Morphological separation of *Trochosa robusta* (Simon, 1876) and *Trochosa ruricola* (De Geer, 1778) females (Araneae: Lycosidae)

Norbert Milasowszky¹, Marie Elisabeth Herberstein² and Klaus Peter Zulka¹

¹Institute of Zoology, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria

²Department of Zoology, University of Melbourne, Parkville, Victoria, 3052 Australia

Summary

Adult female individuals of the wolf spiders *Trochosa robusta* (Simon, 1876) and *Trochosa ruricola* (De Geer, 1778) from the Seewinkel area of Eastern Austria were morphometrically analysed. Continuous data of various epigyne and carapace dimensions were subjected to Principal Components Analysis and categorical data to Hierarchical Cluster Analysis. In Principal Components Analysis individuals could clearly be separated along one PC axis. Variables highly correlated with this axis were: (1) the maximum distance between the arches of the anterior transverse pockets, (2) the medial distance between the transverse pocket arches, (3) the maximum width of the triangular septum, and (4) the width of the epigynal plate. Categorical characters of the septal margins are convergent in *Trochosa robusta* and divergent in *Trochosa ruricola*, and (2) the dark marks anterior to the transverse pockets and the outer walls of the transverse pockets form a distinct concave contour line in *Trochosa robusta*. These results are discussed with regard to their limitation to a local area.

Introduction

Within the wolf-spider genus *Trochosa* (C. L. Koch, 1848), five species occur in Central Europe: *T. hispanica* Simon, 1870; *T. robusta* (Simon, 1876); *T. ruricola* (de Geer, 1778); *T. spinipalpis* (F. O. P.-Cambridge, 1895); *T. terricola* Thorell, 1856 (see Hänggi *et al.*, 1995). These five species are very similar in their morphology (size, genitalia) and are thus considered sibling species (Engelhardt, 1964).

Separation of the females is particularly difficult and several attempts to distinguish preserved material have been undertaken (e.g. Chrysanthus, 1955; Buchar, 1959; Engelhardt, 1964). On the basis of his comprehensive study, Engelhardt (1964) concluded that the high variability of epigyne and vulva characters makes it impossible to use these organs for an exact differentiation of the species. Engelhardt (1964) regarded body coloration of living specimens, especially in the females, as the only reliable character to distinguish the species. However, in recent determination keys, genital characters are used to separate the *Trochosa* females to some extent (e.g. Tanaka, 1988; Roberts, 1995). Roberts (1985) pointed out that the "overall impression" of the epigyne structure is more informative than comparison of single parts. The same is true for differences in body morphology.

In the present study, we attempt a morphological separation of *T. robusta* and *T. ruricola* females using multivariate statistical analyses. We used Principal Components Analysis as a tool to condense interrelated variables into a small number of components that can be used to quantify the "overall impression" mentioned above in terms of size and shape.

Material and methods

The female *Trochosa* specimens come from two separate studies (Löffler, 1993;



Milasowszky & Zulka, 1994), both carried out on the shores of saline pans in the Seewinkel area in Eastern Austria. Here, only males of *T. robusta* and *T. ruricola* have been collected and therefore only the corresponding females were expected. All spiders were preserved in 70% alcohol.

A total of 18 characters were examined on each of 79 individuals including 12 continuous Fig. 1: Schematic drawing of a Trochosa epigyne showing the continuous (A-I) and categorical (L-M) variables used in this study. Arrows point at categorical characters. A = height of epigynal plate, B = width of epigynal plate, C = maximum width of triangular septum, D = distance between the posterior end of the septum and the posterior end of the spermatheca, E =outer distance between the septal margins before extending posteriad into the triangular septum, F = distance between the distal part of the transverse pockets and the distal transverse edge of the epigvnal plate, G = maximum distance between the arches of the anterior transverse pockets, H = medial distance between the transverse pocket arches, I = distance between the inner edges of the distal part of the transverse pockets, L = distal part of the septal marginsconvergent or divergent, M = dark marks anterior to the transverse pockets and the outer walls of the transverse pockets form a distinct concave contour line or not.

(interval-scaled) and six categorical (ordinal-scaled) characters.

The continuous characters (Table 1) comprised three variables on the carapace and nine on the epigyne (Figs. 1A–I; 2–3). Measurements (exclusively performed by M. E. Herberstein) were made using a dissecting microscope fitted with an eyepiece micrometer. Data were subjected to Principal Components Analysis (PCA)

Trochosa robusta

Trochosa ruricola

Character	Mean±CL	Range	Mean ± CL	Range	% Overlap
Α	0.562 ± 0.019	(0.500 - 0.675)	0.500 ± 0.011	(0.375 - 0.600)	77.2
В	0.564 ± 0.016	(0.500 - 0.675)	0.490 ± 0.010	(0.400 - 0.625)	75.1
С	0.382±0.013	(0.300 - 0.450)	0.303±0.009	(0.200 - 0.375)	67.2
D	0.096 ± 0.006	(0.050 - 0.125)	0.098±0.003	(0.075 - 0.125)	98.1
Е	0.099 ± 0.002	(0.075 - 0.100)	0.097±0.003	(0.075 - 0.125)	98.1
F	0.248±0.017	(0.200 - 0.375)	0.229±0.010	(0.150 - 0.325)	95.3
G	0.318±0.013	(0.250 - 0.425)	0.207±0.005	(0.175 - 0.250)	6.6
Н	0.061 ± 0.007	(0.025 - 0.100)	0.028±0.003	(0.000 - 0.050)	80.5
I	0.073 ± 0.007	(0.050 - 0.100)	0.096±0.003	(0.050 - 0.125)	99.0
Carapace length	4.161±0.131	(3.475-4.900)	3.999±0.104	(3.200 - 4.800)	94.3
Carapace width	3.069±0.129	(2.400 - 3.700)	2.919±0.088	(2.350 - 3.625)	97.2
Fovea length	0.602 ± 0.034	(0.400 - 0.800)	0.564 ± 0.020	(0.400 - 0.725)	98.1

Table 1: Means (in mm), confidence limits (95%) and range (minimum-maximum) of morphological measurements for *Trochosa robusta* (n = 27) and *T. ruricola* (n = 52) specimens. Measuring accuracy is ± 0.0125 mm. The overlap was calculated as the percentage of specimens lying within the same range in comparison to the total.



Figs. 2–3: Epigynes. **2** *Trochosa robusta* ("Kleine Neubruchlacke", 30 April 1994, leg. Milasowszky & Zulka); **3** *T. ruricola* ("Untere Fuchslochlacke", 7 June 1993, leg. Milasowszky & Zulka). Scale line = 0.5 mm.

using the correlation matrix and varimax rotation (James & McCulloch, 1990; Norušis, 1993). Only principal components that accounted for variances greater than 1 (Kaiser-Guttman criterion) were used to represent the data.

Among the six categorical characters, four variables were obtained from the dentition of the inner and outer row of the left and the right cheliceral margin and two variables (Fig. 1L–M) were obtained from the epigyne. These six variables were subjected to Hierarchical Cluster Analysis (HCA) using the squared euclidian distance and the average linkage between groups (UPGMA) as cluster method (Norušis, 1993).

All statistical analyses were performed using SPSS for Windows, Version 6.0 (Norušis, 1993).

Results

Hierarchical Cluster Analysis

In the first step, HCA separated the 79 *Trochosa* females into two subgroups comprising 27 and 52 specimens. The first group was tentatively assigned to *T. robusta* and the second

group to *T. ruricola*. This assignment of the specimens was maintained in the subsequent Principal Components Analysis.

Principal Components Analysis

In PCA, three principal components with eigenvalues greater than 1 were extracted (Table 2). A clear separation of the *T. robusta*-group and *T. ruricola*-group was possible along PC 2 which can be interpreted as an epigyne width axis (Table 2; Figs. 4, 6). Characters highly correlated with this axis were (1) G, the "maximum distance between the arches of the anterior transverse pockets"; (2) H, the "medial distance between the transverse pocket arches"; (3) C, the "maximum width of the triangular septum"; and (4) B, the "width of the epigynal plate" (Fig. 1; Table 2).

In contrast, large overlaps between the species groups resulting from HCA were found along PC 1 and PC 3 (Figs. 4–6). PC 1 was highly correlated with carapace characters (Table 2), PC 3 was highly associated with the epigyne character D, the "distance between the posterior end of the the septum and the posterior end of the spermatheca" (Fig. 1; Table 2).

Character	PC 1	PC 2	PC 3
Carapace length	0.87	0.13	0.08
Carapace width	0.85	0.11	0.16
Fovea length	0.80	0.11	-0.07
Α	0.64	0.52	-0.21
E	0.54	0.04	0.01
B	0.53	0.72	0.04
С	0.49	0.74	0.07
F	0.42	0.15	0.09
D	0.14	0.02	0.92
G	0.14	0.93	0.01
Η	0.11	0.79	-0.22
I	0.06	-0.67	-0.28
% Variance			
explained	43.4	15.3	8.9

Table 2: Correlation coefficients resulting from Principal Components Analysis using varimax rotation and eigenvalues greater than 1.

Single characters

Descriptive statistics for each of the 12 morphological measurements of the two *Trochosa* species are presented in Table 1. In most characters *T. robusta* is larger than *T. ruricola*.

Within the morphometric data set, the character G, the "maximum distance between the arches of the anterior transverse pockets" had the smallest range of overlap with 6.6%(Table 1). The overlap was due to two specimens of *T. robusta* and three of *T. ruricola* having the same size in this character. The same character showed the highest correlation with the separating PC axes in the PCA (see above).

No single morphometric characters, but two single categorical characters, separated the species completely. These are (1) L, the "distal part of the septal margins" (Fig. 1) that are convergent in *T. robusta* (Fig. 2) and divergent in *T. ruricola* (Fig. 3); and (2) M, the "dark marks anterior to the transverse pockets" which form a distinct concave contour line with the outer walls of the transverse pockets in *T. robusta* (Figs. 1–2).

The four dentition characters on the cheliceral margins show large overlaps, measured as the percentage of the individuals of the two species groups possessing the same feature: 57.1% for the inner row of the left cheliceral margin, 58.7% for the inner row on the right cheliceral margin, 100% for the outer row left cheliceral margin and 98.1% for the outer row right cheliceral margin. All T. robusta specimens had a 3+3 dentition (sensu Engelhardt, 1964, table 4) at the inner cheliceral margin. In T. ruricola, the combinations of the dentition were 2+2 (n = 38, 71.1%), 2+3 (n = 6, 11.5%), 3+2 (n = 5, 9.6%) and 3+3 (n = 3, 5.8%). Thus, only 5.8% of T. ruricola specimens had the same combination as T. robusta specimens.

Discussion

The morphological analysis of somatic and genitalic characters of *T. robusta* and *T. ruricola* from Eastern Austria showed that separation of the species is possible in the studied material.



Figs. 4–6: Scatter plots of scores resulting from Principal Components Analysis with morphometric characters representing *Trochosa* females on the three principal components axes (PC 1–3). **4** PC 1 versus PC 2; **5** PC 1 versus PC 3; **6** PC 2 versus PC 3. Solid squares = *T. robusta* (n = 27), open squares = *T. ruricola* (n = 52).

The multivariate separation displayed on a two-dimensional plot was possible along the second principal component. In contrast no single continuous character considered in the present study permitted a reliable separation. This confirms the findings of previous authors (e.g. Locket *et al.*, 1974).

The character G, the "maximum distance between the arches of the anterior transverse pockets", is highly correlated with the second principal component. Character G also showed the lowest range of overlap (6.6%) between the two species. It can be recommended with some reservation for the separation of *T. robusta* and *T. ruricola*, in contrast to other single characters that show large overlaps.

Considering the categorical characters, the dentition on the cheliceral margin in our material agrees with the data given in the literature to some extent. However, considering the 3+3 dentition, the percentage of *T. robusta* females possessing this combination was higher in our study (100%) than those given in Buchar (1959, table 1, 80%) and Engelhardt (1964, table 4, 87.8%); whereas in *T. ruricola* females, a 3+3 dentition in 5.8% of our material confirms the findings of Buchar (1959, table 1, 7%) but is not in accordance with Engelhardt (1964, table 4, 26.4%). In any case, dentition is an unreliable feature for identification.

Roberts (1995) has already mentioned that the dark marks anterior to the arches are different between *T. robusta* and *T. ruricola*. We found that this feature (= character M, the "dark marks anterior to the transverse pockets" in our study) always forms a distinct concave contour line with the outer walls of the transverse pocket in *T. robusta*, but not in *T. ruricola*.

With regard to character L, the "distal part of the septal margins", drawings of *T. robusta* and *T. ruricola* epigynes in the literature are quite ambiguous. In contrast to our findings, Simon (1937) presented this feature as clearly divergent in *T. robusta* (fig. 1743) and convergent in *T. ruricola* (fig. 1737), as did Fuhn & Niculescu-Burlacu (1971, figs. 110a, 111a,b). Also, the drawings by Tanaka (1988, figs. 13, 5) do not clearly conform to our findings in this character. Only the drawings of Locket & Millidge (1951, fig. 136a, b) and Roberts (1995, 101, 100) seem to agree with our results. This study was based on specimens from a geographically limited area. It remains to be confirmed whether the suggested separating characters will also hold in other European regions. Moreover, since our comparison only considered two out of five Central European *Trochosa* sibling species, the application of our findings might also be limited in areas where *T. robusta* and/or *T. ruricola* co-occur with other *Trochosa* species.

Acknowledgements

This study was financed by the Ministry of Science and the Federal Government of Burgenland. We are grateful to Dr Michael Rasser and Dr Christine Exner for the photographs. We also wish to thank Professor Hannes F. Paulus and Doz. Karl Sänger for providing laboratory equipment.

References

- BUCHAR, J. 1959: Beitrag zur Bestimmung der mitteleuropäischen Arten der Gattung *Trochosa* (C. L. Koch) (Araneae: Lycosidae). *Acta Univ. Carol., Biol.* 3: 159–164.
- CHRYSANTHUS, F. 1955: Notes on spiders II. About some females of the genus *Trochosa* (C. L. Koch). *Ent. Ber. Berl.* **15**: 515–520.
- ENGELHARDT, W. 1964: Die mitteleuropäischen Arten der Gattung *Trochosa* C. L. Koch, 1848 (Araneae, Lycosidae). Morphologie, Chemotaxonomie, Biologie, Autökologie. Z. Morphol. Ökol. Tiere 54: 219–392.
- FUHN, I. E. & NICULESCU-BURLACU, F. 1971: Fauna Republicii Socialiste România. Arachnida 5 (3) Fam. Lycosidae. Bucureşti: Editura Academiei Republicii Socialiste România.
- HÄNGGI, A., STÖCKLI, E. & NENTWIG, W. 1995: Lebensräume mitteleuropäischer Spinnen. *Miscnea faun. helv.* 4: 1–460.
- JAMES, F. C. & McCULLOCH, C. E. 1990: Multivariate analysis in ecology and systematics: panacea or Pandora's box? A. Rev. Ecol. Syst. 21: 129–166.
- LOCKET, G. H. & MILLIDGE, A. F. 1951: British spiders, I. London: Ray Society.
- LOCKET, G. H., MILLIDGE, A. F. & MERRETT, P. 1974: *British spiders*, **III**. London: Ray Society.
- LÖFFLER, B. 1993: Einfluß der Beweidung auf die Arthropodenfauna der Trockenwiesen im Seewinkel (Burgenland). Diploma thesis, University of Vienna.

- MILASOWSZKY, N. & ZULKA, K. P. 1994: Arthropodenzönosen der Salzlacken im Seewinkel als Grundlage für die Naturschutzarbeit. Unpublished Report.
- NORUŠIS, M. J. 1993: SPSS for Windows. Professional Statistics. Release 6.0. Chicago: SPSS Inc.
- ROBERTS, M. J. 1985: *The spiders of Great Britain* and Ireland, **1**. Colchester, Essex: Harley Books.
- ROBERTS, M. J. 1995: Collins field guide. Spiders of Britain & Northern Europe. London: HarperCollins.
- SIMON, E. 1937: *Les arachnides de France*, **6**. Paris: Roret.
- TANAKA, H. 1988: Lycosid spiders of Japan II. The genus *Trochosa* C. L. Koch. *Acta arachn. Tokyo* 36: 93–113.