OXFORD

# An Assessment of Old and New Characters Using Traditional and Geometric Morphometrics for the Identification of *Dendroctonus approximatus* and *Dendroctonus parallelocollis* (Curculionidae: Scolytinae)

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## Abstract

There have been numerous changes in the nomenclature of species of *Dendroctonus*. The case of *Dendroctonus parallelocollis* Chapuis and *Dendroctonus approximatus* Dietz has been particularly noteworthy. These bark beetles colonize pine trees but are not aggressive species. The high morphological similarity, their overlapping geographical distribution, and the limited knowledge of these species have resulted in taxonomic confusion. The aim of the present study was to assess morphological characters reported in the literature and to scrutinize new characters that might be useful for the separation of these species. We evaluated 26 morphological attributes and performed geometric morphometrics analysis of the antenna, pronotum, spermatheca, and seminal rod to test if the variations in the shape of these structures allow the recognition of additional discrete characters to differentiate them. Our results show that five double-state characters of external morphology are useful to identify these species, and the shape of antenna, spermatheca, and seminal rod.

**Key words:** seminal rod shape, geometric morphometry, *Dendroctonus frontalis*

The genus *Dendroctonus* Erichson has been revised several times and consequently the species included has changed with each reviews (Hopkins 1909; Wood 1963, 1982). These reviews were mainly based on external and internal morphological characters (e.g., beetle size and color, and features of the head, pronotum, elytra, and male genitalia), behavioral characteristics (e.g., galleries, position and arrangement of egg niches, aspects of larval mines and feeding, and pupation), and biological attributes (e.g., life cycles, preferential hosts, and distribution range). Subsequent studies, on particular species incorporated attributes such as chromosome numbers, pheromones, cross breeding, molecular markers, and cuticular hydrocarbons. These attributes confirmed some species, described new species or suggested the presence of cryptic species (Lanier and Wood 1969; Lanier 1981; Bentz and Stock 1986; Lanier et al. 1988; Pajares and Lanier 1990; Furniss 1996; Kelley et al. 1999; Ruíz et al. 2009; Sullivan et al. 2012; Armendáriz-Toledano et al. 2014a, 2015). Currently, 20 species are recognized in *Dendroctonus*, one of them with two subspecies (Furniss 2001) of which 18 inhabit the American continent (Wood 1982; Armendáriz-Toledano et al. 2015, 2017) and two are native to Europe and Asia (Grégoire 1988).

Whereas some *Dendroctonus* species have been widely studied because they are major natural disturbing agents of coniferous forests during outbreaks, others such as *Dendroctonus parallelocollis* Chapuis and *Dendroctonus approximatus* Dietz are little known because they are not aggressive species (Six and Bracewell 2015). As currently understood in the most recent review (Wood 1982), these species are well defined. However, due to confusion of types (Wood 1969), most earlier publications have used incorrect identifications (Hopkins 1909, Schedl 1956, Wood 1963). *Dendroctonus parallelocollis* was described in 1869 for specimens from Durango, Mexico, whereas *D. approximatus* was recognized in 1890 for specimens from New Mexico and Colorado. Wood (1969) concluded that all previous usage of these names except for the original descriptions was incorrect. *Dendroctonus approximatus* has been treated as a member of the *Dendroctonus frontalis* complex (Wood 1982).

Since then, a minimal character set has been available to identify both species, including frontal sculpture, pronotum shape, elevation of epistomal process, and the elytral vestiture (Wood 1982). In addition, phylogenetic and chromosomal analyses have confirmed and recognized both taxa as independent and valid species (e.g., Lanier

1981, Kelley and Farrell 1998, Zúñiga et al. 1998, Víctor and Zúñiga 2016). Nevertheless, the broad morphological similarity of both species, the geographical variation of some morphological attributes (e.g., shape of pronotum), and the overlapping of their range distribution (Salinas-Moreno et al. 2004, 2010) frequently lead to erroneous taxonomic identification of both species.

In this study, we assess the consistency of morphological attributes previously used to identify these species (Lanier 1981, Wood 1982, Armendáriz-Toledano and Zúñiga 2017) using traditional morphometrics. In addition, we look for new discrete characters, as well as their usefulness in the identification of both taxa. Further, we performed a geometric morphometric analyis to determinate whether the variation in shape of the antenna, pronotum, seminal rod, and spermatheca is useful for this purpose.

## Materials and Methods

In total, 144 specimens 39 *D. approximatus* and 105 *D. parallelocollis* were analyzed from 12 and 17 geographical sites, respectively (Table 1). Specimens were collected directly from naturally infested pines. Sex of the specimens was determined by the presence of stridulatory apparatus in males (Hopkins 1909, Lyon 1958). The preliminary identification of species was performed using Wood's (1982) key. The removal, dissection, and mounting of the antenna, elytra, seventh tergite, and genitalia were performed following López et al. (2014) and Armendáriz-Toledano et al. (2015).

The external morphology and specific microstructures (antenna, spermatheca, and seminal rod) of each specimen were examined and photographed with a Nikon DXM1200F digital camera on a NIKON SMZ800 stereo microscope. Measurements of quantitative characters of the body were taken with an optical micrometer at 20–63× and those of mounted structures with a optical micrometer at 400× directly from slides. The images of the pronotum of each insect were obtained by placing the insects in dorsal view in a Petri dish with fine sand and 70% alcohol and photographed at 40× with an Olympus C-5060 camera. The seminal rod (distal process pointing up), spermatheca, (cornu oriented down), and antenna (scape pointing down) were always arranged in the same position. These structures were photographed with a Panasonic DMC-FS3 camera with a phase contrast microscope (Carl Zeiss, Germany) at 400×.

### Characters Analyzed

In total, 26 morphological characters from head, antenna, pronotum, elytral declivity, and genitalia were selected, of which 10 were double-state (DB, binary characters that have two states: presence/absence, yes/no, male/female) and 16 quantitative (QC) characters. The coding and description of these characters are listed below from anterior to posterior.

1. Pubescence on head (PH): The surface of the frons in *Dendroctonus* species is covered with pubescence that varies in density and distribution (Hopkins 1909, Wood 1982). *Dendroctonus parallelocollis* has inconspicuous pubescence in this region (Wood 1963, 1982), whereas *D. approximatus* has a rather long and sparse pubescence (Supp Table 1 [online only]; Figs. 1 and 2)

2. Frontal sculpture of males (FS): Males of some species of *Dendroctonus* have numerous and prominent granular tubercles

**Table 1.** Country, reference acronym, locality, coordinates, altitude, and host of *D. parallelocollis* (*par*) and *D. approximatus* (*app*) specimens examined in this study

| Species | Locality | Coordinates | Altitude (m) | Host |
|---|---|---|---|---|
| **Mexico** | | | | |
| *app* | Coahuila, Saltillo, Sierra de Arteaga (CoSA) | 25°25′42.79″, −100°50′34.66″ | 1,750 | *Pinus rudis* |
| *app* | Durango, Guanacevi (DGU) | 25°55′53.89″, −105°57′16.83″ | 2,069 | *Pinus* sp. |
| *app* | Jalisco, Atenquique, El llanito (JAE) | 19°31′56.97″, −103°26′41.01″ | 2,230 | *Pinus teocote* |
| *app* | Jalisco, Mpio Tapalpa, Alpuyeque (JTA) | 19° 59′ 5.87″, −103° 40′ 8.16″ | 2,270 | *Pinus michoacana* |
| *app* | México, Axapusco, Francisco I. Madero (MAF) | 19°46′44.62″, 98°38′19.52″ | 2,870 | *Pinus rudis* |
| *app* | México, Villa Allende, Buenavista 23 (MVB) | 19°28′37.02″, −100°8′8.014″ | 2,846 | *Pinus pseudostrobus* |
| *app* | Querétaro, La Pingüica (QPI) | 20°22′7″, −100°0′45″ | 1,963 | *Pinus* sp. |
| *app* | Querétaro, Pto Oro (QPO) | 21°8′6.67″, −99°37′31″ | 2,351 | *Pinus arizonica* |
| *app* | Oaxaca, Portillo, Cajones (OPC) | 16°14′20.21″, −96°31′21.08″ | 2,182 | *Pinus* sp. |
| *both* | Durango, San Quintin Barrancas (DSQ) | 23°50′44.62″, −104°14′32.8″ | 1,741 | *Pinus* sp. |
| *both* | Jalisco Gómez Farías (JAL) | 19°46′49″, −103°29′30.99″ | 1,521 | *Pinus* sp. |
| *both* | Oaxaca, San Juan Tepeuxila (OST) | 17°44′08″, −96° 49′53.2″ | 2,329 | *Pinus* sp. |
| *par* | Michoacán Zirahuén (MZH) | 19°26′40.09″, −101°43′32.39″ | 2,140 | *Pinus* sp. |
| *par* | Michoacán, Ario, Puerta Pesada (MPP) | 19°12′7.63″, −101°43′43.67″ | 1,974 | *Pinus* sp. |
| *par* | Michoacán, Tingambato, El Ciprés (MTC) | 19°30′33.8″, −101°54′7.3″ | 2,074 | *Pinus* sp. |
| *par* | Michoacán, Uruapan, km 10, Carr Uruapan (MUU) | 19°24′23″, −102°2′35″ | 1,615 | *Pinus pringlei* |
| *par* | Michoacán, Uruapan, San Lorenzo (MUL) | 19°31′32.70″, −102°6′40.53″ | 2,107 | *Pinus* sp. |
| *par* | Michoacán, Zacapu, La Cofradía (MZC) | 19°48′45.04″, −101°47′44.41″ | 2,600 | *Pinus leiophylla* |
| *par* | México, Amatepec, La Goleta (MAG) | 18°40′40.14″, −100°5′58.63″ | 2,200 | *Pinus leiophylla* |
| *par* | México, Atlautla, 3 km NE San Miguel (ASM) | 19°1′36.57″, −98°46′46.8″ | 2,367 | *Pinus* sp. |
| *par* | Tlaxcala, Temetzontla (TTE) | 19°21′1″, −98°17′19″ | 2,571 | *Pinus* sp. |
| *par* | Veracruz, Jalacingo, Tepeyac (VJT) | 19°47′45.09″, −97°18′22.76″ | 1,939 | *Pinus oaxacana* |
| *par* | Oaxaca, Concepción, Cuicatlán (OBL) | 17°51′04,8″, −96°54′56.5″ | 1,810 | *Pinus pringlei* |
| *par* | Querétaro, El Tepozán (QET) | 20°11′9″, −99°59′44″ | 2,383 | *Pinus leiophylla* |
| **Guatemala** | | | | |
| *par* | Guatemala, Zacapa, San Antonio El Chico (GZUAC) | 14°57′ 25.11″, −89°46′53.56″ | 321 | *Pinus* sp. |
| **Honduras** | | | | |
| *par* | Honduras, Potrerillos, Zihuatepeque (HPZI) | 14°35′13.161″, −87°49′48.32″ | 1,075 | *Pinus* sp. |
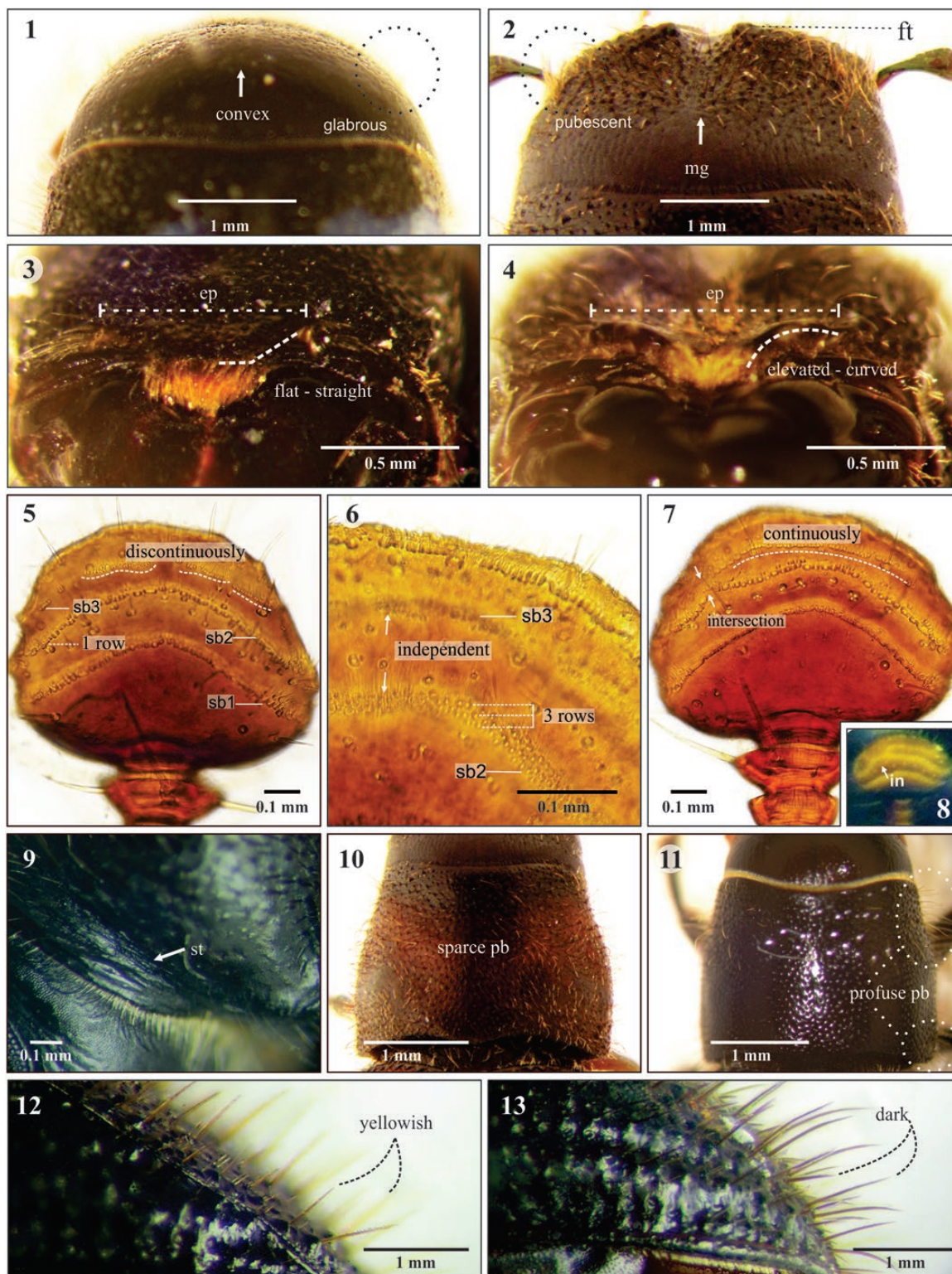
**Figs. 1–13.** Anatomical structures of *D. parallelocollis* (1, 3, 5, 7, 8, 9, 11, and 13) and *D. approximatus* (2, 4, 6, 10, and 13). (1 and 2) male head in dorsal view, (3 and 4) male frons in frontal view, (5–8) anterior side of antennal club, (9) preepisternal area, (10 and 11) pronotum in dorsal view, (12 and 13) color of vesture on elytral declivity. ep, epistomal process; ft, frontal tubercles; in, intersected; mg, median groove; pb, pubescence; sb, sensorial band; st, striae.

on the lateral areas of the frons (Wood 1982, Lanier et al. 1988). The frons in *D. parallelocollis* is deeply punctured with rather abundant, isolated, and scattered granules (Wood 1963, 1982), whereas in *D. approximatus* the frons is coarsely granulate with a pair of prominent lateral tubercles (Dietz 1890; Wood 1963, 1982) (Supp Table 1 [online only]; Figs. 1–4).

3. Head surface (HS). Species of *D. frontalis* complex show a median groove in this region, but not the other species from this genus (Wood 1963, 1982). The head of *D. parallelocollis* is strongly and evenly convex, whereas the *D. approximatus* head displayed a deep median groove (Dietz 1890, Hopkins 1909, Wood 1982) (Supp Table 1 [online only]; Figs. 1 and 2).

4. Shape of epistomal process (SEP): The epistomal process is a structure composed of a pair of lateral elevations (arms) along of the epistomal margin. The relative width of the elevation from the epistomal margin and curvature of the arms show differences that are useful for the identification of *Dendroctonus* species or species groups (Hopkins 1909; Wood 1963, 1982). The epistomal process of *D. parallelocollis* is broad and flat, without elevated arms (Wood 1963, 1982), whereas *D. approximatus* has arms elevated and oblique almost 40° from horizontal (Dietz 1890; Wood 1963, 1982; Supp Table 1 [online only]; Figs. 3 and 4).

5. Distribution of sensillae on third sensorial band of the antennal club (DST): The distribution of sensillae has been taxonomically informative in some *Dendroctonus* species (López et al. 2014, 2018; Armendáriz-Toledano et al. 2017). This character had not been evaluated in *D. parallelocollis* and *D. approximatus* (Supp Table 1 [online only]; Figs. 5 and 7).

6. Organization of sensillae in the sensorial bands on the antennal club (OSB): The organization of sensillae on sensorial bands differs among members of the genus *Dendroctonus* (López et al. 2014, 2018). This character had not been evaluated in the species studied here (Supp Table 1 [online only]; Figs. 5 and 7).

7. Disposition of sensorial bands on antennal club (DSA): Differences in the curvature of the sensorial bands have been recognized in several species of *Dendroctonus* (Hopkins 1909). This character had not been previously evaluated in species studied here (Supp Table 1 [online only]; Figs. 5, 7, and 8).

8. Surface of preepisternal area (SPA): The antero-lateral area of the pronotum is divided into pre-episternal, episternal, and epimeral areas. In some members of the *D. frontalis* complex, the small grooves on the pre-episternal area differentiate some species (Armendáriz-Toledano and Zúñiga 2017). This feature has not been evaluated before in the species studied here (Supp Table 1 [online only]; Fig. 9).

9. Abundance of pronotal vestiture (PV): The vestiture of the pronotum varies in density and distribution. The vestiture in *D. parallelocollis* is generally scanty but more abundant and coarse in the anterolateral region of pronotum, where it becomes longer, and rather coarse. The vestiture on both regions of the pronotum of *D. approximatus* is sparse and long (Wood 1963, 1982; Supp Table 1 [online only]; Figs. 10 and 11).

10. Color of the vestiture on elytral declivity (VCD): This character was considered in the phylogenetic analysis of the genus *Dendroctonus*. All species had yellowish setae except for *D. approximatus* with dark setae (Víctor and Zúñiga 2016); however, the character has not been evaluated geographically in *Dendroctonus* species (Supp Table 1 [online only]; Figs. 12 and 13).

Quantitative characters include the following: (11) width of epistomal process (WEP), (12) width of antennal club (WAC, Fig. 14), (13) length of antennal club (LAC, Fig. 14), (14) eye width (EW), (15) distance between eyes (DBE), (16) head width (HW), (17) head length (HL), (18) pronotum length (PL), (19) pronotum width (PW), (20) elytral length (EL), (21) total length of the body (TLB), (22) nodulus width of the spermatheca (NW, Fig. 16), (23) cornu width of the spermatheca (CW, Fig. 16), (24) distance between nodulus and cornu (DCN, Fig. 16), (25) seminal rod length (SRL, Fig. 17), and (26) seminal rod width (SRW, Fig. 17). Head (WEP, EW, DBE, HW, HL), pronotal (PL, PW), elytral (EL), and TLB attributes were measured according to Armendáriz-Toledano et al. (2017; Figs. 1, 3, and 4).

## Traditional Morphometric Analysis

### Univariate Statistics

Because the statistical tests that are used assume a normal distribution of the data, the homogeneity of variances and normal distribution for continuous quantitative characters were evaluated with the *F*-test and Shapiro–Wilk test, respectively (Zar 2010). Differences between quantitative characters were estimated by comparing mean values with the Student's *t*-test (Zar 2010) and a Guillaumin profile (Guillaumin 1972). Statistical differences between qualitative characters of both species were estimated using the Mann–Whitney *U*-test (Zar 2010).

### Multivariate Statistics

Pairwise similarities among females, males, and both sexes of each species were calculated using Gower index (Gower 1971). To explore whether the variation pattern in a multidimensional space segregates specimens according to their species, we performed separated principal coordinates analyses (PCoAs) using the pairwise similarity matrices of females, males, and females–males of both species. Likewise, to test whether specimens of one species are more similar to each other than with any member of another species, cluster analyses with the unweighted pair-group method with arithmetic average were carried out using the corresponding similarity matrices (Legendre and Legendre 1998).

The characters included in each analysis were different due to the differences in numbers of sex-specific characters examined. The comparison between females of both species was performed on 50 *D. parallelocollis* and 21 *D. approximatus* using 23 characters (PH, HS, SEP, DST, OSB, DSA, SPA, PV, VCD, WEP, WAC, LAC, EW, DBE, HW, HL, PL, PW, EL, TLB, NW, CW, and DCN); the comparison between males on 55 *D. parallelocollis* and 18 *D. approximatus* using 23 characters (PH, FS, HS, SEP, DST, OSB, DSA, SPA, PV, VCD, WEP, WAC, LAC, EW, DBE, HW, HL, PL, PW, EL, TLB, SRL, and SRW) and the comparison of both sexes were performed on 105 *D. parallelocollis* and 39 *D. approximatus* using 20 characters (PH, HS, SEP, DST, OSB, DSA SPA, PV, VCD, WEP, WAC, LAC, EW, DBE, HW, HL, PL, PW, EL, and TBL).

## Geometric Morphometrics

Patterns of two-dimensional shape variation of antennae, pronotum, seminal rod, and spermatheca of both bark beetles were quantified using appropriate landmarks (lm) and semilandmarks (sml) (Figs. 14–17; Bookstein 1991, Zelditch et al. 2004). The antenna club shape was defined by eight type I lm, and 26 sml (Fig. 14), the pronotum with two type I lm, two type II lm, and 38 sml (Fig. 15), the spermatheca with two type II lm and 24 sml (Fig. 16), and the seminal rod with five type II lm and 16 sml (Fig. 17).

Semilandmarks were defined using fans (on spermatheca) and combs (on pronotum, seminal rod, and antenna) on photographs of these structures with MakeFan6 of Integrated Morphometrics Package (IMP; Sheets 2003). Semilandmarks were specific sites located in every intersection between outline of each structure and the projected lines from reference points (putative homologous points) easily recognized in all images. In the antenna, the intersection points between the second sensorial band and their respective lateral margins were used to project nine perpendicular and equidistant lines (Fig. 14). Two combs of 12 parallel lines each were located in the pronotum; the first (perpendicular) located from the points of maximum curvature of posterior sides of pronotum and second (longitudinal) from points of maximum curvature of anterior and posterior median curvatures (Fig. 15). Points of maximum curvature in spermatheca, both nodulus and cornu, were
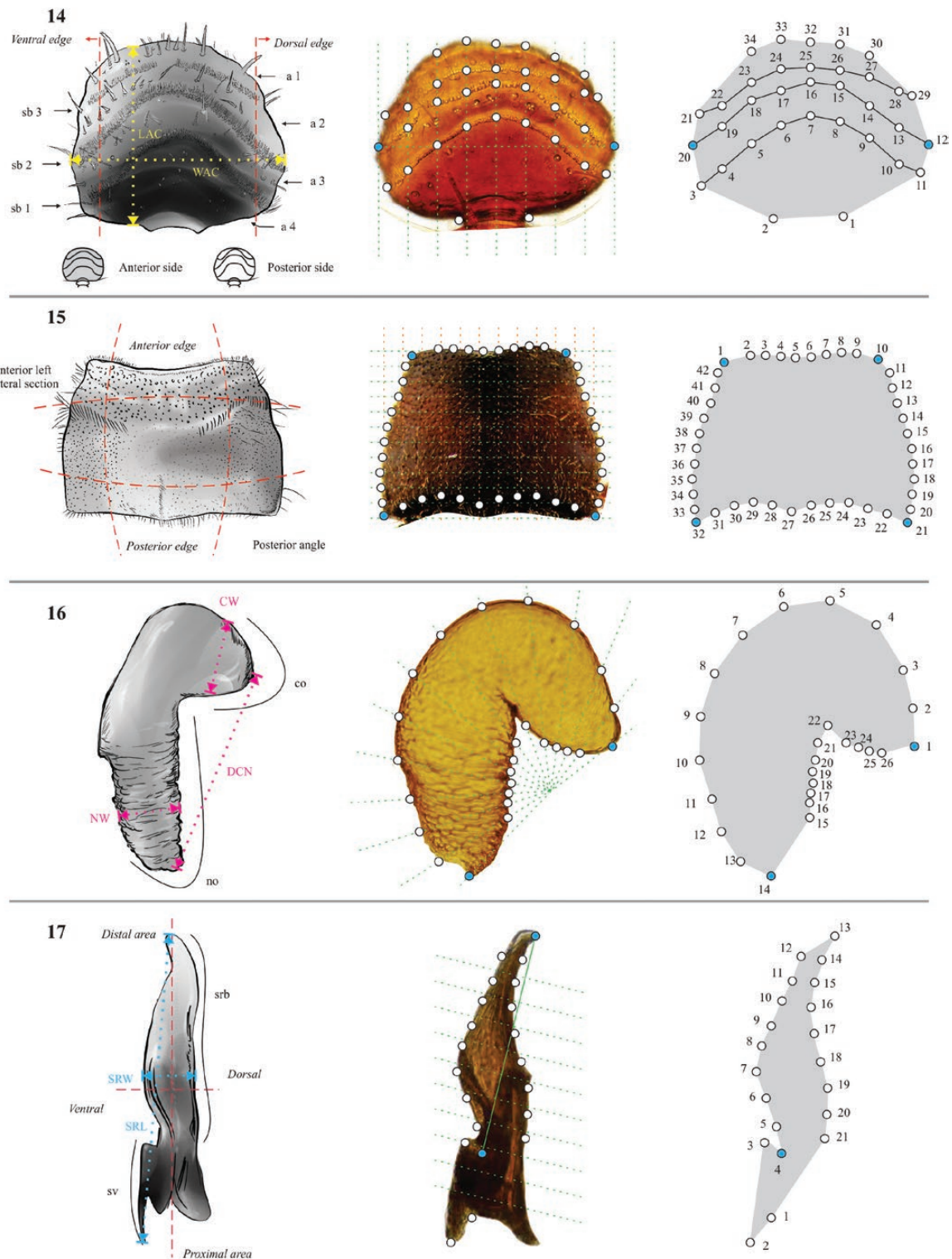
**Figs. 14–17.** Anatomy, measures, position of landmarks with respective combs or fans, and resulting configuration of the structures evaluated, that apply to both species. (14) Antenna, (15) pronotum, (16) spermatheca, and (17) seminal rod. a, antenomere; co, cornu; no, nodulus; sb, sensorial band; srb, seminal rod body; and sv, seminal valve.

used to project a fan of 25 radiating lines at equal angular displacements (Fig. 16). In seminal rod, 10 perpendicular lines were projected from the point of maximum curvature of distal process and the point of maximum curvature between the seminal valve and seminal rod body (Fig. 17).

Landmarks and semilandmarks were digitalized as two-dimensional coordinates with tpsDig 1.40 software (Rohlf 2004) and together constituted the shape configurations. A generalized procrustes analysis (Zelditch et al. 2004) was performed to remove scale effects, position, and orientation with CoordGen6 program of IMP (Sheets

2003). To minimize the tangential variation of curvatures in the shape configurations produced by the use of sml, the minimum Procrustes distance criterion (Perez et al. 2006) was used in all morphological structures, allowing an optimal superimposition with SemiLand6 of IMP (Sheets 2003). These coordinates were transformed to new variables (relative warps, RW) by means of relative warps analysis (Zelditch et al. 2004), which quantify highest variation of shape. Shape variation of each structure was analyzed with the first five RWs and shape changes were visualized by mean of deformation grids obtained with Thin-Plate Spline technique. Patterns of shape variation between species were visualized by means of scatter plots between RW1 and RW2 with the help of their respective deformation grid diagrams.

A multivariate analysis of variance (MANOVA) with the first five RWs was performed for each morphological structure. In particular, for pronotum shape analysis, a canonical variate analysis (CVA, Legendre and Legendre 1998) was performed with these RWs using species and sex as a priori groups. The resulting groups were contrasted with a MANOVA test and its respective pairwise Hotelling's *T*-squared comparisons.

## Results

### Analysis of Characters

Five double-state characters showed differences between *D. approximatus* and *D. parallelocollis*: FS (Figs. 1 and 2), HS (Figs. 1 and 2), SEP (Figs. 3 and 4), OSB (Figs. 5 and 6), and DSA (Figs. 5 and 7). Males of *D. parallelocollis* do not have frontal tubercles (Fig. 1), and both sexes have a uniformly convex frons (Fig. 1). The lateral arms of their epistomal process are flat and straight (Fig. 3), and the antennal club has only one row of sensillae on each sensorial band (Fig. 6). In some specimens, the sensorial bands intersect at some point on the club (Fig. 7). Males of *D. approximatus* have prominent frontal tubercles (Fig. 2), and in both sexes, the frons has a median groove from the upper level of the eyes to the epistomal area (Fig. 2). The lateral arms of their epistomal process are strongly elevated and curved (Fig. 4); the antennal club has three rows of sensillae in the first and second sensorial bands (Fig. 6); and all sensorial bands are distributed independently, i.e., they do not intersect in anterior surface (Fig. 5).

Student's *t*-test showed differences in 12 quantitative continuous characters between these species (Supp Table 2 [online only]): WEP, WAC, LAC, EW, DBE, HW, PL, EL, TLB, NW, SRL, and SRW. The characters WEP, WAC, LAC, EW, HW, NW, SRL, and SRW had higher mean values in *D. approximatus* than in *D. parallelocollis*, whereas DBE, PL, EL, and TLB values were higher in the second species than in the first (Supp Table 1 [online only], Fig. 18). The Guillaumin profile showed that the most different attributes were WEP, LAC, NW, and SRW (Fig. 18); however, despite these differences, their characters were overlapping in their maximum and minimum values between species.

### Multivariate Analysis

The first three principal coordinates of PCoAs of each morphological comparisons explained >59% of variation: males–females (PCo1$_{\male\female}$ = 44.4%, PCo2$_{\male\female}$ = 8.1%; PCo3$_{\male\female}$=7.1%; Fig. 19), females (PCo1$_{\female}$ = 48.0%; PCo2$_{\female}$= 12.0%; PCo3$_{\female}$= 6.6%; Fig. 20), and males (PCo1$_{\male}$ = 44.14%; PCo2$_{\male}$= 11.83%; PCo3$_{\male}$=7.42%; Fig. 21). Scatterplots of the first two principal coordinates (PCo1 vs PCo2) of these comparisons showed the special segregation of discrete phenotype groups according to each species (Figs. 19–21). Likewise, dendrograms showed clustering of *D. parallelocollis* specimens separated of those of *D. approximatus*. However, in the case of female–male and female comparisons, the specimens of *D. approximatus* were separated in two clusters, one of them more similar with respect to the cluster of *D. parallelocollis* (Figs. 19–21).

### Geometric Morphometrics

**Antenna**

The superimposition of antenna configurations of *D. approximatus* (13 ♀ and 11 ♂) and *D. parallelocollis* (33 ♀ and 34 ♂) specimens showed that shape variation is located on the sensorial bands and the relative length of the antennal club. The first two RW explained 62.7% of total variation (RW1 = 35.07%; RW2 = 21.37%). The respective two-dimensional scatterplot of these RWs revealed two discrete groups corresponding to each species (Fig. 22). The deformations in the components RW1 and RW2 were related with the elongation of antennal club and the curvature of sensorial bands (Fig. 22). The MANOVA supported statistically significant differences in antennal shape between species ($\lambda_{Wilk's}$ = 0.19; $F$ = 42.42; $P \geq 0.001$).

**Pronotum**

The superimposition of pronotum configurations of *D. approximatus* (23 ♀ and 21 ♂) and *D. parallelocollis* (29 ♀ and 25 ♂) specimens showed that the sites with a high variation are the lateral profiles. The
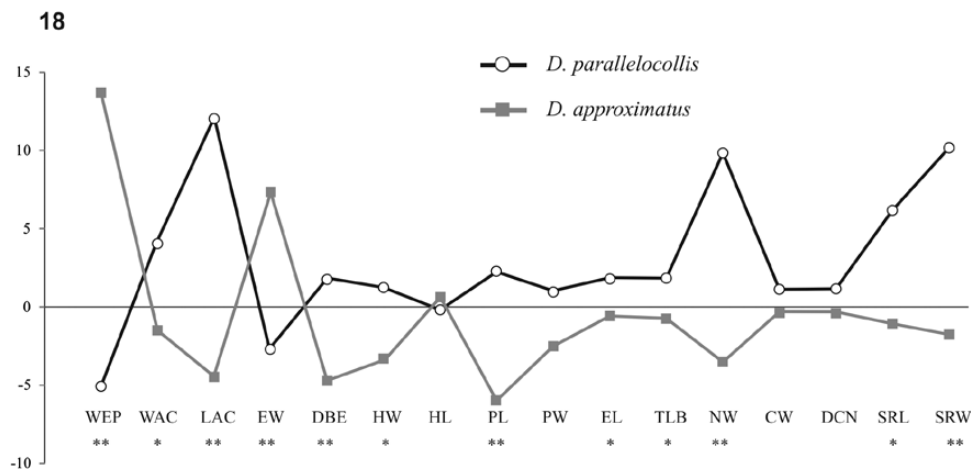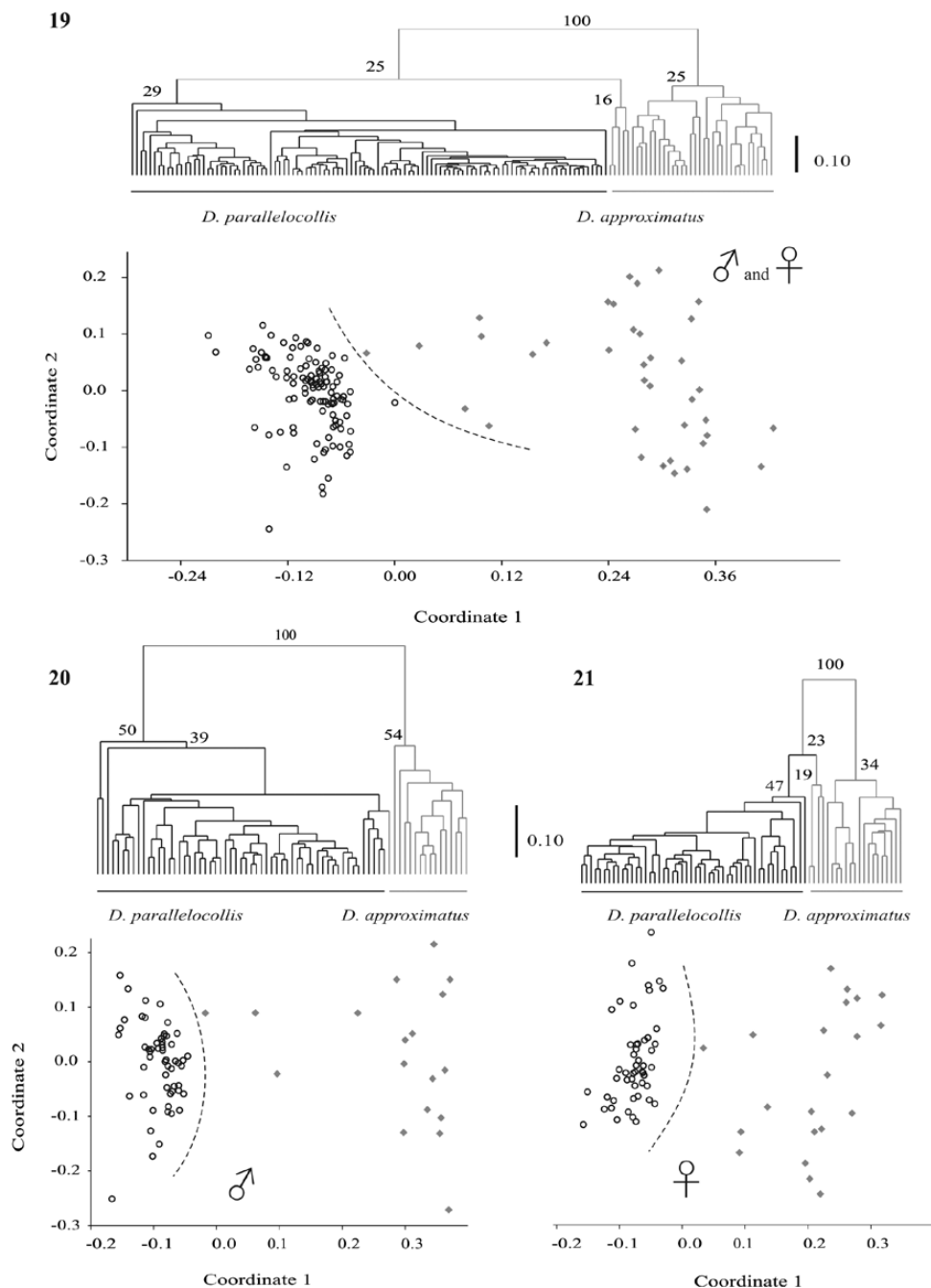


**Fig. 18.** Guillaumin profile based on quantitative continuous characters. *t*-test: * $P \leq 0.05$; ** $P \leq 0.001$. For each variable the points correspond to standarized differences between species (circle for *D. parallelocollis* and square are represented to *D. approximatus*).

**Figs. 19–21.** Principal coordinate analysis and dendograms from cluster analysis of morphological characters of *D. parallelocollis* and *D. approximatus*. (19) Males and females combined. (20) Males. (21) Females. Bootstrap values after 1,000 pseudoreplicates are indicated at the nodes. The scale of the cluster of male and cluster of female is shared (downward).

first two components of the relative warp analysis explained 97.02% of variation (RW1 = 81.55%; RW2 = 15.87%). Two-dimensional scatterplot of these RWs showed the segregation of four overlapped groups corresponding to species and sexes (Fig. 23). Deformations in the two first components RW1 and RW2 were related to the variation in the curvature of lateral sides, which can be strongly curved or straight (Fig. 23). The MANOVA with its respective post hoc pairwise comparisons using Hotelling's test showed statistically significant differences in pronotum shape ($\lambda_{Wilk's}$ = 0.25; $F$ = 7.54; $P \geq$ 0.001) between sexes within each species and between species ($P \leq$ 0.05). The CVA from first five relative warps among sexes and the species (scatter plot of CV1 = 67.76% vs CV3 = 7.35%) recovered

the specimens clustered in four discrete groups corresponding to sexes and species (Fig. 23).

**Spermatheca**

The superimposition of spermatheca configurations of *D. approximatus* and *D. parallelocollis* females showed that the sites with high variation are the curvature of spermatheca and length–width of nodulus and cornu. The first two components of the RWs explained 61.7% of variation (RW1 = 45.02%; RW2 = 21.72%). Two-dimensional scatterplots of these RWs segregated two discrete groups corresponding to each species (Fig. 24). The RW1 deformation was associated with the curvature of spermatheca and RW2

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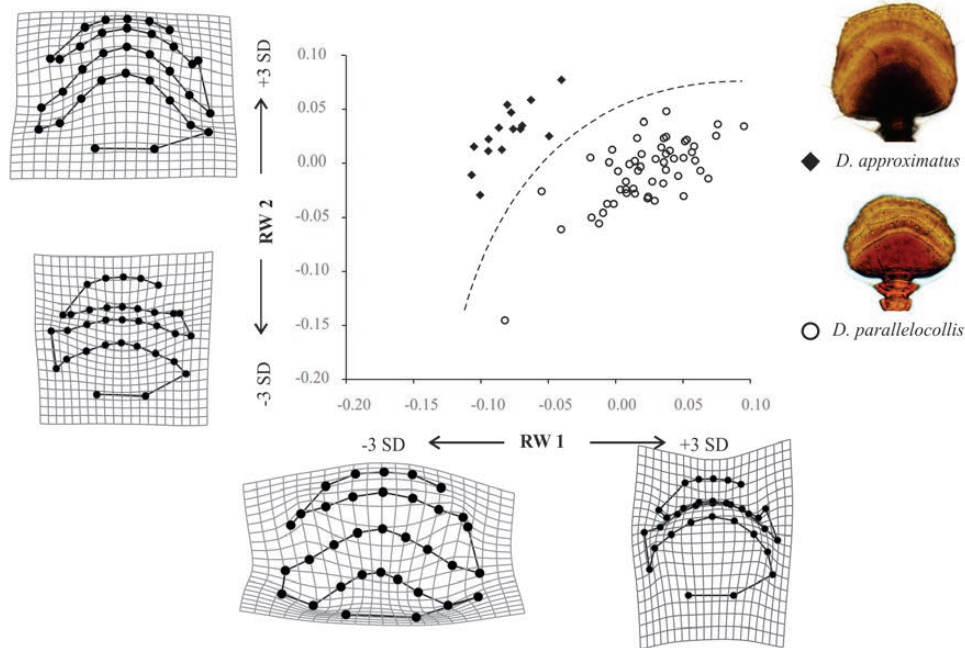


23
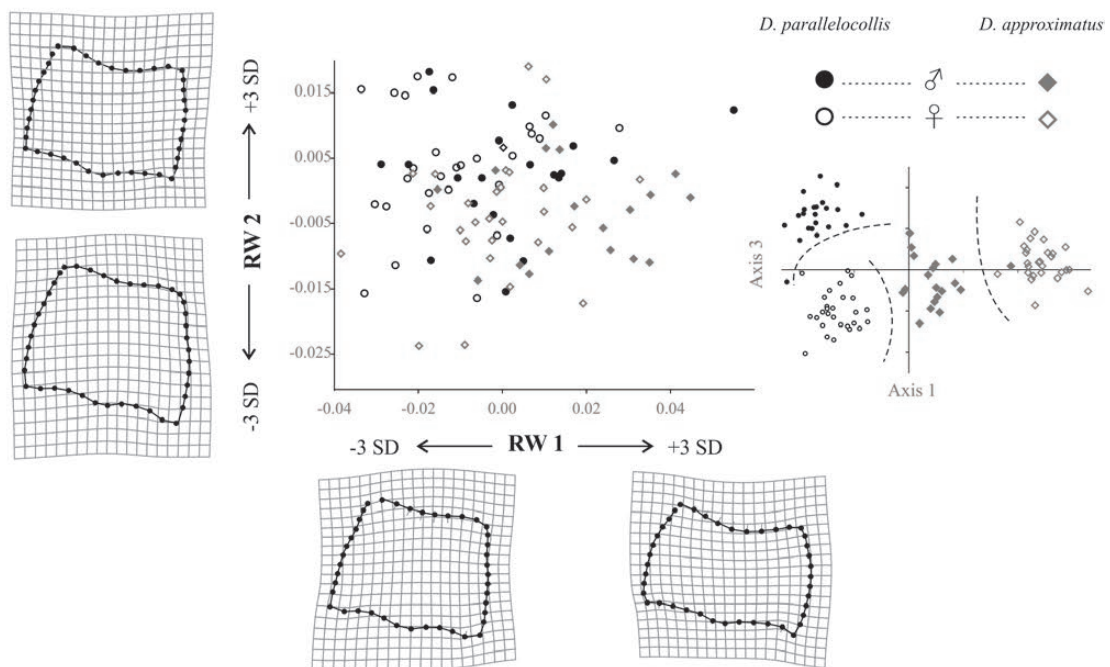


**Figs. 22–23.** Scatter plots between the first and second relative warps with its respective deformation grids ±3 SD, corresponding to shape analysis of antennal club (22) and pronotum in dorsal view (23). A scatter plot between the first and third axis obtained from CVA using 10 first relative warps is shown to the right of the scatter plot of pronotum (RW1 versus RW2).

deformation with the width of the entire spermatheca (Fig. 24). The MANOVA showed statistically significant differences in spermatheca shape between the species ($\lambda_{\text{Wilk's}}$ = 0.17; $F$ = 30.07; $P \leq 0.001$).
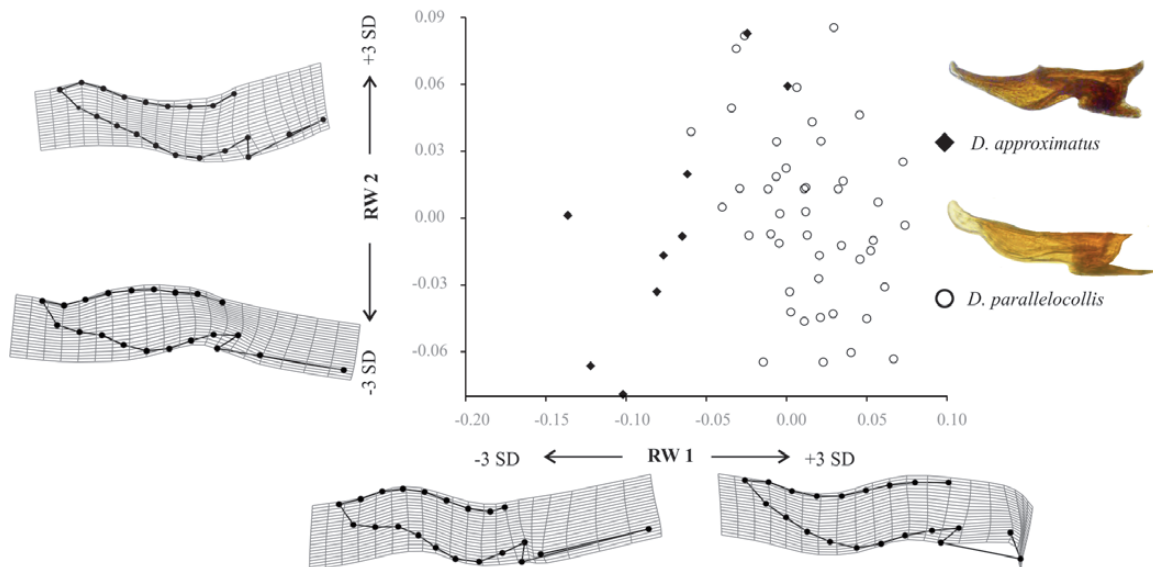
**Seminal Rod**
The superimposition of seminal rod configurations of *D. approximatus* and *D. parallelocollis* males showed that sites with

concentrated variation were the seminal rod body and seminal valve. The first two relative warps explained 57.8% of the variation (RW1 = 34.03%; RW2 = 23.83%). The two-dimensional scatterplot between RWs separated specimens according to species (Fig. 25). The MANOVA supported statistically significant differences in seminal rod shape between species ($\lambda_{\text{Wilk's}}$ = 0.38; $F$ = 10.52; $P \leq 0.001$).
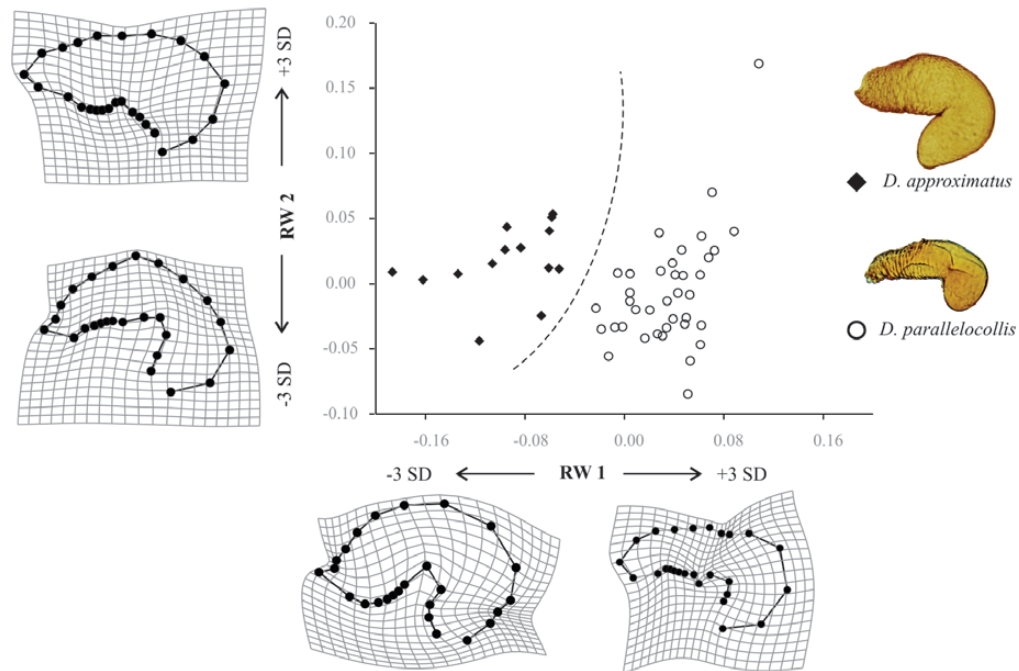
**Figs. 24–25.** Scatter plots of the first and second relative warps with respective deformations grids at ±3 standard deviation (SD). (24) Spermatheca. (25) Seminal rod.

## Discussion

### Analysis of Characters

Our results support that 5 of the 10 double-state characters of external morphology are useful in identifying these species (FS, HS, SEP, OSB, and DSA). OSB and DSA are noteworthy because they are unamiguous characters, which have not been previously reported. Antennal characters have also been reported as diagnostic in other sibling species of the genus (e.g., *Dendroctonus mexicanus* Hopkins–*D. vitei* Wood, and *D. rhizophagus* Thomas and Bright–*D. valens* LeConte) (López et al. 2014, Armendáriz-Toledano et al. 2017).

Our results indicate that characters FS and HS, previously reported in other studies to identify these species (Wood 1982, Lanier et al. 1988, Armendáriz-Toledano and Zúñiga 2017), are useful for discriminating males of *D. approximatus* from *D. parallelocollis*. The presence of prominent frontal tubercles in males is not exclusive of *D. approximatus*, because this character is shared with other members of the *D. frontalis* complex species (Víctor and Zúñiga 2016, Armendáriz-Toledano and Zúñiga 2017). However, the tubercles and median groove upper level of eyes to epistomal area are useful for distinguishing *D. approximatus* from *D. parallelocollis*.
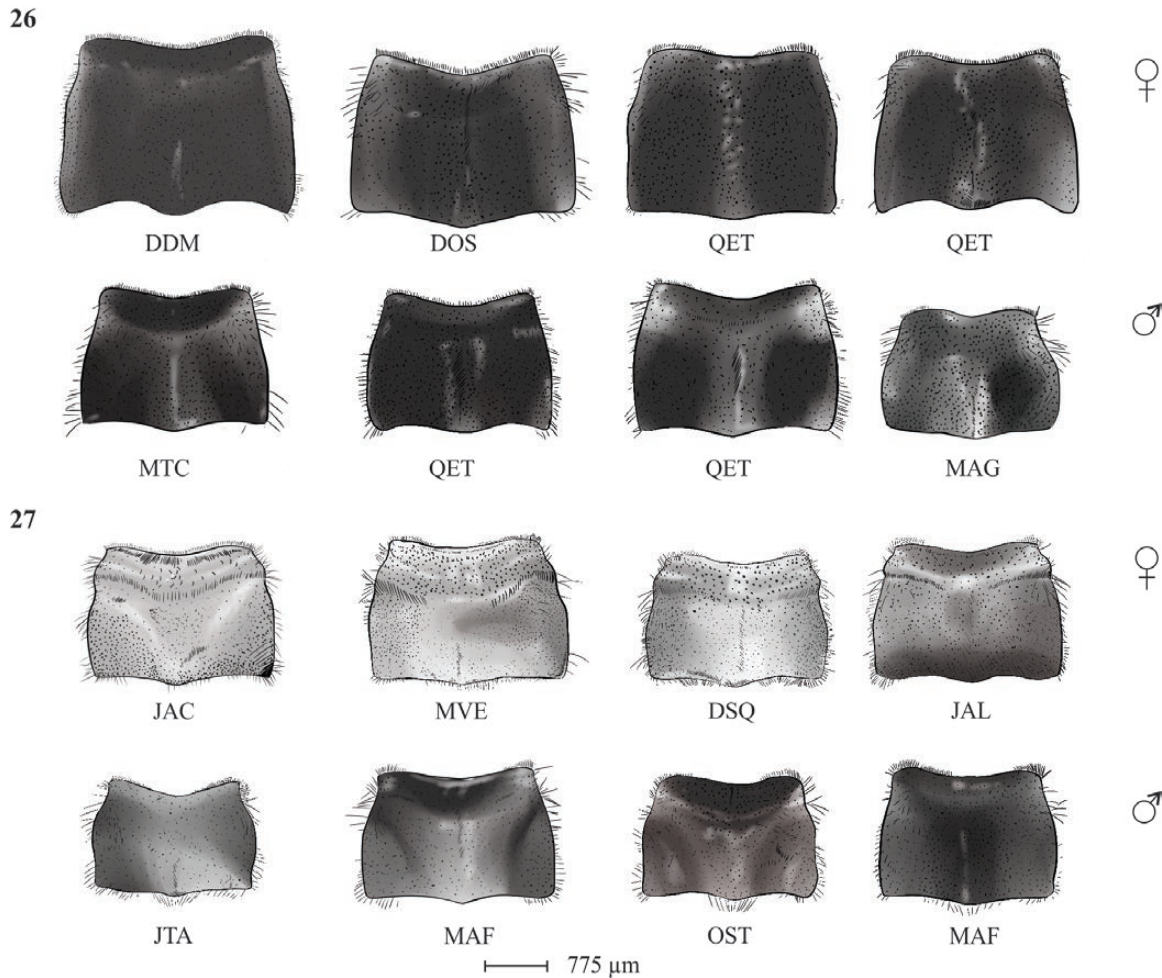
**Figs. 26–27.** Variation in profiles of dorsal pronotum from different localities. (26) *Dendroctonus parallelocollis.* (27) *Dendroctonus approximatus.* Acronyms for localities below images are from Table 1.

Whereas the amplitude of epistomal process (WEP) and the elevation of margins (SEP) have been recognized and proposed as diagnostic characters to identify *D. parallelocollis* (Wood 1982), our results show that only the elevation of margins is useful for differentiating these species , because the WEP overlaps frequently between specimens of both species.

Other characters that were considered nondiagnostic, such as OSB, SPA, PV, and VCD, showed significant differences between species; however, excluding OSB, they are not recommended for the discrimination of these species, because character states overlap between them. OSB can be partially used to identify specimens of *D. parallelocollis*, because about 43% specimens analyzed sensorial bands intersect at some point on the club (Supp Table 1 [online only], Fig. 7). This is a novel character absent in other species of the genus (Payne et al. 1973, Dickens and Payne 1978, Chen et al. 2010, López et al. 2014, Armendáriz-Toledano et al. 2017).

These bark beetles also show marked differences in 12 quantitative continuous characters. *Dendroctonus approximatus* has WAC, LAC, HW, EW, WEP, and SRL, SRW, and wider female genitalia (NW) than *D. parallelocollis* has. Despite this, the latter species is larger in body size than *D. approximatus* (PL, EL). These quantitative measures of almost all these characters have been successfully used in the identification of other *Dendroctonus* species, such as *D. ponderosae* Hopkins and *D. jeffreyi* Hopkins (Lanier and Wood 1968), *D. mesoamericanus* Armendáriz-Toledano and Sullivan and *D. frontalis*

Zimmermann (Armendáriz-Toledano et al. 2014a), *D. mexicanus* Hopkins and *D. vitei* Wood (Armendáriz-Toledano et al. 2017), and other members of *D. frontalis* complex (Lanier et al. 1988).

## Multivariate Analysis

Multivariate analyses of the quantitative continuous and discrete characters (PCoA) show that females, males, and females–males of these bark beetles present strongly differentiated phenotypes (Figs. 19–21). These analyses also show that the males and females of *D. approximatus* display much greater phenotypic variation than the sexes of *D. parallelocollis* (Figs. 19–21), which suggest phenotypic limitations operate less severely in *D. approximatus* than those present in *D. parallelocollis*. One of these factors may be the presence of sibling or phylogenetically close species in the same habitat. For instance, it is very common to find *D. parallelocollis* and *D. valens* in a Mexican forest in the same tree, but it not so for *D. approximatus* and other species of the *D. frontalis* complex.

## Geometric Morphometrics

Quantitative analyses of the shape of the antennal club, pronotum, spermatheca, and seminal rod have not been performed previously in these bark beetles. However, the previous geometric morphometric analyses have shown that both antennal club (e.g., *D. valens* vs *D. rhizophagus*) and seminal rod shape (e.g., *D. frontalis* complex
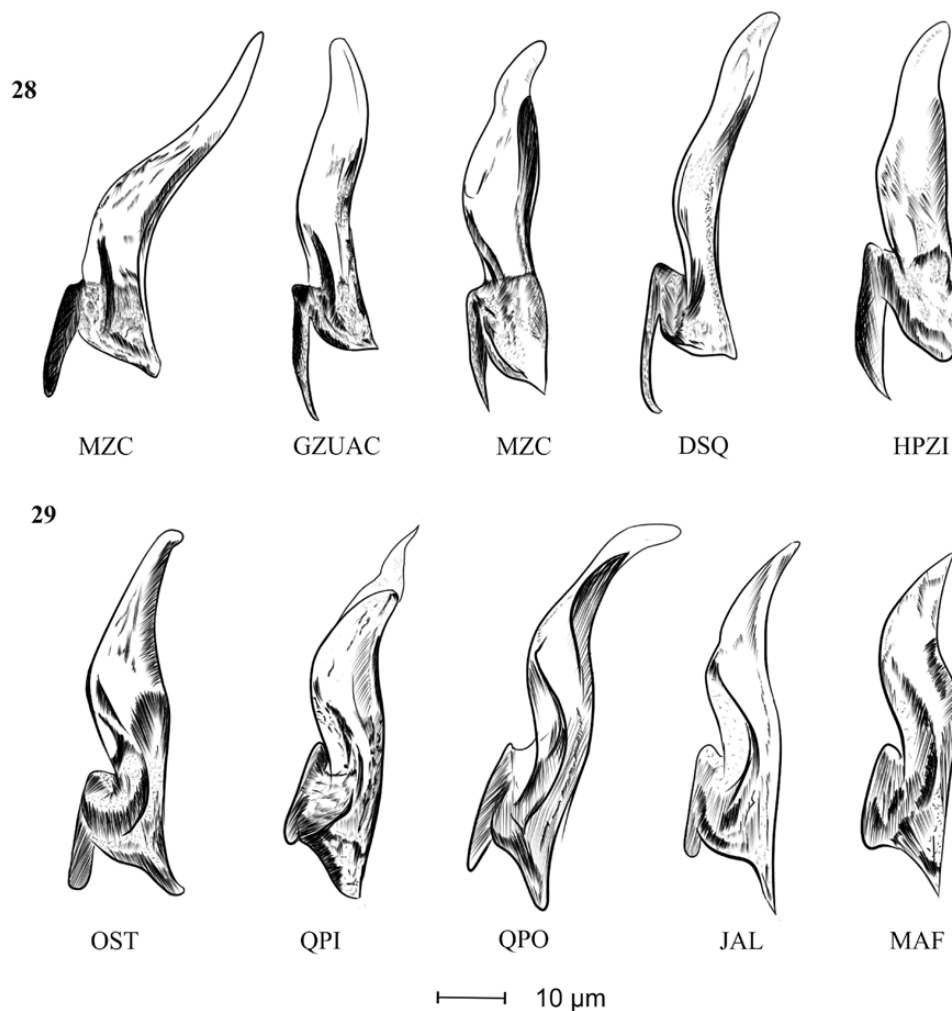
**Figs. 28–29.** Variations in lateral view of seminal rod from different localities. Acronyms for localities below images are from Table 1. (28) *Dendroctonus parallelocollis*. (29) *Dendroctonus approximatus*.

species) differed significantly between sibling species despite their wide range distribution (Armendáriz-Toledano 2014b, López et al. 2014, Valerio-Mendoza et al. 2017), which supports the utility of these characters for identification.

Our findings of the geometric morphometric analysis indicate that the shape of the structures differ among species. The lack of overlap in the shape configurations of the antennal club (Fig. 22), spermatheca (Fig. 24), and seminal rod (Fig. 25) of both species confirms that they constitute a robust diagnostic character useful in their identification. The shape pattern of *D. approximatus*' antennal club is quadrangular with sensorial bands almost equally distributed, whereas the *D. parallelocollis*' antennal club dorso-ventrally elongates with sensorial bands very closely spaced. The spermatheca shape in *D. approximatus* is wide and with a pronounced curvature between nodulus and cornu, nodulus and cornu oblique, and length of the cornu equal or more than a half of nodulus whereas, *D. parallelocollis*' spermatheca is narrow with a poorly developed curvature between nodulus and cornu, the nodulus and cornu perpendicular, and the length of the cornu shorter than of nodulus, approximately a third of nodulus. Lastly, the seminal rod of *D. approximatus* is slightly curved or straight in its distal-ventral edge with a short seminal valve, whereas seminal rod *D. parallelocollis* is sinuous in its distal-ventral edge with a long prolongation of the seminal valve.

With respect to the pronotum, our findings show that the shape variation is complex between the sexes and the species. The scatterplot between RW1 vs RW2 do not segregate discrete groups based on their shape configurations according to their sex and species due to high intraspecific variation (Fig. 23). However, since the first five relative warps are included in this analysis, the species and sexes are different statistically and segregated as discrete groups (Fig. 23). Based on the inclusion of more deformation components, our results indicate that *D. parallelocollis*' males have the basal lateral margins of pronotum slightly curved to almost parallel, whereas the margins in females are parallel from the median half to the anterior region and the anterior third is slightly narrower than posterior third (Fig. 26). *Dendroctonus approximatus*' males have shapes similar to those of both males and females of *D. parallelocollis* and the pronotum of females of that species is shorter than that of *D. parallelocollis* due to the presence of mycangium (Fig. 27), an attribute absent in latter species. The etymology of *D. parallelocollis*, as have been referred by some authors (Chapuis 1869; Wood 1963, 1982), assumes that this species has lateral margins less curved and almost parallel; however, our results indicate that this assertion is only partly true, because only the females have this pattern (Fig. 26). In addition, since *D. approximatus*' males do not have mycangium (Dietz 1890; Hopkins 1909; Wood 1963, 1982), they can be confused with males and females of *D. parallelocollis*, because the variation in shape of this structure overlaps in these species.

## Geographical Variation

Among specimens of *D. parallelocollis* from different locations, we found some differences in shape of antenna, pronotum, and seminal rod. In particular, the antenna in southern locations (Oaxaca and Central America) show pronounced variation in the sensorial bands, because specimens from these regions present a third sensorial band in discontinuous form and in some cases, these intersect at some point. Specimens from the northern locations (states of Coahuila and Durango) do not display these features. We also found marked differences in the pronotum because in specimens from southern locations, the lateral edges are more arched, and in some cases, more similar to *D. approximatus* (Fig. 26). Lastly, we found that the seminal rod from the specimens from the northern locations, has the distal-ventral edge of this structure almost straight as compared with those from the central locations (Jalisco, Michoacán, Queretaro, Tlaxcala, Veracruz, and México states). These present a seminal rod body elongate with a bigger seminal valve and with southern localities and Guatemala specimens, which have the distal-ventral edge with two maximum points of curvature (Fig. 28).

These morphological differences among geographical regions suggest the presence of a cryptic species within *D. parallelocollis*. In fact, a previous phylogenetic analysis found that the phylogenetic position of this species is ambiguous (Víctor and Zúñiga 2016) suggesting that other species might have not been discovered. Furthermore, we have observed behavioral differences in the colonization pattern of *D. paralelocollis* in the northern and southern populations. Specimens from different localities in the Trans-Mexican Volcanic Belt arrive frequently to galleries built previously by *D. valens* but the specimens from other localities in Oaxaca and Guatemala colonize both old and young trees. In the latter ones, *D. parallelocollis* is able to build galleries in the roots. Taxonomic integrative analyses need to be conducted and a more intensive collective effort must be undertaken to evaluate the existence of possible cryptic species in *D. parallelocollis*.

In brief, this study defines a set of old and new features that facilitate the discrimination of *D. approximatus* and *D. parallelocollis*. These features are consistent and robust thorough of the distribution range of these species and provide a more precise framework of intra- and interspecific morphological variation. In the case of *D. parallelocollis*, the inclusion of molecular markers should establish the basis to test the presence or not of a new taxon within this species.

## Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

## Acknowledgments

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