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Phyllobaeis crustacea sp. nov. from China

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Abstract — The lichen-forming fungus genus, *Phyllobaeis,* is reported for the first time from China. A new crustose species, *P. crustacea*, is described and illustrated.

Key words — *Ascomycota*, *Baeomycetaceae*, chemistry, molecular systematics, morphology

Introduction

The lichen-forming fungus genus *Phyllobaeis* is known from five squamulose species (Gierl & Kalb 1993, Index Fungorum 2013) occurring in the Neotropics. During our studies on the lichen flora of China we encountered a species that showed affinities with this genus but deviated conspicuously by its crustose (rather than squamulose) thallus. We present our analysis of the morphology, anatomy, chemistry, and phylogeny of this species to clarify its taxonomy.

Materials & methods

The material used in this study was collected from Hainan, Yunnan, and Qinghai provinces and Xizang (Tibet) autonomous region of China in 2010 and 2012, and from the Antarctica in 2011. The collections examined are preserved in the lichen section of the Herbarium Mycologicum Academiae Sinicae (HMAS-L). A specimen of *Phyllobaeis imbricata* was borrowed from the former Herbarium Universitatis Amstedamensis, now in Museum Naturalis in Leiden (L). A compound microscope (Zeiss Axioskop 2 plus) and a dissecting microscope (Motic SMZ-168) were used for the study of morphology and anatomy. A 10% solution of potassium hydroxide (KOH), a 5% bleaching solution (sodium hypochlorite, NaOCl), concentrated alcoholic *p*-phenylenediamine (PD), Lugol's solution of Iodine, and thin-layer chromatography (TLC) (Culberson & Kristinsson 1970; Culberson 1972; White & James 1985) were used for the detection of lichen substances.

Table 1. Twenty-two nrDNA ITS sequences used in phylogenetic analysis.

Species	Locality; voucher specimen *	GenBank no. ^
Baeomyces placophyllus	Xizang, China; HMAS-L 124223	**KC414621**
	Xizang, China; HMAS-L 124222	**KC414620**
	China; —	DQ001274
Baeomyces rufus	Qinghai, China; HMAS-L 124225	**KC414623**
	Yunnan, China; HMAS-L 124226	**KC414622**
	France; —	AF448457
	France; —	AF448458
Dibaeis absoluta	Hainan, China; HMAS-L 118071	**KC414625**
	Hainan, China; HMAS-L 118073	**KC414626**
Dibaeis baeomyces	—	DQ782844
Dibaeis sorediata	Hainan, China; HMAS-L 118090	**KC414627**
	Hainan, China; HMAS-L 118097	**KC414628**
Icmadophila japonica	Japan; —	AB623070
Phyllobaeis crustacea	Hainan, China; HMAS-L 118086	**KC414614**
	Hainan, China; HMAS-L 118087	**KC414615**
	Hainan, China; HMAS-L 118089	**KC414616**
	Hainan, China; HMAS-L 118095 (holotype)	**KC414617**
	Hainan, China; HMAS-L 118096	**KC414618**
Phyllobaeis imbricata	Carchi, Ecuador; L 0790053	**KC414619**
	—	HQ650635
Placopsis contortuplicata	Antarctica; HMAS-L 124227	**KC414624**
	Antarctica; —	DQ534479

* Missing data indicated with "—". ^ New sequences are shown in bold font.

DNA extraction, PCR amplification and sequencing

The extraction procedure followed the modified CTAB method (Wang et al. 2011). PCR amplifications were performed using a Biometra T-Gradient thermal cycler. The primer pair ITS5 and ITS4 (White et al. 1990) was used to amplify the nrDNA ITS region. Reactions were carried out in 50 µl reaction volume and the components used were 1 µl total DNA, 2 µl each primer (10 µM), 1 µl Taq polymerase (rTaq DNA Polymerase, 5 U/µl), 4 µl dNTP (2.5 mM each), 5 µl amplification buffer (10×, 25 mM $MgCl_2$ contained), 35 µl ddH_2O. Cycling parameters were set to an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 40 s, extension at 72°C for 2 min, and a final extension at 72°C for 10 min. Negative control, without DNA template, was prepared in every series of amplification in order to minimize the possibility of contamination. Finally, PCR products were purified by gel purification kit (Biocolor BioScience & Technology Co. Ltd.). Then, PCR products were sequenced using ABI 3730 XL DNA Sequencer.

Altogether 22 nrDNA ITS sequences belonging to nine species were used for the phylogenetic analysis (Table 1). Fifteen samples representing seven species were

sequenced by the authors, and another seven samples (belonging to six species) were downloaded from GenBank.

Phylogenetic analysis

All sequences were aligned using ClustalW 1.6 (Higgins et al. 1994). The phylogenetic analysis was executed with software Mega5.10 (Tamura et al. 2011). The Kimura-2-parameter was selected as the nucleotide substitution model, and gaps or missing data were set as pairwise deletion. The maximum likelihood (ML) method was used in constructing the phylogenetic tree and the reliability of the inferred tree was tested by 1000 bootstrap replications.

Results

The ML-tree (Fig. 1) of the ITS rDNA sequences shows that the five *Phyllobaeis crustacea* individuals cluster with 100% bootstrap support onto a separate branch, which is most closely related to *P. imbricata*. Together these two species form a common *Phyllobaeis* branch with 79% bootstrap support. *Baeomyces placophyllus* and *B. rufus* cluster together with 98% bootstrap support. *Phyllobaeis* and *Baeomyces* represent *Baeomycetaceae* with 59%

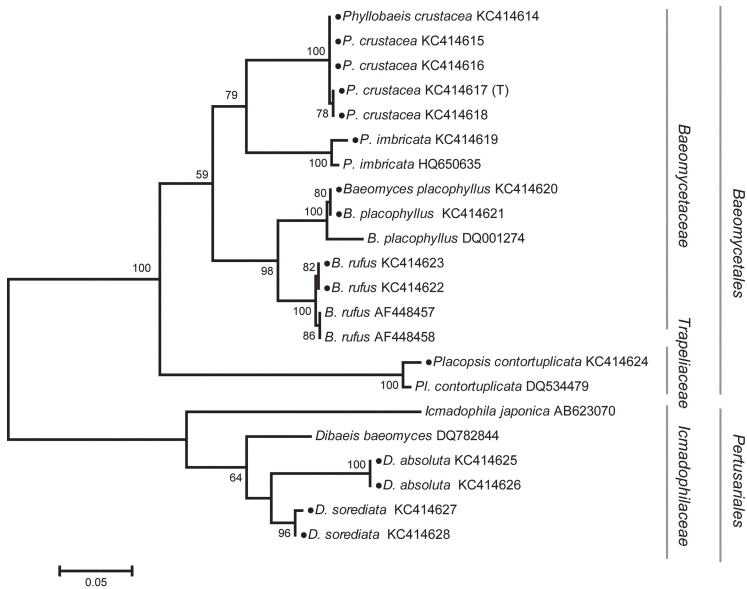

Fig. 1. ML tree based on nrDNA ITS region sequences. The species in the tree marked with "●" were sequenced by the authors. Nucleotide: K2 model, gaps or missing data were partial deletion, bootstrap = 1000. Genetic distance scale = 0.05. Numbers at nodes present the bootstrap support value (numbers <50 not shown).

bootstrap. Meanwhile, the *Baeomycetales,* containing the *Baeomycetaceae* and *Trapeliaceae* (represented by *Placopsis*), is supported by a bootstrap value of 100%, and the *Dibaeis* species (formerly included in *Baeomyces*) are clearly shown to belong to the outgroup, *Icmadophilaceae*.

Summarizing, the ITS rDNA sequences of *P. crustacea* differ significantly from the other species of *Baeomycetaceae* that are most closely related to the *Phyllobaeis* group.

Taxonomy

Phyllobaeis Kalb & Gierl, Herzogia 9:610.1993; emend. S.N. Cao & J.C. Wei

Original diagnosis (Gierl & Kalb 1993: 610): *Genus novum a genere* Baeomyces *differt thallo squamuloso, superne et infra corticato acido norstictico continente; regionibus tropicis distributum*.

Emended diagnosis: Differs from *Baeomyces* by its production of norstictic acid and its tropical distribution.

Phyllobaeis crustacea S.N. Cao & J.C. Wei**, sp. nov.** Fig. 2

Fungal Name FN570052

Differs from the other *Phyllobaeis* species by its crustose thallus.

Type: China, Hainan: Changjiang County, Mt. Bawangling, 19°16' N 109°03' E, alt. 300 m, on rock, 25 Nov. 2010, S. N. Cao CSN047 (**Holotype**, HMAS-L 118095, GenBank KC414617; **Isotype**: HMAS-L 127984).

Etymology: Latin *crustaceus*, referring to the crustose thallus.

Thallus crustose, grayish green, matt, varnish-like, tightly attached to the substrate, forming a patch of 2.5–5 cm in diameter, lacking cortical layers, irregularly delimited; algae layer continuous, algal cells green, ovoid or ellipsoid, single, 5–7.5 × 3.75–5 µm.

Apothecia pale reddish brown to brownish, round and plump, 0.3–0.5mm in diameter, short-stiped, without clear margin, scattered over the thallus; podetia whitish, 0.1–0.5 mm tall, 0.5 mm in diameter, lacking algae; hymenium 112.5–125 µm thick, I–; paraphyses simple, non-septated; asci long-clavate, 8-spored, with apex I–, 80–87.5(–92.5) × 7.5 µm; ascospores oblong or fusiform, hyaline, one-septate, 10–12.5 × 5 µm.

Chemistry: Spot tests: Thallus K+ yellow turning red, C–, KC–, P+ yellow. All specimens contain norstictic acid (TLC).

Additional material examined: **China**, **Hainan**: Changjiang County, Mt. Bawangling, 19°16' N 109°03' E, alt. 300 m, on rock, 25 November 2010, S.N. Cao CSN048 (HMAS-L 118096, GenBank KC414618); CSN049 (HMAS-L 118089, GenBank KC414616); CSN050 (HMAS-L 118087, GenBank KC414615); CSN051 (HMAS-L 118086, GenBank KC414614).

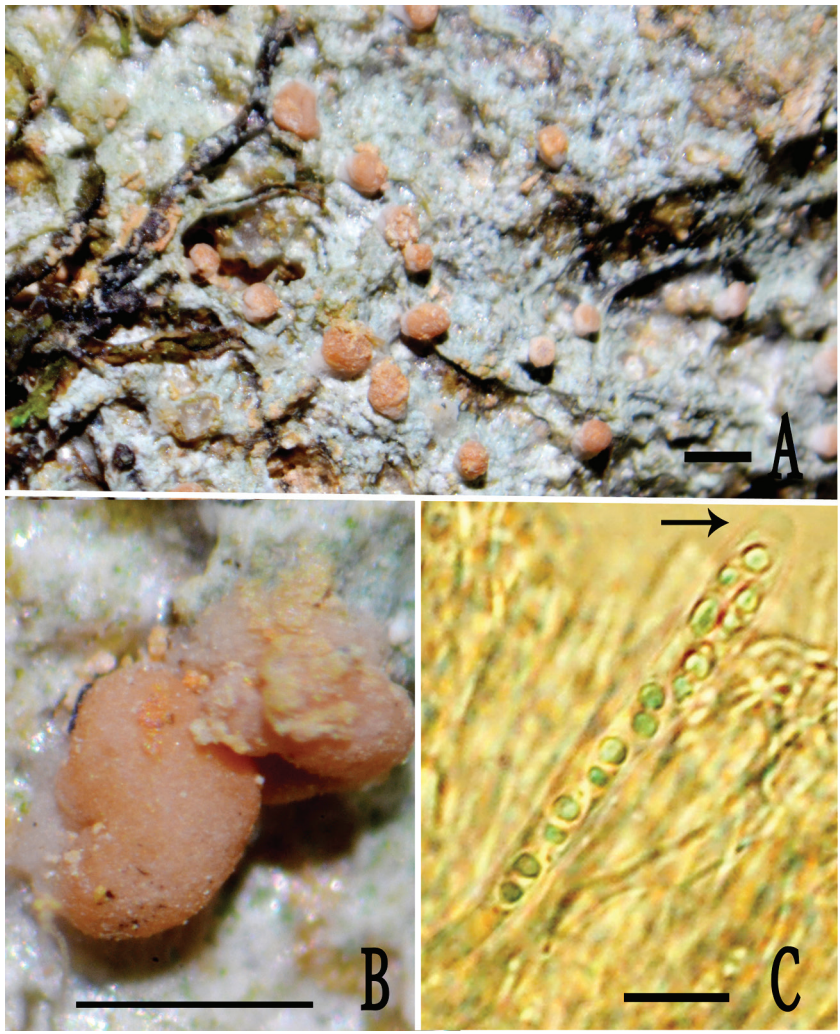

Fig. 2. *Phyllobaeis crustacea* (holotype): A, habit; B, apothecia; C, ascus with eight ascospores, apex I– (arrow). Scale bars: A, B = 0.5 mm; C = 10 µm.

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