Phylogeny of the *Pardosa lugubris* species group (Arachnida, Aranei)

Evgenia Propistsova,
Entomology Department
Biological faculty
Moscow State University
Moscow 119992, Russia
+79032693822
Email: evgenya.jeny@yandex.ru, evgenya.jeny@gmail.com
WWW: https://istina.msu.ru/profile/evgenya.jeny/

Pardosa lugubris species group

- Includes seven species: *Pardosa alacris* (C.L. Koch, 1833); *P. baehrorum* Kronestedt 1999; *P. caucasica* Ovtsharenko, 1979; *P. lugubris* (Walckenaer, 1802); *P. pertinax* (Helversen, 2000); *P. koponeni* Nadolny et al., 2016 and *P. saltans* Töpfer-Hofmann, 2000.
- Five species are found in Europe, *P. koponeni* is found in eastern Europe and Asia, and only *P. lugubris* is distributed throughout the Palaearctic and covers the ranges of other species, except for *P. koponeni*
- The identification of the species of the group is carried out according to the genitals of males, the coloring of the limbs, the ratio of the lengths of the segments of the legs and carapace are used as additional signs
- The species of the group differ well in mating dances, size and genitalia of males, with the exception of the almost identical *P. koponeni* and *P. lugubris*
- The females of the species of the group do not differ in morphological characteristics; the determination is made by males caught in the same area

Pardosa alacris https://araneae.nmbe.ch/

Aim

The aim of our work was to clarify the phylogenetic relationships between the species of the *Pardosa lugubris* group using molecular phylogenetics and morphometric methods

Tasks

- Evaluate the effectiveness of various molecular markers for separating species within a group
- Check the effectiveness of linear and geometric morphometry methods for separating species within a group
- Clarify the status of the group species based on the obtained molecular and morphological data

Materials and methods

- 183 spiders of the genus *Pardosa* were examined
- The work was carried out with our own collections, the zoological collections of Munich, the Zoological Museum of Frankfurt, the Zoological Museum of Vienna, the Zoological Museum of the Lomonosov Moscow State University and the Zoological Institute of the Russian Academy of Sciences
- Type specimens of the species *Pardosa lugubris* and *Pardosa baehrorum* were also processed

Zoological Museum of Vienna

Materials and methods

Molecular genetics:

- Working with two genes: COI, 12S (mitochondrial)
- The selection of author's primers was carried out in the UGENE program
- DNA isolation was performed by boiling, PCR was performed (primer annealing temperature 50 ° C), then horizontal electrophoresis in agarose gel
- Forward and reverse sequences were combined in the SeqMan program, the General Time Reversible model was calculated in the MrModeltest program, alignments and trees were built in the Mega6 program using the ML method (Maximum Likelihood)

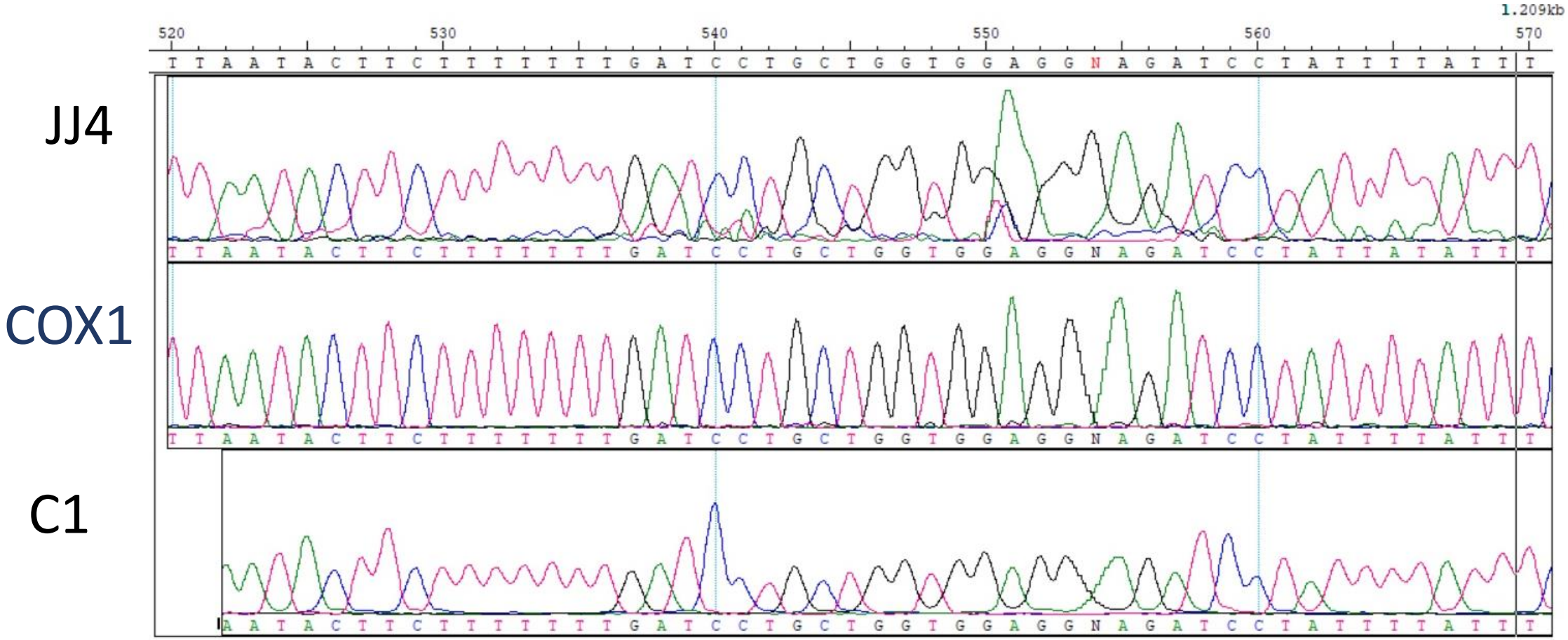
Linear morphometry:

- The length of the Carapace, the segments of all walking legs of males and females, and the male genitals were measured using an MC-4-ZOOM LED binocular micrometer with an eyepiece micrometer
- The results were processed in the Statistica6 program using discriminant analysis

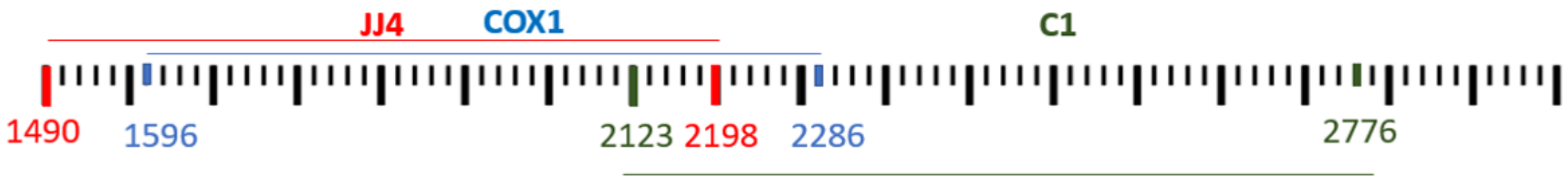
Geometric morphometry of female genitalia:

- Landmarks were placed in the tpsDig program, processed in the MorphoJ program using Procrustes coordinates

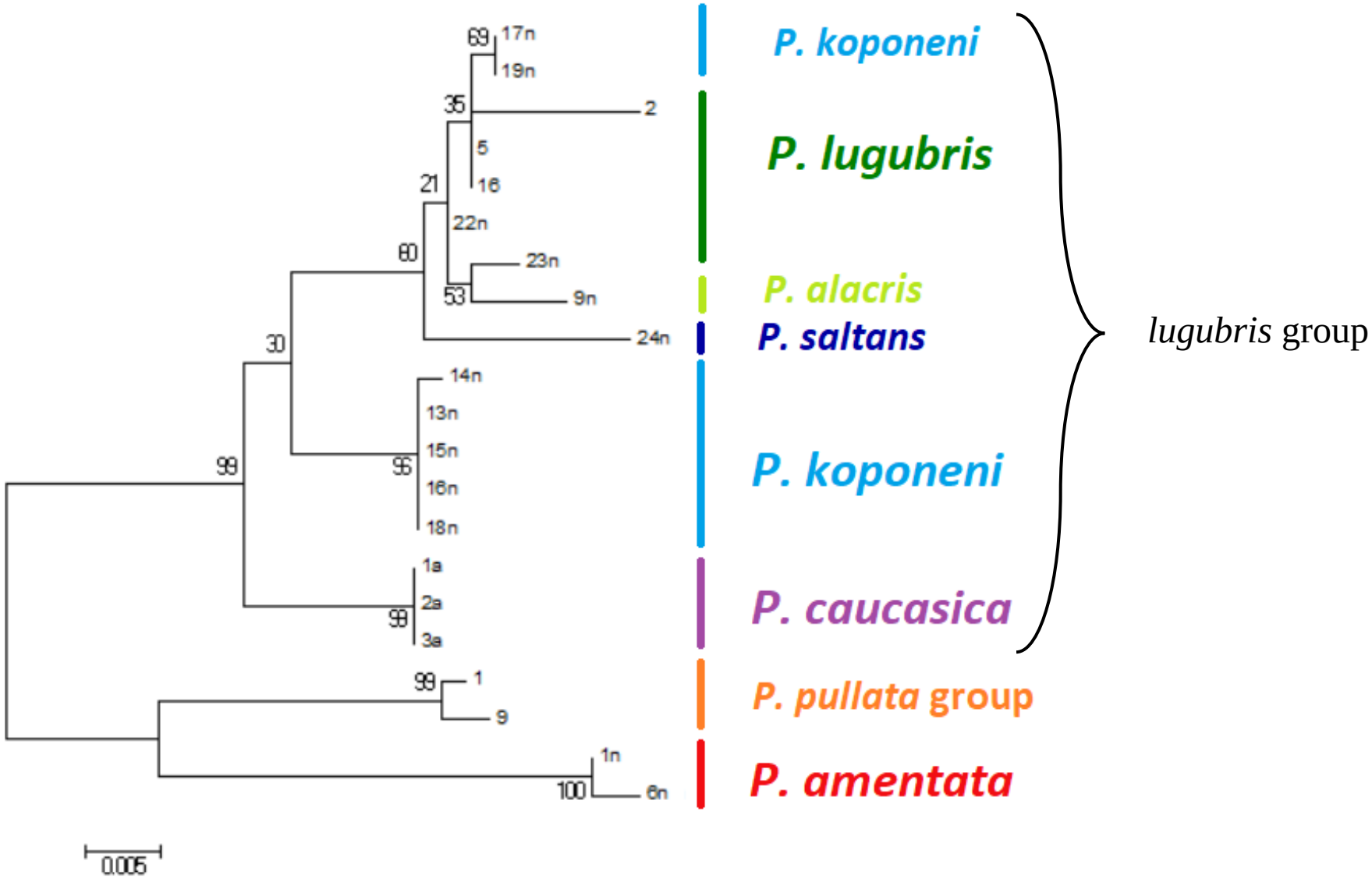
Aligned chromatograms of three regions of the COI gene from three pairs of primers



The most stable results were shown by a pair of primers COX1-F-1596 and COX1-R-2286

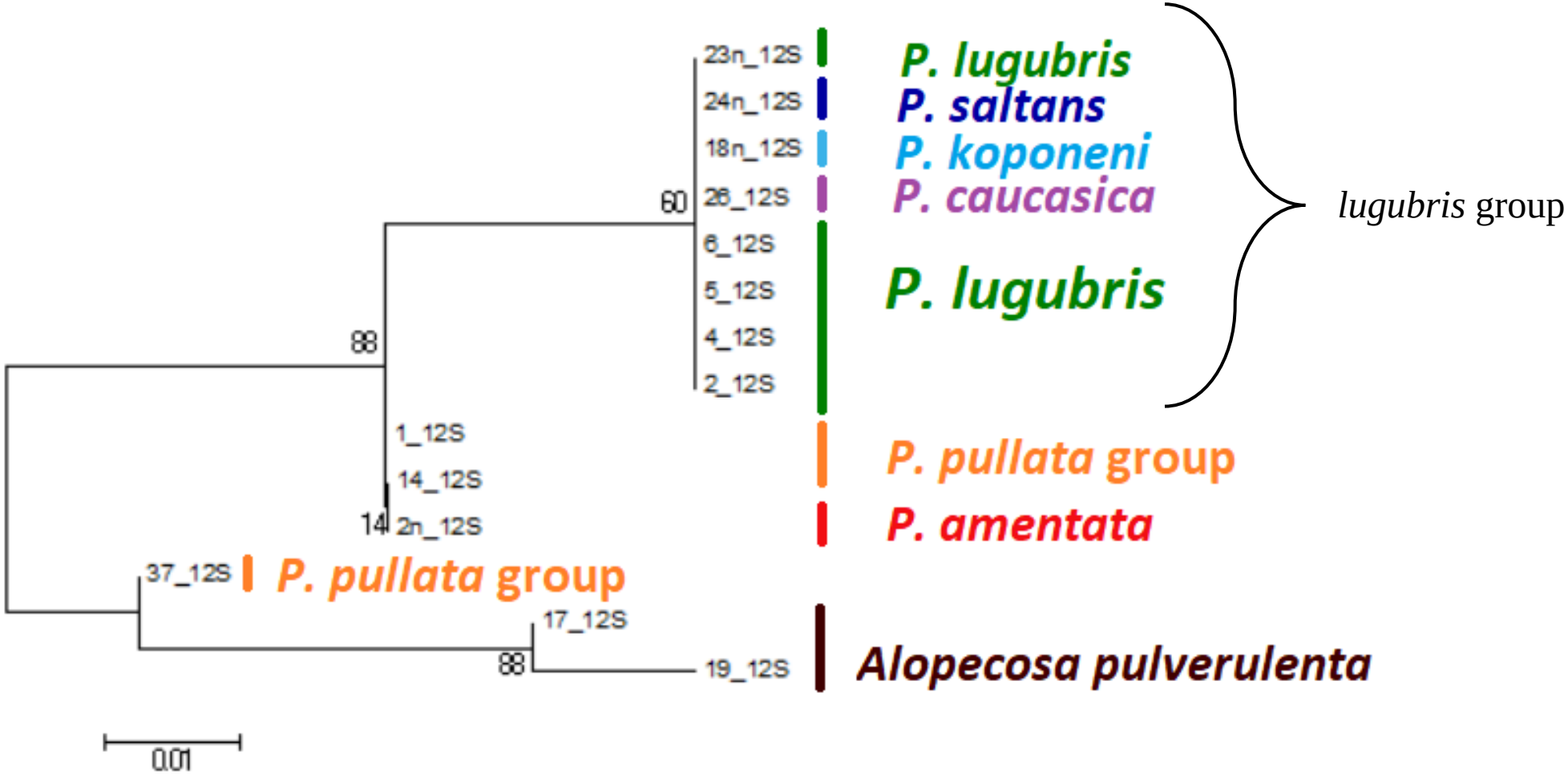

JJ4
COX1
C1
1490
1596
2123
2198
2286
2776

Phylogeny of the *Pardosa lugubris* species group based on COI gene sequences (total of three primer pairs)



Alignment length 1286 bp

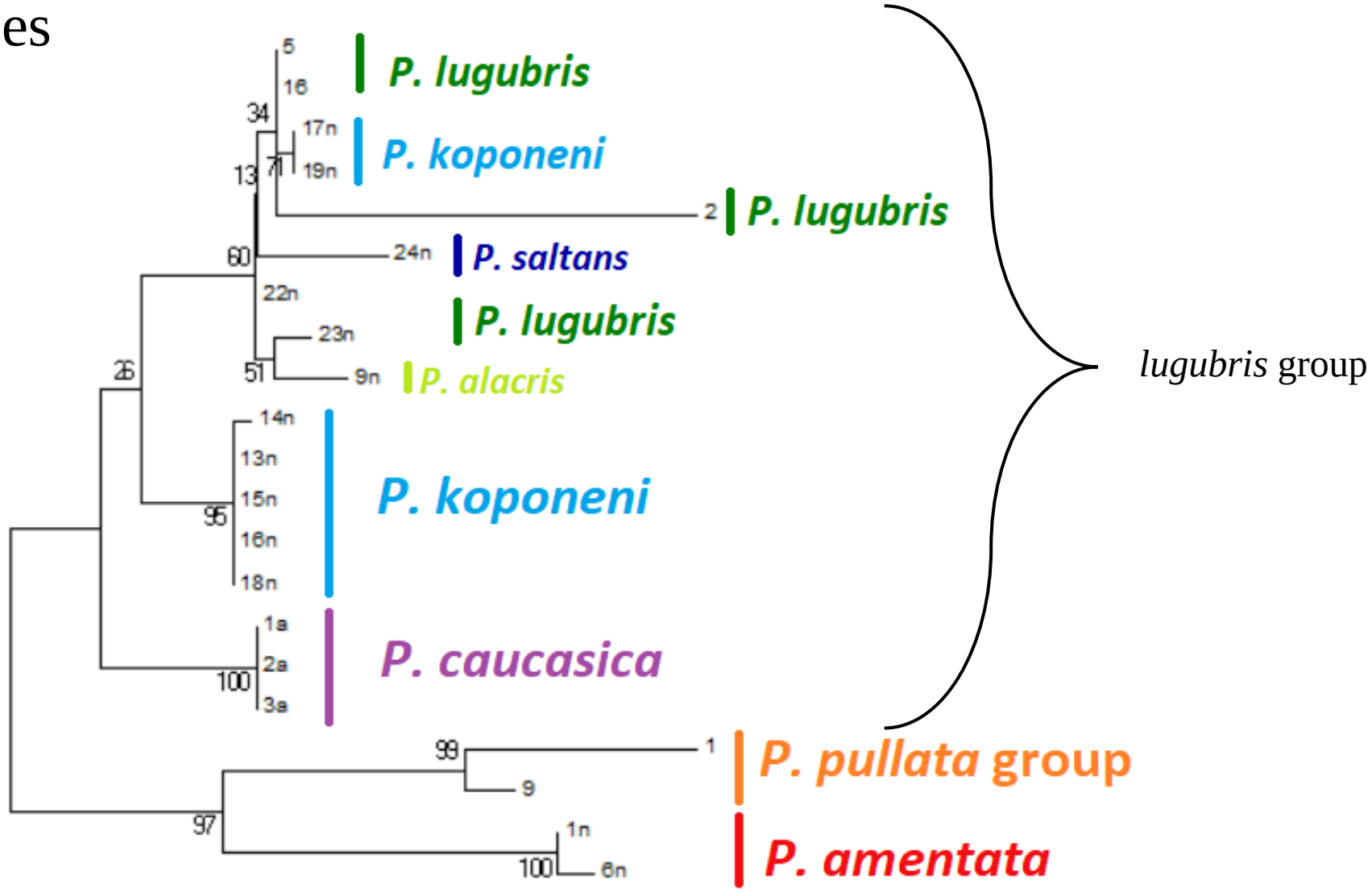
Phylogeny of the *Pardosa lugubris* species group based on 12S gene sequences



Alignment length 313 bp

Primers 12St-L, 12S-bi are taken from Murphy et al, 2006

Phylogeny of the *Pardosa lugubris* species group based on COI and 12S gene sequences

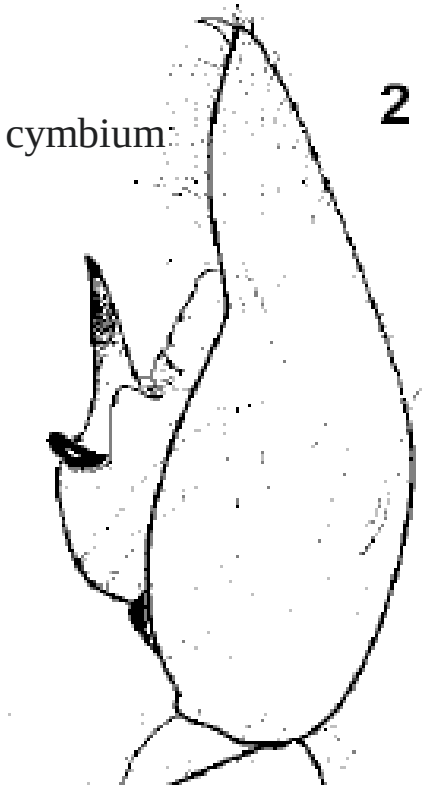


Alignment length 1599 bp

0.005

Genitalia of males of the group, lateral view

Pardosa lugubris



Pardosa koponeni

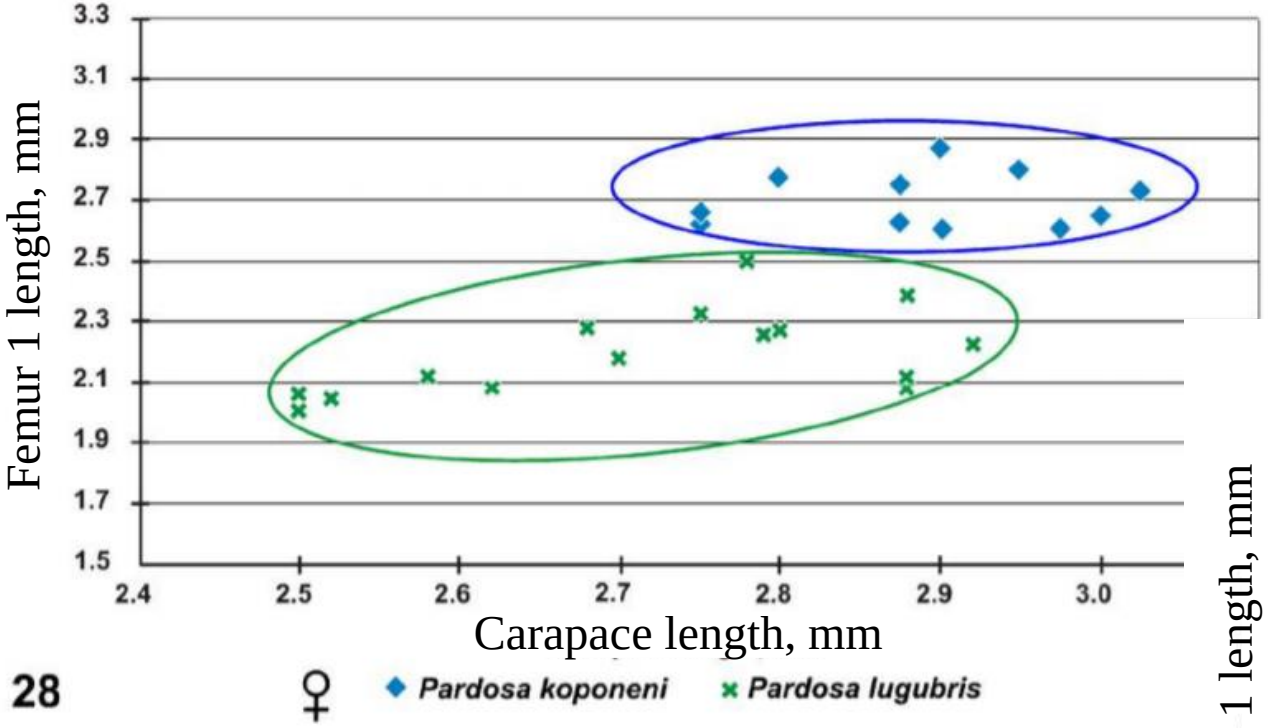
Pardosa alacris

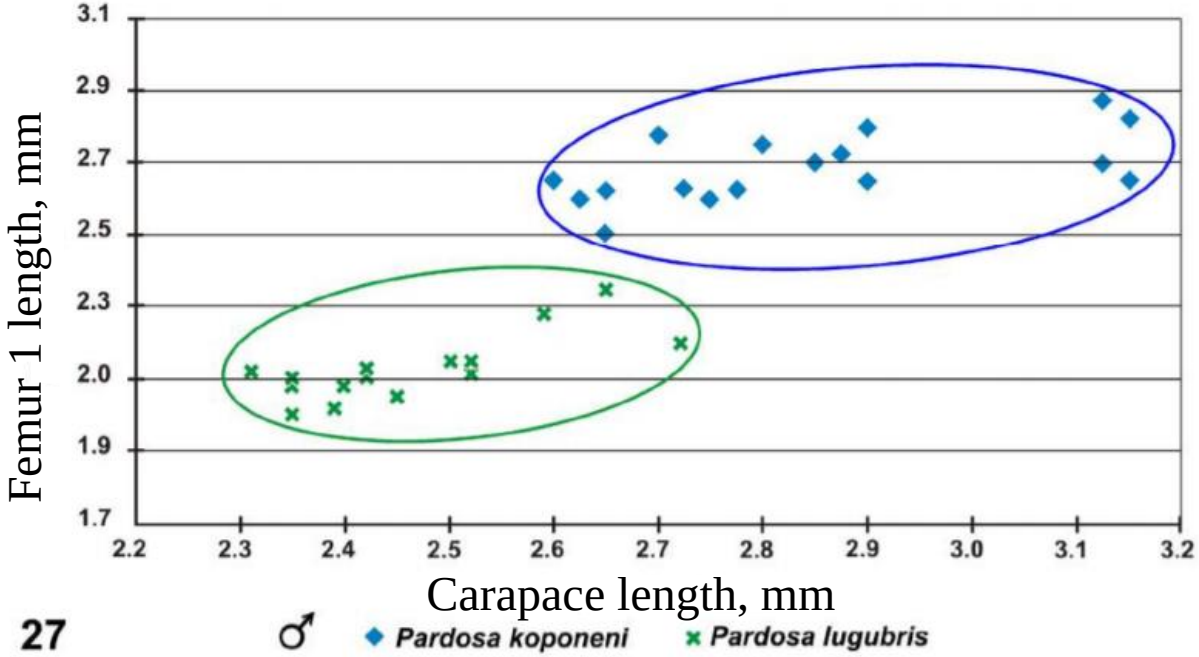
Pardosa saltans

Pardosa caucasica

https://arachno.piwigo.com/
https://araneae.nmbe.ch/

Morphometry of legs and Carapace





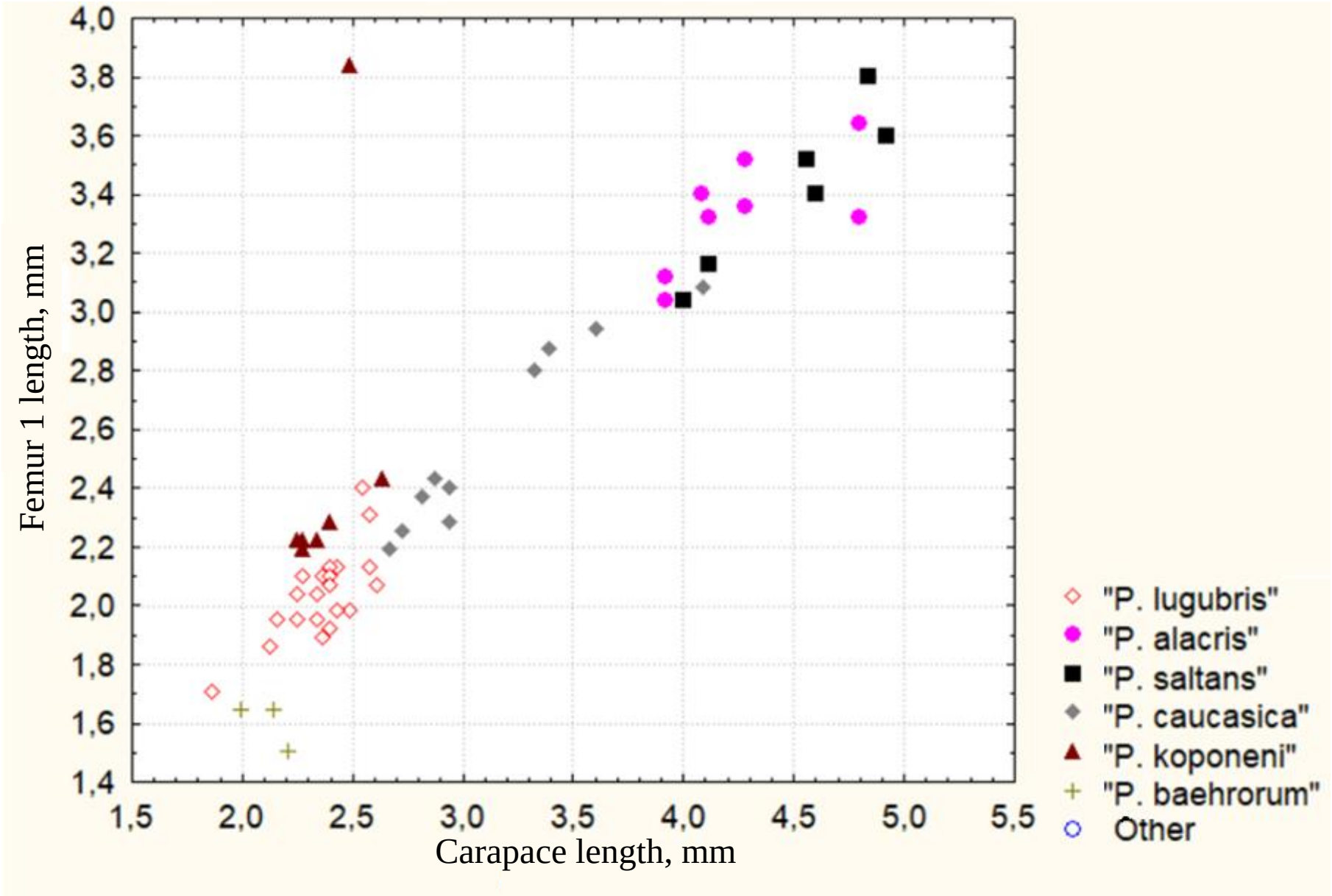
Nadolny A. A. et al. A new species of spider belonging to the Pardosa lugubris-group (Araneae: Lycosidae) from Far East Asia //Zootaxa. – 2016. – Т. 4072. – №. 2. – С. 263-281.


carapace

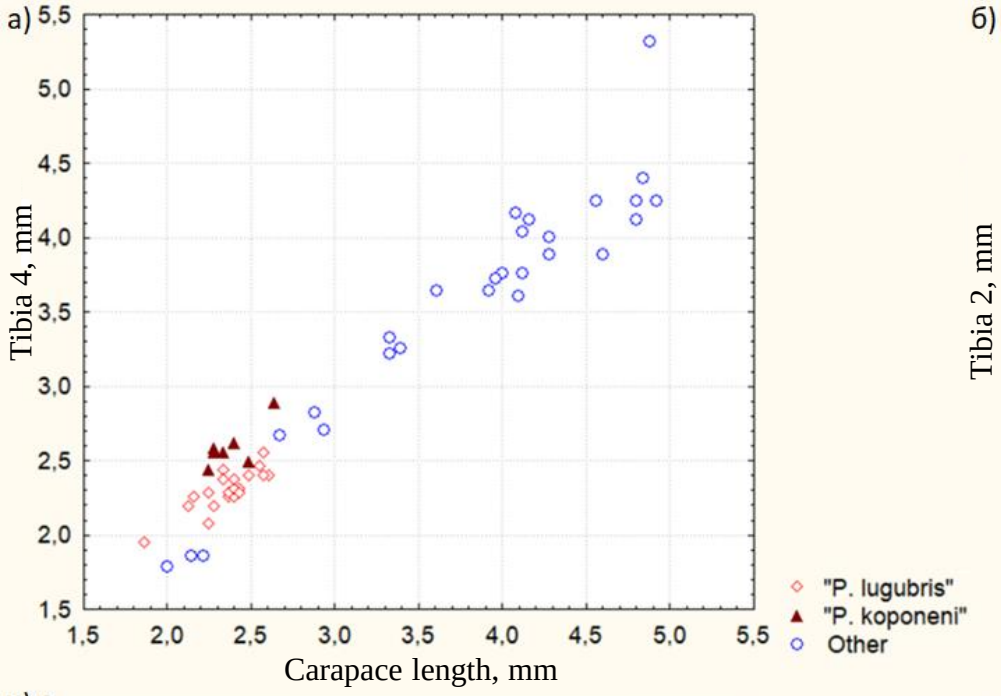

tarsus
metatarsus
tibia
patella
femur

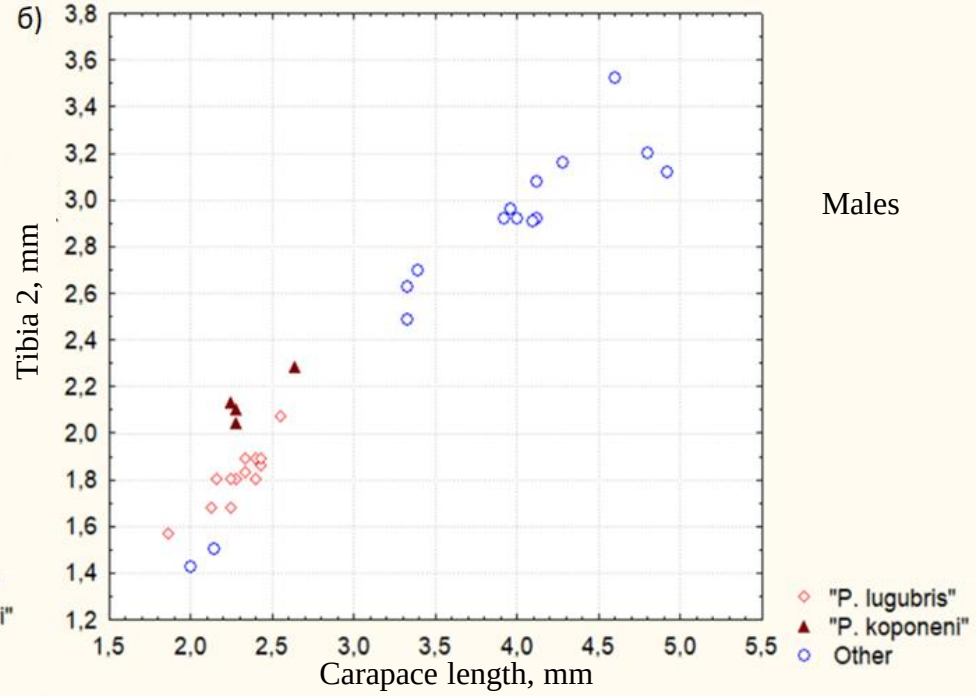

coxa

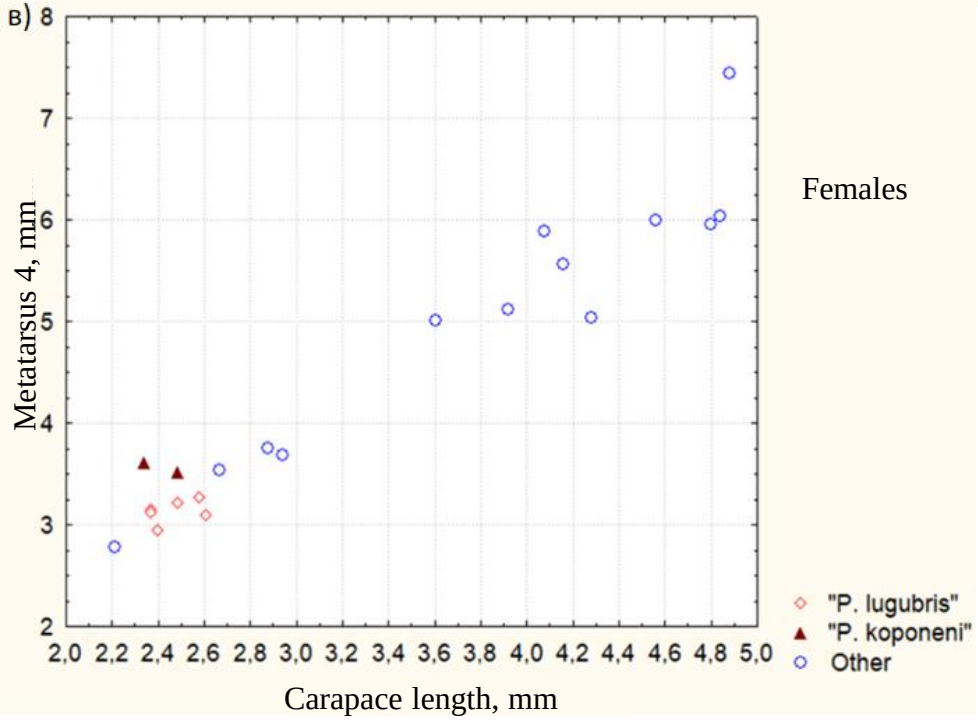
Scatterplot for the ratio of the carapace length to the thigh length of the first leg

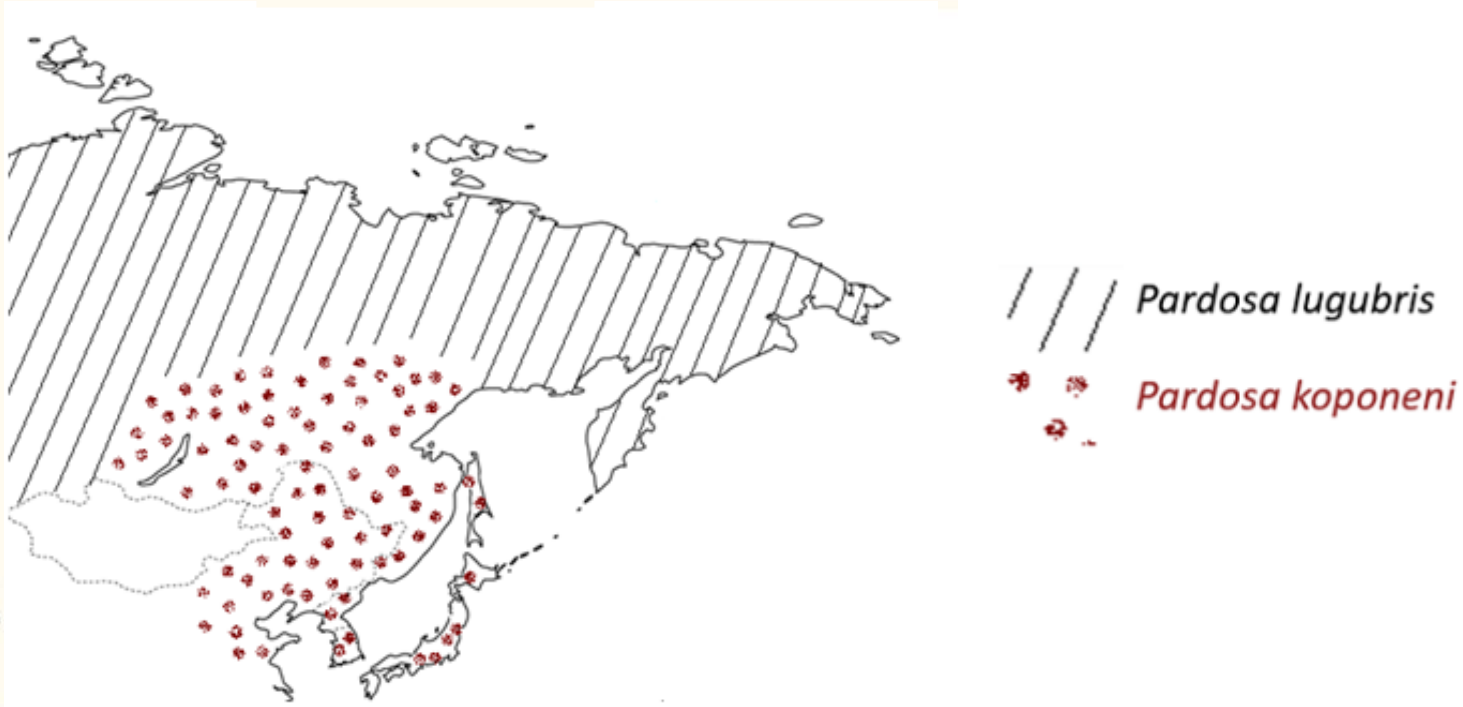



Pardosa lugubris
Pardosa koponeni
Pardosa alacris
Pardosa saltans

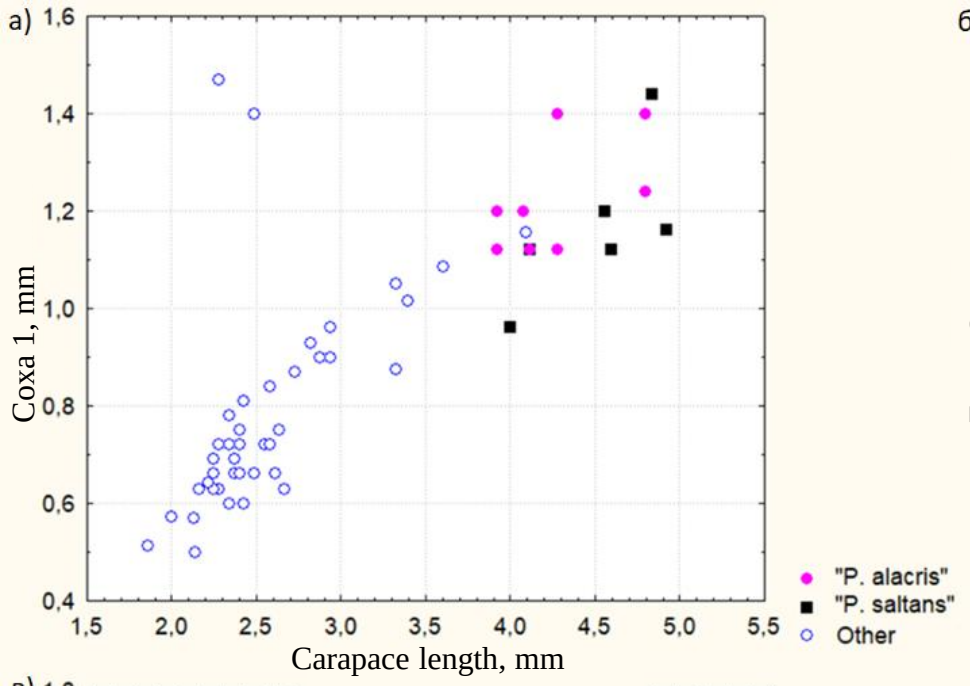


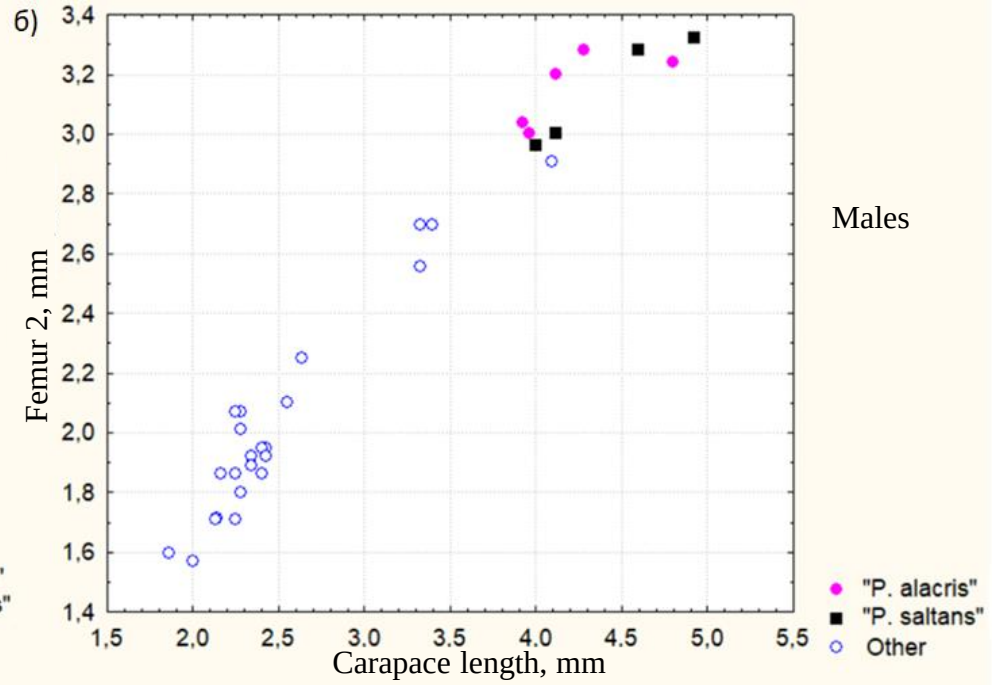


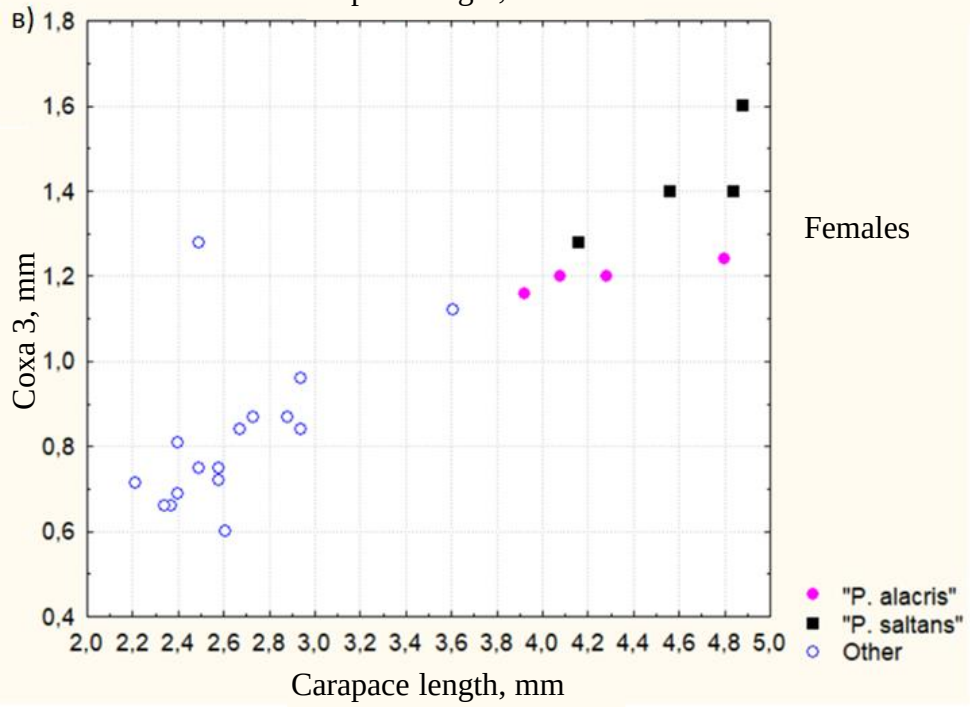




Scatterplots for the ratios of the Carapace length to the measurements of walking legs in a pair of *P. lugubris* and *P. koponeni* species







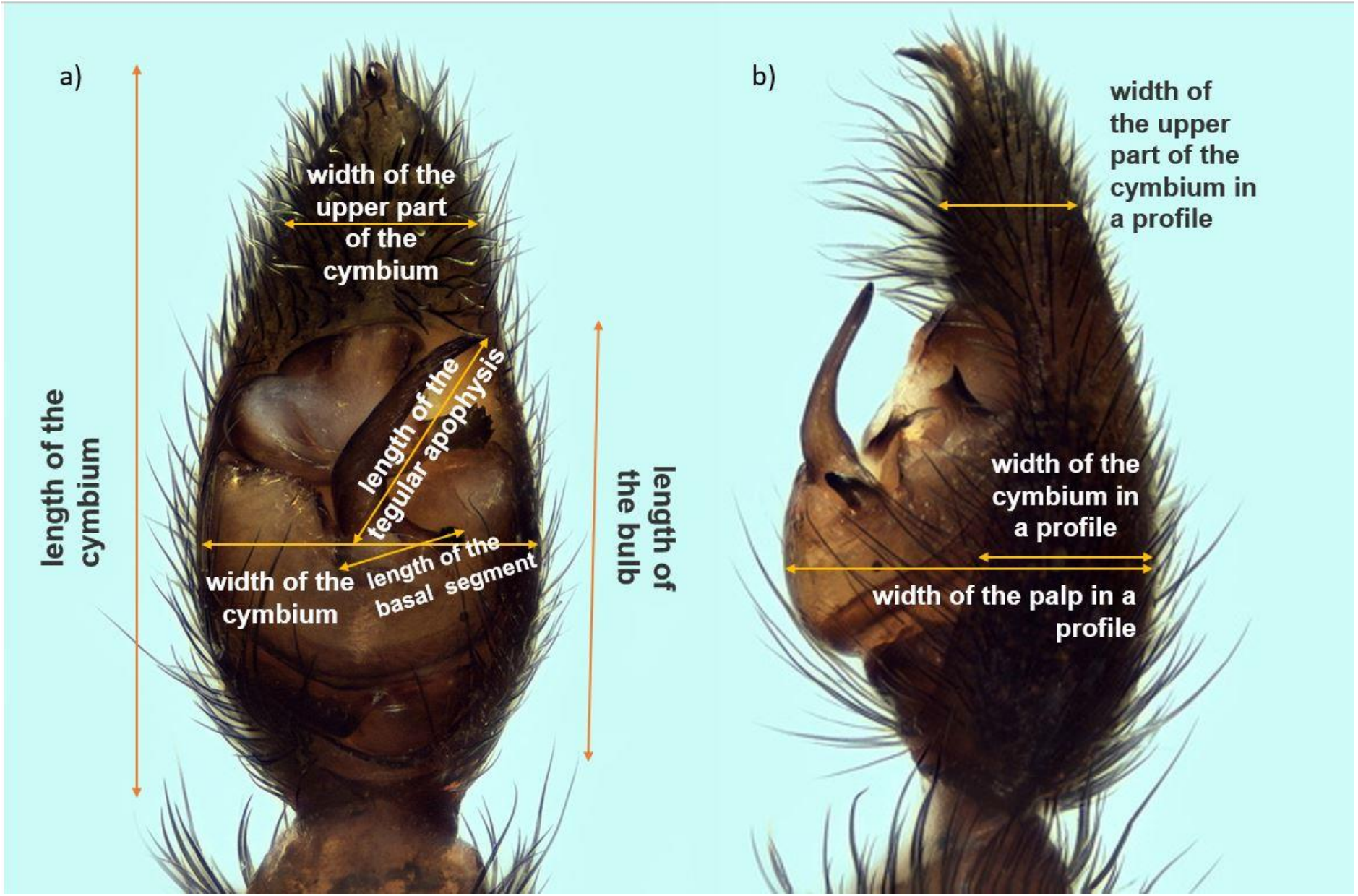
Scatterplots for the ratio of the carapace length to the measurements of walking legs in a pair of *P. alacris* and *P. saltans* species

Pardosa lugubris

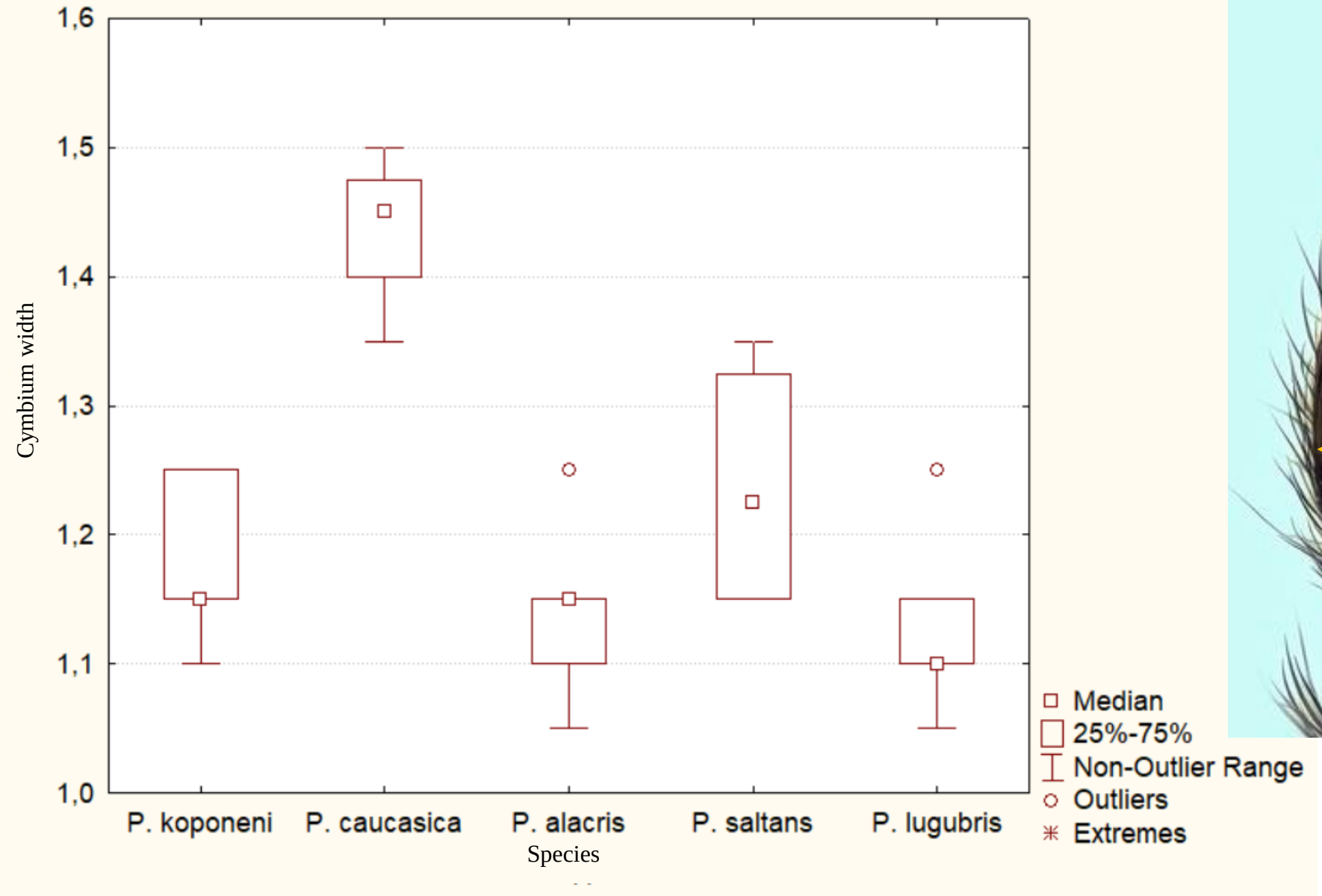
Pardosa alacris

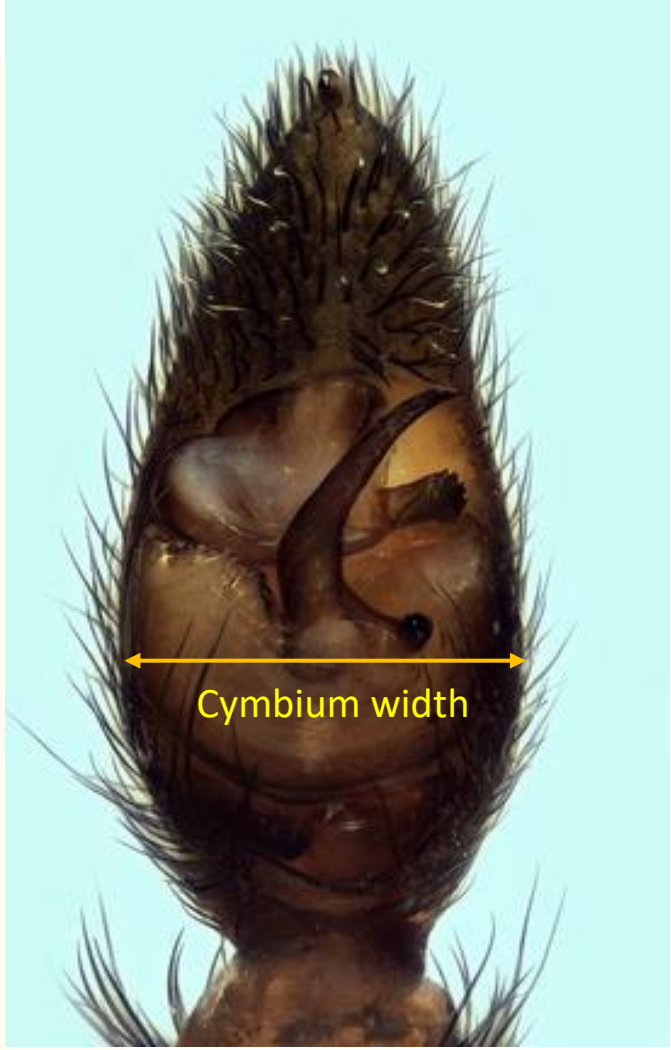
Pardosa saltans

Measurements of male genitalia front view (a) and in profile (b)



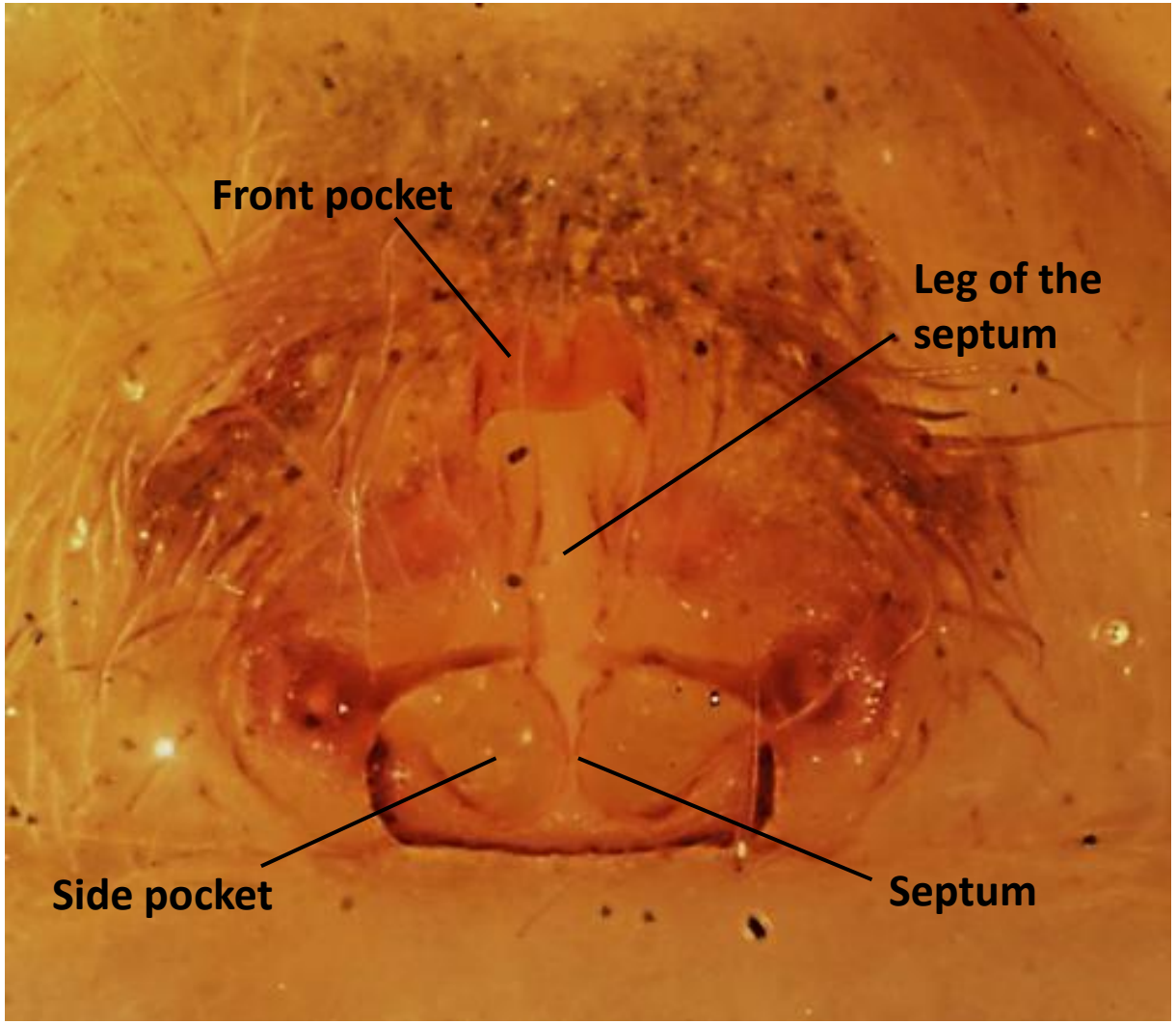
Boxplot showing the width of the cymbium of the male genitalia (*P. lugubris* species group)



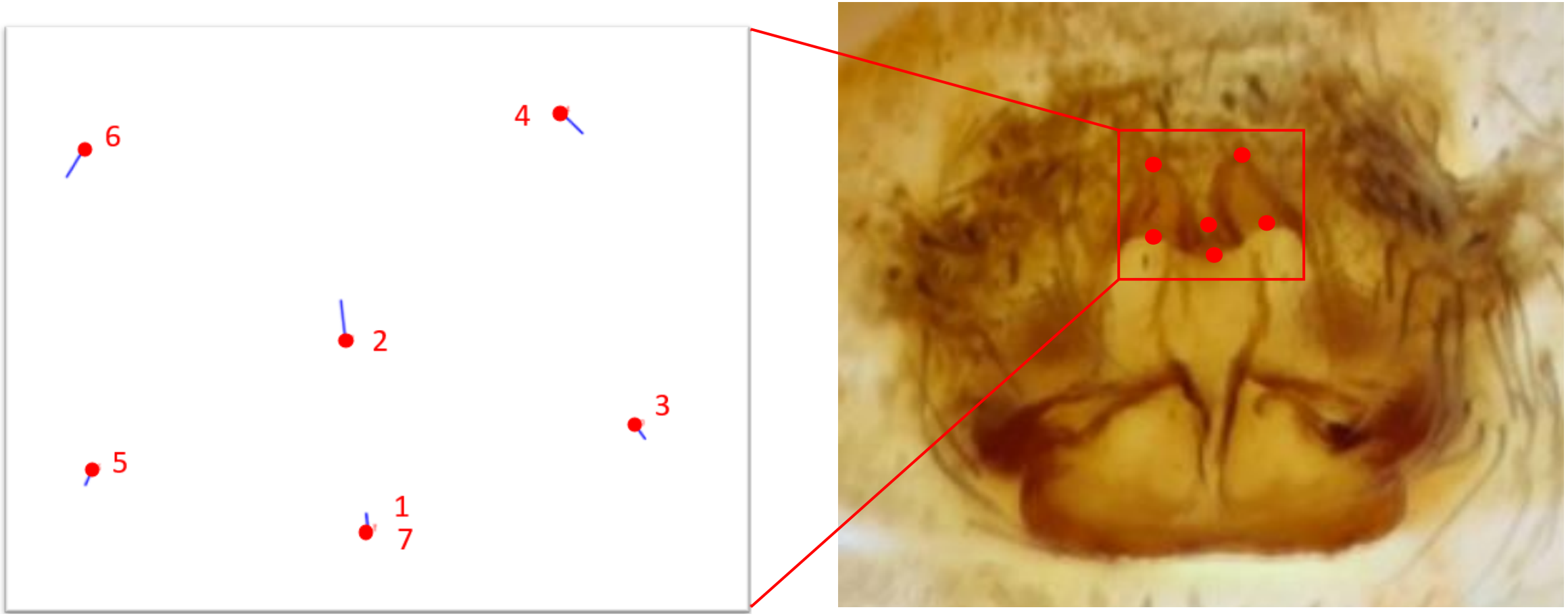


Geometric morphometry

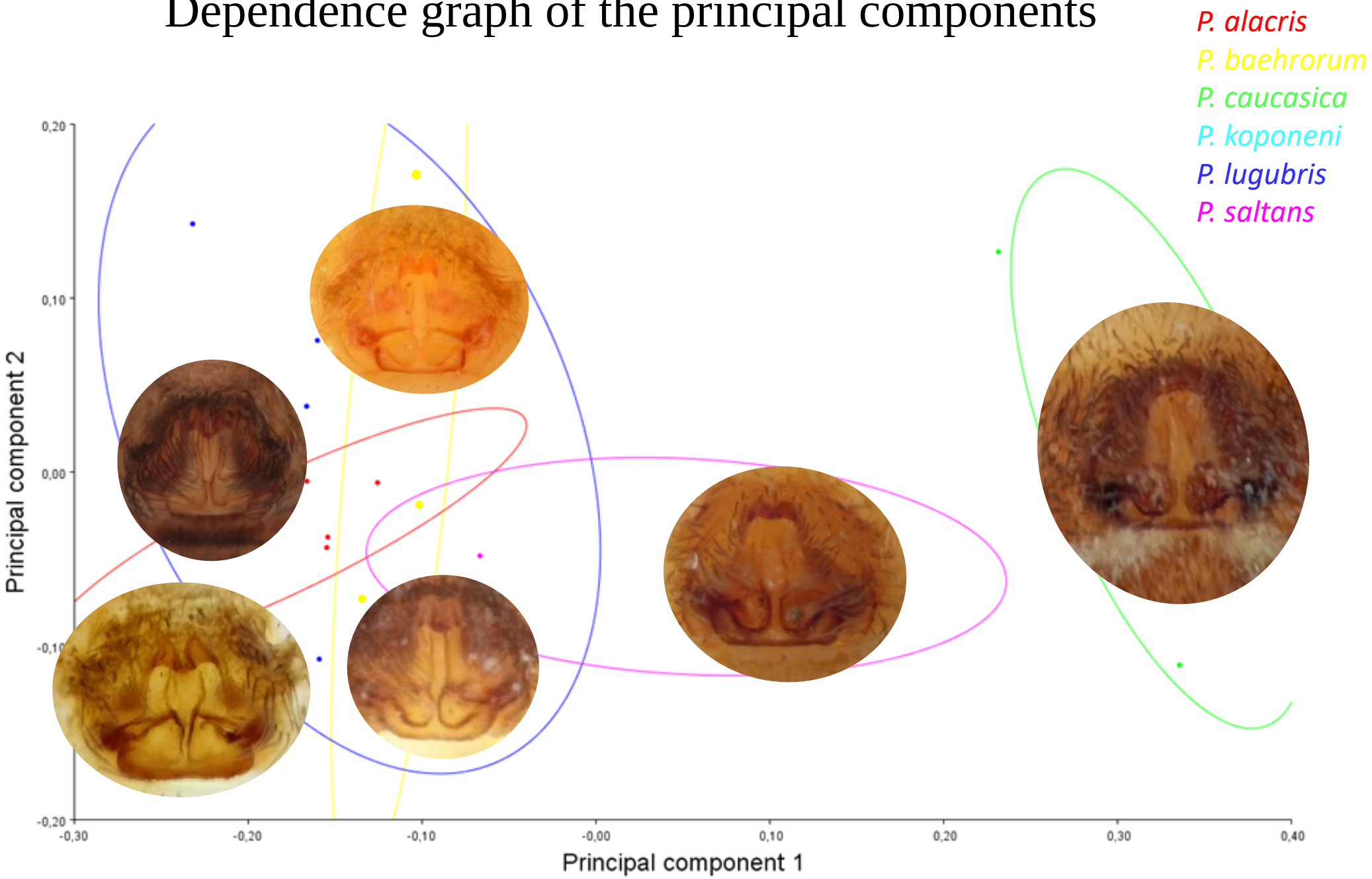
Structure of female genitalia of *P. baehrorum*



Position of the landmarks in the photo of *P. alacris* epygine

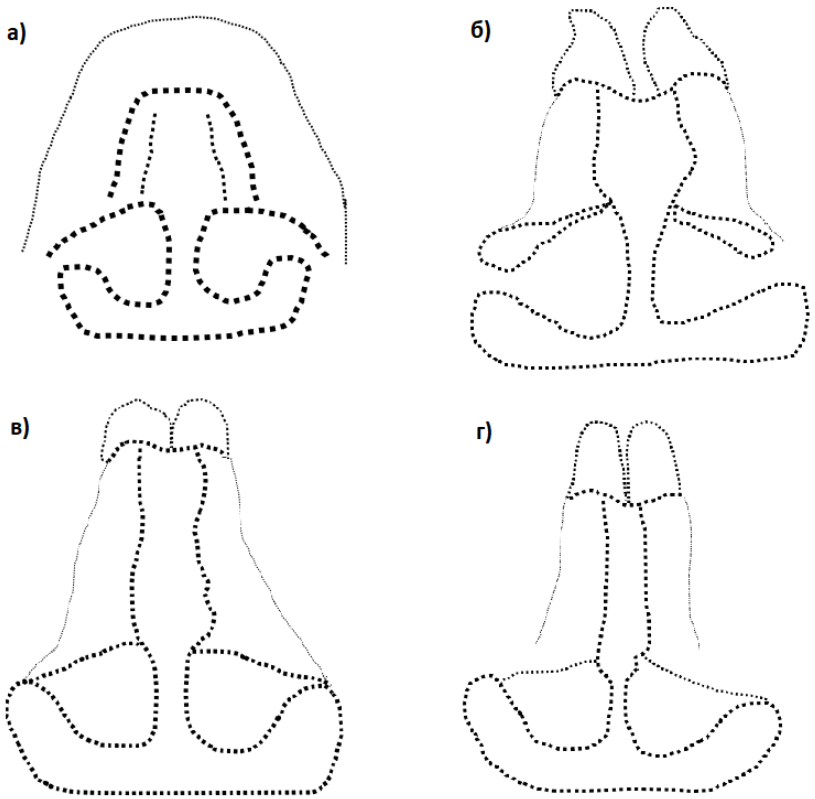


Dependence graph of the principal components



Preliminary key on the females of the 6 species of the group

1. Small species. The length of the carapace is 1.5-2.4 mm. Prosoma brown with light brown median band, lateral bands less distinct, laterally with dark and bright hairs on prosomal margin. Chelicerae and sternum yellowish to brownish. Legs yellowish with weak annulations. Germany, Austria, Switzerland, Romania..... *P. baehrorum*
- Large species. Length of carapace 2.2-5.0 mm..... 2
2. One front pocket of an epygine. The length of the carapax is 2.6-4.2 mm. Prosoma dark brown with small dark bristles, median stripe distinct, white haired. Chelicerae dark brown. Sternum light brown with spots. Coxa to femur dark brown with long dark hairs, distal part of femora to tarsi pale yellow. Caucasus..... *P. caucasica*
- Two front pockets of epigina..... 3
3. The front pockets of the epygine are strongly inclined to each other, their tops are directed in opposite directions. The leg of the septum is often wide. The length of the carapax is 3.9-4.9 mm. Prosoma dark brown, densely covered with black hairs, white hairs on median stripe, no lateral markings. Sternum light brown with dark hairs. Femora black proximally, light yellow distally like remaining segments. Southwest Russia, Sweden, central Europe..... *P. alacris*



Epygines of *P. caucasica* (a), *P. alacris* (b), *P. saltans* (c), *P. lugubris* (d)

Conclusions

- The COI molecular marker is effective for distinguishing *P. caucasica* and is not effective for the other species of the group; the 12S molecular marker is not effective for distinguishing the species of the group.
- For species of *P. lugubris* and *P. koponeni*, it was found that males differ in the ratio of the length of the tibia of the second pair of legs to the carapace, and females differ in the ratio of the length of the metatarsus of the fourth pair of legs to the carapace
- In sympatric species of *P. saltans* and *P. alacris*, males do not differ in linear measurements, females differ in the ratio of the length of the tibia of the third pair of legs to the carapace
- The geometrical morphometry of epygines as well as linear morphometry of cymbium width of spiders is a good method to distinguish sympatric species of *Pardosa alacris*, *P. saltans* as well as *P. caucasica*
- The first identification key for females of *P. lugubris* group was compiled