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The spermatheca in the genus *Dendroctonus* (Curculionidae: Scolytinae): morphology, nomenclature, potential characters for taxonomic use and phylogenetic signal

La espermateca en el género Dendroctonus (Curculionidae: Scolytinae): morfología, nomenclatura, caracteres potenciales para su uso taxonómico y señal filogenética

Jazmín García-Román ^{a, b}, Tonatiuh Ramírez-Reyes ^b
y Francisco Armendáriz-Toledano ^{b, *}

^a Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Prol. de Carpio y Plan de Ayala s/n, Col. Santo Tomás, Miguel Hidalgo, 11340 Ciudad de México, Mexico

^b Universidad Nacional Autónoma de México, Instituto de Biología, Circuito Exterior s/n, Ciudad Universitaria, Coyoacán, 04510 Ciudad de México, Mexico

*Corresponding author: farmendariztoledano@ib.unam.mx (F. Armendáriz-Toledano)

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Abstract

In most members of the genus *Dendroctonus*, the usefulness of the spermatheca for taxonomic purposes has not been evaluated in depth; therefore, the aim of this study is to describe and compare the elements that integrate the female reproductive apparatus among *Dendroctonus* species, clarify their nomenclature and evaluate their interspecific variation to propose useful characteristics for their identification. In addition, we evaluated whether there is a structure between the shape of the receptacle and the phylogenetic relatedness of the species. The spermatheca consists of a sperm duct, a receptacle, and a gland; of these elements, the receptacle is the most taxonomically informative structure. The most closely related species of the *D. frontalis* complex show wide intraspecific variation in the shape and size of this structure, which precludes recognition of diagnostic features. However, there are differences among species groups in both shape and size in less closely related species such as *D. approximatus*, *D. mexicanus*, *D. micans*, *D. parallelocolis* and *D. rhizophagus*. A phylogenetic signal was detected in receptacle shape, and patterns of morphological similarity and phylogenetic relatedness of species were evident in the *D. frontalis* complex.

Keywords: Receptacle; Receptacle gland; Geometric morphometrics

Resumen

En la mayoría de los miembros del género *Dendroctonus*, la utilidad de la espermateca con fines taxonómicos no ha sido evaluada en profundidad; por ello, el objetivo de este estudio es describir y comparar los elementos que integran el aparato reproductor femenino entre las especies de *Dendroctonus*, aclarar su nomenclatura y evaluar su variación interespecífica para proponer características útiles para su identificación. Además, evaluamos si existe una estructura entre forma del receptáculo y el parentesco filogenético de las especies. La espermateca consta de un conducto espermático, un receptáculo y una glándula; de estos elementos, el receptáculo es la estructura taxonómicamente más útil. Las especies más estrechamente relacionadas del complejo *D. frontalis*, presenta una amplia variación intraespecífica en la forma y el tamaño de esta estructura, lo que impide el reconocimiento de características diagnósticas. Sin embargo, hay diferencias entre los grupos de especies tanto en la forma como en el tamaño, en especies menos relacionadas, como *D. approximatus*, *D. mexicanus*, *D. micans*, *D. parallelocollis* y *D. rhizophagus*. Se detectó una señal filogenética en la forma del receptáculo, y los patrones de similitud morfológica y el parentesco filogenético de las especies fueron evidentes en el complejo *D. frontalis*.

Palabras clave: Receptáculo; Glándula del receptáculo; Morfometría geométrica

Introduction

The taxonomy of the genus *Dendroctonus* Erichson is traditionally based on external morphological attributes, such as the abundance, distribution, and size of cuticular ornamentation and pubescence on the different body parts (Hopkins, 1909; Wood, 1982). The use of these characteristics has shown that some of them are not useful to separate closely related species, because they present wide intraspecific geographic variation (Armendáriz-Toledano et al., 2014; Lanier et al., 1998; Valerio-Mendoza et al., 2019).

Recent studies on the analysis of the morphological variation in the species of *Dendroctonus* have revealed new diagnostic characteristics for their identification, from the antennae, stridulatory apparatus, and genitalia (Armendáriz-Toledano et al., 2015, 2017; Armendáriz-Toledano & Zúñiga, 2017; López et al., 2014). Regarding the latter, the elements of the seminal capsule of the male genitalia (anchor and seminal rod) have proved very useful, as they present species-specific morphology for all members, allowing reliable identification (Lanier et al., 1988; Armendáriz-Toledano & Zúñiga, 2017). The morphology of the female genitalia has been used in fewer cases to differentiate species, even though it has allowed improved identification in other genera of the subfamily Scolytinae, as is the case for the genus *Xyleborus* Eichhoff, 1864 (Pérez-Silva et al., 2015).

Of the 21 species recognized for the genus (Valerio-Mendoza et al., 2019; Víctor & Zúñiga, 2016; Wood, 1982), the anatomy of the female genitalia is only known in detail in *D. ponderosae* Hopkins (Cerezque, 1964) and for 7 more species (*D. approximatus* Dietz, *D. brevicomis* LeConte, *D. frontalis* Zimmerman, *D. mesoamericanus* Armendáriz-Toledano & Sullivan, *D. mexicanus* Hopkins,

D. parallelocollis Chapuis, and *D. vitei* Wood) the studies have only focused on describing and comparing qualitative and quantitative attributes of the “spermatheca” (= seminal receptacle, *sensu* De Marzo, 2009) as well as the patterns of variation in shape, all for taxonomic purposes (Armendáriz-Toledano et al., 2014, 2017; García-Román et al., 2019; Ríos-Reyes et al., 2008; Valerio-Mendoza et al., 2019).

Morphometric analyses support that the shape of the seminal receptacle allows the recognition of distinct geographic groups in *D. brevicomis*, information that contributed to removing *D. barberi* Hopkins from synonymy (Valerio-Mendoza et al., 2019). In other congeners, this structure presents a species-specific shape, as is the case of *D. approximatus*-*D. parallelocollis* (García-Román et al., 2019) and *D. mexicanus*-*D. vitei*, whereas, in others such as *D. frontalis* and *D. mesoamericanus*, the shape of the spermatheca has only been partially differentiated (Armendáriz-Toledano et al., 2014). According to the most recent phylogenetic hypotheses for the genus (Godefroid et al., 2019; Víctor & Zúñiga, 2016), the most species in which the spermatheca has been characterized belong to the *D. frontalis* complex *sensu* Lanier et al. (1988), which is supported by genomic data (Godefroid et al., 2019) and several corresponding synapomorphies (Víctor & Zúñiga, 2016). Within this group, pairs of sister species (*D. frontalis*-*D. mesoamericanus* and *D. mexicanus*-*D. vitei*) show distinct morphological character states and shape differences in the seminal receptacle, a situation that suggests that the evolutionary pattern of the female genitalia is divergent in these taxa and, therefore, could present a phylogenetic signal or structure (Klingenberg & Guidaszewski, 2010).

However, in the rest of the members of the genus, the patterns of variation of the female genitalia have not

been described, and, in general terms, their usefulness for taxonomic purposes has not been evaluated in depth. For this reason, it has not been possible to study from a phylogenetic point of view the evolution of the female genitalia and, in particular, the seminal receptacle that apparently has evolved divergently in some groups of species of *Dendroctonus*.

Therefore, the aim of this work is to describe and compare the elements that integrate the female reproductive apparatus, with emphasis on the spermatheca, among *Dendroctonus* species, clarify its nomenclature, and evaluate its interspecific variation to propose taxonomically useful characteristics for the identification of females. Furthermore, we reconstruct the evolutionary history of the shape of the seminal receptacle (spermatheca *sensu* Armendáriz-Toledano et al., 2014, 2017; García-Román et al., 2019; Ríos-Reyes et al., 2007; Valerio-Mendoza et al., 2019) by detecting its phylogenetic signal, thus determining whether there is a structure between the patterns of the shape variation of receptacle and the phylogenetic relatedness of species.

Materials and methods

The material examined included more than 200 female specimens corresponding to 16 species of the genus *Dendroctonus* (*D. adjunctus* Blandford, *D. approximatus*, *D. brevicornis*, *D. frontalis*, *D. jeffreyi* Hopkins, *D. mesoamericanus*, *D. mexicanus*, *D. micans* Kugelann, *D. parallelocolis*, *D. ponderosae*, *D. pseudotsugae* Hopkins, *D. rhizophagus* Thomas and Bright, *D. rufipennis* Kirby, *D. simplex* LeConte, *D. valens* LeConte, and *D. vitei*), collected at localities representative of their geographic distribution (Table 1). The sex of the specimens was determined by observation of the stridulatory apparatus on the seventh abdominal tergite, which in females has an entire edge and is without scrapers (present in males) (Hopkins, 1909). The species from the USA and *D. micans* were identified using the taxonomic key of Labonte and Valley (2013), while for those from Mexico and Central America, we used the key of Armendáriz-Toledano and Zúñiga (2017).

Females were dissected for the extraction of the female genitalia, which was clarified, mounted, and examined, following the protocol described by Armendáriz-Toledano et al. (2014). Details of the spermatheca were observed under a compound microscope at 400x, and measurements were taken with a micrometer. Moreover, to refine the initial observations, a subset of this material was processed for scanning electron microscopy (SEM). For this, the genitalia were immersed in 5% sodium hypochlorite to digest the tissues and then rinsed in distilled water.

Samples were mounted on metal plates, coated with gold, and examined under a Hitachi S-2469N scanning electron microscope.

The nomenclature used to describe the general anatomy of the spermatheca was based on the study by De Marzo (2009), and the attributes used for their characterization were taken from articles corresponding to other subfamilies of Curculionidae (Anderson, 1984; Brizzola-dos Santos & Rosado-Neto, 2010; Erbey et al., 2010; Lanteri & del Río, 2008; Omar, 2012; Sanders, 1960; Velázquez-de Castro et al., 2007) and species of Scolytinae (Pérez-Silva & Equihua-Martínez, 2016; Román-Ruiz et al., 2017; Rubio et al., 2008), including those performed on *Dendroctonus* sp. Since, anatomically, all species of the genus studied presented the same elements of female genitalia (see results), the comparison among species was focused on the seminal receptacle, as it was the element with the greatest interspecific variation and the one that provided the greatest number of characteristics for the purposes of the study.

To analyze the morphological variation of the receptacle, 6 continuous attributes were established in the 16 species studied: diameter of the nodulus (ND), diameter of the cornu (CD), diameter of the receptacle in its median region (APM), length of the nodulus (NL), length of the cornu (CL), and distance between the cornu and the nodulus (CND) (Fig. 1). Two discrete characteristics were also analyzed: the portion of the receptacle covered by striae (PCG): (0) 1/3 of the receptacle, (1), 1/2 of the receptacle, (2), 2/3 of the receptacle; and the anatomy of the posterior region of the cornu (APR): (0), protuberance absent and (1), protuberance present (Fig. 1).

With these attributes, the receptacles of all specimens were characterized. For continuous characteristics, the mean and standard deviation per species were calculated, while for discrete ones, frequencies were calculated for each character state. The multidimensional patterns of variation of the characteristics were analyzed by ordination analyses from 2 matrices: (a) continuous and discrete attributes considering the specimens as operational taxonomic units (OTUs) and (b) discrete characteristics considering species as OTUs. Matrix (a) was analyzed by principal coordinate analysis (PCoA) using a Gower distance matrix, meanwhile, matrix (b) was analyzed by principal component analysis (PCA) from a variance-covariance matrix; since the first component (PC1) quantified almost 80% of the variation, it was used for further analysis as a variable representing receptacle morphometric variation among species.

To determine whether the body size of *Dendroctonus* species was associated with the morphological differences found in the seminal receptacle, a regression analysis was

Table 1

Species, locality, and coordinates of *Dendroctonus* species examined in this study.

Species	Locality	Coordinates	
		Latitude	Longitude
<i>D. adjunctus</i>	Tecpan, Chimaltenango, Guatemala	14.8	-90.96
	Desierto de los Leones, CDMX, Mexico	19.33	-99.63
<i>D. approximatus</i>	Ejido El Largo, Maderas, Chihuahua, Mexico	29.2	-108.11
	El Gallo, Guerrero, Mexico	18.27	100.50
	Zempoala, Huitzilac, Morelos, Mexico	19.05	-99.31
<i>D. brevicomis</i>	Deer Creek Rd., 14.9 km S Nelson, British Columbia, Canada	49.45	-117.33
	Panguitch, Oregon, United States	37.82	-112.43
<i>D. frontalis</i>	Buenavista, Motozintla, Chiapas, Mexico	15.36	-92.25
	El Picacho, Cerro Tomabu, Chinandega, Nicaragua	13.03	-86.3
	La Mesa, Charo, Michoacán, Mexico	19.6	-100.93
<i>D. jeffreyi</i>	San Pedro Mártir, Baja California, Mexico	30.95	-115.37
<i>D. mesoamericanus</i>	Campestre Alta Montaña, Guatemala, Guatemala	14.78	-90.92
	Guaimaca, Francisco Morazán, Honduras	14.53	-86.81
<i>D. mexicanus</i>	Teloloapan, Guerrero, Mexico	18.35	-99.85
	Jacala, Hidalgo, Mexico	21.01	-99.18
<i>D. micans</i>	Rue des Quatre Saisons, Peyrat le Chateau, France	45.81	1.77
<i>D. parallelocollis</i>	Chinandega, Nicaragua	19.78	-103.49
	San Rafael, Tlalmanalco, Mexico State, Mexico	19.23	-98.75
<i>D. ponderosae</i>	Pinaleno Mtns. Graham, Arizona, USA	32.65	-109.87
<i>D. pseudotsugae</i>	Arteaga, Coahuila, Mexico	25.24	-100.44
<i>D. rhizophagus</i>	Chihuahua, Mexico	27.97	-107.55
<i>D. rufipennis</i>	Aklavik, Northwest Territories, Canada	68.22	-135.00
<i>D. simplex</i>	Belgrade, Kennebec, Maine, United States	44.44	-69.83
<i>D. valens</i>	Álvaro Obregón, CDMX, Mexico	14.8	-90.96
<i>D. vitei</i>	Ejido Aquiles Serdán, Montemorelos, Nuevo León, Mexico	25.2	-99.88
	Hidalgo, Tamaulipas, Mexico	24.13	-99.06

performed between the total body length of the species (mean value) and the principal component one (PC1) obtained from the PCA as dependent variable.

Geometric morphometrics. The variation in the shape of the seminal receptacle was studied in 16 of the 21 described species of the genus. For each species, 2 to 4 photographs were used, for a total of 48. The capture and processing of the images, as well as the morphometry protocol, were performed with some modifications, following the protocol of Armendáriz-Toledano et al. (2014), who studied the variation of the receptacle shape in *D. frontalis* and *D. mesoamericanus* through semilandmarks. To facilitate the

positioning of the semilandmarks, a fan of 20 radios was used, taking as reference points the middle part of the nodulus and the cornu (Fig. 2). Semilandmarks were placed at each intersection point of each radio and the receptacle contour. In total, 26 points were digitized in the program tpsDig Version 1.40 (Rohlf, 2004), of which 3 were landmarks type I and the remaining semilandmarks *sensu* Bookstein (1991) (Fig. 2). Subsequently, the coordinates were adjusted to decrease the tangential variation of the semilandmarks, using the criterion of minimum procrustes distances (Pérez et al., 2006) in the program SemiLand6 of the IMP (Integrated Morphometrics Package) (Sheets,

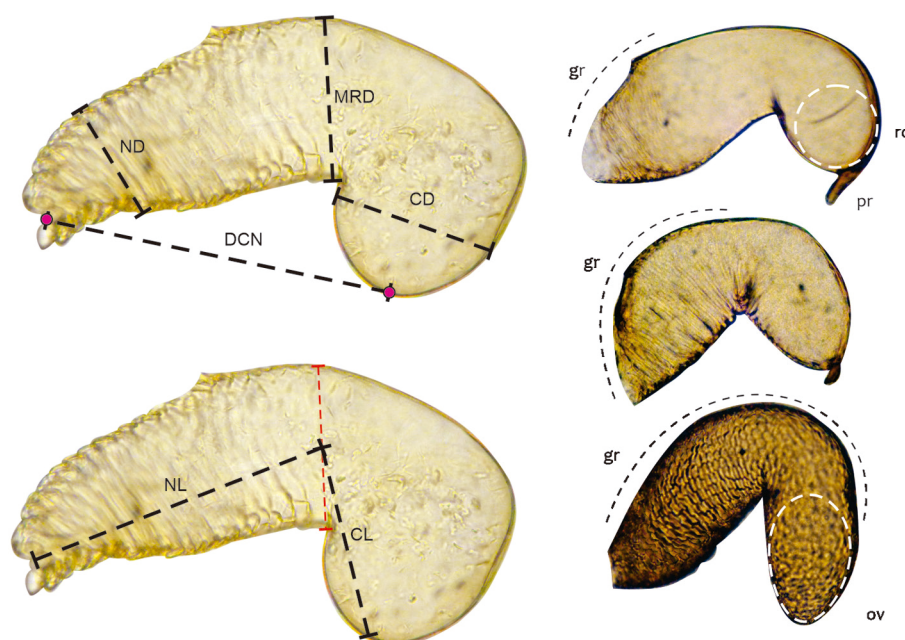


Figure 1. Attributes used in the characterization of the receptacle of 16 species of the genus *Dendroctonus*. The pink nodes indicate the maximum points of curvature of both the nodulus and cornu. ND, Diameter of the nodulus; CD, diameter of the cornu; MRD, diameter of the receptacle in its median region; NL, length of the nodulus; CL, length of the cornu; CND, distance between the cornu and the nodulus; gr, grooves; pr, protuberance; ro, rounded cornu; ov, oval cornu.

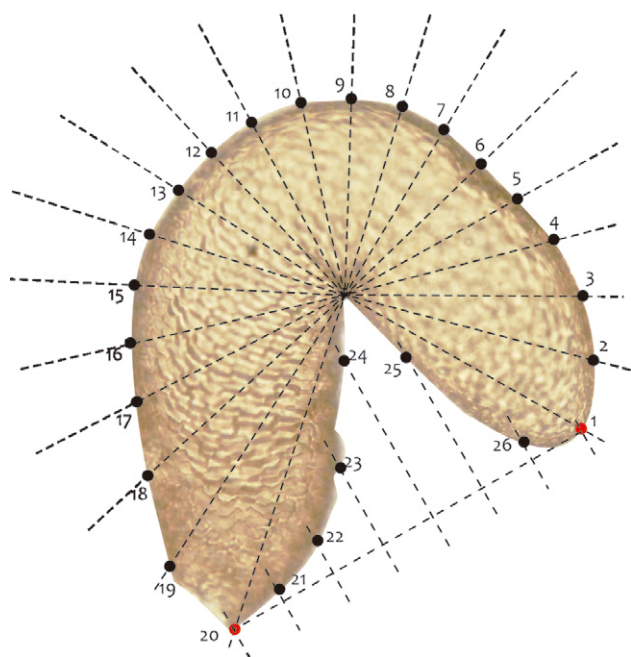


Figure 2. Diagram of the landmarks (red dots) and semilandmarks (black dots) used in the analysis of the receptacle shape.

2003), as well as to eliminate the effects of position, scale, and orientation, using a generalized procrustes analysis in the program CoordGene6f of the IPM (Sheets, 2003). From the adjusted shape coordinate matrices, the average shape configurations of each species were calculated, and a relative warps analysis (RWA) was performed in the PAST 1.95 program (Hammer et al., 2001). Patterns of variation in receptacle shape among species were evaluated by projecting the first 2 components of relative deformation.

Phylogenetic signal. We obtained 2 phylogenetic hypotheses for the genus *Dendroctonus*, the first one based on molecular data and the second one using both molecular and morphological data. To obtain the first phylogeny, 34 sequences of the mitochondrial COI gene fragment were downloaded from GenBank, 33 for *Dendroctonus* and 1 for *Hypothenemus* as an outgroup (Table 2). From the sequence alignment in MAFFT (online: <https://www.ebi.ac.uk/Tools/msa/mafft/>), a matrix of 1,233 characteristics was obtained. We then inferred a maximum likelihood tree in RaxmlGUI 2.0.5 (Edler et al., 2021). We indicated ML+ rapid bootstrap analysis (1,000 replicates), with a GTR+GAMMA model. Subsequently, the resulting

Table 2

Accession numbers and length of cytochrome oxidase gene fragment used to obtain the molecular phylogenetic hypothesis of genus *Dendroctonus*.

Species	Accession number	Length (bp)
<i>D. frontalis</i>	KC855262.1, KC855261.1, KC855260.1	771, 771, 771
<i>D. mesoamericanus</i>	KT364536.1	1,013
<i>D. mexicanus</i>	MK734375.1, MK734374.1, MK734373.1	755, 791, 793
<i>D. vitei</i>	KT364538.1	505
<i>D. approximatus</i>	MK734350.1, MK734349.1, MK734348.1	797, 793, 819
<i>D. brevicornis</i>	MK734343.1, MK734345.1	767, 790
<i>D. adjunctus</i>	AF068001.1, AF067992.1	1,129, 1,177
<i>D. rhizophagus</i>	MK734365.1, MK734364.1, AF067993.1	766, 1,233, 1,009
<i>D. valens</i>	MK734363.1, MK734361.1	764, 735
<i>D. ponderosae</i>	MK734357.1	770
<i>D. jeffreyi</i>	MK734353.1, MK734352.1, MK734351.1	752, 749, 784
<i>D. simplex</i>	AF067985.1	996
<i>D. pseudotsugae</i>	MK840763.1	670
<i>D. rufipennis</i>	MK840771.1	670
<i>D. parallelocollis</i>	KT364537.1, JQ005147.1	977
<i>D. terebrans</i>	AF068003.1	1,077
<i>D. micans</i>	KJ964805.1	658
<i>Hypothenemus obscurus</i>	KX818284.1	667

maximum likelihood tree was pruned with the “drop. tip” function with the ape (Paradis & Schliep, 2019) and phytools (Revell, 2012) packages in RStudio, in order to have one representative for each species and to import it for further analysis. In the second hypothesis, we implemented a total evidence strategy (morphology and mitochondrial COI), for which we downloaded 16 COI gene sequences corresponding to *Dendroctonus* taxa from GenBank (Table 3). The sequences were aligned with the MAFFT program (online version: <https://www.ebi.ac.uk/Tools/msa/mafft/>). The aligned molecular matrix presented 1,545 characters. A morphological dataset corresponding to the 16 *Dendroctonus* species studied was obtained from Víctor and Zúñiga (2016); the morphological matrix contained 31 characters from adult, male genitalia, and larval morphology, in addition, 2 chromosomal characters and 3 behavior traits were added (Table S1 in Víctor & Zúñiga et al., 2016). With these matrices, we inferred a phylogenetic hypothesis through the maximum likelihood (ML) approach estimated in RaxmlGUI 2.0.5 (Edler et al., 2021). We indicated a ML+rapid bootstrap analysis (100 replicates), with a GTRCATI surrogate model.

To evaluate the phylogenetic signal in the receptacle, changes in the shape of this structure were mapped onto both phylogenetic trees, using the first 3 components of relative deformation, by means of the parsimony method of weighted squared change (Klingenberg & Gidaszewski, 2010). The hypothesis of no phylogenetic signal was tested with a permutation test with 10,000 replicates in the program Morpho J 1.01c (Klingenberg, 2011). The *p*-value was calculated as the percentage of permutations resulting in a tree equal to or shorter than the initial tree (Chursina & Negrobov, 2018; Gidaszewski et al., 2009; Klingenberg, 2011; Klingenberg & Gidaszewski, 2010).

Results

In all species studied, the female reproductive apparatus consisted of 2 ovaries, each with a pair of ovarioles converging into a lateral oviduct, 2 oviducts connecting into a middle oviduct, a bursa copulatrix, a pair of accessory glands, and a spermatheca, the last 3 connected to the vagina (Fig. 22 in Cerezke, 1964).

The bursa copulatrix in some species such as *D. ponderosae*, *D. valens*, *D. rufipennis*, and *D. adjunctus*

Table 3

Accession numbers and length of cytochrome oxidase gene fragment used to obtain the total evidence phylogenetic hypothesis of genus *Dendroctonus*.

Species	Accession number	Length (bp)
<i>D. frontalis</i>	AF067986	1,125
<i>D. mesoamericanus</i>	KT364536	1,013
<i>D. mexicanus</i>	AF067988	1,164
<i>D. vitei</i>	KT364538	505
<i>D. approximatus</i>	AF068000	1,178
<i>D. brevicomis</i>	AF068002	1,179
<i>D. adjunctus</i>	AF067992	1,177
<i>D. rhizophagus</i>	JQ005115	1,545
<i>D. valens</i>	JQ005145	1,545
<i>D. ponderosae</i>	AF067987	1,054
<i>D. jeffreyi</i>	AF067994	1,208
<i>D. simplex</i>	AF067985	996
<i>D. pseudotsugae</i>	AF067995	995
<i>D. rufipennis</i>	AF067996	1,034
<i>D. parallelocollis</i>	KT364537	977
<i>D. micans</i>	AF067991	1,096

present inside a pair of very small, sclerotized plates with several spines that apparently are different among species (Fig. 3). These plates were only recovered in 4 species because they are easily lost in the mounting process.

Three elements could be recognized in the spermatheca: a sperm duct, a receptacle, and an accessory gland (Figs. 4, 5). The sperm duct is a thin tube with a transparent and slightly chitinated cuticle, connecting the vagina to the receptacle (Figs. 4, 5a-d); the receptacle is a highly sclerotized kidney-shaped sac associated with muscle tissue, which stores spermatozoa; and the gland is a spherical mass of tissue that can be from 1/3 (Fig. 5f) to slightly larger than the entire receptacle (Fig. 4). The receptacle on the lateral side of the external curvature (Fig. 4) has an opening through which it connects to the accessory gland (Fig. 5g, h).

Of these 3 structures, the seminal receptacle was the one that presented differences among the species studied; the results of the evaluation of continuous and discrete characteristics of this structure in all species are presented in tables 4 and 5, respectively. Although the receptacle does not have areas delimited by sutures, it is possible to distinguish at least 2 regions, the nodulus and the cornu; which are recognized based on a pronounced curvature

that gives the receptacle the appearance of a kidney (Figs. 4, 5a, b). The nodulus is the proximal portion, starting from where the sperm duct ends, up to the curvature of the receptacle, while the cornu corresponds to the distal portion, beyond the curvature (Fig. 4).

The curvature can be in the middle or in the last third of the receptacle; for example, in *D. pseudotsugae*, *D. adjunctus*, *D. approximatus*, *D. brevicomis*, *D. mexicanus*, *D. frontalis*, and *D. mesoamericanus*, it is located approximately in the middle of the receptacle, while in the remaining species it is in the last third (Fig. 6).

Depending on the position of the curvature of the receptacle, the relative length of the cornu and nodulus can be variable; for example, species with curvatures at the middle of the receptacle have cornu and nodulus similar in length (e.g., *D. adjunctus*, *D. approximatus*, *D. mexicanus*, *D. frontalis*, and *D. pseudotsugae*) and species with curvatures in the last third have longer nodulus than cornu (e.g., *D. jeffreyi*, *D. micans*, *D. parallelocollis*, *D. ponderosae*, *D. simplex*, *D. rhizophagus*, and *D. valens*) (Fig. 6; Table 4).

Another variable characteristic among species is the degree of curvature of the receptacle. *Dendroctonus parallelocollis* and *D. simplex* presented receptacles with less pronounced curvatures, so the distance between the nodulus and cornu was relatively larger than the rest of their measurements compared to the other species (Table 4), while *D. adjunctus* and *D. mexicanus* presented the most evident receptacle curvature (Fig. 5). Therefore, the distance between the cornu and nodulus was smaller (Table 4).

The diameter of the receptacle was variable, depending on the region in which it was measured. According to this attribute, taxa may have nodulus and cornu of similar diameter along the receptacle (e.g., *D. mexicanus* and *D. approximatus*), nodulus of larger diameter than cornu (e.g., *D. ponderosae*, *D. rhizophagus*, *D. valens*, and *D. micans*) or cornu of larger diameter than nodulus (e.g., *D. simplex* and *D. parallelocollis*) (Table 4; Fig. 6). Likewise, some species show a reduction in diameter in the region of the curvature of the receptacle (constriction in the curvature), as is the case of *D. jeffreyi*, *D. rhizophagus*, *D. simplex*, *D. valens*, and *D. micans* (Fig. 6).

The surface of the receptacle is ornamented by transverse striae that completely or partially surround it; the density of these striae is much higher in the proximal region of the nodulus and decreases towards the cornu. In the taxa studied, the density of striae and the portion of the receptacle covered by them were an intraspecifically variable characteristic, since most species presented striae covering from one-third to one-half of the receptacle (Fig. 6, Table 5). Only in *D. brevicomis*, *D. frontalis*

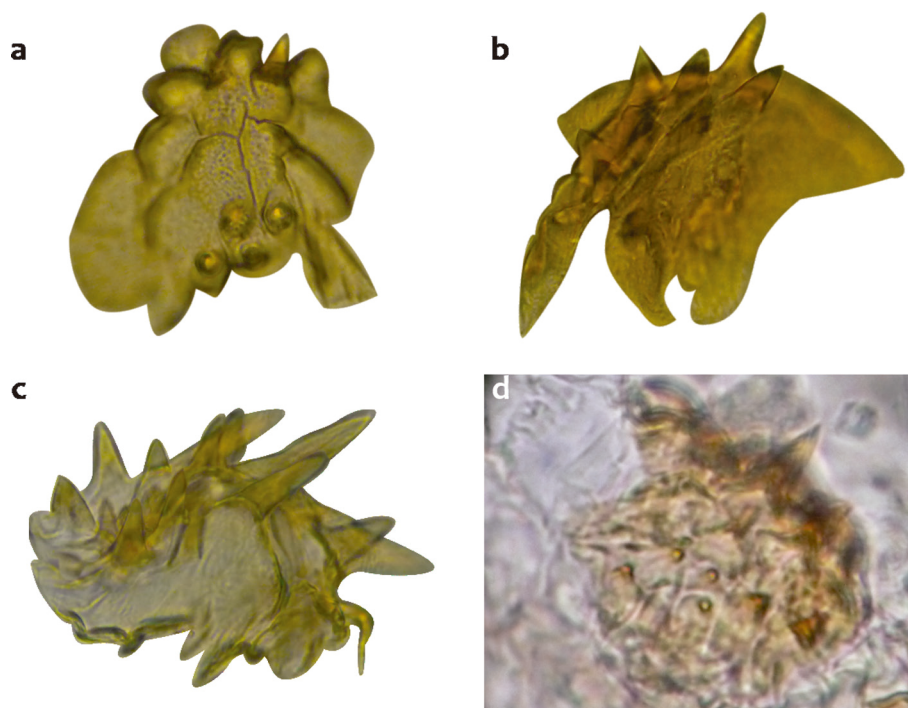


Figure 3. Sclerotized plates of the bursa copulatrix in 3 species of *Dendroctonus*. a) *D. ponderosae*, b) *D. valens*, c) *D. rufipennis*, d) *D. adjunctus*.

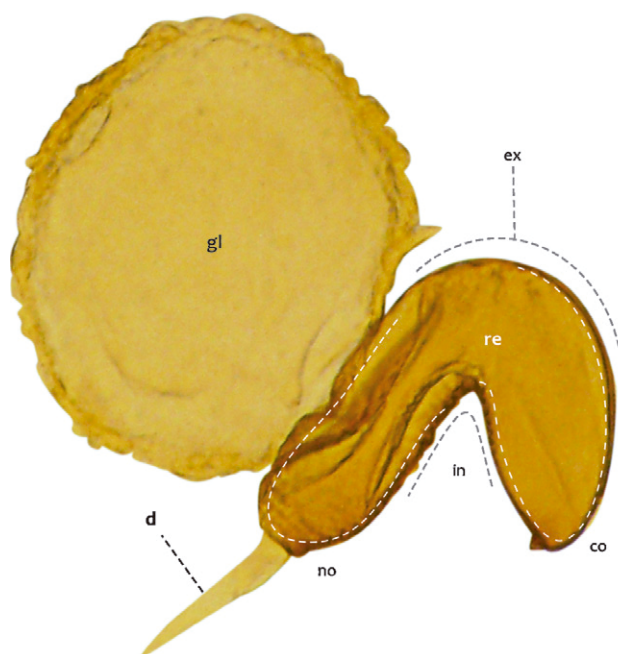


Figure 4. Anatomy of the spermatheca of members of the genus *Dendroctonus* under light microscopy. gl, Gland; re, receptacle; ex, external curvature; in, internal curvature; d, duct; co, cornu; no, nodulus.

and *D. pseudotsugae*, were there some specimens with receptacles entirely covered by striae. In addition to the striae, some species may present a cuticular projection on the surface of the cornu (e.g., *D. micans*, *D. valens* and *D. ponderosae*) (Fig. 6, Table 5), which has been associated with the insertion of the contractor muscles of the receptacle (Cerezque, 1964).

Multivariate analysis of receptacle measurements. The PCoA of the 6 continuous attributes and the 2 discrete attributes recovered 82.65% of the total variation in the first 2 coordinates (PCo1: 69.62%, PCo2: 13.03%); the scatter plot of these coordinates displayed the OTUs in multivariate space in discrete clusters corresponding to most species: *D. approximatus*, *D. brevicornis*, *D. frontalis*, *D. micans*, *D. mesoamericanus*, *D. parallellocollis*, *D. pseudotsugae*, *D. rhizophagus*, and *D. vitei* (Fig. 7). In the cases of *D. adjunctus*, *D. simplex*, and *D. valens*, the specimens showed a wide dispersal and, therefore, overlapped with other ones (Fig. 7). The intraspecific variation of *D. rufipennis* and *D. ponderosae* could not be contrasted, since only 1 receptacle per species was recovered (Fig. 7).

The PCA from the mean values per species of the 8 continuous attributes recovered 90.05% of the total variation in the first 2 principal components (PC1: 79.03%,

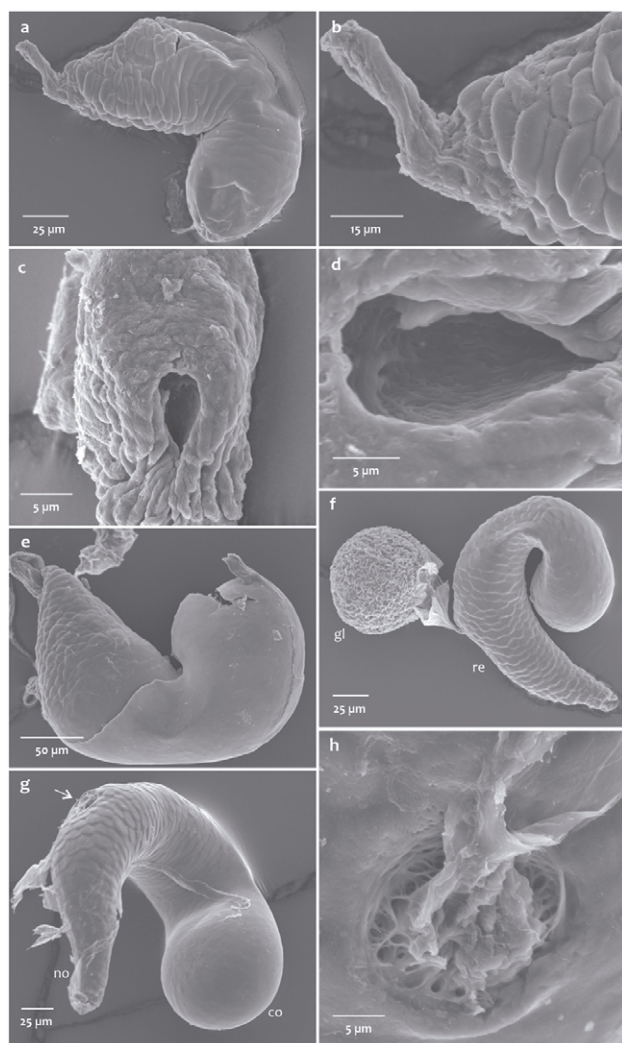


Figure 5. Anatomy of the spermatheca of members of the genus *Dendroctonus* under electron microscopy. a), Receptacle of *D. vitei*; b), outside of the sperm duct of *D. vitei*; c-d), inside of the sperm duct of *D. micans*; e), receptacle of *D. mesoamericanus*; f), receptacle and gland of *D. adjunctus*; g), receptacle of *D. adjunctus*; h), gland duct of *D. adjunctus*. gl, Gland; re, receptacle; no, nodulus; co, cornu.

PC2: 11.02%); the scatter plot between these PCs showed the segregation of the OTUs into 3 groups according to the “size” of the species’ receptacles: “small”, “medium”, and “large”. The attributes that contributed the greatest amount of variation in these components were diameter and length of nodulus (DN, LN) and distance between nodulus and cornu (DCN).

Taxa with small receptacles were *D. adjunctus*, *D. brevicomis*, *D. frontalis*, *D. mesoamericanus*, *D. mexicanus*, *D. simplex*, and *D. vitei* (Fig. 8a), which had

nodulus diameter between 13 and 18.6 µm, nodulus length from 26.8 to 38.3 µm, and nodulus-cornu distance from 23.8 to 39.7 µm (Table 4). Species with medium receptacles were *D. approximatus*, *D. jeffreyi*, *D. parallelocollis*, *D. ponderosae*, *D. pseudotsugae*, *D. rhizophagus*, and *D. rufipennis* (Fig. 8a), which had nodulus diameter between 18.5 and 25.4 µm, nodulus length from 37 to 50.8 µm, and nodulus-cornu distance from 42.8 to 48.8 µm (Table 4). Members with large receptacles were *D. micans* and *D. valens* (Fig. 8a), which had nodulus diameters greater than 26 µm, nodulus length greater than 46 µm, and nodulus-cornu distance greater than 56 µm (Table 4).

Differences in receptacle size among species were correlated with differences in size of the respective adults (Fig. 8b). A linear regression analysis, taking as the independent variable the body length of the adults studied and as the dependent variable the PC1 of the continuous receptacle characteristics, supported that the relationship between adult body size and receptacle dimensions was directly proportional ($r = 0.94$, $r^2 = 0.88$, $t = 10.44$, $p \leq 0.001$).

Geometric morphometrics. Superimposition of the 48 receptacle configurations of the 16 *Dendroctonus* species showed that the sites with the greatest variation were those describing the internal curvature of the receptacle. The analysis of relative deformations from the average configurations per species quantified 74.94% of the total variation in the first 2 components (RW1: 56.05%, RW2: 18.9%). Deformations in RW1 occurred in the degree of constriction at the height of the receptacle curvature and in the distance between the nodulus and cornu. Deformations in RW2 occurred in the internal and external curvatures of the receptacle, as well as in the diameter of the nodulus (Fig. 9).

The scatter plot of the first 2 RWs showed the segregation of the species into several sets according to similarities in receptacle shape (Fig. 9). The first of these was composed of *D. adjunctus*, *D. approximatus*, *D. brevicomis*, *D. frontalis*, *D. mesoamericanus*, *D. mexicanus*, and *D. vitei*, which were characterized by the absence of a constriction in the receptacle curvature, by presenting nodulus and cornu close to each other, as well as receptacles with pronounced curvature (Fig. 6). The receptacle of *D. mexicanus* was the most distinct of this set of species, which has the nodulus and cornu closer together and a more pronounced receptacle curvature compared with the other species (Figs. 6, 9).

Seven of the remaining species (*D. jeffreyi*, *D. micans*, *D. parallelocollis*, *D. rhizophagus*, *D. rufipennis*, *D. simplex*, and *D. valens*) had a constriction in the curvature of the receptacle (the diameter in this area was evidently smaller than that of the nodulus and cornu), nodulus and

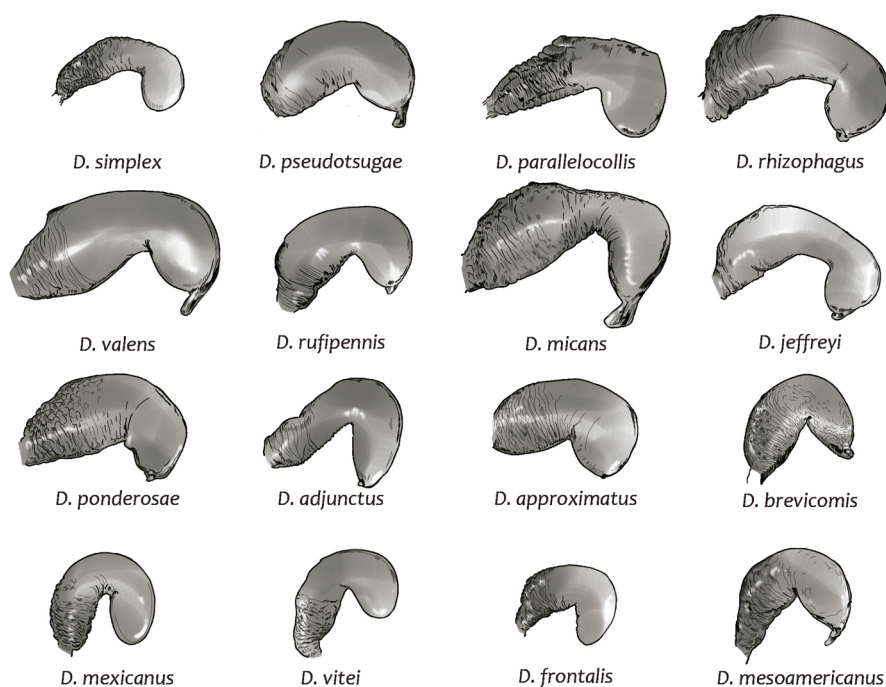


Figure 6. The receptacle of 16 species of *Dendroctonus*.

Table 4

The mean and standard deviation of the quantitative characteristics used to compare 16 of the 21 species of *Dendroctonus*.

Characteristics (μm)	CD	ND	MRD	NL	CL	CND
<i>D. adjunctus</i>	17.5 ± 1.99	15.48 ± 1.03	18.21 ± 0.36	32.98 ± 9.42	25.71 ± 6.8	39.76 ± 5.82
<i>D. brevicomis</i>	17.21 ± 1.79	18.64 ± 2.28	18.71 ± 2.21	31.21 ± 1.22	27.5 ± 2.7	33.93 ± 5.5
<i>D. frontalis</i>	14.29 ± 1.67	13.09 ± 1.6	15.53 ± 4.93	26.84 ± 5.57	20.83 ± 4.32	25.89 ± 2.46
<i>D. mesoamericanus</i>	15.36 ± 2.96	15.36 ± 2.04	14.38 ± 2.11	35.27 ± 4.33	25.89 ± 6.27	37.05 ± 3.38
<i>D. mexicanus</i>	15.12 ± 0.9	16.07 ± 1.78	15 ± 1.24	32.14 ± 3.57	27.38 ± 5.45	23.81 ± 1.03
<i>D. simplex</i>	15.89 ± 0.25	15.89 ± 5.30	17.86 ± 5.05	38.39 ± 1.26	24.82 ± 6.31	41.96 ± 6.31
<i>D. vitei</i>	14.76 ± 0.41	15.95 ± 1.97	13.1 ± 1.25	35.48 ± 2.63	19.17 ± 6.40	30.36 ± 1.79
<i>D. approximatus</i>	22.05 ± 2.32	23.93 ± 4.16	20.98 ± 1.07	43.03 ± 9.32	35.71 ± 11.3	42.96 ± 1.78
<i>D. jeffreyi</i>	18.21	20.71	13.21	46.43	21.43	44.64
<i>D. parallellocollis</i>	19.05 ± 1.03	15.48 ± 1.25	17.14 ± 1.24	47.02 ± 5.74	23.21 ± 3.09	48.81 ± 4.12
<i>D. ponderosae</i>	19.64	26.07	22.14	42.86	27.50	46.43
<i>D. pseudotsugae</i>	24.40 ± 3.72	25.48 ± 1.97	21.67 ± 3.32	41.67 ± 2.73	25.36 ± 4.34	45.83 ± 4.12
<i>D. rhizophagus</i>	21.43 ± 0	24.82 ± 2.27	17.68 ± 1.26	50.89 ± 1.26	25 ± 0	46.43 ± 0
<i>D. rufipennis</i>	19.7 ± 2.05	18.57 ± 3.74	17.44 ± 1.02	37.08 ± 4.32	25.54 ± 3.65	42.86 ± 3.91
<i>D. micans</i>	17.86 ± 0	26.78 ± 2.52	13.75 ± 0.76	59.82 ± 1.26	28.57 ± 5.05	58.03 ± 6.31
<i>D. valens</i>	22.5 ± 3.53	26.25 ± 3.28	22.14 ± 1.01	46.43 ± 5.05	28.93 ± 4.54	56.25 ± 13.89

Table 5

Frequency of each discrete character state in each *Dendroctonus* species.

Species	Portion of receptacle covered by grooves			Anatomy of the posterior region of the cornu	
	(0) one third	(1) half	(2) two thirds	(0) with protuberance	(0) without protuberance
<i>D. adjunctus</i>	33.33%	66.66%	0%	100%	0%
<i>D. approximatus</i>	50%	50%	0%	100%	0%
<i>D. brevicornis</i>	0%	80%	20%	40%	60%
<i>D. frontalis</i>	33.33%	66.66%	0%	80%	20%
<i>D. jeffreyi</i>	100%	0%	0%	0%	100%
<i>D. mesoamericanus</i>	25%	25%	50%	0%	100%
<i>D. mexicanus</i>	100%	0%	0%	100%	0%
<i>D. micans</i>	0%	0%	100%	0%	100%
<i>D. parallelocolis</i>	0%	100%	0%	100%	0%
<i>D. ponderosae</i>	100%	0%	0%	0%	100%
<i>D. pseudotsugae</i>	66.66%	33.33%	0%	0%	100%
<i>D. rhizophagus</i>	100%	0%	0%	0%	100%
<i>D. rufipennis</i>	66.66%	33.33%	0%	33.33%	66.66%
<i>D. simplex</i>	100%	0%	0%	100%	0%
<i>D. valens</i>	100%	0%	0%	0%	100%
<i>D. vitei</i>	100%	0%	0%	100%	0%

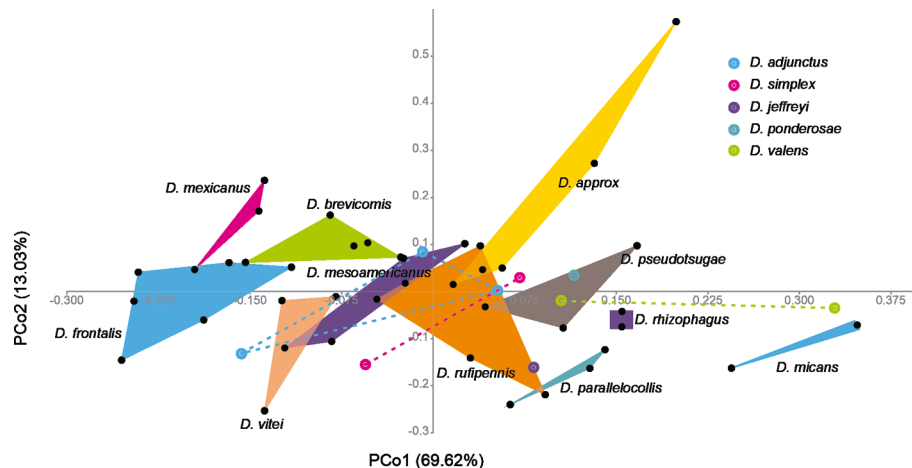


Figure 7. Scatter plot of Principal Coordinate Analysis of continuous and discrete characteristics. The shaded areas of different colors represent species with mostly well-defined characteristics that prevent overlapping groups.

cornu separated from each other, as well as receptacles with less evident curvature (Figs. 6, 9). Within this set, *D. jeffreyi* and *D. rhizophagus* presented very similar shaped receptacles, with narrow nodulus in their proximal region

and spherical shaped cornu in their distal portion (Figs. 6, 9). *Dendroctonus ponderosae* and *D. pseudotsugae* presented intermediate forms to the previously described groups, with receptacles absent of constriction in the

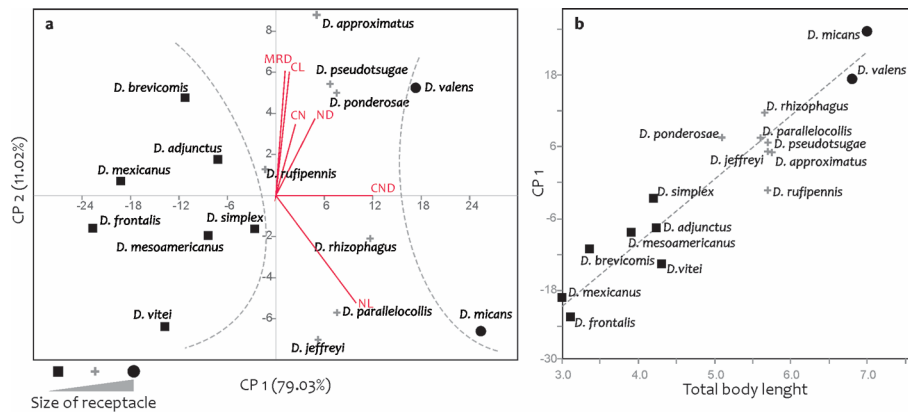


Figure 8. Scatterplot of the first 2 principal components produced in the analysis of the 6 continuous receptacle traits (a) and regression plot between the first total adult length and the first principal component (b); both including 16 species of *Dendroctonus*.

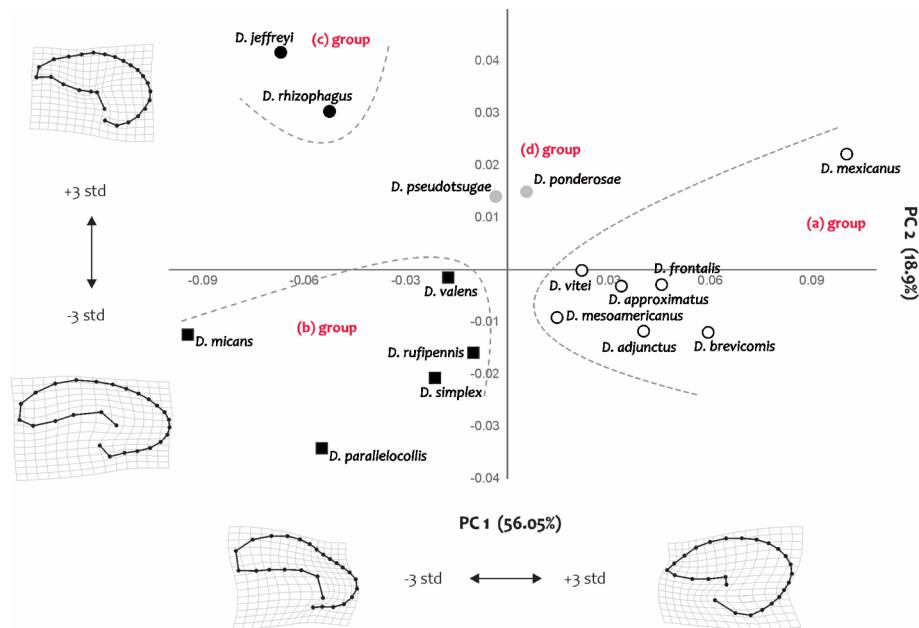


Figure 9. Relative warps analysis (RWA) of the receptacle shape, with their respective deformations, +3 and -3 standard deviations.

curvature, as well as nodulus and cornu far from each other (Figs. 6, 9).

Phylogenetic signal. Both phylogenetic trees constructed from fragments of the mitochondrial COI gene and with total evidence strategy showed an arrangement like those previously reported by Godefroid et al. (2019), Reeves et al. (2012), and Víctor and Zúñiga (2016), (Figs. 10, 12). Similar to previous phylogenetic hypothesis, we recognized 4 groups, the first one corresponding to the *frontalis* group including: *D. adjunctus*, *D. approximatus*, *D. brevicomis*, *D. vitei*, *D. mexicanus*, *D. frontalis* and *D.*

mesoamericanus. The second group is composed by the *valens* group (*D. rhizophagus*, *D. valens*); the third one corresponds to the *pseudotsugae* group (*D. pseudotsugae*, *D. simplex*), and the fourth one to the *ponderosae* group (*D. jeffreyi*, *D. ponderosae*), the remaining species (*D. parallellocollis*, *D. rufipennis*, and *D. micans*) were not grouped within any clade. Both phylogenies differed in the relative position of *D. parallellocollis*, *D. rufipennis*, *D. adjunctus* within the *frontalis*, *ponderosae* and *valens* groups. In the molecular hypothesis, *D. adjunctus* is the sister of the remainder species of the *frontalis* group; the

frontalis group is closely related to *D. parallellocollis*; the *valens* group is the sister clade of *D. parallellocollis-frontalis* group; *D. rufipennis* is the sister of *valens* group, *D. parallellocollis* and *frontalis* group; *D. ponderosae* is a sister group of the most species of *Dendroctonus* less *D. micans*. In the total evidence hypothesis, *D. adjunctus* is the sister of *D. brevicomis*-*D. approximatus*; the *ponderosae* group is sister of the *frontalis* group; the *pseudotsugae* group is the sister of *ponderosae-frontalis* groups; the *valens* group is the sister of *D. parallellocollis*.

Phylogenetic signal based on molecular hypothesis. The principal component analysis to assess the shape change in phylogeny with molecular data quantified 92.61% of the variance in the first 3 PCs (PC1: 69.97%, PC2: 17.13%, and PC3: 5.51%). The mapping of the principal components of shape in the phylogenetic tree obtained provided knowledge of the changes in shape of the seminal receptacle in the hypothetical ancestors of the different nodes of the tree. The most recent common ancestor (MCRA) of *Dendroctonus* was characterized by a larger nodulus than the cornu, an oval-shaped cornu, and a slightly pronounced median constriction (diameter of the median constriction greater than that of the nodulus and equal to that of the cornu). The receptacle corresponding to the ancestor of the *frontalis* group presented a nodulus of the same size as the cornu, the latter of spherical shape, without median constriction (diameter of the median constriction equal to that of the nodulus and cornu) and the inner curvature more pronounced, so, the nodulus and cornu were close to each other. The ancestor of the *valens* group presented receptacles with a larger nodulus than cornu, the latter oval, with a slight median constriction (diameter of the median constriction smaller than that of the nodulus and cornu), and the internal curvature little pronounced, so, the nodulus and cornu were distant from each other. The receptacle of the ancestor of the *pseudotsugae* group presented a larger nodulus than cornu, the latter of spherical shape, without median constriction (diameter of the median constriction equal to that of the nodulus and cornu), and the internal curvature little pronounced, so that the nodulus and cornu were distant from each other. The ancestor of the *ponderosae* group had a larger nodulus than cornu, the latter being oval, with evident median constriction (diameter of the median constriction smaller than that of the nodulus and equal to that of the cornu) and the internal curvature not very pronounced, so, that the nodulus and cornu were distant from each other (Fig. 10).

The phylogenetic signal was statistically significant ($p < 0.05$; $p = 0.049$); mapping changes in shape onto phylogeny yielded a tree length of 0.15, which compared to the initial tree was shorter (37.23). In the

phyломorphospace, the members of the genus were relatively dispersed and separated into 2 distinct non-overlapping groups, one composed of the members of the *frontalis* complex and the other with the remaining groups and species (Fig. 11). However, within each group, the pattern of species dispersal was partly consistent with the molecular phylogenetic hypothesis obtained here (Fig. 10). In the *frontalis* group, overlapping of the terminal branches of *D. adjunctus* with (*D. approximatus*, *D. brevicomis*) and of *D. frontalis* with the group of (*D. mexicanus*, *D. vitei*) were observed. In species not belonging to the *frontalis* complex, many changes in direction in the reconstructed trajectories of evolutionary change and overlapping between terminal branches were observed, indicating the presence of homoplasy in most of them.

Phylogenetic signal based on total evidence hypothesis. Principal component analysis to assess shape change in phylogeny from molecular and morphological data quantified 84.39% of the variance in the first 3 PCs (PC1: 55.99%, PC2: 18.88%, and PC3: 9.61%). The mapping of the principal components of shape in the phylogenetic tree supported similar morphological trajectories among the ancestor of all groups of the genus, which were described in the anterior section (Fig. 12).

The phylogenetic signal was statistically significant ($p = 0.014$); mapping changes in shape onto phylogeny yielded a tree length of 0.036, which compared to the initial tree was shorter (56). In the phyломorphospace of PC1 and PC2, members of the genus were relatively dispersed and separated into 2 distinct non-overlapping groups, one composed of the members of the *frontalis* group and the other with the remaining groups and species (Fig. 13). However, in the PC2 and PC3 phyломorphospace the members of the genus did not segregate, keeping the groups overlapping (Fig. 13). However, within each group, the pattern of species dispersal was only partially consistent with the phylogenetic hypothesis from molecular and morphological data obtained here (Fig. 12). In the *frontalis* group, overlap of the terminal branches of *D. adjunctus* with *D. approximatus* was observed. In species not belonging to the *frontalis* complex, many directional changes in the reconstructed trajectories of evolutionary change and overlap between terminal branches were observed, indicating the presence of homoplasy in most of them.

Discussion

In Coleoptera 5 patterns of morphological organization of the spermatheca have been recognized based on the presence and absence of 3 elements: duct, receptacle, and gland (De Marzo, 2009). The study of 16 of the 21 species

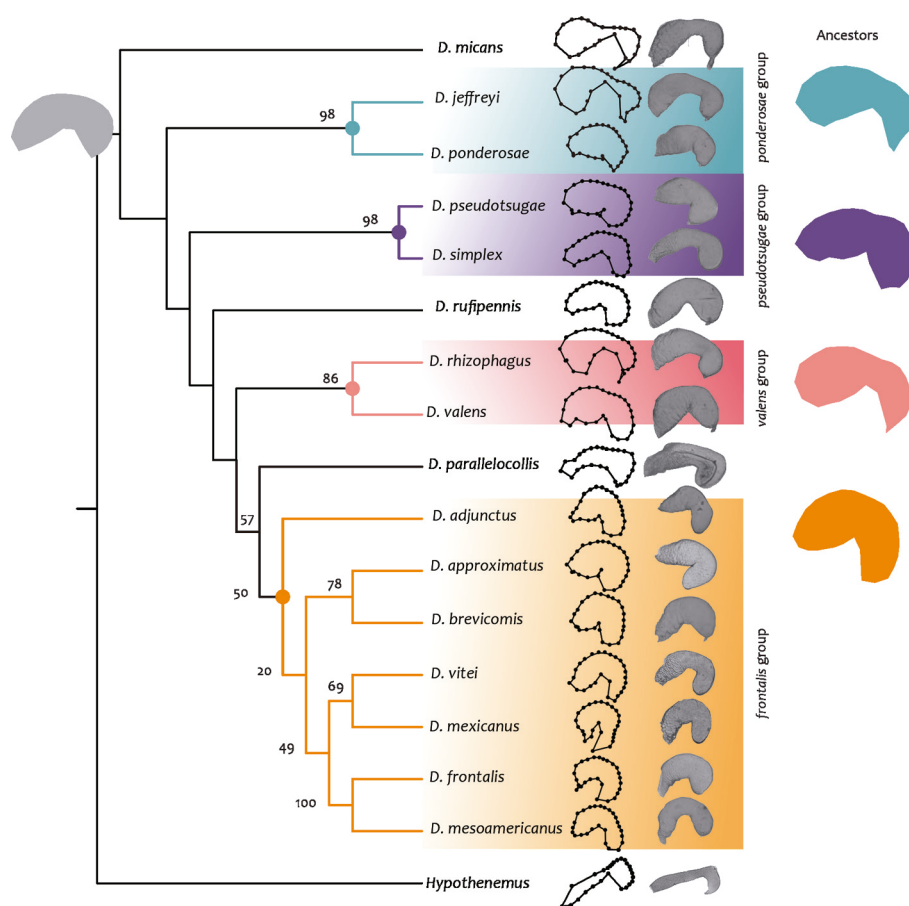


Figure 10. Phylogenetic tree from COI I mitochondrial DNA gene sequences.

of *Dendroctonus* showed that the type of spermatheca in the genus corresponded to the most common type in Coleoptera.

The anatomical descriptions of the male and female reproductive apparatus in *Dendroctonus*, Cereque (1964) also recognized 3 parts in the spermatheca: a spermathecal duct, a driving organ (with compressor muscles), and a sac. The driving organ was described as a U-shaped sclerotized capsule, the spermathecal duct as a tube connecting in the proximal portion to the driving organ, and the sac as a mass of tissue connected at the outer curvature of the driving organ. The sac was attributed glandular function by its histological characteristics. According to these descriptions, the spermathecal duct would correspond to the sperm duct, the driving organ to the receptacle, and the spermathecal sac to the gland *sensu* De Marzo (2009).

Although the spermatheca has been studied to diagnose different groups of Curculionidae, because it presents characteristics with high taxonomic value (Aslam, 1961; Sanders, 1960), the literature presents inconsistencies in

its nomenclature (Tables 4-7). A few studies recognize the spermatheca as an organ integrated by different elements (Erbey et al., 2010; Sanders, 1960), and in most of them (Anderson, 1984; Brizzola dos Santos & Rosado-Neto, 2010; Lanteri & del Río, 2008; Omar, 2012; Velázquez-de Castro et al., 2007), including those focused on species of Scolytinae (Pérez-Silva & Equihua-Martínez, 2016; Román-Ruiz et al., 2017; Rubio et al., 2008), particularly within *Dendroctonus* (Armendáriz-Toledano et al., 2014, 2017; García-Román et al., 2019; Ríos-Reyes et al., 2008), the term spermatheca is used to refer only to the receptacle (Table 6). Therefore, we suggest that in the future the nomenclature proposed by De Marzo (2009) be used, which can be applied to a greater number of species and larger taxonomic scales, facilitating the comparison of this organ for taxonomic and phylogenetic purposes.

Generally, most studies recognize 2 regions, the nodulus and the cornu, which are defined as the proximal and distal part of the receptacle, respectively (Anderson, 1984; Armendáriz-Toledano et al., 2014; García-Román

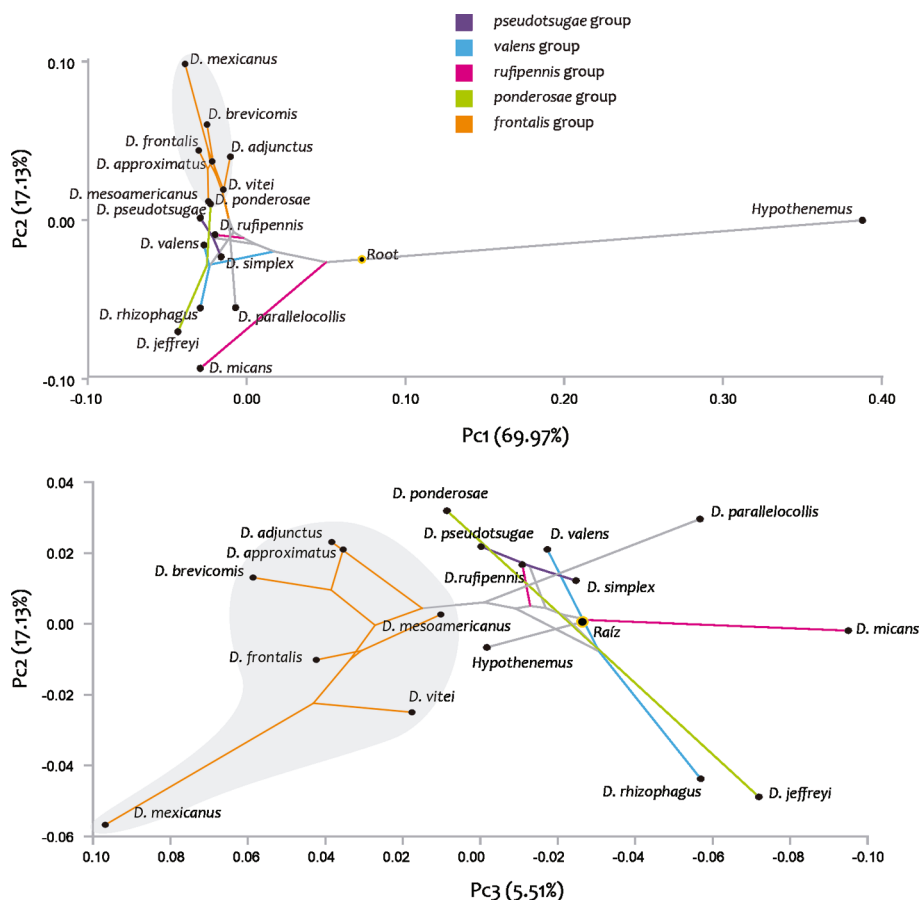


Figure 11. Changes in receptacle mapped onto molecular phylogeny comparing the first 3 deformation components.

et al., 2019; Lanteri & del Río, 2008; Pérez-Silva & Equihua-Martínez, 2016; Román-Ruiz et al., 2017; Rubio et al., 2008; Sanders, 1960; Velázquez-de Castro et al., 2007). In addition, other parts are named, such as the body or collum, which is an intermediate region between the nodulus and the cornu (Brizzola-dos Santos & Rosado-Neto 2010; Lanteri & del Río, 2008; Omar, 2012; Velázquez-de Castro et al., 2007), and the ramus, which is the junction point between the accessory gland and the receptacle (Brizzola-dos Santos & Rosado-Neto, 2010; Lanteri & del Río, 2008; Omar, 2012; Sanders, 1960). The comparison of these elements in our study allowed us to recognize that the cornu was formed only by a slight curvature of the receptacle, and the latter was not differentiated from the ramus, which has been recognized as the simplest morphological pattern compared to other families of Coleoptera (Sanders, 1960).

The characteristics associated with the striae of the receptacle were not very useful for the identification of the species of *Dendroctonus*; since in all evaluated members,

there were individuals with different proportions of the receptacle covered by striae. Previous works, comparing the number, density, and proportion of striae covering this structure, found high intraspecific variation, which did not allow the establishment of diagnostic characteristics in species with great morphological similarity such as *D. frontalis*-*D. mexicanus* (Ríos-Reyes et al., 2008), *D. approximatus*-*D. parallelocolis* (García-Román et al., 2019), as well as between the sister species *D. frontalis*-*D. mesoamericanus* (Armendáriz-Toledano et al., 2014) and *D. mexicanus*-*D. vitei* (Armendáriz-Toledano et al., 2017). Therefore, it is suggested that characteristics associated with receptacle striae should be used with caution.

The 6 measures used to characterize the receptacle (size) (Table 4), as well as the variables to analyze its shape, showed wide intraspecific variation in the members of the genus, which prevented the recognition of discrete species-specific characteristics in most taxa. However, both continuous quantitative and discrete qualitative variables supported that receptacle morphology has been

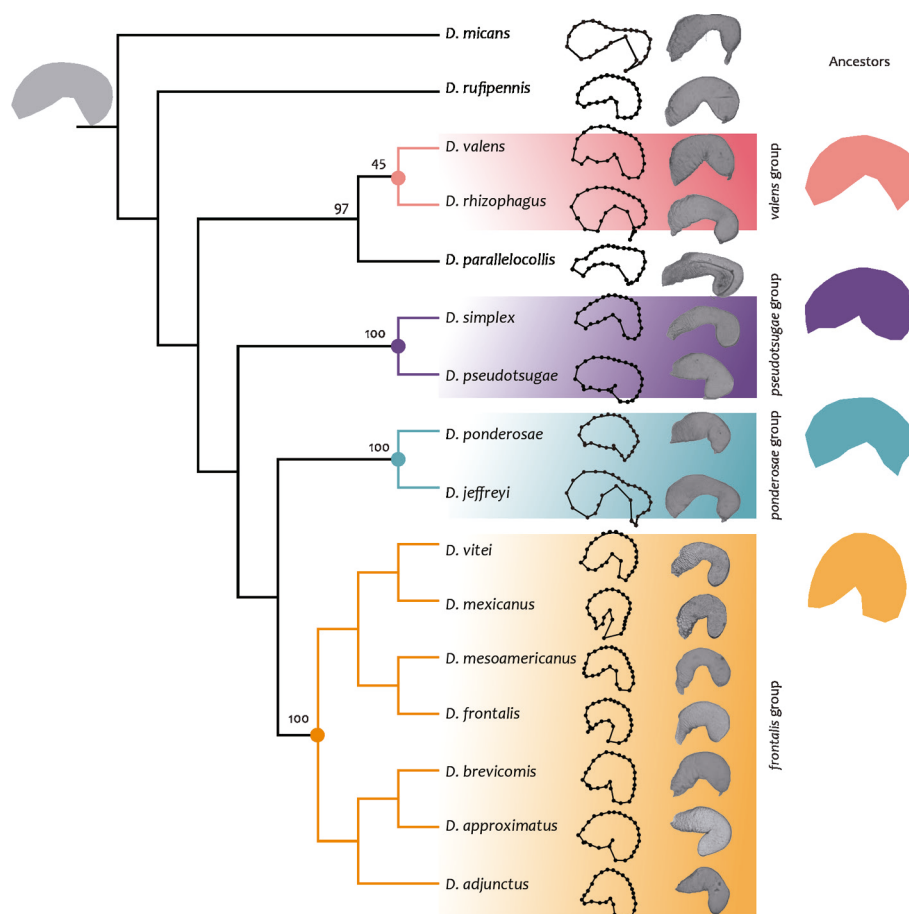


Figure 12. Phylogenetic tree from total evidence (molecular and morphological data).

differentiated in some species and groups, as shown by PCoA in which 11 species were recovered as discrete groups.

With respect to continuous measurements, it was possible to recognize at least 3 groups based on the size of their receptacles: small (*D. adjunctus*, *D. brevicornis*, *D. frontalis*, *D. mesoamericanus*, *D. mexicanus*, *D. simplex*, and *D. vitei*), medium (*D. approximatus*, *D. jeffreyi*, *D. parallellocollis*, *D. ponderosae*, *D. pseudotsugae*, *D. rhizophagus*, and *D. rufipennis*), and large (*D. micans* and *D. valens*) (Fig. 8a). Species in these groups showed more pronounced differences in nodulus diameter, nodulus length, and the distance between the nodulus and cornu (Table 4). Although these measures do not overlap among the groups, they are recommended to be used with caution for taxonomic purposes, because receptacle size (PC1) showed a positive relationship with insect size, a measure that also exhibits wide intraspecific variation (Fig. 8b).

The shape variables also allowed us to recognize that the receptacle has been differentiated into 4 large

groups within the genus: one integrated by species of the *D. frontalis* complex, which present receptacles with pronounced curvature and without constriction (Fig. 9, “(a) group”), a second one by the species *D. micans*, *D. parallellocollis*, *D. rufipennis*, *D. simplex*, and *D. valens* that present receptacles with less pronounced curvature and constriction (Fig. 9, “(b) group”), another one integrated by the species *D. jeffreyi* and *D. rhizophagus* that have narrow nodulus and cornu of spherical shape (Fig. 9, “(c) group”). Finally, another group conformed by *D. ponderosae* and *D. pseudotsugae* with receptacles absent of constriction in the curvature, as well as the nodulus and cornu far from each other (Fig. 9, “(d) group”).

Interestingly, within these groups there were cases in which the shape of the receptacle was species-specific, such as *D. mexicanus*, *D. micans*, and *D. rhizophagus*, allowing them to be identified from the rest of the congeners. *D. mexicanus* showed a small receptacle with very pronounced curvature, no constriction, a cornu and nodulus of similar length and diameter, and without

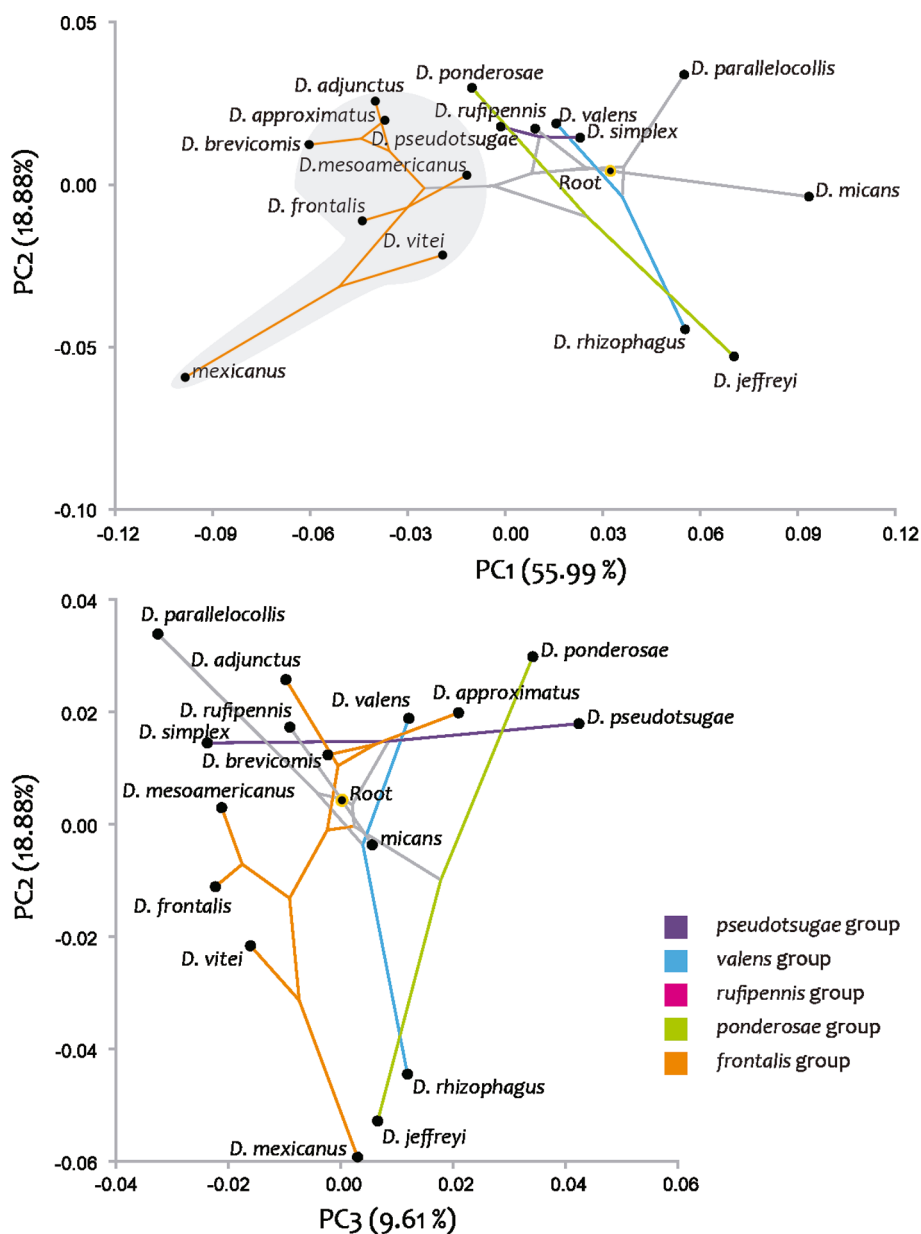


Figure 13. Changes in receptacle mapped onto molecular and morphological phylogeny comparing the first 3 deformation components.

prolongation of the cornu; *D. micans* presented a larger receptacle, less evident curvature, constriction in the curvature, a nodule of greater length and diameter than the cornu, and a long and thick prolongation on the surface of the cornu; *D. rhizophagus* had a medium-sized receptacle, with less obvious curvature, constriction in curvature, a nodule of similar length and diameter as the cornu, and a short prolongation on the surface of the cornu.

Phylogenetic hypothesis. This hypothesis based on mitochondrial data was similar to previous studies

(Godefroid et al., 2019; Reeves et al., 2012; Víctor & Zúñiga, 2016), as it recovered 4 of the 5 groups proposed for the genus, *frontalis* (*D. adjunctus* ((*D. approximatus*, *D. brevicomis*) ((*D. vitei*, *D. mexicanus*) (*D. frontalis*, *D. mesoamericanus*)))), *pseudotsugae* (*D. pseudotsugae*, *D. simplex*), *ponderosae* (*D. jeffreyi*, *D. ponderosae*), and *valens* (*D. rhizophagus*, *D. valens*). Although the species *D. micans* and *D. rufipennis* were included, these taxa were not recovered in a monophyletic group as in previous phylogenies (Godefroid et al., 2019; Reeves et al., 2012;

Table 6

Nomenclature and alternative terms of the spermatheca in Curculionidae, proposed by different authors.

Taxa		Conduct	Receptacle	Gland	Reference
Dryophthorinae	<i>Sitophilus oryzae</i>	Spermatic duct	Spermatheca	-	Omar, 2012
Entiminae	Naupactini	Spermathecal conduct	Spermatheca	Spermathecal gland	Lanteri and del Río, 2008
Entiminae	Sitonini	-	Spermatheca	-	Velázquez-de Castro de Castro et al., 2007
Leptopiinae	<i>Connatichela artemisiae</i>	Spermatic duct	Spermatheca	Spermathecal gland	Anderson, 1984
Lixinae	<i>Lixus nordmanni</i>	Spermathecal duct	Receptaculum seminis	Accessory gland	Erbey et al., 2010
Molytinae	<i>Heilus</i> spp.	Spermathecal duct	Spermatheca	Spermathecal gland	Brizzola dos Santos and Rosado-Neto, 2010
Polyphaga	Rhynchophora	Spermathecal duct	Ductus receptaculi	Spermathecal gland	Sanders, 1960
Scolitynae	<i>Hypotenemus hampei</i>	Spermatic duct	Spermatheca	Spermathecal gland	Román-Ruiz et al., 2017
Scolitynae	<i>Hypotenemus hampei</i>	-	Spermatheca	Accessory gland	Rubio et al., 2008
Scolitynae	<i>Xyleborus volvus</i>	-	Spermatheca	-	Pérez-Silva and Equihua-Martínez, 2016
Scolitynae	<i>Dendroctonus monticolae</i> (= <i>ponderosae</i>)	Spermathecal duct	Driving organ	Sac	Cerezque, 1967
Scolitynae	<i>Dendroctonus mexicanus</i>	-	Spermatheca	-	Ríos Reyes et al., 2008
Scolitynae	<i>Dendroctonus frontalis</i>	-	Spermatheca	-	Ríos Reyes et al., 2008
Scolitynae	<i>Dendroctonus frontalis</i>	-	Spermatheca	-	Armendáriz-Toledano et al., 2014
Scolitynae	<i>Dendroctonus mesoamericanus</i>	-	Spermatheca	-	Armendáriz-Toledano et al., 2014
Scolitynae	<i>Dendroctonus parallelocollis</i>	-	Spermatheca	-	García-Román et al., 2019
Scolitynae	<i>Dendroctonus approximatus</i>	-	Spermatheca	-	García-Román et al., 2019

Victor & Zúñiga, 2016). Similarly to the topology from Victor and Zúñiga (2016), *D. parallelocollis* turned out to be a distinctive taxon that did not cluster within the major clades and was more closely related to the *frontalis* group in our COI hypotheses, in contrast to Víctor and Zúñiga (2016) who relate it with the *valens* group (Fig. 8) and with our total evidence analysis. Another difference was the position of *D. adjunctus* within the *frontalis* group, a taxon that was recovered as a sister species to (((*D. frontalis*, *D. mesoamericanus*) (*D. mexicanus*, *D. vitei*))

(*D. brevicomis*, *D. approximatus*) in our molecular tree, while in previous work and our total evidence phylogeny it constituted the sister species of the latter 2 taxa.

The relationships between the groups also showed different patterns with respect to previous phylogenies. In our tree, the *valens* group was sister to *D. parallelocollis* + *frontalis* group, while other authors related it to the *rufipennis* group (Reeves et al., 2012), to the *frontalis* group (Godefroid et al., 2019) and with *D. parallelocollis* (Victor & Zúñiga, 2016), the last agree with our total

Table 7

Nomenclature of the receptacle in members of Curculionidae, proposed by different authors.

Taxa	Regionalization of the receptacle	Reference
<i>Sitophilus oryzae</i>	Cornu - Collum - Ramus - Apex	Omar, 2012
Naupactinii	Cornu - Body - Ramus - Nodulus	Lanteri and del Río, 2008
Sitonini	Cornu - Corpus- Nodulus	Velázquez-de Castro de Castro et al., 2007
<i>Connatichela artemisae</i>	Cornu - Ramus - Nodulus	Anderson, 1984
<i>Lixus nordmanni</i>	Apex - Basal part	Erbey et al., 2010
<i>Heilus</i> spp.	Cornu - Collum - Ramus	Brizzola dos Santos and Rosado-Neto, 2010
Rhynchophora	Cornu - Ramus - Nodulus	Sanders, 1960
<i>Hypotenemus hampei</i>	Not mentioned	Román-Ruiz et al., 2017
<i>Hypotenemus hampei</i>	Not mentioned	Rubio et al., 2008
<i>Xyleborus volvus</i>	Cornu - Ramus - Nodulus	Pérez-Silva and Equihua-Martínez, 2016
<i>Dendroctonus mexicanus</i>	Not mentioned	Ríos Reyes et al., 2008
<i>Dendroctonus frontalis</i>	Not mentioned	Ríos Reyes et al., 2008
<i>Dendroctonus frontalis</i>	Cornu - Nodulus	Armendáriz-Toledano et al., 2014
<i>Dendroctonus mesoamericanus</i>	Cornu - Nodulus	Armendáriz-Toledano et al., 2014
<i>Dendroctonus parallellocollis</i>	Cornu - Nodulus	García-Román et al., 2019
<i>Dendroctonus approximatus</i>	Cornu - Nodulus	García-Román et al., 2019

evidence tree. The *pseudotsugae* group of our molecular tree was sister to *D. rufipennis* + *valens* group + *D. parallellocollis* + *frontalis* group, and in the total evidence analysis was sister to *ponderosae* group + *frontalis* group, while Reeves et al. (2012) recovered it as sister to *D. armandi*, Víctor and Zúñiga (2016) as sister of the *valens* + *rufipennis* group, and Godefroid et al. (2019) related with the *valens* + *rufipennis* + *frontalis* group. Finally, the *ponderosae* group in previous studies was sister to the *frontalis* group (Godefroid et al., 2019; Reeves et al., 2012; Víctor & Zúñiga, 2016), while in our tree, the *ponderosae* group was sister to the *pseudotsugae* group.

Phylogenetic signal. Hypothesis testing for the absence of a phylogenetic signal indicated that relatedness among *Dendroctonus* members was associated with interspecific variation in seminal receptacle shape; however, the data showed a relatively low phylogenetic signal. Using the phylogenetical tree hypothesis with molecular data, the phylogenetic signal within the *frontalis* group is graphically clearer than when using a total evidence hypothesis. The shape components of this structure mapped onto the phylogeny, allow us to observe that the distribution of species in the phylomorphospace, corresponded in part, to the phylogenetic pattern, since only one of the groups supported by the phylogeny was recovered, which corresponded to the *frontalis* group. Within the *frontalis*

group, most of the species presented a clear relationship between relatedness and morphology, since the terminal branches were short, close, and did not overlap each other, except in 2 members of the complex. The terminal branches corresponding to the other groups and species of the genus presented different directions of evolutionary change in the reconstructed trajectories and overlapping of branches, indicating the presence of homoplasy (Figs. 11, 13).

The absence of a phylogenetic signal in more than half of the species of the genus may be due to the use of an incomplete phylogenetic hypothesis, which included only 16 of the 21 species recognized for the genus. Our tree only allowed us to recover 4 of the 5 groups supported by previous studies and differed in the relationships between them. In fact, the only group in which a topology partially concordant with other works was recovered was the *frontalis* group, whose members have a clearer morphology relationship. Although to date there are several hypotheses of ancestry-descent in these bark beetles, all of them differ in the composition of the groups and their relative position, because they were estimated with different types and sizes of character matrices. For example, the phylogeny that included the most robust data set (Rad seq), did not include 3 members of the genus, most notably *D. parallellocollis* (Godefroid et al., 2019),

a taxon that obscures the relationships among species, when it is included in the phylogeny (Victor & Zúñiga, 2016). The topology of the latter authors was based on a “total evidence” approach combining morphological and molecular attributes; however, it gave weight to larval characteristics that evolved independently and, therefore, proved homoplasy considering the more recent “resolved” phylogeny of Godefroid et al. (2019).

Other factors obscuring the phylogenetic signal may be the plastic environmental response of species, the emergence of parallel functional adaptations (Ge et al., 2015), as well as considerable differences in the age of common ancestors in phylogeny, as older common ancestors tend not to exhibit a phylogenetic signal (Caumul & Polly, 2005). However, these factors cannot be contrasted until we have a phylogenetic hypothesis of the group that includes all members of the genus and with a greater number of genetic attributes.

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