volsellar anteroventral process. In all species, the penial apodeme is an anterolateral horn-like curved structure arising from the mid-valve ridge. All species with serrate longitudinal carinae or edges on the penisvalva have the teeth curved anteriorly with the tips pointing posteriorly (serrated edge = se, Fig. 6).

The morphometric analysis of the penisvalvae evidenced that shape is significantly influenced by allometry (Procrustes Manova, $P < 0.001$). Overall, the centroid size is different between *Dinoponera* species (Procrustes, MANOVA, $P < 0.001$). We found centroid size sorts into three groups of species, it is more similar between *D. gigantea* and *D. quadriceps* than *D. lucida* (Fig. 7). *D. australis* has a wide central size spectrum, being different only from *D. quadriceps*. Additionally, there is no difference between the centroid size of *D. snellingi* and *D.* sp 1 and the other species. The discriminant analysis evidenced that *Dinoponera* species differ significantly in penisvalva shape (Procrustes MANOVA, $P < 0.001$) (PC1 accounted for 49% and PC2 accounted for 23%, Fig. 8). Positive PC1 scores indicate a curved

ventral margin, whereas negatives scores correspond with a slightly straight margin. Positive PC2 scores are related to a s-shaped ventral margin with an acute apex, whereas negative scores indicate a slightly curved ventral margin with rounded apex. The species differ according to the penisvalva with the exception of *D.* sp 1, which does not differ from *D. australis* ($p = 0.11$), *D. gigantea* ($p = 0.55$), *D. quadriceps* ($p = 0.08$), and *D. snellingi* ($p = 0.17$) (Table 2).

4. Discussion

Our findings show that male genitalia of *Dinoponera* offer valuable morphological information for taxonomy and species delineations. The most informative parts were found to be the basal ring, penisvalva and volsella, which varied most conspicuously among the species, and some within putative species, such as *D. australis*. Gonostylus width has been used to support species differences, such as in *D. australis bucki* and *D. australis nigricolor* (Borgmeier, 1937) but we found its relative width varied according to the degree of basal bending, a situation that diminishes its value for alpha taxonomy.

Our morphometric analysis allowed separation of species using penisvalvae shape and also quantify continuously intraspecific variation within a species, providing a useful tool for studying genital morphological data (Mutanen and Pretorius, 2007). Relying mostly on the penisvalva and complementing with the volsella can be enough to differentiate most putative species/populations.

Among the species, *D. australis* has the most variable genital shape and size. Interestingly, this species also has the widest known distribution for the genus and the greatest number of named subspecies, both valid and synonymized (Lenhart et al., 2013). Kempf (1971) called attention to the morphological variability within the "*australis*-complex", regarding it as an unsolved problem and highlighting the need of more males from different localities to permit better resolution of the situation. The examined *D. australis* individuals agree in several characters and have a general shape by which they can be separated from the other species, however the specimens from Goiás State have several morphological differences with specimens from Paraná State, such as their volsella, which is more similar to that of *D. snellingi*. Our results also showed considerable size differences between the specimens. It has been shown that genital size variation is stabilized by sexual or natural selection (Tadler, 1999; Bonduriansky and Day, 2003) and regularly has negative allometric values in relation to body size. Thus, genital size can also be valuable in differentiating species (Eberhard et al., 1998; Mutanen et al., 2006; Fumi and Friberg, 2019).

The *D. snellingi* male from Itirapina, SP, shows similar basal ring and penisvalva morphology with that from Itaum, MT, except that the former has the posteroventral process of the penisvalva with a more rounded apex than the latter. Volsella morphology, however, is quite different amongst the two (Fig. 3). The males from both localities also differed strongly in ocellar diameter and the development of the ocellar promontory; the Itaum male having a condition similar to that in the description for the species (Lenhart et al., 2013), but the Itirapina male has much larger ocelli and a higher promontory, unlike the *D. snellingi* types. In the course of ongoing revisionary studies of *Dinoponera*, A. Martins (unpubl. ms.) found that worker ants belonging to what is presently known as *D. australis* are readily recognizable from all other conspecifics, but that within them, there is a high degree of variability that suggests the presence of cryptic species. Martins compared workers collected in the type locality of *D. snellingi*, plus other nearby localities, with workers collected from Itirapina and could not find any differences for defining two species. Worker morphology alone does not seem to provide clear characters for recognizing forms within *D. australis*, but our results suggest males may provide valuable information for the taxonomy and perhaps for the phylogeny of this group. The similarities found in the basal ring, penisvalva, and volsella for *D. australis* and *D. snellingi* separate them from *D. gigantea* and *D. quadriceps*, both of which share many similarities. Our results suggest what is presently known as *D. australis* can be differentiated into discrete populations, perhaps some representing cryptic species. Molecular evidence in this case could help determine how much genital morphological change accompanies reproductive isolation and could also help pair males with conspecific females. Even though we have had the privilege of examining more *Dinoponera* males than any previous study, most males are associated with just a few species, making this a preliminary assessment for most species.

Our results help to explain the mechanism behind suicidal mating in *D. quadriceps*, whereupon separation of the mating pair is initiated by the female chewing off the posterior end of the male's gaster (Monnin and Peeters, 1998). The directionality of the serrated edges of the penisvalva in this species indicates ease of insertion into the female genital tract, but impossibility of non-traumatic removal (Fig. 6). *D. gigantea* and *D. sp*. 1 also have similar jagged lateral edges and we predict that mating in these two species is also suicidal for the male. In *D. lucida* and *D. australis* the jagged edge is along the ventral margin and in *D. snellingi* the serration of the triangular process seems ambiguous as to the directionality of the teeth and its relation to intrusion/extrusion, so mating may not be traumatic in these species.

Our data represent an example of intraspecific variation within insect genitalia which does not disagree with the predictions of the sexual selection hypothesis (Arnqvist, 1997; Langerhans et al., 2016). Recently, many studies have revealed intraspecific variation in insect male genitalia, for example related to summer and spring generations (Fumi and Friberg, 2019), altitudes (Kiss et al., 2017) and geographical scales (Varcholová et al., 2016). Because sexually selected characters tend to evolve rapidly and divergently, the divergence of genital shape can lead to differences among populations and could quickly enhance mechanical isolation, leading to reproductive isolation and specialization (Hosken and Stockley, 2004). Nevertheless, for most *Dinoponera* species the intraspecific differences of penisvalva and lateropenite seem to be more quantitative rather than qualitative. Since reproductive females are gamergates, colony reproduction in the genus occurs via fission with the new alpha female leaving the nest with workers to found an incipient colony, thus dispersion in this genus can be restricted (Monnin and Peeters, 1998; Peixoto et al., 2008; Lenhart et al., 2013). In this sense, the variability amongst individuals could be the result of its reproductive strategy along habitat fragmentation by humans (Franklin et al., 2002) isolating populations and rearranging their distribution (Mariano et al., 2008). In *Dinoponera* this could play a role, as several species have had their habitat severely reduced and fragmented in the past decades.

Understanding the variability of ant male genital morphology relies not only on understanding morphology comparatively, but also on the comprehension of the role in speciation and genital evolution. We clearly need more comparative studies with quantitative approaches, with an extensive use of geometric morphometric tools in taxonomy, and for testing hypotheses of phenotype–environment associations for genitalia, and more broadly, studies that elucidate how ecological factors influence genital morphologies.

Author contributions

Leonardo Tozetto: Conceptualization, Methodology, Formal analysis, Data Curation, Writing and Reviewing. **John Lattke:** Conceptualization, Methodology, Writing and Reviewing.

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