

Peltigera serusiauxii (Lecanoromycetes, Ascomycota), a new species from Papua New Guinea and Malaysia

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Abstract. *Peltigera serusiauxii* is proposed here as a new species from Papua New Guinea and Sabah, northern Borneo (Malaysia). The species belongs to the polydactyloid clade of section *Polydactylon*. Because of its large thalli with a glabrous upper surface, this species was previously identified as *P. dolichorhiza*, but it differs by its polydactylon-type lower surface and the high amount of dolichorrhizin. It appears to be a strict specialist in its association with *Nostoc* phylogroup IX throughout its known distribution. This is one of many undescribed species remaining to be formally described within the genus *Peltigera*, especially in Asia and Australasia.

Key words: *Fungi*, lichens, *Nostoc*, phylogeny, *rbcLX*, systematics

Introduction

Peltigera is a genus of ascomycetes that mainly form lichen thalli with cyanobacteria of the genus *Nostoc*. This group of cyanolichens is present on all continents and often develops large and conspicuous thalli. It is particularly diverse in boreal and tropical mountain forests (Vitikainen 1994; Martinez et al. 2003). Recent studies based on multi-locus phylogenies and species delimitation methods revealed a largely underestimated level of biodiversity throughout its distribution (Jüriado et al. 2017; Magain et al. 2017a, b, 2018; Miadlikowska et al. 2018; Pardo-De la Hoz et al. 2018), such that half of the currently accepted 150 species remain to be formally described. Undescribed species are distributed across all sections recognized by Miadlikowska & Lutzoni (2000).

In section *Polydactylon*, the name *P. dolichorhiza* (holotype collected in Colombia) has been widely applied to specimens forming large, brittle thalli with a glabrous upper surface in many parts of the world, including Africa (Swinscow & Krog 1988), Asia (e.g., Wei et al. 2009) and Australasia (e.g., Kantvilas & Mitchin 1989). Slightly over

a decade ago, Sérusiaux et al. (2009) demonstrated that specimens morphologically identified as *P. dolichorhiza* in Papua New Guinea potentially represented at least four distinct evolutionary lineages. Species delimitations based on DNA sequences subsequently revealed that none of these four lineages include specimens from the Americas (Magain et al. 2017a, b), where *P. dolichorhiza* was originally described (Nylander 1860). Therefore, specimens previously identified as *P. dolichorhiza* in Asia and Australasia (including Papua New Guinea) represent distinct species (Magain et al. 2017a, b).

In fact, *Peltigera* in Papua New Guinea includes three putative species belonging to the dolichorhizoid clade of section *Polydactylon* (sensu Magain et al. 2017a), as well as a fourth putative species belonging to the polydactyloid clade. This last species, referred to as *P. dolichorhiza* s.lat. D by Sérusiaux et al. (2009), was subsequently recognized as *Peltigera* sp. 11 by Magain et al. (2017a, b) and shares a most recent common ancestor with *P. polydactylon* and *P. nana*. However, it has consistently been shown to be on a long branch, and is therefore considered to be phylogenetically distinct from all currently recognized *Peltigera* lineages (Magain et al. 2017a, b). *Peltigera* sp. 11 is one of the oldest lineages within section *Polydactylon* (Magain et al. 2017a). It also appears to be a strict specialist, having so far been found exclusively in association with *Nostoc* phylogroup IX (Magain et al. 2017a). It is fairly widespread in Papua New Guinea, where it is known from three provinces (Sérusiaux et al. 2009). Members of the polydactyloid clade occur mostly in Asia and Australasia, with the

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exceptions of the North American species *Peltigera seneca* and the primarily circumboreal species *P. polydactylon* (Magain et al. 2016, 2017a).

Here we describe a new species, *Peltigera serusiauxii*, to accommodate the taxon previously reported as *P. dolichorhiza* s.lat. D sensu Sérusiaux et al. (2009) and *P. sp. 11* (Magain et al. 2017a, b). Additionally, we show that a recent collection from Mount Kinabalu, Sabah (northern Borneo, Malaysia), represents a distinct population of *P. serusiauxii*.

Materials and methods

Taxon sampling, additional sequencing and phylogenetic analyses

We extracted DNA from a single thallus of *Peltigera* (DNA number N8001) collected from Mount Kinabalu, Sabah (northern Borneo, Malaysia) in 2018, using a CTAB-based protocol (Cubero et al. 1999). We sequenced the complete internal transcribed spacer of the nuclear ribosomal RNA genes (ITS), β -tubulin, and the largest subunit of RNA polymerase II (*RPB1*) for the mycobiont, as well as the *rbcLX* region of the *Nostoc* photobiont from this thallus, following Magain et al. (2017a). Mycobiont sequences were added to a dataset including all available sequences of these three loci for *Peltigera serusiauxii* (*P. dolichorhiza* s.lat. D), as well as representatives of all known species from the polydactyloid clade (Magain et al. 2017a, b) retrieved from GenBank (Table 1). *Peltigera dolichorhiza* s.str. and three other putative species from Papua New Guinea from the dolichorhizoid clade were chosen for the outgroup (Table 1).

ITS, *RPB1* and β -tubulin alignments were assembled using Mesquite v. 3.11 (Maddison & Maddison 2011) and aligned using MAFFT v7.305b (Katoh & Standley 2013) with default parameters. A 3-locus dataset was assembled using the combine.pl script of the PLEXUS package (Magain 2018). The dataset was divided into eleven subsets (ITS1, 5.8S, ITS2, β -tubulin 1st, 2nd and 3rd codon positions and introns, *RPB1* 1st, 2nd and 3rd codon positions and intron), and the best partition scheme for the phylogenetic analyses was determined using PartitionFinder 2.1.1 (Lanfear et al. 2016) by implementing the corrected Akaike information criterion (AICc) and the greedy algorithm. Maximum likelihood (ML) phylogenetic searches were implemented with RAxML version 8.2.12 (Stamatakis 2006; Stamatakis et al. 2008) using the CIPRES portal (Miller et al. 2010), with the GTRGAMMA model and the best partition scheme retrieved from the PartitionFinder analysis. Bootstrap support values were obtained based on 1000 pseudoreplicates.

Using ITS sequences, we provide DNA barcodes for the unambiguous recognition of *P. serusiauxii* from all other *Peltigera* species. The *rbcLX* sequence from the specimen collected on Mount Kinabalu was added to the *rbcLX* dataset from Magain et al. (2017a, Matrix 4). The dataset was partitioned by codon positions, and the ML phylogeny was generated using the same parameters as for the mycobiont.

Morphological and chemical studies

Ascospores were measured and photographed under a Nikon Eclipse 80i light microscope at 400 \times , mounting the material in 3% KOH. Thin-layer chromatography (TLC) was performed in phase C following Orange et al. (2010) for three specimens of *P. serusiauxii*: the type of the species, an additional representative from Papua New Guinea, and the specimen from Borneo.

Results and discussion

Phylogeny of the polydactyloid clade

The PartitionFinder analysis suggested that the mycobiont dataset should be divided into two subsets: one composed of ITS1, ITS2, *RPB1* 3rd codon position, β -tubulin 3rd codon position and β -tubulin introns; the other composed of 5.8S, the 1st and 2nd codon positions of both protein-coding genes, and the intron in *RPB1*. The phylogeny of the polydactyloid clade (Fig. 1A) is similar to the topology obtained by Magain et al. (2017a, b). Within this clade, *Peltigera sp. 9*, which likely corresponds to *Peltigera dolichospora* based on its unique chemistry (absence of tenuiorin and methyl gyrophorate; Magain et al. unpublished), is sister to the remaining taxa in the clade, whereas *P. serusiauxii* (*P. sp. 11* in Magain et al. 2017a, b) represents the second split. Within the *P. serusiauxii* clade, the specimen from Borneo is sister to a strongly supported group composed of all specimens from Papua New Guinea. All interspecific relationships and clades representing species or putative species are highly supported (BS \geq 90), with the exception of the unresolved relationships within the *nana* group (Fig. 1A). The specimen from Mount Kinabalu, Borneo (N8001), is assigned to *P. serusiauxii* until more material from this lineage becomes available. Additional phenotypic information will allow a re-evaluation of the taxonomic status of this population.

In the *rbcLX Nostoc* tree (Fig. S1), all *P. serusiauxii* symbionts, including the newly sequenced cyanobiont from the Malaysian specimen, belong to a strongly supported clade (Fig. 1B), which corresponds to phylogroup IX sensu Magain et al. (2017a). To date, all known representatives from *Nostoc* phylogroup IX have been found in association with the mycobiont of *P. serusiauxii* and vice versa, representing another rare case of one-to-one reciprocal specificity in the genus (Magain et al. 2017a, 2018). The strict specialist profile of *P. serusiauxii* is confirmed in its current expanded geographic range. Therefore, previous findings do not seem to be an artifact caused by the limited sampling of *P. serusiauxii* exclusively from Papua New Guinea (Magain et al. 2017a).

Chemistry, morphology and biogeography

Peltigera dolichorhiza s.lat. D was distinguished chemically by the abundance of dolichorrhizin, but was otherwise indistinguishable from *P. dolichorhiza* s.lat. A–C (Sérusiaux et al. 2009). In Papua New Guinea, *P. dolichorhiza* s.lat. D occurs as an epiphyte (including dead wood) between 1800 and 2400 meters. The specimen

Table 1. Voucher information and GenBank accession numbers for specimens included in the phylogenetic analyses of the polydactyloid clade of the genus *Peltigera*, section Polydactylon (Fig. 1A). Newly acquired sequences are bolded.

Taxon	Voucher	ITS	β -tubulin	<i>RPB1</i>	<i>rbcLX</i>
<i>Peltigera dolichorhiza</i> (outgroup)	Reunion Island, DNA N999, N. Magain & E. Sérusiaux s.n. (LG)	KX897180	KX880093	KX880193	KX9222887
<i>Peltigera dolichorhiza</i> s.lat. A (outgroup)	Papua New Guinea, P. Diederich 11052 (herb. Diederich)	FJ527294	–	–	–
<i>Peltigera nana</i>	China, Yunnan, DNA P1281, J. Miadlikowska s.n. (DUKE)	KX897212	–	KX880208	KX922915
<i>Peltigera nana</i>	Japan, Kochi, DNA P1666, G. Thor 21255 (UPS)	KX897213	–	KX880209	KX922916
<i>Peltigera</i> cf. <i>nana</i> 1	Papua New Guinea, E. Sérusiaux 13981 (LG)	FJ527286	–	–	–
<i>Peltigera</i> cf. <i>nana</i> 2	New Zealand, DNA P609, S. Stenroos 6037 (H)	KX897217	–	KX880212	KX922919
<i>Peltigera oceanica</i>	Papua New Guinea, E. Sérusiaux s.n. (LG)	FJ527283	–	–	–
<i>Peltigera polydactylon</i> ssp. <i>polydactylon</i>	Norway, DNA P385, N. Magain s.n. (LG)	KX365448	KM005820	KM005994	KX922979
<i>Peltigera polydactylon</i> ssp. <i>udeghe</i>	Russia, Khabarovsk, DNA P3033, J. Miadlikowska et al. s.n. (DUKE)	KX365430	KX365424	KX365440	MH986783
<i>Peltigera seneca</i>	USA, Pennsylvania, DNA P450, J. Lendemer 16792 (NY)	KX365451	–	KX365463	KX923080
<i>Peltigera serustauxii</i>	Papua New Guinea, DNA N1532, E. Sérusiaux s.n. (LG)	KX897391	KX880180	KX880287	KX923081
<i>Peltigera serustauxii</i>	Papua New Guinea, DNA N1547, E. Sérusiaux 13758 (LG)	KX897394	–	–	KX923084
<i>Peltigera serustauxii</i>	Papua New Guinea, DNA N1537, E. Sérusiaux s.n. (LG)	KX897393	–	–	KX923083
<i>Peltigera serustauxii</i>	Malaysia, Sabah, DNA N8001, N. Magain GoLife-204 (BORH)	MN886245	MN883170	MN883169	MN883171
<i>Peltigera serustauxii</i> TYPE	Papua New Guinea, DNA N1533, E. Sérusiaux s.n. (LG)	KX897392	KX880181	–	KX923082
<i>Peltigera</i> sp. 4 (outgroup)	Papua New Guinea, DNA N1534, E. Sérusiaux s.n. (LG)	KX897353	KX880168	KX880273	KX923043
<i>Peltigera</i> sp. 5 (outgroup)	Papua New Guinea, DNA N1545, E. Sérusiaux s.n. (LG)	KX897354	MF946769	KX880274	KX923044
<i>Peltigera</i> sp. 8	Taiwan, DNA P1237, A. Mikulin T71 (A. Mikulin Private Coll.)	KX897378	KX880178	KX880284	KX923067
<i>Peltigera</i> sp. 8	China, Yunnan, DNA N1898, B. Goffinet 10063 (CONN)	KX897373	KX880174	KX880280	KX923062
<i>Peltigera</i> sp. 8	Russia, Khabarovsk, DNA P3013, J. Miadlikowska et al. s.n. (DUKE)	MF908498	MF946780	MF946737	MF947118
<i>Peltigera</i> sp. 8	China, Jilin, DNA P1272, M. Sohrabi 16572 (M. Sohrabi Private Coll.)	KX897369	KX880173	KX880279	KX923058
<i>Peltigera</i> sp. 9 (<i>Peltigera dolichospora</i>)	China, Yunnan, DNA N1921, B. Goffinet 10064 (CONN)	KX897386	MF946782	MF946739	KX923075
<i>Peltigera</i> sp. 9 (<i>Peltigera dolichospora</i>)	China, Yunnan, DNA N1923, B. Goffinet 10073 (CONN)	KX897387	KX880179	KX880285	KX923076
<i>Peltigera sumatrana</i>	Papua New Guinea, DNA P884, E. Sérusiaux 16402 (LG)	KX880182	KX880182	–	–
<i>Peltigera weberi</i>	Papua New Guinea, H. Sipman 39314 (B)	FJ527281	–	–	–

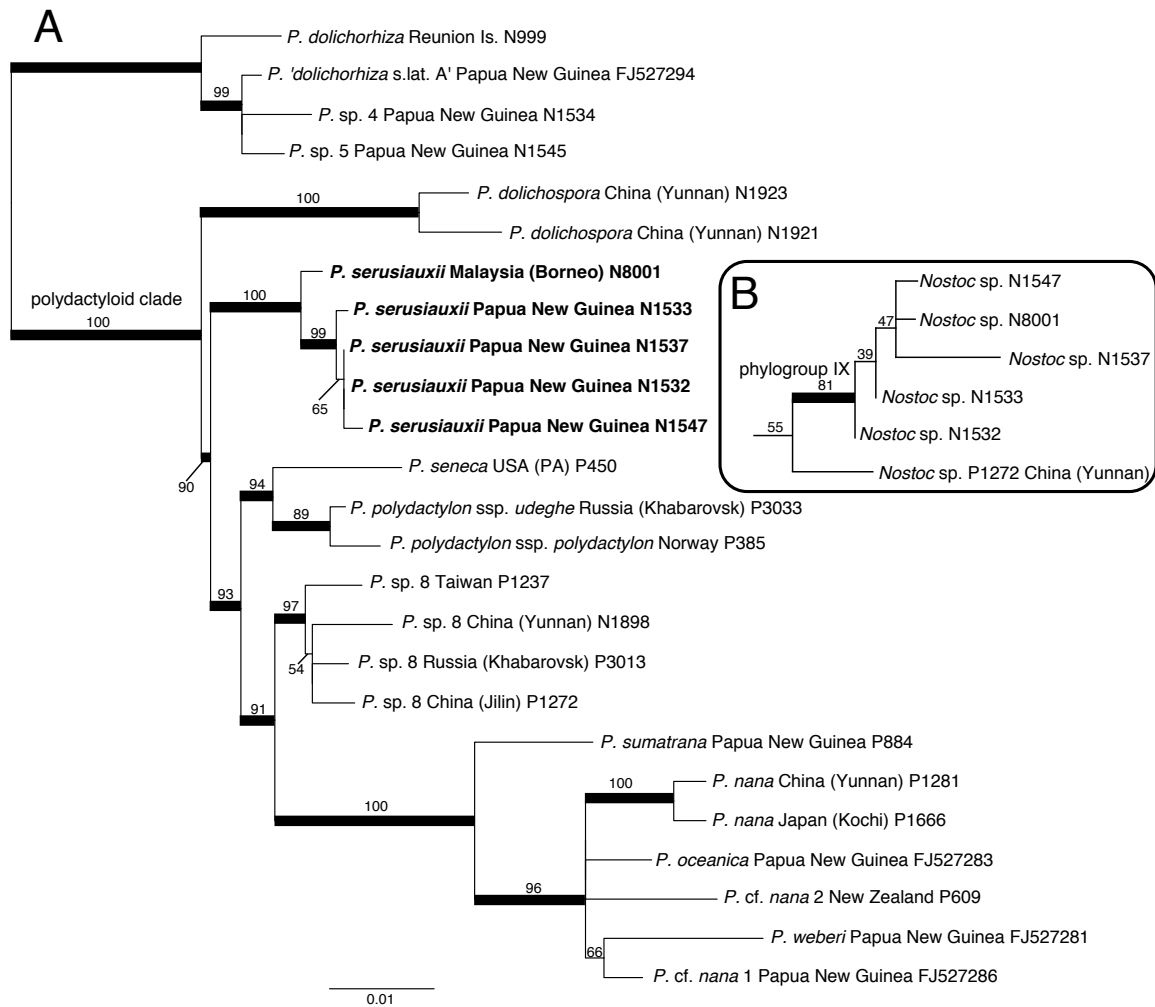


Figure 1. A – phylogenetic relationships (ML) within the polydactyloid clade of the genus *Peltigera*, section *Polydactylon*, based on a 3-locus dataset (ITS, *RPB1*, β -tubulin). The rooting of this tree follows Magain et al. (2017b). Bootstrap values are shown above branches. Thick branches received bootstrap support ≥ 70 . *Peltigera serusiauxii* is bolded. The scale represents nucleotide substitutions per site; B – clade representing *Nostoc* phylogroup IX from specimens of *P. serusiauxii* (part of the *rbcLX* phylogeny shown in Supplementary figure S1). Bootstrap values are shown above branches. Thick branches received bootstrap support ≥ 70 .

from Mount Kinabalu was collected on a mossy slope at ~3235 m elevation.

The lower surface of specimens from Papua New Guinea is variable, but in most cases it resembles the lower surface of *P. polydactylon*, with dark brown veins reaching the end of lobes, but well spaced and not delimiting small white oval interstices (Figs 2B & 3B). The lower surface of the specimen from Borneo has very dark and well-delimited veins, sometimes forming a dense network defining small white oval interstices, reminiscent of the lower surface of *P. occidentalis*. In some specimens with more widely spaced interstices, the lower surface resembles *P. polydactylon* (Fig. 3D).

Specimens of *P. dolichorhiza* s.lat. A and C (the latter shown as *P. sp. 4* in Figure 1A), from the dolichorhizoid clade, generally belong to the dolichorhiza morphotype with thinner and more brittle thalli and paler veins. However, a relatively high degree of plasticity is present, with overlaps between species, preventing these character states from being diagnostic at species level. The upper surface of specimens of *P. dolichorhiza* s.lat. B (*P. sp. 5* in Fig. 1A) is greenish brown to dark brown

when dry, dark brown when wet, smooth and typically shiny, and the lobe margins are brittle and usually fragmented in herbarium specimens. The lower surface is pale orange brown near the margins, becoming black towards the thallus center where the black veins become fused and tend to cover the entire surface. The network of orange veins with rather small interstices is clearly visible towards the thallus margins but becomes more diffused, with large white interstices in the intermediary zone between the margins and the thallus center, similar to *P. serusiauxii*.

Strong affinities between lichens of Mount Kinabalu and Papua New Guinea are common, as many species such as *Pertusaria epitheciifera*, *Thelotrema subweberi* and *Placopsis auriculata* occur in both areas (Sipman 1993). The genus *Sporopodiopsis* (Sérusiaux 1997) includes two species, one from Papua New Guinea (*S. mortimeriana*) and one from Mount Kinabalu (*S. sipmanii*).

The chemistry of the *P. serusiauxii* specimen from Mount Kinabalu is similar to that of the Papua New Guinea specimens, with the presence of tenuiorin, methyl glyrophosphate, dolichorrhizin and zeorin (Fig. S2).

Taxonomy

Peltigera serusiauxii Magain, Miadl. & Goffinet, sp. nov.
(Figs 2–4)

Mycobank MB 833930

= *Peltigera dolichorhiza* s.lat. D (Sérusiaux et al. 2009).

= *Peltigera* sp. 11 (Magain et al. 2017a, b).

Diagnosis: *P. serusiauxii* differs from other glabrous species in Asia and Papua New Guinea by its *P. polydactylon*-like lower surface (in which the veins reach the lobe tips, and the interstices exceed the width of the veins toward the thallus center

but become narrower than the veins towards the lobe tips), its abundant production of dolichorrhizin, and its ITS barcodes GTAGCTGTG in ITS1 and AAATAG in ITS2.

Type: Papua New Guinea, Eastern Highlands Province, Mount Gahavisuka Provincial Park, 11 km N of Goroka, 2300 m, S 6°01'E 145°25', 5 Nov 1995. Little-disturbed mossy mountain forest dominated by *Castanopsis*, E. Sérusiaux s.n. with H. Sipman (UPNG – holotype, LG – isotype).

Reference sequence: KX897392

Description. Thallus large, 6–8(–10) cm across, with lobes 1–2 cm across, upper surface grey, light brown

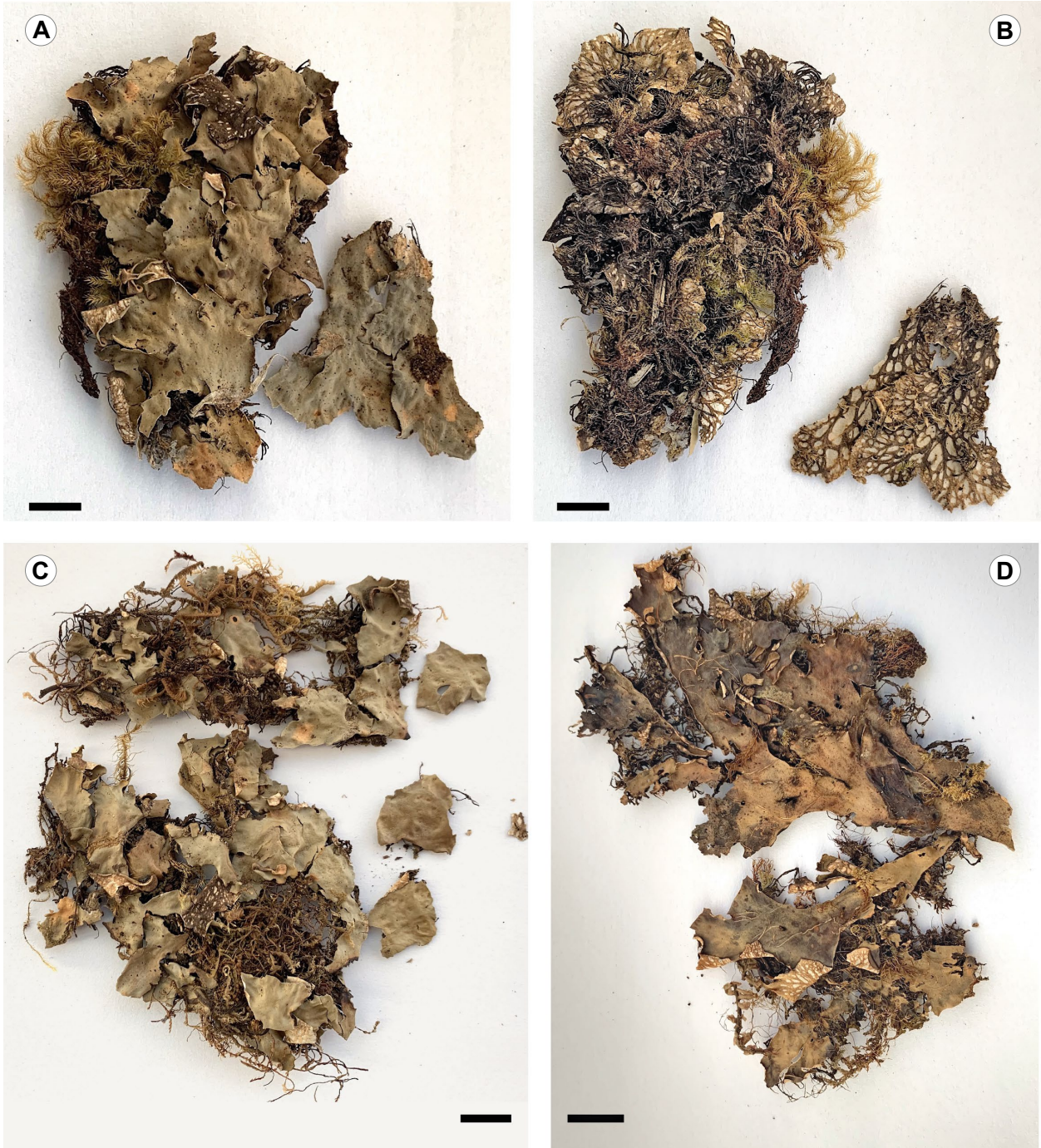


Figure 2. A–D – *Peltigera serusiauxii*. A–B – type specimen from Eastern Highlands Province, Papua New Guinea (E. Sérusiaux s.n., UPNG, Dupl. LG), upper surface (A) and lower surface (B); C – upper surface of specimen from Central Province, Papua New Guinea (DNA N1532, E. Sérusiaux s.n., LG); D – upper surface of specimen from Morobe Province, Papua New Guinea (DNA N1538, H. Streimann 33227 CANB, Dupl. LG). Scale bars = 1 cm.

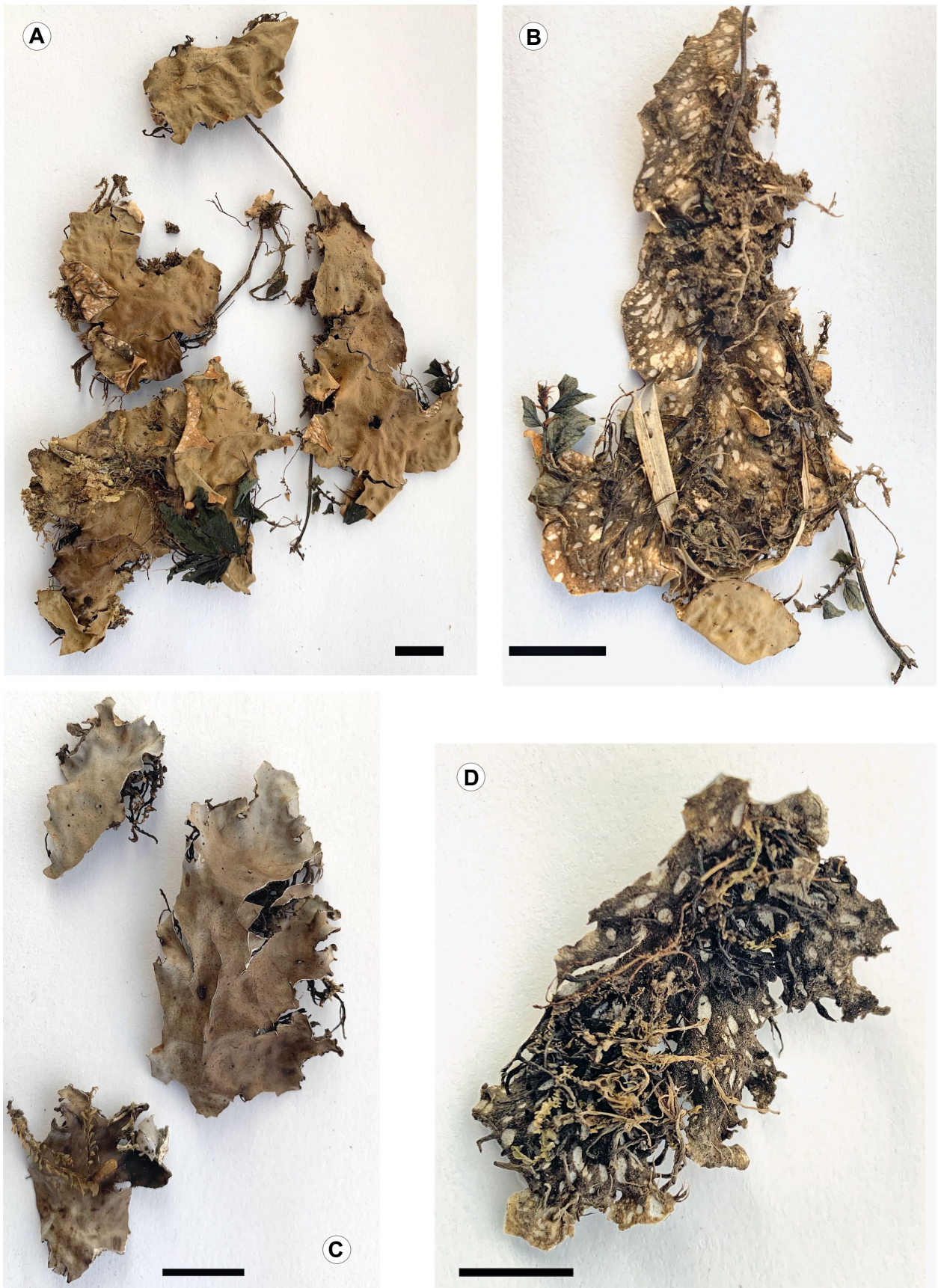


Figure 3. A–D – *Peltigera serusiauxii*. A–B – specimen from Eastern Highlands Province, Papua New Guinea (DNA N1547, E. Sérusiaux 13758, LG), upper surface (A) and lower surface (B); C–D – specimen from Sabah Province, Malaysia (DNA N8001, N. Magain GoLife-204, BORH), upper surface (C) and lower surface (D). Scale bars = 1 cm.

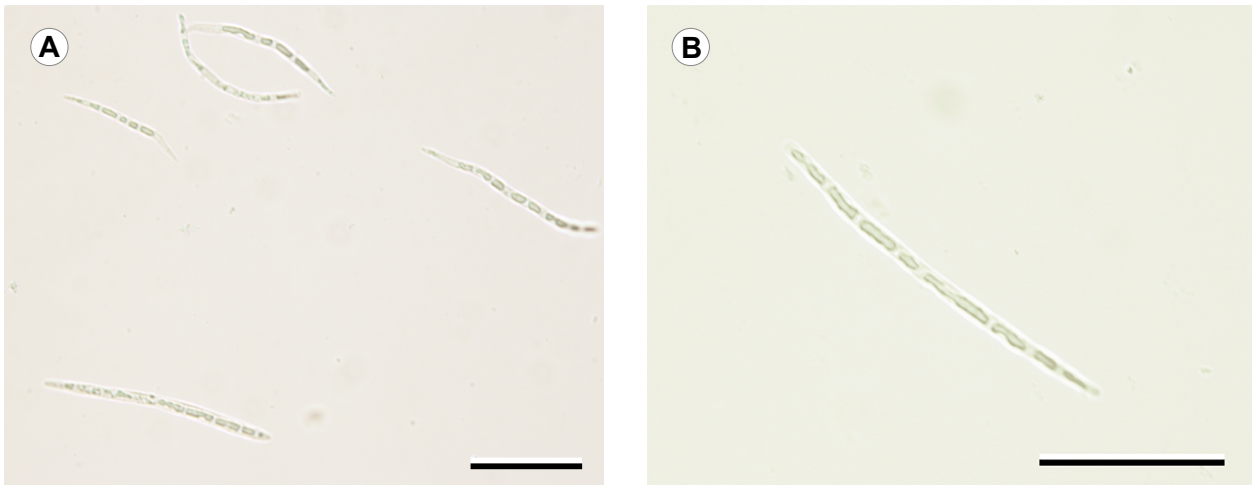


Figure 4. Ascospores of *P. serusiauxii* from Papua New Guinea. A – ascospores from the type specimen (E. Sérusiaux s.n., DNA N1533); B – ascospore from specimen (E. Sérusiaux s.n., DNA N1532). Scale bars = 50 µm.

or dark brown when dry, dark grey to dark brown when wet, smooth but undulating, rather thin and fragile. Margins regular, flat or slightly raised. Vegetative propagules not seen. Lower surface with whitish interstices strongly contrasting with light brown to dark brown veins almost always extending to margins, becoming darker towards thallus center; veins forming a well-delimited network with large white interstices, veins rather narrow (0.5–1 mm), slightly raised, usually much narrower than the white interstices (2–)3–4 mm wide, especially towards center of thallus, whereas veins become broad and often wider than the interstices towards the margins (~1 mm wide each). Rhizines sparse, usually located near margins, quite long (0.5–)0.8–1(–1.2) cm, dark brown to black, simple to fasciculate. Apothecia abundant when present; brown, small and somewhat circular, usually 1–2 mm long and 1.5–2.5 mm wide, but up to 6 mm long and 4 mm wide, usually on top of a somewhat triangular raised lobe, usually folding on itself on upper surface when dry. Ascospores 8 per ascus, fusiform to acicular, (60–)80–110 × 3.5–5 µm, 4–11 septate, hyaline. Pycnidia not seen.

Photobiont. *Nostoc* phylogroup IX (see Figure 5 of Magain et al. 2017a).

Chemistry. Tenuinorin, methyl gyrophorate, dolichorrhizin (abundant), zeorin and peltidactylin (sometimes absent).

Barcodes. ITS1 (GGGCA)GTAGCTGTG(TGGG); ITS2 (AATT)AAATAG(TTGG).

Etymology. The epithet honors Prof. Emmanuël Sérusiaux, who substantially contributed to our understanding of the world-wide diversity of *Peltigera*, especially in Papua New Guinea where he collected the type material among several other specimens of this new species.

Ecology. Epiphytic or on mossy slopes in tropical montane forests, from 1800 to 3235 m a.s.l.

Distribution. Papua New Guinea (Eastern Highlands, Central and Morobe provinces), Malaysia (Borneo, Sabah Province).

Selected specimens examined. PAPUA NEW GUINEA. Eastern Highlands Province, Mount Gahavisuka Provincial Park, 11 km N of Goroka, along trail to lookout, 2300–2450 m, 6°01'S, 145°25'E, 11 Aug. 1992, little-disturbed mossy mountain forest, E. Sérusiaux 13758 (LG); Ibid., Central Province, Owen Stanley Range, Trail from Myola to Naduri, 1800 m, 9°08'S, 147°41'E, In montane forest remnants (with, e.g., *Lithocarpus*) along trail from the Gap downwards, 20 Oct. 1995, E. Sérusiaux DNA1532, with A. Aptroot, P. Lambley & H. Sipman (LG); Ibid., Morobe province, Mt. Kaindi, 5 km W of Wau, 7°21'S, 146° 41'E, 2300 m, disturbed montane forest (*Nothofagus*) on ridge. On rotting tree branch. H. Streimann 33227 (CANB); MALAYSIA. Borneo, Sabah Province, Kinabalu Park, 6°3'16.5594"S, 116°33'55.224"E, trail to summit of Mount Kinabalu, mossy slope, 3235 m, 1 June 2018, N. Magain GoLife-204 (BORH).

Notes. No validly published epithet could be found for this species delimited as distinct in the species delimitation analyses by Magain et al. (2017b). *Peltigera serusiauxii* represents an independent lineage in the polydactyloid clade (Fig. 1A). Usually, thalli of *P. serusiauxii* are more robust than thalli of *P. sp. 4* and *P. dolichorhiza* s.lat. A. As compared to *P. sp. 5*, thalli of *P. serusiauxii* have darker veins that extend to the margins and form a well-delimited brown network with large white interstices. However, ITS barcodes are needed to reliably distinguish *P. serusiauxii* from other species in Papua New Guinea. On Mount Kinabalu (Malaysia), *P. serusiauxii* differs in the vein pattern from other co-occurring species, including *P. neopolydactyla* 3 and an unnamed species related to *P. sp. 4* and *P. sp. 5* (Magain unpublished). *Peltigera serusiauxii* is endemic to Southeast Asia where it has so far been reported exclusively from Malaysia and Papua New Guinea.

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Supplementary electronic material

Figure S1. Phylogenetic relationships of the *Nostoc* symbionts from *P. serusiauxii* specimens based on *rbcLX* Matrix 4 from Magain et al. (2017a). Roman numerals refer to phylogroups following Magain et al. 2017a. Phylogroup IX is bolded and highlighted in light grey. Bootstrap support values are shown above branches and branches with values ≥ 70 are thickened. Clade names follow Otálora et al. (2010). The scale represents nucleotide substitutions per site. [Download file](#)

Figure S2. TLC in phase C of three specimens of *P. serusiauxii*: two specimens from Papua New Guinea (N1532 and type N1533) and the specimen from Malaysia (N8001). [Download file](#)

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