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A morphometric study of the genitalia of *Phyllotreta dilatata*, *P. flexuosa*, *P. obchripes* and *P. tetrastigma*

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Abstract

In the genus *Phyllotreta* genitalia have been successfully used for the separation of closely related species. Between the species *Phyllotreta dilatata* Thomson, 1866, *P. flexuosa* (Illiger, 1794) and *P. tetrastigma* (Comolli, 1837) however, neither in aedeagus nor in spermatheca, morphological differences have been found. This study looks for biometric differences between their genitalia, and those of a fourth closely related species, *P. ochripes* (Curtis, 1837). We took three measures on the genitalia of 88 males and 104 females for the four species.
Our results show that the measures of the aedeagus allow separation of all species. For the spermatheca *P. tetrastigma* can be separated from all others and *P. flexuosa* can be separated from *P. ochripes*. Measurements taken on the genitalia thus offer additional information for the identification of the four species studied, but the aedeagus is more suited for this purpose than the spermatheca.

Keywords: morphology – genitalia – *Phyllotreta* – Chrysomelidae

Samenvatting

In het genus *Phyllotreta* worden genitalia met succes gebruikt ter onderscheiding van nauwverwante soorten. Tussen de soorten *Phyllotreta dilatata* Thomson, 1866, *P. flexuosa* (Illiger, 1794) en *P. tetrastigma* (Comolli, 1837) werden tot nu toe nog geen morfologische verschillen in de aedeagus of de spermatheca gevonden. Hier bestuderen we biometerische verschillen tussen de genitalia van deze soorten en een vierde nauwverwante soort *P. ochripes* (Curtis, 1837). We namen drie maten op de genitalia van 88 mannetjes en 104 vrouwtjes voor de vier soorten.
Onze resultaten tonen aan dat de maten van de aedeagus toelaten alle soorten te scheiden. Voor de spermatheca kan *P. tetrastigma* van de andere soorten gescheiden worden, en *P. flexuosa* van *P. ochripes*. Maten van de genitalia geven dus bijkomende informatie voor de identificatie van de vier bestudeerde soorten, maar de aedeagus is meer geschikt voor dit doel dan de spermatheca.

Trefwoorden: morfologie – genitalia – *Phyllotreta* – Chrysomelidae

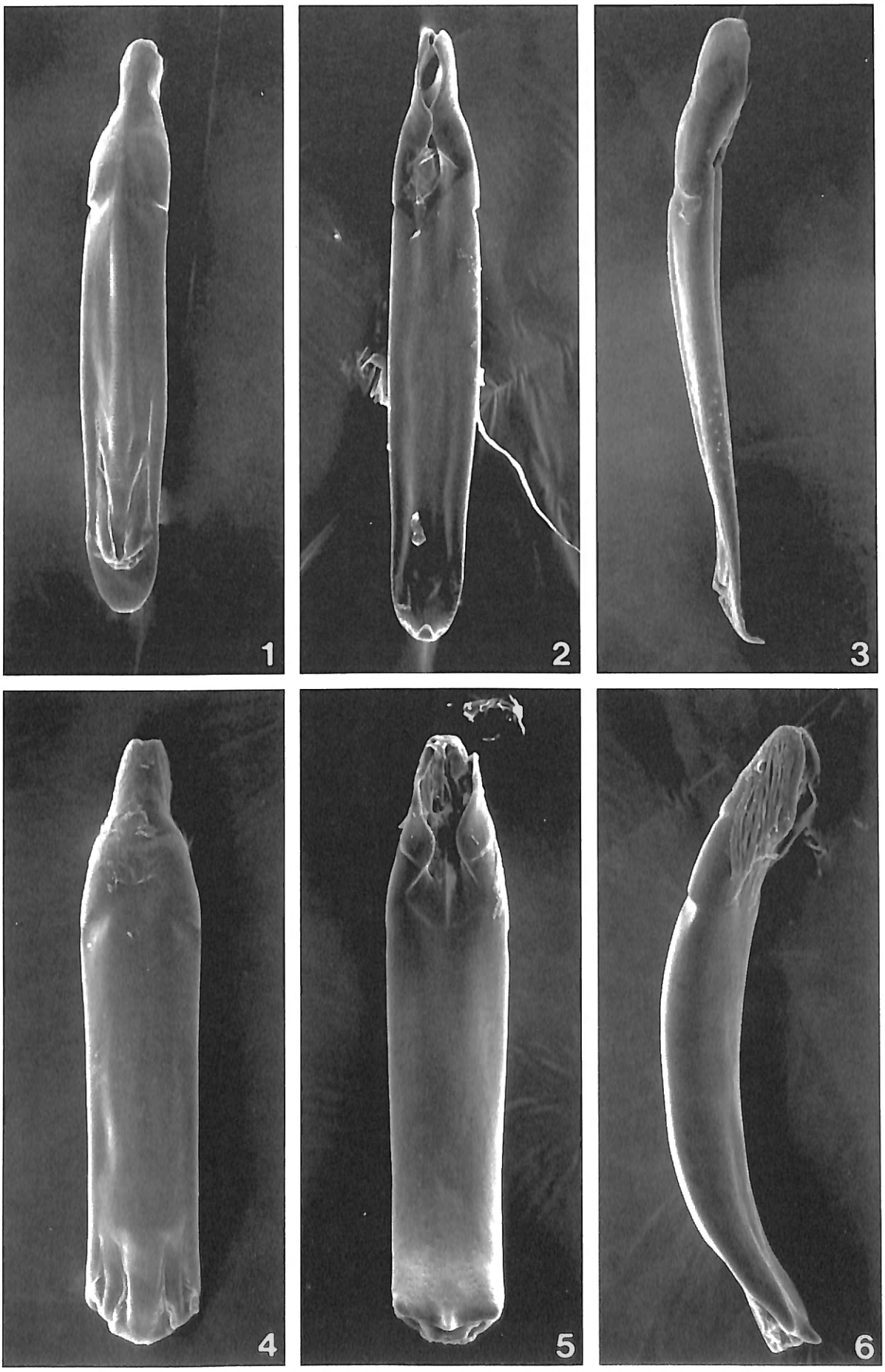
Introduction

In many sibling Coleoptera species, characters provided by the male and female genitalia enable a correct identification. This is especially the case in Alticinae, containing many groups of morphologically very similar species. Although earlier authors only used the male aedeagus for systematic purposes (e.g. HEIKERTINGER, 1941; MOHR, 1966), more recently several authors demonstrated that morphological differences in the female spermatheca also are informative for the discrimination of species (e.g. DÖBERL, 1991, 1994; DOGUET, 1984, 1995) .

In the genus *Phyllotreta* genitalia have been successfully used for the separation of closely related species (e.g. HEIKERTINGER, 1941; MOHR, 1966; SMITH, 1985; DÖBERL, 1983, 1987; DOGUET, 1984, 1995; KONSTANTINOV & LOPATIN, 1992). Between the species *Phyllotreta dilatata* Thomson, 1866, *P. flexuosa* (ILLIGER, 1794) and *P. tetrastigma* (COMOLLI, 1837) however, neither in aedeagus nor in spermatheca, morphological differences have been found and species are mainly diagnosed using elytral colour patterns (DOGUET, 1986, 1995). In these species the aedeagus is slender (figs. 1-2-3), the apex rounded acute (fig. 7) and at the dorsal side a washboard is present (fig. 9). The spermatheca (fig. 11) is elongate pear-shaped, tapering towards the pump, the collar is not developed. In a fourth closely related species, *P. ochripes* (CURTIS, 1837), the aedeagus is relatively broad (figs. 4-5-6) with a very blunt, brace like apex (fig. 8) and has no dorsal washboard (fig. 10). The spermatheca (fig. 12) has an elongate receptacle and the ring collar is more developed (all terminology used as in SMITH, 1985). This study looks for biometric differences between their genitalia.

Material & Methods

We determined the sex of the *Phyllotreta* specimens by examining the fifth visible abdominal sternite (apical ventral plate), which in males shows paired indentations delimiting a median lobe (the apical margin being surrounded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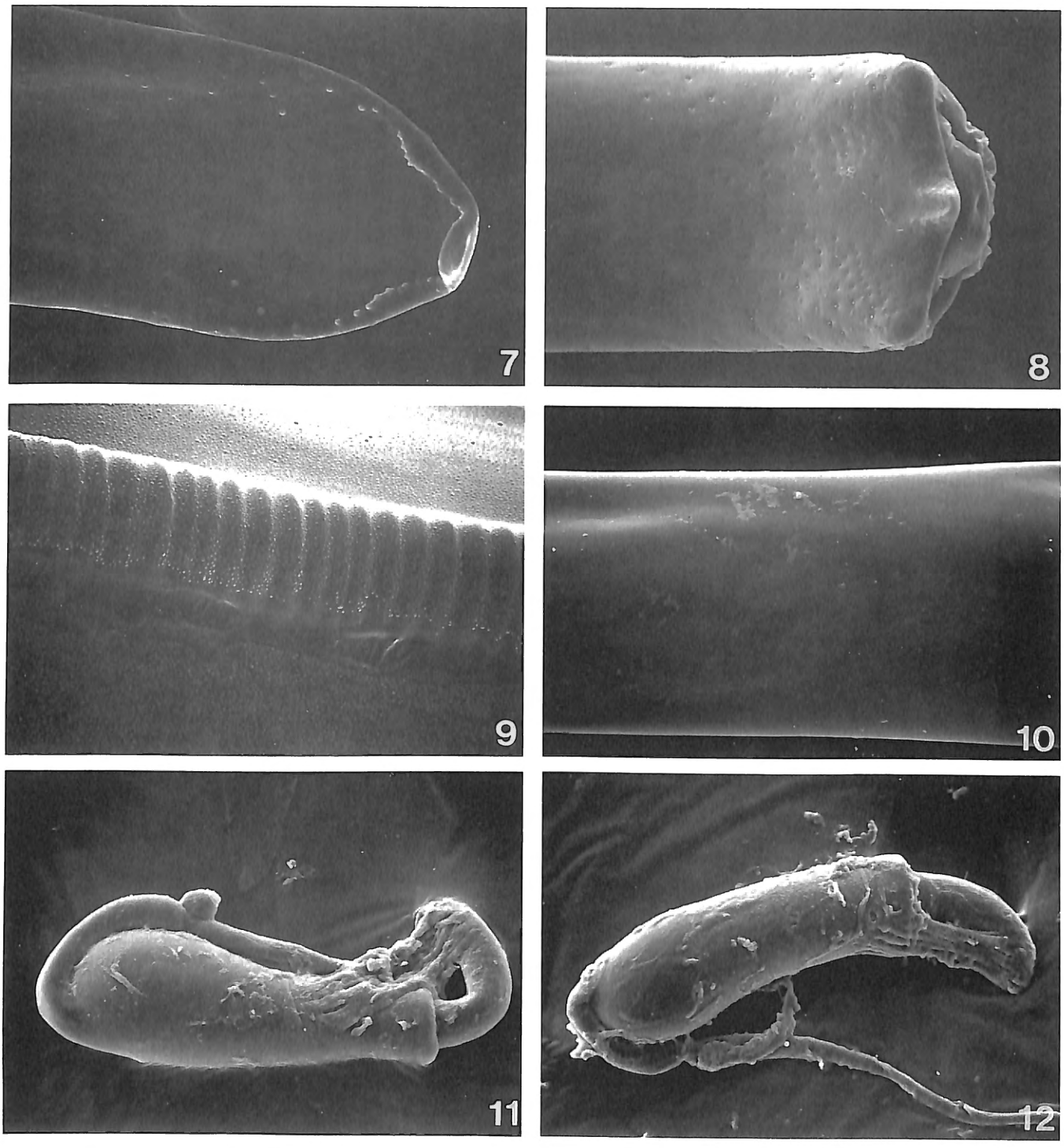

Figs. 7-12. – detail apex of aedeagus *P. dilatata* (x 300); 8. detail apex of aedeagus *P. ochripes* (x 240); 9. detail dorsal washboard of aedeagus *P. tetrastigma* (x 850); 10. detail dorsal side aedeagus without washboard *P. ochripes* (x 235); 11. spermatheca *P. dilatata* (x 190); 12. spermatheca *P. ochripes* (x 145)

←

Figs. 1-6. – dorsal view of aedeagus *P. flexuosa* (x 69.5); 2. ventral view of aedeagus *P. dilatata* (x 67); 3. lateral view of aedeagus *P. tetrastigma* (x 62); 4. dorsal view of aedeagus *P. ochripes* (x110); 5. ventral view of aedeagus *P. ochripes* (x 100); 6. lateral view of aedeagus *P. ochripes* (x 100)

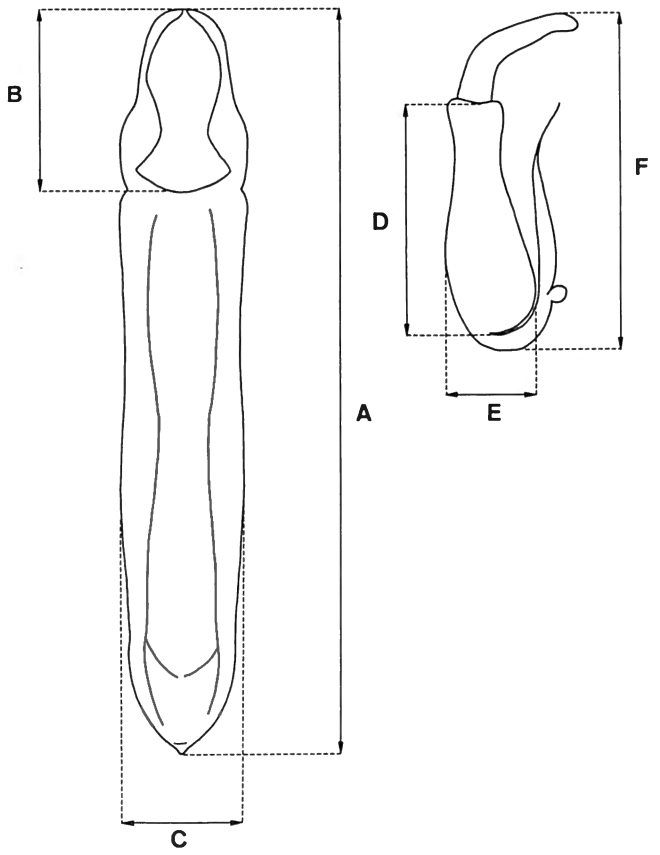

Fig. 13. – measures taken on aedeagus and spermatheca; A = ATOTAL, B = ABASAL, C = AWIDTH, D = SHEIGHT, E = SWIDTH, F= STOTAL

by the apical margin of the pygidium). In females the apical margin is evenly rounded (and not surrounded by the pygidium) (SMITH, 1979). Aedeagus and spermatheca of Alticinae usually are heavily chitinized, enabling dissection and manipulation without damaging the structures or without deformation due to shrinkage. Genitalia were dissected according to the technique described in SMITH (1979). After dissection they were mounted in Hoyer's on small pieces of transparencies (which were pinned under the insect on the same needle).

For both aedeagus and spermatheca we took the measures indicated on figure 13. For the aedeagus, ABASAL (the length of the basal foramen) and ATOTAL (the total length of the aedeagus, including apex and basal foramen) are measured, holding the aedeagus in such position that both its ends are in the same horizontal field. This is necessary to standardize the measures in the sometimes slightly curved aedeagus. AWIDTH is the maximum width of the aedeagus. For the spermatheca the measure SHEIGHT is the height of the receptacle, not including the basis of the spermathecal duct and the pump. STOTAL is measured from the bottom of the sclerotized spermathecal duct to the top of the pump. SWIDTH measures the broadest part of the receptacle. Measurements were taken using the same apparatus and procedures as for the measures of the external habitus (VERDYCK et al., 1996), with an accuracy of 0.01.

Table 1. – Populations and numbers studied

species	locality	country	sex	# measured
P. dilatata	Deurne	Belgium	male	20
			female	49
P. flexuosa	Lund	Sweden	male	5
			female	3
P. flexuosa	Rijmenam	Belgium	male	2
			female	4
P. flexuosa	Tilburg	Netherlands	male	22
			female	13
P. ochripes	Deurne	Belgium	male	13
			female	14
P. tetrastigma	Geisenfeld	Germany	male	23
			female	19
P. tetrastigma	Zoersel	Belgium	male	3
			female	2

Table 2. – Means and standard deviations (in parentheses) for the original aedeagal characters measured

ATOTAL	AMIDDLE	ABASAL	valid #	*P. dilatata*
1.171 (0.038)	0.185 (0.014)	0.308 (0.017)	20	*P. flexuosa*
0.996 (0.039)	0.153 (0.010)	0.266 (0.016)	29	*P. ochripes*
0.831 (0.039)	0.185 (0.008)	0.269 (0.013)	13	*P. tetrastigma*
1.238 (0.036)	0.186 (0.010)	0.330 (0.016)	26	

Table 3. – Means and standard deviations for the original spermathecal characters measured

	SHEIGHT	STOTAL	SWIDTH	valid #
P. dilatata	0.280 (0.018)	0.391 (0.022)	0.125 (0.011)	49
P. flexuosa	0.292 (0.015)	0.375 (0.019)	0.118 (0.012)	20
P. ochripes	0.269 (0.022)	0.385 (0.020)	0.132 (0.016)	14
P. tetrastigma	0.323 (0.013)	0.446 (0.021)	0.143 (0.008)	21

As for several data a positive correlation between the means and variances was observed, data were multiplied by 1000 and logtransformed (as recommended by SOKAL & Rohlf, 1995). For each of the transformed measures ANOVA was performed to check for significant differences between the species. Tukey honest significant difference test (HSD) for unequal sample sizes was used as a post

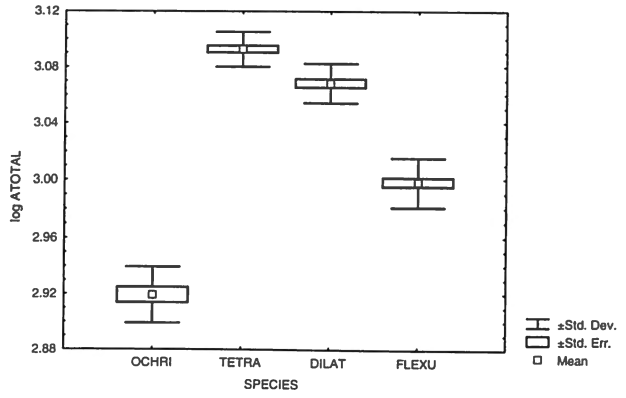

Fig. 14. – Box-Whisker plot (means, standard errors and standard deviations) for ATOTAL

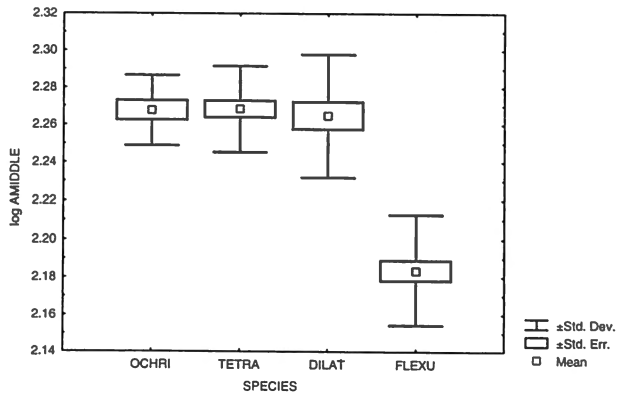

Fig. 15. – Box-Whisker plot (means, standard errors and standard deviations) for AMIDDLE

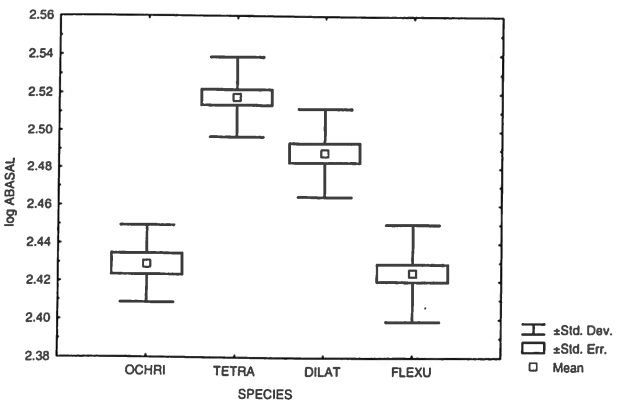

Fig. 16. – Box-Whisker plot (means, standard errors and standard deviations) for ABASAL

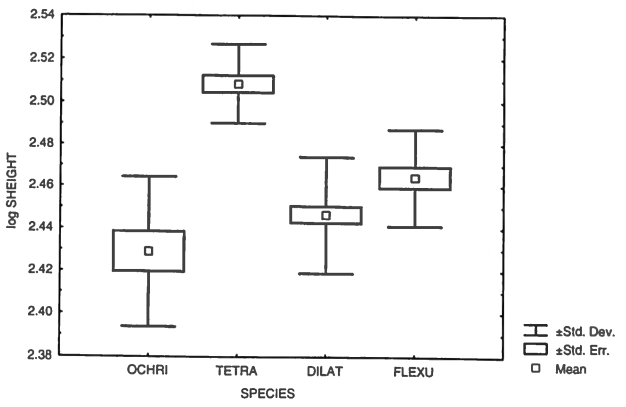

Fig. 17. – Box-Whisker plot (means, standard errors and standard deviations) for SHEIGHT

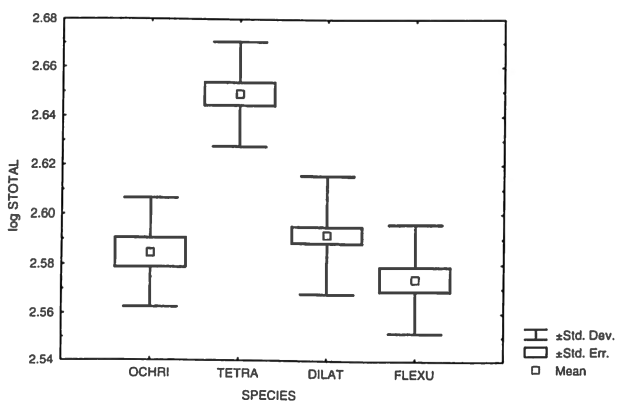

Fig. 18. – Box-Whisker plot (means, standard errors and standard deviations) for STOTAL

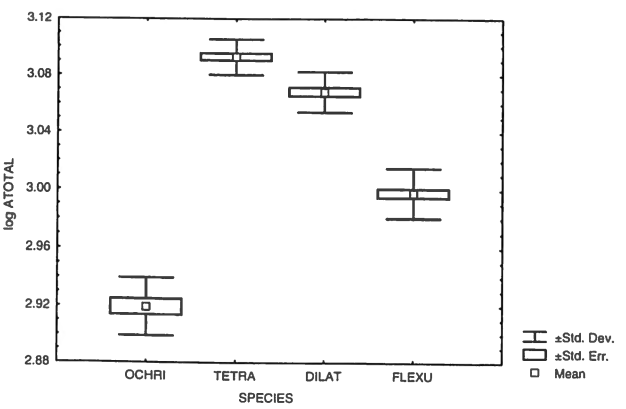

Fig . 19. – Box-Whisker plot (means, standard errors and standard deviations) for SWIDTH

Table 4. – Variables showing significant differences according to Tukey HSD test males (logtransformed measures, unequal n, Bonferroni corrected)

	P. flexuosa	*P. ochripes*	*P. tetrastigma*
P. dilatata	ATOTAL AMIDDLE ABASAL	ATOTAL ABASAL	ATOTAL ABASAL
P. flexuosa		ATOTAL AMIDDLE	ATOTAL AMIDDLE ABASAL
P. ochripes			ATOTAL ABASAL

Table 5. – Variables showing significant differences according to Tukey HSD test females (logtransformed measures, unequal n, Bonferroni corrected)

	P. flexuosa	*P. ochripes*	*P. tetrastigma*
P. dilatata	-	-	SHEIGHT STOTAL SWIDTH
P. flexuosa		SHEIGHT SWIDTH	SHEIGHT STOTAL SWIDTH
P. ochripes			SHEIGHT STOTAL

hoc test to find out which of the means showed significant differences. Sequential Bonferroni correction (HOLM, 1979; RICE, 1989) was applied to correct for multiple testings.

Results

Genitalia of 88 males and 104 females were measured for the four species. Table 1 lists the localities and numbers studied for each species. Tables 2 (males) and 3 (females) show the means and standard deviations for all characters.

For both aedeagi and spermathecae all ANOVA's show highly significant differences ($\alpha < 0.01$) between the species. The results of the Tukey HSD tests for unequal sample sizes are summarized in tables 4 and 5. All species differ significantly from each other for the total length of the aedeagus (ATOTAL), and except for *P. flexuosa* and *P. ochripes*, they also differ for the length of the basal foramen (ABASAL). Only *P. flexuosa* differs significantly from all other species for the width of the aedeagus (AMIDDLE). The females of *P. tetrastigma* differ significantly from the other three species for all spermatheca characters. *P. flexuosa* differs in two characters from *P. ochripes* and *P. dilatata* does not show any differences with *P. flexuosa* and *P. ochripes*.

The Box-Whisker plots in figures 14 to 19 show the means, standard errors and standard deviations of the transformed measures for all species. The aedeagus of *P. ochripes* is as wide as these of *P. dilatata* and *P. tetrastigma*, but is much shorter than these of the other three species. *P. flexuosa* has a shorter aedeagus than *P. dilatata* and *P. tetrastigma* with a shorter basal foramen. Between *P. dilatata* and *P. tetrastigma* there is a small but significant difference in total length and length of the basal foramen. The spermatheca of *P. tetrastigma* is longer and broader than those of the other species. The spermatheca of *P. flexuosa* is higher (SHEIGHT) and less broad than this of *P. ochripes*.

Discussion

To our knowledge this is the first morphometric study on the genitalia of *Phyllotreta* species, and even in other Coleoptera little work of this kind has been done. Our results show that the measures of the aedeagus allow separation of all species. For the spermatheca *P. tetrastigma* can be separated from the other species studied and *P. flexuosa* can be separated from *P. ochripes*. Measurements taken on the genitalia thus offer additional information for the identification of the four species studied, but the aedeagus is more suited for this purpose than the spermatheca. This work confirms the importance of the study of genital differences in the discrimination of sibling species. And even in cases were no differences in general morphological structure of the genitalia is observed, morphometric analysis may still detect differentiation in the genus *Phyllotreta* as well as in other taxa. Morphometric differences between genitalia of different species also can be used for the correct identification of these species.

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