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Morphological and morphometric variability in *Pergamasus decebali* and *P. scorilai* (Acari: Parasitidae), with comments on other species of the *P. crassipes* species-group

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Abstract: Intra- and interspecific variability of *Pergamasus scorilai* Juvara-Balş, 1973 and *P. decebali* Juvara-Balş, 1973 from Romania is investigated through morphological comparison and morphometric analyses. An analysis of morphological differences is used to show intraspecific variability and to establish the taxonomic limits of each species. We studied the proximal apophysis of tibia II in males and the endogynium of females. Using multivariate analyses based on 10 and 9 measurements for males and females, respectively, we demonstrate that the two species can be distinguished unambiguously. Relying on a variable selection approach, we also determine the most useful characters for the identification of these two very similar species. In this case only two tibial measurements allowed to correctly identify all male individuals, while at least six different measurements are needed for the discrimination of female specimens. Finally, we provide an exhaustive overview of male characteristics for the entire species-group, and we place *P. instipatus* Athias-Henriot, 1967 in the synonymy of *P. caninatellus* Athias-Henriot, 1967.

Keywords: Gamasid mites - species recognition - discriminant analysis - Romania.

INTRODUCTION

The genus *Pergamasus* Berlese, 1903 is one of the most common predatory mites in leaf litter and soil in the Northern Hemisphere. The genus was considered by Berlese (1903) as a subgenus of *Gamasus* Latreille, 1802 and raised by Hull (1918) to the generic level. The taxonomy of the genus was studied and revised by several authors (Bhattacharyya, 1963; Schweizer, 1961; Micherdzinski, 1969; Athias-Henriot, 1967, 1971). The classification established by Athias-Henriot (1971) is the one currently recognized (Juvara-Balş, 1976; Karg, 1993; Hruzová & Fend'a, 2018). Athias-Henriot (1971) split *Pergamasus* s. lat. into three subgenera: *Thenargamasus*, *Triadogamasus* and *Pergamasus* s. str. The species included in *Pergamasus* s. str. are currently divided into four species-groups: the *crassipes*-group, the *beklemischevi*-group, the *alpinus*-group and the *athiasae*-group (Athias-Henriot, 1968; Juvara-Balş, 1970). The identification of species in the *Pergamasus crassipes* species-group, especially *P. crassipes* (Linnaeus, 1758), is difficult because of the lack of information on the variability of the main characteristics and because of incomplete descriptions. Micherdzinski (1969) enumerated all localities in Europe from where true *P. crassipes* were identified and pointed out that

a revision was necessary because the occurrence of a complex of multiple species could not be excluded. *Pergamasus crassipes* and *P. longicornis* Berlese, 1906 were the first species to be assigned to the *crassipes* group (Bhattacharyya, 1963). Athias-Henriot (1967) subsequently added seven additional species and Juvara-Balş (1970, 1973) another six species from Romania. Karg (1993) only included six taxa of this species-group from central Europe in his identification key. He also considered *P. crassipes* and *P. longicornis* to belong to the same species with a great variability in the shape of the endogynium (Karg, 1993: fig. 315). Dielmann (1991) drew attention to the importance of better taxonomic descriptions of females but did not propose an updated diagnosis to separate the females of the different species. Considerable morphological variability within the *Pergamasus crassipes* species-group is regarded by some acarologists as mainly intraspecific (Karg, 1993), whereas others (Bhattacharyya, 1963; Athias-Henriot, 1967; Juvara-Balş, 1976) think that it warrants the separation of additional species. This confusing situation still prevails because the species-group has recently received little attention. In many ecological and biodiversity studies species identification is restricted to "*Pergamasus* sp.". This led us to investigate the level of variation between and within two closely related species, i.e. *P. scorilai*

and *P. decebali*. These two species are morphologically similar to one another, although well-differentiated from the other species of the *P. crassipes* species-group in Romania (Juvara-Balş, 1976). Their identification based on discrete characters is difficult, and we wanted to assess whether morphometrics could provide an effective tool for species differentiation.

Collecting was conducted in the southern and western Carpathian Mountains of Romania where several populations of both species can be found in forest soils. Based on a sample of 257 individuals we assess and describe the variability of morphological and morphometric characters in *P. scorilai* and *P. decebali* to determine if it can be attributed to intraspecific polymorphism or to the presence of an overlooked species diversity.

MATERIAL AND METHODS

Morphological analyses

Two populations of *Pergamasus scorilai* and one of *P. decebali* were selected for morphological and morphometric analyses. The mites were collected by sifting leaf litter from two forests in western Romania, from the surface area of about one square meter each. Only adult individuals were used for the analyses. The specimens examined are:

- *P. decebali* (type series): 41 males, 31 females; spruce litter, Scarisoara Village, Apuseni Mountains, Alba County, 1100 m a.s.l.; 26.AUG.1970; leg. I. Juvara-Balş.
- *P. scorilai* (type series): 48 males, 47 females; beech forest, Sucu Valley, Maru Village, Caras-Severin County, 600 m a.s.l.; 29.SEP.1970; leg. I. Juvara-Balş.
- *P. scorilai*: 45 males, 45 females; spruce forest, Mount Mic, Caras-Severin County, 1800 m a.s.l.; 1.OCT.1970; leg. I. Juvara-Balş.

The holotypes and other specimens were deposited in the Emil Racovitza Institute of Speleology of the Romanian Academy, Bucharest, Romania. Four female *P. decebali* specimens, and one male and two female *P. scorilai* specimens from Mount Mic were deposited in the Natural History Museum of Geneva, Switzerland. All mites were cleared in lactic acid, and measurements were taken from dissected and slide-mounted specimens. Identification of species requires a special examination of males, which possess more distinct diagnostic characters than females. It is important to keep tibia II in a horizontal position (Fig. 1D) to observe the morphological characteristics and take measurements as established by Athias-Henriot (1967). The three following measurements were taken from both sexes: LI - length of idiosoma; t1 - length of tarsus I (Fig. 1C); t4 - length of tarsus IV. In males we additionally measured: AB - length of tibia II; AC - length of proximal apophysis of tibia II; GH - distance between base of distal apophysis and distal tibial margin;

EF - distance between insertion of seta all1 and distal tibial margin; all1, all2 and pv1 - length of all1, all2 and pv1 setae, respectively, from insertion to tip (Fig. 1D). Finally, we selected the following morphometric characters in order to differentiate the females: LE - length of epigynium measured along its midline; WEB - width of posterior margin of epigynium; LSS - length of sternal shield; WSS - width of sternal shield (measured at the st2 setae level); W/2 - length of right half of posterior sternal margin; st5-st5 - distance between the insertions of the pair of setae st5 (Fig. 1A-B). All the measurements are in micrometres. All individual measurements are available online as Supplementary Material (<https://doi.org/10.5281/zenodo.5082959>). In addition to these standard measurements, we calculated two new variables: t1/LI - ratio between length of tarsus I and length of idiosoma; t4/LI - ratio between length of tarsus IV and length of idiosoma. The latter two variables were not included in most analyses, but were only used to compare the relative length of limbs in each of the three populations, in order to determine their correlation with the altitude as postulated by "Allen's rule" (Ray, 1960; Bidau & Martí, 2008).

The nomenclature of different structures of the endogynium follows Athias-Henriot (1967). The endogynium comprises a sack which has ventrally a pair of reticulated sphaerules that are separated by the stipula ("endogynial process" according to some authors) and are flanked by a pair of trabeculae. In species where these structures are variable (*P. crassipes*, *P. pinguicrus* Athias-Henriot, 1967, *P. scorilai*, *P. decebali*, *P. buerebistai* Juvara-Balş, 1973), identification based on the examination of a large series of specimens is advised.

Statistical analyses

First, a quick analysis of the morphometric male characters for all species of the *Pergamasus crassipes* species-group was done. This analysis allowed us to validate the reliability of these characters for species discrimination, and to identify where the two studied species (*P. decebali* and *P. scorilai*) lie among other members of the species-group. To this end we aggregated measurements used in Athias-Henriot (1967) and Juvara-Balş (1970, 1973) for all 15 species currently known in the *crassipes* species-group. We then carried out a principal component analysis (PCA) using the package *ade4* (Dray & Dufour, 2007) available in the R environment (R Core Team, 2020).

For *P. decebali* and *P. scorilai*, both sexes were analysed separately and all analyses were conducted in R (R Core Team, 2020). Normality of data was tested using Shapiro tests. Means, standard deviation and coefficient of variability were calculated for each of the measured characters. Differences in mean measurements were assessed between *P. scorilai* and *P. decebali*, and

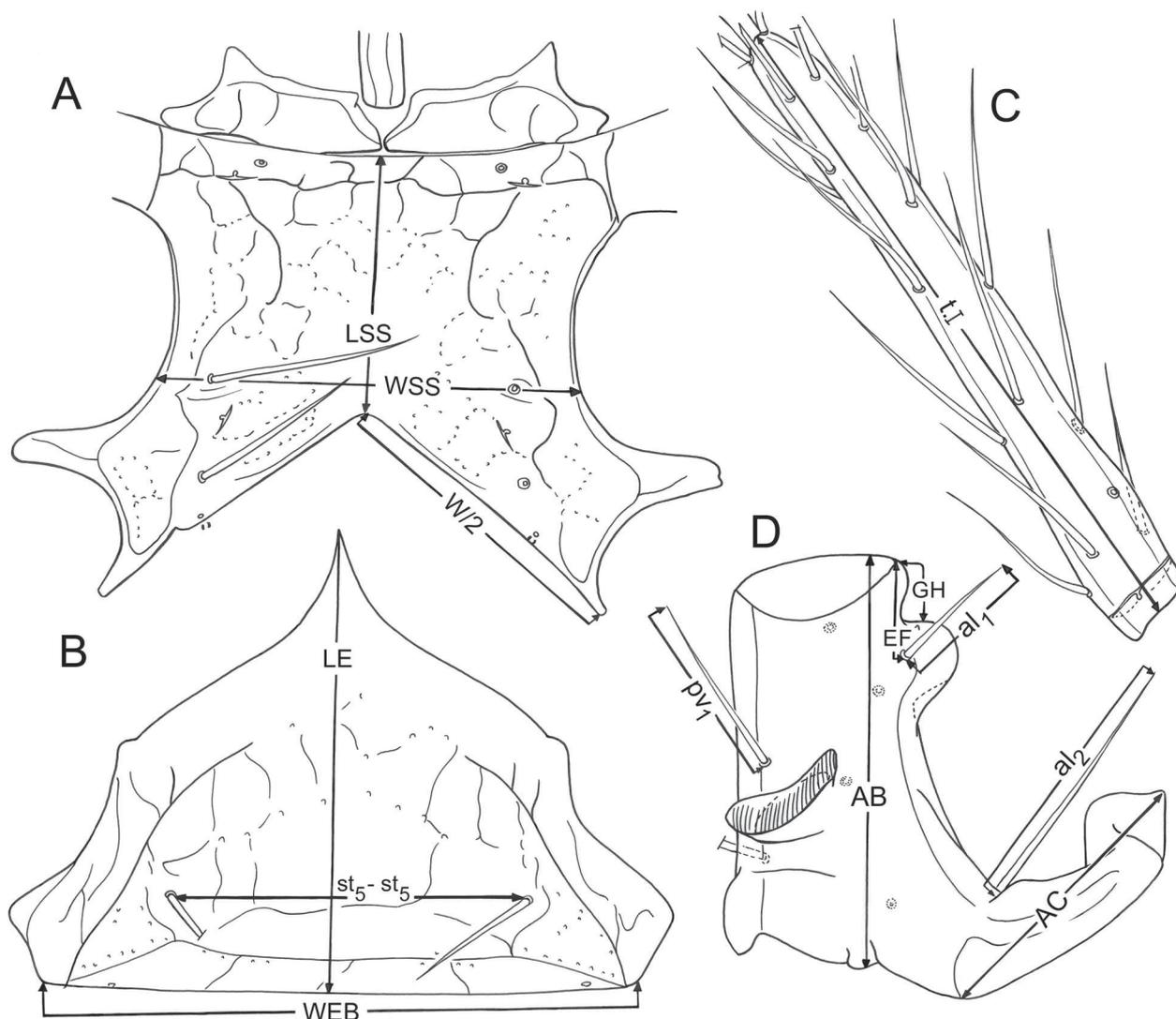


Fig. 1. *Pergamasus scorilai*; explanation of measurements taken from females (A-B) and males (C-D). (A) Sternal shield. (B) Epigynum. (C) Tarsus I. (D) Tibia II. For abbreviations see text and Table 1.

among each pair of populations using Welch's t-tests. For each sex we carried out a principal component analysis (PCA) as described before. This analysis allows representing the maximum of the multivariate variance in a reduced number of dimensions, each of them being a linear combination of the original variables (here: morphological measurements). The generalized squared Mahalanobis distance (D2) was calculated for all pairs of groups. This distance measures the separation of two groups in multidimensional space where all variables are scaled to have unit variance.

In an attempt to reduce the number of measurements necessary for species recognition, we implemented a variable selection process to retain only the most informative ones. To this end we compared the performance of different models (which included from one to all the morphological traits measured) using the

Akaike information criterion (AIC). In particular, we used a backward selection approach, starting from a generalized linear model including all morphometric variables and removing one variable after the other until the model with the lowest AIC value possible was reached (Venables & Ripley, 2002). This stepwise procedure was carried out using the stepAIC function available in the MASS R package (Venables & Ripley, 2002).

RESULTS

Our analysis of all male morphometric characters for the 15 species in the *P. crassipes* species-group indicates that the morphometric characters traditionally used for species recognition does allow the separation of these taxa (Fig. 2). On the left side of the PCA, seven

species (*P. crassipes*, *P. dumitrescui* Juvara-Balş, 1970, *P. longicornis*, *P. palatortus* Athias-Henriot, 1967, *P. pinguicrus*, *P. buerebistai* and *P. similicornis* Athias-Henriot, 1967) exhibit relatively large AB, AC, t1, t4 and al2 measurements. Among the remaining species, three small-sized species (*P. caninatellus*, *P. laetus* Juvara-Balş, 1970 and *P. biharicus* Juvara-Balş, 1973) are isolated from others in the top-right corner of the PCA, while *P. truatellus* Athias-Henriot, 1967, in the bottom-right corner, is characterized by larger EF, GH, pv1 and all1 measurements. Finally, morphometric characters of *P. aequicornis* Athias-Henriot, 1967, *P. primorellus* Athias-Henriot, 1967, *P. decebali* and *P. scorilai* are quite similar (Fig. 2). However, these four species differ by morphological characteristics of tibia II and of the male chelicerae (Fig. 3A, F, L, M).

Morphological variability within species

Males: Variation is striking in the male tibia II of *P. decebali* and *P. scorilai*. A series of variants of the tibia

II distal and proximal apophyses has been illustrated in *P. primorellus* (Athias-Henriot, 1967; fig. 250) and *P. longicornis* (Athias-Henriot, 1967; fig. 251). Juvara-Balş (1973, 1976) briefly described the variability of the proximal tibial apophysis in *P. scorilai*, *P. decebali*, *P. buerebistai* and *P. dumitrescui*.

In *P. scorilai* (n=91) the apex of the tibial apophysis, with a slightly invaginated spatula (n=15), is always more or less square. The form of the apical lobe is also subject to some variation: the upper edge is straight or weakly concave (n=19; Fig. 4A-B, I); the spatula possesses an acute external angle (n=31; Fig. 4C-D) or is trapezoidal with an elongated antiaxial edge (n=12; Fig. 4E-F); the antiaxial edge is considerably extended and the apex is rounded (n=11; Fig. 4G-H).

In *P. decebali* (n=31) the apex of the proximal apophysis, with a non-invaginated spatula, usually has the shape of a triangle that is more or less pointed (Fig. 3F), but in some specimens it is more trapezoidal (Juvara-Balş, 1973: fig. XI E'-E''). The diverse forms of species closely related to *P. scorilai* and *P. decebali* are illustrated in Fig. 3.

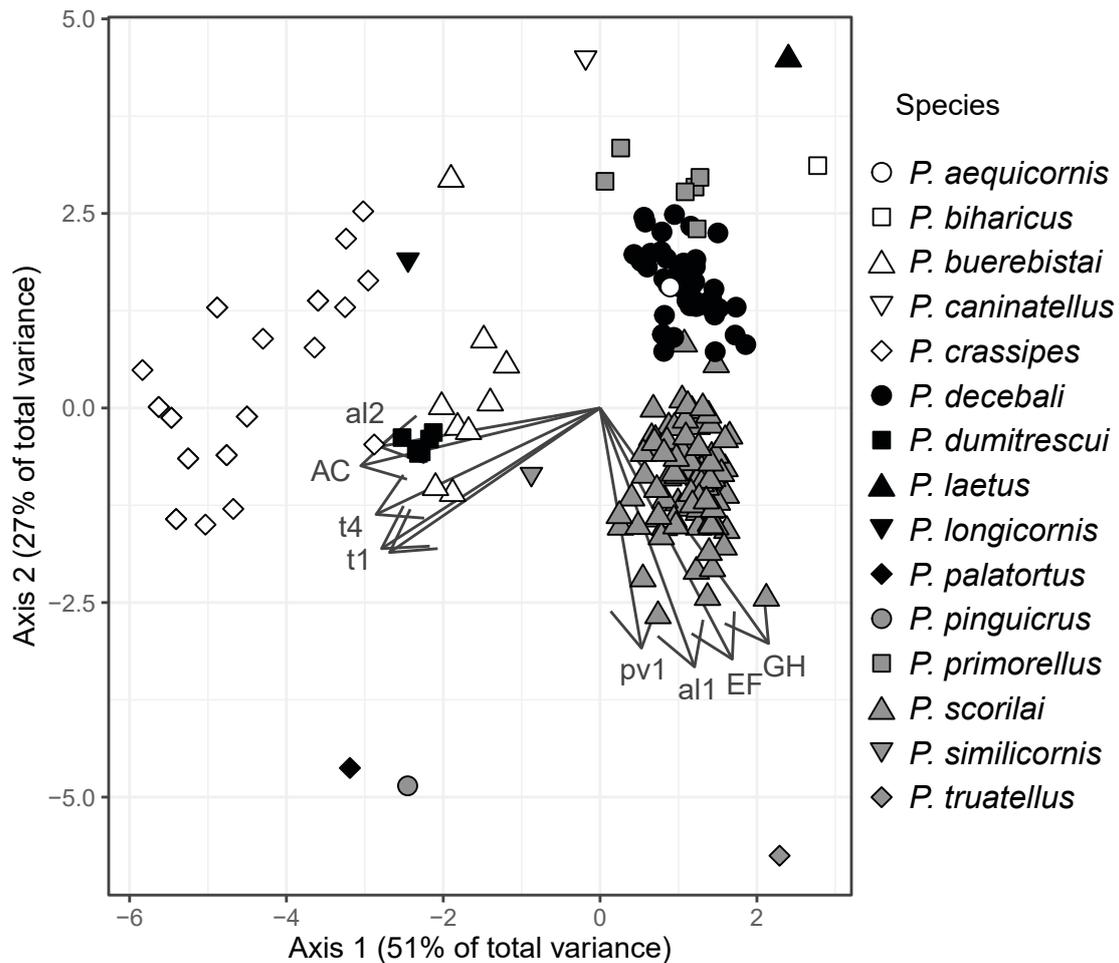


Fig. 2. Principal component analyses based on all morphometric characters of male individuals of the 15 species in the *Pergamasus crassipes* species-group. Individuals are represented by different symbols according to species.

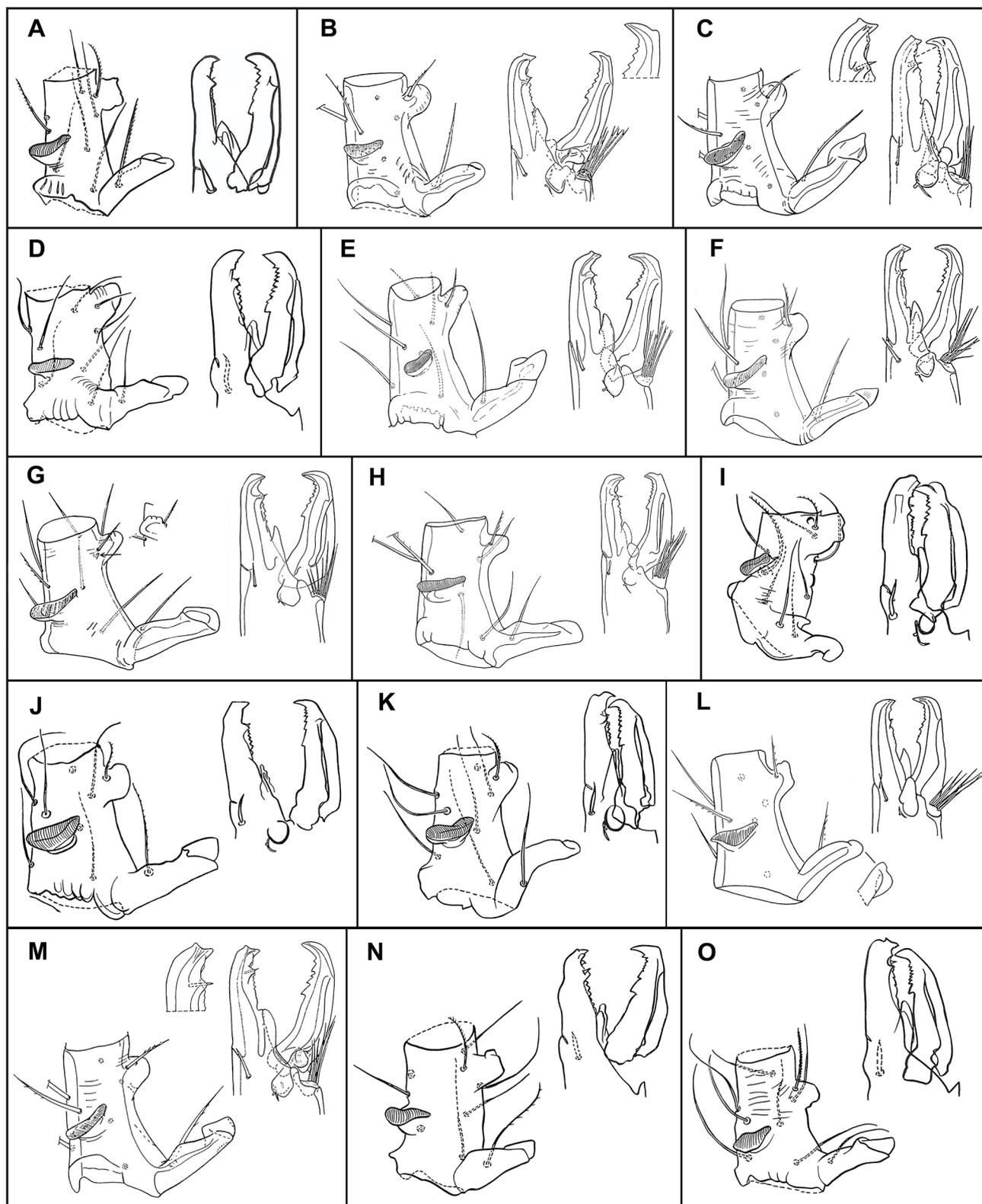


Fig. 3. Male tibia II (on the left) and male chelicerae (on the right) of *Pergamasus* spp. (A) *P. aequicornis*. (B) *P. biharicus*. (C) *P. buerebistai*. (D) *P. caninatellus*. (E) *P. crassipes*. (F) *P. decebali*. (G) *P. dumitrescui*. (H) *P. laetus*. (I) *P. longicornis*. (J) *P. pinguicrus*. (K) *P. palatortus*. (L) *P. primorellus*. (M) *P. scorilai*. (N) *P. similicornis*. (O) *P. truatellus*. Drawings in thick lines modified from Athias-Henriot, 1967 and drawings in thin lines from Juvara-Balș, 1970, 1973 or original.

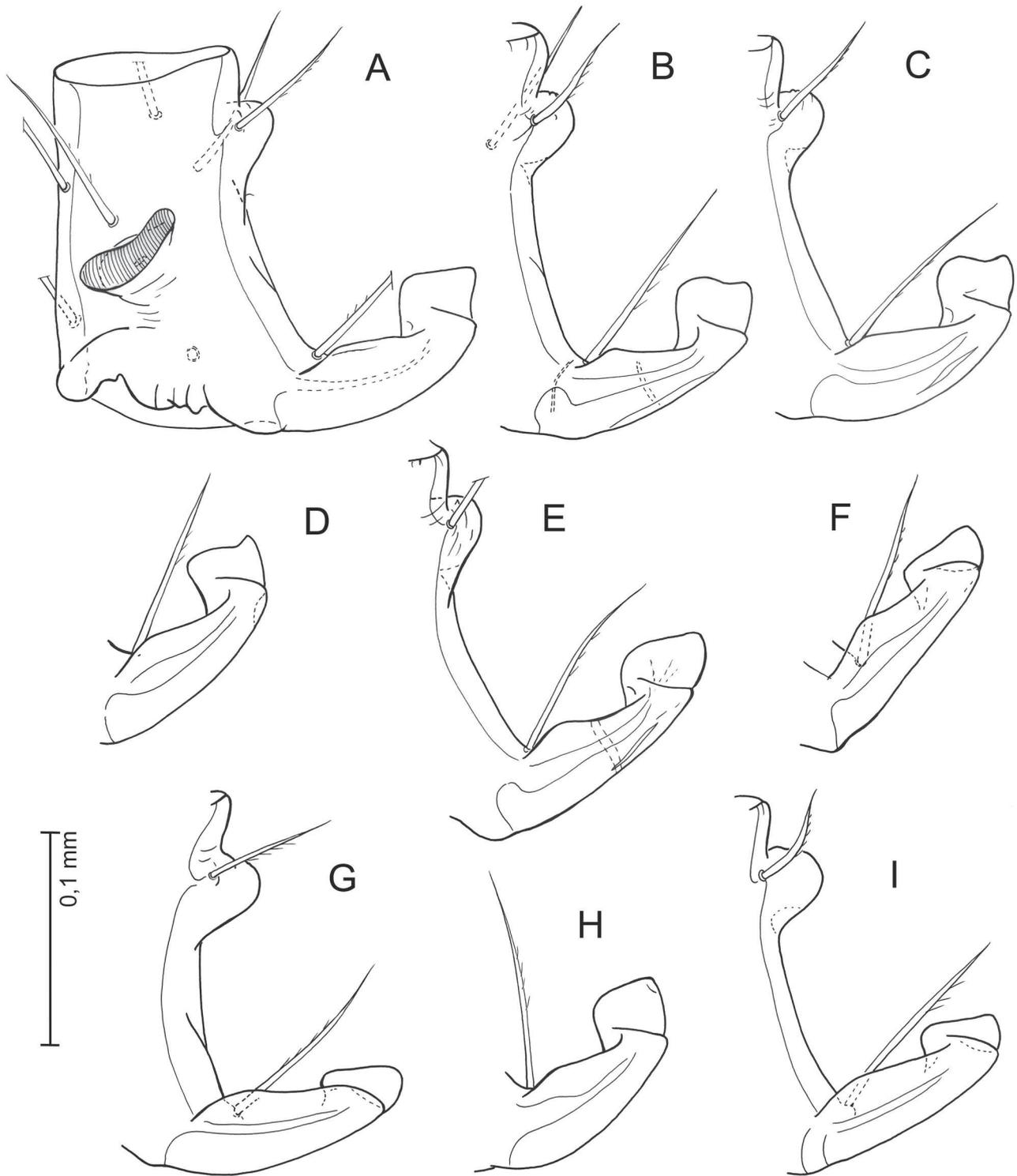


Fig. 4. Variations in the shape of the apex of the proximal apophysis of tibia II in males of *Pergamasus scorilai* from the type locality (Sucu Valley). (A) Tibia II. (B-I) The right side and apophysis of tibia II. Modified from Juvara-Balş, 1973.

In *P. buerebistai* the apical lobe is sub-quadrangular, with a pointed or mucronate apex (Fig. 3C), while in *P. dumitrescui* it is quadrangular (Fig. 3G), and in *P. biharicus* it constantly shows the shape of a club (Fig. 3B).

The form of the gnathotectum is equally variable in *P. scorilai*, *P. decebali* and *P. buerebistai*. In some individuals of these three species the five prongs are at the same level, while in other specimens the three middle prongs are more protruding than the lateral ones (Juvara-Balş, 1973: figs IV G-H, VIII B-C, XI B-C).

Females: An important character in the identification of females is the morphology of the endogynium. Variation can be observed in the size of the triangular process of the trabeculae, in the shape and size of the stipula, as well as in the number of denticles of the endogynial sack.

Variability in endogynial characteristics in *P. scorilai* is so pronounced that two morphotypes in the type series can be differentiated based on the following characteristics (Juvara-Balş, 1973: fig. VII):

- stipula with two branches, very large trabecular process, endogynial sack with denticles;
- stipula digitiform, only slightly enlarged trabeculae, endogynial sack devoid of denticles.

Among the 49 females of the type series, 23 have the stipula with two branches, 18 have a digitiform stipula and 8 have a stipula with intermediate forms. Intermediate forms are those with a stipula possessing less differentiated, smaller, or regressed branches. These forms are the extremes of a series of variants detected in many specimens from two large samples. In all studied populations of *P. scorilai* the form of the endogynium can significantly diverge from the typical morphology (Juvara-Balş, 1973: fig. VII A, F): on one hand by reduction of the two branches of the stipula to a digitiform one (Juvara-Balş, 1973: fig. VII D-F, I), and on the other by the triangular process of the trabeculae being reduced to form a simple edge paraxially (Juvara-Balş, 1973: fig. VII C, F). The latter polymorphism was observed at the individual level, with a specimen having one unarmed trabecula on one side and a triangular process on the other side (Juvara-Balş, 1973: fig. VII B).

Variation in the stipula is evident in *P. decebali* specimens collected in the western and southern Carpathians. Among the 31 specimens from the type-series, only four have an almost digitiform stipula with regressed branches, while 27, including the holotype, have a stipula with two short branches (Fig. 5E-G). The number of denticles of the endogynial sack is variable, and they may even lack in some specimens of *P. decebali* (Fig. 5G).

Pergamasus scorilai shows marked variation in the ornamentation of the sternal and epigynial shields. Some specimens have a marked reticulation and few dimples, whereas others have a strongly reduced reticulation with numerous dimples (Fig. 5C-D). Intermediate forms were found and all combinations of patterns are possible.

In *P. decebali* the reticulation of the sternal shield is accompanied by small dimples located below lyrifissure 2 and towards the lateral edges of this shield. The epigynial reticulation can be pronounced, but always is with dimples (Fig. 5A-B).

Morphometric variability across the three populations examined

Males: Some measurements differ between the two sampled populations of *P. scorilai* (Table 1 and Fig. 6). In particular, the length of tibia II (AB) is significantly greater in individuals from the Sucu Valley beech forest compared to those from Mt Mic spruce forest (Table 2). The length of the idiosoma, and distances EF and GH also differ significantly for both *P. scorilai* populations, but for these measurements the values were lower in individuals from Sucu Valley than in those from Mt Mic. The GH measurement of *P. scorilai* specimens from Sucu Valley have a smaller coefficient of variation (5.54%) than measurement of conspecific specimens from Mt Mic and specimens of *P. decebali* from the same biotope (spruce forest). The squared Mahalanobis distance (D₂) calculated from eight characters leads to larger interspecific distances than intraspecific ones. Moreover, the distance between centroids of the type series of *P. decebali* and *P. scorilai* (D₂ = 5.19), collected in different biotopes, is higher than the one measured between *P. decebali* and *P. scorilai* from the same biotope (D₂ = 4.21). The distance between the two populations of *P. scorilai* is the smallest (D₂ = 2.92).

Females: The morphological measurements of *P. scorilai* and *P. decebali* females show variation in several characters (Table 1 and Fig. 6). In particular, the length of idiosoma, epigynium and tarsi I and IV significantly differ between all populations (Table 2). *Pergamasus decebali* exhibits smaller values than *P. scorilai* for all these measurements. Within *P. scorilai*, the population from Sucu Valley beech forest has a smaller idiosoma and epigynium but larger tarsi than the population sampled in the Mt Mic spruce forest. The Mahalanobis distance is greatest (D₂ = 4.87) for the type series of *P. decebali* and *P. scorilai* (Sucu Valley), intermediate (D₂ = 4.50) for *P. decebali* and *P. scorilai* collected from the same biotope (at Mt Mic), and the lowest (D₂ = 2.72) for the two populations of *P. scorilai*.

Morphometric discrimination of *P. scorilai* and *P. decebali*

Males: All morphological measurements, except the length of setae al₂, significantly differ between males of *P. decebali* and *P. scorilai* (Table 2), the latter species being generally larger than the former (Table 1). However, there is no single morphological

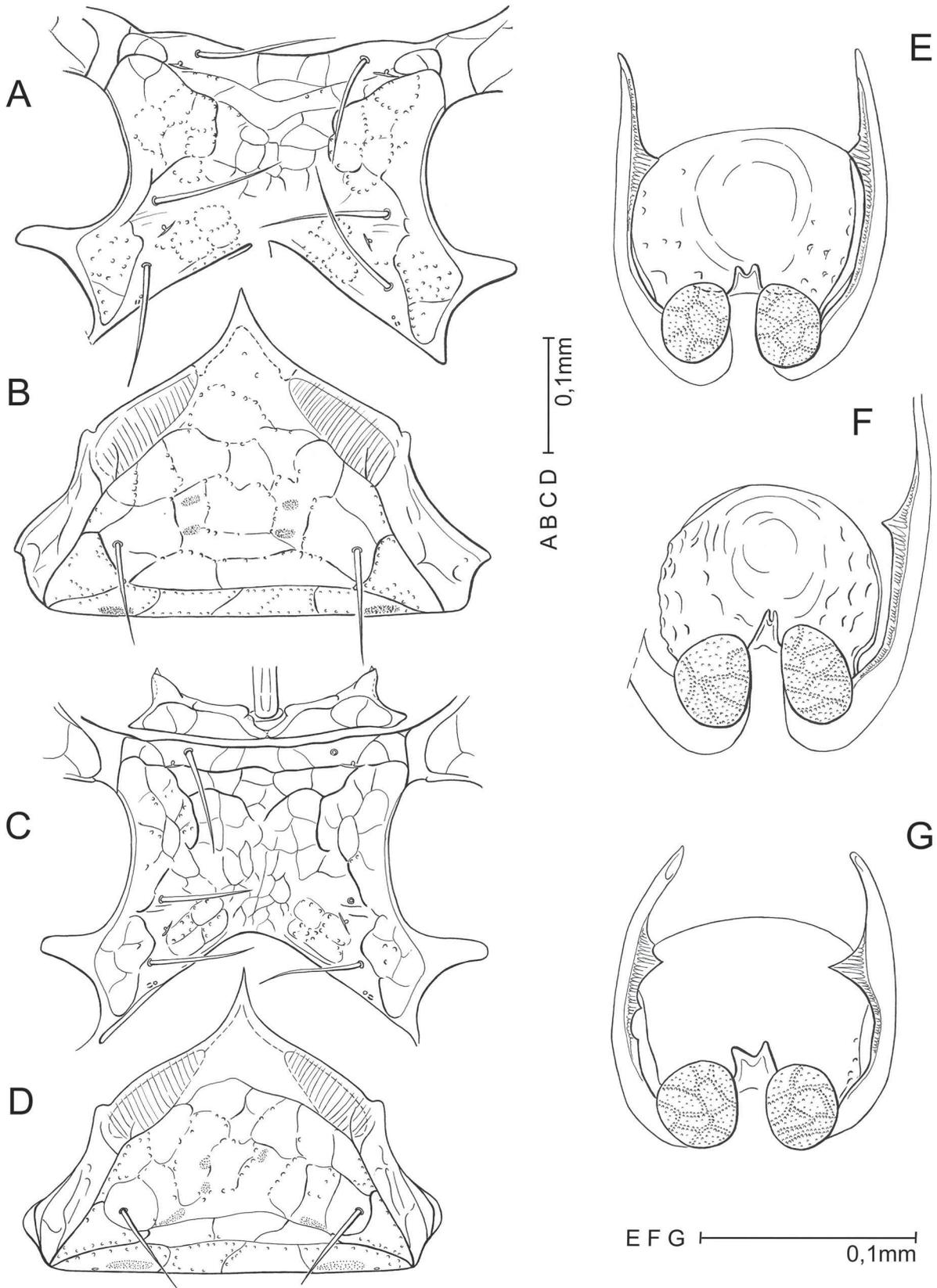


Fig. 5. Females of *Pergamasus decebali* (A-B, E-G) and of *P. scorilai* (C-D). (A, C) Sternal shield. (B, D) Epigynum. (E-G) Endogynium. Modified from Juvara-Balş, 1973.

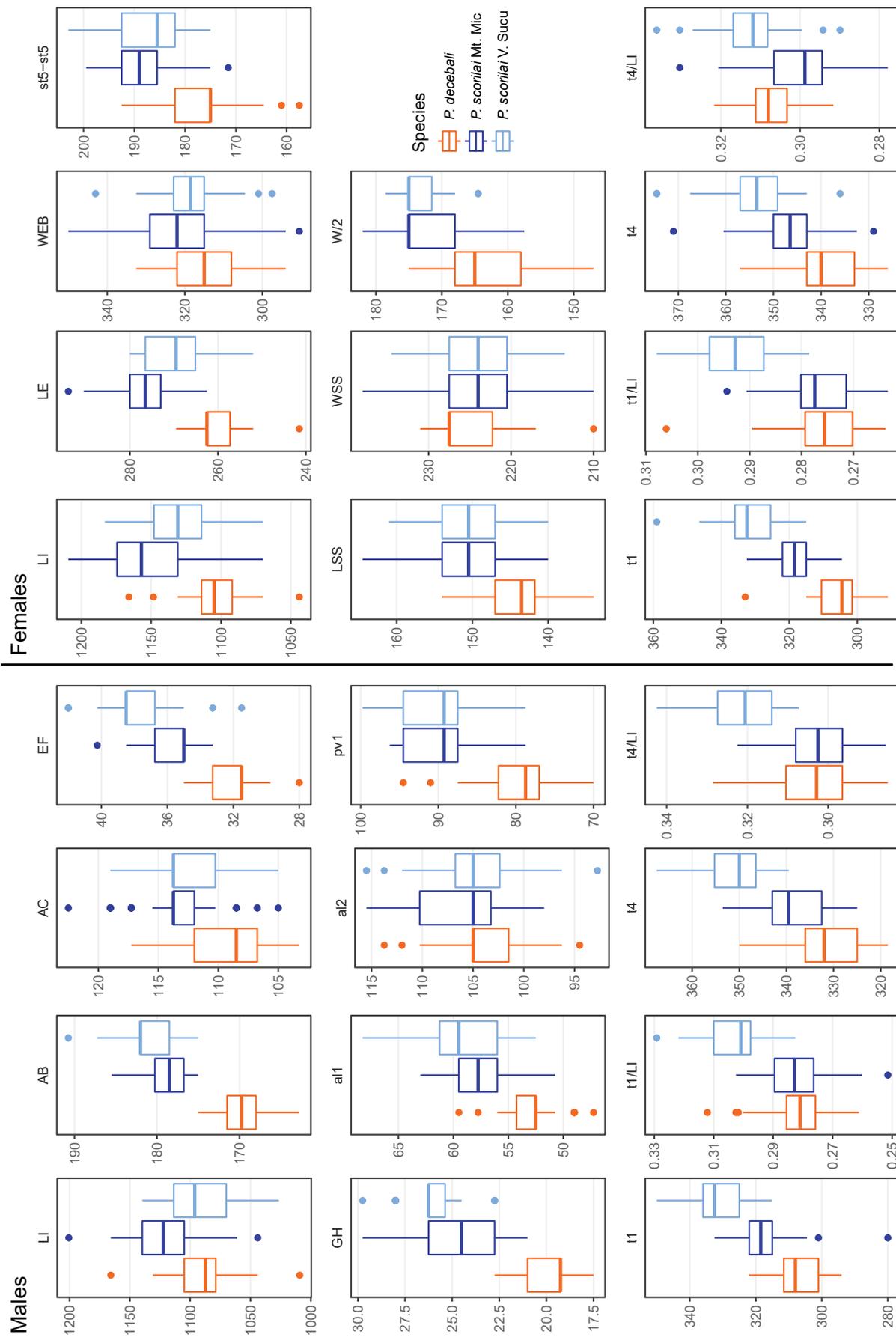


Fig. 6. Statistics of measurements taken from 133 males (left half) and 124 females (right half) of *Pergamasus decebali* (from one population) and *P. scoritai* (from two populations). Thick horizontal lines indicate the median, the vertical lines indicate the 25-75% range, the vertical lines indicate the 10-90% range; outliers are displayed as dots.

Table 1. Measurements (in µm) of *Pergamasus scoritai* and *P. decebali*. For each population and species the mean (M), standard deviation (SD), coefficient of variation (CV), minimum (min) and maximum (max) values are given.

FEMALES	Measurement	Abbreviation	<i>P. scoritai</i> from Sucu Valley (n=48)						<i>P. scoritai</i> from Mt Mic (n=45)						<i>P. scoritai</i> (n=93)						<i>P. decebali</i> (n=31)					
			M	SD	CV	min	max	M	SD	CV	min	max	M	SD	CV	min	max	M	SD	CV	min	max				
	Length of idiosoma	LI	1132.07	24.54	2.17%	1070.00	1183.20	1152.99	28.12	2.44%	1070.00	1209.30	1142.19	28.22	2.47%	1070.00	1209.30	1103.90	23.52	2.13%	1044.00	1166.00				
	Length of epigynum	LE	269.28	7.59	2.82%	252.00	280.00	276.11	6.93	2.51%	262.50	294.00	272.59	8.01	2.94%	252.00	294.00	260.47	6.05	2.32%	241.50	269.50				
	Width of epigynum basis	WEB	318.79	8.78	2.75%	297.50	343.00	321.07	11.85	3.69%	290.50	350.00	319.89	10.39	3.25%	290.50	350.00	314.77	10.92	3.47%	294.00	332.50				
	Distance st5-st5	st5-st5	187.76	7.27	3.87%	175.00	203.00	189.16	7.00	3.70%	171.50	199.50	188.44	7.14	3.79%	171.50	203.00	176.24	7.37	4.18%	157.50	192.50				
	Length of sternal scutum	LSS	149.70	5.22	3.49%	140.00	161.00	150.97	5.15	3.41%	140.00	164.50	150.31	5.20	3.46%	140.00	164.50	144.44	4.25	2.94%	134.00	154.00				
	Width of sternal shield	WSS	224.15	5.05	2.25%	213.50	234.50	224.16	5.73	2.56%	210.00	238.00	224.15	5.36	2.39%	210.00	238.00	224.45	4.93	2.20%	210.00	231.00				
	Half width of posterior sternal scutum	W/2	173.03	3.38	1.95%	164.50	178.50	171.89	5.39	3.14%	157.50	182.00	172.48	4.48	2.60%	157.50	182.00	163.42	6.73	4.12%	147.00	175.00				
	Tarsus I	tI	331.23	8.39	2.53%	315.00	359.00	319.12	6.17	1.93%	304.50	332.50	325.37	9.55	2.93%	304.50	359.00	304.71	9.56	3.14%	291.00	333.00				
	Tarsus IV	t4	353.72	7.99	2.26%	336.00	374.50	346.19	8.20	2.37%	329.00	371.00	350.08	8.89	2.54%	329.00	374.50	339.23	7.47	2.20%	326.00	357.00				
MALES																										
	Measurement	Abbreviation	<i>P. scoritai</i> from Sucu Valley (n=47)						<i>P. scoritai</i> from Mt Mic (n=45)						<i>P. scoritai</i> (n=92)						<i>P. decebali</i> (n=41)					
			M	SD	CV	min	max	M	SD	CV	min	max	M	SD	CV	min	max	M	SD	CV	min	max				
	Length of idiosoma	LI	1092.13	26.22	2.40%	1026.60	1139.70	1121.72	30.33	2.70%	1044.00	1200.60	1106.60	31.84	2.88%	1026.60	1200.60	1089.83	24.84	2.28%	1009.20	1165.80				
	Length of tibia II	AB	181.22	3.00	1.66%	175.00	190.75	178.58	2.77	1.55%	175.00	185.50	179.93	3.17	1.76%	175.00	190.75	169.96	2.60	1.53%	162.75	175.00				
	Length of proximal apophysis	AC	112.56	3.54	3.14%	105.00	119.00	113.52	3.59	3.16%	105.00	122.50	113.03	3.58	3.17%	105.00	122.50	109.23	3.27	3.00%	103.25	117.25				
	Distance between seta a11 insertion and tibial margin	EF	37.83	1.98	5.24%	31.50	42.00	36.13	1.63	4.52%	33.25	40.25	37.00	2.00	5.41%	31.50	42.00	32.05	1.85	5.78%	28.00	35.00				
	Distance between distal apophysis and tibial margin	GH	26.03	1.44	5.54%	22.75	29.75	24.85	1.92	7.74%	21.00	29.75	25.45	1.79	7.02%	21.00	29.75	19.72	1.52	7.69%	17.50	22.75				
	Length of seta a11	a11	58.72	3.30	5.62%	52.50	68.25	57.79	2.75	4.77%	50.75	63.00	58.26	3.06	5.26%	50.75	68.25	53.18	2.68	5.04%	47.25	59.50				
	Length of seta a12	a12	104.85	4.66	4.44%	92.75	115.50	106.17	4.57	4.30%	98.00	115.50	105.49	4.64	4.39%	92.75	115.50	104.27	3.95	3.79%	94.50	113.75				
	Length of seta pv1	pv1	90.03	4.97	5.52%	78.75	99.75	89.72	4.71	5.25%	78.75	96.25	89.88	4.82	5.37%	78.75	99.75	79.43	4.68	5.89%	70.00	94.50				
	Tarsus I	tI	330.87	8.55	2.58%	315.00	350.00	317.09	8.41	2.65%	280.00	332.50	324.13	10.92	3.37%	280.00	350.00	306.98	6.97	2.27%	294.00	322.00				
	Tarsus IV	t4	351.49	6.48	1.84%	339.50	367.50	339.41	6.42	1.89%	325.00	353.50	345.58	8.83	2.56%	325.00	367.50	331.10	7.56	2.28%	318.50	350.00				

Table 2. Welch's *t*-tests for equality of means. Statistical tests were performed for all pairs of species and populations.

FEMALES		<i>P. scorilai</i> from Sucu Valley vs. <i>P. scorilai</i> from Mt Mic	<i>P. scorilai</i> from Sucu Valley vs. <i>P. decebali</i>	<i>P. scorilai</i> from Mt Mic vs. <i>P. decebali</i>	<i>P. scorilai</i> vs. <i>P. decebali</i>
Measurement	Abbreviation				
Length of idiosoma	LI	<0.001	<0.001	<0.001	<0.001
Length of epigynium	LE	<0.001	<0.001	<0.001	<0.001
Width of epigynium basis	WEB	0.29	0.08	<0.05	<0.05
Distance <i>st5-st5</i>	st5-st5	0.35	<0.001	<0.001	<0.001
Length of sternal scutum	LSS	0.24	<0.001	<0.001	<0.001
Width of sternal shield	WSS	0.99	0.79	0.82	0.77
Half width of posterior sternal scutum	W/2	0.22	<0.001	<0.001	<0.001
Tarsus I	t1	<0.001	<0.001	<0.001	<0.001
Tarsus IV	t4	<0.001	<0.001	<0.001	<0.001
Tarsus I/length of idiosoma	t1/LI	<0.001	<0.001	0.67	<0.001
Tarsus IV/length of idiosoma	t4/LI	<0.001	<0.01	<0.001	0.68
MALES		<i>P. scorilai</i> from Sucu Valley vs. <i>P. scorilai</i> from Mt Mic	<i>P. scorilai</i> from Sucu Valley vs. <i>P. decebali</i>	<i>P. scorilai</i> from Mt Mic vs. <i>P. decebali</i>	<i>P. scorilai</i> vs. <i>P. decebali</i>
Measurement	Abbreviation				
Length of idiosoma	LI	<0.001	0.67	<0.001	<0.01
Length of tibia II	AB	<0.001	<0.001	<0.001	<0.001
Length of proximal apophysis	AC	0.20	<0.001	<0.001	<0.001
Distance between seta al1 insertion and tibial margin	EF	<0.001	<0.001	<0.001	<0.001
Distance between distal apophysis and tibial margin	GH	<0.01	<0.001	<0.001	<0.001
Length of seta al1	al1	0.15	<0.001	<0.001	<0.001
Length of seta al2	al2	0.17	0.53	<0.05	0.12
Length of seta pv1	pv1	0.76	<0.001	<0.001	<0.001
Tarsus I	t1	<0.001	<0.001	<0.001	<0.001
Tarsus IV	t4	<0.001	<0.001	<0.001	<0.001
Tarsus I/length of idiosoma	t1/LI	<0.001	<0.001	0.63	<0.001
Tarsus IV/length of idiosoma	t4/LI	<0.001	<0.001	0.54	<0.001

measurement allowing the distinction of these two species because all overlapped to different extents (Fig. 6). Remarkably, the length of tibia II (AB) barely overlaps between the two species, and only so for the marginal value of 175.0 μm . The length of the proximal apophysis AC also has relatively low coefficients of variability within *P. scorilai* (3.14-3.16%) and *P. decebali* (3.00%), but important overlaps in values exist between both species. The distance GH has the largest coefficients of variation, especially for *P. decebali* and the *P. scorilai* population from Mt Mic (7.74% and 7.69%, respectively). However, this character seems interesting for species recognition as values only marginally overlap. The two ratios (t1/LI and t4/LI) also significantly differ between species,

although this difference disappears when restricting the comparison of *P. decebali* with *P. scorilai* from Mt Mic only (Table 2).

The PCA analysis resulted in a perfect segregation of *P. decebali* from *P. scorilai* along the first multivariate axis, which expresses about half of the total variance (Fig. 7). *Pergamasus scorilai* individuals exhibit larger values for many tibial measurements (AB, GH, EF, pv1 and al1). The length of the idiosoma, although statistically significantly different between the two species, was mostly contributing to the second axis and thus is not very useful for species recognition. The length of seta al2 is also contributing to the second axis, but it mostly represents intraspecific variation as it does not differ between both species.

The best model identified by the algorithm using the AIC measure contains only two measurements: the length of tibia II (AB) and the length of setae all1 (al1). The intercept and coefficient of the selected linear model provided the following discriminant function: $15476.0 - 80.04 \times AB - 26.68 \times al1$. The discriminant score calculated using this function was negative when applied to measurements of male *P. scorilai* specimens, and positive for *P. decebali* males. We propose a simplified, yet effective function inspired from the previous for species discrimination based on male specimens: $155 - 0.8 \times AB - 0.3 \times al1$.

Females: As in males, all morphological measurements but one (the width of the sternal shield) significantly differ between females of the larger *P. scorilai* and those of the smaller species *P. decebali* (Tables 1-2). For the eight measured characters the coefficient of variability ranges from 1.93% to 4.18% and is in general smaller than the values observed in males (1.53%-7.74%). However, all morphological characters overlap considerably between both species, so that no single character or combination of two characters can be used for species recognition. As in males, the ratio between the tarsus I and the idiosomal length (t1/LI) significantly differs between species, but not when only considering *P. scorilai* from Mt Mic (Table 2). The ratio between the tarsus IV and the idiosomal length (t4/LI) does not significantly differ between species, but significant differences were recovered when considering populations, because *P. scorilai* from Mt Mic and from Sucu Valley have lower and greater values, respectively, than *P. decebali* (Fig. 6).

In females species discrimination was not completely achieved in the bidimensional space maximising the total variance (Fig. 7). Similar to the PCA conducted on males, the first axis carries over 40% of the total

variance, with *P. scorilai* individuals having lower values along this axis than those of *P. decebali*. This position on the first axis correlates with higher values of many morphological measurements, including lengths of idiosoma, epigynum, tarsi and sternal scutum (Fig. 7).

The best model identified by the algorithm using the AIC measure contains no less than six measurements. The intercept and coefficient of the selected model provides the following discriminant function: $37020.41735 - 25.30969 \times LI - 113.96783 \times LSS + 143.80938 \times WSS - 73.66982 \times W/2 - 89.42145 \times t1 + 46.84512 \times t4$. The discriminant score calculated using this function is negative when applied to measurements of female *P. scorilai* specimens, and positive for *P. decebali* females. We propose an even more simplified, yet efficient function inspired from the previous one: $370.204 - 0.253 \times LI - 1.14 \times LSS + 1.438 \times WSS - 0.737 \times W/2 - 0.894 \times t1 + 0.468 \times t4$.

DISCUSSION

Overview of the *Pergamasus crassipes* species-group

Based on male characteristics, Athias-Henriot (1967) and Juvara-Balş (1970, 1973) listed as many as 15 described species in the *Pergamasus crassipes* species-group: *aequicornis*, *biharicus*, *buerebistai*, *caninatellus*, *crassipes*, *decebali*, *dumitrescui*, *laetus*, *longicornis*, *pinguicrus*, *palatortus*, *primorellus*, *scorilai*, *similicornis*, and *truatellus*. Males of most species can be easily separated using the nine measurements retained in our analyses (Fig. 2). The measurements of *P. aequicornis* from Connecticut, USA are similar to those of *P. decebali* from Romania (Fig. 2). However, *P. aequicornis* is very different from *P. decebali* by the

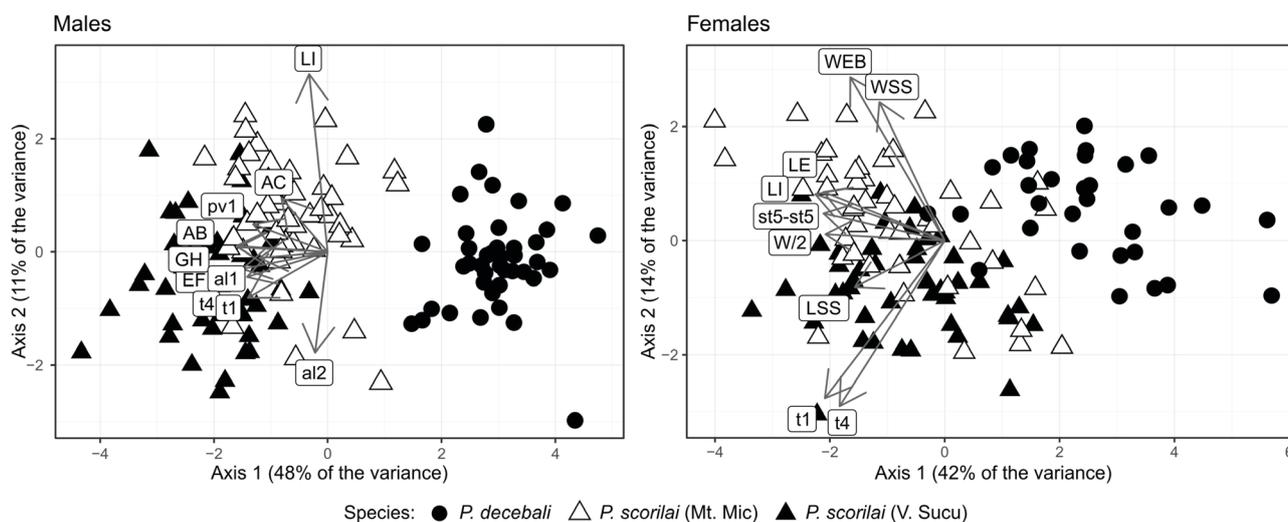


Fig. 7. Principal component analyses based on all morphometric characters of male and female *Pergamasus decebali* and *P. scorilai* individuals examined. Individuals are represented by different symbols according to species and geographical origin.

morphology of the chelicera and of the apophysis of tibia II (Fig. 3A cf. Fig. 3F). *Pergamasus primorellus* also lies close to small *P. decebali* specimens in the ACP representation. Males of the two species differ by the shape of the apophyses of tibia II (Fig. 3L cf. Fig. 3F), as well by the shape of the endogynium in females.

Finally, the case of *P. instipatus*, a species described from a single female, deserves special attention. Athias-Henriot (1967) considered the corresponding male to be unknown and thus the species was not assigned to any of the species-groups based on male characteristics. In the collection of Athias-Henriot held in the Natural History Museum of Geneva three presumably conspecific specimens from the same locality (Seppenbauer, northwestern Carinthia, Austria) were found. One female and one male (AN260 and AN261, respectively) identified as *P. caninatellus* were collected in the same sample under composite flowers, while the third specimen was a female (AK766) collected under *Trifolium* and *Plantago* at the same locality. The latter individual was tentatively identified as “cf. *instipatus*” in Athias-Henriot’s field notebook, which was then corrected as “*caninatellus*”. The comparison of these two *P. caninatellus* female specimens collected at Seppenbauer together with the holotype of *P. instipatus* shows that both nominal species are indistinguishable. Therefore we here place *P. instipatus* Athias-Henriot, 1967 in the synonymy of *P. caninatellus*, a conclusion that Athias-Henriot seemingly reached herself as indicated in her field notebook.

Variability and recognition of *Pergamasus scorilae* and *P. decebali*

Taxa are named and given representative type specimens, but they are also populations and aggregates of populations (Mayr, 1969). Therefore, assessment of the morphological variability and statistical analysis of the morphometric characteristics should be mandatory for taxonomic work. In particular, it is necessary to specify the limits of variability of qualitative and quantitative characters, especially in species with a similar morphology.

The morphological and morphometric analysis of over 250 specimens from Romania revealed that a fair amount of intraspecific morphological variability occurs in two similar species, *P. scorilae* and *P. decebali*. The morphological study of an extensive material allowed us to describe distinctive morphological characters, either found on tibia II or in the endogynium of males and females, respectively. Morphometric values overlap between both species for most of the other morphological measurements, complicating species recognition and giving some credit to previously expressed opinions which considered the two species as conspecific (Karg, 1993). However, multivariate analyses clearly

discriminate two morphometric groups, independent of the biotope they were collected in (Fig. 7) and are consistent with the morphology-based concepts of the two nominal species. Accordingly, the morphometric variation remains smaller at the intraspecific level than between the two species. Based on a combination of a few morphometric characters (two in males, six in females), we demonstrate that the species *P. decebali* and *P. scorilae* can be unambiguously separated. These results show the value of the characters that were established by Athias-Henriot (1967) for the diagnosis of the taxa included in the *Pergamasus crassipes* species-group.

Analysis of the variables showed that most of the retained male characteristics can be used for species distinction, especially the length of tibia II and the length of setae al1. The size of setae al2 does not significantly differ between the two species, and the length of the idiosoma overlaps greatly although the individual means are significantly different. The morphological analysis revealed other essential characters for the identification of males, which could not be included in our morphometric analyses: (i) the shape of the apex of the movable or fixed chelicera digit; (ii) the shape of the proximal tibial apophysis of leg II (AC) and especially its apical lobe, which although variable is showing patterns specific to each species; (iii) trochanter IV protrusions on the dorsal and ventral sides. In females the variable selection approach consistently requires as many as six measurements that allow species differentiation, with a weaker segregation of individuals in the multivariate analysis than observed in males. Interestingly, the width of the sternal scutum was retained in the discriminant function, although it was the only character which did not statistically differ between both species. Hence it appears that many additional morphological measurements should always be accounted for when diagnosing or describing females. Diagnoses of females of *P. scorilae* and *P. decebali* must be emended mainly by accounting for details of the endogynium, the shape of the protrusions of trochanter IV, and to a lesser extent the sterno-genital cuticle ornamentation. In females the phenotypes of the same species are so different that if one chooses a holotype without examining the variation of the endogynium, one would be convinced that other conspecific females belong to different species (Juvara-Balş, 1973: fig. VII A, F). Another feature that characterizes *P. decebali* females from the southern Carpathians lie in the reduced size of the protuberance on trochanter IV. Unfortunately, this character cannot be used in geographical regions where *P. decebali* coexist with *P. scorilae* (e.g., the western Carpathians - Mt Apuseni) since both species have a similar marked protuberance on trochanter IV in these areas (Juvara-Balş, 1973; fig. IV. I and fig. XI. I). The morphometric characters most useful for the identification of females of these two species are the half-length of the posterior sternal margin, and the lengths of sternal scutum, epigynium, tarsus I and tarsus IV.

In males and females *P. decebali* turned out to be more similar to *P. scorilai* from Mt Mic than to *P. scorilai* from Sucu Valley. We assume that this is due to some form of ecological convergence, as the two first mentioned populations were sampled in spruce forests situated above 1000 m a.s.l., while the Sucu Valley population occurs in a lowland beech forest. In particular, relative leg measurements of females (t1/LI and t4/LI) were lower in individuals sampled at higher elevations and higher in the population from lowlands. Although based on two sampling sites, these observations are congruent with the Allen's rule which postulates that "protruding body parts [...] are relatively shorter in cooler parts of the range of a species than in warmer parts" (Ray, 1960). Although originally formulated for thermoregulating animals, this rule was also found to apply to various arthropod groups (Ray, 1960; Bidau & Martí, 2008).

Pending questions regarding other species

Even partial knowledge of variability raises new problems concerning other species. One case involves *P. crassipes* and *P. primorellus*, which are similar and difficult to separate without taking into account the variability of the endogynium of females and that of the proximal apophysis of tibia II as well as other morphometric characters in males. Another case concerns the great resemblance between *P. dimitrescui* and *P. pinguicrus* which casts doubts on the species status of corresponding populations found in Romania. *Pergamasus dimitrescui* specimens from the southern Carpathian (Bucegi Massif; Mount Baiului) are morphologically similar to *P. pinguicrus* specimens described from Austria (Niedere Tauern, Admont).

The corresponding type series justified the description of these two nominal species at the time. In the description of *P. dimitrescui*, Juvara-Balş (1970) specified that this species differs from *P. pinguicrus* "by its smaller dimensions, the number of hypostomial ridges, and the lack of accessory denticles between the prongs of the gnathotectum". Studying other specimens from Austria (in the collection of Athias-Henriot) and Romania (eastern and southern Carpathian; Moldavian Hills) we observed that the variation of the endogynium (size of the stipula, denticles of the endogynial sack) and the degree of ornamentation of the sclerocuticle were similar in these two species. The male characteristics are fairly constant. The differences in males are expressed only in the distal apophysis of the tibia II which have a denticle at the base of setae all in *P. dimitrescui* but lack this denticle in *P. pinguicrus*.

The presented data lead us to consider these two nominal taxa as true species, but the variation of each is primarily statistical, with the studied specimens from populations in Romania being much smaller than those from populations in Austria. The upper limits of values

of many characters does not overlap with the lower limits of *P. pinguicrus* characters (Juvara-Balş, pers. obs.). However, the status of these species or subspecies is unclear until two points are clarified. First, there is a need to establish the variability of *P. pinguicrus* from the type locality in Austria (Leichenberg bei Admont). Second, the geographical ranges of the two taxa still need to be delimited. The Slovenian Alps, the Dinaric Range and the Balkan Peninsula are still "terra incognita" with respect to these taxa. Intergradation zones could exist, and a clinal variation cannot be excluded.

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