Karstenia 56: 39–46

A new species of Maireina on Filipendula ulmaria

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LÆSSØE, T., DAVEY, M.L. & PETERSEN, J.H. 2016: A new species of Maireina on Filipendula ulmaria. – Karstenia 56: 39–46. HELSINKI. ISSN 0453–3402.

Based on material from Sweden and Denmark a new species of *Maireina* is described. It produces tiny, pale yellow brown, cyphelloid basidiomata at the very base of *Filipendula ulmaria* in grazed forest meadows. Phylogenetically it falls within the Niaceae clade with sisterrelations to *Cyphellopsis*/*Merismodes*. It differs from *M. maxima* by habitat and by smaller spores and from *M. monacha* by much smaller spores.

Key words: Niaceae, Agaricales, cyphelloids, taxonomy, phylogeny, *Merismodes*, *Cyphellopsis*

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Introduction

Recent fieldwork in Sweden and Norway has produced many novelties including the subject of this paper – a pale ochraceous cyphelloid found at the very base of standing living stems of *Filipendula ulmaria*. It had the same growth pattern as *Woldmaria filicina* on the fern *Matteucchia* but with smaller fruitbodies. The fungus was discovered in 2014 during a general survey of a grazed forest meadow in Medelpad, central Sweden during the annual autumn foray hosted by the Swedish Mycological Society and the Sundsval Mycological Society. The following year a detailed plot study across Denmark revealed the presence of the same fungus in exactly the same environment – at the base of *F. ulmaria* in a grazed forest meadow rich in plant species and very rich in grassland fungi. The first collection was photographically documented whilst fresh and we obtained DNA sequences from both collections. The morphological and molecular characters of the collections suggested a placement near *Merismodes* Earle / *Cyphellopsis* Donk in the family Niaceae, but the specimens could not be identified using the key included in Bodensteiner (2006). Following this account, the specimens are best accommodated within *Maireina* W.B. Cooke, although we acknowledge that generic delimitations among cyphelloid members of the Niaceae are poorly resolved. Nevertheless, the two collections mentioned above from Sweden and Denmark, respectively, represent a morphologically and phylogenetically distinct species that is described below as *M. filipendula,* and dedicated to our loving memory of the late Juhani Ruotsalainen.

Material and methods

Morphology. Images were taken with a Leica M 420 macroscope equipped with a digital camera. Pictures were stacked using Zerine Stacker and post processed with Adobe Photoshop. Spores and other structures were measured in ca 5 % ammoniacal Congo Red and Melzer's reagent was used to test for amyloid/dextrinoid reactions. Final drawings were prepared from originals made with a drawing tube fitted on an Olympus BX50.

DNA sequencing and phylogenetic analysis. Three fruitbodies from each collection were crushed in a 1.5mL Eppendorf tube using a TissueLyser II bead mill homogenizer (Quiagen, Hilden, Germany). Genomic DNA was extracted using a CTAB-based extraction protocol (Murray & Thompson 1980) with modifications as described in Gardes & Bruns (1993). A continuous stretch of ribosomal DNA spanning the internal transcribed spacer (ITS) region and the D1-D2 domains of the large subunit (LSU) region was amplified using the primers ITS1-F (Gardes & Bruns 1993) and LR3 (Vilgalys & Hester 1990) and illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's instructions. Amplicons were sequenced through the LIGHTRUN sequencing service at GATC Biotech (Cologne, Germany) with the primers ITS1-F, ITS4, LROR, and LR3 (Vilgalys & Hester 1990, White et al. 1990, Gardes & Bruns 1993).

ITSx (Bengtsson-Palme et al. 2013), as implemented on the PlutoF web-based workbench (Abarenkov et al. 2010), was used to extract the ITS and LSU regions from the sequences generated. LSU sequences from *M. filipendula* were then assembled in a data matrix with sequences of niaceous cyphelloid species (*Calathella, Cyphellopsis, Flagelloscypha, Halocyphina, Lachnella, Merismodes, Woldmaria*), and non-cyphelloid members of Niaceae that were retrieved from GenBank. A second data matrix was assembled consisting of ITS sequences of *M. filipendula* and members of the *Cyphellopsis/Merismodes* clade of the Niaceae with a single record of *Flagelloscypha japonica* to serve as an outgroup taxon. Both matrices were aligned using MAFFT version 7 (Katoh & Toh 2008), and the resulting alignments were manually verified before Gblocks v0.91b (Castresana 2000) was used to remove ambiguously aligned bases. The best-fit model of evolution was determined based on the Bayesian information criterion in jModelTest v0.1.1 (Guindon & Gascuel 2003, Posada 2008), which was then implemented in both maximum likelihood and Bayesian analyses. GARLI v 1.0 (Zwickl 2006) was used to determine the most likely tree and maximum likelihood bootstrap support for each dataset. Bayesian analyses were conducted in Mr. Bayes version 3.2.6 (Ronquist et al. 2011). Two independent runs of four Markov Chain Monte Carlo chains with 1.0 x 10^6 generations each were made, with trees sampled every 1000^{th} generation. Convergence was considered to be achieved when a a final standard deviation of <0.01 for the split frequency was attained. The first 25% of sampled trees were discarded as burn-in and posterior probabilities for each node of the 50% majority rule consensus tree were recorded.

Fifty taxa and 601 characters were included in the aligned matrix of LSU sequences, and the TIM3ef+I+G model was selected by jModelTest as the best-fit model of evolution for the data. Results of the maximum likelihood bootstrap analyses and the Bayesian inference are shown on the maximum likelihood tree (-lnL -4230.860) (Fig. 1). The cyphelloid taxa *Cyphellopsis, Merismodes,* and *M. filipendula* form a well supported clade within the *Niaceae* (100% Bayesian Posterior Probability (BPP), 89% Maximum Likelihood Bootstrap Proportion (BP). Within this clade, *M. filipendula* forms a well-supported grouping (100% BPP, 100% BP) that is sister to a poorly supported grouping consisting of all other representatives of *Cyphellopsis* and *Merismodes*. Cyphelloid growth forms do not form a single, highly supported lineage within the *Niaceae* and some show affinities to other growth forms, including the gasteromycete *Nia*. The aligned matrix of ITS sequences included 10 taxa and 666 characters, and the HKY+G model was selected by jModelTest as the best-fit model of evolution for the data. In the ITS maximum likelihood and Bayesian analyses (Fig. 2), *M. filipendula* forms a well supported clade (100% BPP, 100% BP) that is sister to the highly supported *Cyphellopsis/Merismodes* clade (100% BPP, 96%BP).

Maireina filipendula Læssøe, **sp.nova**
–Figs. 1–5

MycoBank no: MB 818045

Diagnosis: Similar to the lignicolous *Maireina maxima* but herbicolous and with subfusiform spores, 5.0–5.9 × 2.5–3.1 µm.

Typus: **Sweden. Medelpad.** Tolvösand-Myckeläng (Natura 2000); alt c. 5 m asl, on dead, small, sheathing leaves at the very base of young, living *Filipendula ulmaria* stems in forest meadow grazed by cattle, 13.IX.2014 *Læssøe TL-14226* (C – holotype).

Basidiomata in loose or dense groups on brown leaf sheaths at soil level, pale brownish yellow (hard to match in Kornerup & Wancher), ± cupulate-tubular-campanulate, 0.25–0.6 mm high and 0.2–0.3 mm wide, covered in somewhat projecting to appressed, whitish to pale brownish hairs; opening fimbriate from external hairs; without subiculum; hymenophore smooth, whitish. *Hairs* almost straight to undulating, occasionally constricted, to at least 250 µm in length, cylindrical and ca 3–3.5 µm wide, hardly septate, covered in crystals and often more so at the slightly inflated (clavate) apex, this 4–4.5 µm wide and rather thin-walled, just below the enlarged part and in full length distinctly thick-walled, walls ca 0.5 µm thick, sometimes with asymmetrical thicken-

ing in the lower part of the inflated apices; hyphae with clamps; large, irregular, angular crystals present in great quantity in trama. Hairs and crystals ± KOH inert and not or hardly dextrinoid and no parts amyloid. No gelatinized tissues noted. *Basidia* clavate, clamped, (2-)4-spored, 14.6–19.0 × 3.9–5.3 µm. *Basidiospores* hyaline, slightly thick-walled, wall slightly uneven but smooth in light microscope, subfusiform, 5.0–5.9 × 2.5–3.1 µm, mean 5.6 × 2.9 µm, Q= 1.6–2.2, Q_{av} =1.9 (n= 10), often gluing to hairs in pairs or tetrads.

The Danish collection cited below does not differ from the above type description, but slightly thicker hairs (up to 1.1 µm basally, measured in Melzer's reagent) were noted.

Additional specimen studied: **DENMARK. Sjælland**. Sorø Sønderskov, Bimosen (Biowide plot 104), lat: 55.3991598289, lon: 11.5924818974, on *Filipendula ulmaria* at soil level in cattle grazed forest meadow, 14.X.2015 *Læssøe, atlas DMS-724890* (C).

Discussion

Bodensteiner (2006, 2007) defines *Maireina* as brownish cyphelloids with smooth, hyaline spores and non-branching, brownish external hyphae that are thick-walled and heavily incrusted with crystals, including the apical parts. The present material falls within this definition. Although phylogenetic analysis of both the LSU and ITS regions suggest a close relationship between the studied collections and the genera *Merismodes* and *Cyphellopsis*, these genera are distinguished Bodensteiner (2006) as having exclusively smooth hair apices and trama that tend to be gelatinized. The ITS phylogeny establishes *M. filipendula* as distinct from, and sister to, *Cyphellopsis* and *Merismodes*, further supporting the accommodation of these specimens within *Maireina*. However, it must be noted that generic delimitations among cyphelloid taxa are the cause of some debate, with Knudsen (2012) accepting only one genus, *Merismodes,* with *Cyphellopsis*, *Maireina* and *Phaeocyphellopsis* W.B. Cooke listed as synonyms. Similarly, Dam & Dam (2012) provide morphology based arguments that *Cyphella ferruginea* P. Crouan & H. Crouan would be better accommodated in the genus *Maireina*. Jagers (2012), likewise, acknowledge the indistinct borders between cyphelloid genera in connection with reporting the first record of *M. afibulata* Bodenst. from the Netherlands. Our phylogenetic analyses, and those presented by Bodensteiner et al. (2004), support a close relationship between *Cyphellopsis, Maireina,* and *Merismodes*. However, analyses by Bodensteiner et al. (2004) find the type species of *Cyphella*, *C. digitalis* (Alb. & Schwein.) Fr., is remote from this grouping.

The sequences generated here represent the first molecular data for any representative of *Maireina*, and taxon sampling within public sequence databases for the genera in question is limited to a single species each of *Cyphella, Cyphellopsis* and *Merismodes,* and no representatives of *Phaeocyphellopsis*. The available data is clearly insufficient to fully resolve generic delimitations among these fungi, and more comprehensive taxon sampling is required to establish a phylogenetic-based framework for the taxonomy of this group.

Nevertheless, the *Filipendula* material studied here clearly represents a unique species that cannot be accommodated among described cyphelloid species. Although the specimens resembled *Woldmaria filicina* in their growth pattern and colouration, they have smaller fruit bodies (Læssøe & Petersen 2016). Morphologically, the collections are most similar to *Maireina maxima* (Massee) W.W. Cooke, as described in the key provided by Bodensteiner (2007). However, Bodensteiner (2006, 2007) list no species or specimens on or from *Filipendula*. Although the hairs depicted for *M. maxima* by Bodensteiner (2007) resemble those of *Maireina filipendula,* the species can be distinguished from *M. filipendula* by its longer, more distally rounded spores, and its habitat preference for woody, mostly angiosperm hosts.

Like *M. filipendula*, *Maireina monaca* (Speg.) W.B. Cooke in the sense of Bodensteiner (= *Merismodes bresadolae* (Grélet) Singer and ss Knudsen 2012 (= *Merismodes granulosa* (Fuck.) Knudsen) occurs on herbaceous stems, but it has much larger spores (10.5–15(–17) × 5.5–8.5(–10) µm sec Bodensteiner 2006; see also Van Ryckegem & Dam 1999) who give an account of this species. In phylogenetic analyses of the LSU and ITS regions, the two specimens of *M. filipendula* are clearly supported as a separate

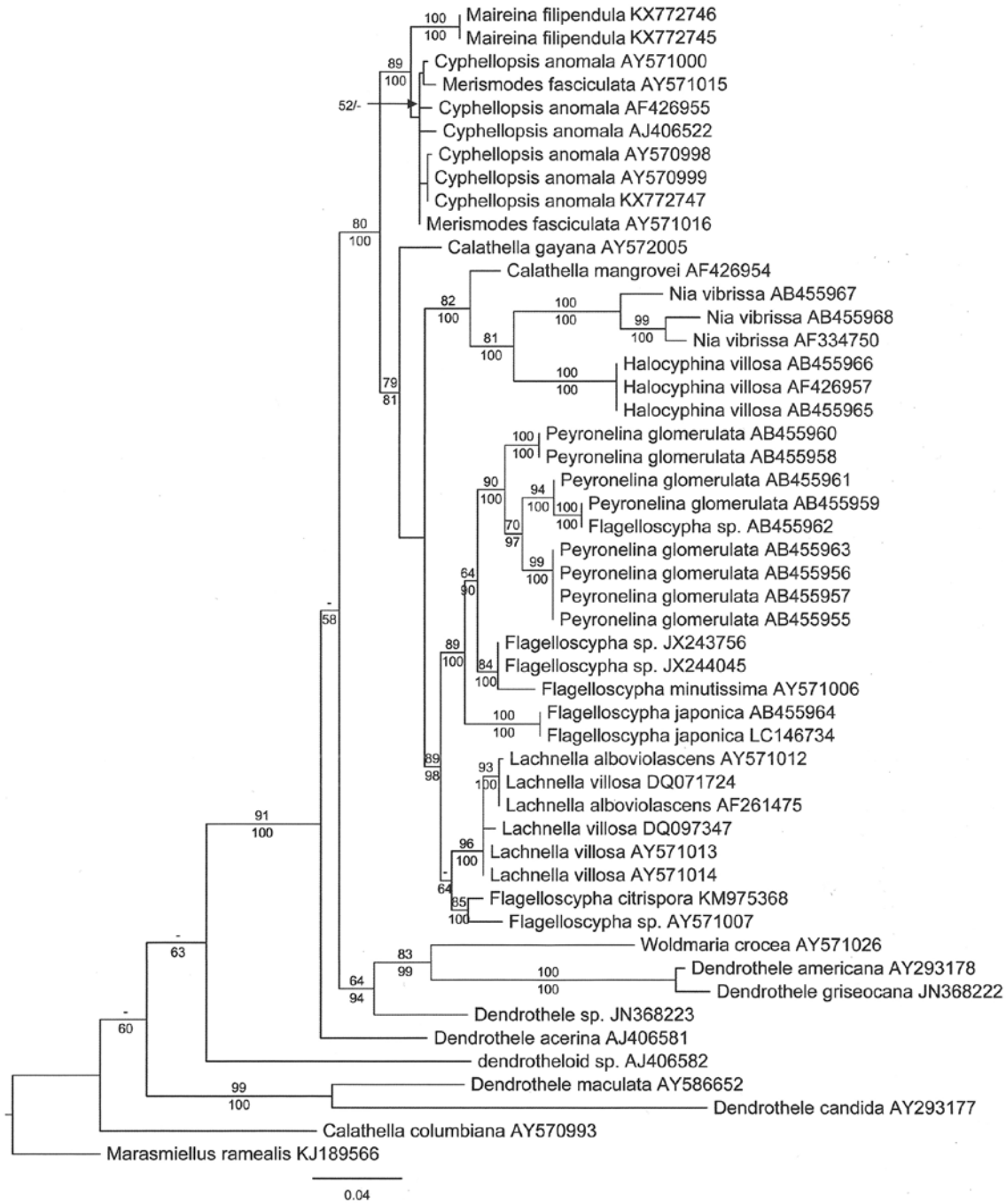

Fig. 1. Maximum likelihood tree (-lnL 4230.86) inferred from a dataset of large subunit rDNA sequences showing the placement of *Maireina filipendula* among members of the Niaceae. Support values above and below the branches indicate boostrap proportions and Bayesian posterior probabilities, respectively. GenBank accession numbers are given following the sequence name.

clade that is distinct from other representatives of cyphelloid taxa, further supporting them as a new species. *Maireina filipendula* is therefore described herein based on both unique morphological and molecular characters, although it is acknowledged that the taxonomic status of the genus *Maireina* may be re-evaluated in the future in light of further phylogenetic analyses with more comprehensive taxon sampling.

Acknowledgements: Sundvalls Mycological Society for supporting TL's field work and to Villum-fonden for financing the Biowide project in Denmark.

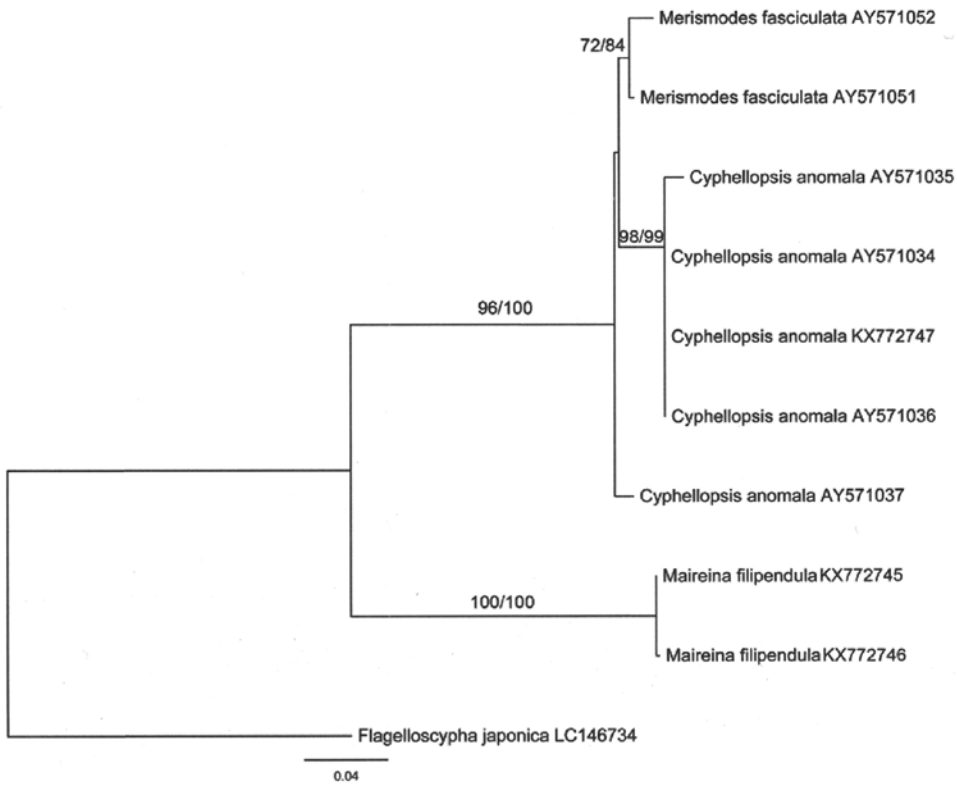

Fig. 2. Maximum likelihood tree (-lnL 2021.11) inferred from a dataset of ITS rDNA sequences showing the placement of *Maireina filipendula* relative to *Cyphellopsis* and *Merismoides*. Support values given above the branches indicate boostrap proportions/Bayesian posterior probabilities. GenBank accession numbers are given following the sequence name.

Fig. 3. *Maireina filipendula.* Holotype. – Photo: J.H. Petersen

Fig. 4. *Maireina filipendula.* Holotype. – Photo: J.H. Petersen

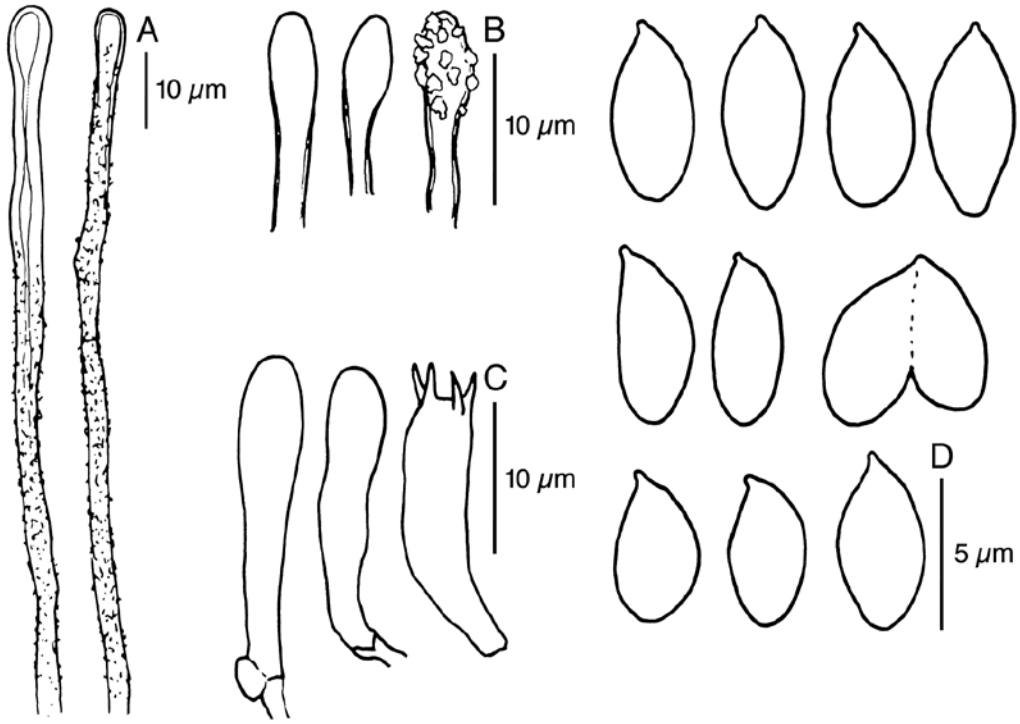

Fig 5. *Maireina filipendula.* Microscopic details from the holotype. – Drawings: J.H. Petersen

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