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Standard Paper

Molecular, morphological and chemical variation of the *Usnea pectinata* aggregate from Tanzania, São Tomé and Príncipe

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Abstract

This study investigated the molecular, chemical and morphological variation in the *Usnea pectinata* aggregate using 42 specimens, 22 from Tanzania and 20 from São Tomé and Príncipe. A total of 31 sequences (13 ITS, 13 nuLSU and 5 *RPB*1) were generated. The results are presented in two phylogenies: first a three-markers 'backbone' phylogeny for the *U. pectinata* aggregate, where six distinct, strongly supported subclades indicate considerable genetic variation in the dataset; and second, an ITS phylogeny with 47 terminals along with a mapping of morphological and chemistry data. Several well-supported monophyletic clades were recovered in both phylogenies and these may well represent separate species in the complex referred to here as the *U. pectinata* aggregate. Three morphotypes characterized by axis pigmentation and four by branch shape were noted. Six chemotypes were observed.

Key words: chemistry, lichen, molecular phylogeny, *Parmeliaceae*, taxonomy

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Introduction

Usnea Adans. is the second largest genus in *Parmeliaceae*, and is considered to comprise *c.* 350 taxa (Thell *et al.* 2012; Lücking *et al.* 2017). *Usnea* species are commonly known by the name 'beard lichens', referring to the appearance of the thallus. Characteristic features of *Usnea* are a fruticose thallus with radially symmetrical branches having an elastic central axis consisting of a cartilaginous strand of longitudinally orientated hyphae, and the presence of usnic acid in the cortex (Wirtz *et al.* 2006). The greatest challenge in this genus is the morphological plasticity of its members, which causes difficulties in species recognition (Clerc 1998). Whereas morphological characters and secondary metabolites have previously been used to characterize *Usnea* species (Swinscow & Krog 1975, 1988; Clerc 1998; Ohmura 2001), species recognition using molecular data has been useful, for example, in the *Usnea cornuta* aggregate (Gerlach *et al.* 2019).

Usnea pectinata Taylor is a species in subgenus *Eumitria* and is characterized by having a pendent thallus with elongated terminal branches, a dark brown base, punctiform maculae on lateral branches, a non-pigmented central axis and the presence of stictic acid as a major substance (Ohmura 2001). The inclusion of *U. pectinata* in *Eumitria* is mainly based on molecular support from the work of Ohmura (2002) and later works have also supported this (Articus 2004; Nadel 2016; Temu *et al.* 2019). Stictic acid, however, was not reported in the recent work of the *U. pectinata* aggregate from Tanzania (Temu *et al.* 2019). In morphology and chemistry, African specimens of the aggregate show a strong resemblance to samples named *Usnea mexicana* Vain. (Herrera-Campos *et al.* 1998). Interestingly, in an unpublished work by Velasquez (2012), *Usnea mexicana* is shown to be closely related to *U. pectinata* with two sequences of *U. pectinata* from Ohmura (2002) included in a group forming the sister clade of *U. baileyi* (Stirt.) Zahlbr. Species such as *U. chloreoides* Motyka, *U. duriuscula* Motyka, *U. gigas* Motyka and *U. himantodes* Stirt. are putative synonyms of *U. mexicana* (Swinscow & Krog 1988; Herrera-Campos *et al.* 1998; Ohmura 2001; Truong *et al.* 2013*b*; Nadel 2016; Lücking *et al.* 2020). Furthermore, species such as *U. africana* Motyka, *U. amaniensis* Dodge, *U. bakongoensis* P. A. Duvign., *U. fernandiae* P. A. Duvign., *U. gigas* and *U. savanarum* P. A. Duvign. are suspected to be closely related to *U. mexicana* (P. Clerc, unpublished data). The chemotypes of these species are similar to the chemotypes reported in the *U. pectinata* aggregate from Tanzania (Temu *et al.* 2019), as listed in Table 1. In Africa, the stictic acid chemotype of *U. pectinata* is currently known only from Madagascar, as represented by the type specimens of *U. contorta* Jatta, *U. eburnea* Motyka and *U. indigena* Motyka (P. Clerc, unpublished data).

Here, we characterize the *U. pectinata* aggregate as having a pendulous thallus, distinct main branches with ±short lateral, perpendicular branches with or without small rounded soralia, a thin and glossy cortex, and a thick and compact medulla with a brittle, mostly brown-pigmented and thick central axis (> 50% of the branch thickness), which in part may be fistulose. It has a quite diverse chemistry (Table 1).

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Table 1. A list of some of the species and their chemotypes putatively belonging to the *U. pectinata* aggregate (Swinscow & Krog 1978; Herrera-Campos *et al*. 1998; P. Clerc, unpublished data).

	Constictic acid	Diffractaic acid	Protocetraric acid	Salazinic acid
U. africana	X			
U. amaniensis			X	
U. chloreoides		X		X
U. gigas	X	X	X	X
U. mexicana	X	X	X	X
U. trichodeoides	X		X	X

The high number of species described in this group is caused by the high morphological and chemical variability and suggests the existence of many poorly understood and closely related species collectively treated here as the *U. pectinata* aggregate, with *U. pectinata* as the oldest available name.

Molecular studies in the *U. pectinata* aggregate are few. Until recently, *U. pectinata* s. lat. had been included in only a small number of molecular investigations (Ohmura 2002; Articus 2004; Truong *et al*. 2013*a*; Nadel 2016; Temu *et al*. 2019; Lücking *et al*. 2020). However, these studies have paid greater attention to the genetic variation rather than morphological and chemical variability. In a recent molecular contribution on African material of the *U. pectinata* aggregate (under '*U. pectinata*'), a varied chemistry and morphology was revealed (Temu *et al*. 2019). African specimens of the *U. pectinata* aggregate are highly variable in secondary chemistry: some have protocetraric acid while others have constictic and diffractaic acids or constictic and salazinic acids as main substances (Temu *et al*. 2019; Table 1). Secondary chemistry is considered an important feature in species recognition, in particular when used in combination with other characters (Clerc 1998). In studies of the *Usnea cornuta* aggregate, the chemistry was found to correlate well with molecular data (Gerlach *et al*. 2019). However, in *Usnea*, the occurrence of several chemotypes in a species has often been reported (Swinscow & Krog 1979; Stevens 1990, 2004; Clerc 2007; Truong *et al*. 2011, 2013*b*; Herrera-Campos 2016).

In this study, we aimed to assess the molecular, morphological and secondary chemistry diversity among specimens of the *U. pectinata* aggregate, primarily from Tanzania and São Tomé and Príncipe. We try to answer the following: 1) do we have one very polymorphic taxon (a hypothesis originally developed primarily by Swinscow & Krog (1978)) or 2) do we have several small strongly supported genetic clades that correlate with morphology and secondary chemistry (see e.g. the *Usnea cornuta* aggregate (Gerlach *et al*. 2019))?

Material and Methods

Sampling

This study was based mainly on material collected by the authors (SGT, LT and ST) in the West Usambara Mountains, north-eastern Tanzania, mostly in the Korogwe District of the Tanga Region. The forests are subject to frequent cloud mists and precipitation due to the high elevation and winds carrying humid air from the Indian Ocean. These forests are rich in rare and endemic species of plants and animals (Myers *et al*. 2000).

Voucher specimens collected during field trips have been deposited in UPS (SGT, LT and ST), with some duplicates in DSM and G.

Specimens examined. **Tanzania:** Tanga, Usambara Mountains, *c*. 16.4 km NEW of Korogwe, 5°03′32.81″S, 38°23′19.59″E, 1152 m, 2016 (SGT01); 5°03′33.04″S, 38°23′19.01″E, on twigs of *Drypetes usambarica, Isoberlinia schefleri* and *Maesopsis eminii*, 1153 m (SGT05, SGT06, SGT07, SGT08, SGT09, SGT10); 5°04′24.42″S, 38°24′24.37″E (SGT24, SGT25, SGT26, SGT42, SGT44); 5°04′15″S, 38°24′02″E, on twigs of *Drypetes usambarica*, *Alablankia stomanii* and *Ficus soningiae*, 1227 m, 2017 (SGT86, SGT87, SGT88); 5°04′15″S, 38°24′02″E, 1307 m (SGT106, SGT107, SGT109, SGT114, SGT115, SGT116, SGT117 (Temu *et al*. 2019)).

DNA samples from specimens collected on the islands of São Tomé and Príncipe (Nadel 2016; samples deposited in CAS) were also used for further study. **São Tomé:** MN0060, MN0063, MN0065, MN0068, MN0070b, MN0125, MN0163, MN0241, MN0556, MN0567, MN0575a, MN0578a, MN0583, MN0585, MN0597, MN0602. **Príncipe:** MN0481, MN0527, MN0540, MN0542 (Nadel 2016).

Morphological and chemical analyses

For each specimen, pigmentation of the axis and branch shape were recorded as observed on longitudinal sections of branches at ×10 magnification.

Chemical analyses of all the studied specimens were performed by thin-layer chromatography (TLC) in solvents A, B and C following Culberson & Ammann (1979), with solvent B modified according to Culberson & Johnson (1982). For each chemotype, main substances and accessory substances were recorded, with chemical substances present in high concentrations considered as 'main substances' while those present only in trace amounts as 'accessory substances'.

Molecular analyses: DNA extraction, amplification and sequencing

Total DNA was extracted from freshly collected material within one month after storage at −20 °C, using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The material for extraction was selected carefully, with a part of the main branch, *c*. 1 cm in length, being used.

Total DNA was used for PCR amplifications of the entire ITS regions (internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2), the nuclear large subunit ribosomal RNA gene (nuLSU), and the protein-coding RNA polymerase II largest subunit (*RPB*1). The primers used were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990); LROR and LR5 (Vilgalys & Hester 1990); gRPB1-A and gRPB1-C

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(Matheny *et al.* 2002). Amplifications were carried out using the AccuPower PCR PreMix (Bioneer, Daejeon, Korea), with the reaction mixture consisting of 3 µl diluted DNA, forward and reverse primers of each marker (10 mM), and water to a total volume of 20 µl. The thermal cycling parameters were: initial denaturation for 4 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 54 °C, 45 s at 72 °C, and a final elongation for 5 min at 72 °C. The PCR products were visualized by electrophoresis on 1.5% agarose gels. Products were purified using the Illustra™ ExoStar buffer diluted 10×, following the manufacturer's protocol. Sequencing was carried out by Macrogen (www.macrogen.com).

Alignment and phylogenetic analyses

The alignments contained all DNA sequences of ITS, nuLSU and *RPB*1 of *Usnea pectinata* available at GenBank NCBI: txid2789761 (with one exception: MW267160.1, last accessed on 27 February 2022), together with newly produced sequences from Tanzania and four newly produced nuLSU from DNA supplied by M. Nadel (Table 2). Sequences of *Usnea baileyi*, a species in *Eumitria* close to *U. pectinata* (Temu *et al.* 2019), were chosen as outgroup for the phylogenetic analyses. The sequences were assembled and edited using AliView (Larsson 2014) and aligned with MAFFT v. 7 (https//mafft.cbrc.jp/alignment/server/).

In this study two datasets were analyzed. The first consisted of a concatenated matrix of three markers (ITS, nuLSU and *RPB*1) whereas the second consisted of ITS sequences only. Phylogenetic relationships were inferred using a Bayesian approach, and additional support values were estimated using maximum likelihood bootstrap support (ML). A conflict among single-locus datasets was considered significant if a well-supported monophyletic group (posterior probability (PP) ≥ 0.95) was found to be non-monophyletic with good support when different loci were used.

For the Bayesian analyses, the most likely models of evolution were estimated using the Akaike Information Criterion (AIC) as implemented in MODELTEST v. 3.7 (Posada & Crandall 1998). The models employed for the first dataset were HKY + I for ITS and nuLSU, and HKY for the *RPB*1, whereas for the second dataset the GTR + G model was used. The Bayesian analysis was performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012), where two analyses of two parallel runs were carried out for 10 M generations. Each run included four chains and trees were sampled every 1000 generations, with 25% discarded as burn-in. All runs converged on the same average likelihood score and topology. Further analyses for a three-marker phylogeny were performed after concatenation using Sequence Matrix (Vaidya *et al.* 2011).

Additional support for each dataset was obtained using maximum likelihood estimates from RAxML v. 8.2.10, with the GTR + G model of site substitution (Stamatakis 2014). The branch support was acquired by maximum likelihood bootstrapping of 1000 replicates (Hillis & Bull 1993), and ML ≥ 70% were considered to be significant.

The trees were visualized in FigTree v. 1.3.1 (Rambaut & Drummond 2010) and edited using Adobe Illustrator CC 2017.0.2.

Results and Discussion

We produced 31 new sequences (13 ITS, 13 nuLSU and 5 *RPB*1; Table 2). The concatenated dataset comprised 24 terminals, with 19 representatives from Tanzania, four from São Tomé and Príncipe, and one from Indonesia; two *Usnea baileyi* sequences served as outgroup. Analyses were performed on each marker separately: 25 sequences for ITS, 24 sequences for nuLSU and 12 sequences for *RPB*1. No significant incongruence among single marker trees was detected, and so the three matrices were concatenated. In total, there were 1932 nucleotide positions (483 bp for ITS, 807 bp for nuLSU and 642 bp for *RPB*1). The second (ITS) dataset consisted of 47 terminals, including two as outgroup.

Three-marker phylogeny of the Usnea pectinata aggregate

A consensus phylogenetic tree of the *U. pectinata* aggregate based on three markers was produced (Fig. 1). In this 'backbone' phylogeny, the *U. pectinata* aggregate forms a well-supported monophyletic group, receiving maximum support from both Bayesian and maximum likelihood analyses. Six distinct subclades (A–F; Fig. 1) receive strong support, indicating considerable genetic variation in the dataset. Subclade A has maximum support (1.00 PP and 100% ML) and consists of three terminals, two from São Tomé and Príncipe and one from Tanzania. The subclades B–F are likewise well supported and contain exclusively representatives from Tanzania.

ITS phylogeny, and the phenotypic and chemical variation in the Usnea pectinata aggregate

An ITS tree of the *U. pectinata* aggregate, in which morphological, chemical and geographical features have been mapped, is presented in Fig. 2. The phylogeny includes all ITS sequences of the *U. pectinata* aggregate specimens in Fig. 1 and an additional 21, of which 13 are newly produced in this study and two represent *U. pectinata* s. str. downloaded from GenBank (Table 2). In the ITS phylogeny, the monophyly of the *U. pectinata* aggregate received maximum support from both Bayesian and maximum likelihood analyses.

All well-supported subclades in Fig. 1 (concatenated analysis) are also well supported in Fig. 2 (ITS analysis). Subclade G (Fig. 2) contains two representatives from Asia and is well supported. Some of the subclades (B, C & F; Fig. 2) have quite a uniform chemistry and morphology. Some branches in Fig. 2 did not receive strong support, meaning that their position on the tree remains unclear.

Usnic acid was present in all specimens. A total of six chemotypes, according to the main substances, were observed (Fig. 2): 1) protocetraric acid; 2) constictic acid; 3) protocetraric and constictic acids; 4) salazinic and diffractaic acids; 5) constictic and diffractaic acids; 6) salazinic acid. The chemotype containing stictic, norstictic, cryptostictic, menegazziaic and constictic acids reported by Ohmura (2001) was not observed in this study. However, in Fig. 2 stictic acid is present only in specimens reported by Ohmura (2002) from Japan and Indonesia. Recently, these specimens (Y2989, Y04373), loaned by Y. Ohmura, were studied by PC and the material corresponds well with the type of *Usnea pectinata*.

The specimens studied here were found to mainly produce two secondary products: protocetraric acid as the only main substance was noted in 13 specimens, 11 from Tanzania and two from São Tomé and Príncipe; constictic acid as the only main substance was observed in 10 specimens (seven from Tanzania and three from São Tomé). Salazinic plus diffractaic acids, and protocetraric plus constictic acids, were recorded in four and six specimens respectively, all originating from São Tomé and Príncipe.

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Table 2. *Usnea* species used in the DNA analyses, with specimen codes and GenBank Accession numbers of sequences. Newly produced sequences are in bold.

		GenBank Accession numbers		
Species	Specimen code	ITS	nuLSU	*RPB*1
U. baileyi	SGT110	MN080250	MN080264	MN098768
U. baileyi	SGT120	MN080246	MN080262	MN098771
U. pectinata	Y2989	AB051655		
U. pectinata	Y04373	AB051656	AB720729	
U. pectinata	SGT01	**ON911477**		
U. pectinata	SGT05	**ON911470**	**ON911485**	**OP006718**
U. pectinata	SGT06	**ON911465**		
U. pectinata	SGT07	**ON911471**	**ON911492**	
U. pectinata	SGT08	**ON911476**	**ON911487**	**OP006716**
U. pectinata	SGT09	**ON911469**		**OP006715**
U. pectinata	SGT10	**ON911468**	**ON911490**	**OP006719**
U. pectinata	SGT24	**ON911466**	**ON911488**	**OP006717**
U. pectinata	SGT25	**ON911473**	**ON911482**	
U. pectinata	SGT26	**ON911474**	**ON911491**	
U. pectinata	SGT42	**ON911467**	**ON911484**	
U. pectinata	SGT44	**ON911475**	**ON911486**	
U. pectinata	SGT86	MN080233	MN080253	MN098765
U. pectinata	SGT87	MN080241	MN080254	MN098766
U. pectinata	SGT88	**ON911472**		
U. pectinata	SGT106	MN080234		
U. pectinata	SGT107	MN080240	MN080255	
U. pectinata	SGT109	MN080236	MN080256	MN098767
U. pectinata	SGT114	MN080235	MN080257	MN098769
U. pectinata	SGT115	MN080237	MN080258	
U. pectinata	SGT116	MN080239	MN080259	
U. pectinata	SGT117	MN080238	MN080260	MN098770
U. pectinata	TNM L00004729	FJ494946		
U. pectinata	MN0060	MW267150		
U. pectinata	MN0063	MW267163		
U. pectinata	MN0065	MW267156		
U. pectinata	MN0068a	MW267147		
U. pectinata	MN0070b	MW267151		
U. pectinata	MN0125	MW267148		
U. pectinata	MN0163	MW267157	**ON911493**	
U. pectinata	MN0241	MW267167		
U. pectinata	MN0481	MW267153	**ON911489**	
U. pectinata	MN0527	MW267154		
U. pectinata	MN0540	MW267155		
U. pectinata	MN0542	MW267152		
U. pectinata	MN0556	MW267164		
U. pectinata	MN0567	MW267165		

(*Continued*)

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Table 2. (*Continued*)

Species	Specimen code	GenBank Accession numbers		
		ITS	nuLSU	*RPB*1
U. pectinata	MN0575a	MW267158	**ON911494**	
U. pectinata	MN0578a	MW267159		
U. pectinata	MN0583	MW267162		
U. pectinata	MN0585	MW267166		
U. pectinata	MN0597	MW267149		
U. pectinata	MN0602	MW267161	**ON911483**	

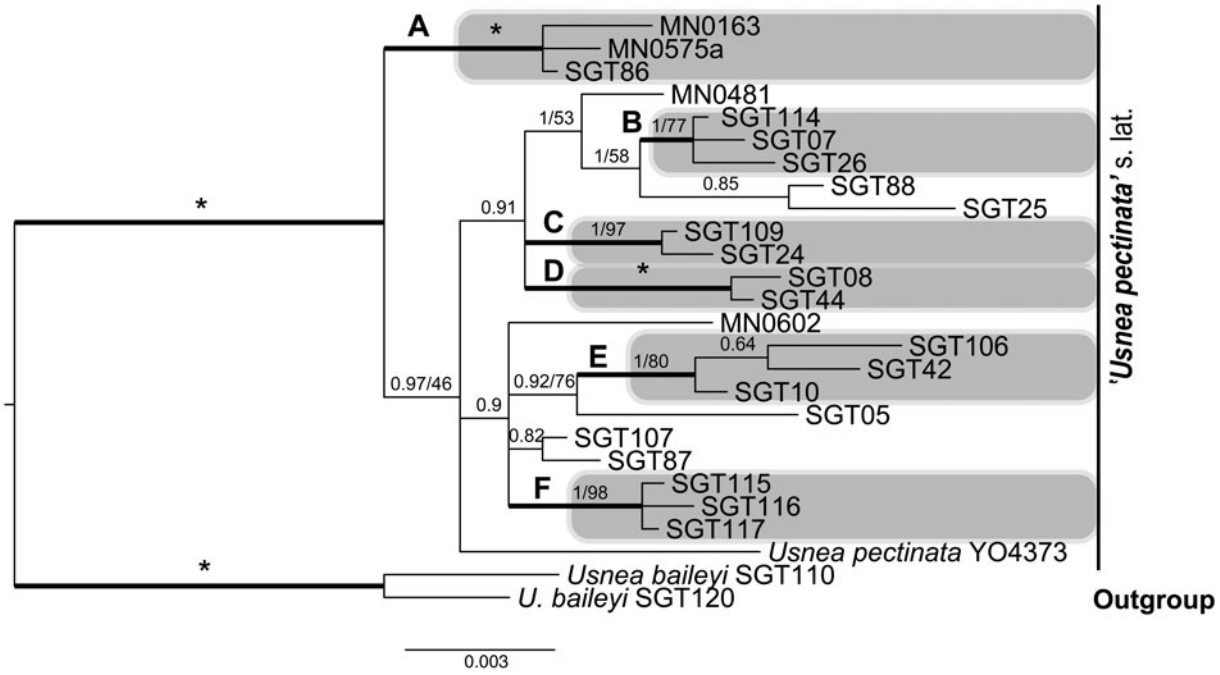

Fig. 1. Consensus tree based on the Bayesian and maximum likelihood (ML) analyses of concatenated ITS, nuLSU and *RPB*1 of the *Usnea pectinata* aggregate. The tree was rooted using *Usnea baileyi*. The two support values associated with each internal branch correspond to posterior probability (PP) and bootstrap support (ML), respectively. Branches in bold indicate a support of PP ≥ 0.95 and ML ≥ 70%. An asterisk on a bold branch indicates that this node has maximum support in both analyses. Subclades A–F are highlighted by shaded boxes and further details for the specimens included are given in Table 2.

Diffractaic and constictic acids were found in five specimens, three originating from Tanzania and two from São Tomé. Salazinic acid as the main substance was recorded in only one specimen from Tanzania. The chemistry of three of the specimens included in the molecular study remains unknown.

Usnea species are known to have a variable secondary chemistry. For example, four chemotypes were reported in *U. gigas*: fumarprotocetraric acid, psoromic acid, salazinic acid and diffractaic acid (see Table 1; Swinscow & Krog 1978).

Axis pigmentation and branch shape are highly variable in the *U. pectinata* aggregate, as studied here (Fig. 3). The specimens displayed three morphotypes with respect to axis pigmentation and four in branch shape (Fig. 2). The variation in axis pigmentation is depicted in Fig. 3 (A–H), which includes a selection of eight representatives (out of 45 terminals; Fig. 2) for two morphotypes (light brown and black) from four subclades. The branch shapes were terete, ridged, and alate to flattened. Twenty specimens were found to have terete branches, while in 13 they were ridged. Both types of branch were found in specimens from Tanzania and São Tomé and Príncipe. The morphotype with flattened branches was represented by three specimens from São Tomé and Príncipe only. Terete/ridged and ridged/alate branches were found in Tanzanian specimens only, in one and five specimens respectively.

The central axis was pale to dark brown and this variation is mapped among the features in Fig. 2. The axis colour of representatives of the subclades B, D, F and C (Fig. 2) was correlated with chemistry and morphology. In subclade A the specimens have a pale central axis (as in SGT86 and SGT06; Fig. 3A & B) and there are two chemotypes present: four specimens contain salazinic and diffractaic acids, while two have protocetraric acid. The branch shape (terete) is uniform. In subclade B the central axis is dark brown (SGT26 and SGT07; Fig. 3C & D) and correlates well with the chemistry (protocetraric acid) and branch shape

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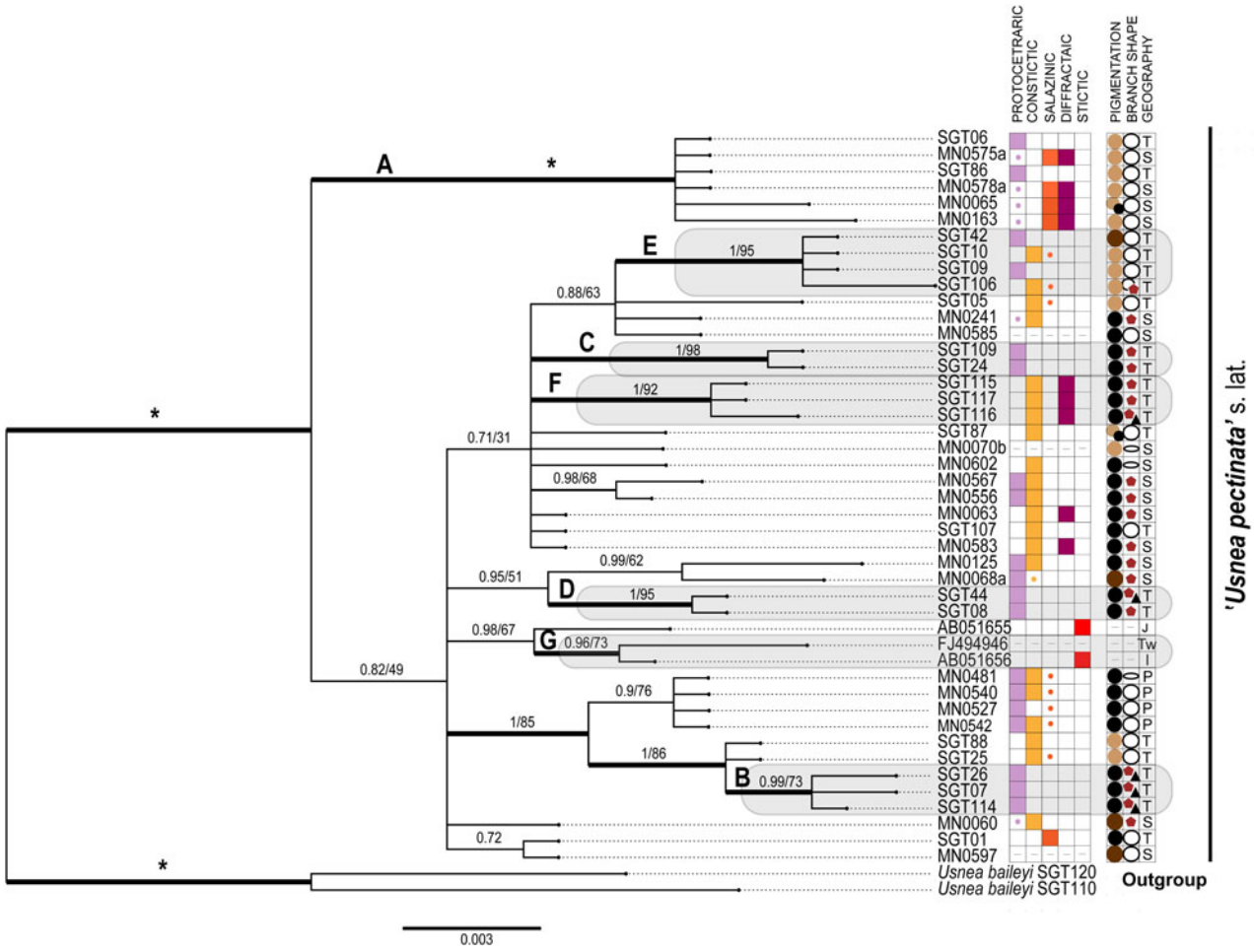

Fig. 2. ITS phylogeny based on Bayesian and ML analyses of the *Usnea pectinata* aggregate along with morphological and chemical data. The two support values associated with each internal branch correspond to posterior probability (PP) and bootstrap support (ML), respectively. Branches in bold indicate a support of PP ≥ 0.95 and ML ≥ 70%. An asterisk on a bold branch indicates that this node has maximum support in both analyses. Full square shade = main chemical substance; small coloured dot = accessory chemical substance; dash = unknown. Usnic acid is found in all specimens and is therefore not indicated. Axis pigmentation: pale brown circle = pale brown pigmentation; brown circle = brown; black circle = dark brown. Branch shape: white circle = terete; flattened white circle = flattened; triangles = alate; pentagon = ridged. Geography: I = Indonesia; J = Japan; S = São Tomé; P = Príncipe; Tw = Taiwan; T = Tanzania. Subclades A–F are indicated and, where necessary, highlighted by a shaded box. Further details for the specimens included are given in Table 2.

(ridged/alate). In subclade C the central axis is likewise dark brown (Fig. 3E), and both chemistry (protocetraric acid) and branch shape (ridged) are uniform. Subclade D is uniform with respect to central axis pigmentation (dark brown) and chemistry (protocetraric acid), and the branch shape is ±uniform (ridged). In subclade E there is variation in both axis pigmentation (Fig. 2, SGT106; Fig. 3F) and chemistry. Out of the four specimens in this subclade, two have constictic acid and two have protocetraric acid as main substances. The branch shape is ±uniform (terete). Subclade F (three specimens from Tanzania) is uniform with respect to pigmentation (dark brown) and chemistry (constictic and diffractaic acid), while branch shape is ±uniform (ridged). Overall, the central axis displays considerable variation in colour and texture (Fig. 3A–H) in this preliminary investigation and further work on morphology with more material of the *U. pectinata* aggregate worldwide is called for.

In our results, a correlation between chemistry, morphology and molecular data was observed in the majority (4 out of 6) of the well-supported subclades. We have used the name *U. pectinata* aggregate as a place-holder for what we see as a species complex that requires extensive taxonomic work for its clarification. This view of the *U. pectinata* aggregate as a species complex might well be in line with the views of Ohmura (2008), Wirtz *et al.* (2008), Kelly *et al.* (2011), Saag *et al.* (2011), Truong *et al.* (2013*a*) and Gerlach *et al.* (2019), who all reported that species described on morphological and chemical characters are usually monophyletic. More specimens from different parts of the world should be studied before making any taxonomic/nomenclatural decisions on this difficult group. Morphological features do warrant closer anatomical studies.

Conclusion

A total of 31 (13 ITS, 13 nuLSU and 5 *RPB*1) sequences for the *U. pectinata* aggregate were generated in this study as the basis for phylogenetic investigations of the group. A phylogeny based on three markers was supplemented by an ITS analysis revealing the occurrence of several well-supported subclades found in both phylogenies. These subclades were also often quite consistent in chemistry and morphology and may well represent separate species in the complex referred to here as the *U. pectinata* aggregate. Therefore, we support our second hypothesis that we do

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Fig. 3. Images of the *Usnea pectinata* aggregate specimens (Table 2) showing a section of the central axis. The specimen code (bottom left) is usually followed by a slash (/) and a subclade indication, with the addition of a pale brown circle (pale brown pigmentation), or black circle (dark brown pigmentation). Scale = 0.1 mm.

have several small, strongly supported genetic clades that correlate with morphology and secondary chemistry. This is in line with previous studies in the group (Gerlach *et al.* 2019), where a clade that is uniform in chemistry and morphology was found to be a species. Here we suggest two sequences to represent *Usnea pectinata* s. str. (GenBank AB051655 and AB051656; Ohmura 2002). We wish to emphasize that this study is a first step towards understanding the *U. pectinata* aggregate and that in order to connect already existing ('historical') species names with potential subclades in the group, comprehensive studies of a geographically wider sampling are needed, and eventually also the sequencing of the type materials.

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