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A new species of spider belonging to the *Pardosa lugubris*-group (Araneae: Lycosidae) from Far East Asia

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Abstract

A new species, *Pardosa koponeni* **sp. n.**, is described. The new species is widely distributed in Far East Asia. It was previously confused with *P. lugubris* (Walckenaer, 1802). The two species have very similar copulatory organs but differ in the colouration of legs II–IV in males and the carapace/femur I ratio in both sexes. The distribution of the new species is mapped using material examined and literature data. To provide a more complete understanding of the boundaries between such closely related species, morphological and DNA barcoding approaches for species discrimination were integrated. Two species of the *Pardosa lugubris*-group (*P. lugubris* and *P. alacris*) were found to share haplotypes, suggesting evidence of hybridization or incomplete lineage sorting, or they are perhaps separate morphotypes of the same species. This is another example of complexity and the value of comparing morphology and DNA barcode data among spiders.

Key words: wolf spiders, Aranei, Pardosinae, barcoding, East Palaearctic

Introduction

Pardosa C.L. Koch, 1847 is the largest genus of wolf spiders with 533 species distributed worldwide (Platnick 2014). Most of its species occur in Eurasia (332), the Nearctic (101), and Africa (77). Despite its broad distribution, *Pardosa* has received unequal taxonomic attention across the globe, being best studied in Europe, North America, Siberia, and Japan (Platnick 2014; World Spider Catalog 2015). Holarctic *Pardosa* species are split into roughly 30 species groups (Zyuzin 1979; Dondale & Redner 1990). In some of these groups, species discrimination is relatively simple (*Pardosa tesquorum*-group (cf. Kronestedt & Marusik 2011), *Pardosa nigra*-group (cf. Kronestedt *et al.* 2014), *Pardosa modica*-group (cf. Kronestedt 1981; Dondale & Redner 1990 and Kronestedt 1975, 1986 as *glacialis*-group)), while other groups pose more difficulty: *Pardosa nebulosa*-group (cf. Marusik & Ballarin 2011), *Pardosa monticola*-group (cf. Tongiorgi 1966; Ballarin *et al.* 2012; Marusik *et al.* 2012), *Pardosa lugubris*-group (cf. Kronestedt 1999; Töpfer-Hofmann *et al.* 2000; Nadolny & Kovblyuk 2012). In several taxonomically difficult groups, females are nearly or completely indistinguishable, such as within the *Pardosa lugubris*-group. This species group was formally established by Töpfer-Hofmann & Helversen (1990). Earlier members of the *Pardosa lugubris*-group were placed either in group II (Locket & Millidge 1951), group III (Fuhn & Niculescu-Burlacu 1971; Wiebes 1959) or in the *Pardosa amentata*-group (Wiebes 1959; Zyuzin 1979). Revisions by Kronestedt (1992, 1999) and Töpfer-Hofmann *et al.* (2000) revealed several cryptic species closely related to *P. lugubris,* which are morphologically distinct from *P. amentata.* Besides morphological differences

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from one another, species belonging to this group also have distinct courtship behaviour (Töpfer-Hofmann *et al.* 2000). Currently, the *Pardosa lugubris*-group includes six named species: *P. alacris* (C.L. Koch, 1833); *P. baehrorum* Kronestedt, 1999; *P. caucasica* Ovtsharenko, 1979; *P. lugubris* (Walckenaer, 1802); *P. pertinax* Helversen, 2000 and *P. saltans* Töpfer-Hofmann, 2000. Of these six species, five are restricted to Europe, and only *P. lugubris* was thought to have a Transpalaearctic distribution (Platnick 2014; World Spider Catalog 2015).

While studying wolf spiders of the eastern Palaearctic, we recognized a distributional gap of *P. lugubris* between central Buryatia and Far East Asia. We observed that *P. lugubris* is rather rare in southern Siberia (Tuva, Western Buryatia) and yet very common in Sakhalin and South Kuril Islands, Maritime Province, northeastern China, Korea, and Japan. Besides differences in abundance between regions, we also recognised differences in habitat preferences: *P. lugubris* in Europe and southern Siberia occurs mainly in meadows and the edges of forests, and alternatively it inhabits dry litter in broadleaf forests in Far East Asia. Additionally, morphological differences were found: European specimens have relatively shorter legs and carapace than specimens in Far East Asia. Thus, we hypothesized that the European and Far Eastern populations of *P. lugubris* may represent two separate species. To address this hypothesis, we applied both morphological and genetic methods to compare *P. lugubris* specimens from Far East Asia with others from Crimea and Finland.

The main goals of this paper are to: 1) describe a new species, 2) trace its distribution and 3) investigate the applicability of DNA barcoding to separate sibling species of *Pardosa.*

Material and methods

Illustrations were made using both reflected and transmitted light microscopes. Illustrations of epigynes and bulbs were made after maceration in 20% KOH solution. Colouration was described from specimens preserved in 75% ethanol. Leg and palp segments were measured after their separation from the prosoma. All measurements are in millimetres. Scanning electron photographs were made using the SEM JEOL JSM-5200 scanning microscope in the Zoological Museum, University of Turku. Abbreviations for spination: a—apical, d—dorsal, p—prolateral, r—retrolateral, v—ventral.

DNA was extracted and sequenced (Table 1) from 70 specimens of nine species of the genus *Pardosa*, with other species used as outgroups in our study. All spiders used in molecular assays were kept in 95% ethyl alcohol. In addition, six specimens of the newly described *P. koponeni* **sp. n.**, represented by three juvenile specimens and three adults (one male and two females), were included in our analyses. All 70 sequences have been submitted to GenBank and the Barcode of Life Data System (BOLD; Ratnasinham & Hebert 2007), and accession numbers are given in Table 1.

The DNA extracts and the outgroup specimens are stored at the Biodiversity Institute of Ontario, University of Guelph, Ontario, Canada, whereas all other specimens are distributed among the following museums:

ISEA	Institute of Systematics and Ecology of Animals, Novosibirsk, Russia;
MMUM	the Manchester Museum, University of Manchester, United Kingdom;
TNU	National Arachnological Collection, Department of Zoology, V.I. Vernadsky Taurida National University, Simferopol, Ukraine (V.I. Vernadsky Crimean Federal University);
ZMMU	Zoological Museum of the Moscow State University, Russia;
ZMUT	Zoological Museum, University of Turku, Finland.

Molecular techniques. DNA barcoding was performed using standard methods employed at the Canadian Centre for DNA barcoding (http://dnabarcoding.ca/pa/ge/research/protocols). DNA extraction was performed according to the glass-fiber protocol for invertebrates (Ivanova *et al.* 2006). The polymerase chain reaction (PCR) was utilized to amplify a 658 bp segment of the target barcode region of the cytochrome *c* oxidase subunit I (COI) gene. We employed a cocktail primer set containing the Lepidoptera (Hebert *et al.* 2004a) and the classic Folmer primers (Folmer *et al.* 1994), in a 1:1 ratio.

Nearest neighbour analyses were performed using the Barcode of Life Data System (BOLD, Ratnasingham & Hebert 2007), including all specimens with sequences greater than 500 bp. The nearest neighbour, or barcode gap analysis, on BOLD plots the maximum pairwise divergence found within a species against its minimum divergence to the closest sequence belonging to a different species. A neighbour-joining (NJ) phenogram (Saitou & Nei 1987) employing the Kimura-2-Parameter (K2P) distance model (Kimura 1980) was constructed for all sequences, with bootstraps calculated based upon 1050 replicates (Fig. 36). The Kimura correction was claimed as the best DNA substitution model for low genetic distances (Casiraghi *et al.* 2010). While more recent literature has advocated the use of p-distances (Collins *et al.* 2012; Srivathsan & Meier 2012), the authors of those studies found that model choice had minimal impact upon conclusions at small genetic distances. Therefore, we retained usage of the more commonly employed K2P model, to enable comparisons with divergences in other barcoding studies (e.g. Blagoev & Dondale 2014). Finally, the distance analyses were conducted in BOLD, and the neighbour joining tree was constructed in MEGA5 (Tamura *et al.* 2007).

Taxonomy

Family Lycosidae Sundevall, 1833

Genus *Pardosa* C.L. Koch, 1847

Pardosa koponeni sp.n.

Figs 1–4, 8–9, 12, 14–23, 29–32.

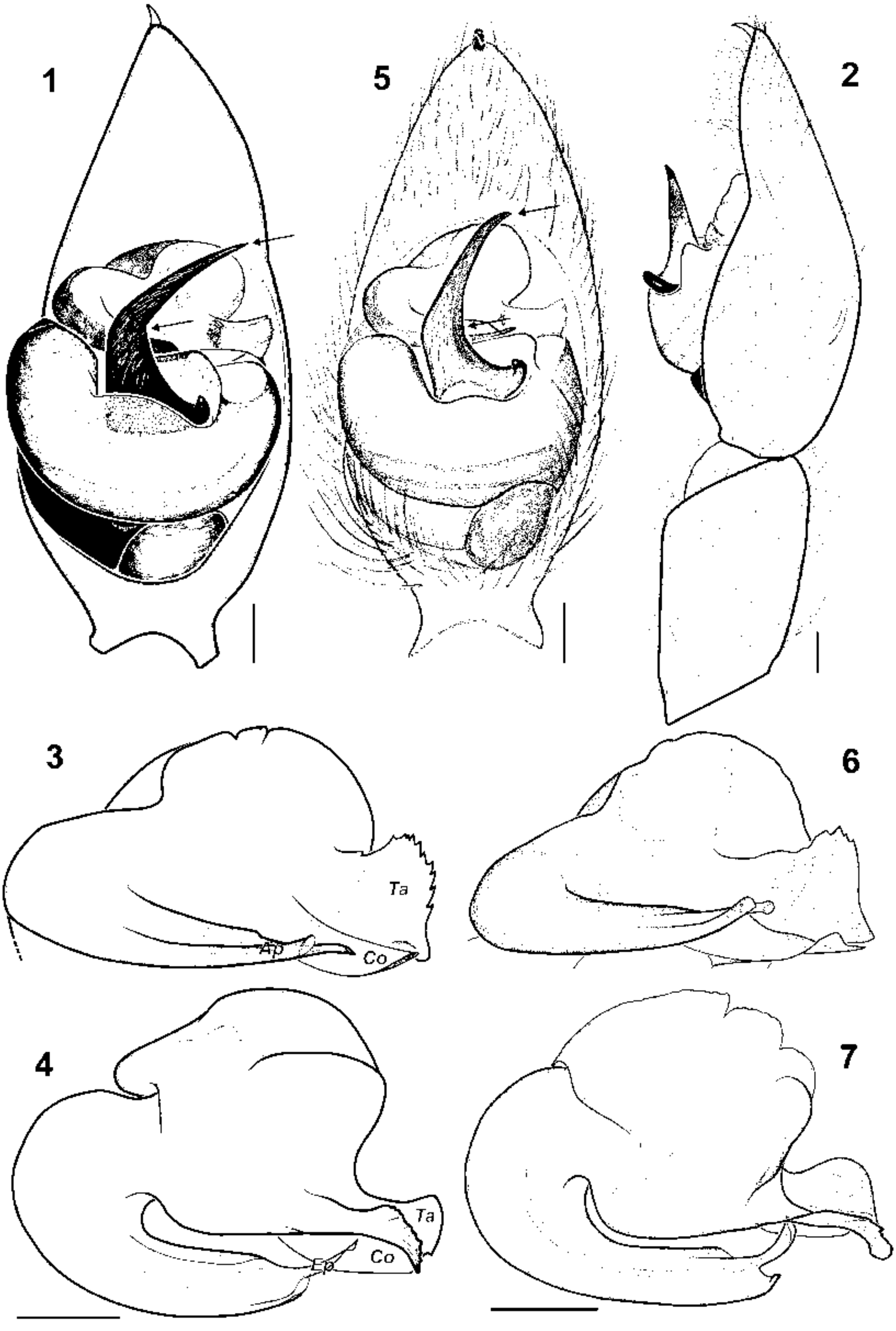
Lycosa lugubris: Yaginuma 1957: 57, f. 2 (♀).

Pardosa lugubris: Yaginuma 1960: 85, f. 76.8 (♀); Yaginuma 1971: 85, f. 76.8 (♀); Namkung *et al.* 1972: 94, f. 14 (♀); Oliger 1981: 7, f. 12–15 (♂♀); Yaginuma 1986: 163, f. 90.5 (♂♀); Chikuni 1989: 116, f. 34 (♂♀); Tanaka 1993: 265, f. 1–4 (♂♀); Kim & Yoo 1997: 35, f. 8, 20–21, 38–39, 50 (♂♀); Yoo & Kim 2002: 28, f. 59 (♂); Namkung 2002: 333, f. 20.27a–c (♂♀); Kim & Cho 2002: 217, f. 475–480 (♂♀); Namkung 2003: 335, f. 20.27a–c (♂♀); Tanaka 2009: 240, f. 101–102 (♂♀); Zhu *et al.* 2010: 59, f. 3a–f (♀).

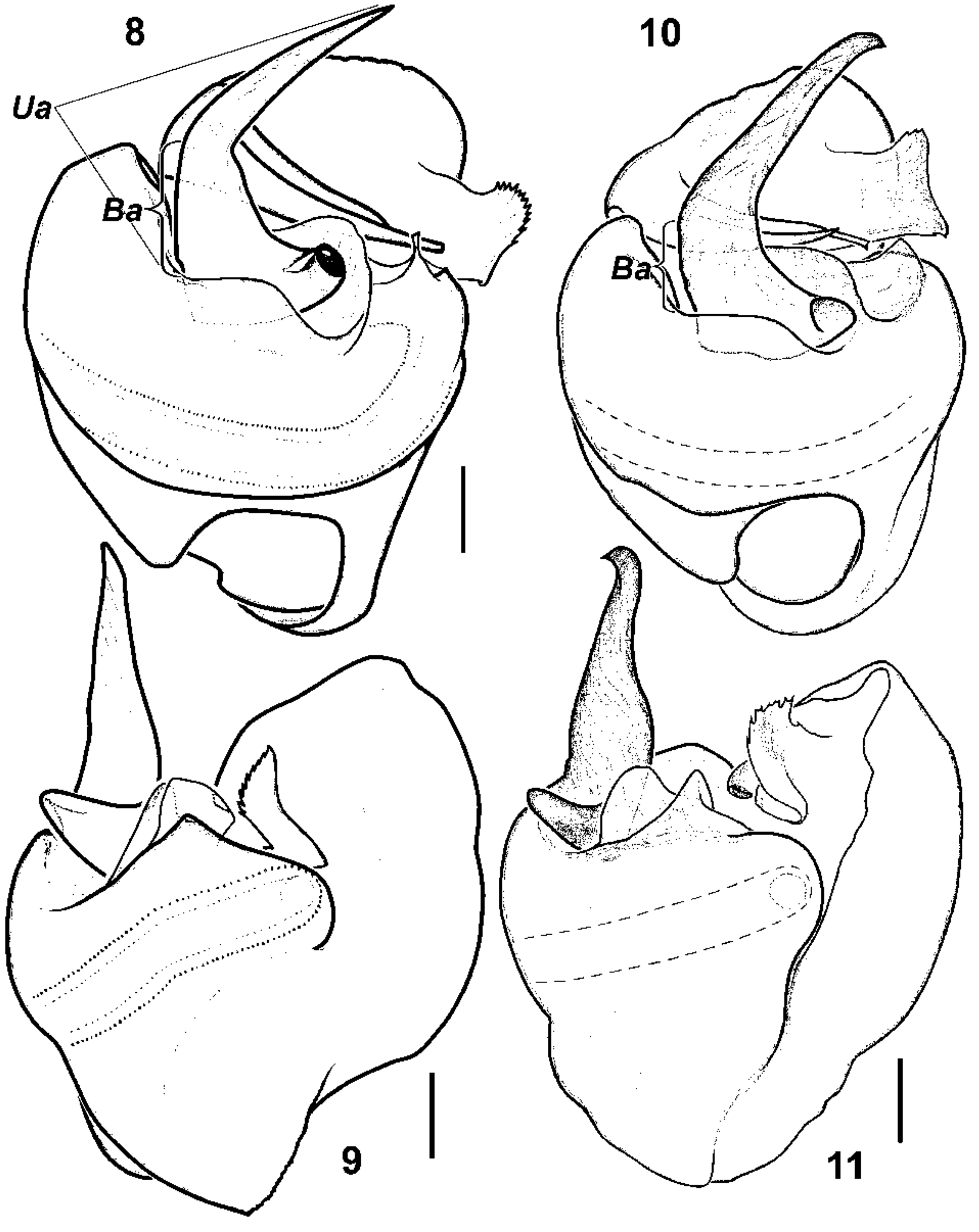
Etymology. The species is named after our colleague and friend Seppo Koponen, who made important contributions to the taxonomy and faunistics of Holarctic spiders, particularly Lycosidae.

Type material. Holotype ♂ (ZMMU), **RUSSIA**, ***Maritime Province***: environs of Ryazanovka Village, 42°47'52"N 131°14'37"E, copses of *Quercus dentatus*, 25–30.06.2009 (M.M. Omelko). Paratypes: **RUSSIA**: ***Maritime Province***: Chuguevski Distr.: 5♀ (ZMMU), S part, Chuguyevka Field Station, ca 43°50'N 134°15'E, 31.07–5.08.1998 (Yu.M. Marusik). Lazo Distr.: 2♂ (ZMMU), Lazo Reserve, Petrova Gorge, valley mixed forest, 27–28.07.1977 (T.I. Oliger); 4♂ 2♀ (ZMMU), Lazo Reserve, Slukhe Gorge, valley forest, 20.05.1979 (T.I. Oliger); 1♂ (ZMMU), Kiyevka Village, oak forest, 7.06.1982 (T.I. Oliger); 4♀ (ZMMU), Lazo Reserve, Korpad' Camp, 43°16'N 124°08'E, 6–9.08.1998 (Yu.M. Marusik); 1♂ (ZMMU), Sikhote-Alin Mt. range, Gorelaya Sopka Mt., ca 43°30'30"N 134°06'08"E, 1300–1470 m, 17–20.06.1999 (Yu. Sundukov); 17♂♀ (ZMMU), Lazo Reserve, Sukhoi River, 11–20.06.2002 (Yu. Sundukov); 13♂ 3♀ (TNU, M194–195), Sestra Mt., 43°31'52.23"N 134°02'49.44"E, low and middle belts of mountain, 16–23.06.2005 (M.M. Omelko); 2♂ 3♀ (TNU, M251), same locality, mixed forest, 500–600 m, 16–23.06.2005 (M.M. Omelko); 4♂ (ZMMU), Lazo Reserve, Korpad' Gorge, 23–30.06.2006 (M. Smirnov); 6♀ (ZMMU), same locality, 1–14.07.2006 (V. Shokhrin); 1♂ (ZMMU), Lazo Village, 1–10.07.2006 (V. Shokhrin); 17♂ 2♀ (ZMMU), S part, Lazo Reserve, Prosyolochnaya Bay, 22–24.06.2002 (Yu. Sundukov); 1♀ (ZMMU), same locality, 8–12.04.2006 (Yu. Sundukov); 12♂ (ZMMU), same locality, 1–7.07.2006 (Yu. Sundukov); 4♂ (ZMMU), Lazo Reserve, Amerika Kordon, 25.08–3.09.2006 (Yu. Sundukov, V. Shokhrin). Khasanski Distr.: 1♂ (ISEA), Kedrovaya Pad' Reserve, 22.05.1977 (B.P. Zakharov); 3♂ (TNU, M55), Ryazanovka Village env., 42°47'52"N 131°14'37"E, copses of *Quercus dentatus*, 25–30.06.2009 (M.M. Omelko). Khorol'ski Distr.: 3♀ (ZMMU), Khanka Lake, S shore, Luzanova Sopka, 44°33'N 132°23'E, 16–17.07.1998 (Yu.M. Marusik). Ussuriiski Distr.: 1♀ (ZMMU), S part, Ussuri Reserve, 43°39'N 132°33'E, 29–31.07.1998 (Yu.M. Marusik); 3♂ 1♀ (ZMMU), Ussuri Reserve, Komarovo-Zapovednoe, 43°38'48"N 132°20'48'E, 21–27.05.1998 (Yu. Sundukov). Terneiski Distr.: 5♂ (ZMMU), Sikhote-Alinski Reserve, kordon Kabaniy, ca 45°08'16"N 135°52'40"E, 650–900 m, 30.06–4.07.1999 (Yu. Sundukov); 5♂ (ZMMU), Sikhote-Alinski Reserve, kordon Blagodatnoye, ca 44°55'45"N 136°32'36"E, 7–12.07.1999 (Yu. Sundukov). ***Khabarovsk Province***: Bolshekhekhtsirski Reserve: 4♂ 4♀ (ISEA), forest on the plain, 2.06.1987 (D.V. Logunov); 2♂ (ISEA, 19), dry litter in *Populus* forest, 150–200 m, 11.06.1987

(D.V. Logunov); 2♂ 1♀ (ISEA, 40), marsh wood, 18.06.1987 (D.V. Logunov); 24♂ 3♀ (ISEA, 47), *Cedrus* & broadleaf forest, 250 m, pitfalls, 22.06.1987 (D.V. Logunov); 6♂ (ISEA, 52), *Cedrus* & broadleaf forest, 350–400 m, pitfalls, 24.06.1987 (D.V. Logunov).

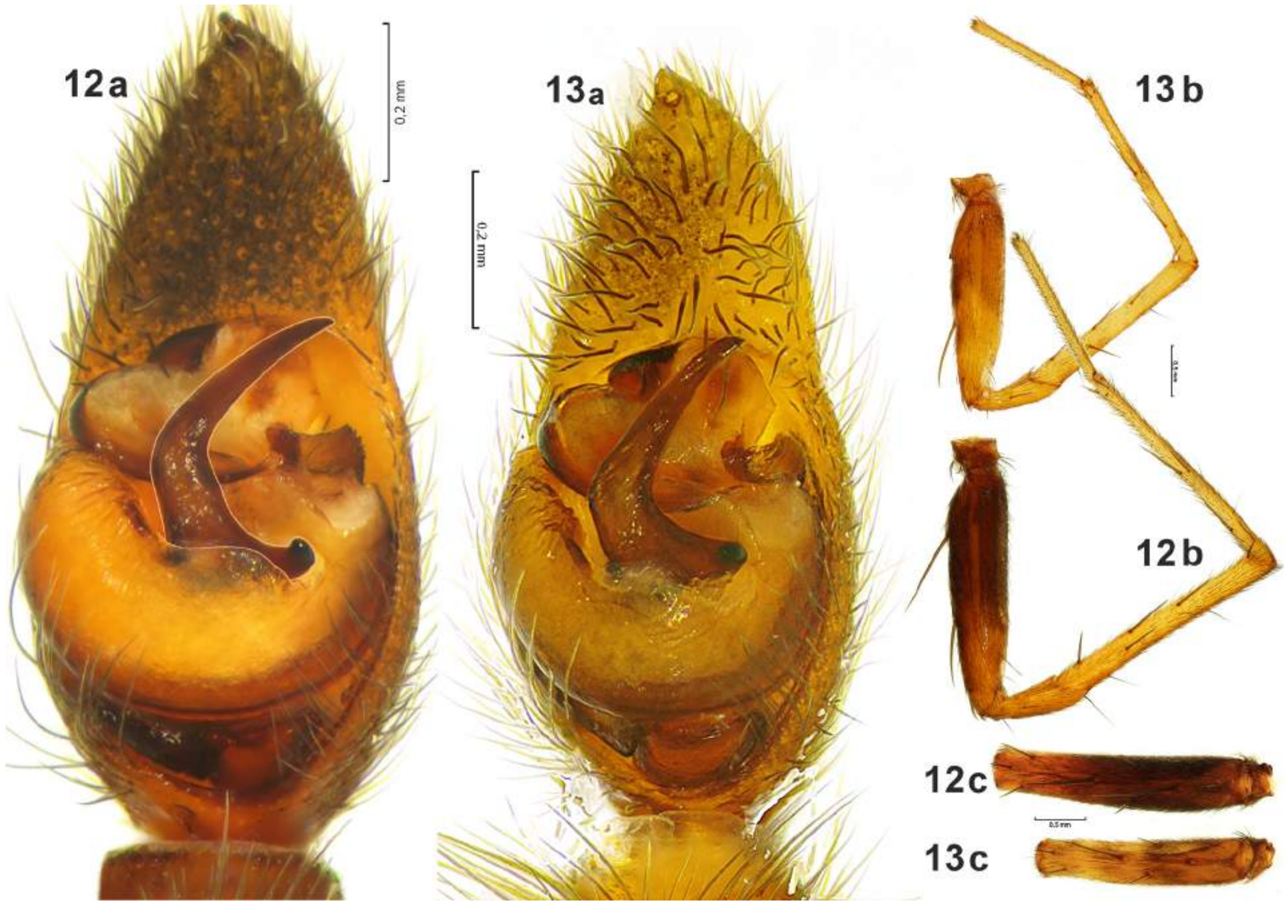


FIGURES 1–7. Male palp of *Pardosa koponeni* **sp. n.** (1–4) from Maritime Province and *P. lugubris* (5–7) from Crimea. 1, 5—ventral; 2—retrolateral; 3, 6—embolic division, ventral; 4, 7—embolic division, from above. Scale bars 0.1 mm. Abbreviations: *Ap* accessorial process of embolus, *Ep* embolus proper, *Co* conductor, *Ta* terminal apophysis.



FIGURES 8–11. Bulb of *Pardosa koponeni* **sp. n.** (8–9) from Maritime Province and *P. lugubris* (10–11) from Crimea. 8, 10—ventral, 9, 11—retrolateral. Scale bars 0.1 mm. Abbreviations: basal part (*Ba*) of the upper arm (*Ua*) of tegular apophysis.

Other material examined: RUSSIA: ***Kuril Islands***: 136♂ 14♀ (ZMMU), Shikotan Island, Tserkovnaya Bay, ca 43°44'N 146°42'E, May–September 2012 (Yu. Sundukov). ***Sakhalin Island***: Aniva Distr.: 1♀ (MMUM), Lugovoe Village, 13.08.1983 (A.M. Basarukin); 5♂ 2♀ (MMUM), Novoaleksandrovsk, 1985 (A.M. Basarukin); 6♀ (MMUM, G7518.162), Susui River, Novoaleksandrovsk, 21.04–2.09.1985 (A.M. Basarukin); 1♀ (MMUM), vicinity Lugovoe Village, Chekhov Mt., 11.05.1985 (A.M. Basarukin); 1♂ (MMUM), 5–7 km E of Lugovoe Village, 30.05.1985 (A.M. Basarukin); 1♂ 3♀ (MMUM), Novoaleksandrovsk, 07.1985 (A.M. Basarukin); 1♂ (MMUM, G7518.167), 5–8 km E of Lugovoe Village, 4.05.1986 (A.M. Basarukin); 2♀ (MMUM, G7518.153), 5–7 km E of Starorusskoe, 20.06.1986 (A.M. Basarukin); 1♀ (MMUM), vicinity Lugovoe Village, Chekhov Mt., 1000 m, 3.07.1986 (A.M. Basarukin); Dolinsk Distr.: 1♂ 2♀ (MMUM), vicinity of Lebyazh'e Lake, 18.05.1985 (A.M. Basarukin). Korsakovo Distr.: 1♂ (MMUM, G7518.192), Utesnoe Village, nr. Ozerskoe Village, 4.06.1986 (A.M. Basarukin); 1♂ 1♀ (MMUM), Utesnoe & Ozerskoe Village, 4.06.1986 (A.M. Basarukin). Tomari Distr.: 1♂ (MMUM), Ainskoe Lake, Ptich'ya River, 13.06.1984 (A.M. Basarukin); 1♂ (MMUM, G7518.161), Ainskoe Lake, 21–22.05.1986 (A.M. Basarukin); 1♀ (MMUM, G7518.206), Baklanie Lake, 2.08.1994 (A.M. Basarukin). Yuzhno-Sakhalinsk Distr.: 3♀ (MMUM, G7518.214.217), park in Yuzhno-Sakhalinsk, 10.05–11.09.1985 (A.M. Basarukin); 1♀ (MMUM, G7518.171), 5–8 km E of Yuzhno-Sakhalinsk, Dolina Turistov, 14.05–25.06.1985 (A.M. Basarukin); 1♀ (MMUM, G7518.147), same locality, 14.05–25.08.1985 (A.M. Basarukin); 3♀ (MMUM), Yuzhno-Sakhalinsk, 3.06.1985 (A.M. Basarukin). **JAPAN**, ***Hokkaido***: 3♂ 3♀ (ZMMU), Aizankei, 10.07.1971 (H. Tanaka).



FIGURES 12–13. Male palps and legs of *Pardosa koponeni* **sp. n.** (12) from Maritime and Khabarovsk Province and *P. lugubris* (13) from Finland and Crimea. a—palp, ventral; b—leg II, retrolateral; c—femur II, dorsal.

Comparative material of *Pardosa lugubris*: **FINLAND**: 12♂ 15♀ (ZMUT ARA 27268), Turku, Kärsämäki, Ponponrahka bog, 19.06.1977 (I. Oksala). **RUSSIA**, ***Buryatia***, Kabansk Distr.: 2♂ 2♀ (ZMMU), Selenginsk (=Beregovaya), 21.06.1983 (S. Danilov); 1♂ 1♀ (ZMMU), ca. 20 km ENE of Vydrino & Osinovka River valley, mixed forest, 460–480 m, 17.06.2001 (D.V. Logunov).**UKRAINE**, ***Crimea***: 966♂ 289♀ (TNU), details see Nadolny & Kovblyuk (2012).

Diagnosis. The new species is very similar to *P. lugubris*. The two sibling species can be separated most easily by the colouration of femora in males: in *P. koponeni* **sp.n.** femora are dark brown (blackish in life), with the distal part being light brown (cf. Figs 12b–c, 31), while in *P. lugubris* they have distinct annulations (cf. Figs 13b–c, 33). Males of *P. koponeni* **sp.n.** can be separated from *P. lugubris* by the shape of tegular apophysis: 1) thinner distal part of upper arm (*Ua*) (wider in *P. lugubris*; cf. Figs 1, 5, 8, 10, 12a, 13a); 2) basal part of the upper arm (*Ba*) 1/3 long of upper arm (*Ua*) (in *P. lugubris*—1/4) (cf. Figs 8, 10, 12a, 13a); 3) tip of tegular apophysis straight (slightly bent in *P. lugubris*; cf. Figs 1, 5, 8–11); 4) tip of tegular apophysis located close to the retrolateral edge of cymbium (closer to the midline of cymbium in *P. lugubris*; cf. Figs 1, 5, 12a, 13a). The two species differ also in size (cf. Figs 27–28). No differences in spination were found between the two species. Female epigynes in *P. koponeni* **sp.n.** and *P. lugubris* are indistinguishable.

Note. Also, males of the new species differ from other species of *P. lugubris*-group: from *P. alacris* it distinguished by coloration of the cymbium (Kronestedt 1992), from *P. baehrorum*—by size of the cymbium and femora, and by coloration of first femora (Kronestedt 1999), from *P. caucasica*—by the shape of tegular apophysis and embolus (Nadolny & Kovblyuk 2012); from *P. pertinax*—by the shape of tegular apophysis (Töpfer-Hofmann *et al.* 2000), and from *P. saltans*—by the size of the cymbium (Roberts 1998; Kronestedt 1999; Töpfer-Hofmann *et al.* 2000). Female epigynes in *P. lugubris*-group are indistinguishable, except *P. caucasica* (Nadolny & Kovblyuk 2012).

Description. Measurements of ♂/♀ from Maritime Province (TNU, M194): total length 5.0 / 6.0; carapace 2.50 / 2.78 long, 1.80 / 2.12 wide.

Length of palps and legs：

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
Palp	1.00/0.97	0.52/0.56	0.52/0.72	-	1.02/1.01	3.06/3.26
Leg I	2.34/2.35	0.88/0.99	2.32/2.08	2.42/2.02	1.48/1.18	9.44/8.62
Leg II	2.19/2.25	0.85/0.96	2.05/1.86	2.40/1.98	1.34/1.15	8.83/8.2
Leg III	2.08/2.10	0.79/0.88	1.88/1.75	2.58/2.30	1.24/1.10	8.57/8.13
Leg IV	2.72/2.28	0.90/1.00	2.60/2.06	3.90/2.04	1.69/1.19	11.81/8.57

Male leg spination.

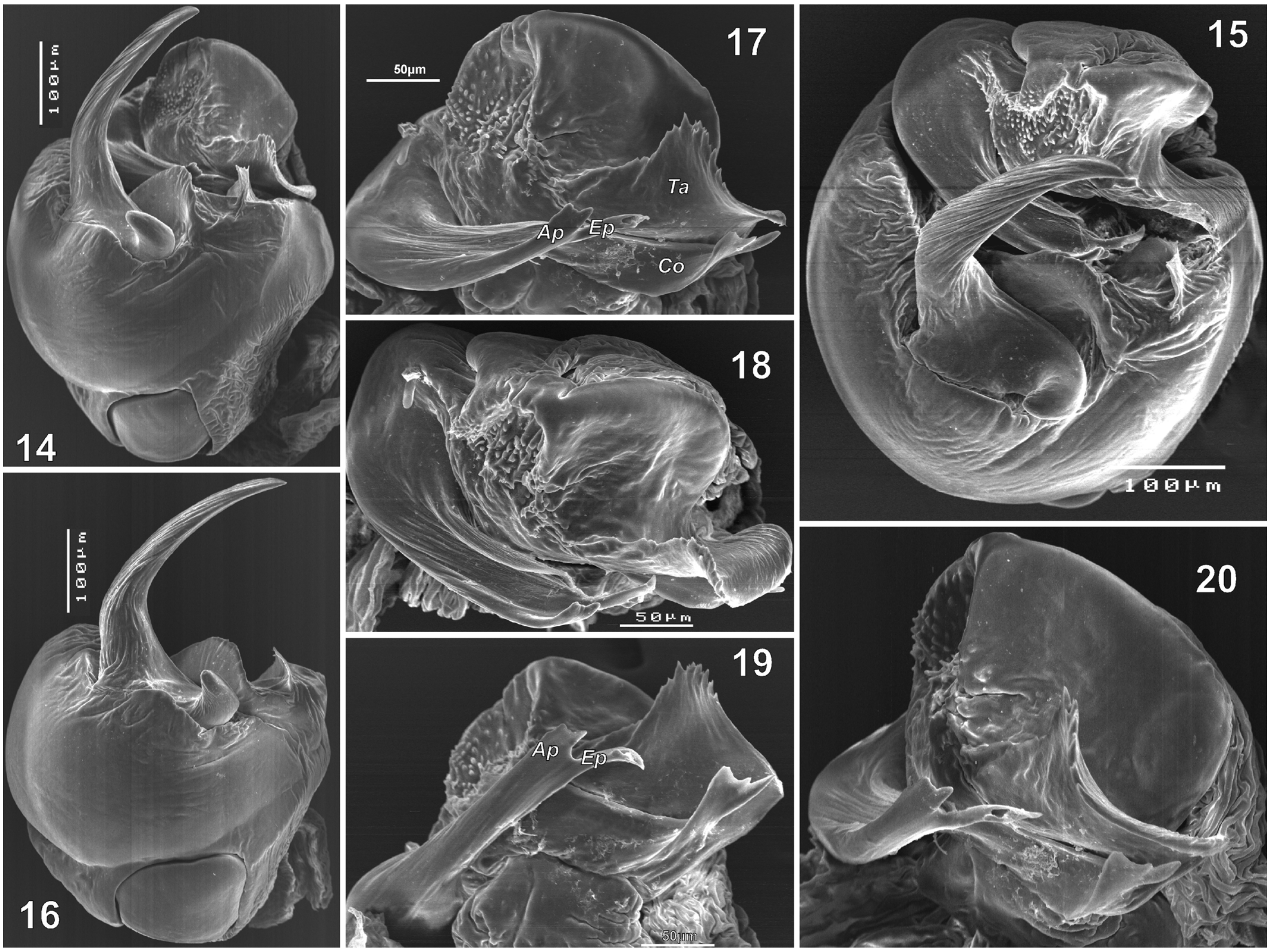
	Femur	Patella	Tibia	Metatarsus
I	d1-1-1(a), p2, r1-1	p1, r1	p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v2-2-3(a)
II	d1-1-1(a), p1, r1-1	p1, r1	p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v2-2-3(a)
III	d1-1-1(a), p1-1, r1-1	d1-1(a), p1, r1	d1-1, p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v2-2-3(a)
IV	d1-1-1(a), p1-1, r1	d1-1(a), p1, r1	d1-1, p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v1-2-2-3(a)

Female leg spination.

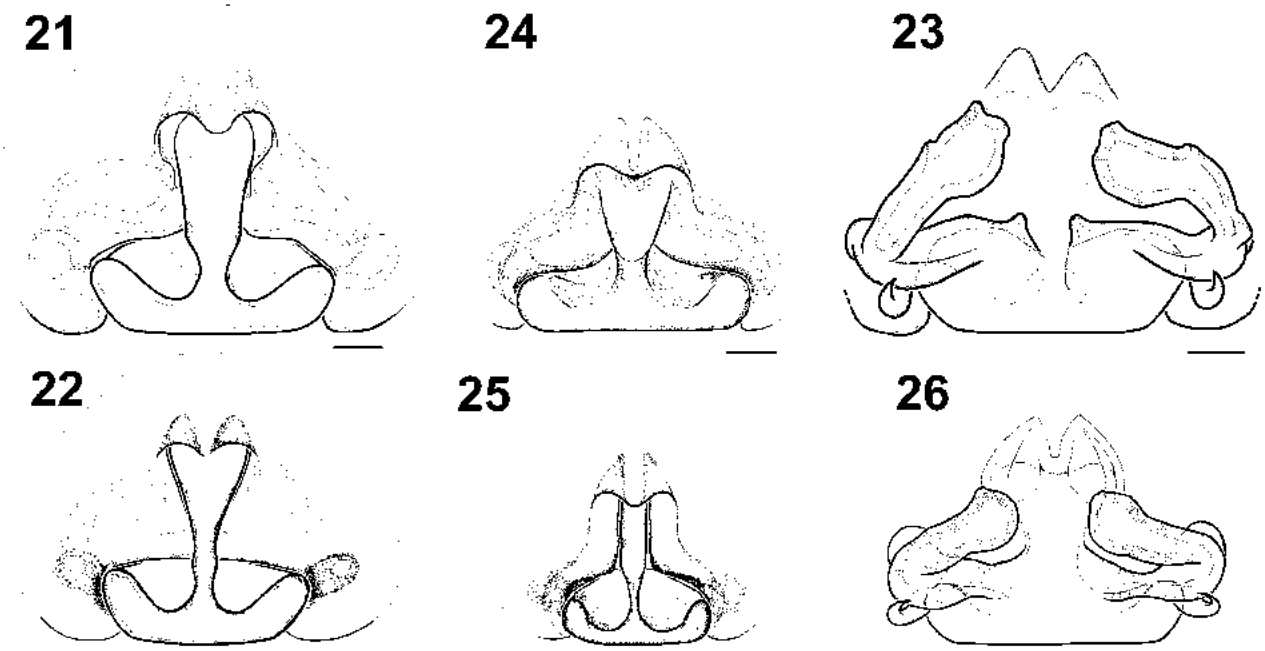
	Femur	Patella	Tibia	Metatarsus
I	d1-1-1(a), p2, r1-1	p1	p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v2-2-2(a)
II	d1-1-1(a), p1, r1-1	p1, r1	p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v2-2-3(a)
III	d1-1-1(a), p1-1, r1-1	d1-1(a), p1, r1	d1-1, p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v2-2-3(a)
IV	d1-1-1(a), p1-1, r2	p1, r1	d1-1, p1-1, r1-1, v2-2-2(a)	p1-1-1(a), r1-1-1(a), v2-2-2(a)

Male. Carapace black; median band and margins covered with white setae (Figs 29–30, 31). Sternum black. Abdomen dark, covered with white setae. Femora of all legs black, distally brown; patella, tibia, metatarsi and tarsi of all legs brown.

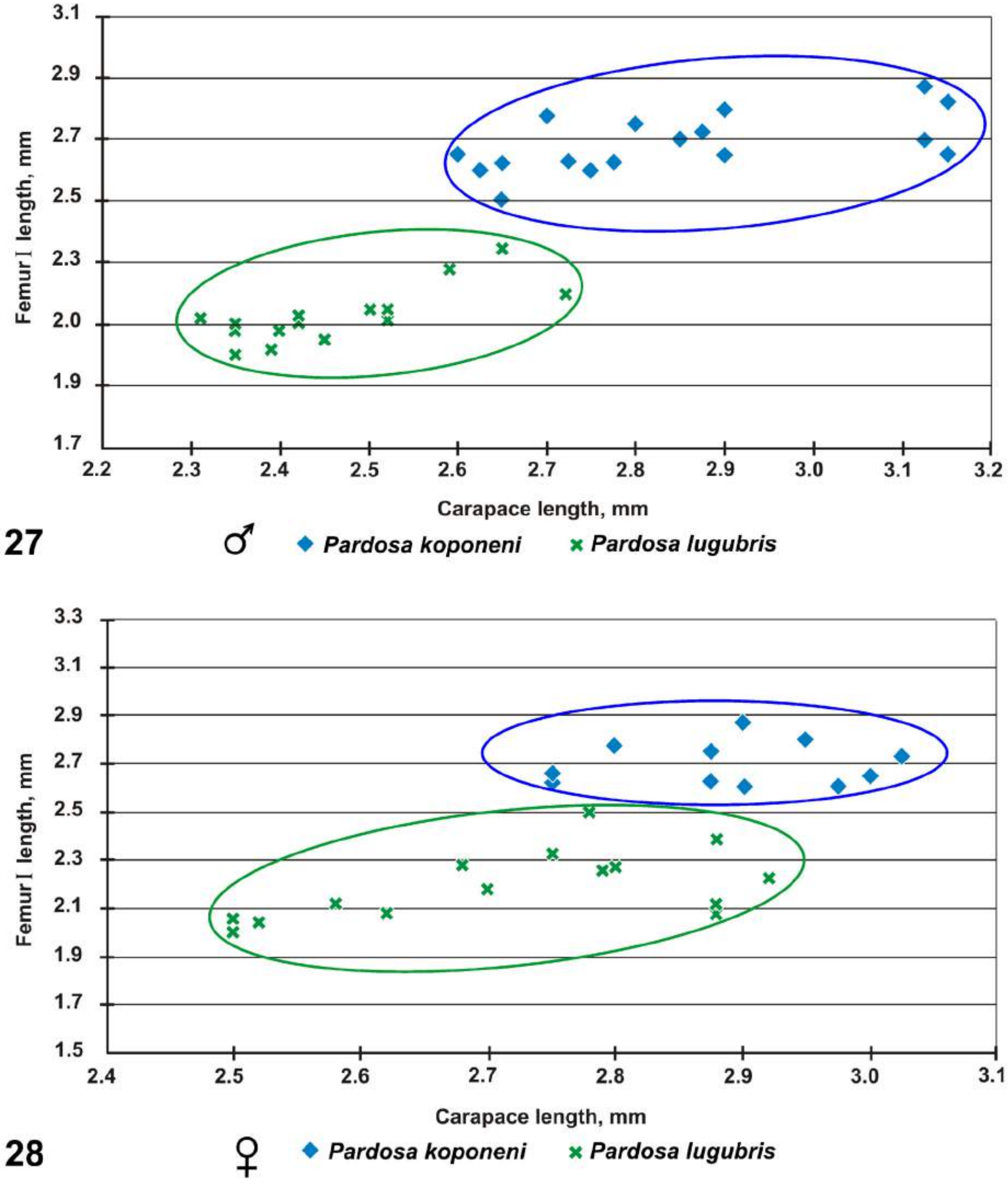
Palp as in Figs 1–4, 8–9, 12a, 14–20; dark-brown, longer than carapace; femur about as long as patella + tibia, and as long as cymbium; patella and tibia equal in length; cymbium with one claw, long, its top part longer than 1/3 of cymbial length, and subequal to height of tegular apophysis; bulb as wide as about 1/2 of cymbium length; tegular apophysis with two well developed arms: long apical one and short basal one; apical arm thin and gradually tapering (*Ua*), bent at the angle about 115°, its tip straight and pointed; tegular apophysis terminates close to the retrolateral edge of cymbium; conductor smaller than terminal apophysis, with subparallel edges, tip abrupt, slightly widened; embolus modified: wide, subdivided into two parts, embolus proper (*Ep*) slightly bent dorsally, and accessorial process of embolus (*Ap*) gently bent apically, its tip somewhat bifurcated on the top.



FIGURES 14–20. Bulb of *Pardosa koponeni* **sp. n.** from Maritime Province. 14—retrolateral; 15—from above; 16—ventral; 17—embolic division, ventral, 18—embolic division, from above; 19—embolic division, from below, 20—embolic division, retrolateral. Abbreviations: *Ap* accessorial process of embolus, *Co* conductor, *Ep* embolus proper, *Ta* terminal apophysis.



FIGURES 21–26. Epigyne of *Pardosa koponeni* **sp. n.** (21–23) from Maritime Province and *P. lugubris* (24–26) from Crimea. 21–22, 24–25—ventral; 23, 26—dorsal. Scale bars 0.1 mm.



FIGURES 27–28. Diagrams showing carapace length / femur I length ratio in males (27) and females (28) of *Pardosa koponeni* **sp. n.** from Maritime Province and *P. lugubris* from Crimea.

Female. Carapace brown; median band and borders covered with light-brown setae. Sternum brown. Abdomen brown, covered with light-brown setae. Legs brown; femora with four light-brown rings; palps brown, Epigyne as in Figs 21–23, septum anchor shaped.

Comments. The new species occurs in large numbers in broadleaf forests. Sometimes it can be found in glades within coniferous forests. Juvenile spiders are numerous in meadows near forest edges at spring time. Males and females were found in the south part of Maritime Province from the end of May. Males occur until the first half of July. Juvenile spiders can be found at snow-free places from the beginning of March. In Sakhalin Island, the first males were found during the beginning of May. Females occur until August.

FIGURES 29–34. Live *Pardosa koponeni* **sp. n.** (29–32) from Maritime Province and *P. lugubris* (33, 34) from Crimea. 29, 30—in copula; 31, 33—males; 32, 34—females holding egg cocoons.

The average length of femur I and carapace of both sexes from Sakhalin, Shikotan and Hokkaido Islands is less than in specimens from Khabarovsk and Maritime Province. Also, the island and mainland populations of *P. koponeni* **sp. n.** have some minor differences in bulb. The island specimens have a thinner upper arm of the tegular apophysis (*Ua*) and shorter cymbium, which may indicate that they are a separate species.

Distribution. So far, the new species has been found from Khabarovsk to Sakhalin Island, south to Korea and Southern Honshu, Japan (Fig. 35). There is only one record of the species from China (eastern part Jilin Province) (Zhu *et al.* 2010).

FIGURE 35. Distribution of *Pardosa koponeni* **sp.n.** One symbol may refer to several localities; square—specimens examined; dot—literature records.

Genetic divergence analysis

Seventy sequences were obtained from ten species listed in Table 1, all with a length of 658 bp. Almost all species were represented by more than one specimen, with the exception of *P. amentata*. *Pardosa koponeni* **sp. n.** was represented by six specimens (Table 1, Fig. 36). The ten *Pardosa* species examined for barcodes belong to five species groups: ***amentata***: *P. amentata*; ***lugubris***: *P. alacris, P. caucasica, P. koponeni* **sp. n.** and *P. lugubris*; ***monticola***: *P. agrestis* and *P. palustris*; ***paludicola***: *P. paludicola*; ***proxima***: *P. hortensis* and *P. proxima*.

Ten divergent haplotype assemblages were present. *Pardosa lugubris* and *P. alacris* were observed to share haplotypes. Thus, all males of these two species were re-examined, and the morphological results support two distinct and valid species.

The mean intraspecific divergence value (MNID) of all *Pardosa* species examined was found to be 0.15%, the average maximum intraspecific divergence (MXID) was 0.62%, while the mean divergence between congeneric species (MDNN) was 5.6%.

Within the *P. lugubris*-group MNID was 0.27%, MXID was 0.61%, and MDNN was 1.21%. More specifically, *P. koponeni* **sp.n.** showed MNID = 0.05%, MXID = 0.15%, and MDNN = 0.92%. *Pardosa caucasica* had an MNID = 0.31%, MXID = 0.46%, and MDNN = 1.38%. And *P. lugubris* and *P. alacris* showed MDNN = 0.4%, MXID = 0.61%, and MDNN = 0%.

Nearest neighbour (NN) analysis showed that all but *P. lugubris* and *P. alacris* fall above the 1:1 line, indicating the presence of a barcode gap (Fig. 37, Meyer & Paulay 2005, Hebert *et al.* 2004b). The four species of the *Pardosa lugubris*-group (*P. lugubris*, *P. alacris*, *P. caucasica*, and *P. koponeni* **sp.n.**) are genetically similar,

TABLE 1. A list of specimens and sequences used along with their associated collection details. Both, latitude and longitude are given in decimal degrees, and the altitude is in meters.

Species	Gender	BOLD ProcessID	Genbank Accession	Country	Date Collected	Latitude	Longitude	Altitude
Pardosa agrestis	Female	SPIEU643-10	HQ975256	Bulgaria	25-Feb-10	42.443	23.58	815
Pardosa agrestis	Female	SPIEU663-10	HQ580726	Bulgaria	25-Feb-10	42.4431	23.5804	815
Pardosa agrestis	Female	SPIEU666-10	HQ580729	Bulgaria	25-Feb-10	42.4431	23.5804	815
Pardosa agrestis	Female	TURAR003-10	HM385668	Turkey	22-Aug-09	38.5046	43.4546	1842
Pardosa agrestis	Female	TURAR006-10	HM385671	Turkey	22-Sep-09	38.4828	43.4147	1776
Pardosa agrestis	Female	TURAR007-10	HM385672	Turkey	22-Sep-09	38.4828	43.4147	1776
Pardosa agrestis	Immature	TURAR1364-10	JN310618	Turkey	25-May-10	39.8794	39.3425	2080
Pardosa alacris	Male	SPIEU616-10	HQ975237	Bulgaria	31-Mar-10	41.37	23.187	701
Pardosa alacris	Female	SPIEU617-10	HQ975238	Bulgaria	31-Mar-10	41.37	23.187	701
Pardosa alacris	Male	SPIEU1238-11	KC130125	Bulgaria	31-May-10	42.9346	24.0127	409
Pardosa alacris	Female	SPIEU1240-11	KC130128	Bulgaria	31-May-10	42.9346	24.0127	409
Pardosa amentata	Male	SPIEU334-10	HQ975128	Bulgaria	7-Mar-10	42.584	23.364	884
Pardosa caucasica	Female	SPIEU1341-11	KC130133	Georgia	15-Jul-09	43.313	40.249	1705
Pardosa caucasica	Female	SPIEU1342-11	KC130119	Georgia	15-Jul-09	43.313	40.249	1705
Pardosa caucasica	Male	SPIEU1343-11	KC130126	Georgia	15-Jul-09	43.313	40.249	1705
Pardosa caucasica	Male	SPIEU1344-11	KC130122	Georgia	15-Jul-09	43.313	40.249	1705
Pardosa hortensis	Male	SPIEU001-09	HM416918	Bulgaria	4-May-09	42.027	24.297	480
Pardosa hortensis	Male	SPIEU002-09	HM416919	Bulgaria	4-May-09	42.027	24.297	480
Pardosa hortensis	Male	SPIEU308-10	HQ975105	Bulgaria	3-Mar-10	41.433	23.52	487
Pardosa hortensis	Female	SPIEU543-10	HQ975204	Bulgaria	23-Sep-09	42.073	22.81	796
Pardosa hortensis	Male	SPIEU556-10	HQ975214	Bulgaria	10-Mar-10	42.696	23.329	558
Pardosa hortensis	Male	SPIEU568-10	HQ975220	Bulgaria	7-Mar-10	42.584	23.364	884
Pardosa hortensis	Female	SPIEU607-10	HQ975230	Bulgaria	31-Mar-10	41.37	23.187	701
Pardosa hortensis	Female	SPIEU988-10	HQ975536	Bulgaria	15-Jun-10	41.6437	25.382	292
Pardosa hortensis	Female	SPIEU1018-10	JF886272	Bulgaria	22-Jun-10	42.0213	28.0114	31
Pardosa hortensis	Female	SPIEU1082-11	JN310129	Bulgaria	17-May-09	41.4132	23.5558	753
Pardosa hortensis	Female	SPIEU1083-11	JN310130	Bulgaria	17-May-09	41.4132	23.5558	753
Pardosa hortensis	Female	SPIEU1085-11	JN310132	Bulgaria	17-May-09	41.4132	23.5558	753
Pardosa hortensis	Female	SPIEU1218-11	KC130118	Bulgaria	24-Apr-11	42.5256	24.1919	620
Pardosa koponeni	Female	SPRMA1008-12	KC130127	Russia	27-Jul-11	43.693	132.164	130
Pardosa koponeni	Female	SPRMA1009-12	KC130135	Russia	27-Jul-11	43.693	132.164	130
Pardosa koponeni	Female	SPRMA1010-12	KC130124	Russia	27-Jul-11	43.693	132.164	130
Pardosa koponeni	Male	SPRMA1012-12	KC130132	Russia	27-Jul-11	43.693	132.164	130
Pardosa koponeni	Male	SPRMA1014-12	KC130123	Russia	27-Jul-11	43.693	132.164	130

……*continued on the next page*

TABLE 1. (Continued)

Species	Gender	BOLD ProcessID	Genbank Accession	Country	Date Collected	Latitude	Longitude	Altitude
Pardosa koponeni	Male	SPRMA1016-12	KC130120	Russia	27-Jul-11	43.693	132.164	130
Pardosa lugubris	Male	SPIEU301-10	HQ975100	Bulgaria	3-Mar-10	41.433	23.52	487
Pardosa lugubris	Female	SPIEU787-10	HQ975343	Bulgaria	18-May-10	42.834	23.9856	577
Pardosa lugubris	Female	SPIEU814-10	HQ975366	Germany	31-Jul-10	49.8673	8.6854	178
Pardosa lugubris	Female	SPIEU815-10	HQ975367	Germany	31-Jul-10	49.8673	8.6854	178
Pardosa lugubris	Female	SPIEU919-10	HQ975469	Poland	14-Jul-10	52.1704	22.2784	150
Pardosa lugubris	Female	SPIEU997-10	HQ975542	Bulgaria	16-May-10	41.3696	23.1871	701
Pardosa lugubris	Female	SPIEU1345-11	KC130131	Poland	14-Jul-10	52.125	23.529	127
Pardosa paludicola	Female	SPIEU877-10	HQ975427	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU956-10	HQ975506	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU957-10	HQ975507	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU958-10	HQ975508	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU959-10	HQ975509	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU960-10	HQ975510	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU961-10	HQ975511	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU962-10	HQ975512	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU963-10	HQ975513	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU964-10	HQ975514	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa paludicola	Immature	SPIEU965-10	HQ975515	Poland	14-Jul-10	54.1716	23.3642	145
Pardosa palustris	Immature	SPIRU660-11	JF886879	Russia	6-Oct-10	59.7352	150.867	165
Pardosa palustris	Immature	SPIRU661-11	JF886880	Russia	6-Oct-10	59.7352	150.867	165
Pardosa palustris	Immature	SPIRU915-11	KC130130	Russia	9-Aug-09	59.0866	159.942	113
Pardosa palustris	Female	SPIRU2131-12	KC130134	Russia	31-Jul-11	62.4542	166.191	81
Pardosa proxima	Female	SPIEU664-10	HQ580727	Bulgaria	25-Feb-10	42.4431	23.5804	815
Pardosa proxima	Female	SPIEU665-10	HQ580728	Bulgaria	25-Feb-10	42.4431	23.5804	815
Pardosa proxima	Male	SPIEU761-10	JF886285	Bulgaria	22-Jun-10	42.0213	28.0114	31
Pardosa proxima	Male	SPIEU983-10	HQ975531	Bulgaria	15-Jun-10	41.6437	25.382	292
Pardosa proxima	Immature	TURAR737-10	HQ982910	Turkey	27-Mar-10	38.5356	27.4586	1130
Pardosa proxima	Male	TURAR740-10	HQ982913	Turkey	27-Mar-10	38.5356	27.4586	1130
Pardosa proxima	Female	TURAR800-10	HQ982956	Turkey	13-Apr-10	38.25	40.0737	836
Pardosa proxima	Male	TURAR830-10	HQ982976	Turkey	20-Jul-10	38.4687	42.4635	1655
Pardosa proxima	Female	TURAR831-10	HQ982977	Turkey	20-Jul-10	38.4687	42.4635	1655
Pardosa proxima	Female	TURAR832-10	HQ982978	Turkey	20-Jul-10	38.4687	42.4635	1655
Pardosa proxima	Female	TURAR834-10	HQ982979	Turkey	20-Jul-10	38.4687	42.4635	1655
Pardosa proxima	Immature	TURAR1506-10	KC130129	Turkey	16-Nov-10	37.5607	38.9299	595
Pardosa proxima	Male	SPIEU1302-11	KC130121	Bulgaria	22-May-11	42.9344	22.9544	702

with relatively low NN distances (Fig. 37), and are genetically distinct from the rest of the *Pardosa* species used as outgroups. However, *P. lugubris* and *P. alacris* lie below the barcode gap zone, consistent with the results of these two species sharing haplotypes (Fig. 36).

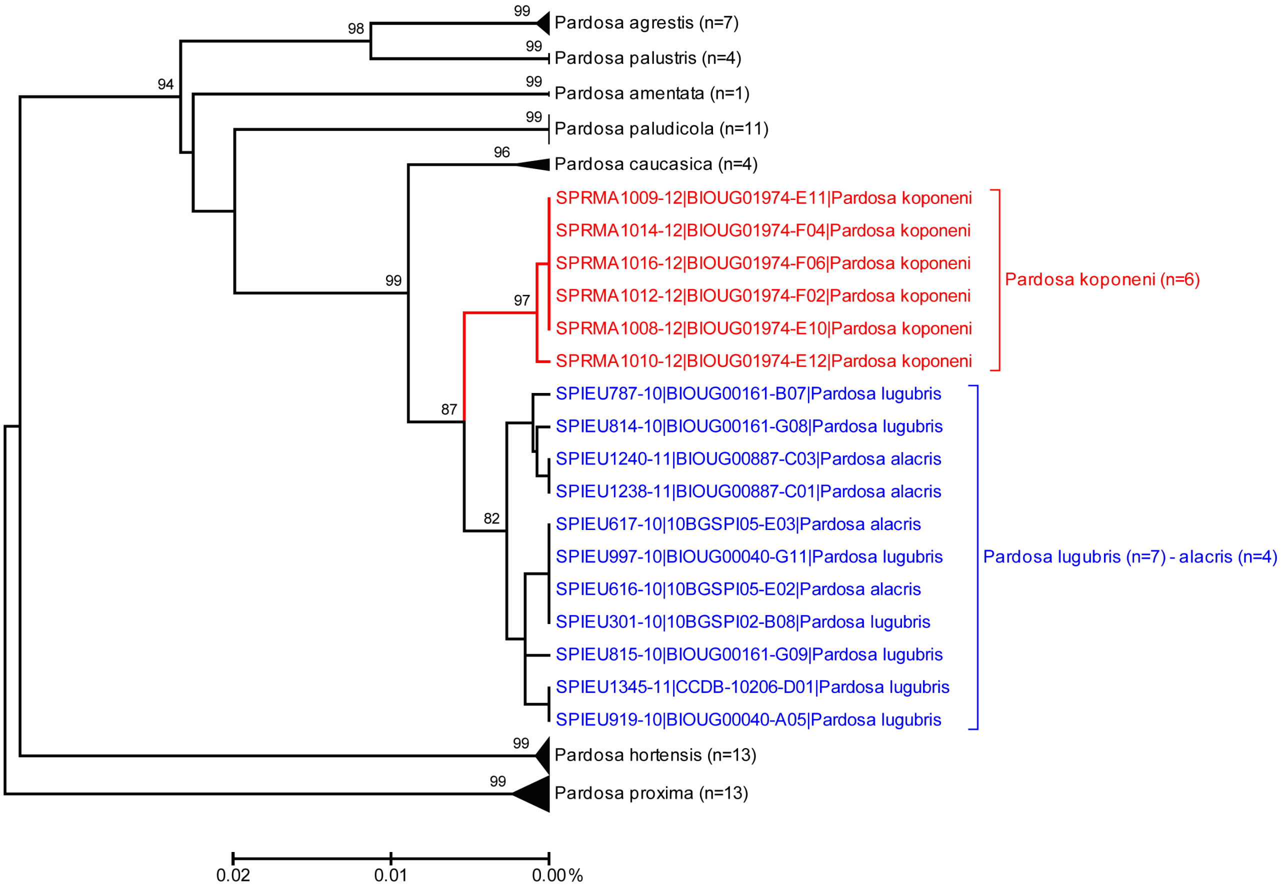


FIGURE 36. Neighbour joining tree generated using K2P distances of COI for ten species of the genus *Pardosa* belonging to five species groups [1—*agrestis*, *palustris*; 2—*amentata*; 3—*paludicola*; 4—*caucasica*, *koponeni*, *lugubris*, *alacris*; 5—*hortensis*, *proxima*]. The sequences are sometimes collapsed into triangles, with the horizontal dimension indicating the genetic divergence within that species. Bootstrap support values are shown above the branches.

Discussion

This study tested the effectiveness of DNA barcoding in distinguishing closely related sibling species of spiders. We examined levels and patterns of barcode divergence among 10 species of *Pardosa* including the new one. DNA barcoding was used to verify its reliability in the delimitation of species boundaries in closely related and young species (Casiraghi *et al.* 2010; Dai *et al.* 2012). These often show limited genetic divergence, and their discrimination may rely solely on classic morphological methods (Mutanen *et al.* 2012). Therefore use of only molecular methods, without morphological confirmation, may lead to mixing the boundaries in some species (Chang *et al.* 2007).

In recent studies some authors reported interspecific divergences less than 2% in different animal groups. Hebert *et al.* (2003) suggest that only 1.9% of the congeneric pairs in groups with normal rates of mitochondrial evolution show less than 2% divergence, which can be a consequence of their short histories of reproductive isolation, but intraspecific sampling was modest in that study. Leaché *et al.* (2009) described their results of the delimitation of species in the coast horned lizard species complex as 0.99%. Later, Jeffery *et al.* (2011) reported cases of recently diverged crustaceans (*Daphnia*) which exhibit less than 2% divergence. Recently, Sauer & Hausdorf (2010) noted 66 sequences of Cretan land snails to be grouped into 58 cluster sets with average interspecific distance of 1%, and Nazari *et al.* (2011) ascertained a low DNA barcode divergence (about 1%)

between two butterfly species living in geographically isolated habitats. Despite these examples, the dominant pattern remains that most interspecific divergences studied to date in animals are found to be >2%, with intraspecific divergence being more constrained. For example, in the analysis of 122 arctic-alpine species of Lepidoptera from the Alps, Fennoscandia and North America (Mutanen *et al.* 2012), 78 species (or 87.6%) of all 89 Fennoscandian species studied possessed less than 1% intraspecific divergence, and 48 of all 67 (71.6%) Alpine species fell below 1% intraspecific divergence. Much more intraspecific variation was shown in the North America lepidopteran species, as just 12 (63%) of all 19 species fell below the 2% threshold in their maximum intraspecific divergence levels (Blagoev *et al.* 2016).

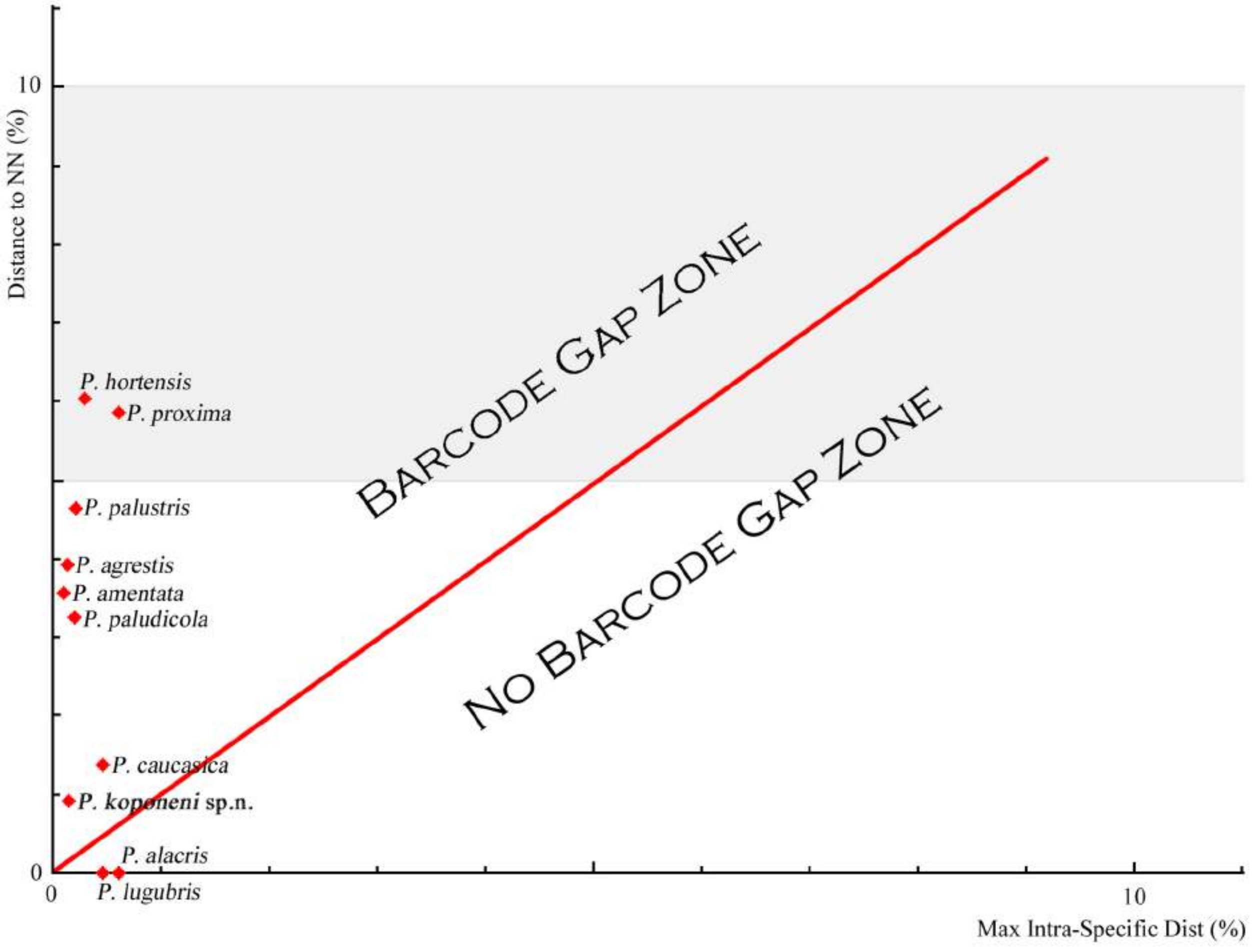


FIGURE 37. A plot of the maximum intraspecific distance compared with distance to nearest neighbour of all species of the genus *Pardosa* used in this study.

The fact that some species have shallow interspecific variation does not discredit the use of DNA barcoding for their identification. Conversely, it allows delineation of the local endemic lineages (Hebert *et al.* 2003, Sauer & Hausdorf 2010). Higher divergences can be expected in geographically isolated populations, which probably reflect their origin in past episodes of gene pool fragmentation.

Our study revealed a case of lower interspecific variation between representatives of the *Pardosa lugubris*-group, which suggested a recent split over the allopatric populations. Töpfer-Hofmann *et al.* (2000) reviewed the *Pardosa lugubris*-group in Central Europe. *Pardosa lugubris* (sensu stricto) and *P. alacris* were separated based largely on particularities of male behaviour during the breeding season (Vlček 1995). The most obvious morphological differences between those two species occur in males (different colour of the cymbium), whereas the females of almost all species of the *Pardosa lugubris*-group are indiscernible (Roberts 1998; Kronestedt 1999; Töpfer-Hofmann *et al.* 2000). However, our DNA barcoding results show a lack of sequence divergence between specimens of *P. alacris* and *P. lugubris* (Fig. 36). The absence of interspecific variation between these species is likely caused by hybridization or incomplete lineage sorting (Funk & Omland 2003; Wiley & Lieberman 2011).

Pardosa lugubris seems to be more ecologically plastic and can be found in a broad range of natural or disturbed habitats, whereas *P. alacris* can occur sympatrically in limited areas with different ecological preferences.

The World Spider Catalog (2015) lists ten junior synonyms within the group *Pardosa lugubris*: *Aranea chelata* Müller, 1764; *A. dorsalis* Fabricius, 1775; *Lycosa silvicola* Sundevall, 1833; *L. silvicultrix* C. L. Koch, 1836; *L. nigriceps* Menge, 1879; *L. nemoralis* Bertkau, 1880; *L. blanca* Wagner, 1894; *L. l. arctica* Strand, 1906; *Pardosa barndti* Wunderlich, 1969; *P. pseudolugubris* Wunderlich, 1984. Upon molecular analysis, usually such complex groups with high numbers of synonyms are revealed to be comprised of many genetic clusters with high levels of "intraspecific" divergences; historical synonymies are often a clue as to the diversity of evolutionary species (Mutanen *et al.* 2012; Nazari *et al.* 2011; Webb *et al.* 2012). Although we found interesting patterns of deep intraspecific differences among geographically disparate populations of some species, they are beyond the scope of this paper and will be treated elsewhere. However, the evidence suggests that the *P. lugubris*-group may be a species complex that can be better resolved by incorporating standard animal barcode approaches and including more representatives of the *P. lugubris*-group, focusing on species that occur in Central Europe.

In conclusion, our results here and results from other studies (Blagoev *et al.* 2013, 2016) indicate that sequence divergences in COI enable the discrimination of most but not all closely allied sibling species among the *Pardosa* species we investigated, and that combining morphological and molecular approaches can unravel many complicated taxonomic cases.

Acknowledgments

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