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Standard Paper

Peltigera (*Lecanoromycetes*) on Mt Kilimanjaro, East Africa

Ulla Kaasalainen[1,2], Lea Biermann[1], Neduvoto P. Mollel[3], Alexander R. Schmidt[1] and Andreas Hemp[4]

[1]Department of Geobiology, University of Göttingen, Goldschmidtstraße 3, 37077 Göttingen, Germany; [2]Finnish Museum of Natural History, P.O Box 7, 00014 University of Helsinki, Helsinki, Finland; [3]National Herbarium, Tropical Pesticides Research Institute, P.O. Box 3024, Arusha, Tanzania and [4]Department of Plant Systematics, University of Bayreuth, Universitätsstraβe 30, 95440 Bayreuth, Germany

Abstract

Tropical mountain forests are hotspots of biodiversity that are widely threatened by human population pressure and climate change. However, the cryptogamic species richness of many tropical mountain regions is insufficiently known, the poorly understood biodiversity of tropical African lichens being a prime example. To study the diversity of the genus *Peltigera* (*Ascomycota*, *Lecanoromycetes*) in East Africa, we studied lichens in a wide range of habitats on the slopes of Mt Kilimanjaro in Tanzania. Ranging from savannah to alpine heath vegetation and from natural forests to agricultural environments, 13 habitat types were sampled for lichens, which were then identified based on the nuITS genetic marker and morphology. We found eight *Peltigera* species on the slopes of Mt Kilimanjaro, including *P. alkalicola* sp. nov., *P. dolichorhiza*, *P. polydactyloides*, *P. praetextata*, *P. rufescentiformis*, *P. seneca*, *P. somediifera* and *P. ulcerata*. *Peltigera* is most common and species-rich in the subalpine *Erica* forest zone, and four of the eight detected species were present only in the subalpine and alpine vegetation zones. *Peltigera alkalicola* was identified as a previously undescribed species, growing on trachybasaltic lava in the subalpine and alpine zones of Mt Kilimanjaro. The species resembles *P. lepidophora* but differs by possessing smaller thalli and peltate isidia that are distinctly dark on the lighter, tomentose lamina. Based on data from the NCBI GenBank, *P. alkalicola* probably also occurs in Alaska (USA) and Ningxia (China). This suggests that even though the species might generally be rare, it may have a global distribution in extreme mountain environments. For the first time, we report *P. somediifera* from Tanzania and *P. seneca* from Africa.

Key words: cyanolichens, Eastern Afromontane biodiversity hotspot, lichenized fungi, montane ecosystems, *Peltigerales*, Tanzania, tropical lichen diversity

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Introduction

Tropical mountains and especially montane forests are hotspots of biodiversity. Together with other East African mountains, Mt Kilimanjaro forms one of the globally acknowledged biodiversity hotspots (Mittermeier *et al.* 2004). Even though only *c.* 10% of the extent of the original habitats is remaining (Mittermeier *et al.* 2004), the Eastern Afromontane biodiversity hotspot still harbours a staggering amount of diversity and endemism (Burgess *et al.* 2007; Harper *et al.* 2010; Mittermeier *et al.* 2011), including lichens and lichen-symbiotic organisms (Suija *et al.* 2018; Kaasalainen *et al.* 2021*a*, *b*; Kantelinen *et al.* 2021).

Peltigera is perhaps among the most studied of lichen genera (Miadlikowska & Lutzoni 2000; Miadlikowska *et al.* 2003; Sérusiaux *et al.* 2009; Zúñiga *et al.* 2015; Jüriado *et al.* 2017; Magain *et al.* 2017*a*, *b*, 2018). The genus has been further divided into eight monophyletic sections, of which sections *Peltigera* and *Polydactylon* are most speciose, together containing over 100 species (Miadlikowska & Lutzoni 2000; Magain *et al.* 2017*a*, 2018). The other sections, such as *Horizontales*, are significantly smaller with less than ten species each (Miadlikowska & Lutzoni 2000). However, as with many lichens, the worldwide sampling of *Peltigera* concentrates on certain non-tropical areas while, for example, tropical Africa is under-represented and only a small number of African *Peltigera* specimens have been included in studies using DNA data.

So far, 11 species of *Peltigera* have been reported from East Africa, including Ethiopia, Kenya, Tanzania and Uganda. These species include *P. canina* (L.) Willd., *P. cichoracea* Delise, *P. didactyla* (With.) J. R. Laundon, *P. dolichorhiza* (Nyl.) Nyl., *P. polydactyloides* Nyl., *P. polydactylon* (Neck.) Hoffm., *P. praetextata* (Flörke ex Sommerf.) Zopf, *P. rufescens* (Weis.) Humb., *P. rufescentiformis* (Gyeln.) C. W. Dodge, *P. somediifera* (Nyl.) Vitik. (as *P. lambinonii* Goffinet) and *P. ulcerata* Müll. Arg.; of these, all but *P. canina*, *P. rufescens* and *P. somediifera* have also been reported from Tanzania (Swinscow & Krog 1988; Goffinet & Hastings 1995; Frisch & Hertel 1998; Krog 2000; Alstrup & Christensen 2006; Kirika *et al.* 2018; Kaasalainen *et al.* 2021*b*). The phylogenetic analysis of genetic markers has revealed previously unknown diversity among *Peltigera* in other parts of the world and, for example, *P. canina*, *P. didactyla*, *P. dolichorhiza*, *P. polydactylon* and *P. rufescens* have been shown to include additional and partly still undescribed species (Goffinet *et al.* 2003; Miadlikowska *et al.* 2003; Sérusiaux *et al.* 2009; Magain *et al.* 2016, 2017*a*, *b*, 2018; Manoharan-Basil *et al.* 2016; Jüriado *et al.* 2017). Here, we

Author for correspondence: Ulla Kaasalainen. E-mail: ulla.kaasalainen@helsinki.fi

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sampled *Peltigera* from various habitats on Mt Kilimanjaro in Tanzania, East Africa, and assessed the species identities and their distribution using DNA-based methods and morphology.

Material and Methods

Study location and sampling

The relatively young (*c.* 2.5 million years old; Nonnotte *et al.* 2008) dormant volcano Mt Kilimanjaro in north-eastern Tanzania, located *c.* 330 km south of the equator, reaches 5895 m above sea level and *c.* 4900 m above the surrounding savannah. The elevational gradient of the mountain slopes supports a wide range of natural vegetation types, as well as many human-modified habitats (Lambrechts *et al.* 2002; Hemp 2005, 2006*b*; Rutten *et al.* 2015; Hemp & Hemp 2018). The windward slopes receive considerable amounts of atmospheric moisture from the Indian Ocean and harbour several types of moist mountain forest with abundant and diverse epiphytes (Hemp 2006*b*; Kaasalainen *et al.* 2021*a*). For this study, 13 natural and disturbed habitat types were sampled on the moist southern and south-eastern slopes of the mountain: 1) natural savannah and 2) maize fields (800–1100 m alt.); 3) lower-montane forests, 4) traditional Chagga home gardens, 5) commercial coffee farms and 6) grasslands (1100–2000 m); 7) montane *Ocotea* forest and 8) selectively logged *Ocotea* forest (2100–2800 m); 9) upper-montane *Podocarpus* forest and 10) *Podocarpus* forest replaced by *Erica excelsa* forest as a result of fire (2800–3100 m); 11) subalpine *Erica trimera* forest and 12) fire disturbed *Erica* forest (3500–4000 m); 13) alpine *Helichrysum* heaths (4000–4600 m). Between the habitats, the mean annual temperature varies from *c.* 23 °C at the base of the mountain to 4 °C in the alpine zone, and night frosts occur above 2700 m (Hemp 2006*a*; Appelhans *et al.* 2016). The relative humidity and precipitation are highest in the montane forest zones within the stable cloud condensation belt (mean annual precipitation over 2000–2400 mm, partly up to 3000 mm), from where the precipitation diminishes both up to the *Helichrysum* heath (*c.* 1000 mm) and down to the savannah (*c.* 600 mm) (Hemp 2006*a*; Appelhans *et al.* 2016). The specimens were collected in 2016–2017 from a total of 65 sampling plots, including five plots in each above-mentioned habitat type. In each plot, specimens were collected from a 5 × 20 m central plot, as well as along two 50 m transects running in parallel 20 m apart on both sides of the central plot.

The lichen substrata were divided into five classes: 1) ground, 2) rock, 3) trunk, 4) canopy and 5) decaying wood. Ground includes specimens collected from the ground layer, except for specimens on rock surfaces and tree roots; rock includes specimens from rock surfaces and stones; trunk includes specimens mainly from tree trunks below the height of *c.* 2 m but also specimens from shrubs and from large tree roots; canopy includes specimens from freshly fallen branches and the canopy of occasional, relatively recently, fallen trees; decaying wood includes specimens from clearly decaying wood, including mainly tree trunks and stumps. Almost all specimens grew together with bryophytes.

Morphology and thin-layer chromatography

A total of 146 *Peltigera* specimens (see Supplementary Material Table S1, available online) from Mt Kilimanjaro were morphologically identified based on existing literature of studies from the area (Swinscow & Krog 1988). Of these 91 specimens were selected for DNA extraction, PCR and sequencing, including 1–3 specimens of each species from each plot.

Thin-layer chromatography was performed with solvent G (toluene/ethyl acetate/formic acid, 139:83:8) following Orange *et al.* (2010). Since the secondary chemistry of most *Peltigera* species is well recorded for East Africa (Swinscow & Krog 1988), the previous observations were confirmed from 1–2 specimens of each species, preferably collected from different habitats. Separate samples were obtained, from the thallus and soralia, of the sorediate species *P. sorediifera* and *P. ulcerata*.

DNA extraction and polymerase chain reaction (PCR)

A small fragment of each selected specimen was used to extract DNA using the GeneJET Genomic DNA Purification Kit (Fisher Scientific, Germany) following the manufacturer's instructions. PCR amplification of the fungal nuclear internal transcribed spacer region (nuITS: ITS1-5.8S-ITS2) was performed with the primers ITS5 and ITS4 (White *et al.* 1990) using GoTaq DNA polymerase (Promega, USA) as follows: 0.2 mM dNTPs, 0.2 μM of each primer, 0.5 mg ml^{-1} BSA and 0.025 U/μl polymerase in a total volume of 50 μl. The cycling conditions were: initial denaturation at 95 °C for 2 min, followed by 35 cycles of 45 s at 95 °C, 45 s at 56 °C, and 1 min at 72 °C, with a final elongation of 5 min at 72 °C. Sequencing was carried out, using the specified PCR primers, by LGC Genomics (Germany). After sequencing, the chromatograms of all DNA sequences were checked and edited using CodonCode Aligner (CodonCode Corporation, USA). The 81 *Peltigera* nuITS sequences generated in this study, together with three previously unpublished *P. lepidophora* nuITS sequences from northern and southern Finland, are deposited in the NCBI GenBank under Accession numbers MZ385613–MZ385696 and listed with specimen and collection information in Supplementary Material Table S1.

Phylogenetic analyses

The obtained sequences were aligned according to *Peltigera* sections *Horizontales*, *Peltigera* and *Polydactylon* (Miadlikowska & Lutzoni 2000) using PhyDE-1 v. 0.997 (Müller *et al.* 2010). Identical ITS variants were combined (see Supplementary Material Table S1: ITS variant). Using the alignments of Jüriado *et al.* (2017) as starting points and utilizing existing studies (Sérusiaux *et al.* 2009; Magain *et al.* 2017*a*, 2018), the newly obtained ITS variants were aligned with sequences from GenBank. Two ambiguous regions within ITS1 in section *Peltigera*, and one ambiguous region within ITS2 in section *Polydactylon* were removed, after which ITS variants identical to the remaining portion were further combined. This resulted in alignments of 661, 627 and 719 nts for *Horizontales*, *Peltigera* and *Polydactylon*, respectively. Phylogenetic analyses were performed using Bayesian inference and the data partitioned according to the marker regions (ITS1-5.8S-ITS2). Substitution models for the regions were selected using jModelTest2 (Posada 2008) and Bayesian information criterion: K80, JC and K80 were selected for section *Horizontales*, and HKY + G, JC and HKY + G for sections *Peltigera* and *Polydactylon*. The analyses were run using MrBayes v. 3.2.3 (Ronquist & Huelsenbeck 2003) according to the principles described by Olsson *et al.* (2012) on the CIPRES Science Gateway (Miller *et al.* 2010), with three runs of four chains each. The convergence of the parallel runs was checked after 10^7 generations and the first 25% was discarded as burn-in using Tracer v. 1.5 (Rambaut *et al.* 2018); the results were visualized using TreeGraph2 v. 2.15 (Stöver & Müller 2010).

Results and Discussion

Most *Peltigera* species detected from Mt Kilimanjaro could be placed within existing taxa based on morphology and nuITS data. These include *P. dolichorhiza* (30 specimens), *P. polydactyloides* (28), *P. praetextata* (10), *P. rufescentiformis* (38), *P. sorediifera* (13) and *P. ulcerata* (7). Of these, *P. sorediifera* has not previously been reported from Tanzania. Additionally, *P. seneca* (18 specimens) is reported for the first time from Africa and a new species, *P. alkalicola*, is described in this study based on two collected specimens. *Peltigera* seems to be absent below the lower-montane forest zone and in the more heavily modified habitats (i.e. in natural savannah, maize fields, coffee plantations, grasslands and home gardens). Of the *Peltigera* species reported in this study, *P. dolichorhiza*, *P. polydactyloides*, *P. praetextata* and *P. ulcerata* had a relatively wide distribution reaching over at least three montane vegetation zones, while *P. alkalicola*, *P. rufescentiformis*, *P. seneca* and *P. sorediifera* were present only above *c.* 3500 m alt. in the subalpine and alpine vegetation zones.

nuITS and phylogenetic analyses

Of the 91 selected specimens from Mt Kilimanjaro, 81 were successfully sequenced. No more than one sequence failed from any single sampling plot, and the failed specimens were not concentrated on any specific species, sections, or habitats. Thirty-six of the 81 sequenced specimens represent five species of *Peltigera* section *Peltigera* (Fig. 1A). The five specimens of *Peltigera praetextata* all represent one ITS variant identical to *P. praetextata* sequences in GenBank originating from different parts of the world. The four *Peltigera ulcerata* specimens represent three different ITS variants and formed a well-supported (posterior probability PP = 1) group with *P. ulcerata* specimens originating from other parts of the world. All specimens morphologically belonging to the *Peltigera didactyla* group (i.e. sorediate and tomentose; Goffinet *et al.* 2003) represent *Peltigera sorediifera*: the six sequenced specimens represent four ITS variants and formed a well-supported (PP = 1) group with *P. sorediifera* and *P. lambinonii* specimens from East Africa and Australia (Miadlikowska *et al.* 2003; Magain *et al.* 2018). *Peltigera lambinonii* was separated from *P. didactyla* and described as its own species from East Africa (Goffinet & Hastings 1995) but was later synonymized with *P. sorediifera* (Vitikainen 2008). Two specimens with identical ITS regions have a general morphological resemblance to *Peltigera lepidophora* but formed a well-supported (PP = 1) group distinct from that species, together with two sequences from GenBank originating from Alaska (USA; MH758352) and Ningxia (China; MH758356). Magain *et al.* (2018) acknowledged that these specimens collected from Alaska and Ningxia represent a species separate from *Peltigera lepidophora* s. str. (*P.* '*lepidophora* 2') based on the analysis of ITS, β-tubulin, COR1b, COR3 and COR16 genetic regions, but the species has not yet been formally described. Based on our material collected from Mt Kilimanjaro, we now describe the new species as *Peltigera alkalicola*. The specimens from the USA and China are also tentatively presumed to represent *P. alkalicola*, even though differences in nine nucleotide sites exist in the ITS1 region. The 17 sequenced *Peltigera rufescentiformis* specimens represent ten ITS variants which formed a well-supported clade (PP = 1) in the phylogenetic analysis. Two variants, 'rufescentiformis 11' and 'rufescentiformis 12', are clearly more variable than the other ITS variants and differ in over ten nucleotide sites from the other *P. rufescentiformis* ITS variants. However, most of this variation is contained within the ambiguous regions of ITS1 that were excluded from the phylogenetic analysis.

Twenty-six sequenced specimens represent the two species of *Peltigera* section *Polydactylon* (Fig. 1B). All the specimens with a morphological resemblance to *Peltigera polydactylon* s. lat. belong to *Peltigera seneca*; the 11 sequenced specimens include three ITS variants which all formed a well-supported (PP = 1) group with *P. seneca* from the USA (KX365472; Magain *et al.* 2016). *Peltigera seneca* is fairly common in the high elevation habitats of Mt Kilimanjaro, Tanzania, extending the distribution of *P. seneca* to Africa. All 15 sequenced specimens of *P. dolichorhiza* represent only one ITS variant, identical to a *P. dolichorhiza* specimen from Kenya (JX195207; Kaasalainen *et al.* 2013). In our analysis, the specimens formed a well-supported (PP = 1) group with a *P. dolichorhiza* specimen from Brazil (KX897187; Magain *et al.* 2017*a*). The *P. dolichorhiza* sequences did not form a monophyletic group in the analysis and the polyphyly of *P. dolichorhiza* has also been reported previously (Sérusiaux *et al.* 2009; Magain *et al.* 2017*b*). In the study of Magain *et al.* (2017*a*), based on a worldwide sampling of *Peltigera* section *Polydactylon*, the specimen from Brazil (KX897187) was placed within *P. dolichorhiza* s. str. but was separate from the bulk of the *P. dolichorhiza* specimens which mainly originated from South and Central America, but also from Rwanda and Reunion Island in Africa and the Indian Ocean.

The only species of *Peltigera* section *Horizontales* on Mt Kilimanjaro, *Peltigera polydactyloides*, was represented in the dataset by 19 sequences and 12 ITS variants, which formed a well-supported (PP = 0.96) group with other *P. polydactyloides* specimens from East Africa downloaded from GenBank (Fig. 1C; Miadlikowska *et al.* 2003; Sérusiaux *et al.* 2009).

Peltigera species on Mt Kilimanjaro

Peltigera alkalicola Kaasalainen sp. nov.

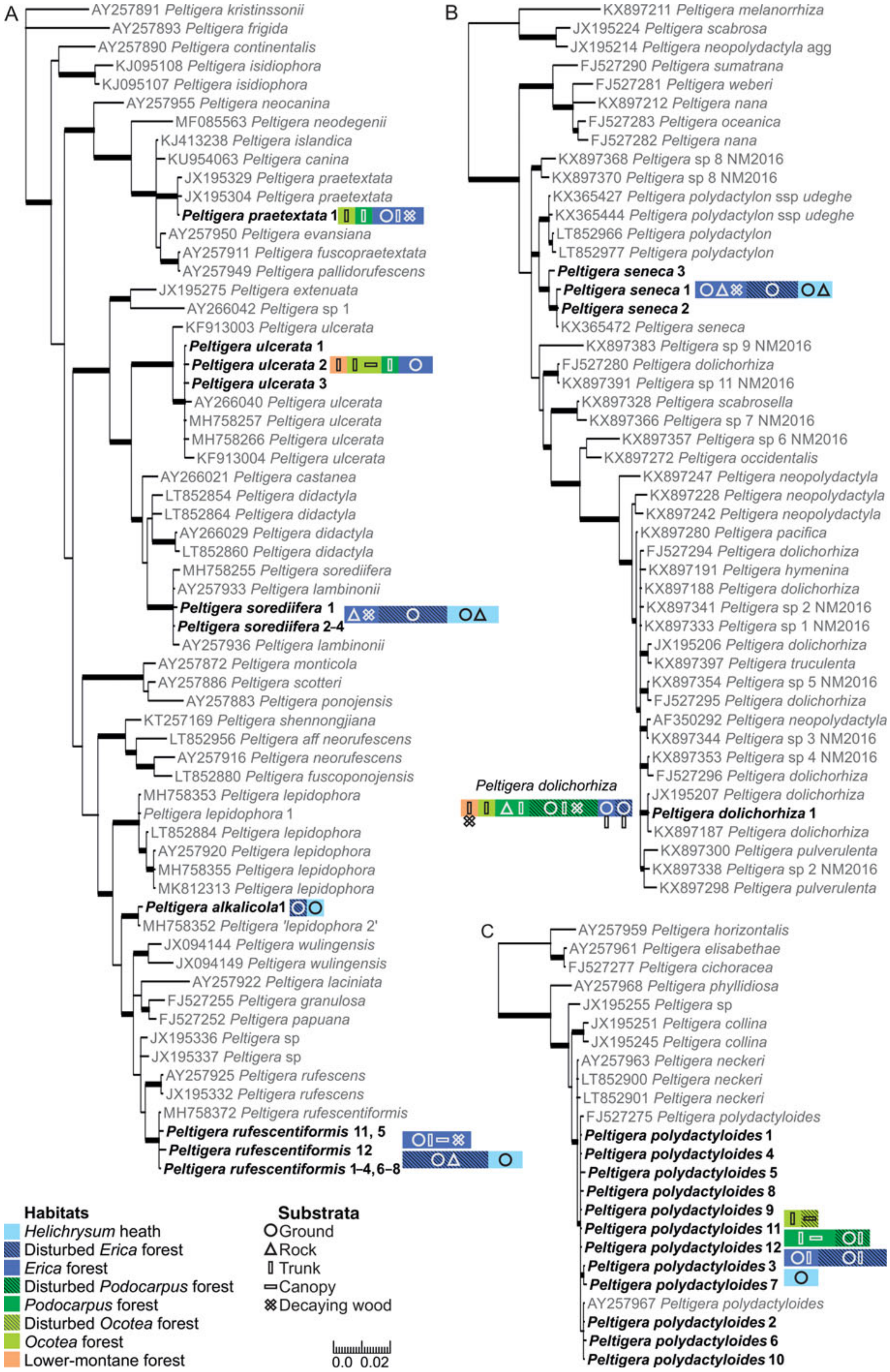
MycoBank No.: MB 840656

Similar to *Peltigera lepidophora* (Vain.) Bitter but differs in having smaller thalli and peltate isidia that are distinctly darker than the tomentose lamina; genetically distinct.

Type: Tanzania, Kilimanjaro Region, Kilimanjaro National Park, between Maua and Marangu routes, on soil in *Helichrysum* heath, 4390 m alt., −3.101144°S, 37.440764°E, 8 June 2017, *U. Kaasalainen* UK171254d (H—holotype). GenBank Accession no.: MZ385614.

(Fig. 2)

Thallus small, up to 5 cm diam. but often smaller (*c.* 1–2 cm). Lobes rounded to elongate, thick, up to 1–2 cm long and 1 cm wide, with rounded ends and upturned margins, occasionally with a slightly crisped appearance. *Upper surface* tomentose, often slightly scabrous, greyish brown. *Lower surface* usually white to pale brown, with concolorous or slightly darker, low to indistinct veins. *Rhizines* more abundant centrally, shorter closer to the margins, simple to confluent, concolorous with the lower surface. *Isidia* peltate, dark brown to almost black, often clearly


A
AY257891 Peltigera kristinssonii
AY257893 Peltigera frigida
AY257890 Peltigera continentalis
KJ095108 Peltigera isidiophora
KJ095107 Peltigera isidiophora
AY257955 Peltigera neocanina
MF085563 Peltigera neodegenii
KJ413238 Peltigera islandica
KU954063 Peltigera canina
JX195329 Peltigera praetextata
JX195304 Peltigera praetextata
Peltigera praetextata 1
AY257950 Peltigera evansiana
AY257911 Peltigera fuscopraetextata
AY257949 Peltigera pallidorufescens
JX195275 Peltigera extenuata
AY266042 Peltigera sp 1
KF913003 Peltigera ulcerata
Peltigera ulcerata 1
Peltigera ulcerata 2
Peltigera ulcerata 3
AY266040 Peltigera ulcerata
MH758257 Peltigera ulcerata
MH758266 Peltigera ulcerata
KF913004 Peltigera ulcerata
AY266021 Peltigera castanea
LT852854 Peltigera didactyla
LT852864 Peltigera didactyla
AY266029 Peltigera didactyla
LT852860 Peltigera didactyla
MH758255 Peltigera soredifera
AY257933 Peltigera lambinonii
Peltigera soredifera 1
Peltigera soredifera 2–4
AY257936 Peltigera lambinonii
AY257872 Peltigera monticola
AY257886 Peltigera scotteri
AY257883 Peltigera ponojensis
KT257169 Peltigera shennongjiana
LT852956 Peltigera aff neorufescens
AY257916 Peltigera neorufescens
LT852880 Peltigera fuscoponojensis
MH758353 Peltigera lepidophora
Peltigera lepidophora 1
LT852884 Peltigera lepidophora
AY257920 Peltigera lepidophora
MH758355 Peltigera lepidophora
MK812313 Peltigera lepidophora
Peltigera alkalicola1
MH758352 Peltigera 'lepidophora 2'
JX094144 Peltigera wulingensis
JX094149 Peltigera wulingensis
AY257922 Peltigera laciniata
FJ527255 Peltigera granulosa
FJ527252 Peltigera papuana
JX195336 Peltigera sp
JX195337 Peltigera sp
AY257925 Peltigera rufescens
JX195332 Peltigera rufescens
MH758372 Peltigera rufescentiformis
Peltigera rufescentiformis 11, 5
Peltigera rufescentiformis 12
Peltigera rufescentiformis 1–4, 6–8
B
KX897211 Peltigera melanorrhiza
JX195224 Peltigera scabrosa
JX195214 Peltigera neopolydactyla agg
FJ527290 Peltigera sumatrana
FJ527281 Peltigera weberi
KX897212 Peltigera nana
FJ527283 Peltigera oceanica
FJ527282 Peltigera nana
KX897368 Peltigera sp 8 NM2016
KX897370 Peltigera sp 8 NM2016
KX365427 Peltigera polydactylon ssp udeghe
KX365444 Peltigera polydactylon ssp udeghe
LT852966 Peltigera polydactylon
LT852977 Peltigera polydactylon
Peltigera seneca 3
Peltigera seneca 1
Peltigera seneca 2
KX365472 Peltigera seneca
KX897383 Peltigera sp 9 NM2016
FJ527280 Peltigera dolichorhiza
KX897391 Peltigera sp 11 NM2016
KX897328 Peltigera scabrosella
KX897366 Peltigera sp 7 NM2016
KX897357 Peltigera sp 6 NM2016
KX897272 Peltigera occidentalis
KX897247 Peltigera neopolydactyla
KX897228 Peltigera neopolydactyla
KX897242 Peltigera neopolydactyla
KX897280 Peltigera pacifica
FJ527294 Peltigera dolichorhiza
KX897191 Peltigera hymenina
KX897188 Peltigera dolichorhiza
KX897341 Peltigera sp 2 NM2016
KX897333 Peltigera sp 1 NM2016
JX195206 Peltigera dolichorhiza
KX897397 Peltigera truculenta
KX897354 Peltigera sp 5 NM2016
FJ527295 Peltigera dolichorhiza
AF350292 Peltigera neopolydactyla
KX897344 Peltigera sp 3 NM2016
KX897353 Peltigera sp 4 NM2016
FJ527296 Peltigera dolichorhiza
JX195207 Peltigera dolichorhiza
Peltigera dolichorhiza
Peltigera dolichorhiza 1
KX897187 Peltigera dolichorhiza
KX897300 Peltigera pulverulenta
KX897338 Peltigera sp 2 NM2016
KX897298 Peltigera pulverulenta
C
AY257959 Peltigera horizontalis
AY257961 Peltigera elisabethae
FJ527277 Peltigera cichoracea
AY257968 Peltigera phyllidiosa
JX195255 Peltigera sp
JX195251 Peltigera collina
JX195245 Peltigera collina
AY257963 Peltigera neckeri
LT852900 Peltigera neckeri
LT852901 Peltigera neckeri
FJ527275 Peltigera polydactyloides
Peltigera polydactyloides 1
Peltigera polydactyloides 4
Peltigera polydactyloides 5
Peltigera polydactyloides 8
Peltigera polydactyloides 9
Peltigera polydactyloides 11
Peltigera polydactyloides 12
Peltigera polydactyloides 3
Peltigera polydactyloides 7
AY257967 Peltigera polydactyloides
Peltigera polydactyloides 2
Peltigera polydactyloides 6
Peltigera polydactyloides 10
Habitats
Helichrysum heath
Disturbed Erica forest
Erica forest
Disturbed Podocarpus forest
Podocarpus forest
Disturbed Ocotea forest
Ocotea forest
Lower-montane forest
Substrata
○ Ground
△ Rock
Trunk
Canopy
Decaying wood
0.0 0.02

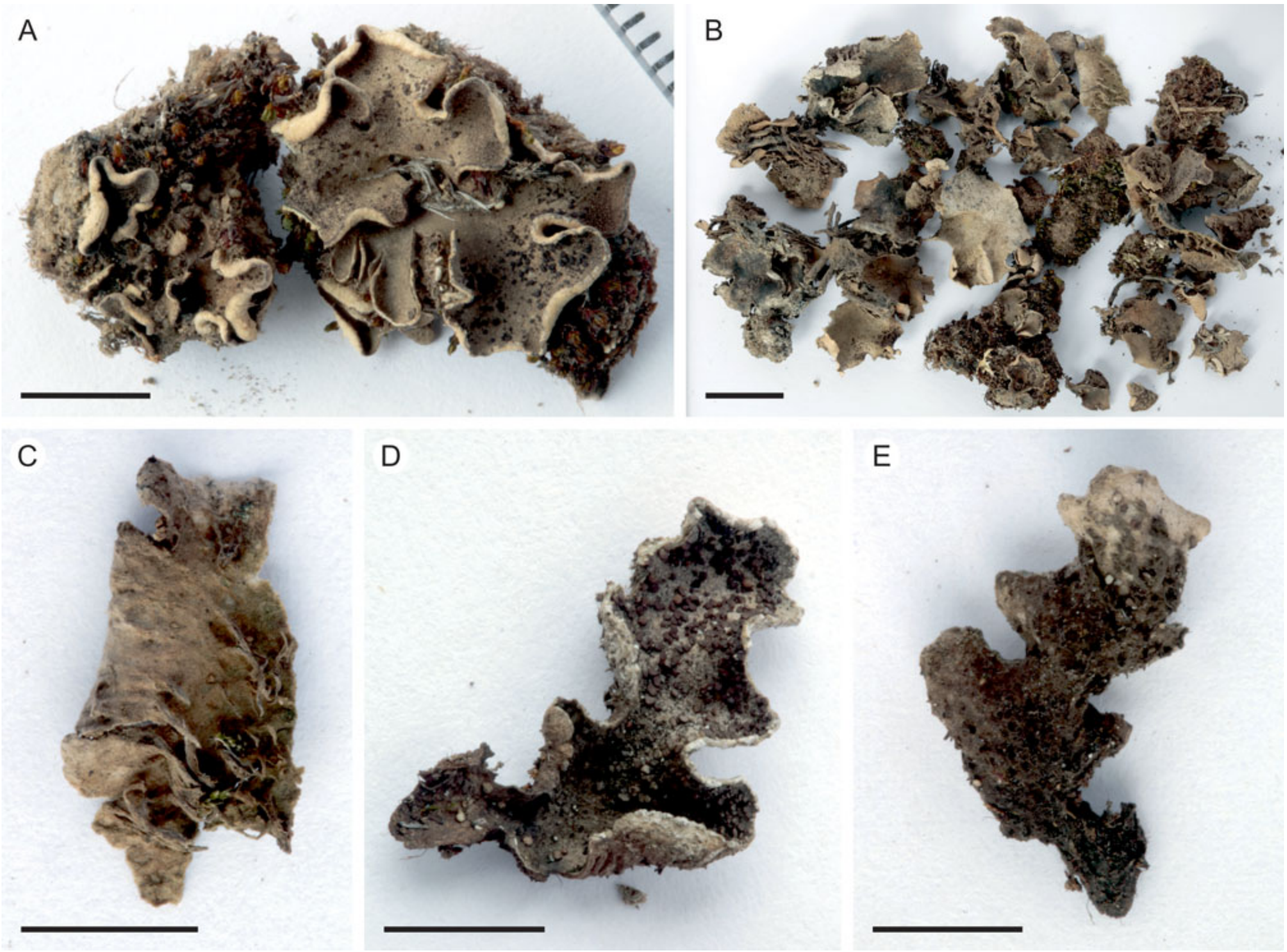


Fig. 2. *Peltigera alkalicola.* A, UK171254d (TYPE) from alpine *Helichrysum* heath, 4390 m alt. B, remainder of the same collection as the type specimen. C, lower surface and rhizines (specimen from the same collection as the type). D & E, UK171041b from disturbed *Erica* forest, 3940 m alt. Scales: A, C–E = 0.5 cm; B = 1 cm. In colour online.

darker than the thallus, 0.1–0.5 mm wide, evenly distributed on the upper lamina, rarely also present on the lower side. *Photobiont Nostoc.*

Apothecia and *pycnidia* not seen.

Secondary chemistry. No secondary compounds detected by TLC.

Etymology. The specific epithet refers to the substrate preference of the species.

Ecology. Peltigera alkalicola is rare on Mt Kilimanjaro. It grows in open, high-altitude habitats including disturbed, formerly burnt subalpine *Erica trimera* forest and alpine *Helichrysum* heath at 3940–4390 m alt. on trachybasaltic lava (Downie & Wilkinson 1972). The two collection locations have 900–1100 mm of annual precipitation, 60–80% relative humidity, and an annual mean temperature of 4 °C (Appelhans *et al.* 2016). *Peltigera alkalicola* grows on soil, occasionally with bryophytes.

Distribution. Based on data from the NCBI GenBank, *Peltigera alkalicola* is probably also present in Alaska, USA and Ningxia, China (Magain *et al.* 2018), extending the known distribution of *P. alkalicola* from Africa to Asia and North America. The extreme habitats and morphological similarity to *Peltigera lepidophora* may have hindered previous observations of the species and *P. alkalicola* may be more common than so far recognized. Based on the existing observations, the distribution is expected to be limited to cold and montane regions of the world. Additionally, the observed preference for alkaline substrata may further delimit the distribution and frequency of the species.

Notes. Of the two similar species, *Peltigera lepidophora* and *P. alkalicola*, the first seems to be more common,

Fig. 1. Bayesian 50% majority trees based on the ITS region of the lichen mycobionts of *Peltigera* sections *Peltigera* (A), *Polydactylon* (B) and *Horizontales* (C). Specimens from Mt Kilimanjaro are shown in bold. The different ITS variants are indicated by the number directly after the species name. The colours and shapes refer to the habitats and substrata, respectively, from which each species was collected (including information from specimens with no ITS obtained), hatched colours distinguish the disturbed and natural forest habitats; one square equals a species' presence in one sample plot. Thicker branches have a posterior probability value ≥ 0.95. Information about the sequences is available in Supplementary Material Table S1 (available online). The scale refers to nucleotide substitutions per site. In colour online.

having a distribution ranging from arctic-alpine to boreal and temperate regions. Martinez *et al.* (2003) reported *P. lepidophora* from every continent except Africa, and DNA-confirmed observations exist at least from Northern Europe, the United Kingdom and North America (Jüriado *et al.* 2017; Magain *et al.* 2018). The specimens from northern and southern Finland included in this study also represent *P. lepidophora* (Fig. 1A). *Peltigera alkalicola* and *P. lepidophora* also share a similar substratum ecology: *P. lepidophora* is reported to be terricolous on slightly calcareous (i.e. alkaline) substrata, somewhat nitrophilous, apophytic or preferring open habitats, and occasionally bryicolous (Vitikainen 1994; Jüriado *et al.* 2017); *P. alkalicola* specimens from Mt Kilimanjaro were terricolous in exposed habitats with only sparse vegetation, and occasionally growing on bryophytes. Additionally, the two study plots from which *P. alkalicola* was collected were the only sampled plots with presumably highly alkalic trachybasaltic lava as the substratum for the species.

Further specimen examined. **Tanzania:** Kilimanjaro Region, Kilimanjaro National Park, near Maua route, on soil with bryophytes in fire-disturbed *Erica trimera* forest at 3940 m alt., −3.126322°S, 37.434386°E, 2017, *U. Kaasalainen* UK171041b (H). GenBank Accession no.: MZ385613.

Peltigera dolichorhiza (Nyl.) Nyl.

Description. A detailed description of *P. dolichorhiza* in East Africa has been given by Swinscow & Krog (1988). On Mt Kilimanjaro, *P. dolichorhiza* can be identified based on its large (up to several dm), glabrous, relatively thin, and clearly foveate thallus with wide (up to 3.5 cm) lobes, brown, low true veins, and simple to confluent rhizines (Fig. 3A & B). It is without isidia and soredia but quite often has apothecia, especially in the *Podocarpus* forest zone.

Secondary chemistry. The main compounds detected by TLC from *Peltigera dolichorhiza* included dolichorrhizin, tenuiorin, peltidactylin, zeorin and methylgyrophorate (in the order of spot intensity). Additionally, gyrophoric acid has previously been reported from *P. dolichorhiza* in East Africa (Swinscow & Krog 1988).

Ecology. On Mt Kilimanjaro, *P. dolichorhiza* can be found from the lower montane forest zone to the subalpine *Erica* forest zone at 1920–3520 m alt., being especially common in the *Podocarpus* forest zone; mostly growing on the ground and on tree trunks, occasionally on rocks or decaying wood, often with bryophytes.

Peltigera polydactyloides Nyl.

Description. A detailed description of *P. polydactyloides* in East Africa has been provided by Swinscow & Krog (1988). On Mt Kilimanjaro, *P. polydactyloides* is morphologically quite variable but is distinguished by the thick brown layer of loosely woven hyphae on the lower side, the lack of true veins, and the always glabrous and often glossy upper surface; the thallus is usually thick and the lobes up to 3 cm wide (Fig. 3D & E). Occasionally a vein-like pattern can be seen on the lower surface, especially in closed forest habitats where the thallus is also often thinner and not as glossy (Fig. 3C). Particularly in the high-altitude open habitats, *P. polydactyloides* may grow as a more narrow-lobed (up to 0.5–1 cm wide), very thick, and dark brown form, often with gnawed-looking margins (Fig. 3F). This may resemble *Peltigera seneca* but is easily identified by the thicker thallus and the thick layer of hyphae and rhizines on the lower surface (Fig. 3G). *Peltigera polydactyloides* also occasionally has phyllidiate margins and/or apothecia.

Secondary chemistry. The main compounds detected by TLC from *Peltigera polydactyloides* included zeorin, tenuiorin, and one unidentified terpenoid. Additionally, methylgyrophorate and gyrophoric acid have previously been reported from *P. polydactyloides* in East Africa (Swinscow & Krog 1988).

Ecology. On Mt Kilimanjaro, *P. polydactyloides* is found from montane *Ocotea* forest to alpine *Helichrysum* heath at 2560–4190 m alt. Common especially in *Podocarpus* and *Erica* forest zones. On tree trunks and canopy branches in *Ocotea* and *Podocarpus* forest, and on soil, often with bryophytes, in *Erica* forest and *Helichrysum* heath.

Peltigera praetextata (Flörke ex Sommerf.) Zopf

Description. A detailed description of *P. praetextata* in East Africa has been given by Swinscow & Krog (1988). On Mt Kilimanjaro, *P. praetextata* can be identified based on the large thallus with wide (up to 4 cm), foveate, scarcely tomentose lobes and long, mainly simple rhizines (Fig. 4A & B). Occasionally with marginal phyllidia, rarely with apothecia.

Secondary chemistry. No secondary compounds detected by TLC.

Ecology. On Mt Kilimanjaro, *P. praetextata* can be found from montane *Ocotea* forest to subalpine *Erica* forest at 2750–3850 m alt., but only in undisturbed forest habitats. *Peltigera praetextata* is most common in *Erica* forest but even there it is not abundant. In *Ocotea* and *Podocarpus* forest, it grows on tree trunks and climbers, in *Erica* forest mainly on the ground and on decaying wood, often with bryophytes.

Peltigera rufescentiformis (Gyeln.) C. W. Dodge

Description. A detailed description of *P. rufescentiformis* in East Africa has been given by Swinscow & Krog (1988). On Mt Kilimanjaro, *P. rufescentiformis* can be identified based on the relatively narrow (up to 2 cm) and smooth lobes mostly covered by thick tomentum, often with a scabrous, flaky, or stripy pattern and dark, simple to confluent rhizines (Fig. 4C & D). Often with apothecia with discs round or wider than long.

Secondary chemistry. No secondary compounds detected by TLC.

Ecology. On Mt Kilimanjaro, *P. rufescentiformis* is found in the subalpine *Erica* forest zone and alpine *Helichrysum* heath at 3500–4190 m alt. Common and abundant, especially in the *Erica* forest zone. Mainly on soil with bryophytes, often in the shelter of rocks or shrubs, occasionally on tree trunks, decaying wood or rocks.

Peltigera seneca Magain, Miadl. & Sérus.

Description. A detailed description of *P. seneca* is provided by Magain *et al.* (2016); however, on Mt Kilimanjaro the species seems to grow slightly larger, with lobes often up to 1(–1.5)

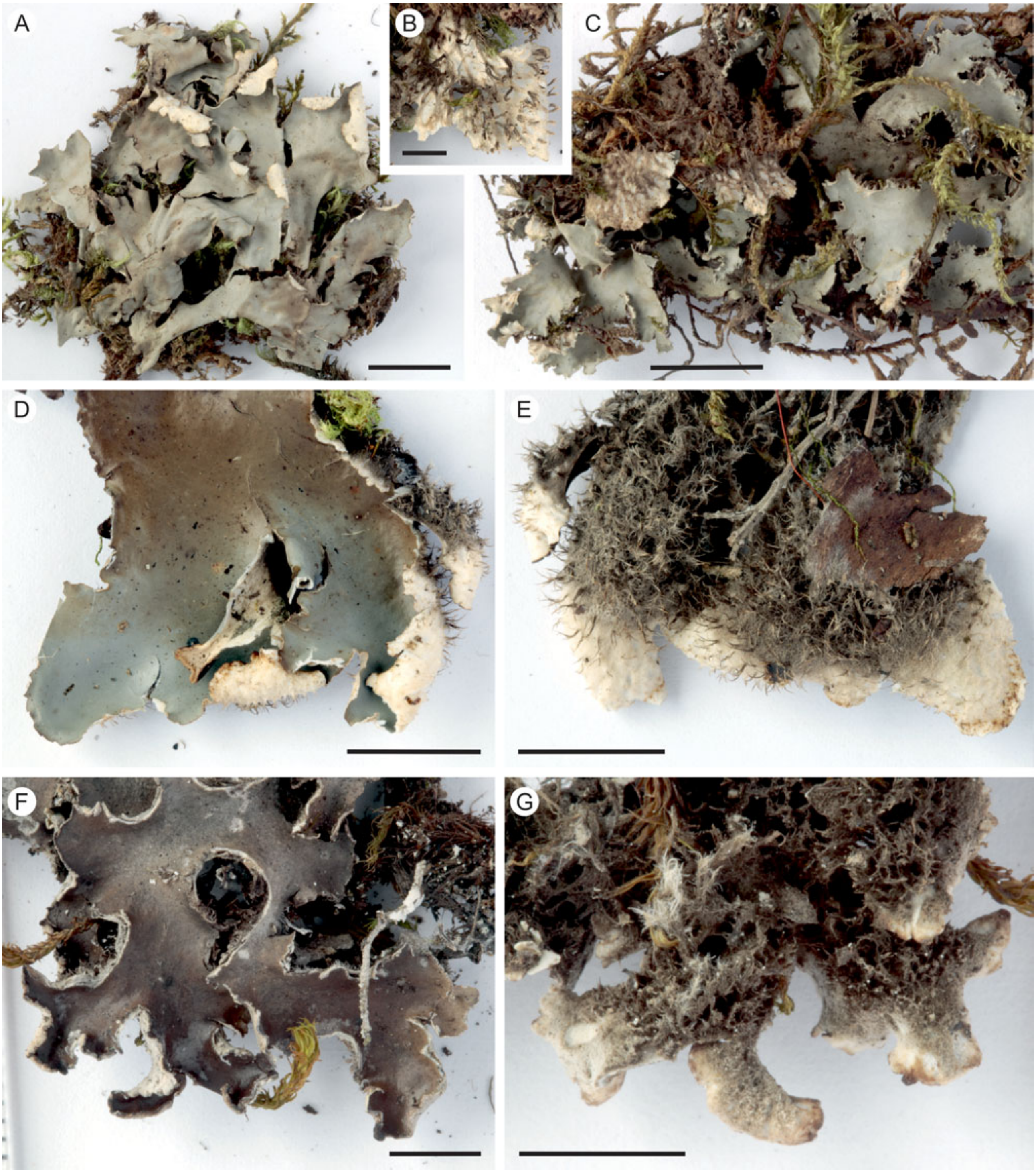


Fig. 3. A & B, *Peltigera dolichorhiza* (UK170942a) from the lower-montane forest, 1920 m alt. C–G, morphological variation in *Peltigera polydactyloides*. C, UK170906e from the closed middle montane *Ocotea* forest, 2560 m alt. D & E, thick and glossy UK171396a from *Erica* forest, 3500 m alt. F & G, brown, thick, and narrow-lobed UK171080a from the open high-altitude disturbed *Erica* forest, 3800 m alt. Scales: A, C–E = 1 cm; B, F & G = 0.5 cm. In colour online.

cm wide. *Peltigera seneca* can be distinguished from the other *Peltigera* species by the relatively small, glabrous, mostly smooth and often glossy thallus with narrow lobes, the dark, low veins and the dark, confluent to simple rhizines (Fig. 4E & F). Occasionally with marginal phyllidia and/or apothecia. Also *P. seneca* often has gnawed-looking margins similar to some *P. polydactyloides* specimens, but the lower surface lacks the thick layer of woolly tomentum and rhizines that identify *P. polydactyloides*.

Secondary chemistry. The main compounds detected by TLC from *Peltigera seneca* included dolichorrhizin, zeorin, tenuiorin, peltidactylin and methylgyrophorate, which is in accordance with what was described by Magain *et al.* (2016).

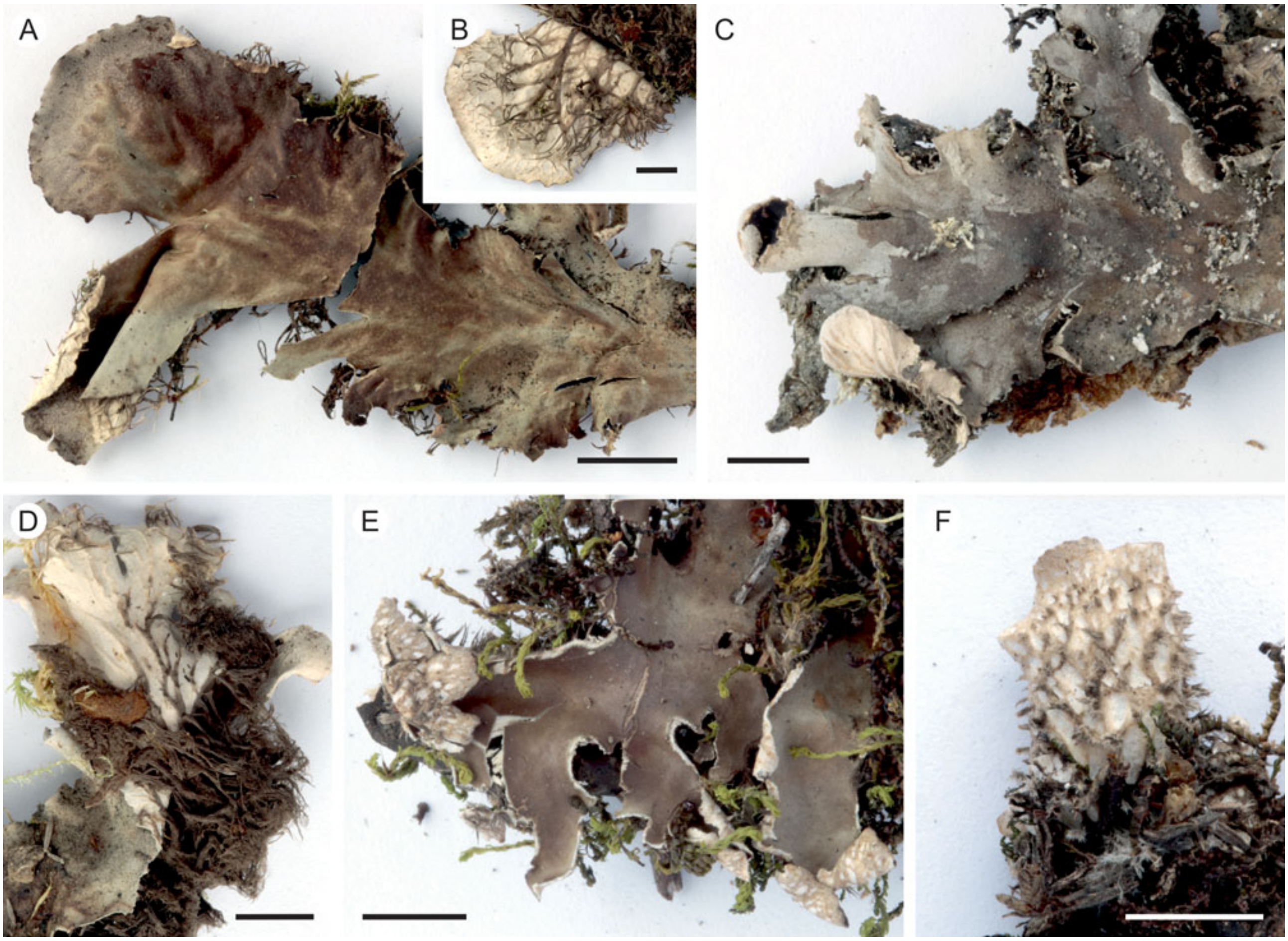


Fig. 4. *Peltigera* from the upper montane *Erica* forest. A & B, *Peltigera praetextata*, with wide lobes, scarce tomentum, and simple rhizines (UK171449e). C, *Peltigera rufescentiformis* with thickly tomentose upper surface and wide apothecia (UK171388b). D, *Peltigera rufescentiformis* with dark confluent rhizines (UK171449c). E & F, *Peltigera seneca* with narrow, glabrous lobes and confluent rhizines (UK171426a). Scales: A = 1 cm; B–F = 0.5 cm. In colour online.

Ecology. On Mt Kilimanjaro, *P. seneca* is found in subalpine *Erica* forest zone and alpine *Helichrysum* heath at 3500–4190 m alt., and it is common especially in the *Erica* forest zone. Growing mainly on the ground with bryophytes, often in the shelter of rocks or shrubs, occasionally on rocks or decaying wood.

Peltigera soredifera (Nyl.) Vitik.

Description. A detailed description of *P. soredifera* in East Africa is given by Goffinet & Hastings (1995) (as *Peltigera lambinonii*). On Mt Kilimanjaro, *P. soredifera* can be identified by the abundantly tomentose upper surface, the rounded to irregular, mainly laminal soralia often placed in thallus depressions, and the very abundant and richly branching rhizines (Fig. 5A & B). Apothecia are not seen. In more exposed or harsher conditions, *P. soredifera* may resemble *P. ulcerata* by having small, rounded lobes without visible tomentum. However, the placement of soralia more clearly on the lamina instead of just along the margin, as well as the richly branched rhizines, identify the species. The non-sorediate specimens with thick tomentum may occasionally resemble *P. rufescentiformis* but the light, richly branched rhizines and occasional furry-looking lobe margins distinguish *P. soredifera*.

Secondary chemistry. Methylgyrophorate was detected from the soralia of *Peltigera soredifera*. Additionally, traces of gyrophoric acid have been reported to be occasionally present in the soralia of specimens from East Africa (Goffinet & Hastings 1995).

Ecology. On Mt Kilimanjaro, *P. soredifera* is relatively common in the subalpine *Erica* forest and rare in the alpine *Helichrysum* heath, at 3520–4550 m alt. Growing mainly on soil or rocks with bryophytes, occasionally on decaying wood.

Peltigera ulcerata Müll. Arg.

Description. A detailed description of *Peltigera ulcerata* in East Africa has been given by Swinscow & Krog (1988). On Mt Kilimanjaro, *P. ulcerata* can be identified based on the small, glabrous thallus with oval to round, often slightly convex soralia usually situated close to the margin of the thallus, and simple to confluent rhizines (Fig. 5C & D; but also see comments below *P. soredifera*). Apothecia are not seen.

Secondary chemistry. No secondary compounds were detected by TLC from the thallus or soralia of the tested *Peltigera ulcerata*

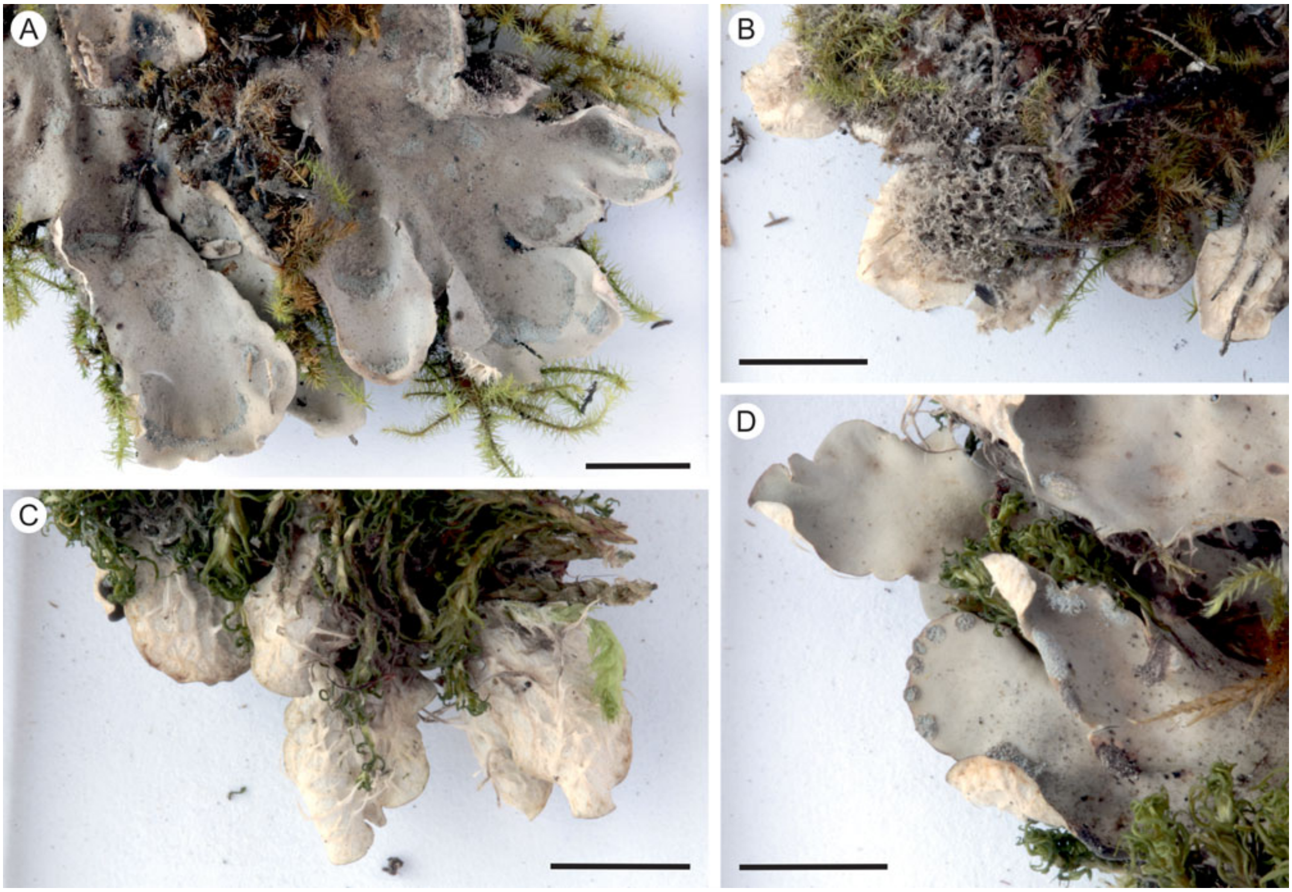


Fig. 5. A & B, a large *Peltigera sorediifera* (UK171341) with tomentose upper surface, laminal soralia, and abundant, richly branched rhizines; from disturbed *Erica* forest, 3520 m alt. C & D, *Peltigera ulcerata* (UK171519c) with glabrous upper surface, rounded soralia along the margin, and mainly simple rhizines; from *Ocotea* forest, 2750 m alt. Scales: A & B = 1 cm; C & D = 0.5 cm. In colour online.

specimen. However, methylgyrophorate and gyrophoric acid have previously been reported from the soralia of *P. ulcerata* in East Africa (Swinscow & Krog 1988).

Ecology. On Mt Kilimanjaro, *P. ulcerata* is found from lower-montane forest to subalpine *Erica* forest at 1920–3830 m alt., but only in undisturbed forest habitats. *Peltigera ulcerata* is not common in any of the studied habitats. It is epiphytic on tree trunks and canopy branches from the lower-montane to *Podocarpus* forest, and grows on the ground in *Erica* forest, occasionally with bryophytes.

Species not observed in this study

Peltigera species previously reported from East Africa but not detected in this study include *P. canina*, *P. cichoracea*, *P. didactyla*, *P. polydactylon* and *P. rufescens*. Of these, *P. canina* and *P. rufescens* have been reported as rare or uncommon in high montane habitats in East Africa but not from Tanzania (Swinscow & Krog 1988; Frisch & Hertel 1998). Whether these represent *P. canina* s. str. and *P. rufescens* s. str. or some more recently discovered morphologically similar taxa (Jüriado *et al.* 2017; Magain *et al.* 2018) remains to be seen.

Peltigera cichoracea is described from Ethiopia and additionally reported as rare in the montane forests of Kenya and Tanzania in East Africa (Jatta 1882; Swinscow & Krog 1988; Sérusiaux *et al.* 2009). Swinscow & Krog (1988) describe *P. cichoracea* as the 'sorediate counterpart of *P. polydactyloides*' and mention that 'after shedding of the propagules the lobe margins take on a gnawed appearance', a character separating it from morphologically quite similar *P. polydactyloides*. Some of our specimens from Mt Kilimanjaro were initially identified as *P. cichoracea* based on morphology and especially the gnawed appearance of the margins (e.g. Fig. 3F). However, in the phylogenetic analysis of the ITS region, all specimens fall within the same clade (Fig. 1C). *Peltigera polydactyloides* on Mt Kilimanjaro is both morphologically and genetically somewhat variable and further studies with additional genetic markers may reveal additional diversity, as has been the case in some other groups of *Peltigera* (Magain *et al.* 2017*b*). However, Sérusiaux *et al.* (2009) reported *P. cichoracea* from Papua New Guinea based on the description of Swinscow & Krog (1988) from East Africa and, according to the phylogenetic analyses, these specimens are not closely related to *P. polydactyloides* (Fig. 1C; Sérusiaux *et al.* 2009). In the same study, Sérusiaux *et al.* (2009) also reported that *P. cichoracea* is absent from the mountains of Rwanda (Africa) and that the type collection of the species could not be located and is presumably lost.

Peltigera didactyla s. lat. has previously been reported from Ethiopia, Kenya, Tanzania and Uganda in East Africa (Swinscow & Krog 1988; Frisch & Hertel 1998). However, many

Table 1. *Peltigera* species in East Africa. Included in 'Distribution' are records from the present study and also observations from other studies which are listed in 'References'. 'Ecology' refers to observations from this study on Mt Kilimanjaro. E = Ethiopia, K = Kenya, R = Rwanda, T = Tanzania, U = Uganda.

Species	Distribution	Ecology	Comments	References
Peltigera alkalicola sp. nov.	T**	Open high-montane habitats, 3940–4390 m alt., rare; on trachybasaltic lava	Fig. 2	
P. canina	E	Not observed in this study		Swinscow & Krog 1988
P. cichoracea	E, K, T	Not observed in this study	See 'Species not observed in this study' for discussion	Swinscow & Krog 1988
P. didactyla	?	Not observed in this study	Some previous reports refer to *P. sorediifera*. See 'Species not observed in this study' for discussion	Swinscow & Krog 1988; Goffinet & Hastings 1995; Frisch & Hertel 1998
P. dolichorhiza	K, R, T, U	Montane forest, 1920–3520 m alt., common; on soil, tree trunks	Fig. 3A & B	Swinscow & Krog 1988; Frisch & Hertel 1998; Alstrup & Christensen 2006; Kaasalainen *et al.* 2013; Magain *et al.* 2017*a*; Kaasalainen *et al.* 2021*b*
P. polydactylon	-	Not observed in this study	The previous observations probably represent *P. seneca*. See 'Species not observed in this study' for discussion	Swinscow & Krog 1988
P. polydactyloides	E, K, R, T, U	Montane forest and open habitats, 2560–4190 m alt., common; epiphytic, on soil	Fig. 3C–G	Swinscow & Krog 1988; Miadlikowska *et al.* 2003; Magain *et al.* 2017*a*; Kirika *et al.* 2018; Kaasalainen *et al.* 2021*b*
P. praetextata	E, K, T, U	Undisturbed montane forest, 2750–3850 m alt., not common; epiphytic, on soil, decaying wood	Fig. 4A & B	Swinscow & Krog 1988; Frisch & Hertel 1998; Krog 2000; Kaasalainen *et al.* 2021*b*
P. rufescens	E, K, U	Not observed in this study		Swinscow & Krog 1988; Frisch & Hertel 1998
P. rufescentiformis	E, K, T, U	High-montane forest and open habitats, 3500–4190 m alt., common; on soil	Fig. 4C & D	Swinscow & Krog 1988; Frisch & Hertel 1998; Magain *et al.* 2018
P. seneca	(K), T**	High-montane forest and open habitats, 3500–4190 m alt., common; on soil, rocks	Presence in Kenya is based on a previous observation of *P. polydactylon*. Fig. 4E & F	Swinscow & Krog 1988
P. sorediifera	(E, K), R, T*, U	High-montane forest and open habitats, 3520–4550 m alt., fairly common; on soil, rocks	Previously reported as *P. didactyla* and *P. lambinonii*; presence in Ethiopia and Kenya probable but remains to be confirmed. Fig. 5A & B	Swinscow & Krog 1988; Goffinet & Hastings 1995; Frisch & Hertel 1998; Miadlikowska *et al.* 2003
P. ulcerata	E, K, T, U	Undisturbed montane forest, 1920–3830 m alt., scattered; epiphytic, on soil, rocks	Fig. 5C & D	Swinscow & Krog 1988; Alstrup & Christensen 2006; Kirika *et al.* 2018

* New species for Tanzania

** New species for Tanzania and Africa

of these observations very probably represent *P. sorediifera* (Goffinet & Hastings 1995; Vitikainen 2008). Goffinet & Hastings (1995) reported that, in East Africa, *P. didactyla* and *P. sorediifera* grow sympatrically, listing collection locations for *P. sorediifera* from Rwanda, Uganda and Zaire. However, they do not provide more specific information on the specimens studied or the collection details for *P. didactyla* s. str. in East Africa, so the exact distribution of the species in the area remains to be confirmed.

Peltigera polydactylon has previously been reported from two upper montane locations in Kenya and Tanzania (Swinscow & Krog 1988). Since then, *Peltigera seneca* has been described as a separate species from *P. polydactylon* s. str. (Magain *et al.* 2016) and in our collections was a common species in the subalpine and alpine zones of Mt Kilimanjaro. *Peltigera seneca* and *P. polydactylon* s. str. can be distinguished chemically and genetically (Vitikainen 1994; Magain *et al.* 2016; Timdal & Rui 2021), and *P. polydactylon* reported by Swinscow & Krog (1988) from East Africa could represent either species. Since we found that *P. seneca* is a common species and did not detect *P. polydactylon* s. str., it is probable that previous reports of *P. polydactylon* from East Africa also represent *P. seneca*. Both species occur in North America and Northern Europe, but *P. seneca* has a much more restricted distribution in the temperate, hemiboreal and boreal zones (Magain *et al.* 2016; Timdal & Rui 2021).

Tropical Peltigera *diversity*

In conclusion, a total of eight species of *Peltigera* are confirmed to be present on Mt Kilimanjaro, Tanzania: *P. alkalicola*, *P. dolichorhiza*, *P. polydactyloides*, *P. praetextata*, *P. rufescentiformis*, *P. seneca*, *P. servdiifera* and *P. ulcerata*. Additionally, *P. cichoracea* has previously been reported from Tanzania but was not detected in this study. *Peltigera polydactylon* s. str. is probably not present in East Africa, and the distribution of *P. didactyla* s. str. remains uncertain. A summary of *Peltigera* in East Africa is provided in Table 1.

The recognized global diversity of *Peltigera* has increased significantly during the last few decades. New species and yet undescribed lineages have been discovered from most continents, and also many of the *Peltigera* species previously reported from East Africa have been divided into several species or shown to contain undescribed diversity (e.g. Miadlikowska & Lutzoni 2000; Goffinet *et al.* 2003; O'Brien *et al.* 2009; Sérusiaux *et al.* 2009; Han *et al.* 2013, 2015, 2018, 2019; Magain *et al.* 2016, 2017*a*, 2018; Manoharan-Basil *et al.* 2016; Jüriado *et al.* 2017; Pardo-De la Hoz *et al.* 2018). *Peltigera* sections *Peltigera* and *Polydactylon*, for example, are both globally very diverse, with tens of yet undescribed species in South, Central and North America, Asia, Australasia and Europe (Jüriado *et al.* 2017; Magain *et al.* 2017*a*, 2018). In the wide range of habitats sampled on Mt Kilimanjaro, however, only seven species from these sections were present and these were mostly well-known and established species. The biogeographical affinities of the *Peltigera* species on Mt Kilimanjaro are diverse and include: 1) widely distributed species from both hemispheres (*P. praetextata* and *P. ulcerata*); 2) disjunct species shared, for example, with South and Central America (*P. dolichorhiza*) and Australia (*P. sorediifera*); 3) African endemics (*P. polydactyloides* and *P. rufescentiformis*); 4) species from warmer (*P. dolichorhiza*) and colder (*P. alkalicola* and *P. seneca*) regions of the world. Even though eight *Peltigera* species appears low for a tropical biodiversity hotspot, the species number resembles observations from another supposed paleotropical hotspot of lichen diversity, Papua New Guinea, where likewise relatively few *Peltigera* species were detected (Sérusiaux *et al.* 2009). Possible reasons why our sampling from Mt Kilimanjaro could underestimate the species diversity of *Peltigera* in East Africa include habitat destruction and the geologically relatively young age of Mt Kilimanjaro. The extensive destruction of the extremely diverse lower-montane forest environments has impacted the observed diversity of vascular plants here (Hemp 2006*b*). The lack of natural lower altitude habitats could also explain the difference in species composition of another Peltigeralean lichen genus, *Leptogium*, between Mt Kilimanjaro and the nearby, much older mountains (Kaasalainen *et al.* 2021*a*). However, unlike *Peltigera*, *Leptogium* is very diverse in most montane habitats in the area, but especially speciose in the lower-montane forests (Kaasalainen *et al.* 2021*a*). Since East African *Peltigera* is concentrated in higher altitude habitats, the scarcity of lower-montane forest and woodlands on Mt Kilimanjaro is an unlikely cause of the observed low diversity. Additionally, in the lower-montane forest and woodland habitats of the nearby, ancient Taita Hills and Mt Kasigau, the same lower-elevation species (*P. dolichorhiza*, *P. polydactyloides*, *P. praetextata* and *P. ulcerata*) exclusively occur (U. Kaasalainen, personal observation).

Key to *Peltigera* species on Mt Kilimanjaro

1 Laminal peltate isidia present (rare, above *c.* 3500 m alt.) . . . . **P. alkalicola** sp. nov.
Laminal peltate isidia absent 2

2(1) Soralia present on the upper surface 3
Soralia absent 4

3(2) Upper surface tomentose; rhizines abundantly branched; soralia usually not restricted to the margins (above *c.* 3500 m alt.) . . . . **P. sorediifera**
Upper surface glabrous; rhizines confluent to simple; soralia restricted to the margins . . . . **P. ulcerata**

4(2) Upper side entirely glabrous 5
Upper side tomentose (occasionally just submarginally) 7

5(4) Lower surface covered by a thick brown layer of loosely woven hyphae; true veins absent . . . . **P. polydactyloides**
Lower side without loosely woven hyphae; with a network of distinct dark veins 6

6(5) Thallus small; lobes narrow (up to 1.5 cm wide), often thick and smooth (not foveate); margins may be phyllidiate (above *c.* 3500 m alt.) . . . . **P. seneca**
Thallus usually large; lobes wide (up to 3.5 cm wide), distinctly foveate; margins usually without phyllidia/lobules . . . . **P. dolichorhiza**

7(4) Lobes wide (up to 4 cm), often clearly foveate; tomentum on the upper surface often sparse and restricted to areas close to the margins . . . . **P. praetextata**
Lobes narrow (up to 2 cm), not clearly foveate; tomentum on the upper surface thick, usually covering large portions of the thallus, tomentum often stripey, flaky or scabrous-looking (above *c.* 3500 m alt.) . . . . **P. rufescentiformis**

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Author Contribution. UK: conceptualization, methodology, formal analysis, investigation, data curation, writing (original draft, review and editing), visualization, supervision, project administration, funding acquisition. LB: investigation, data curation. NPM: writing (review and editing). ARS: writing (review and editing), supervision. AH: methodology, writing (review and editing).

Author ORCIDs. Ulla Kaasalainen, 0000-0001-9899-4768; Neduvoto P. Mollel, 0000-0002-4402-4667; Alexander R. Schmidt, 0000-0001-5426-4667; Andreas Hemp, 0000-0002-5369-2122.

Competing Interests. The authors declare none.

Data Accessibility. The sequences used in this study were submitted to the NCBI GenBank database under Accession numbers MZ385613–MZ385696.

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References

Alstrup V and Christensen SN (2006) New records of lichens with cyanobacteria from Tanzania and Kenya. *Cryptogamie, Mycologie* **27**, 59–68.

Appelhans T, Mwangomo E, Otte I, Detsch F, Nauss T and Hemp A (2016) Eco-meteorological characteristics of the southern slopes of Kilimanjaro, Tanzania. *International Journal of Climatology* **36**, 3245–3258.

Burgess ND, Butynski TM, Cordeiro NJ, Doggart NH, Fjeldså J, Howell KM, Kilahama FB, Loader SP, Lovett JC, Mbilinyi B, *et al.* (2007) The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biological Conservation* **134**, 209–231.

Downie C and Wilkinson P (1972) *The Geology of Kilimanjaro*. Sheffield: University of Sheffield.

Frisch A and Hertel H (1998) Flora of macrolichens in the alpine and subalpine zones of Mount Kenya (Kenya). *Sauteria* **9**, 363–370.

Goffinet B and Hastings RI (1995) Two new sorediate taxa of *Peltigera*. *Lichenologist* **27**, 43–58.

Goffinet B, Miadlikowska J and Goward T (2003) Phylogenetic inferences based on nrDNA sequences support five morphospecies within the *Peltigera didactyla* complex (lichenized *Ascomycota*). *Bryologist* **106**, 349–364.

Han L-F, Zhang Y-Y and Guo S-Y (2013) *Peltigera wulingensis*, a new lichen (*Ascomycota*) from north China. *Lichenologist* **45**, 329–336.

Han L-F, Zhang T-X and Guo S-Y (2015) A new species in the lichen genus *Peltigera* from northern China based on morphology and DNA sequence data. *Bryologist* **118**, 46–53.

Han L-F, Xu X-M, Yang J-Y and Guo S-Y (2018) *Peltigera neodegenii* sp. nov. from Central China. *Mycotaxon* **133**, 323–332.

Han L-F, Yang J-Y, Bei S-Q and Guo S-Y (2019) *Peltigera shennongjiana*, a new cyanolichen from Central China. *Lichenologist* **51**, 561–574.

Harper EB, Measey J, Patrick DA, Menegon M and Vonesh JR (2010) *Field Guide to the Amphibians of the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya*. Nairobi: Camerapix Publishers International.

Hemp A (2005) Climate change-driven forest fires marginalize the impact of ice cap wasting on Kilimanjaro. *Global Change Biology* **11**, 1013–1023.

Hemp A (2006*a*) Continuum or zonation? Altitudinal gradients in the forest vegetation of Mt. Kilimanjaro. *Plant Ecology* **184**, 27–42.

Hemp A (2006*b*) Vegetation of Kilimanjaro: hidden endemics and missing bamboo. *African Journal of Ecology* **44**, 305–328.

Hemp A and Hemp C (2018) Broken bridges: the isolation of Kilimanjaro's ecosystem. *Global Change Biology* **24**, 3499–3507.

Jatta A (1882) Licheni africani raccolti nello Scioà dal Marchese Antinori, illustrati da A. Jatta. *Nuovo Giornale Botanico Italiano* **14**, 169–175.

Jüriado I, Kaasalainen U and Rikkinen J (2017) Specialist taxa restricted to threatened habitats contribute significantly to the regional diversity of *Peltigera* (*Lecanoromycetes*, *Ascomycota*) in Estonia. *Fungal Ecology* **30**, 76–87.

Kaasalainen U, Fewer DP, Jokela J, Wahlsten M, Sivonen K and Rikkinen J (2013) Lichen species identity and diversity of cyanobacterial toxins in symbiosis. *New Phytologist* **198**, 647–651.

Kaasalainen U, Tuovinen V, Kirika PM, Mollel NP, Hemp A and Rikkinen J (2021*a*) Diversity of *Leptogium* (*Collemataceae*, *Ascomycota*) in East African montane ecosystems. *Microorganisms* **9**, 314.

Kaasalainen U, Tuovinen V, Mwachala G, Pellikka P and Rikkinen J (2021*b*) Complex interaction networks among cyanolichens of a tropical biodiversity hotspot. *Frontiers in Microbiology* **12**, 672333.

Kantelinen A, Hyvärinen M-T, Kirika PM and Myllys L (2021) Four new *Micarea* species from the montane cloud forests of Taita Hills, Kenya. *Lichenologist* **53**, 81–94.

Kirika PM, Ndiritu GG, Mugambi GK, Newton LE and Lumbsch HT (2018) Diversity and altitudinal distribution of understorey corticolous lichens in a tropical montane forest in Kenya (East Africa). *Cryptogam Biodiversity and Assessment* **2018**, 47–70.

Krog H (2000) Corticolous macrolichens of low montane rainforests and moist woodlands of eastern Tanzania. *Sommerfeltia* **28**, 1–75.

Lambrechts C, Woodley B, Hemp A, Hemp C and Nnyiti P (2002) *Aerial Survey of the Threats to Mt. Kilimanjaro Forests*. Dar es Salaam: UNDP.

Magain N, Sérusiaux E, Zhurbenko MP, Lutzoni F and Miadlikowska J (2016) Disentangling the *Peltigera polydactylon* species complex by recognizing two new taxa, *P. polydactylon* subsp. *udeghe* and *P. seneca*. *Herzogia* **29**, 514–528.

Magain N, Miadlikowska J, Goffinet B, Sérusiaux E and Lutzoni F (2017*a*) Macroevolution of specificity in cyanolichens of the genus *Peltigera* section *Polydactylon* (*Lecanoromycetes*, *Ascomycota*). *Systematic Biology* **66**, 74–99.

Magain N, Miadlikowska J, Mueller O, Gajdeczka M, Truong C, Salamov AA, Dubchak I, Grigoriev IV, Goffinet B, Sérusiaux E, *et al.* (2017*b*) Conserved genomic collinearity as a source of broadly applicable, fast evolving, markers to resolve species complexes: a case study using the lichen-forming genus *Peltigera* section *Polydactylon*. *Molecular Phylogenetics and Evolution* **117**, 10–29.

Magain N, Truong C, Goward T, Niu D, Goffinet B, Sérusiaux E, Vitikainen O, Lutzoni F and Miadlikowska J (2018) Species delimitation at a global scale reveals high species richness with complex biogeography and patterns of symbiont association in *Peltigera* section *Peltigera* (lichenized *Ascomycota*: *Lecanoromycetes*). *Taxon* **67**, 836–870.

Manoharan-Basil SS, Miadlikowska J, Goward T, Andrésson ÓS and Miao VPW (2016) *Peltigera islandica*, a new cyanolichen species in section *Peltigera* ('*P. canina* group'). *Lichenologist* **48**, 451–467.

Martínez I, Burgaz AR, Vitikainen O and Escudero A (2003) Distribution patterns in the genus *Peltigera* Willd. *Lichenologist* **35**, 301–323.

Miadlikowska J and Lutzoni F (2000) Phylogenetic revision of the genus *Peltigera* (lichen-forming *Ascomycota*) based on morphological, chemical, and large subunit nuclear ribosomal DNA data. *International Journal of Plant Sciences* **161**, 925–958.

Miadlikowska J, Lutzoni F, Goward T, Zoller S and Posada D (2003) New approach to an old problem: incorporating signal from gap-rich regions of ITS and rDNA large subunit into phylogenetic analyses to resolve the *Peltigera canina* species complex. *Mycologia* **95**, 1181–1203.

Miller MA, Pfeiffer W and Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, Louisiana*, pp. 1–8.

Mittermeier RA, Robles GP, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J and da Fonseca GAB (2004) *Hotspots Revisited: Earth's Biologically Richest and Most Endangered Ecoregions*. Mexico City: CEMEX.

Mittermeier RA, Turner WR, Larsen FW, Brooks TM and Gascon C (2011) Global biodiversity conservation: the critical role of hotspots. In Zachos F and Habel J (eds), *Biodiversity Hotspots*. Berlin, Heidelberg: Springer, pp. 3–22.

Müller K, Müller J and Quandt D (2010) *PhyDE. Phylogenetic Data Editor*. [WWW resource] URL www.phyde.de

Nonnotte P, Guillou H, Le Gall B, Benoit M, Cotten J and Scaillet S (2008) New K–Ar age determinations of Kilimanjaro volcano in the North

Tanzanian diverging rift, East Africa. *Journal of Volcanology and Geothermal Research* **173**, 99–112.

O'Brien HE, Miadlikowska J and Lutzoni F (2009) Assessing reproductive isolation in highly diverse communities of the lichen-forming fungal genus *Peltigera*. *Evolution* **63**, 2076–2086.

Olsson S, Kaasalainen U and Rikkinen J (2012) Reconstruction of structural evolution in the trnL intron P6b loop of symbiotic *Nostoc* (*Cyanobacteria*). *Current Genetics* **58**, 49–58.

Orange A, James PW and White FJ (2010) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Pardo-De la Hoz CJ, Magain N, Lutzoni F, Goward T, Restrepo S and Miadlikowska J (2018) Contrasting symbiotic patterns in two closely related lineages of trimembered lichens of the genus *Peltigera*. *Frontiers in Microbiology* **9**, 2770.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256.

Rambaut A, Drummond AJ, Xie D, Baele G and Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**, 901–904.

Ronquist F and Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Rutten G, Ensslin A, Hemp A and Fischer M (2015) Vertical and horizontal vegetation structure across natural and modified habitat types at Mount Kilimanjaro. *PLoS ONE* **10**, e0138822.

Sérusiaux E, Goffinet B, Miadlikowska J and Vitikainen O (2009) Taxonomy, phylogeny and biogeography of the lichen genus *Peltigera* in Papua New Guinea. *Fungal Diversity* **38**, 185–224.

Stöver BC and Müller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**, 7.

Suija A, Kaasalainen U, Kirika PM and Rikkinen J (2018) *Taitaia*, a novel lichenicolous fungus in tropical montane forests in Kenya (East Africa). *Lichenologist* **50**, 173–184.

Swinscow TDV and Krog H (1988) *Macrolichens of East Africa*. London: Natural History Museum Publications.

Timdal E and Rui S (2021) *Peltigera seneca* new to Europe. *Graphis Scripta* **35**, 79–85.

Vitikainen O (1994) Taxonomic revision of *Peltigera* (lichenized *Ascomycotina*) in Europe. *Acta Botanica Fennica* **152**, 1–96.

Vitikainen O (2008) *Peltigera sorediifera* supersedes *P. lambinonii*. *Graphis Scripta* **20**, 56–57.

White TJ, Bruns T, Lee S and Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. New York: Academic Press, pp. 315–322.

Zúñiga C, Leiva D, Ramírez-Fernández L, Carú M, Yahr R and Orlando J (2015) Phylogenetic diversity of *Peltigera* cyanolichens and their photobionts in southern Chile and Antarctica. *Microbes and Environments* **30**, 172–179.