Mycoscience VOL.64 (2023) 150–155

Mycoscience

An open access journal for general mycology

The Mycological Society of Japan

Full paper

Neoerysiphe leontopodii sp. nov., a new powdery mildew from China

Tie-Zhi Liu[a*, #], Li Liu[b, c, #], Jing Wen[a], Shu-Yan Liu[b, c]

[a] College of Chemistry and Life Sciences, Chifeng University, Chifeng 024000, Inner Mongolia Autonomous Region, PR China

[b] Department of Plant Pathology, College of Plant Protection, Jilin Agricultural University, Changchun 130118, Jilin Province, PR China

[c] Engineering Research Center of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University, Changchun 130118, Jilin Province, PR China

ABSTRACT

A powdery mildew was found on *Leontopodium leontopodioides* (*Asteraceae*) in China. Phylogenetic analyses using a combination of internal transcribed spacer and 28S rDNA sequences showed that this species, which clusters as sister to *Neoerysiphe joerstadii*, is allied to *N. galii*, *N. geranii*, and *N. nevoi*. This species differs from the closely allied *N. joerstadii* in the number and size of asci (3–10 asci, 55–75 × 20–40 µm versus 16–32 asci, 40–60 × 20–30 µm). This species is morphologically very similar to *N. gnaphalii*, but clearly differs from this species in having larger chasmothecia and colorless appendages. Therefore, the powdery mildew on *L. leontopodioides* is described as *N. leontopodii* sp. nov.

Keywords: *Asteraceae*, *Erysiphaceae*, Molecular phylogeny, Morphology, New species

Article history: Received 15 August 2023, Revised 21 September 2023, Accepted 24 September 2023, Available online 27 October 2023.

1. Introduction

Leontopodium leontopodioides (Willd.) Beauv. (*Asteraceae*), a perennial herb, is widely distributed in northeast, northwest, north and southwest China, especially in the Qinghai Tibet Plateau (Chen et al., 2011). *Leontopodium leontopodioides* is commonly used in the traditional Chinese medicine for the treatment of albuminuria, nephritis, urinary tract infection, hematuria, vaginitis, bronchopneumonia, gastric ulcer and traumatic bleeding (Li et al., 2012; Zhang et al., 2018; Zhao et al., 2019). Its main chemical components include phenylpropanoids, flavonoids, sesquiterpene, phytosterols, etc. (Pan, 2009). In addition, it plays an important role in the succession of alpine meadows (Zhang et al., 2019).

Leontopodium leontopodioides belongs to *Asteraceae* subtribe *Gnaphalinae* (Chen et al., 2011). Two powdery mildew species of *Neoerysiphe* have been described on host species of subtribe *Gnaphalinae*. *Neoerysiphe joerstadii* V. P. Heluta & S. Takam. parasitizes on the *Phagnalon rupestre* and is distributed in the Golan Heights of Israel in the Mediterranean region (Heluta et al., 2010; Braun & Cook, 2012). *Neoerysiphe joerstadii* has larger chasmothecia and a larger number of asci compared to *N. leontopodii*. The second species, *N. gnaphalii* U. Braun, parasitizes on *Omalotheca norvegica* and is distributed in northern Europe, including Finland, Norway, and Sweden (Braun & Cook, 2012). Its morphology is similar to *N. galeopsidis*, and appendages are geniculate-sinuous to spirally twisted, usually unbranched (Braun & Cook, 2012).

In late Aug of 2012, we found chasmothecia on the surface of leaves of *L. leontopodioides* in the Inner Mongolia Autonomous Region, China. The leaves of *L. leontopodioides* are characterized by having a dense white tomentum, so that the asexual morph of this species is difficult to detect. Morphological and molecular analyses revealed that this morph represents an undescribed species belonging to the genus *Neoerysiphe*. *Neoerysiphe leontopodii* sp. nov is proposed in this study for this fungus, including a detailed morphological description and illustration.

2. Materials and methods

2.1. Morphology

So far, only a single powdery mildew sample on *L. leontopodioides* has been collected in Sumushan Forest Park, Xinghe County, Ulanqab City, Inner Mongolia Autonomous Region, China. The collection is deposited in the Mycological Herbarium of Chifeng University (CFSZ). Conidiophores, conidia, and chasmothecia were stripped from the leaf surface with a clean needle, mounted on a microscope slide, and examined in lactic acid using a light microscope (ZEISS scopeA1, Germany) with phase contrast at 10 ×, 20 ×, and 40 × objectives. Thirty measurements for all structures of taxonomical value were performed.

2.2. Molecular phylogeny

Genomic DNA was extracted from mycelia and chasmothecia

# These authors contributed equally to this work and should be considered co-first authors

* Corresponding author.
College of Chemistry and Life Sciences, Chifeng University, Chifeng 024000, Inner Mongolia Autonomous Region, PR China
E-mail address: tiezhiliu@aliyun.com

using the chelex-100 method (Walsh et al., 1991) as previously described (Hirata & Takamatsu, 1996). PCR and DNA sequencing were conducted according to the procedures described in Liu et al. (2020), except for the primers that were used. PM2/PM10 and PM3/LSU2 primer sets were used to amplify 5'-half of the internal transcribed spacer (ITS) region and 3'-half of ITS and 5'-end of the 28S rDNA gene (including domains D1 and D2), respectively (Bradshaw & Tobin, 2020; Scholin et al., 1994). The newly obtained sequences in this study were deposited in GenBank with the accession number OR269994. The sequences were aligned with closely related sequences of other *Neoerysiphe* species retrieved from GenBank using MUSCLE implemented in the MEGA7 program (Kumar et al., 2016). Alignments were further manually refined and deposited in TreeBASE (https://www.treebase.org/) under the accession number 30579. Phylogenetic trees were obtained from the data using the maximum-parsimony (MP) method in PAUP4.0 (Swofford, 2002) with heuristic search option using the tree-bisection-reconstruction (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweight with gaps treated as missing data. The strength of internal branches of resulting trees was tested with bootstrap analysis (1000 replications) (Felsenstein, 1985). Bootstrap (BS) values of 50% or higher are indicated in the tree (Fig. 3). Tree scores, including tree length, consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated.

3. Results

3.1. Morphology

Neoerysiphe leontopodii T.Z. Liu & L. Liu, sp. nov. Figs. 1, 2.

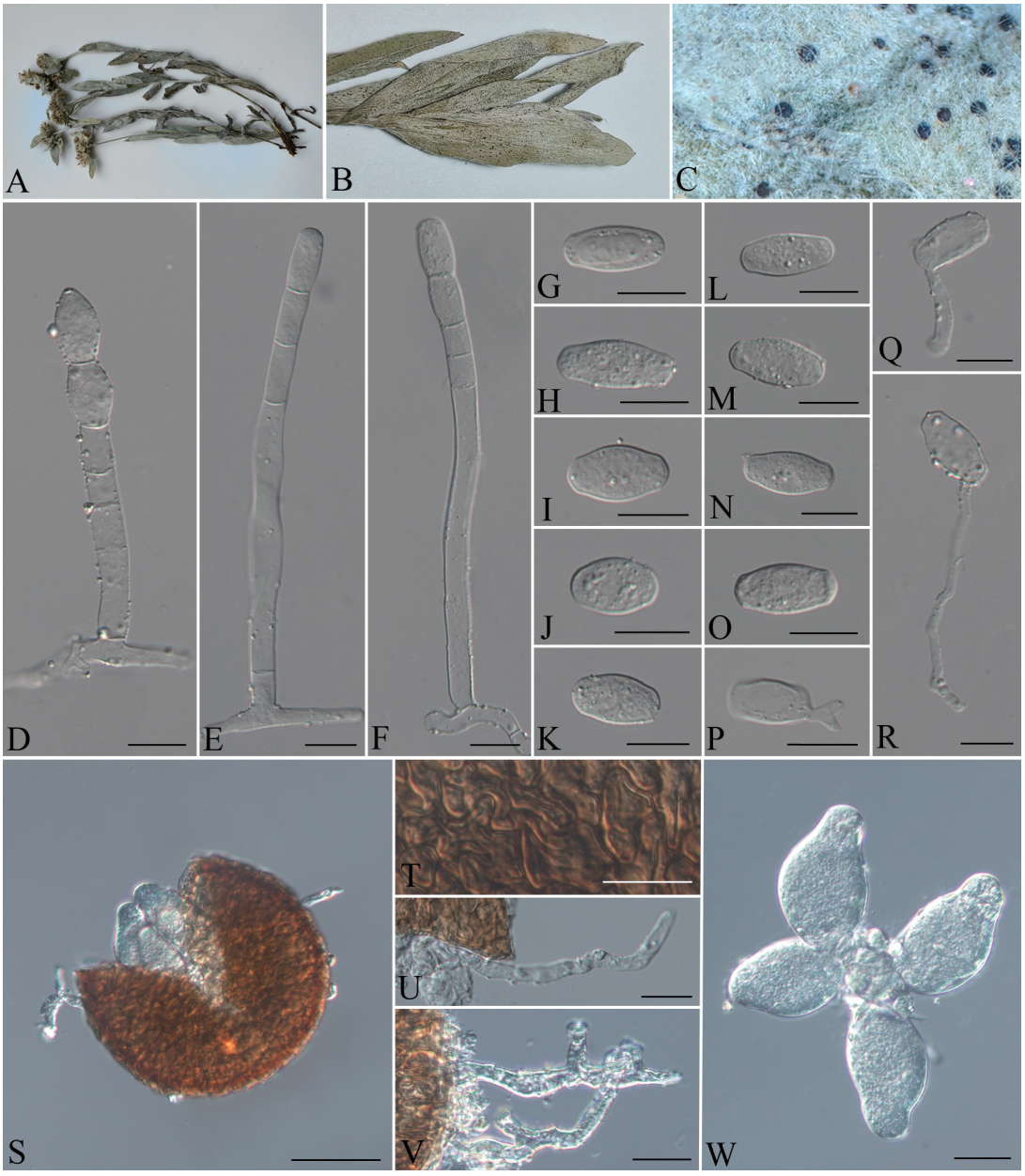

Fig. 1 – *Neoerysiphe leontopodii* on *Leontopodium leontopodioides*. A: Symptoms on infected leaves. B: Chasmothecia scattered on leaf surfaces. C: Close-up view of chasmothecia. D–F: Conidiophores on a leaf. G–O: Conidia. P–R: Conidia with germ tube. S: Open chasmothecium releasing chasmothecia. T: Peridium cells. U–V: Appendages. W: Asci. *Bars*: D–R, T–W 20 μm; S 50 μm.

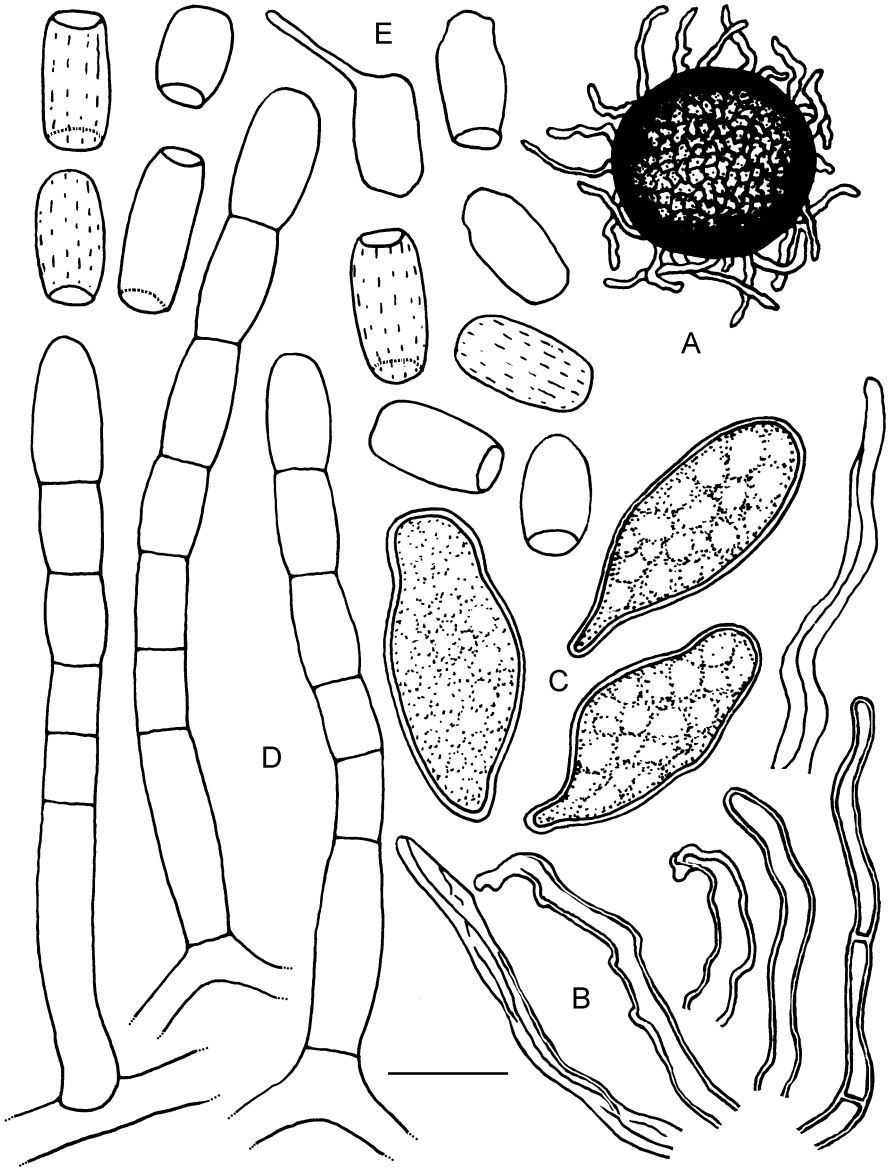

Fig. 2 – *Neoerysiphe leontopodii* on *Leontopodium leontopodioides*. A: Chasmothecium. B: Appendages. C: Asci. D: Conidiophores. E: Conidia. *Bars*: A 100 μm; B–E 25 μm.

MycoBank no.: MB849490.

Diagnosis: Differs from *Neoerysiphe gnaphalii* in having larger chasmothecia (vs. smaller chasmothecia, 85–140 μm diam in *N. gnaphalii*) and colorless appendages (vs. appendages yellowish brown to moderate dark brown in *N. gnaphalii*).

Type: CHINA, Sumushan Forest Park, Xinghe County, Ulanqab City, Inner Mongolia Autonomous Region, on *Leontopodium leontopodioides* (*Asteraceae*), 21 Aug 2012, Tie-Zhi Liu, Zhen-Hua Jia and De-Nan Su (holotype, CFSZ 5854).

Gene sequences ex-holotype: OR269994

Etymology: The specific epithet "*leontopodii*" is derived from the host genus name.

Description: Mycelium on leaves, amphigenous, and stems, white, in irregular patches, sometimes confluent or covering the entire surface of the leaves, evanescent to persistent; hyphal cells 5–10 μm wide, smooth to slightly rough, hyaline; hyphal appressoria and secondary hyphae not observed; conidiophores arising mostly centrally from the upper surface of the hyphal mother cell, erect, about 85–175 μm long, foot-cells 50–100 × 10–15 μm, cylindrical, straight to mostly somewhat flexuous at the base, followed by 1–2 shorter cell, forming catenescent conidia; conidia ellipsoid, doliiform-cylindrical, subcylindrical, 20–35(–37.5) × 10–15(–17.5) μm, with a longitudinal surface wrinkling pattern. Chasmothecia scattered to gregarious, sometimes immersed in the pannose tomentum of the host, 120–180 μm diam, dark brown, depressed globose; peridium cells irregularly polygonal or daedaleoid, 8–25 μm diam; appendages numerous, about 10–40, arising from the lower half, mycelioid, unbranched, rarely irregularly branched, rarely stiff, sometimes poorly developed, sinuous or geniculate, length 20–150 μm, 0.2–1 times as long as the diameter of the chasmothecium, mostly shorter than the chasmothecial diam., 2.5–8 μm wide, thin-walled, walls thick below, becoming gradually thinner towards the tip, smooth or slightly rough, 0–3-septate, hyaline; asci 3–10, ellipsoid, obovoid, with irregular outline, 55–75 × 20–40 μm, sessile or short-stalked, content often yellowish, but always immature in the current season; ascospores not developed before overwintering.

Host range and distribution: On *Leontopodium leontopodioides*.

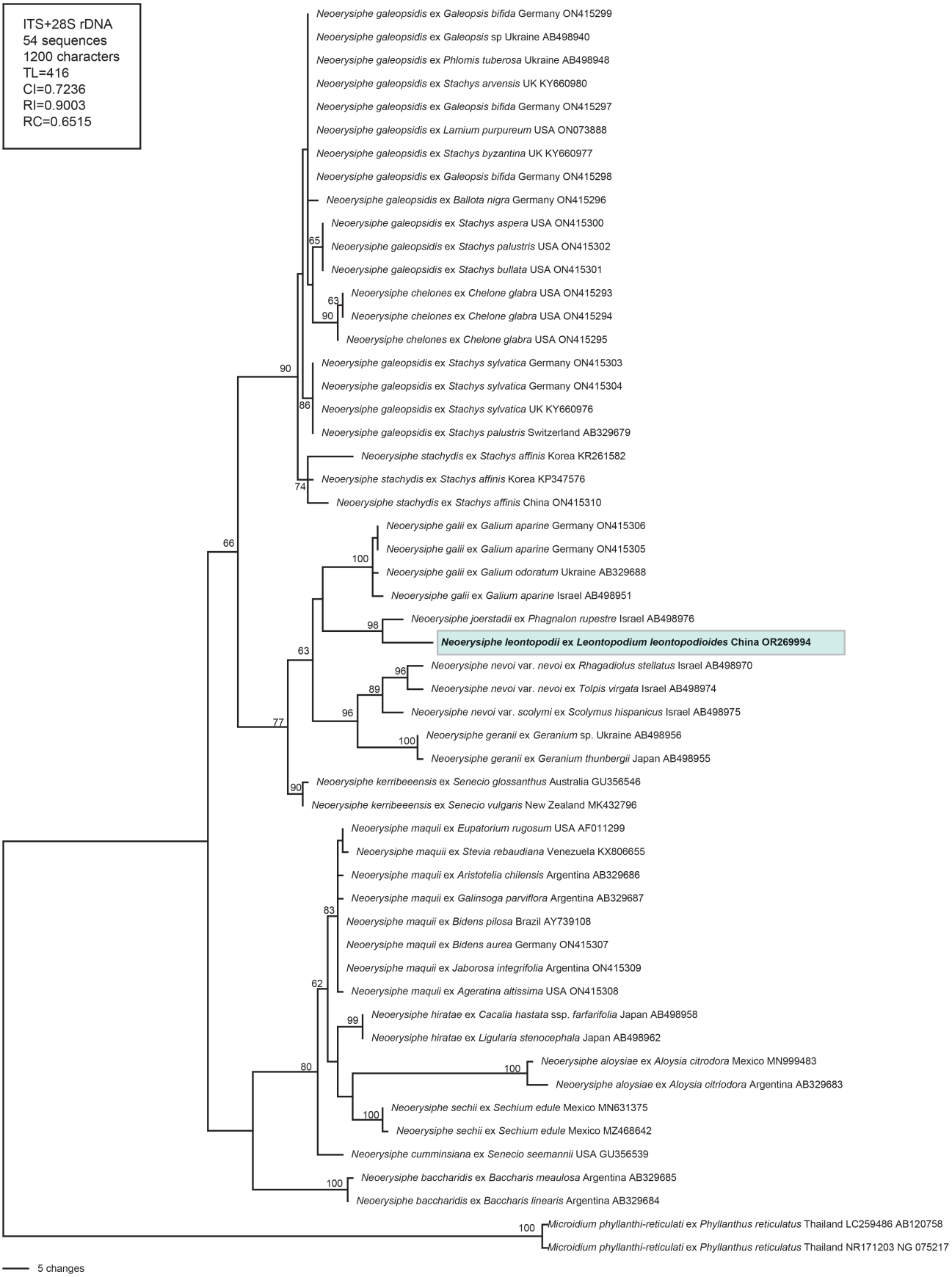

Fig. 3 – Phylogenetic analysis of *Neoerysiphe leontopodii* based on the internal transcribed spacer (ITS) and 28S rDNA sequences. The tree was constructed based on 51 sequences from genus *Neoerysiphe* reported in Bradshaw et al. (2022). The sequence of *N. leontopodii* is highlighted as green box. Two sequences of *Microidium phyllanthi-reticulati* were used as outgroup. Bootstrap values based on 1000 replications are indicated above/below the branches.

Inner Mongolia Autonomous Region, China, only known from the type locality.

3.2. Molecular phylogeny

A phylogenetic tree has been constructed, using a data set of ITS and 28S rDNA sequences, to clarify the phylogenetic affinity of *N. leontopodii*. These sequences were aligned with 51 sequences from closely related species reported in Bradshaw et al. (2022). Two sequences of *Microidium phyllanthi-reticulati* were used as outgroup based on Bradshaw and Tobin (2020). The data-set consisted of 54 sequences. Of the 1200 characters, 28 characters were variable and 228 characters were informative for parsimony analysis. A total of 100 equally parsimonious trees were constructed by the MP analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal groups and branch lengths. One of the trees with the highest likelihood value is shown in Fig. 3. *Neoerysiphe leontopodii* forms an independent cluster with *N. joerstadii* with high BS support (98%).

4. Discussion

The ITS sequence of *Neoerysiphe leontopodii* clusters close to a corresponding sequence of *N. joerstadii* (AB498976), with a similarity of 98.04%. The analysis of the 28S rDNA sequence yielded a maximum similarity of 98.48% to both *N. galeopsidis* (ON415299), *N. galii* (ON415305) and *N. chelones* (ON415293). Based on the phylogenetic tree constructed by ITS and 28S rDNA sequences, *N. leontopodii* is sister to *N. joerstadii*, with *N. galii*, *N. nevoi*, and *N. geranii* as further allied species. The hosts of *N. leontopodii* and *N. joerstadii* both belong to subtribe *Gnaphalinae*, and in the phylogenetic tree, they cluster together, indicating that these two species may come from a common ancestor, reflecting co-evolution with the host plants. Hosts of *N. galii* belong to the genera *Cruciata*, *Galium*, and *Phuopsis* (*Rubiaceae*). The taxonomic-phylogenetic placement of the latter species has been proven by several authors (Takamatsu et al., 2008, 2009). Based on overwintering experiments of this species, it was demonstrated that the asci and ascospores within chasmothecia mature in the next year (Braun & Cook, 2012). *Neoerysiphe nevoi* is parasitic on hosts of the tribe *Cichorieae* of the *Asteraceae*. The host range of this species is still insufficiently known and needs further research. Some of the collections reported as "*Erysiphe cumminsiana*", "*E. galeopsidis*", "*E. galii*", and "*E. cichoracearum*" on *Asteraceae* might belong to *N. nevoi* and need to be microscopically (and/or phylogenetically) re-examined (Amano, 1986; Braun, 1987; Gorter, 1987). *Neoerysiphe geranii* was introduced as a new species by Nomura (1997), and its phylogenetic position was later clarified by Heluta et al. (2010). Originally reported from Japan, this species has later been confirmed for China (Liu, 2010; Liu et al., 2006), India (Braun & Paul, 2009), Russia and Ukraine (Heluta et al., 2010), the United Kingdom, and New Zealand (Braun & Cook, 2012), but it might have a much wider distribution. *Neoerysiphe leontopodii* differs from *N. joerstadii* in having 3–10 asci, 55–75 × 20–40 μm (vs., 16–32 asci, 40–60 × 20–30 μm). *Neoerysiphe leontopodii* is morphologically very close to *N. gnaphalii*, but differs in having larger chasmothecia, 120–180 μm diam, whereas the latter species has smaller chasmothecia, 85–140 μm diam. Furthermore, the appendages of the new species are colorless, in contrast to yellowish brown to moderately dark brown in *N. gnaphalii* (Braun & Cook, 2012). Unfortunately, *N. gnaphalii* has not yet been sequenced, so that the phylogenetic relationship between *N. gnaphalii* and *N. leontopodii* can currently not be elucidated. In general, it is recommendable to base new species at least on two specimens and sequences. However, in the present case it was not possible to find a second collection. The texture of the leaf surface of the host, *Leontopodium leontopodioides*, with a dense white hairiness, strongly impedes the tracing of this powdery mildew, so that the discovery of this powdery happened rather by chance.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 31760004, No. 31970019, No. U21A20177) and First-class disciplines construction project of Chifeng University (No. CFXYYLXKB 202102). The authors sincerely acknowledge Prof. Uwe Braun for reading the full text, for critical comments and for corrections.

References

Amano, K.H. (1986). *Host range and geographical distribution of the powdery mildew fungi.* Japan Scientific Societies Press.

Bradshaw, M., & Tobin, P.C. (2020). Sequencing herbarium specimens of a common detrimental plant disease (Powdery Mildew). *Phytopathology*, *110*(7), 1248–1254. https://doi.org/10.1094/PHYTO-04-20-0139-PER

Bradshaw, M.J., Braun, U., Götz, M., & Pfister, D.H. (2022). Phylogeny and taxonomy of the genera of Erysiphaceae, part 2: *Neoerysiphe*. Mycologia, *114*(6), 994–1007. https://doi.org/10.1080/00275514.2022.2115420

Braun, U. (1987). *A monograph of the Erysiphales (powdery mildews)*, Beihefte zur Nova Hedwigia, *87*, 1–700.

Braun, U., & Cook, R.T.A. (2012). *Taxonomic manual of the Erysiphales(powdery mildews).* CBS Biodiversity series No. 11. CBS-KNAW Fungal Biodiversity Centre.

Braun, U., & Paul, Y. (2009). The Indian Erysiphaceae revisited. Nova Hedwigia, *89*, 371–395.

Chen, Y.S., Zhu, S.X. & Bayer, R.A. (2011). Gnaphalieae. Pp. 774–818 in: Wu, Z.Y., Raven, P.H. & Hong, D.Y., eds., Flora of China Volume 20–21 (Asteraceae). Science Press (Beijing) & Missouri Botanical Garden Press (St. Louis).

Felsenstein, J. (1985). Confidence limits on phylogenetics: An approach using the bootstrap. Evolution, *39*(4), 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Gorter, C.J.M.A. (1987). Relevance of conidial surface structure for identifying *Erysiphe galeopsidis* DC. *South African Journal of Science*, *83*, 112–114.

Heluta, V., Takamatsu, S., Harada, M., & Voytyuk, S. (2010). Molecular phylogeny and taxonomy of Eurasian *Neoerysiphe* species infecting Asteraceae and Geranium. *Persoonia*, *24*(1), 81–92. https://doi.org/10.3767/003158510X501696

Hirata, T. & Takamatsu, S. (1996). Nucleotide diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. *Mycoscience*, *37*(3), 283–288. https://doi.org/10.1007/BF02461299

Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/ 10.1093/molbev/msw054

Li, X., Luo, J.G., Wang, X.B. Luo, J., Wang J.S., & Kong, L.Y. (2012). Phenolics from *Leontopodium leontopodioides* inhibiting nitric oxide production. *Fitoterapia*, *83*(5), 883–887. https://doi.org/10.1016/j.fitote.2012.03.020

Liu, L., Tang, S.R., Takamatsu, S., Braun, U., Li, Y., & Liu, S.Y. (2020). Morphology and molecular phylogeny of a poorly known species- *Erysiphe bulbouncinula* (Erysiphaceae) on *Koelreuteria paniculata*. *Forest Pathology*, *50*, e12621. https://doi.org/10.1111/efp.12621.

Liu, T.Z. (2010). *The Erysiphaceae of Inner Mongolia*. Inner Mongolia Science and Technology Press, Chifeng.

Liu, T.Z., Bai, X.L, & Wang, Y.C. (2006) Four new records of powdery mildews from China. *Mycosystema*, *25*(4), 529–539.

Nomura, Y. (1997). *Taxonomical study of Erysiphaceae of Japan*, Yokendo, Tokyo.

Pan, C.Y. (2009). Study on the chemical constituents of n-BuOH extracts from whole plant of Leontopodium leontopodioides. Master's thesis, Shenyang Pharma-

ceutical University
Scholin, C.A., Herzog, M., Sogin, M., & Anderson, D.M. (1994). Identification of group- and strain-specific genetic markers for globally distributed Alexandrium (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *Journal of Phycology*, *30*(6), 999–1011.
Swofford, D.L. (2002). PAUP*. *Phylogenetic analysis using parsimony (*and other Methods)*. Version 4.0b10. Sinauer Associates.
Takamatsu, S., Havrylenko, M., Wolcan, S.M., Matsuda, S., & Niinomi, S. (2008). Molecular phylogeny and evolution of the genus *Neoerysiphe* (Erysiphaceae, Ascomycota). *Mycological Research*, *112*(6), 639–649. https://doi.org/10.1016/j.mycres.2008.01.004
Takamatsu, S., Heluta, V., Havrylenko, M., & Divarangkoon, R. (2009). Four powdery mildew species with catenate conidia infect *Galium*: molecular and morphological evidence. *Mycological Research*, *113*(1), 117-129. https://doi.org/10.1016/j.mycres.2008.09.006
Walsh, P.S., Metzger, D.A., & Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, *10*(4), 506–513. https://doi.org/10.2144/000114018
Zhang, X.F., Liu, X.D., Jing, W.M., & Cao, J.J. (2019). Characteristics of *Leontopodium leontopodioides* leaf stochiometry with altitude and their relationship with soil nutrients in Qilian Mountains, Northwest China. *Chinese Journal of Applied Ecology*, *30*(12), 4012–4020. https://doi.org/10.13287/j.1001-9332.201912.009
Zhang, Y., Yang, Y.L., Ruan, J.Y., Chen, Q., Li, J., Guo, Y.Q., Han, L.F., & Wang, T. (2018). Isobenzofuranones from the aerial parts of *Leontopodium leontopodioides* (Wild.) Beauv. *Fitoterapia*, *124*, 66–72. https://doi.org/10.1016/j.fitote.2017.10.019
Zhao, Y., You, X.M., Jiang, H., Zou, G.X., & Wang, B. (2019). Spectrum–effect relationships between high-performance liquid chromatography fingerprints and anti-inflammatory activities of *Leontopodium leontopodioides* (Willd.) Beauv. *Journal of Chromatography B*, *1104*, 11–17. https://doi.org/10.1016/j.jchromb.2018.11.001