

***Psathyrella aberdarensis*, a new species of *Psathyrella* (*Agaricales*) from a Kenyan National Park**

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Abstract: *Psathyrella aberdarensis*, collected in the Aberdare National Park, Kenya, is described as a new species. Colour plates and drawings of the microscopic characteristics are presented. Closely related and similar species are compared with the new species and the classification within the genus *Psathyrella* is discussed. *Psathyrella aberdarensis* belongs to the *P. candolleana* alliance and is characterized by small basidiomata, a persistent veil made up of polymorphic elements and very pale spores without a recognizable germ pore.

Zusammenfassung: *Psathyrella aberdarensis*, gesammelt im Aberdare National Park, Kenia, wird als neue Art beschrieben. Farbfotos und Zeichnungen der mikroskopischen Merkmale werden vorgestellt. Eng verwandte und ähnliche Arten werden verglichen und die Klassifizierung innerhalb der Gattung *Psathyrella* wird diskutiert. *Psathyrella aberdarensis* gehört zur *P. candolleana*-Verwandtschaft und ist durch kleine Basidiomata, ein beständiges Velum aus polymorphen Elementen und sehr blasse Sporen ohne Keimporus gekennzeichnet.

The Aberdare National Park is located in Nyeri County in central Kenya and covers an area of 766 km². The park is part of the Aberdares Ranges, comprises the upper forest zone and includes Mount Satima, the third-highest mountain in Kenya. The climate in the ranges is characterized by alternating wet and dry seasons that are linked to the shifting intertropical convergence zone. Due to altitude differences between 1800 and 3600 m s. m., the forest harbors a high diversity of forest types within different vegetation zones. The most common tree species are *Ocotea usambarensis* ENGL., *Juniperus procera* HOCHST. ex ENDL., *Podocarpus latifolius* (THUNB.) R. BR. ex MIRB. and *Ha-genia abyssinica* (BRUCE) J. F. GMEL. To our best knowledge, almost nothing is known about the local Funga.

In 2016, one of the authors (V. W. K.) collected a *Psathyrella* specimen that was growing on small twigs (of undetermined wood). Since an unambiguous identification

was impossible morphologically, we decided to carry out molecular genetic analyses alongside.

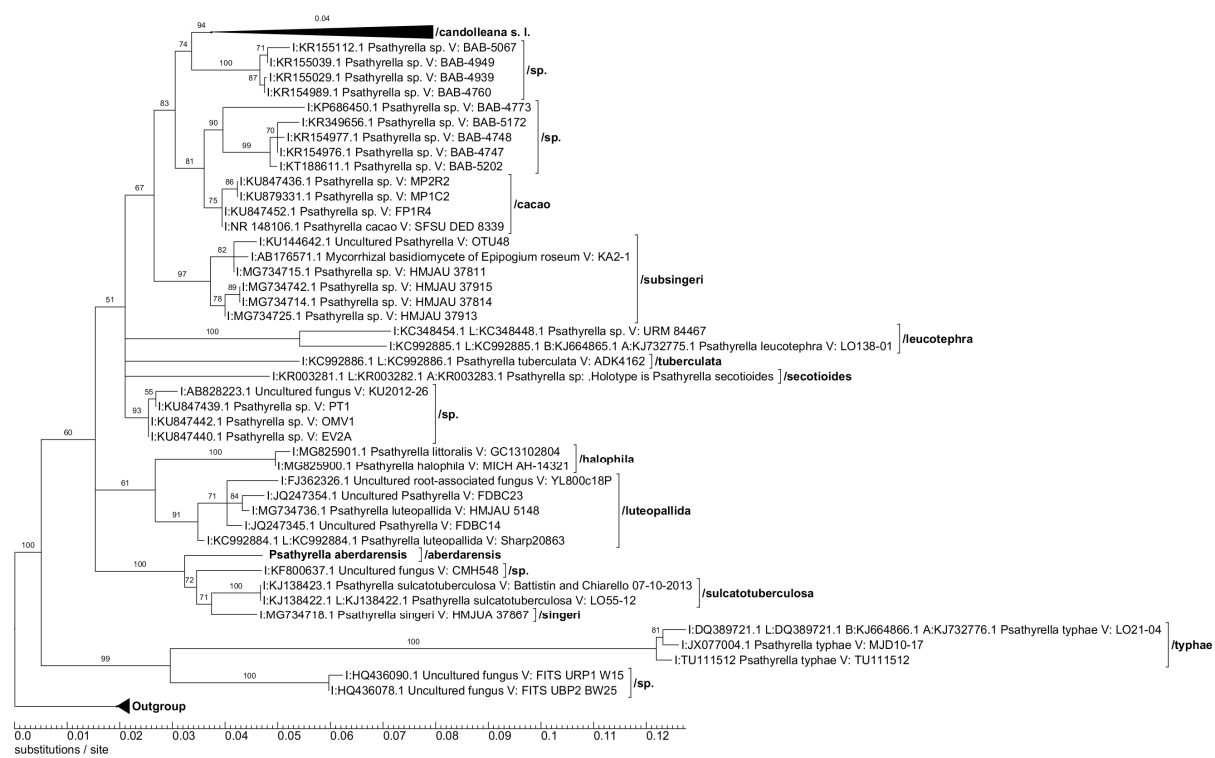


Fig. 1. Maximum Likelihood consensus tree, collapses to 50 %. Bootstrap probabilities in % noted above branches. In front of the species-name, the Genbank numbers are given, finally the name of the voucher. Abbreviations: I: ITS-region, L: LSU-region, B: β -tubulin-region, A: ef-1 α -region.

Material and methods

Morphology:

Macromorphological characteristics were recorded based on fresh specimens; photographs of the basidiomata were taken *in situ*. Micromorphological analyses were performed by light microscopy using hand sections of fresh material as well as of revived exsiccata. The size of mature spores was measured in water after their collection from the stipe apex. Spore colour was assessed in plain water, ammonia solution (10 %), and in potassium hydroxide solution (5 % w/v KOH). Cystidia and other microscopic structures were studied in ammonia solution after staining with Congo red. The colour code is based on KÜPPERS (2007).

Molecular analyses:

DNA extraction was performed according to standard methods. The ITS region (ITS1, 5.8S rRNA gene, ITS2) as well as parts of the 28S rRNA gene region were amplified by PCR using standard primer pairs (WHITE & al. 1990). PCR products were Sanger sequenced by LGC Genomics (Berlin, Germany). For comparison, sequences from the NCBI Genbank were used. As outgroup, representative species of *Cystoagaricus* SINGER, *Kauffmania* ÖRSTADIUS & E. LARSSON, and *Typhrasa* ÖRSTADIUS & E. LARSSON were chosen. The alignment of the individual partitions was performed with Prank Version 140603 (VEIDENBERG & al. 2016), refined by an iterative guide tree method. The best fitting partition scheme and optimum evolution models for the Bayesian analysis were calculated with Partitionfinder (LANFEAR & al. 2016), while the Bayesian information criterion (BIC) was used for scoring. The Maximum-Likelihood analysis was performed with RAxML 8.2.10. (STAMATAKIS 2014). Out of 1000, the best tree was provided with the ML bootstrap support values. The outgroup and the subsequent *P. candolleana*-group were collapsed for better clarity.



Fig. 2. *Psathyrella aberdarensis*, young specimen. Photo: V. W. KIMANI.



Fig. 3. *Psathyrella aberdarensis*, old specimen. Photos: A. KARICH.

Cultivation:

An appropriate twig covered with mycelium was collected in the Aberdare National Park, transferred to Zittau (Germany), and placed under saturation vapour at 23 °C into an incubation chamber. After about six weeks, a few fruiting bodies and some small primordia appeared. From this material, we were able to isolate a pure culture of the fungal strain.

Results**Phylogenetical analysis**

The new species belongs to the *Psathyrella* group without pleurocystidia and with a lamellar edge predominantly composed of utriform to subcylindrical marginal cells. SMITH (1972) summarized the species with such a morphology in subg. *Candolleana* (ROMAGN.) A. H. SM.; KITS VAN WAVEREN (1985) placed them in sect. *Spintrigerae* (FR.) KONR. & MAUBL. emend. KITS V. WAV. Recent molecular genetic studies (NAGY & al. 2010, 2011; ÖRSTADIUS & al. 2015) confirmed that these morphological features largely coincide with phylogenetic analysis. The number of species in this group is certainly larger than previously expected; recently, *P. secotioides* G. MORENO, HEYKOOP, ESQUEDA & OLARIAGA (MORENO & al. 2015), *P. cacao* DESJARDIN & B. A. PERRY (DESJARDIN & PERRY 2016) and *P. subsingeri* T. BAU & J. Q. YAN (YAN & BAU 2018) have been described as new species.

The closest relatives of *P. candolleana* (FR.: FR.) MAIRE comprise very similar and morphologically hardly distinguishable species. Thus, in the NCBI Genbank, many respective sequences are just deposited under "spec.". On the other hand, some more distantly related species can be easily distinguished and determined merely based on macro- and microscopic features, e.g., *P. typhae* (KALCHBR.) A. PEARSON & DENNIS and *P. leucotephra* (BERK. & BROOME) P. D. ORTON; the species described here indeed belongs to this group of *Psathyrella*.

In the phylogenetic tree (Fig. 1) the closest relatives of the new taxon *P. aberdarensis* are *P. singeri* A. H. SM., *P. sulcatotuberculosa* (J. FAVRE) EINHELL., and an unidentified sequence (KF800637) of RITTENOUR & al. (2014) which was generated from spores collected in air and dust samples indoors in Kansas City and thus cannot provide any further characters. *Psathyrella singeri* was described by SMITH (1972) from a wetland area in Florida (USA). He did not mention a veil and described the lamellae as very crowded and strikingly pale. A respective sequence in Genbank (MG734718) was analysed using a specimen from China, Jilin, Changbai Mountain National Nature Reserve (YAN & BAU 2018). It is uncertain that this sequence belongs to *P. singeri* ss. stricto; it does not match with our sequence and may rather represent a separate species. On the other hand, a good match was found between our strain and *P. sulcatotuberculosa*. This species also has small and fragile basidiomata on dead wood; its almost equally sized spores appear very pale and a distinct germ pore is absent or – at best – extremely indistinct. However, there are also significant differences to our *Psathyrella* strain. The cheilocystidia of *P. sulcatotuberculosa* are more polymorphic, the pileus surface is heavily rugulose, the veil is pale yellowish and fibrillose, does not make patches and is made up of subcylindrical to in the middle slightly enlarged, thin-walled cells, some of which are sometimes encrusted. A detailed description is given by BATTISTIN & al. (2014).

Taxonomy

Psathyrella aberdarensis A. MELZER, KIMANI & R. ULLRICH, spec. nov. (Figs. 2–4)

Mycobank no.: MB 827350

Genbank accession no.: MH880928

Latin diagnosis:

Pileus usque ad 10 mm latus, primum conicus vel campanulatus, deinde planus, ad marginem tandem supra involutus, rubro-brunneus vel brunneus, non striatus, medium fuscum. Velum flocculis albis et perseverantibus plenum, usque ad medium pilei. Lamellae mediis intervallis distantes, stipiti adnae, brunneae, in acie albae, non deliquescentes. Stipes usque ad 15 × 1 mm, cylindratus, in parte superiore albus, in parte inferiore pallide brunneus, in parte infima fibrillosus. Basidia 4-sporigera. Sporae 9,4–10,6 × 5–6,3 µm, ellipsoideae, per microscopium pallide brunneae vel flavae, porus germinativus absens. Pleurocystidia nulla. Cheilocystidia 19–33 × 8–12,3 µm, utriformia. Caulocystidia utriformia, cellulis sphaeropedunculatis et clavatis multum immixtis. Cellulae veli subcylindraceae vel subglobosae, non raro crassiparietales et brunneae. Fibulae adsunt. Basidiomata gregaria ad lignum mortuum.

Holotypus: Cultivated in Germany, Zittau, 31. May 2016, type deposited as metabolically inactive dried culture and basidiomata (GLM-F116094); origin: Kenya, Nyeri County, Aberdares National Park, -0.35220 °N; 36.80049 °E; approx. 2400 m s. m., 9. April 2016, leg. V. W. KIMANI.

Etymology: Named after the place of origin.

English description:

Pileus: up to 10 mm wide, at first conical to campanulate, later flattening, old with an up-rolled margin, reddish brown to brown (ca. Y₅₀M₅₀C₂₀ to Y₅₀M₇₀C₃₀), centre darker (ca. Y₅₀M₉₉C₆₀); parts of the universal veil well recognizable and quite persistent as patches.

Lamellae: somewhat distant, adnate, brown, lamellar edge white.

Stipe: up to 15 × 1 mm, cylindrical, in the upper part white, in the lower part brownish, base strikingly white tomentose.

Basidiospores: 7.5–8(–8.8) × 4.4–5 µm, average 7.8 × 4.6 µm, Q=1.6–1.75, Q_{av.}=1.7; in front view ellipsoid, in side view adaxially slightly flattened, often somewhat phaseoliform, apiculus tiny, germ pore not visible; in water and ammonia solution pale yellowish brown, in KOH nearly hyaline.

Basidia: 15–16.5 × 6.8–8 µm, 4-spored, clavate to sphaeropedunculate.

Cheilocystidia: 19–33 × 8–12.3 µm, predominantly utriform, rarely lageniform, numerous but moderately crowded, sometimes with small golden-brown deposits; intermixed with variously frequent clavate and sphaeropedunculate cells (paracystidia), 16.5–27 × 9.5–19 µm; all marginal cells thin-walled and colourless.

Pleurocystidia: absent.

Caulocystidia: 24.5–43.7 × 12.3–16.5 µm, utriform, scattered; sphaeropedunculate and clavate cells also present, these 19–33 × 11–24.5 µm.

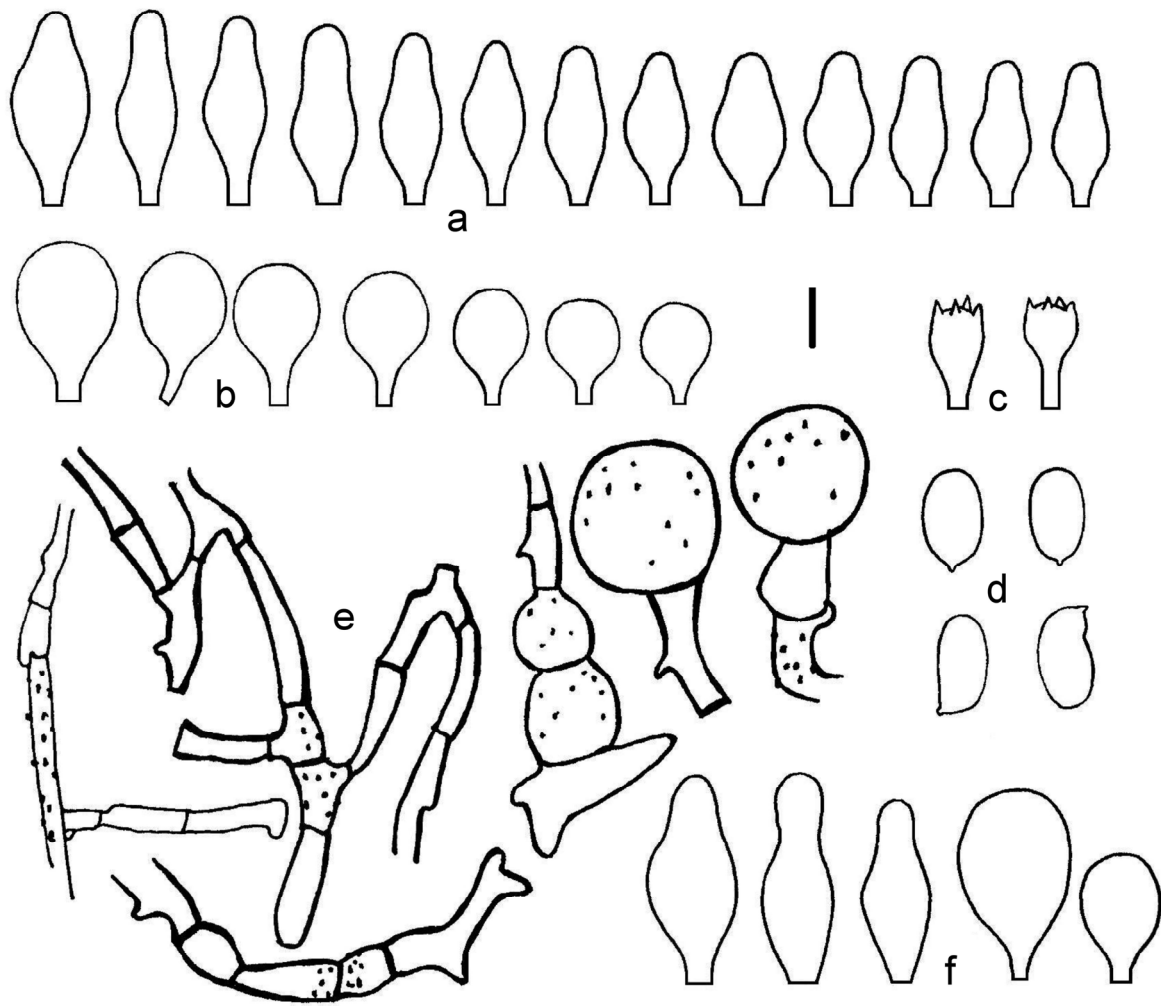


Fig. 4. Microcharacters of *Psathyrella aberdarensis*; a cheilocystidia, b paracystidia, c basidia, d spores, e veil elements, f caulocystidia. Bar: 5 μm (spores), 10 μm (others). – Drawing by A. MELZER.

Veil: made up mainly of heavily branched, slightly diverticulate, often thick-walled and brownish pigmented cells, 13.7–50 \times 4–11 μm , beside globose elements, these 16.5–30 μm in diam.; all cells often slightly to strongly encrusted.

Clamp connections: present, e.g., in mycelium and veil.

Habitat: gregarious on dead wood (fallen twigs).

Discussion

Psathyrella aberdarensis is characterized by small basidiomata, a persistent veil made up of polymorphic elements and very pale spores without a recognizable germ pore. Some other species without pleurocystidia, with pale spores and without germ pore, are mentioned below along with species suggesting a closer relationship to our *Psathyrella* species for other reasons. Since sequences of these species are currently not available in GenBank or Unite, distinctions are made based on other characteristics that - as a whole - appear to be plausible.

Psathyrella aequatoria SINGER was described from Ecuador. According to SINGER (1978) it is a very small species with a pileus diameter of 6–14 mm and without (or with invisible) veil. The lamellae are crowded and only pale brownish.

Psathyrella atroumbonata PEGLER is habitually quite similar but has larger basidiomata with the veil hanging on the margin of the pileus and consisting of hyaline hyphae. The spores are pale brownish, and the germ pore is small and sometimes indistinct. However, the drawings in PEGLER (1966, 1973) show strongly truncate spores.

Psathyrella bivelata CONTU is probably a closely related species due to a similar veil structure (globose elements mixed with branched-cylindrical, slightly thick-walled ones). The spores, however, are larger, darker and have a germ pore. Moreover, the species is currently known only from the Mediterranean region (CONTU 1991, VOTO 2011, SAMMUT & MELZER 2012).

Psathyrella pallidispora DENNIS has 8–11 × 4–5 µm large and slender spores, the cheilocystidia are often capitate (DENNIS 1970: fig. 9G). No records are known outside South America.

Psathyrella varicosa A. PEARSON has relatively large basidiomata with up to 60 mm wide pilei and up to 120 mm long stipes. PEARSON (1950) does not mention a veil. The spores measure (6–)9 × (4–)4.5–5 µm, are frontally ellipsoid to amygdaloid, laterally phaseoliform, yellowish, and the germ pore is absent. This terrestrial species was described from South Africa.

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References

- BATTISTIN, E., CHIARELLO, O., VIZZINI, A., ÖRSTADIUS, L., LARSSON, E., 2014: Morphological characterisation and phylogenetic placement of the very rare species *Psathyrella sulcatotuberculosa*. – *Sydowia* **66**(2): 171–181.
- CONTU, M., 1991: *Psathyrella bivelata* spec. nov., une nouvelle espèce de la section *Cystopsathyra*. – *Bull. Trimest. Soc. Mycol. France* **107**(3): 85–89.
- DENNIS, R. W. G., 1970: Fungus flora of Venezuela and adjacent countries. – *Kew Bull. Addit. Ser.* **3**: 1–531.
- DESJARDIN, D. E., PERRY, B. A., 2016: Dark-spored species of *Agaricineae* from Republic of São Tomé and Príncipe, West Africa. – *Mycosphere* **7**(3): 359–391.
- KITS VAN WAVEREN, E., 1985: The Dutch, French and British species of *Psathyrella*. – *Persoonia, Suppl.* **2**: 1–300.
- KÜPPERS, H., 2007: *DuMont Farbenatlas* (10th edn). – Köln: DuMont.
- LANFEAR, R., FRANDBSEN, P. B., WRIGHT, A. M., SENFELD, T., CALCOTT, B., 2016: Partition Finder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. – *Mol. Biol. Evol.* **34**(3): 772–773.
- MORENO, G., HEYKOOP, M., ESQUEDA, M., OLARIAGA, I., 2015: Another lineage of secotioid fungi is discovered: *Psathyrella secotioides* sp. nov. from Mexico. – *Mycol. Prog.* **14**(6/34): 1–8.
- NAGY, L. G., URBAN, A., ÖRSTADIUS, L., PAPP, T., LARSSON, E., VÁGVÖLGYI, C., 2010: The evolution of autodigestion in the mushroom family *Psathyrellaceae* (*Agaricales*) inferred from Maximum Likelihood and Bayesian methods. – *Mol. Phylogenet. Evol.* **57**(3): 1037–1048.

- NAGY, L. G., WALTHER, G., HAZI, J., VÁGVÖLGYI, C., PAPP, T., 2011: Understanding the evolutionary processes of fungal fruiting bodies: correlated evolution and divergences times in the *Psathyrellaceae*. – *Syst. Biol.* **60**(3): 303–317.
- ÖRSTADIUS, L., RYBERG, M., LARSSON, E., 2015: Molecular phylogenetics and taxonomy in *Psathyrellaceae* (*Agaricales*) with focus on psathyrelloid species: introduction of three new genera and 18 new species. – *Mycol. Progress* **14**(5/25): 1–42.
- PEARSON, A. A., 1950: Cape agarics and boleti. – *Trans. British Mycol. Soc.* **33**(3–4): 276–314.
- PEGLER, D. N., 1966: Tropical African *Agaricales*. – *Persoonia* **4**(2): 73–124.
- PEGLER, D. N., 1973: A preliminary Agaric flora of East Africa. – *Kew Bull. Addit. Ser.* **6**: 1–615.
- RITTENOUR, W. R., CIACCIO, C. E., BARNES, C. S., KASHON, M. L., LEMONS, A. R., BEEZHOLD, D. H., BRETT J. GREEN, B. J., 2014: Internal transcribed spacer rRNA gene sequencing analysis of fungal diversity in Kansas City indoor environments. – *Environ. Sci. Process Impacts* **16**(1): 33–43.
- SAMMUT, C., MELZER, A., 2012: *Psathyrellaceae* from Malta, a preliminary survey. – *Micol. Veget. Medit.* **27**(1): 33–44.
- SINGER, R., 1978: Interesting and new species of *Basidiomycetes* from Ecuador II. – *Nova Hedwigia* **29**(1–2): 1–98.
- SMITH, A. H., 1972: The North American species of *Psathyrella*. – *Mem. New York Bot. Gard.* **24**: 1–633.
- STAMATAKIS, A., 2014: RaxML, version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. – *Bioinformatics* **30**(9): 1312–1313.
- VEIDENBERG, A., MEDLAR, A., LÖYTYNOJA, A., 2016: Wasabi: an integrated platform for evolutionary sequence analysis and data visualization. – *Molec. Biol. Evol.* **33**(4): 1126–1130.
- VOTO, P., 2011: *Psathyrella carinthiaca* sp. nov. e nuove segnalazioni di *P. bivelata*. – *Riv. Micol.* **54**(2): 121–133.
- WHITE, T. J., BRUNS, T., LEE, S., TAYLOR, J., 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: INNIS, M., GELFAND, D., SNINSKY, J., WHITE, T. (Eds.): *PCR protocols: a guide to methods and applications*, pp. 315–322. – Orlando: Academic Press.
- YAN, J.-Q., BAU, T., 2018: The Northeast Chinese species of *Psathyrella* (*Agaricales*, *Psathyrellaceae*). – *MycoKeys* **33**: 85–102.