The northern-most record of Leptanillinae in China with description of *Protanilla beijingensis* sp. nov. (Hymenoptera: Formicidae)

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ABSTRACT. *Protanilla beijingensis* sp. nov. from Beijing, China, is described. The distribution of the new *Protanilla* species is further north than that of any other species in the genus. This also represents the first record of a Leptanillinae species from Palaearctic China. By means of a subterranean pitfall trap (between 30 and 55 cm below ground), we made a collection of three workers and one queen of *P. beijingensis* sp. nov. and 106 individuals of *Leptanilla taiwanensis* Ogata, Terayama *et* Masuko, 1995 in Beijing. The depth of Leptanillinae distribution in Beijing and collection method is discussed. An updated key to the known species of *Protanilla* based on worker and queen castes is presented.

Keywords: Leptanillinae, Protanilla, New species, Beijing, China

INTRODUCTION

Leptanillinae is considered a primitive ant subfamily, and represents one of the early branches of the ant phylogeny (Moreau *et al.*, 2006; Rabeling *et al.*, 2008; Kück *et al.*, 2011). Leptanillinae workers are difficult to collect because of their minute body size and subterranean life history (Masuko, 1990). The Leptanillinae are widely distributed in tropical and warm temperate regions of the Old World and Australian regions (Baroni Urbani, 1977; Bolton, 1990). The genus *Protanilla* Taylor was erected by Bolton (1990), and belongs to the tribe Anomalomyrmini within the Leptanillinae. The type species *Protanilla rafflesi* Taylor from Sri Lanka was re-described by Xu (2012) based on Antweb images. Nine species of *Protanilla* have been described globally, including five species known from China. Xu (2002) described two new species, *P. bicolor* and *P. concolor*, from Yunnan, representing the first records of the genus in China. Terayama (2009) then described *P. lini* from Taiwan, and Xu (2012) described *P. tibeta*



Fig. 1. Sampling sites for Leptanillinae ants in Beijing. *Protanilla beijingensis* sp. nov. was collected at site 1; *Leptanilla taiwanensis* was collected at sites 2 - 10.



Fig. 2. Composition of the subterranean pitfall trap



Fig. 3. Embedding method of the subterranean pitfall trap

from Tibet and *P. gengma* from Yunnan. The other species of the genus include *P. schoedli* from Sri Lanka (Baroni Urbani & De Andrade, 2006), *P. izanagi* from Japan (Terayama, 2013) and *P. wardi* from India (Bharti & Akbar, 2015). Species of *Protanilla* are distributed throughout the South Palaearctic, Oriental and Indo-Australian regions (Baroni Urbani & De Andrade, 2006; Xu, 2012; Bolton, 2016), including China, Japan, India, Sri Lanka, Malaysia, Singapore, and Indonesia (Ant-Maps, 2016).

By means of a subterranean pitfall trap sampling ants between 30 and 55 cm below ground, we collected three workers and one queen of *Protanilla beijingensis* sp. nov. in Xiaolongmen Forest Park (Fig.1), west of Beijing in 2015. Here we present the northernmost record of the genus *Protanilla* and describe *P. beijingensis* sp. nov. as a new species from China. In addition, *P. beijingensis* is the first *Protanilla* species recorded in the Palaearctic of China.

MATERIAL AND METHODS

The worker and queen castes of Protanilla beijingensis sp. nov. were collected using a subterranean pitfall trap. The subterranean pitfall trap was improved based on the device used by Heriberto et al. (2011); we abandoned the bait container because it deteriorates easily during long-term collections, and we changed the preservation solution from propylene glycol to salt water. We collected ant samples using the improved trap device (Figs. 2–3) buried deeply in the soil layer. The subterranean pitfall trap is made of PVC tubing (length 70 cm and inner diameter 15 cm). We made 32 evenly spaced holes (diameter = 1.5cm) along a section of the tube wall that would be 30–55cm below ground when the device was used. In setting up the device (Fig. 3), the tube was buried vertically in soil, and a bucket holding supersaturated salt water as preservation solution was placed at the bottom of the tube. The tube was capped to prevent debris from falling into

the collection bucket. We set the device in the ground in October 2013, and collected samples twice a year, respectively in April and October. Upon collection of the samples, we simultaneously replaced the preservation solution. Samples were then sorted using a stereo microscope and specimens preserved in 100% ethanol.

The total park area is 771 hectares, and geographic coordinates range from $39^{\circ}48'34''N$ to $40^{\circ}10'37''N$ and $115^{\circ}25'00''E$ to $116^{\circ}10'07''E$, with elevation ranging from 68 meters to 1882 meters above sea level. The annual average temperature of Beijing is $13^{\circ}C$, with lowest temperatures reaching -15°C in winter time during recent years (Xu *et al.*, 2015).

Descriptions and measurements were made under a NIKON SMZ1500 stereo microscope with a micrometer. Images were taken with a Leica DFC 450 digital imaging system.

Standard measurements and indices are as defined in Bolton (1987) and Xu (2012):

- TL Total Length: The total outstretched length of the ant from the mandibular apex to the gastral apex.
- HL Head Length: The length of the head in full-face view, excluding the mandibles, measured in a straight line from the midpoint of the anterior clypeal margin to the mid-point of the posterior margin, in fullface view. In species where the posterior margin or the clypeal margin is concave, the measurement is taken from the midpoint of a transverse line spanning the anteriormost or posteriormost projecting points, respectively.
- HW Head Width: The maximum width of the head in full face view, excluding the eyes.
- CI Cephalic Index = $HW \times 100 / HL$.
- SL Scape Length: The maximum straight line length of the antennal scape excluding the basal constriction or neck close to the condylar bulb.

- SI Scape Index = $SL \times 100 / HW$.
- ML Mandibular Length: The straight-line length of the mandible from apex to the base.
- PW Pronotal Width: The maximum width of the pronotum in dorsal view.
- MSL (=AL=WL) Mesosomal Length: The diagonal length of the mesosoma in profile view from the point at which the pronotum meets the cervical shield to the posterior base of the metapleuron.
- PNL Petiolar Node Length: With petiolar node in lateral view, the maximum longitudinal length of the node without its anterior and posterior peduncles.
- PNH Petiolar Node Height: With petiolar node in lateral view, the maximum vertical height of the node from summit to lowermost part of subpetiolar process.
- PNW Petiolar Node Width: The maximum width of the petiolar node in dorsal view.
- PI Petiolar Index = $PNW \times 100 / PNL$
- PPNL Postpetiolar Node Length: With postpetiolar node in lateral view, the maximum longitudinal length of the node without its anterior and posterior peduncles.
- PPNH Postpetiolar Node Height: With postpetiolar node in lateral view, the maximum vertical height of the node from summit to lowermost part of subpostpetiolar process.
- PPNW Postpetiolar Node Width: The maximum width of the postpetiolar node in dorsal view.
- PPI Postpetiolar Index = $PPNW \times 100 / PPNL$
- All measurements are expressed in millimeters.



Figs 4 – 6. Holotype worker of *Protanilla beijingensis* sp. nov. 4. Head in full-face view; 5. Body in profile view; 6. Body in dorsal view.

DESCRIPTION OF NEW SPECIES

Protanilla beijingensis Man, Ran, Chen & Xu, sp. nov.

(Figures 4-9)

Type materials. Holotype. Worker. China: Beijing, Mentougou District, Xiaolongmen National Forest Park, N39°58'25", E115°25'30", 1247m, collected by subterranean pitfall trap in monsoon deciduous forest, 2015.X.15, Pei Man leg., No. IOZ(E) 227911. The holotype worker is deposited in the Institute of Zoology, Chinese Academy Of Sciences, Beijing, China. Paratypes: 2 workers and 1 queen; the paratypes were collected by the same method and with the same data. One queen (No. IOZ(E) 227912) is deposited in the Institute of Zoology, Chinese Academy Of Sciences, Beijing, China. One worker (No. IOZ(E) 227913) is deposited in the Insect Collection, Guangxi Normal University, Guilin, Guangxi Region, China. The other worker (No. IOZ(E) 227914) is deposited in the Insect Collection, Southwest Forestry University, Kunming, Yunnan Province, China.

Holotype worker (Figs. 4-6): TL 4.0, HL 0.70, HW 0.68, CI 97, SL 0.61, SI 90, ML 0.51, PW 0.44, AL 1.21, PNL 0.25, PNH 0.44, PNW 0.29, PI 116, PPNL 0.28, PPNH 0.40, PPNW 0.29, PPI 104.

In full face view (Fig. 4), head roughly trapezoidal and slightly longer than broad, anterior 1/3 of the head distinctly narrowed anteriorly and strongly constricted at antennal socket position, lateral margins evenly convex. Posterior margin weakly concave, posterior corners rounded. Mandibles elongate and curving downwards apically, lateral surface with a longitudinal groove, basal corners prominently round, masticatory margin with 19 peg-like teeth. Clypeus nearly trapezoidal, with a depressed longitudinal central furrow, anterior margin weakly concave. Apex of labrum moderately convex, with a peglike tooth and a pair of stout long hairs. Antennae 12-segmented, apex of scape surpassed posterior head corner by about 1/6 of its length, flagella segments 4-9 about as broad as long.

In profile view (Fig.5), dorsum of mandible strongly convex. Mesosoma strongly constricted at middle position. Dorsum of pronotum weakly convex. Promesonotal suture complete and weakly depressed. Dorsum of mesonotum straight, weakly sloping down posteriorly. Metanotal groove moderately impressed. Dorsum of propodeum weakly convex, posterodorsal corner rounded; declivity slightly convex, about 1/2 length of the dorsum. Petiolar node nearly trapezoidal and narrowed dorsally, anterior face weakly convex, posterior face nearly straight, dorsal face roundly convex; anterodorsal corner rounded, posterodorsal corner relatively prominent. Subpetiolar process large and triangular, with an elliptical semitransparent fenestra, anteroventral corner blunt, anterior and posteroventral margins weakly convex. Postpetiolar node roughly rectangular and weakly widened dorsaly, dorsal face weakly convex, anterior face strongly convex, anterodorsal corner rounded, posterodorsal corner blunt. Subpostpetiolar process large and lobe-like, anteroventrally pointed and rounded at apex. Gaster roughly elliptical, first gastral segment occupies about 1/2 length of gaster. Sting well-developed and extruding.

In dorsal view (Fig. 6), pronotum wide with strongly convex sides. Mesonotum strongly constricted and nearly square. Propodeum relatively narrow and rectangular, with weakly convex sides. Petiolar node nearly rectangular, slightly broader than long, sides evenly convex, anterior face almost straight, posterior face slightly concave. Postpetiolar node trapezoidal and widened posteriorly, as broad as long; anterior face, sides and posterior face weakly convex. Anterior margin of gaster weakly concave.

Mandibles finely retirugose. Head and body smooth and shining. Body dorsum with sparse subdecumbent hairs and abundant decumbent pubescence. Scapes with sparse subdecumbent hairs and abundant decumbent pubescence. Tibiae with abundant decumbent pubescence. Mandibles and clypeus with relatively abundant stouter and longer hairs, apex of each mandible with a very long stout hair on ventral portion (Fig. 5). Body color reddish brown with the exception of the black parts of the posterior half of mesothorax and anterior half of the metathorax Mandibles, antennae, pronotum, legs, and posterior 2/3 of gaster brownish yellow.



Figs 7 – 9. Paratype queen of *Protanilla beijingensis* sp. nov. 7. Head in full-face view; 8. Body in profile view; 9. Body in dorsal view.

Paratype workers: TL 3.9-4.0, HL 0.65-0.70, HW 0.63-0.68, CI 96-97, SL 0.61-0.63, SI 90-98, ML 0.47-0.51, PW 0.43-0.44, AL 1.21-1.23, PNL 0.31-0.35, PNH 0.42-0.44, PNW 0.28-0.30, PI 86-90, PPNL 0.25-0.28, PPNH 0.38-0.40, PPNW 0.28-0.30, PPI 107-112 (2 individuals measured). As holotype, but slightly vary in total length and body color darker.

Paratype queen (Figs. 7-9): TL 4.6, HL 0.78, HW 0.73, CI 94, SL 0.66, SI 90, ML 0.50, PW 0.55, AL 1.46, PNL 0.28, PNH 0.49, PNW 0.35, PI 125, PPNL 0.30, PPNH 0.48, PPNW 0.36, PPI 120 (1 individual measured).

In full face view (Fig. 7), head similar to the holotype worker, but posterior margin almost straight. Masticatory margin of mandible with approximately 34 peg-like teeth. Anterior margin of clypeus concavity more pronounced than in worker caste. Antennae relatively shorter, apex of scape surpasses posterior head corners by about 1/8 of its length. Compound eyes present and moderately large, located slightly behind midlength of head side, with 14 ommatidia in the longest diameter and 11 ommatidia in the shortest diameter, weakly convex with sparse interommatidial pilosity. Vertex with 3 distinct ocelli.

In profile view (Fig. 8), body basically similar to holotype worker, but dorsal outline of mesosoma moderately arched. Promesonotal suture and metanotal groove weakly impressed, mesometanotal suture narrowly notched. Mesopleuron with a distinct oblique furrow. Posterodorsal corner of propodeum very bluntly angled, dorsum short and as of similar length to declivity, the latter straight. Petiolar node weakly narrowed dorsally, anterior and posterior faces almost straight, dorsal face weakly convex; anterodorsal and posterodorsal corners bluntly angled; subpetiolar process similar to worker caste. Dorsum of postpetiolar node strongly convex, subpostpetiolar process similar to worker caste.

In dorsal view (Fig. 9), body basically similar to holotype worker, but mesonotum massive and complex, scutum with a pair of posteriorly convergent longitudinal furrows, transverse furrow posteriorly arched, scutellum rounded posteriorly. Metanotum transverse and very short, posteriorly arched. Propodeum short, posterior corners rounded. Both petiolar node and postpetiolar node broader than long. Sculpture and pilosity similar to holotype worker. Body color yellowish brown. Ocellar area, mesothorax, metathorax, propodeum, petiole and postpetiole brownish black. Antennae, legs and gastral apex brownish yellow. Eyes grey.

Remarks

The new species is close to *P. lini* Terayama, but its petiolar node is relatively low, with rounded anterodorsal corner; dorsum of postpetiolar node almost straight, subpostpetiolar process longer with anteroventral corner rounded; body size larger with TL 3.9 to 4.0 and HL 0.65 to 0.70; body color reddish brown, posterior half of mesothorax and anterior half of metathorax black.

Etymology:

The new species is named after the type locality "Beijing".

REVISED KEY TO KNOWN SPECIES OF *PROTANILLA* OF THE WORLD BASED ON WORKER CASTE

1) In profile view dorsal face of mandible with a large roughly rectangular lobe which occupied about 4/5 of the dorsal margin. Petiolar node roughly triangular with a distinct summit. Postpetiolar node inclined posteriorly, subpostpetiolar process not developed with almost straight ventral margin. In dorsal view petiolar node distinctly narrower than postpetiolar node. Head, pronotum and mesonotum microreticulate (Japan)......*P. izanagi* Terayama

- In profile view dorsal face of mandible without a large roughly rectangular lobe. Petiolar node roughly tapezoidal with a distinct dorsal margin. Postpetiolar node nearly vertical or inclined anteriorly, subpostpetiolar process developed with distinct ventral lobe. In dorsal view petiolar node about as broad as postpetiolar node. Head, pronotum and mesonotum smooth and shining.......2

5) In profile view anterodorsal corner of petiolar node broadly angled and formed a blunt angle. Postpetiolar node inclined anteriorly with posterodorsal corner rounded. Body color brownish yellow (Singapore, Malaysia)....*P. rafflesi* Taylor

- In profile view anterodorsal corner of petiolar node narrowly angled and formed a right angle. Postpetiolar node vertical with posterodorsal corner bluntly angled. Body color blackish brown (India......*P. wardi* Bharti & Akbar

6) In dorsal view petiolar node nearly square, almost as broad as long. In profile view postpetiolar node vertical, not inclined anteriorly........7

7) In profile view petiolar node relatively higher with bluntly angled anterodorsal corner. Dorsum

of postpetiolar node moderately convex, subpostpetiolar process longer with anteroventral corner rounded. Body size smaller with TL 2.9 and HL 0.60. Body color yellowish brown (China: Taiwan Province)......*P. lini* Terayama

- In profile view petiolar node relatively lower with rounded anterodorsal corner. Dorsum of postpetiolar node almost straight, subpostpetiolar process longer with anteroventral corner rounded. Body size larger with TL 3.9 to 4.0 and HL 0.65 to 0.70. Body color reddish brown, posterior half of mesothorax and metathorax black (China: Beijing*P. beijingensis* sp. nov.

- In full face view lateral margin of head with a small tooth at antennal socket position. In profile view petiolar node relatively thicker, dorsal face long, about as long as anterior face. Anteroventral corner of subpostpetiolar process obliquely truncated (China: Tibet Region)......*P. tibeta* Xu

KEY TO KNOWN SPECIES OF *PROTANIL-LA* OF THE WORLD BASED ON QUEEN CASTE

1) In profile view dorsal face of mandible with a large roughly rectangular lobe which occupied about 4/5 of the dorsal margin. Petiolar node roughly triangular with a distinct summit. Postpetiolar node inclined posteriorly, subpostpetiolar process not developed with almost straight ventral margin. In dorsal view petiolar node distinctly narrower than postpetiolar node. Head, pronotum and mesonotum microreticulate (Japan)......*P. izanagi* Terayama

- In profile view dorsal face of mandible without a large roughly rectangular lobe. Petiolar node roughly tapezoidal with a distinct dorsal margin. Postpetiolar node nearly vertical or inclined an- In full face view scapes surpassing posterior head corners by its apical width. In profile view posterodorsal corner of propodeum bluntly angled. Petiolar node slightly narrowing dorsally, subpetiolar process well developed. Postpetiolar node widening dorsally, subpostpetiolar process well developed. Body color reddish brown, mesosoma, petiole and postpetiole blackish brown (China: Beijing)......*P. beijingensis* sp. nov.

COLLECTION OF *Leptanilla taiwanensis* Ogata, Terayama & Masuko IN BEIJINBG

By using the subterranean pitfall trap, we collected 116 workers of Leptanilla taiwanensis Ogata, Terayama & Masuko from nine sample plots in Beijing (Fig.1). Sample plots 2 to 4 are situated in the northwest suburban of Beijing, where the habitat is a mountainous forested area close to a road and surrounded by farmland. Plots 5 to 10 are located in urban parks and green belts along roads, and are significantly disturbed by human activities. Geographic coordinates and elevation of the sites where L. taiwanensis were collected are as follows. Site 2: 40°22'26"N, 115°58'18"E, 550 m; Site 3: 40°8′59″N, 116°1′32″E, 167 m; Site 4: 40°1'13"N, 115°52'5"E, 269 m; Site 5: 40°2'7"N, 116°15′8″E,68m;Site6:40°0′18″N,116°18′17″E, 51 m; Site 7: 39°57'12"N, 116°12'49"E, 60 m; Site 8: 39°56'38"N, 116°18'47"N, 61 m; Site 9: 39°55'8"N, 116°18'37"E, 75 m; Site 10: 39°52'13"N, 116°20'53"E, 47 m.

DISCUSSION

In the 26 years since establishment of the genus Protanilla by Bolton (1990), nine species have been described, eight of which were recorded in the Oriental and Indo-Australian regions, and only P. izanagi Terayama from the Palaearctic region. No species of the genus has ever been reported in the Palaearctic region of China. Several different methods have been used to collect Leptanillinae species, as pointed out by Ward & Sumnicht (2012) and Wong & Guénard (2016). Robertson (2000) gathered males of nine species of Leptanilla from the Brandburg Massif, Namibia with the use of light traps, pan traps and Malaise traps. Lopez and collaborators (1994) collected a large number of individuals of L. charaonea and L. zaballosi by means of the lavage de terre method. Belshaw & Bolton (1994) also collected 30 workers of L. boltoni Baroni Urbani, 1977 with a Winkler extraction of soil samples of 25×25 square centimeters taken to a depth of 5 centimeters. Wong & Guénard (2016) also collected a single Leptanilla worker by using the similar method, which represented the first record of a Leptanillinae species from southeastern mainland China. Meanwhile, by means of small (50 ml) baited subterranean pitfall traps, Wong & Guénard (2016) collected L. hypodracos in Singapore, and by measuring the depth of the trap's entrance they confirmed that L. hypodracos occurs between 10cm to 15cm below ground. The advantage of our improved subterranean pitfall trap is that we can keep track over long periods of time because samples are immersed in supersaturated salt water and will thus only decompose very slowly. In addition, the subterranean pitfall trap does not require artificial monitoring but simply the occasional collection of samples, thus saving a substantial amount of labor. The frequency of sample collection should depend on local environmental patterns and the specific habitat; samples should be gathered more frequently if the study area is rich in rainfall and loose soil, because that rainfall could change the concentration of brine and flush salt into the pitfall trap.

The discovery of *Protanilla beijingensis* sp. nov. and *Leptanilla taiwanensis* Ogata, Terayama & Masuko in Beijing broadens the known distribution range of Leptanillinae species; thus far Beijing is the northern-most region in China where Leptanillinae species have been found. From previous records, Leptanillinae has been recorded in only six provinces or regions of China such as Hubei, Hunan, Yunnan, Tibet, Hong Kong and Taiwan (Xu, 2002; Xu, 2012; Wong & Guénard, 2016; Terayama 2009). As part of the Oriental region, the climatic conditions of these regions are largely different from that of Beijing, which is located in the Palaearctic. Although P. tibeta was collected from Medog County, Tibet, the collection site is located at 1200 meters above sea level on the south slope of Mt. Himalaya, which is within the Oriental region. With a lowest winter temperature of -13°C in recent years and the lowest recorded temperature of -27°C in 1966 (Administration China Meteorological, 2015), Beijing is colder than other distribution areas of Leptanillinae in China. The depth of the subterranean pitfall trap (i.e. 35-55cm below ground) at which we collected samples is much deeper than the conventional depth for collecting Leptanillinae. Therefore, it is likely that we will enrich the knowledge about the distribution region of Leptanillinae in future research by collecting Leptanillinae at a greater depths in the Palaearctic.

We envisage that more Leptanillinae species may perhaps be found in the Palaearctic region with the use of this subterranean pitfall trap in areas further north. The usage of underground trap devices is not confined to myrmecology; for example, similar devices have been used to sample other soil animals such as Carabidae (Ortuño et al., 2014), Gastropoda, Oligochaeta, Nematoda and Arachnida, etc. (Ortuño et al., 2013). In general, increased sampling of subterranean fauna with underground trapping devices may yet make important contributions to knowledge on biological distributions.

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