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Chlorovibrissea korfii sp. nov. from northern hemisphere and *Vibrissea flavovirens* new to China

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Abstract

A new species, *Chlorovibrissea korfii*, is described and illustrated, which is distinct from other members of the genus by the overall pale greenish apothecia 0.8–2.0 mm in diam. and 0.6–1.5 mm high, J+ asci 70–83 × 4.5–5.5 µm, and non-septate ascospores 44–52 × 1.2–1.5 µm. This is the first species of *Chlorovibrissea* reported from northern hemisphere. *Vibrissea flavovirens* is reported from China for the first time. Sequence analyses of the internal transcribed spacer of nuclear ribosomal DNA are used to confirm the affinity of the two taxa.

Key words

morphology, sequence analysis, taxonomy, Vibrisseaceae

Introduction

Vibrisseaceae was erected by Korf in 1990 to accommodate the genera *Vibrissea* Fr., *Chlorovibrissea* L.M. Kohn and *Leucovibrissea* (A. Sánchez) Korf. Members of the family are characterized by aquatic or semi-aquatic habitat, apothecia sessile to long stipitate, disc color range from whitish, yellowish, brownish, olivaceous to blackish green, ectal excipulum composed of textura globosa, textura angularis to textura prismatica, and filiform ascospores (Korf 1990). The three genera are distinguishable by the color of apothecia, structure of ectal excipulum and ascal apex apparatus (Korf 1990; Sandoval-Leiva et al. 2014). Besides the above three genera, *Acephala* Grünig

& T.N. Sieber and *Phialocephala* W.B. Kendr. known only by asexual states, are also included in the family (Kirk et al. 2008; Johnston et al. 2014). *Acephala* was erected for the non-sporulating species of *Phialocephala* that contain a small group of dark septate root endophytes (Grünig and Sieber 2005; Grünig et al. 2008; Grünig et al. 2009; Münzenberger et al. 2009; Wang et al. 2009; Day et al. 2012). About 80 species are currently accepted in the family, among which two species are in *Acephala*, six in *Chlorovibrissea*, one in *Leucovibrissea*, 33 in *Phialocephala*, and 36 in *Vibrissea* (Grünig et al. 2008; Kirk et al. 2008; Grünig et al. 2009; Münzenberger et al. 2009; Wang et al. 2009; Day et al. 2012; Johnston et al. 2014; Sandoval-Leiva et al. 2014; Index Fungorum 2017). The previous phylogenetic studies on the vibrisseaceous fungi showed that *Chlorovibrissea* and *Vibrissea* are not closely related, and the family was presumably polyphyletic (Wang et al. 2006; Sandoval-Leiva et al. 2014).

In China, only two Vibrisseaceous fungi were recorded, i.e. *V.* cf. *sporogyra* (Ingold) A. Sánchez from Hainan Province (Zhuang et al. 2002) and *V. truncorum* (Alb. & Schwein.) Fr. from Guizhou Province (He 1988). During our studies of the helotialean fungi from China, two newly collected specimens fit the circumscription of Vibrisseaceae and represent two species. Based on morphology and molecular data, one is proposed as a new species of *Chlorovibrissea*, and the other is identified as *V. flavovirens* (Pers.) Korf & J.R. Dixon which is new to China.

Materials and methods

Specimens were collected from Beijing and Yunnan Province in 2016, and apothecial gross morphology when fresh was photographed by a Canon PowerShot G16 digital camera. Dried apothecia were rehydrated with distilled water and sectioned at a thickness of 15 μm with a Yidi YD-1508A freezing microtome (Jinhua, China). Measurements were taken from longitudinal sections and from squash mounts in lactophenol cotton blue solution using an Olympus BH-2 microscope (Tokyo, Japan). Iodine reactions of ascus apparatus were tested in Melzer's reagent and Lugol's solution with or without 3% KOH solution pretreatment according to Baral (2009). Images were captured using a Canon G5 digital camera (Tokyo, Japan) attached to a Zeiss Axioskop 2 Plus microscope (Göttingen, Germany) for anatomical structure. Voucher specimens were deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS). Name of the new species was formally registered in the database Fungal Names, one of the three official nomenclatural repositories for fungal names (Redhead and Norvell 2012).

Genomic DNA was extracted from dried apothecia using Plant Genomic DNA Kit (TIANGEN Biotech. Co., Ltd., Beijing, China). Materials were crushed in liquid nitrogen before extraction. The internal transcribed spacer of nuclear ribosomal DNA (ITS) were amplified and sequenced using primer pair ITS1 and ITS4 (White et al. 1990) according to Zheng and Zhuang (2016). Newly generated sequences were deposited in GenBank, and other sequences used in the phylogenetic analyses were retrieved from GenBank (Table 1). *Holwaya mucida* (Schulzer) Korf & Abawi was selected as

Table 1. Sequences used in this study.

Species	Strain/specimen	ITS
Acephala applanata Grünig & T.N. Sieber	CBS 109314	AY078146
A. macrosclerotiorum Münzenb. & Bubner	BB11_301_Ah_Pi_150506 (DNA46)	HM189696
Ascocoryne cylichnium (Tul.) Korf	VL272	JF440583
A. sarcoides (Jacq.) J.W. Groves & D.E. Wilson	CBS:309.71	HM152550
Chlorovibrissea albofusca (G.W. Beaton) Sandoval-Leiva, A.I. Romero & P.R. Johnst.	PDD 75692	AY789384
C. albofusca	PDD:88457	JN809648
C. bicolor (G.W. Beaton & Weste) L.M. Kohn	ICMP19895	KF924737
C. chilensis Sandoval-Leiva, A.i. Romero & P.R. Johnst.	PDD 99891	NR_132892
C. phialophora Samuels & L.M. Kohn 1989	PDD:83226	KF429261
C. korfii H.D. Zheng & W.Y. Zhuang	HMAS 275652	**KY924871***
Chlorovibrissea sp.	PDD70070	DQ257353
Hyaloscypha aureliella (Nyl.) Huhtinen	M235	EU940229
H. vitreola (P. Karst.) Boud.	M39	EU940231
Lachnum bicolor (Bull.) P. Karst.	ARON3113.P	AJ430394
L. virgineum. (Batsch) P. Karst.	3706	JF937586
Phialocephala aylmerensis J.B. Tanney & B. Douglas	DAOM C 250106	NR_136124
P. catenospora J.B. Tanney & B. Douglas	DAOM C 250108	NR_136122
P. dimorphospora W.B. Kendr.	CBS 300.62	NR_135931
Vibrissea filisporia (Bonord.) Korf & A. Sánchez	JLF2084	JX415338
V. flavovirens (Pers.) Korf & J.R. Dixon	MBH39316	AY789427
V. flavovirens	N/A	KF429257
V. flavovirens	HMAS 275653	**KY924872**
V. truncorum (Alb. & Schwein.) Fr.	PDD 99892	KF429259
V. truncorum (Alb. & Schwein.) Fr.	PDD 99893	KF429260
Uncultured *Vibrissea*	WPD-OTU-39	KT581681
Uncultured *Vibrissea*	WPW-OTU-33	KT581735
Chlorociboria aeruginosa (Oeder) Seaver ex C.S. Ramamurthi, Korf & L.R. Batra	PDD 81292	AY755360
Chlorociboria sp.	HMAS 273905	KY498614
Cudoniella clavus (Alb. & Schwein.) Dennis	BM18#13	AY789374
Dicephalospora aurantiaca (W.Y. Zhuang) W.Y. Zhuang & Z.Q. Zeng	HMAS 81363	DQ986483
Holwaya mucida (Schulzer) Korf & Abawi	B 70 0009352	DQ257357
Hymenoscyphus scutula (Pers.) W. Phillips	MBH29259	AY789432
Hyphodiscus hymeniophilus (P. Karst.) Baral	TNS:F-31802	AB546951
Ombrophila violacea (Hedw.) Fr.	WZ0024	AY789366
Roseodiscus rhodoleucus (Fr.) Baral	ARON2329.P	AJ430395

* Numbers in bold indicate sequences produced by this study

outgroup taxon. Alignment was generated and manually edited with BioEdit 7.0.5.3 (Hall 1999). Maximum parsimony (MP) and Neighbor-joining (NJ) analyses were carried out using PAUP*4.0b10 with parameters used by Zheng and Zhuang (2014). The

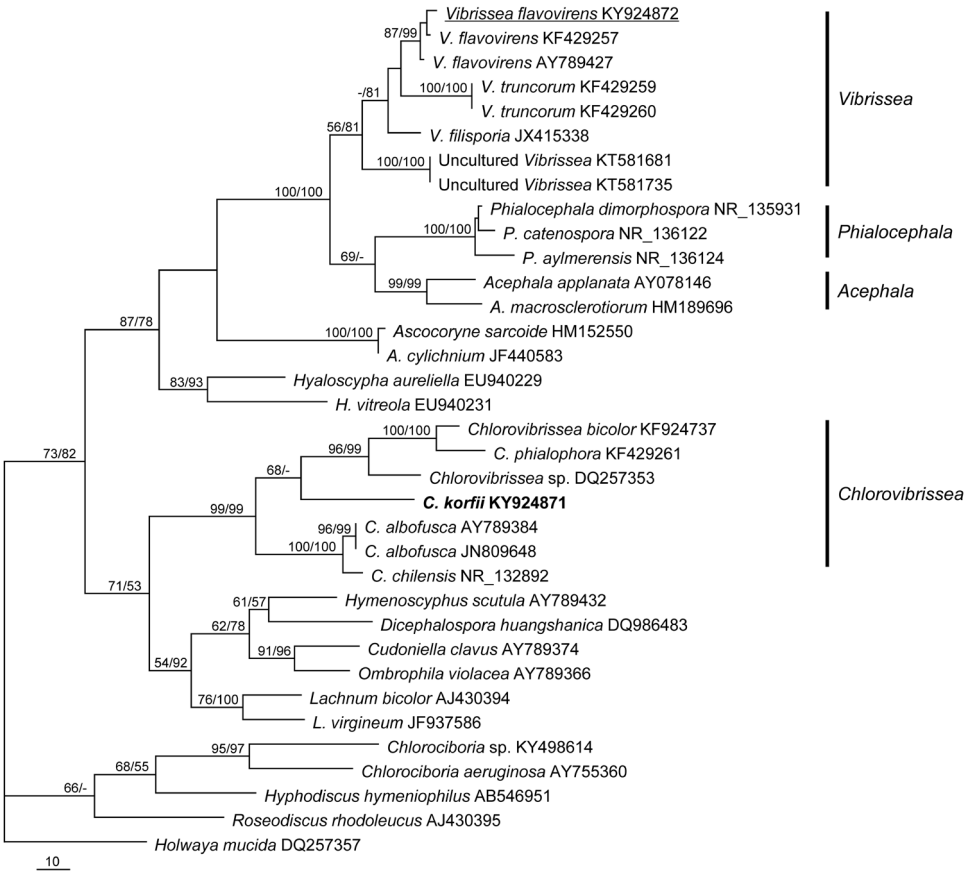

Figure 1. Phylogenetic affinity of *Chlorovibrissea korfii* and *Vibrissea flavovirens* inferred from maximum parsimony analysis of ITS sequences. Bootstrap values (≥50%) of MP and NJ are shown at nodes from left to right.

topological confidence of the NJ and MP trees was assessed with bootstrap analysis using 1,000 replications, each with 10 replicates of random stepwise addition of taxa. The resulting trees were viewed in TreeView 1.6.6 (Page 1996).

Results

Phylogenetic analyses

The ITS alignment consisted of 574 characters including gaps, of which 292 were constant, 52 were variable and parsimony-uninformative, and 230 were parsimony-informative. Forty-five equally most parsimonious trees were yielded by MP analysis (Tree length = 1085, Consistency index = 0.4719, Homoplasy index = 0.5281, Retention

index = 0.6739, Rescaled consistency index = 0.3180) and one of them was shown in Fig. 1. MP and NJ bootstrap proportions (BP) greater than 50% were labeled at the nodes. In the phylogenetic tree (Fig. 1), species of *Acephala*, *Chlorovibrissea*, *Phialocephala* and *Vibrissea* formed four well-supported clades corresponding to each genus, and three of them further clustered together with high supporting values except for *Chlorovibrissea*, which showed a distant relationship with others. The undescribed species appeared as a distinct terminal lineage within *Chlorovibrissea*. ITS sequences of *V. flavovirens* from the Chinese, North American and New Zealand materials were of high similarity (99%) and formed a well-supported terminal branch.

Taxonomy

Chlorovibrissea korfii **H.D. Zheng & W.Y. Zhuang, sp. nov.**
Fungal Names FN570451
Figure 2

Type. CHINA, Yunnan Province, Maguan County, 23°6.22'N, 104°19.75'E, alt. 1450 m, on moist rotten twig, 14 August 2016, X.H. Wang, S.H. Li, H.D. Zheng & S.C. Li YN16-151 (holotype: HMAS 275652).

Etymology. The specific epithet is in memory of the late distinguished mycologist Dr. R.P. Korf.

Description. Apothecia scattered or in cluster, discoid to slightly convex, stipitate, light greenish yellow, with hymenium and receptacle surface concolorous when fresh, 0.8–2.0 mm in diam. and 0.6–1.5 mm high, dried apothecial tissues with exudation of light yellow pigment in water; stipe cylindrical, with base dark, 0.4–1.0 mm high. Ectal excipulum of textura prismatica, non-gelatinous, 30–100 µm thick, hyphae parallel or lying at a low angle to receptacle surface, cells hyaline, with walls slightly thickened, terminal cells of external hyphae thin-walled, 8–25 × 3–7 µm. Medullary excipulum of textura porrecta to textura intricata, 30–300 µm thick, hyphae hyaline, 3–5 µm wide. Subhymenium not distinguishable. Hymenium 95–110 µm thick. Asci arising from simple septa, 8-spored, cylindrical, J+ in Melzer's reagent and Lugol's solution without KOH pretreatment, visible as two short blue lines, 70–83 × 4.5–5.5 µm. Ascospores filiform, tapering slightly from rounded apex to pointed base, hyaline, smooth, multiguttulate, non-septate, in fascicle, 44–52 × 1.2–1.5 µm. Paraphyses filiform, hyaline, unbranched, 1.5–2.0 µm wide, not exceeding the asci.

Notes. Six species have been recorded in the genus *Chlorovibrissea*. *Chlorovibrissea korfii* is different from any known species of the genus in both morphological and phylogenetic evidences. Morphologically, the diagnostic characteristic of the new species is the overall light greenish yellow apothecia. *Chlorovibrissea phialophora* Samuels & L.M. Kohn has ascospores of similar shape and size, but differs in apothecia stipitate-capitate, larger (2–5 mm in diam.) and dark green to nearly black, stipe longer (1–2.5 mm), asci larger (100–123 × 5–6 µm), and apical cells of ascospores forming subglobose to cylindrical phialides within the asci. The new fungus resembles *C. albofusca*

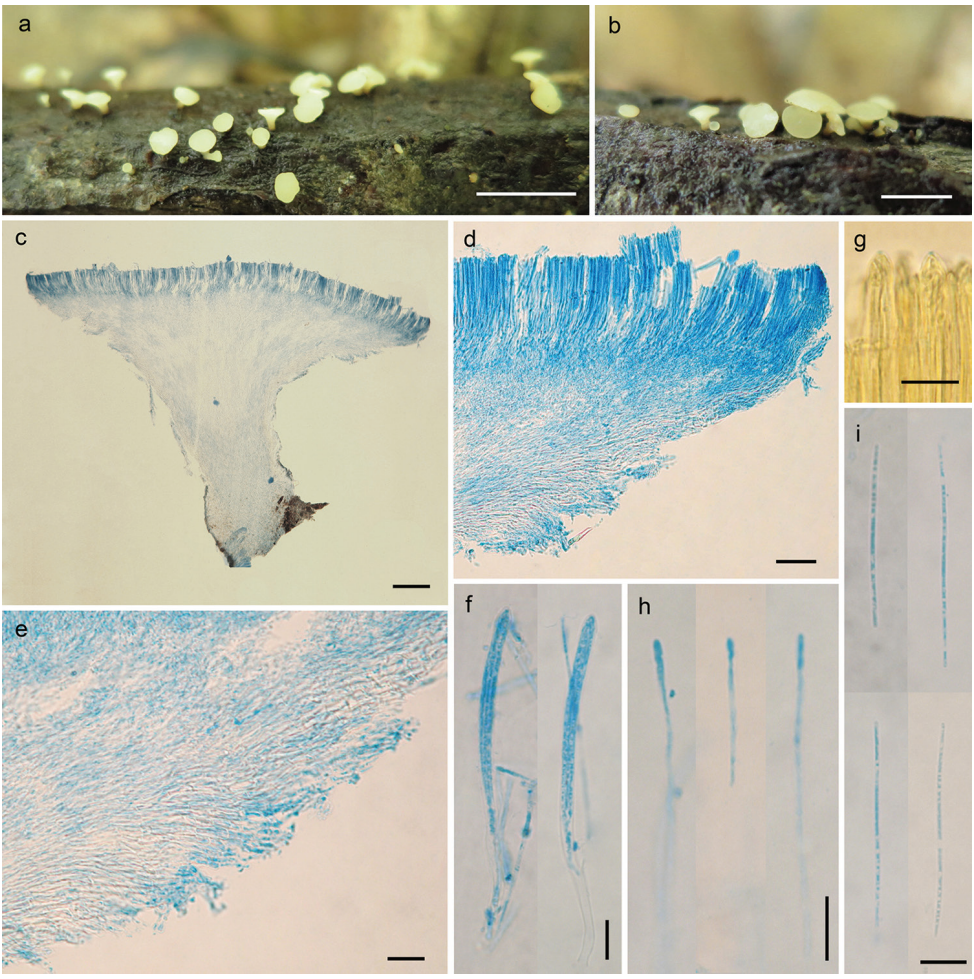

Figure 2. *Chlorovibrissea korfii* H.D. Zheng & W.Y. Zhuang (HMAS 275652, holotype). **a, b** Fresh apothecia on natural substrate **c** Longitudinal section of apothecium **d** Structure of margin, flank and hymenium **e** Excipular structure of flank **f** Asci **g** IKI reaction of apical rings **h** Paraphyses **i** Ascospores. Mouting media: **c–f, h, i** lactophenol cotton blue, **g** Lugol's solution. Scale bars: **a** = 5.0 mm, **b** = 2.0 mm, **c** = 200 μm, **d** = 40 μm, **e** = 20 μm, **f, h, i** = 10 μm, **g** = 5 μm.

(G.W. Beaton) Sandoval-Leiva, A.I. Romero & P.R. Johnst. in having pale colored apothecia, but the latter has larger apothecia (up to 4 mm diam. when dry) with hemispherical cap, larger ectal excipular cells (up to 35 × 15 μm) lying at a high angle to the outer surface, larger asci (195–225 × 7.5–8.5 μm), and larger ascospores (95–115 × 1.5–2 μm). Phylogenetically, *C. korfii* appeared to be related to *C. bicolor*, *C. phialophora* and *Chlorovibrissea* sp. (DQ257353) in the ITS tree (Fig. 1). *Chlorovibrissea bicolor* is different from the new species in apothecia with yellow, subglobose or lobed head up to 4 mm in diam. and a dark green tomentose stalk, larger asci (132–155 × 5–6.5 μm) and ascospores (53–60 × 1.5–2 μm).

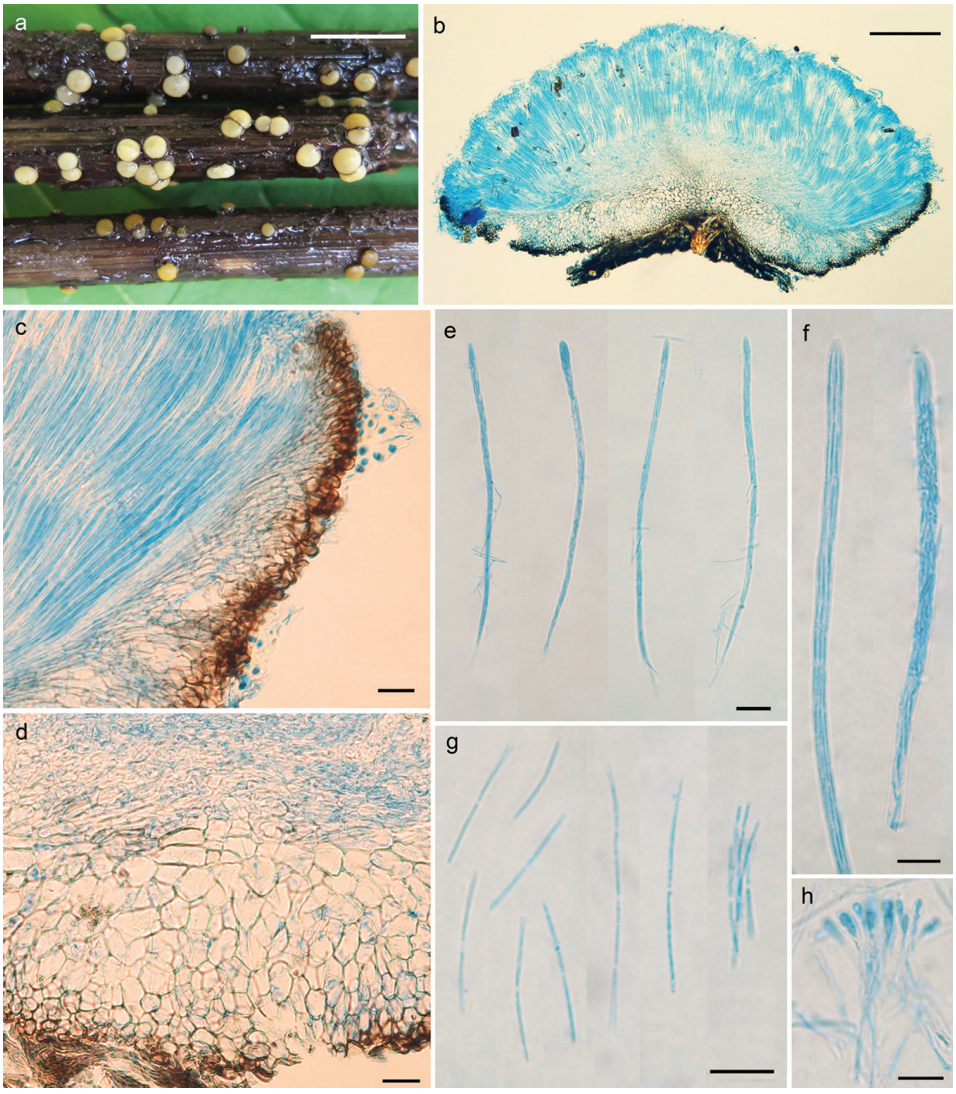

Figure 3. *Vibrissea flavovirens* (Pers.) Korf & J.R. Dixon (HMAS 275653). **a** Fresh apothecia on natural substrate **b** Longitudinal section of apothecium **c** Structure of margin and upper flank **d** Excipular structure of lower part and near base **e, f** Asci **g** Fragments of ascospores **h** Paraphyses. Mouting medium: **b–h** lactophenol cotton blue. Scale bars: **a** = 5.0 mm, **b** = 200 µm, **c–e** = 20 µm, **f–h** = 10 µm.

***Vibrissea flavovirens* (Pers.) Korf & J.R. Dixon, Mycotaxon 1(2): 134, 1974.**
Figure 3

≡ *Peziza flavovirens* Pers., Mycol. Eur. (Erlanga) 1: 323, 1822.

Description. Apothecia scattered or in cluster, slightly convex, sessile, hymenium surface light yellow, 0.5–1.5 mm in diam., receptacle surface brownish. Ectal excipulum

of textura angularis to textura prismatica, non-gelatinous, lying at a high angle to the outer surface, 30–140 μm thick, inner cells subhyaline to light brown and outer cells brown, 15–30 × 9–14 μm. Medullary excipulum of textura angularis to textura prismatica, 50–150 μm thick, cells hyaline, 5–16 × 4–6 μm. Subhymenium not distinguishable. Hymenium 280–290 μm thick. Asci arising from simple septa, 8-spored, cylindrical, J– in Melzer's reagent and Lugol's solution with or without KOH pretreatment, 227–241 × 5–6 μm. Ascospores filiform, hyaline, smooth, multiguttulate, multi-septate, break into several pieces, in fascicle, 192–208 × 1.5–2.0 μm. Paraphyses filiform, slightly enlarged at the apex, hyaline, branched at upper portion, 3.0–5.0 μm wide at apex and 2.0–2.5 μm wide blow, exceeding the asci by 20–35 μm.

Specimen examined. CHINA, Beijing, Yunmeng Moutain, 40°33.00"N, 116°40.80'E, alt. 800 m, on herbaceous stem of unidentified plant submerged in water, 10 July 2016, H.D. Zheng, Z.Q. Zeng, X.C. Wang, K. Chen & Y.B. Zhang 10660 (HMAS 275653).

Notes. This is the first report of *V. flavovirens* from China. The fungus was originally described from France and currently known in Denmark, Germany, Madeira, New Zealand, UK and USA (Korf 1974; Iturriaga 1995; Sandoval-Leiva et al. 2014). The Chinese collection agrees with the description of *V. flavovirens* by Iturriaga (1995). The ITS sequence of the Chinese specimen shared high similarity (99%) with those of North American and New Zealand materials, and the sequences of materials from different geographic regions formed a strongly supported terminal branch (Fig. 1).

Discussion

The three sexual genera in Vibrisseaceae are mainly differentiated by color of apothecia and structure of ectal excipulum. The excipular cells of *Vibrissea* are more or less angular to globose and lying at a high angle to the receptacle surface, while those of *Chlorovibrissea* and *Leucovibrissea* are rectangular and axes of cells are nearly parallel to the receptacle surface. *Chlorovibrissea* is different from *Leucovibrissea* in having greenish apothecia instead of the whitish ones. Differences between *Chlorovibrissea* and *Vibrissea* were found in ascal apex, which is round to somewhat truncate and with the apical ring placed subapically in the former genus, while that of the latter is acute with apical ring placed at the tip (Sandoval-Leiva et al. 2014). The ascal apex of *C. korfii* is somewhat conical and with apical ring placed apically, broader at tip and narrower downward (Fig. 2g), unlike that of other known *Chlorovibrissea* species. Further studies are needed to evaluate whether ascal apical apparatus is phylogenetically informative in Vibrisseaceae.

Chlorovibrissea was assumed to have a southern hemisphere distribution because species of the genus was never reported from north hemisphere (Kohn 1989; Sandoval-Leiva et al. 2014). The discovery of *C. korfii* from China expands the distribution of the genus to north hemispheres, which might break the assumption "the origin of the southern hemisphere vibrisseaceous fungi could be independent from the northern hemisphere representative" (Wang et al. 2006).

In the family Vibrisseaceae, sequence data of *Leucovibrissea* are not available. The two asexual genera Vibrisseaceae are *Phialocephala* and *Acephala*. Due to the heterogeneity of *Phialocephala*, only the type species, *P. dimorphospora*, and two closely related taxa were included in our phylogenetic analyses. *Vibrissea*, *Acephala* and *Phialocephala* clustered as a highly supported group (MPBP/NJBP = 100%/100%, Fig. 1), while *Chlorovibrissea* were distantly related (Fig. 1). The results coincided with those of the previous studies (Wang et al. 2006; Sandoval-Leiva et al. 2014), in which *Chlorovibrissea* and *Vibrissea* appeared as two separate clades. As to relationships among species of *Chlorovibrissea*, *C. albofusca* and *C. chilensis* were very closely related (MPBP/NJBP = 100%/100%), and *C. korfii* is associated with the rest species of the genus, which did not receive a reasonable support (MPBP = 81%). The interspecific relationships of *Vibrissea* were hardly demonstrated (Fig. 1) since very few species were sampled.

The two asexual genera are recognizable and associated each other with low supports (Fig. 1). The two existing species of the genus *Acephala* were sisters (Fig. 1, MPBP/NJBP = 99%/99%). *Acephala* was thought to be congeneric with *Phialocephala* by some authors, and differentiated only by the lack of observed sporulation in culture (Grünig et al. 2009; Münzenberger et al. 2009; Tanney et al. 2016). *Phialocephala* species are commonly isolated as dark-septate endophytes from coniferous tree roots or from decomposing wood (Menkis et al. 2004), and attributed to Vibrisseaceae mainly based on sequence data (Wang et al. 2006; Kirk et al. 2008). Connections of some *Phialocephala* species with *Mollisia* or mollisioid sexual states were reported recently (Grünig et al. 2009; Tanney et al. 2016). It seems that a lot of work needs to be done to establish the generic concepts.

In conclusion, Vibrisseaceae established based on morphology is quite possibly polyphyletic. Sequence data of *Leucovibrissea* are desirable to get a more comprehensive outline of the family. *Phialocephala* s.l. is heterogeneous. Its generic concept needs to be clarified. As more sequences of vibrisseaceous fungi are available, the circumscription of the family will become monophyletic.

Acknowledgements

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