

Hyphodontia juniperi (Basidiomycota) newly recorded from China

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ABSTRACT

Hyphodontia juniperi (*Xylodon juniperi*) was recorded for the first time for China in Jilin Province, Hebei Province, and in Beijing Municipality. General distribution of the species, its ecological and morphological differences from *H. crustosa* (*Xylodon crustosus*) are discussed. Bayesian reconstruction of phylogeny based on ITS1, 5.8S, and ITS2 nuclear ribosomal DNA sequences demonstrated that both *H. crustosa* and *H. juniperi* belong to a species complex, which requires richer molecular sampling for separation.

Key words: Corticiaceae s.l., geography, host range, subulate cystidia

Introduction

Hyphodontia juniperi is a corticioid fungus, the member of Schizoporaceae (Hymenochaetales). The name *Xylodon juniperi* (Bourdot & Galzin) Hjortstam & Ryvar den is applied to this taxon after splitting *Hyphodontia* s.l., which was based on morphological characters (Hjortstam and Ryvar den, 2009). The species was previously reported from Europe, North and South America, Africa, and two localities in Asia (Iran and Japan). It is fairly common in some parts of its range, like Azores and Italy, but rarely recorded in other regions, and thus it remains poorly studied in many respects. In this study, three specimens of this species were found for the first time in mainland China. Chinese specimens of *H. juniperi* and their relation to *H. crustosa*

(Pers. : Fr.) J. Erikss. [*Xylodon crustosus* (Pers.) Chevall.] are discussed below.

Material and methods

Specimens of *H. juniperi* were examined in the course of a critical study of *Hyphodontia*-like fungi from China. Reference specimens are deposited in herbaria TNM and MSK (acronyms follow Index Herbariorum, <http://sweetgum.nybg.org/ih>). The morphology description is based on dry basidiocarps. Micromorphology measurements were carried out in the medium of 3% KOH water solution; color for hyphae and other structures was observed in the same solution. Average spore size was calculated as arithmetical mean for 30 spores, randomly selected for each specimen in basidioma sections or squash preparations.

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Spore quotient (Q) was calculated as spore length/width ratio. Crystalline material morphology was examined in Melzer's reagent;

additional studies of spore morphology were carried out in this medium also.

Table 1. Reference ITS1-5.8S-ITS2 sequences used in this research*

Species	Country / host	Isolate / Specimen voucher	GenBank accession no.
<i>Hyphodontia crustosa</i>	Germany / <i>Fraxinus excelsior</i>	GEL 2325	DQ340313
<i>H. crustosa</i>	Germany / <i>Alnus incana</i>	GEL 5336	DQ340314
<i>H. crustosa</i>	Germany / <i>Alnus incana</i>	GEL 5360	DQ340315
<i>H. crustosa</i>	Finland / angiosperm	/ KHL 11731 (GB)	DQ873614
<i>H. crustosa</i>	Taiwan / not identified	Wu 0711-90 / TNM F22360	KC460312
<i>H. crustosa</i>	China / angiosperm	Wu 1007-31 / TNM F25228	KC460313
<i>H. hastata</i> (Litsch.) J. Erikss.	Norway / <i>Picea abies</i>	GEL 2143	DQ340323
<i>H. juniperi</i>	China / <i>Thuja orientalis</i>	Wu 0910-95 / TNM F23863	JX175047
<i>H. juniperi</i>	China / angiosperm	Wu 0308-26 / TNM F15237	KC460311
<i>H. juniperi</i>	Réunion Island / <i>Cyathea excelsa</i>	GEL 4940	DQ340316
<i>H. pruni</i> (Lasch) Svrček	Sweden /	/ Ryberg 021018 (GB)	DQ873624
<i>H. rimosissima</i> (Peck) Gilb.	Sweden /	/ Ryberg 021031 (GB)	DQ873627
<i>Resinicium bicolor</i> (Alb. & Schwein.) Parmasto	Lithuania / <i>Pinus mugo</i>	Z-3-4 /	FJ872065

* Taxa in bold indicate original sequences from this study

For molecular phylogeny study, DNA was extracted from cultural mycelium as described earlier in Wu *et al.* (2007). Living cultures are preserved in TNM culture collection. ITS region of nuclear ribosomal DNA was amplified using primer pair ITS1/ITS4 (White *et al.*, 1990). Amplicons were sequenced as described in Wu *et al.* (2010). Sequences were submitted to GenBank (Tab. 1). To assess phylogenetic distances, ITS sequences of *H. juniperi*, *H. crustosa* and some other allied species with subulate cystidia were involved in this research. *Resinicium bicolor* (Hymenochaetales) was

selected as the outgroup.

Sequences were aligned with MAFFT software online (<http://mafft.cbrc.jp/alignment/server>), using Q-INS-i strategy. Final hand cut of the aligned sequences was done in BioEdit 7.0.4.1 software. Ready data matrix was deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S14991>). MrModeltest 2.3 by J.A.A. Nylander (<http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html>) with the Akaike information criterion (Posada and Buckley, 2004) was used to assess the best-fit

models of nucleotide evolution for ITS1+ITS2 and 5.8S sub-regions separately. The suggested models were implemented in partitioned Bayesian reconstruction of phylogeny using MrBayes vers. 3.2.1 (Ronquist and Huelsenbeck, 2003). Eight Metropolis-coupled Markov chain Monte Carlo chains with temperature 0.2 were initiated, and these were run for 1M generations, in two independent runs, with tree and parameter sampling every 100 generations. The initial burn-in was set to 0.5. A majority-rule consensus phylogram was computed from the remaining 5000 trees.

Results

Three specimens of *Hyphodontia juniperi* was identified from different localities in N and NE China (see below in Specimens examined). Morphological description of *H. juniperi* based on Chinese material, is given below.

Hyphodontia juniperi (Bourdot & Galzin) J. Erikss. & Hjortstam Figs. 1, 2

Basidioma effused, subceraceous or subcretaceous, continuous or minutely fissured with age, extending from 2–10 mm to about 10 cm, 50–120(–150) μm thick in section. **Hymenophore** smooth to sparsely grandinioid, with 4–10 granules/cm. **Hymenial surface** pale cream to cream. **Margin zone** white, somewhat thinning out and powdery, up to 0.5 mm broad. **Hyphal system** monomitic, hyphae clamped at all septa, colorless. **Subiculum** moderately loose, rich of mostly mid-sized to coarse crystals. **Subicular hyphae** moderately to loosely branched, thin- or slightly thick-walled, (1–)1.5–2.5(–3) μm wide, the ones close to the

substratum up to 3.5 μm wide. **Subhymenium** little differentiated from subiculum; subbasidial hyphae moderately branched, 2.5–3 μm wide, thin-walled, fairly richly encrusted. **Hymenium** moderately dense. **Cystidia** of one type, in hymenium, clamped at base, flexuous-subulate, slightly constricted, apically attenuate, seldom almost capitate, 20–30(–50) \times 2.5–4.5 μm , colorless, thin- or almost thin-walled, smooth to loosely encrusted, some ones projecting over the hymenium up to 15–20 μm . **Basidia** clamped at base, subcylindrical to utriform, with 1–2 constrictions, 20–25 \times 3.5–5 μm , colorless, thin-walled, smooth or slightly encrusted, with four sterigmata 3–4 \times 0.3–0.5 μm . Linear repetition of basidia seldom. **Basidiospores** ellipsoid to oblong, 5–7.5 \times 2.5–3.5 μm (rarely up to 8.5 μm long), colorless, thin-walled, with one or more oily drops, with a small or unclear apiculus.

Specimens examined. China. Jilin Province, Antu County, in the vicinity of Erhtao town, Shangbaishan, 42.11°N, 128.10°E, alt. 1,050 m, on dead, partly corticated twig of *Abies* sp. about 1 cm diam, coll. S.H. Wu and S.Z. Chen, 4.IX.2003, *Wu 0309-130* (TNM F15343). Hebei Province, Wulingshan, 20.36°N, 117.29°E, alt. 1,900 m, on twig of angiosperm species, coll. S.H. Wu and S.Z. Chen, 29.VIII.2003, *Wu 0308-26* (TNM F15237; duplicate in MSK). Beijing Municipality, Tanzhesi west of Beijing, 39.54°N, 116.00°E, alt. 340 m, on dead corticated twigs about 1–1.5 cm diam of *Thuja orientalis* L., coll. S.H. Wu, 15.X.2009, *Wu 0910-95*, *0910-98*, *0910-103* [TNM F23863 (duplicate in MSK), 23865, 23868, accordingly].

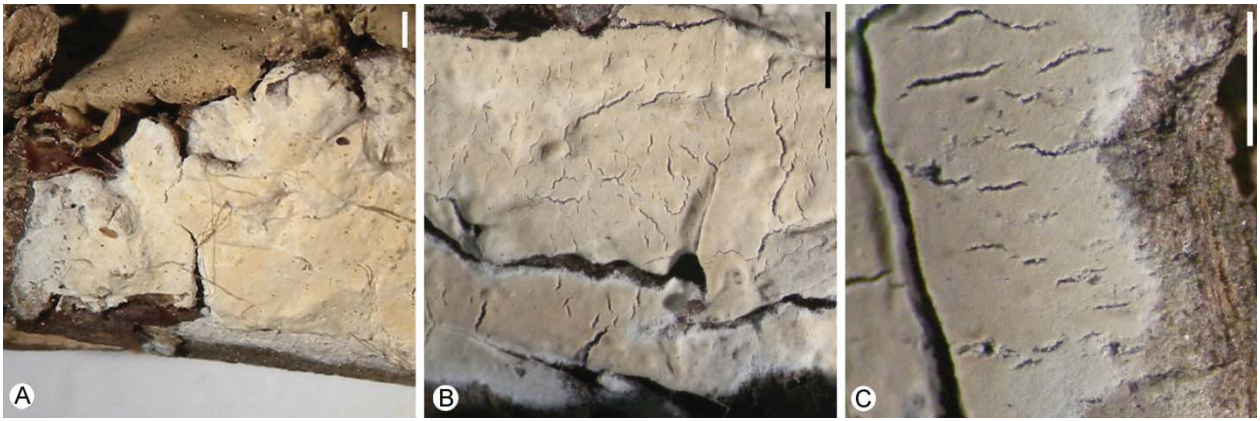


Fig. 1. Hymenial surface and margin zone in *Hyphodontia juniperi* basidiomata. A. TNM F15343 (Jilin Province). B & C. TNM F23863 (Beijing). Scale bars = 1 mm.

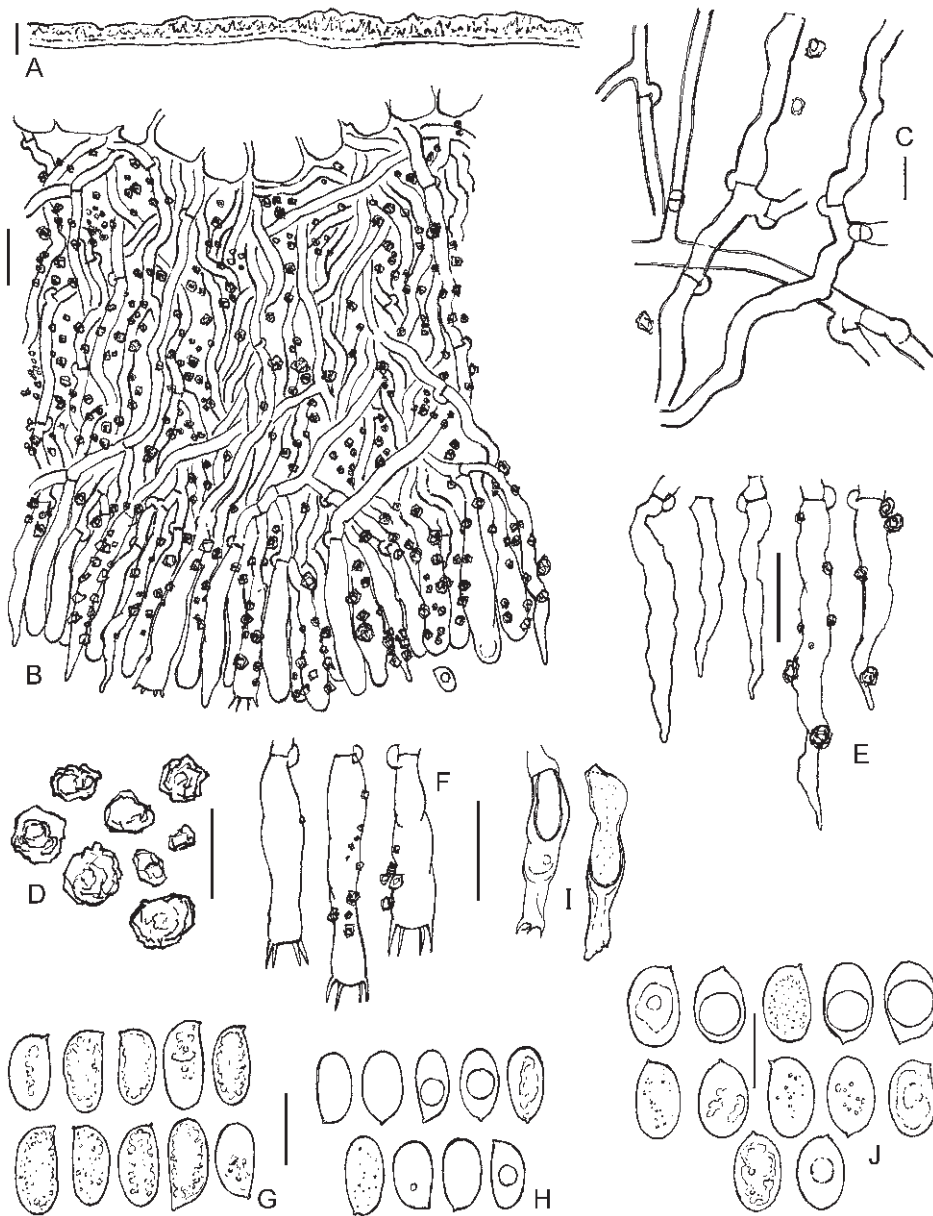


Fig. 2. Micromorphology of *Hyphodontia juniperi* from China. TNM F15343 (Jilin Province). A & B. Vertical sections through basidioma. C. Subicular hyphae. D. Crystals from subiculum. E. Cystidia. F. Basidia. G. Spores in KOH solution. H. Spores in Melzer's reagent. TNM F23863 (Beijing). I. Linear repetition in basidia. J. Spores. Scale bars: for A = 100 μm , for B, E, F & I = 10 μm , for C, D, G, H & J = 5 μm .

Basidiospore variation. Notable spore size and shape variation was observed among specimens of *H. juniperi* (Tab. 2). The largest spores were observed in specimen from the

northernmost locality (Jilin Province). The smallest Q was in specimens from Beijing vicinity.

Table 2. Spore size and shape in *Hyphodontia juniperi*

Specimen ref. no.	Locality	Spore size range,* μm	Mean spore size, μm	Q range	Mean Q
TNM F15343	Jilin, Erhtao	(5–)5.5–6.5(–7.5) \times (2.5–)3–3.5	5.87 \times 3.20	1.5–2.3	1.84
TNM F15237	Hebei, Wulingshan	5–6(–8.5) \times 2.8–3.2(–3.5)	5.59 \times 3.08	1.4–2.7	1.82
TNM F23863	Beijing, Tanzhesi	5–6.5(–7) \times 3–3.5	5.71 \times 3.32	1.4–2.1	1.73

* Size for 10% of spores in brackets

Phylogenetical study. The data matrix of ITS region was composed of 13 sequences and 652 nucleotide positions, including introduced gaps. Best models of evolution selected for Bayesian reconstruction were HKY+G for ITS1+ITS2 and SYM+G for 5.8S. The obtained Bayesian phylogram (Fig. 3) shows that *H. crustosa* together with *H. juniperi* constitute a separate cluster (B–F) apart of three other *Hyphodontia* species with subulate cystidial elements (A). There are three sister groups in *crustosa-juniperi* clade, with Bayesian posterior probably (BPP) value 0.77: phylogenetically distant specimen of *H. juniperi* from Réunion (F), *H. juniperi* from Beijing vicinity (E), and *H. crustosa* subclade (B, C, D). Thus, there is no an

independent subclade of *Hyphodontia juniperi* within *crustosa-juniperi* clade. Moreover, specimens *Wu 0910-95* and GEL4940 look like two separate species. Within *H. crustosa* phylogenetic distances are very short, but two subclades can be distinguished here with BPP=0.91: *H. crustosa* from Europe (B, C) and *H. crustosa* from China (D). A specimen of *H. juniperi* from Wuling Mountains (*Wu 0808-26*) demonstrates remarkably high similarity in ITS sequence with *H. crustosa* from Finland (B). Specimen from Wulingshan was collected on wood of angiosperm species, which is seldom substratum for *H. juniperi*. However, spores in this specimen are ellipsoid, not cylindrical as in *H. crustosa*.

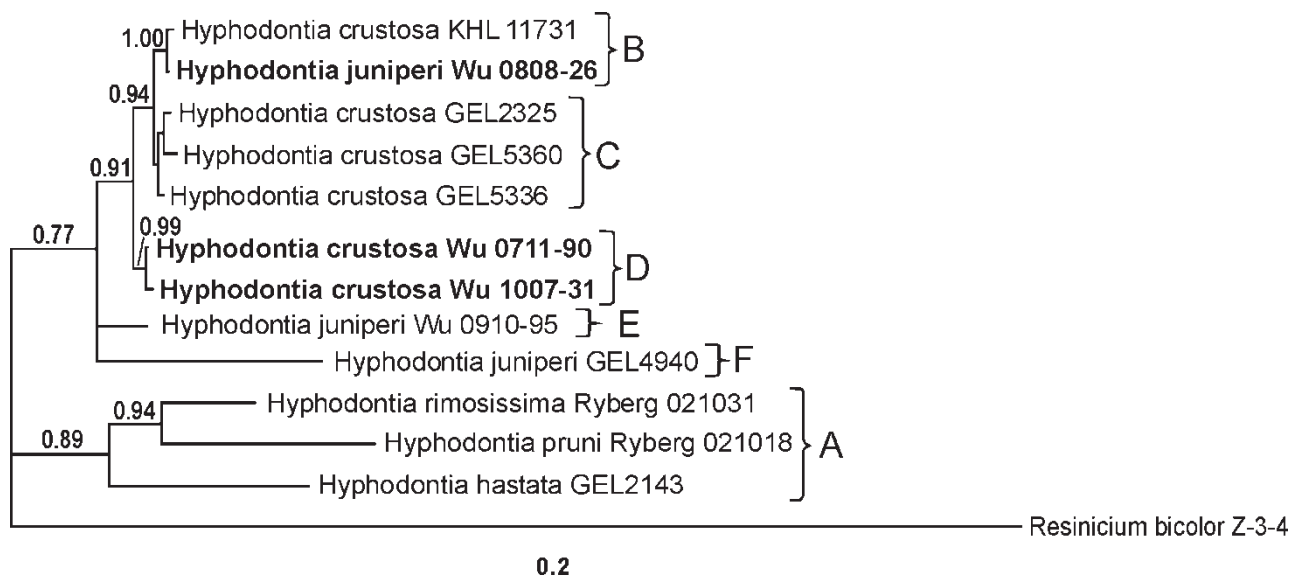


Fig. 3. Bayesian phylogram of phylogenetic relationships of *Hyphodontia* species with subulate cystidia inferred from internal transcribed spacer (ITS) sequences. Numbers above branches indicate Bayesian posterior probabilities (PP > 0.75).

Discussion

According to the published records (Eriksson *et al.*, 1981; Hallenberg, 1981; Gilbertson and Blackwell, 1985; Tellería, 1990; Langer, 1994; Rodríguez-Armas and Beltrán Tejera, 1995; Melo and Tellería, 1992, 1997; Hjortstam and Ryvarden, 1997, 2004; Vesterholt, 1997; Ghobad-Nejhad *et al.*, 2009; Telleria *et al.*, 2009; Gorjón *et al.*, 2009; Bernicchia and Gorjón, 2010; Dämon *et al.*, 2010), *H. juniperi* has predominantly temperate-holarctic distribution, as below.

Europe: Norway, Sweden (Gotland Island), Estonia, Denmark, Germany (southwest), France (south-central part – type locality for *Corticium serum* (Pers.) Fr. var. *juniperi* Bordot & Galzin), Austria, Portugal, Spain (north and central part), Italy, Croatia, Macedonia, Greece (Crete). **Macaronesia:** Canary Islands, Azores. **Caucasus:** Russia (Krasnodar krai, Black Sea coast). **Asia:** Iran (north), Japan (center of

Honshu Island). **Africa:** Ethiopia (central part), Kenya (central part), Tanzania (north). **Islands of south-west Indian Ocean:** Réunion (E. Langer, pers. comm.). **North America:** USA (Texas), Canada (southwest, Vancouver Island). **South America:** Colombia.

Hyphodontia juniperi occurs in boreonemoral, nemoral and mediterranean types of biomes. The identity of the collections made from tropical regions (Africa, Canary Islands, South America, Réunion) with *H. juniperi* needs further study. As shown in its distribution, *H. juniperi* prefers to grow in warm and moderately dry regions, more often on low mountains or stony areas. The findings in China correspond with ecogeographical pattern of the species. One specimen from China was on *Abies*, which is a new host for this species.

Smooth or almost smooth hymenial surface, apically tapering cystidia, and ellipsoid spores are the main diagnostic features of *H. juniperi*.

Nevertheless, there are variations both in specimens examined by us and in the published diagnoses. Hymenial surface is described as even [Jülich and Stalpers, 1980, under the name *Kneiffiella juniperi* (Bourdot & Galzin) Jülich & Stalpers], or initially smooth, then subtuberculate or minutely odontoid in some areas (Rodríguez-Armas and Beltrán Tejera, 1995; Melo and Tellería, 1992; Eriksson *et al.*, 1981; Hjortstam and Ryvarde, 2004). Hymenial surface in Chinese specimens of *H. juniperi* is completely smooth or very poorly tuberculate.

Spores in *H. juniperi* are described as ellipsoid (Jülich and Stalpers, 1980; Eriksson *et al.*, 1981; Melo and Tellería, 1992; Langer, 1994; Rodríguez-Armas and Beltrán Tejera, 1995; Bernicchia and Gorjón, 2010), whereas Hjortstam and Ryvarde (1997; 2004) characterized spores as broadly ellipsoid. Spores are depicted as narrowly ellipsoid and oblong in Langer (1994, Abb. 57). The Chinese materials bear spores for the most part narrowly ellipsoid.

Hyphodontia juniperi belongs to *H. crustosa* complex, and variation in spore shape makes the hiatus between *H. juniperi* and *H. crustosa* somewhat diffuse. We believe that in all controversial cases an additional diagnostic criterion of *H. juniperi* is the ecology, namely a gymnosperm host and the preference of moderately dry, mountain habitats. Based on DNA sequence data we conclude that *H. crustosa* plus *H. juniperi* is a complicated species complex, requiring more molecular sampling for species delimitation. Until such kind of research will be done, we propose the working taxonomical concepts for the two

species as described in the key below.

* Hymenial surface typically whitish and smooth, not cracking into polygons; cystidia often with attached crystals, never distinctly papillate or capitulate apically; basidia 15–25 μm long; spores ellipsoid to oblong, (2.5–)3–3.5(–4) μm broad, straight, convex or seldom barely pressed-in adaxially; on Cupressaceae (*Juniperus*, *Cupressus*, *Thuja*, *Cryptomeria*), occasionally on other gymnosperm and angiosperm genera ... *H. juniperi*

— Hymenial surface usually cream-colored, with small, distinctly separated aculei when young, with age becoming odontoid with closer, sometimes crowded aculei; old and dry specimens often becoming cracked into small polygons; cystidia usually naked, some ones apically with conidium-like segment; basidia 20–35 μm long; spores narrowly ellipsoid to cylindrical, sometimes subballantoid, 2.5–3 μm broad, a part of spores pressed-in adaxially; on angiosperms, occasionally on gymnosperms ... *H. crustosa*

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刺柏絲齒菌 (*Hyphodontia juniperi*)，中國新紀錄擔子菌

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摘 要

刺柏絲齒菌 (*Hyphodontia juniperi*) 首次發現於中國吉林省、河北省和北京市。本文並討論刺柏絲齒菌的分佈，以及它與殼絲齒菌 (*H. crustosa*) 生態與形態的差異。經由貝葉氏導出式分析核糖體內轉錄間隔區 (ITS region) 序列，結果顯示刺柏絲齒菌與殼絲齒菌屬於同一個複合種。需要更多標本納入研究，始能區別這兩種。

關鍵詞：皮殼菌、地理學、寄主範圍、錐狀囊狀體。