

Phylogeny and taxonomy of *Staurothele* (Verrucariaceae, lichenized ascomycetes) from the karst of northern Vietnam

Cécile GUEIDAN, Truong VAN DO and Ngan Thi LU

Abstract: The crustose genus *Staurothele* (Verrucariaceae, Ascomycota) is a common component of the lichen flora from subneutral to alkaline silicate rocks in temperate to cold-temperate climates. Our field study in the karst system of northern Vietnam showed that it is also common on dry to humid limestone in the wet tropics. Molecular data revealed that species of *Staurothele* from Vietnam belong to an unnamed clade sister to the genus *Endocarpon*, together with the tropical Australian species *Staurothele pallidopora* and *Staurothele diffractella*, a North American species recently transferred to *Endocarpon* based on molecular data. The genus *Willeya* is here resurrected for this clade of crustose epilithic *Staurothele* with pale ascospores. Eight new combinations are proposed and three new species of *Willeya* are described from Vietnam. Sampling tropical members of a lichen family previously mostly known from temperate areas contributed significantly to improving its generic classification.

Key words: classification, *Endocarpon*, generic delimitation, lichens, Verrucariales, *Willeya*

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Introduction

Staurothele Norman is a genus of lichenized fungi forming crustose thalli on subneutral to alkaline silicate rocks. The species are found both in dry and amphibious conditions and are common in habitats including dry limestone outcrops or alkaline rocks along rivers and streams. This genus belongs to the Verrucariaceae Zenker, a fungal family whose members are predominantly lichen-forming. Although members of Verrucariaceae occur on various substrata, rock surfaces constitute the most common habitat. As with many genera within Verrucariaceae, *Staurothele* is most diverse in temperate climates, including the Mediterranean region, cold-temperate to subarctic regions (e.g., Alaska, Scandinavia) and high elevations (e.g., the

Alps). It has occasionally been reported from wetter and warmer areas of the world, for example, Indonesia, (Groenhart 1954), southern China (Harada & Wang 1996, 2006), but its diversity in subtropical and tropical regions remains understudied.

Staurothele currently comprises 72 species (Kirk *et al.* 2008), all characterized by a crustose thallus and muriform ascospores. They also all possess a peculiar feature: algal cells, usually present only in the thallus in other lichens, are also found in the perithecia, between the asci. These stichococcoid algae, recently shown with molecular data to belong to the green-algal genus *Diplosphaera* Bial. (Thüs *et al.* 2011), are co-dispersed with large muriform fungal ascospores. This character is not unique to this genus, but is also found in *Endocarpon* Hedw., another genus of Verrucariaceae. *Staurothele* is morphologically similar to *Endocarpon*, but differs in the habit (squamulose in *Endocarpon* and crustose in *Staurothele*) and the structure of the upper cortex (eucortex in *Endocarpon* and pseudocortex in *Staurothele*). Within *Staurothele*, species-diagnostic features in-

C. Gueidan: Natural History Museum, Department of Life Sciences, Cromwell Road, London, SW7 5BD, UK. Email: c.gueidan@nhm.ac.uk

T. Van Do and N. T. Lu: Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam.

T. Van Do: Institute of Botany, Technical University of Dresden, Zellescher Weg 20b, 01062 Dresden, Germany.

clude the structure of the thallus, which can either be epilithic (growing on the rock surface) or endolithic (growing within the superficial layer of the rock), and the ascospore colour, which varies from pale to darkly pigmented. Other differences include the size and structure of the perithecia and the size of the ascospores. The shape of the algal cells present in the hymenium has also been used for species delimitation in the past (Malme 1919; Vainio 1921). However, culture experiments showed that their shape may vary depending on the culture conditions and developmental stages (Ahmadjian & Heikkilä 1970). This character was therefore suggested as being inappropriate for species delimitation in *Staurothele* and *Endocarpon* (Thomson 1991).

In the past, a number of genera have been segregated from *Staurothele*. Müller Argoviensis (1883) placed the species *Staurothele diffractella* (Nyl.) Tuck. in the new genus *Willeya* Müll. Arg. as its ascospores remain pale at maturity whereas they become dark brown in many other species of *Staurothele*. Later, the same author described a second species of *Willeya*, *W. rimosa* Müll. Arg., based on a specimen with pale ascospores collected by the French botanist P. Hariot in Tonkin, northern Vietnam (Müller Argoviensis 1889). Clements (1909) described the genus *Phalostauris* Clem. for *Staurothele* with pale ascospores. These two genera were, however, not accepted by most authors and were considered as synonyms of *Staurothele* by Thomson (1991) and Brodo *et al.* (2001). Other synonyms of *Staurothele* listed in Species Fungorum (<http://www.speciesfungorum.org>, 27/05/2011) are *Goidanichia* Tomas. & Cif., *Goidanichiomyces* Cif. & Tomas., *Paraphysorma* A. Massal., *Polyblastiomyces* E. A. Thomas, *Sphaeromphale* A. Massal. and *Stigmatomma* Müll. Arg.

More recently, molecular phylogenetic studies have allowed the traditional generic delimitation in *Verrucariaceae* to be tested (Gueidan *et al.* 2007, 2009; Savić & Tibell 2008; Savić *et al.* 2008; Muggia *et al.* 2010; Prieto *et al.* 2010, 2012). In studies supported by a good sampling of *Staurothele* species, the genus was shown to be polyphyletic

(Gueidan *et al.* 2007, 2009; Savić *et al.* 2008). One well-supported monophyletic lineage composed exclusively of crustose epilithic species of *Staurothele* is closely related to the genera *Catapyrenium* and *Placidiopsis*. This lineage includes *Staurothele clopima* (Wahlenb.) Th. Fr., the type of the genus, and is now considered as *Staurothele* s. str. Crustose endolithic species of *Staurothele* for which molecular data were obtained [*S. immersa* (A. Massal.) Dalla Torre & Sarnth. and *S. rupifraga* (A. Massal.) Arnold] did not cluster in this lineage, but in another group including species of *Thelidium* A. Massal. and *Polyblastia* A. Massal. Finally, one crustose epilithic species, *Staurothele diffractella*, was sister to the genus *Endocarpon*. This species, found in North America, was transferred to the genus *Endocarpon*. As a result, the definition of this genus was emended to include both squamulose and crustose species (Gueidan *et al.* 2007).

The genus *Staurothele* has been studied in various parts of the world. In North America, Thomson provided the most complete revision of the temperate and arctic species (Thomson & Murray 1988; Thomson 1991, 2002). It has also been well studied in Japan (Bouly de Lesdain 1921; Harada & Iwatsuki 1989; Harada 1992), Australia (McCarthy 1995, 2001) and Europe (Malme 1919; Servit 1955; Swinscow 1963; Clauzade & Roux 1985; Thüs & Schultz 2009). Few species of *Staurothele* have been described from South-East Asia: *S. australis* Groenh. from Java (Groenhart 1954), *S. malayensis* Zahlbr. from Java and Sumatra (Zahlbruckner 1934), and *S. rimosa* (Müll. Arg.) Zahlbr. from Vietnam (Müller Argoviensis 1889). Several species have been described from karst in southern China: *S. chlorospora* Zahlbr., *S. honghensis* H. Harada & Li S. Wang, *S. kwapiensis* Zahlbr., *S. microlepis* Zahlbr., *S. muliensis* Zahlbr., *S. ochroplaca* Zahlbr., *S. sinensis* Zahlbr., and *S. yunnana* H. Harada & Li S. Wang (Zahlbruckner 1930; Harada & Wang 1996, 2006). However, the species diversity of the genus in this part of the world remains understudied, as only a few localities have been explored. In the large karstic system of northern Vietnam, exposed calcareous out-

crops and cliffs are very abundant and constitute a favourable habitat for *Staurothele*. Three main localities were therefore explored in this part of Vietnam in order to investigate the diversity of the genus in a wet tropical region. Two phylogenetic analyses were also carried out to investigate the placement of these Vietnamese species of *Staurothele* within the *Endocarpon*-group (as defined in Gueidan *et al.* 2007), as well as their species delimitation.

Materials and Methods

Morphological study

Twenty-six specimens of *Staurothele* were collected from limestone in three nature reserves in northern Vietnam: Bắc Mê (Hà Giang Province), Na Hang (Tuyên Quang Province) and Hang Kia-Pà Cò (Hòa Bình Province). No comprehensive identification key is available for tropical species of *Staurothele*. We therefore first used several floras and keys from Europe (Clauzade & Roux 1985; Smith *et al.* 2009), North America (Thomson 1991, 2002) and Australia (McCarthy 2001) to identify our collections. Except for *Staurothele pallidopora* P. M. McCarthy, a species from Australia (McCarthy 1995),

our material did not match any previously described species. We then created a partial key from original descriptions of various earlier described but overlooked tropical to subtropical species of *Staurothele*, including also some more recently described species from Japan (Harada 1992) and China (Harada & Wang 2006). To create this key, we selected all species previously described from Asia or other subtropical to tropical regions of the world from the list of species names available for this genus in Index Fungorum (<http://www.indexfungorum.org>, 27/05/2011). These 19 species were *S. acarosporoides* Vain. (St. Vincent, Caribbean), *S. arenaria* Malme (Paraguay), *S. australis* (Java), *S. chlorospora* (Southern China), *S. fauriei* B. de Lesd. (Taiwan), *S. honghensis* (Southern China), *S. iwatsukii* H. Harada (Japan), *S. japonica* B. de Lesd. (Japan), *S. kwapiensis* (Southern China), *S. malayensis* (Java and Sumatra), *S. microlepis* (Southern China), *S. muliensis* (Southern China), *S. ochroplaca* (Southern China), *S. pachystroma* Müll. Arg. (Brasilia), *S. pallidopora* (Australia), *S. paraguayensis* Malme (Paraguay), *S. rimosa* (Vietnam), *S. sinensis* (Southern China), *S. yunnana* (Southern China). We classified them using the following morphological and anatomical characters: 1) thallus structure, 2) ascospore size, 3) the number of ascospores per ascus, 4) ascospore pigmentation. A complete key was not attempted because data obtained from original diagnoses were often vague or incomplete. However, the preliminary key presented below was an efficient tool for selecting taxa to compare to our material from Vietnam.

Preliminary key to tropical to subtropical *Staurothele* species based on their original descriptions

Staurothele australis appears twice in the key (as marked with asterisk) because the number of ascospores per ascus is not known.

1	Thallus endolithic.	S. chlorospora, S. muliensis
	Thallus entirely or mostly epilithic	2
2(1)	Ascospores >35 µm	3
	Ascospores <35 µm	6
3(2)	Ascospores 1–3 per ascus	4
	Ascospores 6–8 per ascus	5
4(3)	Ascospores pale	S. arenaria
	Ascospores dark.	S. pachystroma
5(3)	Ascospores pale	S. yunnana
	Ascospores dark.	S. sinensis, S. ochroplaca
6(2)	Ascospores 1–4 per ascus	7
	Ascospores 6–8 per ascus, pale	
 S. australis* , S. iwatsukii, S. japonica, S. malayensis, S. microlepis,	
 S. pallidopora, S. rimosa	
7(6)	Ascospores dark.	S. acarosporoides var. acarosporoides
	Ascospores pale	
 S. acarosporoides var. pallescens, S. australis*, S. fauriei,	
 S. honghensis, S. kwapiensis, S. paraguayensis	

Seven species with a crustose epilithic thallus, 8-spored asci and pale ascospores less than 35 µm long, a set of features shared with all our material from Vietnam, were selected for comparison with our collections: *S. australis*, *S. iwatsukii*, *S. japonica*, *S. malayensis*, *S. microlepis*, *S. pallidopora* and *S. rimosa*. Type specimens were borrowed from G (*S. rimosa*), L (*S. australis*), HIRO (*S. iwatsukii*), KYO (*S. japonica*), MEL (*S. pallidopora*), W (*S. malayensis*) and WU (*S. microlepis*). The type material of *S. diffractella* was also requested from H as our molecular results showed that our Vietnamese specimens were closely related to this species.

Morphological and anatomical characters were studied using a Zeiss Axioskop light microscope and illustrated using a drawing chamber. Sections were prepared by hand and mounted in water. Photographs of specimens were taken in the Sackler Biodiversity Imaging Laboratory at the Natural History Museum using a Zeiss Stemi SV11 stereomicroscope coupled with a Canon EOS imaging system. For a better depth of field, images were stacked using the software Helicon Focus (Helicon Soft, Kharkov, Ukraine). Characters studied were 1) thallus colour, 2) degree of cracking of thallus, 3) degree of immersion of perithecia, 4) size of the perithecia, 5) pigmentation of the excipulum, 6) structure of the involucrellum, 7) number of ascospores per ascus, 8) ascospore size, 9) ascospore pigmentation, 9) size of hymenial algae, 10) shape of hymenial algae, and 11) presence or absence of a black basal layer. These observations allowed us to classify the Vietnamese specimens into four morphological groups (Table 1), which were then compared to the seven previously selected species of *Staurothele*, as well as the North American species *E. diffractellum* (Table 2). Representatives of each morphological group were then selected for molecular study for a total of 17 specimens. The remaining eight specimens were not used for DNA extraction because of their small size or poor condition (e.g. old or covered with epiphytic algae or lichenicolous fungi). For the species descriptions, categories of plectenchymas followed Yoshimura & Shimada (1980). Thallus colours were described according to the Methuen Handbook of Colour (Kornerup & Wanscher 1961). For the new species, the size of the ascospores or hymenial algae was based on 25 to 50 measurements and extreme values are indicated in parentheses.

Taxon and gene sampling

Seventeen specimens of *Staurothele* collected from Vietnam were used for molecular work. Additionally, two recent specimens of the Australian species *S. pallidopora* were borrowed from CANB and used in this study (Table 3). Recent material was not available for the six other species sharing morphological and anatomical similarities with our Vietnamese material. These 19 specimens were subjected to molecular analyses. For the first phylogenetic analysis, 15 taxa previously shown to belong to the *Endocarpon*-group (as defined in Gueidan *et al.* 2007) were added to the taxon sampling (six from *Endocarpon*, two from *Involucropyrenium*, two from *Neocatapyrenium* and five from *Verrucaria*). For these

taxa, some sequences are newly published here and others were already available in GenBank (Table 3). Two nuclear ribosomal markers were used: 1) the internal transcribed spacer (ITS) region, which includes the intergeneric transcribed spaces 1 and 2 and the 5.85 subunit of the RNA gene, and 2) the large subunit of the RNA gene (nuLSU).

DNA extraction, amplification and sequencing

Material was removed from dry specimens with a sterile razor blade and transferred to an Eppendorf tube. Genomic DNA was obtained using a protocol modified from Zolan & Pukkila (1986), as described in Gueidan *et al.* (2007). DNA extracts were checked with gel electrophoresis and for each sample the band intensity was used to choose the appropriate genomic DNA dilution for amplification. For the two gene regions, 1 µl of a 1/10 or 1/100 dilution of genomic DNA was added to the following PCR mix: 2.5 µl PCR buffer 10× NH₄ (Biolone, London, UK), 1.5 µl of MgCl₂ (50 mM), 0.5 µl dNTP (100 mM), 1 µl primers (10 µM), 0.5 µl DNA polymerase Bioline BioTaq (5 U µl⁻¹), and water to a total volume of 25 µl. PCR was performed on a Techne TC-4000 PCR machine (Bibby Scientific Ltd, Stone, UK). The ITS region was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). The marker nuLSU was amplified using LR0R (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990). For ITS, the PCR program was as follows: 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 53°C, 1 min at 72°C, and finally 7 min at 72°C. For nuLSU, the PCR program was: 1 min at 95°C, 35 cycles of 45 s at 95°C, 40 s at 52°C, 2 min 30 s at 72°C, followed by 10 min at 72°C. PCR product clean-up and sequencing were carried out by the sequencing facility of the Natural History Museum in London using PCR Clean-up Filter Plates (Millipore, Billerica, MA), BigDye chemistry and an ABI 3730xl sequencing machine (Applied Biosystems, Carlsbad, CA, USA). The internal primers ITS2 and ITS3 (White *et al.* 1990) were used to sequence ITS, and LR3, LR5, LR6, LR3R, LR5R and LR6R (Vilgalys & Hester 1990) to sequence nuLSU.

Phylogenetic analyses

DNA sequences were edited and assembled using Sequencher version 4.8 (Gene Codes Corporation, Ann Arbor, MI). Sequences were manually aligned in MacClade version 4.08 (Maddison & Maddison 2003). BLAST searches in GenBank (<http://www.ncbi.nlm.nih.gov/genbank>) suggested that all Vietnamese specimens belonged to the *Endocarpon*-group (as defined in Gueidan *et al.* 2007). Two phylogenetic analyses were therefore carried out: the first to investigate the placement of the Vietnamese *Staurothele* within the *Endocarpon*-group, and the second to reconstruct the phylogenetic relationships between the Vietnamese specimens and their related taxa. The first analysis included 29 taxa for which two gene regions, ITS and nuLSU, were available (Table 3). Ambiguous regions were delimited according

TABLE 1. Main morphological and anatomical characteristics of specimens of *Staurothele* collected in Vietnam. Based on these characters, the material can be classified into four morphogroups, which correspond to four species of *Willeya*.

Morphogroups	Thallus		Perithecia	Centrum size	Excipulum	Involucrellum	Ascus	Ascospores		Hymenial algae		Black basal layer
	colour	structure						size	colour	size	shape	
morphogroup 1 = <i>W. pallidopora</i> comb. nov.	greenish grey to pale olive-grey	finely rimose to areolate	immersed to 1/2 immersed	0.2–0.3 mm	pale to brown or black	spreading laterally, not appressed	8-spored	25–29 × 10–13 μm	pale	3.5–8.5 × 1.5–3.0 μm	elongated to cylindrical	present where perithecia densely aggregated
morphogroup 2 = <i>W. protrudens</i> sp. nov.	greyish green to olive-brown	rimose to subareolate around the perithecia	protruding, entirely or only partly covered by the thallus	0.3–0.4 mm	brown to black	spreading laterally, not appressed	8-spored	22–30 × 10–14 μm	pale	4.0–9.0 × 1.5–3.0 μm	elongated to cylindrical	present where perithecia densely aggregated
morphogroup 3 = <i>W. fusca</i> sp. nov.	dark greyish brown to black	rimose-areolate	immersed in areoles	0.25–0.30 mm	dark brown to black	appressed to upper part to entire	8-spored	22–28 × 10–13 μm	pale	3.5–7.0 × 2.0–2.5 μm	elongated to cylindrical	generally present
morphogroup 4 = <i>W. laevigata</i> sp. nov.	greyish to brownish green	continuous to rimose here and there	entirely immersed	0.4–0.6 mm	pale	appressed to upper part	8-spored	25–29 × 11–15 μm	pale	2.0–8.0 × 1.5–3.0 μm	elongated to cylindrical	absent

TABLE 2. Main morphological and anatomical characteristics of nine tropical or subtropical *Staurothele* taxa with ascospore characters similar to our material from Vietnam. The North American species *Endocarpon diffractellum* was also included for comparison. Most data were obtained from original species descriptions. Data marked with a star were modified or completed from the original descriptions by studying the type material. W = Willeya

Type specimens	Thallus		Perithecia	Centrum size	Excipulum	Involucrellum	Ascus	Ascospores		Hymenial alga		Black basal layer
	colour	structure						size	colour	size	shape	
<i>S. australis</i> (<i>W. australis</i> comb. nov.)	shabby green	rimose to areolate*	immersed	0.28–0.32 mm	rust brown	spreading laterally*	unknown	19–26 × 11–13 μm	pale	5–10 × 1.5–2.0 μm	elongated	present
<i>E. diffractellum</i> (<i>W. diffractella</i>)	ashy to yellowish or pale brownish, sometimes olive-brownish	areolate, with contiguous to partly dispersed areoles	immersed in areoles, with ostiole raised and prominent	0.2–0.4 mm*	pale*	spreading laterally*	8-spored	18–23 × 10–11 μm	pale	3 × 1.5–2.0 μm	globose to elongated	absent*
<i>S. iwatsukii</i> (<i>W. iwatsukii</i> comb. nov.)	yellowish grey to greyish beige*	rimose to subareolate*	immersed to slightly raised	0.17–0.41 mm	pale to brown	spreading laterally	6–8-spored	20–33 × 8–12 μm	pale	2–9 × 2 μm	globose to elongated	present
<i>S. japonica</i> (<i>W. japonica</i> comb. nov.)	yellowish grey to greyish beige*	areolate, with contiguous to partly dispersed areoles	immersed	0.22–0.31 mm	black*	appressed to upper part of perithecium*	8-spored	21–27 × 8–11 μm	pale	2–3 μm diameter	globose	present
<i>S. malayensis</i> var. <i>malayensis</i> (<i>W. malayensis</i> comb. nov. var. <i>malayensis</i>)	yellowish beige to grey*	continuous, with few cracks here and there, mostly around the perithecia	± immersed to at last not entirely covered by the thallus	0.2–0.3 mm*	brown to black	spreading laterally*	8-spored	23–25 × 10 μm	pale	4.0 × 1.5 μm	elongated	absent
<i>S. malayensis</i> var. <i>vegetior</i> (<i>W. malayensis</i> var. <i>vegetior</i> comb. nov.)	light to darker yellowish grey*	rimose to irregularly subareolate, with large and deep cracks	immersed	0.2–0.3 mm*	brown to black	appressed to upper part of perithecium to entire*	8-spored	23–25 × 10 μm	pale	4.0 × 1.5 μm	elongated	absent
<i>S. malayensis</i> var. <i>sulphurea</i> (syn. <i>W. malayensis</i>)	sulfhur-yellow green	thin, continuous and smooth	± immersed to at last not entirely covered by the thallus	0.2–0.3 mm*	brown to black	spreading laterally*	8-spored	23–25 × 10 μm	pale	4.0 × 1.5 μm	elongated	absent
<i>S. microlepis</i> (<i>W. microlepis</i> comb. nov.)	greyish beige to brownish grey*	areolate, with contiguous to partly dispersed areoles	immersed in areoles, with ostiole raised and prominent	0.2–0.4 mm	brown to black*	entire	8-spored	18–26 × 8–10 μm	pale	± 2 μm diameter	globose	present*
<i>S. pallidopora</i> (<i>W. pallidopora</i> comb. nov.)	pale grey to pale grey-brown	richly rimose to areolate (mainly around perithecia)	2/3 immersed to almost entirely immersed	0.22–0.36 mm	medium to dark brown	spreading vertically	8-spored	18–28 × 9–14 μm	pale	4–12 × 1.5–2.5 μm	elongated	present*
<i>S. rimosa</i> (<i>W. rimosa</i>)	clay olivaceous green	continuous to rimose at the centre of the thallus	immersed to 1/2 immersed*	± 0.3 mm*	black	spreading laterally*	8-spored	20–23 × 11–12 μm	pale	5–7 × 1.3–1.5 μm	elongated	absent*

TABLE 3. Collection number, locality and sequence data for the 34 taxa used in our molecular analyses. Corresponding herbaria are indicated in parentheses after the collection number (abbreviation as in *Index Herbariorum*). GenBank numbers highlighted in bold indicate sequences generated in this study. Missing sequences are represented by a dash.

Species	Collection number	Locality	GenBank Acc. Number	
			ITS	nuLSU
<i>Verrucaria submersella</i> Servit	CG 726 (DUKE)	Switzerland, Canton St-Gallen, Lutertannen	KF959776	EF643797
<i>Endocarpon adscendens</i> (Anzi) Müll. Arg.	CG 671 (DUKE)	Switzerland, canton Schwyz, Würzli	KF959777	EF643751
<i>E. pallidulum</i> (Nyl.) Nyl.	SJ 4028 (DUKE)	USA, North Carolina, Jones County, Croatan National Forest	DQ826735	DQ823097
<i>E. petrolepideum</i> (Nyl.) Nyl.	U-492F (DUKE)	USA, Maryland, Prince Georges County	KF959778	EF643752
<i>E. psorodeum</i> (Nyl.) Th. Fr.	CG 684 (DUKE)	Estonia, Harjümaa, Kostivere	KF959779	EF643753
<i>E. pusillum</i> Hedw.	CG 470 (MARSSJ)	France, Bouches-du-Rhône, Fos-sur-Mer	JQ927447	EF643754
<i>Involutocyprenium waltheri</i> (Kremp.) Breuss	OB s.n. (LI)	Austria, Steiermark, Steirisches Randgebirge, Stubalpe	KF959780	KF959808
<i>I. waltheri</i> (Kremp.) Breuss	JH 59126 (GZU)	Austria, Steiermark, Niedere Tauern	KF959781	KF959809
<i>Neocatapyrenium rhizinosum</i> (Müll. Arg.) Breuss	OB s.n. (LI)	Greece, Crete, Gorge of Samaria	KF959782	EF643757
<i>N. rhizinosum</i> (Müll. Arg.) Breuss	VV s.n. (LI)	Turkey, Denizli, Haybey	KF959783	KF959810
<i>Verrucaria nigrescens</i> Pers.	CG 475 (MARSSJ)	France, Bouches-du-Rhône, Mouriès, les Alpilles, le Destet	KF959784	EF643804
<i>V. polysticta</i> Borrer	CG 689 (MARSSJ)	Switzerland, Zugerberg, Choellenroeid	KF959785	EF643807
<i>V. viridula</i> (Schrad.) Ach.	CG 587b (MARSSJ)	France, Bouches-du-Rhône, St-Mitre-les-Remparts, Mauvegeane	KF959786	EF643814
<i>V. weddellii</i> Servit	CG 460 (MARSSJ)	France, Bouches-du-Rhône, Ste-Victoire	KF959787	EF643812
<i>Willeya diffractella</i> (Nyl.) Gueidan comb. nov.	CG 585 (DUKE)	USA, Missouri, Perry County, Seventy-Six Conservation Area	KF959788	EF643773
<i>W. pallidopora</i> (P. M. McCarthy) Gueidan comb. nov.	PMC 2546 (CANB)	Australia, Queensland, Cook District, Atherton Tableland, Millaa Millaa Falls	KF959797	–
<i>W. pallidopora</i> (P. M. McCarthy) Gueidan comb. nov.	PMC 2612 (CANB)	Australia, Queensland, Cook District, Wooroonooran National Park, above Wallicher Falls	KF959796	–
<i>Willeya</i> sp. 1a (<i>Willeya pallidopora</i> s. lat.)	CG 1908 (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, Na Hang Nature Reserve, Ban Bung village	KF959790	KF959811
<i>Willeya</i> sp. 1a (<i>Willeya pallidopora</i> s. lat.)	CG 1926 (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, Na Hang Nature Reserve, Ban Bung village	KF959791	KF959812
<i>Willeya</i> sp. 1a (<i>Willeya pallidopora</i> s. lat.)	CG 1927 (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, Na Hang Nature Reserve, Ban Bung village	KF959792	KF959813
<i>Willeya</i> sp. 1b (<i>Willeya pallidopora</i> s. lat.)	CG 1940b (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, near the lake after the dam	KF959793	–
<i>Willeya</i> sp. 1b (<i>Willeya pallidopora</i> s. lat.)	CG 1941 (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, near the lake after the dam	KF959794	KF959820
<i>Willeya</i> sp. 1b (<i>Willeya pallidopora</i> s. lat.)	CG 1948 (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, near the lake after the dam	KF959795	KF959821
<i>Willeya</i> sp. 1b (<i>Willeya pallidopora</i> s. lat.)	CG 1865 (BM)	Vietnam, Hà Giang Prov., Bắc Mê District, Lạc Nông County, forested trail along the river	KF959789	KF959819
<i>Willeya</i> sp. 2 (<i>Willeya protrudens</i> sp. nov.)	CG 1885 (BM)	Vietnam, Hà Giang Prov., Bắc Mê District, Lạc Nông County	KF959798	KF959814
<i>Willeya</i> sp. 2 (<i>Willeya protrudens</i> sp. nov.)	CG 1922 (BM)	Vietnam, Hà Giang Prov., Bắc Mê District, Lạc Nông County	KF959799	–
<i>Willeya</i> sp. 2 (<i>Willeya protrudens</i> sp. nov.)	CG 1940a (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, near the lake after the dam	KF959800	KF959815
<i>Willeya</i> sp. 2 (<i>Willeya protrudens</i> sp. nov.)	CG 1943 (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, near the lake after the dam	KF959801	KF959816
<i>Willeya</i> sp. 2 (<i>Willeya protrudens</i> sp. nov.)	CG 1945 (BM)	Vietnam, Tuyên Quang Prov., Na Hang District, Na Hang Nature Reserve	KF959802	KF959817
<i>Willeya</i> sp. 2 (<i>Willeya protrudens</i> sp. nov.)	CG 1957a (BM)	Vietnam, Hòa Bình Prov., Mai Châu District, Pà Cò County, Hang Kia-Pà Cò Nature Reserve	KF959803	KF959818
<i>Willeya</i> sp. 2 (<i>Willeya protrudens</i> sp. nov.)	CG 1957b (BM)	Vietnam, Hòa Bình Prov., Mai Châu District, Pà Cò County, Hang Kia-Pà Cò Nature Reserve	KF959804	–
<i>Willeya</i> sp. 3 (<i>Willeya fusca</i> sp. nov.)	CG 1877 (BM)	Vietnam, Hòa Bình Prov., Mai Châu District, Pà Cò County, Hang Kia-Pà Cò Nature Reserve	KF959805	KF959822
<i>Willeya</i> sp. 3 (<i>Willeya fusca</i> sp. nov.)	CG 1912 (BM)	Vietnam, Hòa Bình Prov., Mai Châu District, Pà Cò County, Hang Kia-Pà Cò Nature Reserve	KF959806	KF959823
<i>W.</i> sp. 4 (<i>Willeya laevigata</i> sp. nov.)	CG 1852 (BM)	Vietnam, Hòa Bình Prov., Mai Châu District, Pà Cò County, close to the limit with Sơn La Province	KF959807	KF959824

to Lutzoni *et al.* (2000) and excluded from the alignments. Congruence between the two datasets was tested using a 70% reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996): the two matrices (ITS and nuLSU) were analyzed separately using 1000 rapid bootstrap pseudoreplicates and a GTRCAT model of molecular evolution with RAxML VI-HPC v. 7.4.4 (Stamatakis *et al.* 2005, 2008) on the Cipres Web Portal (<http://www.phylo.org>; Miller *et al.* 2010). After comparing the two resulting topologies, no conflicts were detected and the two datasets were combined. For this first analysis, *Verrucaria submersella* was selected as outgroup based on previous studies (Gueidan *et al.* 2007, 2009). The second analysis included only the ITS region from 20 taxa shown to belong to *Willeya* in the first analysis (Table 3), and two species of *Endocarpon* as an outgroup (*E. petrolepideum* and *E. psorodeum*). For this dataset, all characters were included as no ambiguously aligned regions were present.

For both analyses, phylogenetic relationships were investigated using a Bayesian approach with MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003), as implemented on the Cipres Web Portal. Models of molecular evolution were estimated for both ITS and nuLSU using the Akaike Information Criterion, as implemented in Modeltest version 3.7 (Posada & Crandall 1998): a GTR+I+G model was selected for both partitions. For each dataset, two analyses of four chains were run for 5 million generations and trees were sampled every 500 generations. All runs converged on the same average likelihood score and topology. A burn-in sample of 5000 trees was discarded for each run. The remaining 10 000 trees were used to estimate the posterior probabilities with the 'compute consensus' command in PAUP* version 4.0b10 (Swofford 1999). The most likely tree was computed with the sumt command in MrBayes and visualized in PAUP*. Additional support values were obtained using a maximum likelihood (ML) approach with the software RAxML VI-HPC version 7.4.4 as implemented on the Cipres Web Portal. The two-gene dataset (with the two partitions ITS and nuLSU) and the single gene dataset (ITS) were analyzed using a GTRCAT model. Support values were obtained using a fast bootstrap analysis of 1000 pseudo-replicates.

Results

Morphological study

All 26 Vietnamese specimens had 8-spored asci, pale muriform ascospores and crustose epilithic thalli. They also had ascospores with similar and overlapping size ranges, and elongated to cylindrical hymenial algae. However, several differences could be used to classify them into four morphological groups (or morphogroups, Table 2). Morphogroup 1 (CG1850, CG1865, CG1868, CG1870,

CG1908, CG1926, CG1927, CG1938, CG1940b, CG1941, CG1948; Fig. 1A–C) had a greenish grey to pale olive-grey thallus rather similar to morphogroup 2, but differing by immersed to semi-immersed perithecia (never protruding) and a thallus rimose to areolate up to the margin (morphogroup 2 tends to be rimose-areolate only in the centre of the thallus). Morphogroup 2 (CG1871, CG1874, CG1878, CG1885, CG1909, CG1922, CG1940a, CG1943, CG1945, CG1947, CG1957a, CG1957b; Fig. 1D–F) was characterized by protruding perithecia. Morphogroup 3 (CG1877 and CG1912; Fig. 1G & H) differed from all other morphogroups by its dark greyish brown to black and clearly areolate thallus, and its perithecia with an involucrellum appressed to the excipulum and covering the upper part of the perithecium down to half of its height or entirely surrounding the perithecium by fusing with the black basal layer. Morphogroup 4 (CG1852; Fig. 1I) had a continuous and rather thick thallus, whereas all other morphogroups had rimulose to areolate thalli.

The Vietnamese specimens were compared to the type material of the following eight species of *Staurothele*: *S. australis*, *S. diffractella* [syn. *Endocarpon diffractellum* (Nyl.) Gueidan & Cl. Roux], *S. iwatsukii*, *S. japonica*, *S. malayensis*, *S. microlepis*, *S. pallidopora*, *S. rimosa*. Morphogroup 2, with its dark brown areolate thallus, and morphogroup 3, with its protruding perithecia, did not match any of these species. Morphogroup 4 was rather similar to *Staurothele malayensis* (both var. *malayensis* and var. *sulphurea* Zahlbr.). These two species have immersed perithecia and a similar continuous thallus, only occasionally thinly cracked here and there. However, in *S. malayensis*, the perithecia were smaller (centrum only up to 0.2 mm wide for *S. malayensis* and up to 0.6 mm for morphogroup 4), the ascospores smaller (23–25 × 10 µm for *S. malayensis* and 25–29 × 11–15 µm for morphogroup 4), the involucrellum was spreading laterally and not appressed to the excipulum (closely appressed to the excipulum wall in morphogroup 4) and the thallus was thinner (50–100 µm for *S. malayensis* and 150–600 µm

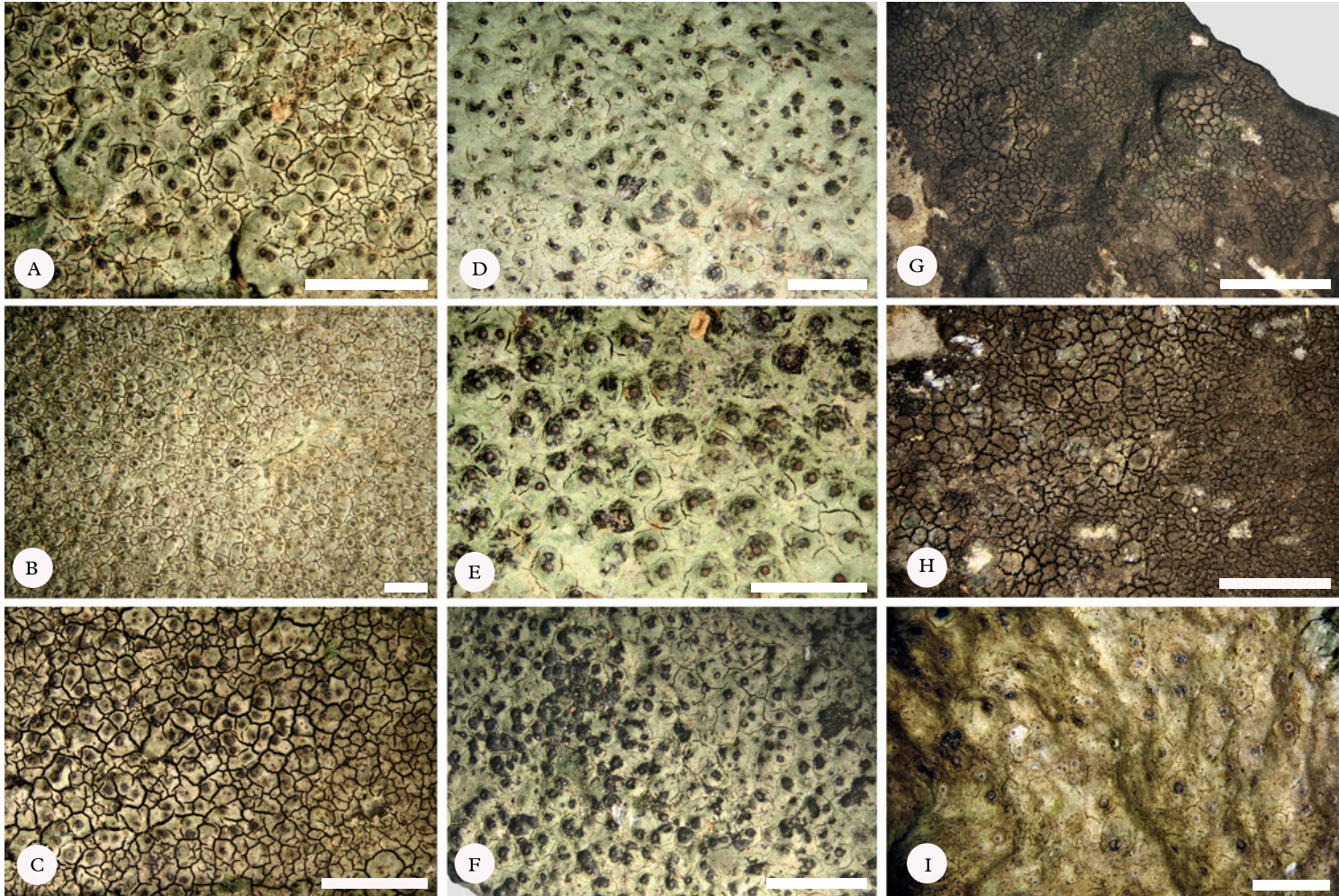


FIG. 1. Photographs showing the colour and structure of the thallus upper surface for the four morphogroups. A–C, greenish grey rimose to areolate thallus in morphogroup 1 (*W. pallidopora*); A, CG1926; B, CG1927; C, CG1865. D–F, greyish green rimose to subareolate thallus with protruding perithecia in morphogroup 2 (*W. protrudens*); D, CG1943; E, holotype CG1945; F, CG1885. G & H, dark greyish brown areolate thallus in morphogroup 3 (*W. fusca*); G, CG1912; H, holotype CG1877. I, greyish to brownish green continuous thallus with entirely immersed perithecia in morphogroup 4 (*W. laevigata*, holotype CG1852). Scales: A–I = 3 mm.

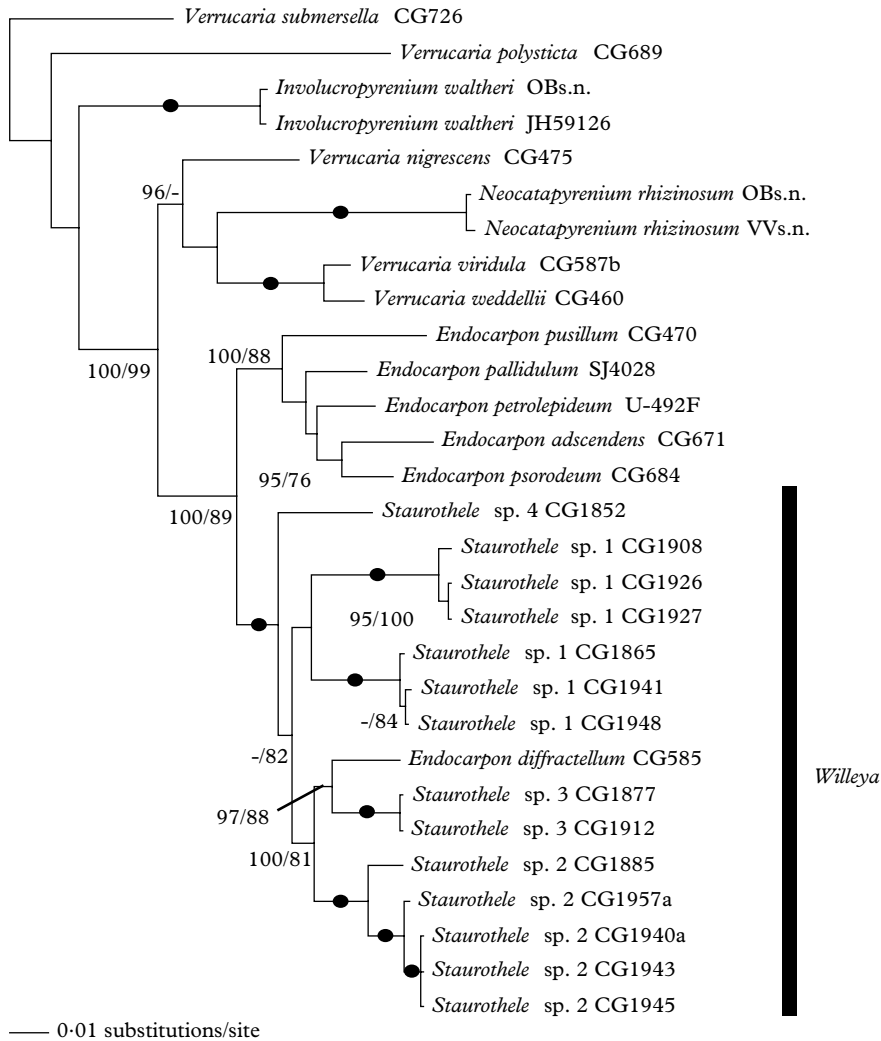


FIG. 2. Most likely tree of the *Endocarpon*-group obtained with a two-gene (ITS-nuLSU) dataset and a Bayesian approach (MrBayes). All specimens of *Staurothele* from Vietnam (morphogroups 1 to 4) belong to a clade sister to *Endocarpon*, thereafter named *Willeya*. *Verrucaria submersella* was selected as the outgroup. High support values (100% PP and BS) are indicated by a dot on branches, and other support values in the following order: PP/BS.

for morphogroup 4). Finally, morphogroup 1 could be matched with the Australian species *S. pallidopora*. In the same way as the types of *S. diffractella*, *S. microlepis* and *S. japonica* have a thallus with contiguous to partly dispersed areoles and globose hymenial algae.

Molecular study

The combined dataset (ITS-nuLSU; first analysis) included 2165 characters (653 for ITS and 1512 for nuLSU), of which 1748 were constant and 295 were parsimony-informative. The most likely tree is presented in Figure 2 with posterior probabilities (PP)

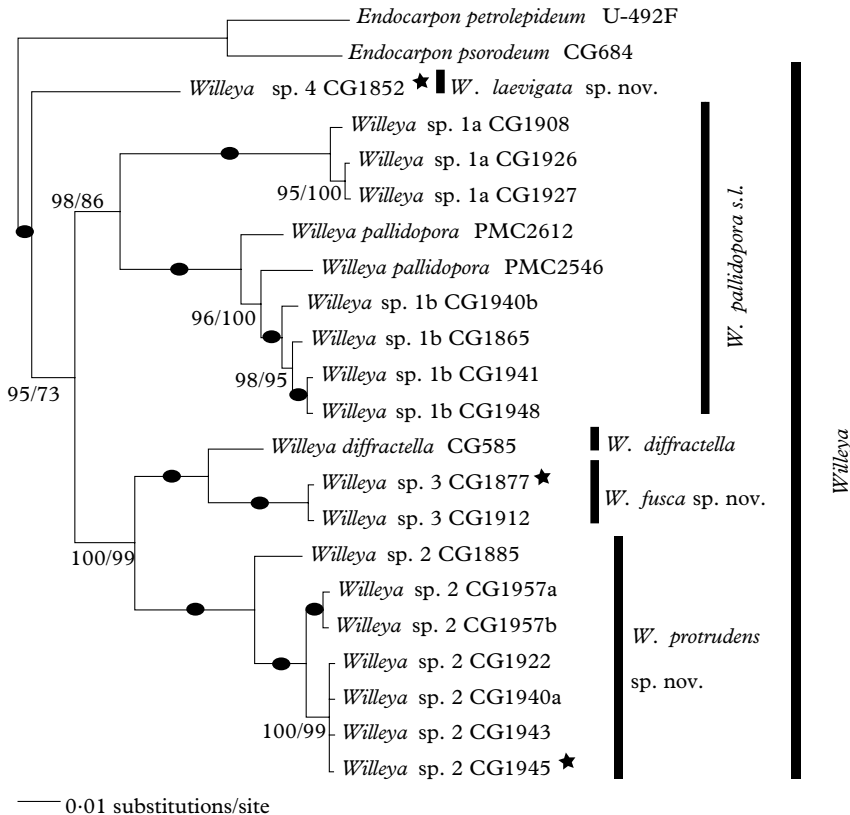


FIG. 3. Most likely tree of *Willeya* obtained with a single-gene (ITS) dataset and a Bayesian approach (MrBayes). Five species can be delimited, including *W. pallidopora*, *W. diffractella* and three new species from Vietnam. Two species of *Endocarpon* (*E. petrolepideum* and *E. psorodeum*) were used as the outgroup. High support values (100% PP and BS) are indicated by a dot on branches, and other support values in the following order: PP/BS. Type specimens are highlighted by a star.

and RAxML bootstrap values (BS). All Vietnamese specimens cluster in a well-supported monophyletic group (100% PP and BS), together with *E. diffractellum*. This group is well supported as sister to the genus *Endocarpon* (100% PP and 89% BS). As *E. diffractellum* (previously *S. diffractella*) is the type of *Willeya* (Müller Argoviensis 1883), the Vietnamese specimens are referred below to this genus.

The ITS dataset (second analysis) included 852 characters, of which 191 were parsimony-informative and 598 were constant. The most likely tree is presented in Figure 3 with posterior probabilities and RAxML bootstrap values. The genus *Willeya* forms a well-supported

monophyletic group (100% PP and BS). Although a few basal nodes were not supported, most other relationships obtained both high Bayesian and bootstrap support. Specimens attributed to morphogroup 1 formed a well-supported monophyletic lineage together with the two Australian specimens of *S. pallidopora* (98% PP and 86% BS). This group was divided into two well-supported and genetically distant groups, 1a and 1b (each with 100% PP and BS). The two Australian specimens of *Staurothele pallidopora* sequenced here are nested within group 1b. However, with our current taxon sampling, we were not able to clearly distinguish members of the two groups 1a and 1b

using morphological and anatomical characters. Moreover, morphological variation was found amongst the four specimens of *S. pallidopora* studied (*P. M. McCarthy* 2612, *P. M. McCarthy* 2546, *P. M. McCarthy* 760 and the holotype *P. M. McCarthy* 768). This species therefore most probably corresponds to a species complex, which we will refer to as *S. pallidopora* s. lat. All specimens from morphogroup 2 cluster together in a well-supported monophyletic group (100% PP and BS). The two specimens of morphogroup 3 also cluster together (100% PP and BS). Finally, morphogroup 4 forms the earliest diverging species in *Willeya*.

Discussion

Recent studies on the lichen family *Verrucariaceae* have focused on testing generic boundaries using molecular data (Gueidan *et al.* 2007, 2009; Savić & Tibell 2008; Savić *et al.* 2008; Muggia *et al.* 2010; Prieto *et al.* 2010, 2012). Most of the taxa studied were collected from temperate to cold-temperate areas, where they are known to be particularly diverse. However, several taxonomic studies have suggested that some genera of *Verrucariaceae* are also common in tropical and subtropical areas (McCarthy 1995; Harada & Wang 1996, 2006). Our work on the lichen flora in Vietnam confirms that *Verrucariaceae* are present on limestone in northern Vietnam. In particular, the genus *Staurothele* s. lat. was commonly found on small calcareous rock outcrops, either exposed or under forest cover.

Molecular data obtained from these tropical *Staurothele* changed part of the current generic classification of the *Verrucariaceae*. Our results show that *E. diffractellum* is not the only *Staurothele*-like (and therefore crustose) species closely related to the genus *Endocarpon*. In fact, all Vietnamese specimens of *Staurothele* for which we obtained molecular data in this study cluster with *E. diffractellum*. Together with *Staurothele pallidopora*, these Vietnamese *Staurothele* and *E. diffractellum* form a well-supported monophyletic group, which is genetically rather diverse and sister to all other species of *Endocarpon*.

As the type of *Staurothele* (*S. clopima*) belongs to a different group within *Verrucariaceae* (the *Staurothele*-group; Gueidan *et al.* 2007, 2009), it is necessary to rename *S. pallidopora*. It would be possible to transfer all these *Staurothele* to the genus *Endocarpon*, but because the two clades obtained in our phylogeny are both well supported by molecular data and morphologically well characterized by their thallus structure (crustose vs squamulose) and their upper cortex (pseudocortex vs eucortex), it is preferable to find a different genus name for the crustose clade. Because the genus *Endocarpon* was not officially emended when the crustose species *Staurothele diffractella* was transferred to this genus (Gueidan *et al.* 2007, 2009), no further emendation will be done here as this species is now placed in a different genus.

Several synonyms of *Staurothele* are available, but most of them were based on type material with dark ascospores (*Goidanichia*, *Goidanichiomyces*, *Paraphysorma*, *Sphaeromphale* and *Stigmatomma*). The type of *Polyblastiomyces* (*P. catalepae* Ach.) was shown to correspond to *Verrucaria aethiobola* and is therefore not available. *Willeya*, a genus described for *Staurothele* with pale ascospores based on *S. diffractella* (Müller Argoviensis 1883), is available. *Phalostauris* was also based on *S. diffractella* (Clements 1909), and is thus a later synonym of *Willeya*. We therefore decided to resurrect the genus *Willeya*, reinstate the name *Willeya diffractella* for *E. diffractellum*, and propose a new combination for *S. pallidopora* and for the seven other taxa for which the type material was studied. We also describe here three new species of *Willeya* from Vietnam and provide a key to Vietnamese species of *Willeya*.

Taxonomy

Willeya Müll. Arg.

Müll. Arg. (1883): 345; type: *Willeya diffractella* (Nyl.) Müll. Arg.

Phalostauris Clem. (1909): 39, 173; type: *Phalostauris diffractella* (Nyl.) Clem.

Description. The genus *Willeya* includes saxicolous lichenized species with a crustose epilithic thallus, a pseudocortex, perithecia

characterized by the presence of algal cells in the hymenium and pale ascospores. As in all members of the family *Verrucariaceae*, the hamathecium consists of short pseudoparaphyses bordering the upper part of the perithecial cavity, and periphyses in the ostiolar canal. Based on one observation of *Willeya iwatsukii* (Harada 1992), pycnidia are of the *Endocarpon*-type (*sensu* Janex-Favre & Wagner 1986). Species of *Willeya* are associated with stichococcoid algae from the green-algal genus *Diplosphaera*.

Notes. *Willeya* differs from the squamulose *Endocarpon* species by its crustose thallus and pseudocortex, and from other epilithic *Staurothele* by the hyaline to pale yellow or pale brown ascospores. Ascospores in *Staurothele* s. str. can be pale in the early stages of development, but become dark brown at maturity. We currently attribute the following 13 taxa to *Willeya*, but other epilithic *Staurothele* with pale ascospores (e.g., *S. effigurata* Thomson, *S. lecideoides* B. de Lesd., *S. polygonia* B. de Lesd.) might also belong to this genus. These species are not transferred to *Willeya* here because their type material was not studied. Except for *Willeya diffractella*, all species here placed within *Willeya* are from Asia or Australia. Although the biogeographical context of this group is poorly known, it is unlikely that *Willeya* is restricted to this part of the world. Similarly, although most species of *Willeya* studied here were found on calcareous rock, it is possible that some species can grow on other types of rock. A world revision of this group will be necessary to clarify the substratum preference, ecology and distribution of species within this genus.

***Willeya australis* (Groenh.) Gueidan comb. nov.**

Mycobank No.: MB807218

Staurothele australis Groenh., *Reinwardtia* 2: 390 (1954); type: East Java, Malang, falls of the Brantas River near Sengguruh, c. 200 m, October 1936, *Groenhardt* 94 (L—holotype!).

Note. Groenhardt (1954) described this species as a *Staurothele* but also suggested that it might belong to *Willeya*.

***Willeya diffractella* (Nyl.) Müll. Arg. var. diffractella**

Flora 66: 345 (1883).—*Verrucaria diffractella* Nyl., *Mém. Soc. Acad. Maine-et-Loire* 4: 33 (1858).—*Staurothele diffractella* (Nyl.) Tuck., *Gen. Lich.*: 258 (1872).—*Endocarpon diffractellum* (Nyl.) Gueidan & Cl. Roux, *Mycological Research* 111: 1157 (2007); type: Nova Anglia, ad schistes micaceas, *Frost* 44, ex *Tuckerman* 134 (H-NYL 3645—lectotype!).

Note. For a description of this species and additional taxonomic information, see Thomson (1991) and Gueidan *et al.* (2007).

Additional specimen examined. USA: Missouri: Perry County, Seventy-Six Conservation Area, 37°42'58"N, 89°36'59"W, alt. 125–150 m, on calcareous rocks, 2003, C. Gueidan 585 (NY).

***Willeya diffractella* (Müll. Arg.) Gueidan var. flavicans comb. nov.**

Mycobank No.: MB807219

Staurothele diffractella var. *flavicans* Müll. Arg., *Flora, Jena* 71: 548 (1888); type: Brasilia, Iporanga ad fluvium Iguape, 1888, *Puiggari* s.n. (G—holotype!).

Note. The variety *flavicans* differs from the variety *diffractella* by its rimose to sub-areolate thallus. A further study of the species delimitation of *W. diffractella* is needed to confirm the status of these two varieties.

Additional specimens examined. Brazil: São Paulo: Apiahy, 1880, *Puiggari* s. n., (G); Apiahy, os Pinheyros, Outubro 1879, *Puiggari* s. n. (G); Iporanga ad fluvium Iguape, 1888, *Puiggari* s. n. (G 2227); Iporanga ad fluvium Iguape, 1888, *Puiggari* s. n. (G).—Costa Rica: dans le Rio Grande à Boruca, 1893, *Tonduz* s. n. (G).—USA: New York, 1976, *H. Willey* 1872 (G).—Tonkin: 1889, *Hariot* s. n. (G).

***Willeya fusca* Gueidan sp. nov.**

Mycobank No.: MB807220

Differing from other species of *Willeya* by its dark brown areolate thallus.

Type: Vietnam, Hòa Bình Province, Mai Châu District, Pà Cò County, Hang Kia-Pà Cò Nature Reserve, on calcareous outcrops in a deforested area, 24 February 2011, C. Gueidan 1877 (BM—holotype). ITS barcode: KF959805

(Fig. 4A–C)

Thallus crustose, epilithic, determinate, matt, sometimes greyish-pruinose due to the presence of an epinecral layer, smooth, dark greyish brown to black, becoming slightly

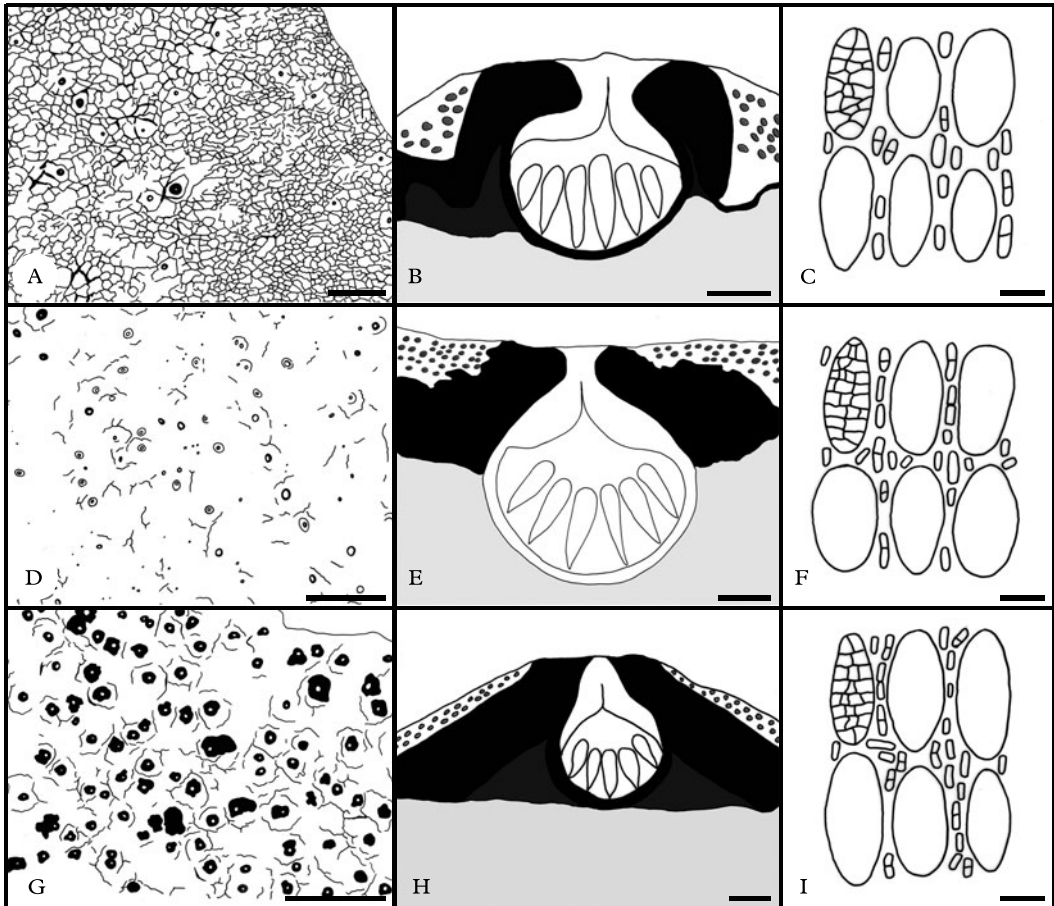


FIG. 4. Drawings of the main diagnostic characters of the three new *Willeya* species from Vietnam. *Willeya fusca* CG1877 (holotype): A, areolate thallus; B, perithecium immersed in an areole; C, muriform ascospores and elongated to cylindrical hymenial algal cells. *Willeya laevigata* CG1852 (holotype): D, continuous to slightly rimose thallus; E, entirely immersed perithecium; F, muriform ascospores and elongated to cylindrical hymenial algal cells. *Willeya protrudens* CG1945 (holotype): G, rimose to subareolate thallus; H, protruding perithecia; I, muriform ascospores and elongated to cylindrical hymenial algal cells. Scales: A, D & G = 3 mm; B, E & H = 100 μ m; C, F & I = 10 μ m.

darker when wet, rimose-areolate, 0.10–0.25 mm thick, areoles 0.1–0.5 mm diam., often larger when fertile (0.5–1.0 mm). *Upper cortex* absent to thin (<10 μ m) and weakly differentiated from the algal layer (pseudocortex, as defined in Gueidan *et al.* 2007), with hyaline to pale brown rounded cells (4–6 μ m diam.) and a thin (5–10 μ m) epinecral layer. *Algal layer* 25–100 μ m thick, with a green *Diplosphaera*-like alga, \pm globose, 6–8 μ m diam., single to clustered into

pairs, scattered throughout the algal layer. *Medulla* mostly absent, but sometimes present, and then undifferentiated and formed of hyaline cells similar to those in the algal layer and upper cortex (5.0–7.5 μ m diam.). Carbonaceous layer often present below the algal layer and the medulla and on the side of the areoles, as well as forming columns within the areoles. *Prothallus* sometimes apparent at the thallus margin, dark brownish to greenish grey.

Perithecia immersed in the thallus, 0.4–0.6 mm, one per areole, forming only slight projections, with only the black ostiole visible at the thallus surface. *Involucrellum* black, appressed to the excipulum wall and covering the upper part of the perithecium down to half the height to entirely covering the perithecium by fusing with the black basal layer, 50–150 µm thick, contiguous with the excipulum to spreading laterally at the base (space between involucrellum and excipulum then filled with cells with thick melanized walls, 4–7 µm diam.). *Centrum* ± globose, 250–300 µm diam. *Excipulum* dark brown to black, 10–25 µm thick. *Periphyses* present in ostiolar canal, pale brown, unbranched, septate, 30–40 × 2–3 µm. *Pseudoparaphyses* lining the upper part of the perithecial cavity, unbranched, septate, sometimes swollen at the apex, 20–45 × 1.5–3.0 µm. *Interascal filaments* absent at maturity, reduced to a KI+ blue hymenial gel. *Hymenial algal cells* elongated to cylindrical (2.5–)3.5–7.0 (–8.0) × (1.8–)2.0–2.5(–3.0) µm. *Asci* clavate, fissitunicate, 8-spored, 75–90 × 15–20 µm. *Ascospores* colourless to pale yellow, narrowly to broadly ellipsoid, muriform, (20–)22–28(–30) × (9–)10–13(–15) µm.

Pycnidia not seen.

Etymology. The epithet *fusca* refers to the dark brown to black colour of the upper surface of this species.

Note. Two rather old perithecia from specimen CG1912 did not have hymenial algal cells.

Additional specimen examined. **Vietnam:** Hòa Bình Province: Mai Châu District, Pà Cò County, Hang Kia-Pà Cò Nature Reserve, on calcareous rocks, 2011, *C. Gueidan* 1912 (BM, VNMN).

***Willeya iwatsukii* (Harada) Gueidan comb. nov.**

Mycobank No.: MB807222

Staurothele iwatsukii Harada, *Natural History Research* 2: 39 (1992); type: Japan, Shikoku, Kôchi-ken, Takaokagun, Yusushara-chô, Jôsei, 450 m alt., on rock at the edge of the stream, 2 August 1985, *H. Harada* 3401 (HIRO—holotype!; NMW—isotype; CBM—istotype).

***Willeya japonica* (B. de Lesd.) Gueidan comb. nov.**

Mycobank No.: MB807223

Staurothele japonica B. de Lesd., *Bull. Soc. Bot. Fr.* 68: 494 (1921); type: Japan, Hokkaido, Kamuikotan, on rock, September 1904, *U. Faurie* 6213 (KYO—lecto-type!).

***Willeya laevigata* Gueidan sp. nov.**

Mycobank No.: MB807224

Differing from *Willeya malayensis* in having larger ascomata and spores, a thicker thallus and an involucrellum closely appressed to the excipulum wall, as opposed to not appressed to the excipulum wall and laterally spreading.

Type: Vietnam, Hòa Bình Province, Mai Châu District, Pà Cò County, close to the limit with Son La Province, on shaded calcareous outcrops within the rainforest, 25 February 2011, *C. Gueidan* 1852 (BM—holotype). ITS barcode: KF959807

(Fig. 4D–F)

Thallus crustose, semi-endolithic, determinate, matt, ± smooth, greyish to brownish green, becoming olive-green when wet, continuous to rimose here and there, thick (0.15–0.60 mm). *Upper cortex* thin (5–15 µm) and weakly differentiated from the algal layer (pseudocortex), with hyaline to pale brown rounded to angular cells (2–6 µm diam.). *Algal layer* 25–60 µm thick, with a green *Diplosphaera*-like alga, ± globose, 4–8 µm diam., single or clustered in pairs, ± organized in columns. *Medulla* thick (250–500 µm), endolithic, interspersed with rock crystals throughout, prosoplectenchymatous to paraplectenchymatous, with cells 5.0–7.5 µm diam. Basal carbonaceous layer absent. *Prothallus* not apparent.

Perithecia entirely immersed in the thallus, not forming projections, large (c. 0.6 mm wide), with an ostiole visible on the thallus surface. Ostiole pale brown, sometimes surrounded by a black involucrellar ring. In older perithecia, an additional brown ring, resulting from the pigmentation of the upper part of the excipulum, can also be seen inside the black involucrellar ring. *Involucrellum* black, appressed to the excipulum wall and covering the upper part of the perithecium

down to a third or half of its height, enlarging laterally at the lower extremities, interspersed with rock crystals, up to 200–300 µm thick, scleroplectenchymatous, with melanized and thick-walled cells (7.5–10.0 µm diam.). *Centrum* globose, 400–600 µm diam. *Excipulum* pale, but becoming dark brown around the ostiole in older perithecia, 10–20 µm thick. *Periphyses* present in ostiolar canal, pale brown, unbranched, septate, 25–40 × 2–3 µm. *Pseudoparaphyses* lining the upper part of the perithecial cavity, unbranched, septate, 40–50 × 1.5–3.0 µm. *Interascal filaments* absent at maturity, reduced to a KI+ blue hymenial gel. *Hymenial algal cells* elongated to cylindrical, sometimes in pairs or short filaments, 2.0–8.0 × 1.5–3.0 µm. *Asci* clavate, fissitunicate, 8-spored, 90–100 × 20–30 µm. *Ascospores* colourless to pale, narrowly to broadly ellipsoid, muriform, (20–)25–29(–31) × 11–15 µm.

Pycnidia not seen.

Etymology. The epithet *laevigata* refers to the rather smooth appearance of the upper surface of this species.

Willeya malayensis* (Zahlbr.) Gueidan comb. nov. var. *malayensis

Mycobank No.: MB807225

Staurothele malayensis Zahlbr. var. *malayensis*, *Archiv für Hydrobiol. vol. suppl.* 12: 732 (1934); type: Mittel-Java, Wasserfall des Kali Djumok bei Sarangan am Südost-Hang des Gunung Lawu, 1450 m, 10 December 1928, *F. Ruttner* s. n. (W 6035—lectotype designated here!).

Staurothele malayensis var. *sulphurea* Zahlbr., *Archiv für Hydrobiol. vol. suppl.* 12: 733 (1934); type: West-Java, Bach Kali Tjiwalen bei Tjibodas, 1350 m, auf Urgestein, 10 July 1929, *F. Ruttner* s. n. (W 6040—holotype!).

Note. The type material included five morphologically homogeneous specimens, except for two that had old and partially eroded thalli with deep cracks (W 6042, W 6037).

Additional specimens examined. Indonesia: West Java: Bach Kali Tjiwalen bei Tjibodas, 1350 m, 10 vii 1929, *F. Ruttner* s. n. (W 6042). *Süd-Sumatra:* Ranau-See, Felsblock am Hauptzufluss (Wai Warku), 550 m, 4 ii 1929, *F. Ruttner* s. n. (W 6036); Stromgebiet des Musi, Bachbett unterhalb des Wasserfalls Kapala Tjurup, 500 m, 5 v 1929, *F. Ruttner* s. n. (W 6039); Musi bei Moara Klingi, c. 50 m, Konglomeratbank am Ufer. an allen Standorten auf Urgestein, 10 v 1929, *F. Ruttner* s. n. (W 6037).

***Willeya malayensis* var. *vegetior* (Zahlbr.) Gueidan comb. nov.**

Mycobank No.: MB807226

Staurothele malayensis var. *vegetior* Zahlbr., *Archiv für Hydrobiol. vol. suppl.* 12: 733 (1934); type: Süd-Sumatra, Musi, an Blöcken knap punter Wasser bei Aër Simpang, 700 m, 6 May 1929, *F. Ruttner* s. n. (W 6038—lectotype designated here!).

Note. This taxon is still recognized here as a variety of *Willeya malayensis* but might need to be raised to the species level in the future if molecular data confirm that it is a different species.

Additional specimen examined. Indonesia: Süd-Sumatra: Ranau-See, Felsblock am Hauptzufluss (Wai Warku), 550 m, 4 ii 1929, *F. Ruttner* s. n. (W 6041).

***Willeya microlepis* (Zahlbr.) Gueidan comb. nov.**

Mycobank No.: MB807227

Staurothele microlepis Zahlbr. apud Handel-Mazzetti, *Symbol. Sinic. pars III:* 15 (1930); type: China, Yunnan, Kalksteine in der wtp. St. bei Hsinyingpan zwischen Yungbei und Yungning, 2750 m, 27 June 1914, *A. Zahlbruckner* 3247 (WU—holotype!).

***Willeya pallidopora* (P. M. McCarthy) Gueidan comb. nov.**

Mycobank No.: MB807228

Staurothele pallidopora P. M. McCarthy, *Muelleria* 8: 275 (1995); type: Australia, Queensland, Darling Downs district, Bunya Mountains National Park, 50 m below Little Falls, 26°52'S, 151°35'E, on dry shaded siliceous rocks, 5 September 1993, *P. M. McCarthy* 768 (MEL—holotype!; BRI—isotype).

Note. High genetic and morphological diversity suggest that *W. pallidopora* is a species complex, and is therefore in need of revision.

Additional specimens examined. Australia: Queensland: Moreton district, Bunya Mountains National Park, between Paradise Falls and Little Falls, 26°52'S, 151°35'E, on deeply shaded aquatic and semi-aquatic rocks, 1993, *P. M. McCarthy* 760 (MEL); Cook district, Atherton Tableland, car park at Millaa Lillaa Falls, 750 m altitude, 17°29'44"S, 145°36'41"E, on damp, steep rock face, 2006, *P. M. McCarthy* 2546 (CANB); Cook district, Wooroonooran National Park, tributary of North Johnstone River, above Wallicher Falls, 35 km W of Innisfail, 300–350 m altitude, 17°36'18"S, 145°46'21"E, on moderately shaded riverside siliceous rocks, 2006, *P. M. McCarthy* 2612 (CANB).—**Vietnam:** *Tuyên Quang Province:* Na Hang District, Na

Hang Nature Reserve, Ban Bung village, on calcareous outcrops, 2011, *C. Gueidan* 1908, 1926, 1927 (BM), 1938 (VNMN); Na Hang Nature Reserve, near the lake after the dam, on calcareous outcrops, 2011, *C. Gueidan* 1940b, 1941, 1948 (BM). *Hà Giang Province*: Bắc Mê District, Lạc Nông County, forested trail along the river, on calcareous outcrops, 2011, *C. Gueidan* 1865, 1868 (BM), 1870 (VNMN). *Hòa Bình Province*: Mai Châu District, Pà Cò County, close to the limit with Sơn La Province, on shaded calcareous outcrops within the rainforest, 2011, *C. Gueidan* 1850 (BM).

Willeya protrudens Gueidan sp. nov.

Mycobank No.: MB807229

Differing from other species of *Willeya* by its perithecia characteristically forming projections.

Type: Vietnam, Tuyên Quang Province, Na Hang District, Na Hang Nature Reserve, on calcareous outcrops, 4 March 2011, *C. Gueidan* 1945 (BM—holotype; VNMN—isotype). ITS barcode: KF959802

(Fig. 4G–I)

Thallus crustose epilithic, determinate, matt, smooth, greyish green to olive-brown, green to olive-green when wet, continuous to rimose at the periphery and rimose to sub-areolate at the centre. Irregular areoles and deeper cracks mostly found around the perithecia, with one perithecium per areole. Areoles 0.2–1.0 mm diam. Thallus thin at the margin (50–100 µm), but thicker around the perithecia (200–300 µm) due to the presence of a black basal layer. *Upper cortex* thin (10–20 µm) and weakly differentiated from the algal layer (pseudocortex), with hyaline cells rounded to angular, 4–6 µm diam. *Algal layer* 40–60 µm thick, with a green *Diplosphaera*-like alga, ± globose, 4–10 µm diam., single or by pair, scattered throughout the algal layer. *Medulla* absent. Black basal layer present at the centre of the thallus when perithecia densely aggregated, 200–250 µm thick, possibly deriving from laterally spreading involucrellae. *Prothallus* pale but becoming dark brown when contiguous with other lichen thalli.

Perithecia protruding, entirely or only partly covered by the thallus, 0.3–0.8 mm, characteristically forming projections. Ostiole visible, brown, often surrounded by a ± large black involucrellar ring. *Involucrellum* black, spreading laterally and not contiguous with the excipulum, 75–175 µm thick. Space between involucrellum and excipulum filled with cells with thick melanized walls, cells 4–8 µm diam. *Centrum* ± globose, 300–400 µm diam. *Excipulum* brown to black, 10–25 µm thick. *Periphyses* present in the ostiolar canal, pale brown, unbranched, septate, 30–40 × 2–3 µm. *Pseudoparaphyses* lining the upper part of the perithecial cavity, unbranched, septate, 25–100 × 2–3 µm. *Interascal filaments* absent at maturity, reduced to a KI+ blue hymenial gel. *Hymenial algal cells* elongated to cylindrical, sometimes in pairs or short filaments, (3–)4–9(–12) × 1.5–3.0 µm. *Asci* clavate, fissitunicate, 8-spored, 60–90 × 20–30 µm. *Ascospores* colourless to pale, narrowly to broadly ellipsoid, muriform, (20–)22–30(–32) × (9–)10–14(–15) µm.

Pycnidia not seen.

Etymology. The epithet *protrudens* refers to the projecting perithecia characteristic of this species.

Additional specimens examined. **Vietnam:** *Tuyên Quang Province*: Na Hang District, Na Hang Nature Reserve, Ban Bung village, on calcareous outcrops, 2011, *C. Gueidan* 1909 (BM); Na Hang Nature Reserve, near the lake after the dam, on calcareous outcrops, 2011, *C. Gueidan* 1940a, 1943, 1947 (BM). *Hòa Bình Province*: Mai Châu District, Pà Cò County, Hang Kia-Pà Cò Nature Reserve, on calcareous outcrops in small deforested valley, 2011, *C. Gueidan* 1957a, 1957b, 1871 (BM), 1874, 1878 (VNMN). *Hà Giang Province*: Bắc Mê District, Lạc Nông County, on calcareous outcrops, 2011, *C. Gueidan* 1885, 1922 (BM).

Willeya rimosa Müll. Arg.

Flora 72: 146 (1889).—*Staurothele rimosa* (Müll. Arg.) Zahlbr., *Cat. Lich. Univers.* 1: 176 (1921); type: ad saxa compacto-calcareo in territorio asiatico Tonkin, benevole commun. cl. *Hariot* sub n. 3463 (G—holotype!).

Key to species of *Willeya* from Vietnam

- 1 Thallus dark brown, rimose-areolate **W. fusca**
 Thallus greenish grey to olive-brown, usually pale, continuous to areolate 2
- 2(1) Thallus continuous, rimose only here and there. Perithecia entirely immersed, 0.4–0.6 mm diam **W. laevigata**
 Thallus finely rimose to sub-areolate or areolate. Perithecia immersed to protruding, 0.2–0.4 mm diam. 3
- 3(2) Perithecia projecting above the thallus level, with an involucrellum often only basally covered by the thallus **W. protrudens**
 Perithecia immersed to half immersed, with involucrellum covered by the thallus up to the ostiole. **W. pallidopora**

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