

Letter to the editor

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## Tip of the iceberg: species diversity of *Pholcus* spiders (Araneae, Pholcidae) in the Changbai Mountains, Northeast China

Despite 250 years of taxonomic classification and over 1.2 million species already catalogued, known species diversity is only a small part of true species diversity on Earth, and thus, the known species are only the tip of iceberg. Here, we investigated the genus *Pholcus* Walckenaer, 1805 of the family Pholcidae C. L. Koch, 1850 in the Changbai Mountains, Northeast China, which provides an excellent case of high species diversity. Previously, only 14 endemic *Pholcus* spiders, all belonging to the *P. phungiformes* species group, and two introduced species *P. manueli* Gertsch, 1937 and *P. zichyi* Kulczyński, 1901 from the *P. crypticolens* species group, have been recorded from this area. Our study confirmed 11 new species of the *P. phungiformes* species group based on morphology and three methods of molecular species delimitation: *P. gaizhou* Yao & Li, **sp. nov.**, *P. guanshui* Yao & Li, **sp. nov.**, *P. jiguanshan* Yao & Li, **sp. nov.**, *P. longxigu* Yao & Li, **sp. nov.**, *P. luquanbei* Yao & Li, **sp. nov.**, *P. shenshi* Yao & Li, **sp. nov.**, *P. tianmenshan* Yao & Li, **sp. nov.**, *P. wangjiang* Yao & Li, **sp. nov.**, *P. xingqi* Yao & Li, **sp. nov.**, *P. yaoshan* Yao & Li, **sp. nov.**, and *P. yuhuangshan* Yao & Li, **sp. nov.** This study brings the fauna of the *P. phungiformes* species group from the Changbai Mountains to 25 species, approximately two times more than previously known, which could indicate that species diversity in the area is underestimated for all arthropod fauna.

The genus *Pholcus* is mainly distributed in the Palaearctic, Indo-Malayan, Afrotropical, and Australasian Regions (Huber, 2011). It is the most diverse genus in Pholcidae and currently includes 343 species belonging to 21 species groups (Huber, 2011; Huber et al., 2018; WSC, 2021). A recent molecular phylogenetic study (Eberle et al., 2018) indicated that the *P. phungiformes* species group is closely related to the *P. bidentatus* species group, although this relationship is not

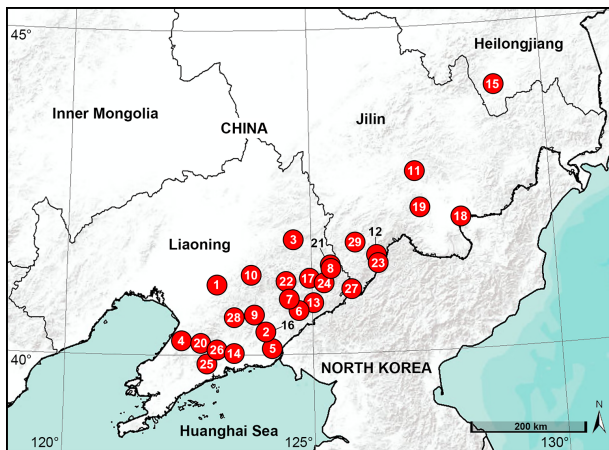
supported. Species of the *P. phungiformes* group mainly occurs on rock walls in three large mountain ranges: the Taihang Mountains in southern North China, the Changbai Mountains at the border between Northeast China and North Korea, and the Taebaek Mountains on the Korean Peninsula (Figure 1; Supplementary Figure S1; Wang et al., 2020). Only *P. phungiformes* Oliger, 1983 occurs further east (Maritime Territory, Sakhalin Island, and Kurile Islands, Russia), probably as a result of human transport (Huber, 2011). The species group has recently been studied by several authors. Zhang and collaborators (Peng & Zhang, 2011, 2013; Zhang & Zhang, 2000; Zhang et al., 2005, 2016; Zhang & Zhu, 2009) reported 13 new species, of which nine were collected from the Taihang Mountains and four from the Changbai Mountains. Li and colleagues (Liu & Tong, 2015; Tong & Ji, 2010; Tong & Li, 2010; Wang et al., 2020; Yao & Li, 2012; Yao et al., 2012; Zhu et al., 2018) described nine and eight new species from the above two areas, respectively. For the Taebaek Mountains, 17 species have been recorded (e.g., Paik, 1978; Seo, 2004, 2014), although several species need to be further illustrated. In addition, Huber (2011) described five new species from the Taebaek Mountains and redescribed nine known species, with two from this region. To date, 59 species of the *P. phungiformes* species group have been recorded from these three mountain ranges. Nevertheless, surveys of the species group in each area have not been uniform. The highest diversity is concentrated in the Taihang Mountains ( $n=22$  spp.) and the Taebaek Mountains ( $n=22$  spp.). In contrast, only 14 species have been recorded from the neighboring Changbai Mountains. This pattern indicates that additional species diversity in the Changbai Mountain region remains undiscovered (Wang et al., 2020).

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**Figure 1** Distribution records of the *Pholcus phungiformes* species group from the Changbai Mountains, China

1: *P. decorus*; 2: *P. fengcheng*; 3: *P. foliaceus*; 4: *P. gaizhou* sp. nov.; 5, 6: *P. gaoi* (5 from type locality); 7: *P. guanshui* sp. nov.; 8: *P. hamatus*; 9: *P. jiguanshan* sp. nov.; 10: *P. jiuwei*; 11, 12: *P. lingulatus* (11 from type locality); 13: *P. longxigu* sp. nov.; 14: *P. luoquanbei* sp. nov.; 15: *P. ningan*; 16: *P. phoenixus*; 17: *P. shenshi* sp. nov.; 18, 19: *P. sublingulatus* (19 from type locality); 20: *P. tianmenshan* sp. nov.; 21: *P. tongji*; 22: *P. wangi*; 23: *P. wangjiang* sp. nov.; 24: *P. wangtian*; 25, 26: *P. xianrendong* (25 from type locality); 27: *P. xingqi* sp. nov.; 28: *P. yaoshan* sp. nov.; 29: *P. yuhuangshan* sp. nov.

For this reason, in 2020, a one-month expedition to the Changbai Mountains was undertaken.

Specimens were examined and measured with a Leica M205 C stereomicroscope. Left male palps were illustrated (exceptions are indicated in figure legends). Epigynes were photographed before dissection. Vulvae were treated in a 10% warm solution of potassium hydroxide (KOH) to dissolve soft tissues before illustration. Images were captured with a Canon EOS 750D wide zoom digital camera (24.2 megapixels) mounted on the stereomicroscope mentioned above and assembled using Helicon Focus 3.10.3 image stacking software (Khmelik et al., 2005). All measurements are given in millimeters (mm). Leg measurements are shown as: total length (femur, patella, tibia, metatarsus, tarsus). Leg podomeres were measured on their dorsal side. The distribution map was generated with ArcGIS 10.2 (Esri Inc.). The specimens studied are preserved in 75% ethanol and deposited in the College of Life Science, Shenyang Normal University (SYNU) in Liaoning, China, the Museum of Hebei University (MHBHU) in Hebei, China, and the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing, China. Terminology and taxonomic descriptions follow Huber (2011) and Yao et al. (2015). Morphological descriptions of all the species from the Changbai Mountains can be found in the supplementary materials. All the new species are registered in Zoobank at <http://zoobank.org/>.

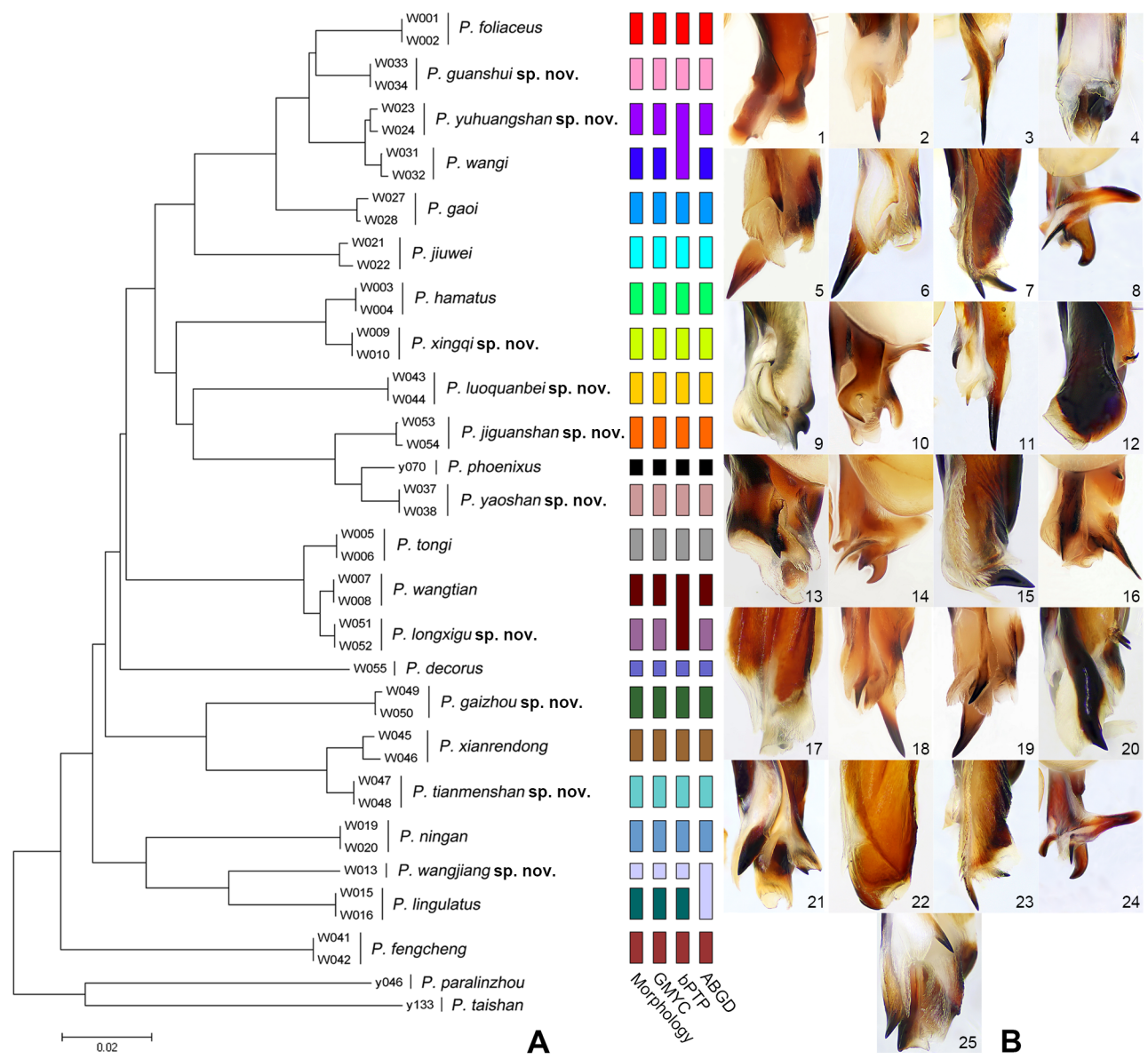
DNA barcodes of *COI* were obtained for 45 samples, including two samples of two species *P. paralinzhou* Zhang & Zhu, 2009 and *P. taishan* Song & Zhu, 1999 as outgroups, using the following primers: forward: LCO1490-oono (5'-CWACAAAYCATARRGATATTGG-3') and reverse: C1-N-2776

(5'-GGATAATCAGAATANCGNCGAGG-3'). The dataset was analyzed using MEGA 5 (Tamura et al., 2011), and a neighbor-joining tree was constructed for molecular species delimitation. DNA extractions are preserved in TE buffer and stored at  $-20^{\circ}\text{C}$ . For additional information on extraction, amplification, and sequencing procedures, see Yao et al. (2016).

We applied three methods for molecular species delimitation. (1) The general mixed Yule coalescent (GMYC) model, which delimits species from an ultrametric tree of individual genes without prior definitions of species. The GMYC method identifies a time point on the best tree (the tree with the highest likelihood) where the branching rate shifts from speciation to the population coalescent process. This analysis was performed under the single-threshold model using the R 4.0.3 package SPLITS (Species Limits by Threshold Statistics) (R Development Core Team, 2020). (2) The Bayesian implementation of the Poisson Tree Processes (bPTP) model tests species boundaries based on phylogenetic trees of individual genes. The bPTP method uses nucleotide substitution information and implements a model assuming phylogenetic tree branch lengths are generated by two classes of Poisson processes (intra- and interspecific branching events). This analysis was conducted on a web server (<http://species.h-its.org/ptp/>) using individual gene trees. The MCMC was run for 100,000 generations, with a thinning of 100 and burn-in of 0.2 (Zhang et al., 2013). (3) The automatic barcode gap discovery (ABGD) online version examines species delimitation with recursive partitioning using a range of prior intraspecific divergence and relative gap widths, estimating the threshold between intra- and interspecific genetic variation to generate species-level groupings. The ABGD analyses were conducted using both Jukes-Cantor and Kimura 2-P distance matrices with options: Pmin=0.001, Pmax=0.1, Steps=10, X=0.5, Nb bins=20 (Puillandre et al., 2012).

A total of 45 *COI* sequences from 43 ingroup members and two outgroup members were generated. The alignment did not include any gaps, and 640 base pairs (bp) were recovered. The sequences are deposited in GenBank under accession Nos. MW721782–MW721826 (Supplementary Table S1). The GMYC analysis identified 23 provisional species, and the result was fairly consistent with morphology (Figure 2; Supplementary Figure S2). The bPTP analysis identified 21 species. Compared to morphology and the GMYC, *P. yuhuangshan* sp. nov. and *P. wangi* Yao & Li, 2012 were recognized as a single species, and *P. wangtian* Tong & Ji, 2010 and *P. longxigu* sp. nov. were considered one species (Figure 2; Supplementary Figure S3). The result of the ABGD analysis was also highly consistent with the morphology and the GMYC (Figure 2), except for *P. wangjiang* sp. nov. and *P. lingulatus* Gao, Gao & Zhu, 2002 which were recognized as a single species.

Considering all of the evidence, we conclude that there are 25 provisional species, of which two, *P. shenshi* sp. nov. and *P. sublingulatus* Zhang & Zhu, 2009 were not included in the molecular analyses due to the lack of DNA data. The GMYC analysis supports speciation events among the 23 species, and the result is fairly consistent with morphology. Although



**Figure 2 A simplified phylogenetic tree and the results of species delimitation analyses**

A: The neighbor-joining tree constructed based on *COI* barcode sequences (different species delimitation methods are presented on the right, different colors of the bars represent the different species); B: Distal part of procurus in prolateral view of the *P. phungiformes* species group from the Changbai Mountains, China (1: *P. decorus*; 2: *P. fengcheng*; 3: *P. foliaceus*; 4: *P. gaizhou sp. nov.*; 5: *P. gaoi*; 6: *P. guanshui sp. nov.*; 7: *P. hamatus*; 8: *P. jiguanshan sp. nov.*; 9: *P. jiuwei*; 10: *P. lingulatus*; 11: *P. longxigu sp. nov.*; 12: *P. luoquanbei sp. nov.*; 13: *P. ningan*; 14: *P. phoenixus*; 15: *P. shenshi sp. nov.*; 16: *P. sublingulatus*; 17: *P. tianmenshan sp. nov.*; 18: *P. tongi*; 19: *P. wangi*; 20: *P. wangjiang sp. nov.*; 21: *P. wangtian*; 22: *P. xianrendong*; 23: *P. xingqi sp. nov.*; 24: *P. yaoshan sp. nov.*; 25: *P. yuhuangshan sp. nov.*).

the bPTP collapsed 2 species pairs each to a single species, both ABGD and GMYC results clearly support their separate species status. Furthermore, *P. yuhuangshan sp. nov.* can be easily distinguished morphologically from *P. wangi* by the procurus with a short, angular ventro-distal apophysis and a blunt distal apophysis, by the uncus distally strongly curved, by the epigyne not curved posteriorly, and by the vulval anterior arch without dorsal sclerites; *P. longxigu sp. nov.* can be easily distinguished from *P. wangtian* by the procurus with a pointed prolatero-subdistal apophysis, a curved, pointed

ventro-subdistal apophysis, and a long, spine-shaped distal apophysis, and by the epigyne which is very slightly curved posteriorly, and the vulval anterior arch which is large and strongly sclerotized. The ABGD analysis recognized *P. wangjiang sp. nov.* and *P. lingulatus* as one species; however, a speciation event between those two species is well supported by the bPTP and GMYC analyses. In addition, *P. wangjiang sp. nov.* can be easily distinguished morphologically from the latter by the procurus with a prolatero-distal membranous process with a long, nearly s-

shaped apophysis and three short dorso-subdistal spines, without the dorso-subdistal apophysis and the distal membranous lamella, and by the uncus distally narrow and not curved. Finally, although *P. shenshi* sp. nov. and *P. sublingulatus* are not included in our molecular analyses, their diagnostic morphological characters (Supplementary Text S1) clearly suggest that they are separate species. For detailed morphological descriptions, diagnoses, illustrations, and an identification key of all species of the *P. phungiformes* group from the Changbai Mountains, see Supplementary Text S1 and Figures S4–S30.

Our study revealed high diversity of the *P. phungiformes* group members from the Changbai Mountains, China, which now contains 25 species, approximately two times more than previously recorded. We believe that a large amount of arthropod diversity is yet to be discovered in this area. The Changbai Mountain area presents a very interesting study system, and further research in this area will shed more light on true species diversity and diversification processes.

#### NOMENCLATURE ACTS REGISTRATION

The electronic version of this article in portable document format represents a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone (see Articles 8.5–8.6 of the Code). This published work and the nomenclature acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information can be viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>.

Publication LSID:

urn:lsid:zoobank.org:pub:386FF05A-85F6-4862-AD3F-B388937EAFDD

*Pholcus gaizhou* LSID:

urn:lsid:zoobank.org:act:FE2BF057-E074-4776-8888-0D513717DBA4

*Pholcus guanshui* LSID:

urn:lsid:zoobank.org:act:91D36863-3494-486D-A63A-40E21B9B41A3

*Pholcus jiguanshan* LSID:

urn:lsid:zoobank.org:act:39513F66-36D7-44B7-B24A-4E85185E0F00

*Pholcus longxigu* LSID:

urn:lsid:zoobank.org:act:80F3F37D-1F1E-4478-8675-3D3573AF1716

*Pholcus luoquanbei* LSID:

urn:lsid:zoobank.org:act:CF55577A-53F1-4EDF-AEB7-C221CBDF0601

*Pholcus shenshi* LSID:

urn:lsid:zoobank.org:act:9BC89C62-4147-47D2-A9DB-F104730E1BBC

*Pholcus tianmenshan* LSID:

urn:lsid:zoobank.org:act:683CA3E6-B023-44E8-9861-35D4E1801479

*Pholcus wangjiang* LSID:

urn:lsid:zoobank.org:act:06B5B24A-8F2C-4072-9E30-93F1F4A2E338

*Pholcus xingqi* LSID:

urn:lsid:zoobank.org:act:F245A38E-FF93-415B-94A6-25871F3DD098

*Pholcus yaoshan* LSID:

urn:lsid:zoobank.org:act:03E39090-3B07-4E19-B642-942EADDCC706

*Pholcus yuhuangshan* LSID:

urn:lsid:zoobank.org:act:8988CC5F-8D87-4E0D-A152-2B7FFA6C19C8

#### SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Permission for field surveys in Liaoning, Jilin, and Heilongjiang was granted by the National Forestry and Grassland Administration.

#### SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

#### COMPETING INTERESTS

The authors declare that they have no competing interests.

#### AUTHORS' CONTRIBUTIONS

Z.Y.Y. and S.Q.L. designed the study. Z.Y.Y. and X.W. contributed the fieldwork, performed the molecular experiments, and finished the descriptions. Z.Y.Y. performed the morphological species identification and the analytical work of the molecular data. X.W. finished taking photos. Z.Y.Y. and S.Q.L. drafted and revised the manuscript. All authors read and approved the final version of the manuscript.

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