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Phylogeography of *Hyphoderma setigerum* (*Basidiomycota*) in the Northern Hemisphere

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Previous studies of morphological variation in the homobasidiomycete *Hyphoderma setigerum* have lead to suspicions of a species complex. This study explores variation in DNA sequences from the nuclear ribosomal ITS region of 45 specimens from America, Asia, and Europe in a phylogeographic context. Based on molecular analysis, morphological studies, and crossing tests, nine preliminary taxa are shown to exist inside the species complex, and the two previously described segregate species *H. subsetigerum* and *H. nudicephalum* are confirmed. The molecular analysis shows evidence of allopatric differentiation over intercontinental distances. Only one of the nine well-supported clades has a geographic distribution spanning more than one continent, probably indicating the importance of vicariance in the evolution of this species complex. The basionym of *H. setigerum*, *Thelephora setigera*, is neotypified to fix the application of that name.

INTRODUCTION

Hyphoderma setigerum (Fr.) Donk 1957 (*Basidiomycota*, *Homobasidiomycetes*) is a white-rotting corticioid fungus known from all tree-bearing continents. Its main habitat is fallen decorticated wood and stumps of deciduous trees; it is less frequently collected on coniferous wood and dead but still attached branches (Eriksson & Ryvarden 1975, Yurchenko & Zmitrovich 2001). The species is easily identified microscopically by its multiseptate, clamped, and encrusted cystidia, but substantial variation exists in both micro- and macromorphology in specimens from north Europe (Eriksson & Ryvarden 1975) and from Belarus and north-west Russia (Yurchenko & Zmitrovich 2001). Cultural studies in *H. setigerum* show that the mating system is heterothallic and bipolar, but homothallism has also been recorded. Homothallism in *H. setigerum* is probably primary as basidiospores even in homothallic specimens have been shown to be uninucleate (Boidin & Lanquetin 1984). There seems to be reason to believe that *H. setigerum* as presently understood represents several distinct biological species, in what is termed a species complex. It has proved difficult to delimit the units of the complex by morphological criteria alone, and the present study aims to advance the understanding of the complex through canvass of a widely sampled collection of specimens using molecular analysis in addition to morphological studies and intercompatibility tests. A further aim of the study is to investigate if heterothallic and homothallic specimens in *H. setigerum* are phylogenetically divergent.

We also examine the status of two recently recognized segregates from *H. setigerum*. Gilbertson & Blackwell (1988) described *H. nudicephalum* based on material from the North American Gulf Coast. The observation of *setigerum*-like cystidia suggested to Gilbertson & Blackwell (1988) a close affinity of *H. nudicephalum* to *H. setigerum*, but the additional presence of clavate cystidia with a swollen, bulbous apex prompted them to describe a new species. Wu (1997) described *H. subsetigerum*, which is morphologically very close to *H. setigerum*, but distinguished by smaller and narrower basidiospores (*H. subsetigerum*, 6–8 × 2.8–3.2 μm; *H. setigerum*, 7.5–12.5 × 3.5–5 μm; Wu 1997) and by negative crossing tests. The *H. subsetigerum* holotype used by Wu (1997), and one specimen identified as *H. nudicephalum*, were included in our study in the hope of addressing their status as distinct species in the complex.

Finally, the question whether or not *H. setigerum* contains several biological species in North America,

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Asia, and Europe can also be cast in terms of speciation processes. A scenario where only one distinct biological species is found per continent would hint that the main pattern of speciation in the species is allopatric speciation; previous studies have indicated that both sympatric and allopatric speciation may occur within species complexes of corticioid fungi (Hallenberg 1991). Corticioid fungi are generally believed to have high dispersal capacities (Hallenberg 1995, Hallenberg & Küffer 2001, James & Vilgalys 2001), and speciation events within continents are commonly thought to involve host specialisation. Due to the efficient dispersal abilities of many species, it is probable that only very large distances, like those between continents, act as dispersal barriers. Thus, the wide geographical scope of the *H. setigerum* specimens in this study not only makes possible detailed studies of the species itself, but it also provides a potential to beget and test biogeographical hypotheses.

MATERIALS AND METHODS

Sampling

The specimens of the study (Table 1) were selected from the culture collection of Göteborg University (FCUG), the National Museum of Natural Science (NMNS, Taipei), and the Tottori Mycological Institute (TM, Tottori). The cultures from 45 ingroup and two outgroup specimens were used for DNA sequencing and, where applicable, crossing tests; their associated vouchers, where available, were used for morphological comparison.

DNA extraction, amplification, and sequencing

For crossing tests and as a source of DNA extraction, single-spore mycelium was isolated, cultivated on malt agar plates (1.25% malt extract), and subsequently placed in malt liquid solution (malt extract as above) for 3 wk. When single-spore mycelium was not available, polyspore mycelium was used. Mycelia were harvested and dried between sheets of sterile filter paper; approximately 2 mg d.w. of input mycelium were used per specimen. DNA extraction was accomplished using the DNeasy® Plant Mini Kit (QIAGEN®, Hilden); during this and the following steps of the DNA preparation, purification, and sequencing, the recommendations of the respective manufacturer were followed.

The polymerase chain reactions were carried out using Ready-To-Go™ PCR Beads kits (Amersham Pharmacia Biotech, Uppsala), a Biometra TRIO-Thermoblock (Biometra, Göttingen), the PCR primers ITS1F and ITS4B, and the PCR set-up of Gardes & Bruns (1993). The PCR product was purified using the QIAquick™ Spin procedure (QIAGEN®) and the sequence reactions were conducted using 100 ng of template DNA and the CEQ 2000 Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter, Fullerton). Sequences were obtained using the CEQ 2000XL DNA Analysis System (Beckman Coulter).

Alignment and phylogenetic analysis

The obtained sequences (ITS1 save its 19 first bases, 5.8S, ITS2, and 16 bases of nuc-LSU) were edited in Sequencher® 4 (GeneCodes, AnnArbor) and aligned in ClustalX 1.81 (Thompson *et al.* 1997) using factory settings. The alignment was adjusted manually in PAUP* 4.0b10 (Swofford 2002). To better account for indel events, 16 indels were coded and added as artificial characters at the end of the matrix while keeping the original indel events aligned in the matrix. All ambiguous characters and the coding operations are annotated in the matrix file (available upon request). All further analysis methods employed outgroup rooting: *H. definitum* (H. S. Jackson) Donk 1957 appeared a suitable outgroup due to basidiome morphology which put it close to yet separate from *H. setigerum*. Additionally, an unidentified *Hyphoderma* was included as outgroup by virtue of having basidiome micro- and macromorphology which suggest a position close to the *H. setigerum* complex, but which was clearly distinguished from *H. setigerum* by its cystidia morphology and basidiome colour.

Heuristic parsimony analysis was performed in PAUP* using 5000 random addition sequences with ten trees held at each step and TBR branch swapping with no more than two trees saved per round. All characters were unordered and gaps were treated as missing data. A strict consensus tree was calculated from the resulting trees. Clade support was estimated through 1000 jack-knife replicates of 37% character exclusion, 100 random addition sequences with one tree held per step, and TBR branch swapping with no more than two trees saved per round.

Crossing tests

The crossing tests were restricted to specimens for which non-clamped single spore isolates were available. Single-spore mycelia from different specimens were placed in pairs on malt-extract agar (1.25% malt extract) and left in room temperature for three weeks. From each specimen, two to four single-spore mycelia were used. Paired cultures were checked for clamp formation in three different regions: at the immediate contact zone and on opposite sides of the inocula, some 20 mm from respective inoculum. Plates with negative results were re-checked after an additional three weeks. Di-mon tests were normally not done because it would be impossible to distinguish a negative reaction caused by incompatibility between different biological species from a negative reaction caused by crossings between a heterothallic and a homothallic strain. Several of the cultures used in this study were homothallic or were

Table 1. Details of the studied specimens; the substratum is specified to the extent known.

Taxon	FCUG no.	Locality	Substratum	Other no.	EMBL no.
H. setigerum IA					
	997	Germany: Hessen	Angiosperm	NH 4742	AJ534250
	1202[a]	Norway: Sogn og Fjordane	*Pinus*	NH 8309	AJ534248
	1521	Romania: Covasna	Angiosperm	NH 9277	AJ534251
	2398[a]	Russia: Krasnodar	*Abies*	NH 12108	AJ534249
H. setigerum IB					
	2351[a]	Greenland: Quinqua valley	*Betula*	NH 11888	AJ534256
	2355[a]	Greenland: Narssarssuaq	*Betula*	NH 11801	AJ534254
	2356[a]	Greenland: Tasermiut fjord	*Larix*	NH 11844	AJ534255
	2357[a]	Greenland: Quinqua valley	*Salix*	NH 11813	AJ534252
	2361[a]	Greenland: Quinqua valley	*Betula*	NH 11865	AJ534253
H. setigerum II					
	476	Canada: BC	*Abies*	NH 6748	AJ534259
	691	Canada: BC	*Alnus*	NH 7110	AJ534258
	2003	Canada: BC	*Alnus*	NH 10819	AJ534257
H. setigerum IIIA					
	551	Canada: Que.	*Fagus*	NH 6121	AJ534261
H. setigerum IIIB					
	2872	USA: NC	Angiosperm	NH 14263	AJ534260
H. setigerum IV					
	922	UK: Perth	*Betula*	NH 7799	AJ534262
	1426	Norway: Östfold	*Quercus*	NH 8917	AJ534263
H. nudicephalum (*Group V*)					
	WU 9307-29	Taiwan: Nantou	Angiosperm	FCUG 2926	AJ534269
	WU 9508-225	China: Yunnan	Angiosperm	FCUG 2934	AJ534268
	TMIC 33708	Japan: Tokyo	Angiosperm	FCUG 2942, TMI 20531	AJ534264
	TMIC 30479	Japan: Tottori	*Castanopsis*	FCUG 2943, NM 875	AJ534267
	TMIC 50049	Japan: Tottori	*Quercus*	FCUG 2945, TMI 6755	AJ534270
	TMIC 50048-1	Japan: Tottori	*Quercus*	FCUG 2946, TMI 6730	AJ534265
	2949	Japan: Tottori	*Castanopsis*	J 011102-1	AJ534266
H. setigerum VI					
	593	Canada: Que.	Angiosperm	NH 6339	AJ534271
H. setigerum s. str. (*Group VIIA*)					
	1200	Norway: Oppland	*Alnus*	NH 8211	AJ534273
	1688	Finland: Pohjois-Häme	*Alnus*	NH 9468	AJ534272
H. subsetigerum (*Group VIIB*)					
	WU 9508-155	China: Yunnan	Angiosperm	FCUG 2927	AJ534275
	WU 9507-3	China: Yunnan	Coniferous	FCUG 2928	AJ534274
	WU 9202-15	Taiwan: Pingtun	Angiosperm	FCUG 2930	AJ534278
	WU 9304-18[b]	Taiwan: Nantou	Angiosperm	FCUG 2931	AJ534277
	TMIC 33552	Japan: Nagano	Angiosperm	FCUG 2935, TMI 20023	AJ534276
H. setigerum VIII					
	2016	Canada: BC	*Alnus*	NH 10662	AJ534290
	2360	Greenland: Narssarssuaq	*Betula*	NH 11951	AJ534282
	2707	Russia: Krasnodar	Angiosperm	NH 13089	AJ534287
	WU 9506-5	Taiwan: Taichung	Angiosperm	FCUG 2925	AJ534289
	WU 9506-6	Taiwan: Taichung	Angiosperm	FCUG 2932	AJ534286
	TMIC 33546	Japan: Nagano	Angiosperm	FCUG 2936, TMI 20017	AJ534288
	TMIC 31889	Japan: Tottori	*Fagus*	FCUG 2937, TMI 12789	AJ534279
	TMIC 31208	Japan: Tottori	*Quercus*	FCUG 2938, TMI 2831	AJ534285
	TMIC 31205	Japan: Ishikawa	Angiosperm	FCUG 2939, TMI 6993	AJ534280
	TMIC 31206	Japan: Okayama	*Quercus*	FCUG 2940, TMI 7434	AJ534281
	TMIC 30476	Japan: Tottori	Angiosperm	FCUG 2944, NM 879	AJ534283
	2948	Japan: Tottori	Angiosperm	J 011024-1	AJ534284
	2947	Japan: Tottori	*Pinus*	J 011021-2	AJ534291
H. setigerum IX					
	1264	Sweden: Skåne	*Carpinus*	NH 8544	AJ534292
H. definitum (outgroup)					
	2426	Russia: Krasnodar	*Abies*	NH 12266	AJ534293
Hyphoderma sp. (outgroup)					
	2860	USA: NC	*Quercus*	NH 14195	AJ534294

[a] Homothallic specimens.
[b] Culture ex-holotype of *H. subsetigerum*.

only available as polyspore isolates and were consequently left out from the crossing tests.

Morphological studies

Studies of macro- and micro-morphology were carried out under a dissection microscope (×12) and light microscope (×1000). According to the literature, *Hyphoderma setigerum* has 0.1–5(–10) mm thick, white to creamish fruit bodies, with a smooth to tuberculate hymenium; smooth, thin-walled spores which are narrowly ellipsoid to suballantoid and (5.5–)7–10 (–14) × (2–)3–4.5(–6) μm; cylindrical cystidia which are projecting, thick-walled, multiseptate, clamped, often encrusted, and (30–)70–280 × (5.5–)10–15 μm; and subclavate to clavate basidia 25–30(–45) × 5–6(–7) μm with four sterigmata and a basal clamp (Christiansen 1960, Eriksson & Ryvarden 1975, Jülich & Stalpers 1980, Breitenbach & Kränzlin 1986, Yurchenko & Zmitrovich 2001). Spore measurements were undertaken from spore prints where available, and from basidiomata in the remaining cases. For each specimen, 30 spores were measured for length and width.

RESULTS

Alignment and phylogenetic analysis

Nucleotide sequence data from the rDNA region of the 47 studied *Hyphoderma* specimens was obtained and processed as described above. The regions ITS1 (minus its first 19 bases, which were incompletely read in some specimens), 5.8S, and ITS2 had been read well and were included in the analysis. Additionally, the first 16 bases of nuclear large subunit ribosomal DNA (nuc-LSU) were included. After the recoding of 16 indel events and the leaving out of 58 characters due to alignment ambiguities, the analysis matrix consisted of 568 characters, 377 of which were constant and 46 of which were uninformative with respect to parsimony, leaving 145 (26%) informative characters.

The parsimony analysis yielded 8908 most parsimonious trees of 381 steps with a CI of 0.6273 and an HI of 0.3727. Fig. 1 shows one of the most parsimonious trees together with branch support of the corresponding strict consensus tree clades as estimated through jackknife methodology.

Crossing tests

The crossing tests and their outcome are summarised in Table 2. Compatibility was found inside, but not amongst, clades. Full compatibility was achieved inside the *Hyphoderma nudicephalum* clade (clade 5), but no compatibility with any other clade was found. Similar results were obtained for clade *H. setigerum s. str.* (7A); the *H. subsetigerum* (7B) clade was found to be composed solely of dikaryotic specimens, rendering compatibility tests troublesome. A dimon mating test was set up between representatives of the 7A and 7B clades, but the haploid mycelium was not dikaryotized. As mentioned above, such incompatibilities are difficult to evaluate.

Morphological analysis

The morphological analysis reveals a certain variation among the different clades. The differences that were noted were, however, minor, and do not support a further division with accompanying descriptions of new taxa. Distinguishing characters between the clades are summarised in Table 3, and the following morphological description of the complex can be added to the general species description given in the introduction:

Hymenium dense, smooth to grandinioid with scattered tubercles or regularly grandinioid with small to minute tubercles. *Cystidia* cylindrical, 70–220 × 7–12 μm, septate with clamps at the septa, walls slightly thickened to distinctly thick-walled, partly naked and partly covered with small or coarse crystals. Cystidium apex obtuse, slightly capitate, or more distinctly capitate (*H. nudicephalum*); cystidium base abruptly narrowing towards the hypha or more gradually tapering (*H. nudicephalum*). Cystidia regularly or irregularly distributed over the hymenial layer; in *H. subsetigerum* and its sister-clade, cystidia typically found in the centre of hymenial tubercles. *Basal hyphae* with thickened to thick walls, not differentiated or differentiated into a distinct layer with thick-walled hyphae. *Basidiospores* narrowly ellipsoid, cylindrical, suballantoid, 8–14 × 3–5 μm; ranges for individual clades are indicated in Table 3.

Typification

A designated type has been lacking for *Thelephora setigera* Fr. 1828, the basionym of *Hyphoderma setigerum*; no authentic material is left today. Fries' (1828) morphological description is of little value when discriminating amongst the clades revealed in this study. Fries notes that he saw the species himself, which means that it should occur in the Femsjö area of southwest Sweden. There are 11 specimens labelled as *T. setigera* or *H. setigerum* from Femsjö in UPS, most of which were collected by Nannfeldt and Lundell during the first part of the last century. We re-examined those specimens, and, from morphological characters, conclude that more than one of the preliminary taxa outlined in this study were present in the Femsjö area. To maintain taxonomic stability it is necessary to anchor the name *Hyphoderma setigerum* to one of the clades revealed in this study. We select clade 7A to be that taxon and make the following neotypification: *Thelephora setigera* Fr., *Elench. Fung.* **1**: 208 (1828); typus: **Finland**: North Häme, Konnevesi, Siikakoski, on branch of *Alnus* by a lake shore, 19 Aug. 1986, *N. Hallenberg NH 9468* (GB – ***neotypus hic designatus***; FCUG 1688 – cultura viva).

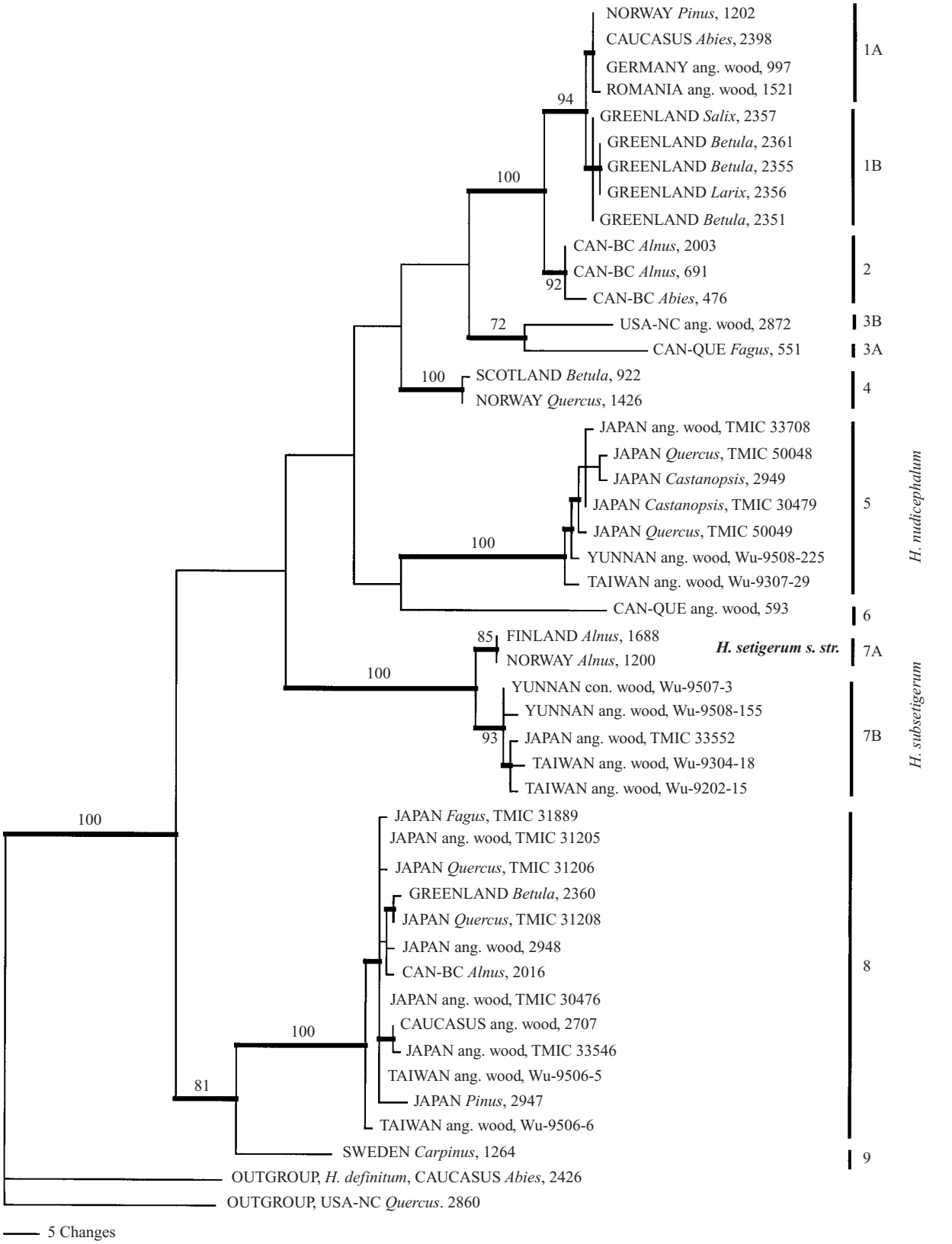

Fig. 1. One of the 8908 most parsimonious phylogenetic trees. Numbers above branches denote branch support from jack-knife analysis, and branches in bold are present in the strict consensus tree. For each specimen, information on geographical location, substrate, and identification number is given (*cfr* Table 1).

Table 2. Summary of the crossing tests performed. The number of tests performed for each combination is indicated by a superscript.

Taxon	Origin	Crossing test		2945	2946	2949	1200	1688	2935	2003	476	1426	922	2016	2360	2707	2948	2936	1264
nudicephalum	Japan	2945	2945		$+^{2}$	$+^{2}$	$-^{2}$					$-^{2}$							$-^{2}$
nudicephalum	Japan	2946	2946	$+^{2}$		$+^{2}$													
nudicephalum	Japan	2949/2,3,5,6	2949	$+^{2}$	$+^{2}$		$-^{4}$					$-^{4}$		$-^{8}$		$-^{8}$			$-^{8}$
setigerum s. str.	Norway	1200/3	1200	$-^{2}$		$-^{4}$		$+^{2}$	$-^{1}$	$-^{2}$		$-^{2}$							$-^{2}$
setigerum s. str.	Finland	1688/1,2	1688				$+^{2}$		$-^{1}$	$-^{4}$		$-^{2}$		$-^{4}$	$-^{4}$				$-^{2}$
subsetigerum	Japan	2935, dimon	2935				$-^{1}$	$-^{1}$											
setigerum 2	Canada, BC	2003/1,4	2003				$-^{2}$	$-^{4}$			$+^{4}$			$-^{4}$	$-^{4}$				$-^{2}$
setigerum 2	Canada, BC	476/1,4	476							$+^{4}$				$-^{4}$					
setigerum 4	Norway	1426/2,3	1426	$-^{2}$		$-^{4}$	$-^{2}$	$-^{2}$					$+^{4}$	$-^{4}$					$-^{4}$
setigerum 4	Scotland	922/4,6	922									$+^{4}$							
setigerum 8	Canada, BC	2016/1,5	2016			$-^{8}$		$-^{4}$		$-^{4}$	$-^{4}$	$-^{4}$			$+^{4}$	$+^{4}$	$+^{2}$		$-^{4}$
setigerum 8	Greenland	2360/1,5	2360					$-^{4}$		$-^{4}$				$+^{4}$					
setigerum 8	Russia	2707/3,4	2707			$-^{8}$								$+^{4}$			$+^{2}$	$+^{1}$	
setigerum 8	Japan	2948	2948											$+^{2}$		$+^{2}$			
setigerum 8	Japan	2936, dimon	2936													$+^{1}$			
setigerum 9	Sweden	1264/1,3	1264	$-^{2}$		$-^{8}$	$-^{2}$	$-^{2}$		$-^{2}$		$-^{4}$		$-^{4}$					

Table 3. Summary of morphological variation from comparisons of the specimens in the clades. Note that group 5 = *Hyphoderma nudicephalum*, 7A = *H. setigerum s. str.*, and that group 7B = *H. subsetigerum*.

Clade	1	1	2	3	3	4	5	6	7	7	8	9
Subgroup	A	B		A	B				A	B		
Hymenophore												
Scattered tubercles	×	×							×			
Regularly grandinioid			×	×	×	×	×	×		×	×	×
Cystidia												
Cylindrical	×	×	×	×	×	×		×	×	×	×	×
Cylindrical, or with capitate apex and tapering towards the base							×					
Basal hyphae												
Distinct to thickened walls, not in a distinguished layer	×	×	×	×	×	×		×	×	×		×
Thick-walled, forming a distinct, subicular layer							×				×	
Thin-walled, densely branched						×						
Spores (μm)												
12–14.5 × 4.5–5		×										
9.5–13 × 3.5–4.5	×		×	×	×				×			×
8.5–10 × 3.5–4.5						×		×			×	
8–9.5 × 3–4							×			×		

Description of *Hyphoderma setigerum s. str.*: *Basidiome* resupinate, adnate, hymenium partly smooth, partly irregularly tuberculate, cream-coloured. *Hyphal system* monomitic, hyphae distinct, with slightly thickened walls, 2.5–4.5 μm wide, loosely interwoven in subiculum but densely branched in subhymenial parts, regularly provided with clamps at the septa. *Cystidia* cylindrical and partly projecting, 120–180 × 7.5–10 μm, septate with clamps at the septa, walls distinctly thickened, partly naked and partly covered with coarse crystals, apex obtuse, thin-walled, base abruptly narrowing towards the hypha. Cystidia typically found in the centre of hymenial tubercles. *Basidia* subclavate to clavate, 25–40 × 6.5–7 μm, with 4 sterigmata and a basal clamp. *Basidiospores* subcylindrical, 9.5–11.5 × 3.5–4.5 μm, thin-walled, smooth, non-amyloid, with oildrops in the protoplasm.

In the present study, *H. setigerum s. str.* is represented by two specimens, both collected on branches of *Alnus* trees. If the preference for *Alnus* or the *Betulaceae* is a characteristic of the taxon remains to be evaluated. *H. setigerum* is morphologically clearly distinguished from its sister taxon, *H. subsetigerum*, by the distinctly larger spores. On the other hand, specimens from clade 7A are hardly distinguishable by morphology alone. A representative illustration of *H. setigerum s. str.* is found in Eriksson & Ryvarden (1975), fig. 245.

Geographical origin

Table 4 was constructed by sorting specimens in the clades of the phylogenetic tree according to their geographical origin. Most clades appear to have a limited distribution, with the notable exception of clade 8, the specimens of which were found in Europe, East Asia, and western and eastern North America. The number of clades per specimen is highest in Europe and eastern North America and lowest in East Asia.

DISCUSSION

While the *Hyphoderma setigerum* complex was known to show considerable heterogeneity in its morphological characters, the substantial variation found in the nuclear ITS region was less expected. Altogether 58 nucleotide sites had to be left out from the analysis due to uncertain alignment, the phylogenetic tree retrieved shows a monophyletic but heterogeneous ingroup. The basal branching order of the phylogenetic tree is not fully supported in the jack-knife analysis, but most terminal and subterminal clades receive strong support; the tree also features specimens on comparatively long branches (clades 3A, 3B, 6, and 9; Fig. 1). It

Table 4. Number of specimens in the phylogenetic analysis related to clades and geographic origin.

Clade number\No. of specimens	Europe[a]	East Asia	W. North Am.	E. North Am.[b]
1	4			5
2			3	
3				2
4	2			
5 (*nudicephalum*)		7		
6				1
7 (*setigerum s. str.*, *subsetigerum*)	2	5		
8	1	10	1	1
9	1			
Total number of specimens	10	22	4	9
Total number of clades	5	3	2	4
Clades/specimen	0.5	0.136	0.5	0.444

[a] To Europe, adjacent areas are added, i.e. Tenerife and the Caucasus.

[b] East North America includes Greenland.

appears irrefutable that a taxon sampling of far more than 50 specimens and of a much wider geographical scope than the present must be considered if the *H. setigerum* complex is to be understood in detail. Limiting further discussion to terminal and subterminal clades and treating single specimens on long branches as representatives of separate and largely unsampled clades, the present study still allows for several conclusions to be established.

The *H. subsetigerum* holotype of Wu (1997) is inside the well-supported east Asian 7B clade, which has an equally well supported European sister clade (7A, *H. setigerum s. str.*). A morphological synapomorphy ties the specimens of 7A and 7B clades together: all cystidia originate in the centre of tubercles in such a way that hymenial tissue is formed around the cystidia. However, 7B is distinguished from 7A by significantly smaller spores (7–8.5 × 3 μm), and by slightly narrower basal hyphae; additionally, no compatibility was found between 7A and 7B. This suggests that 7A and 7B represent closely related yet distinct species. Since the distribution areas of *H. setigerum s. str.* and *H. subsetigerum* do not appear to intersect, divergence as a result of geographic isolation (i.e. allopatric speciation) appears to have been a vital component in the speciation process. The divergence may have taken place 10–15 Myr ago when a coherent broad-leaved forest zone existed throughout northern parts of Eurasia (Kornas 1972). The ancestral species was distributed throughout Eurasia or conceivably circumpolar.

The specimens in clade 5 all have a distinct type of cystidia with a bulb-like, capitate apex which fits the description of *H. nudicephalum* well (Gilbertson & Blackwell 1988). Crossing tests reveal compatibility inside this clade, and refusal of its specimens to cross with specimens of other clades, which suggests the specimens are biologically conspecific. The type specimen of *H. nudicephalum* originates from the southern USA, whereas all *H. nudicephalum* specimens in this study were collected in east Asia, leaving the genetic distance between the two populations to be evaluated. Present taxonomy suggests that the name *H. nudicephalum* be attached to this clade, but the *H. nudicephalum* type and additional specimens from the southern USA should be examined and sequenced before this can be verified. Interestingly, all specimens were collected on angiosperm wood, in accordance with the description of *H. nudicephalum* (Gilbertson & Blackwell 1988).

The other clades of the phylogenetic tree are not as easily approached, although there is proof that they represent distinct and hitherto not formally described species. The results obtained from morphological analysis suggest that it is possible to distinguish more taxa in the complex than those recognised today. To do this properly, a thorough revision of the abundant herbarium material available would be necessary, an undertaking for which the preliminary morphological descriptions of the clades given in this study should serve as efficacious starting points.

The clade 1A–B contains two strongly supported subclades. The specimens from Greenland (1B) are phylogenetically divergent from the European specimens (1A) in spite of being morphologically similar. The Greenland specimens can, however, be separated from the European ones by the larger spores (12–14.5 × 4.3–5 μm *vs.* 9.5–13 × 3.5–5 μm) and being exclusively homothallic. Geographical isolation appears to be correlated with the divergence. Extending the subterminal group to also include clade 2, a strongly supported clade, (1A, 1B and 2), is retrieved. Its ancestral species may have been circumboreal; British Columbia (clade 2), Greenland (clade 1B), and Europe (clade 1A) have been phytogeographically isolated for up to 40 Myr (Lickey, Hughes & Petersen 2002). The geographical scope of the specimens suggests the taxa are distributed in eastern Canada/northeastern USA as well, and if this is the case, it would be valuable to see if those populations are genetically closer to those from Greenland (and Europe) than to British Columbia. If so, the barrier in central North America has probably been more efficient than the north Atlantic.

The moderately supported clade (3A, 3B) consists of two specimens which differ somewhat in morphology; one (FCUG 2872) is homothallic, rendering crossing tests troublesome. Clade 4 consists of two compatible specimens from northern Europe; all crossing tests with these and specimens from outside clade 4 were negative, suggesting clade 4 represents a distinct biological species. Based on an extended study, it may be possible to delimit clade 4 from other specimens by the characteristically denser ramification of the subicular and subhymenial hyphae. Clade 8 includes specimens from Greenland, the Caucasus, and eastern Asia. In this study, this was the most widely distributed taxon, and it is likely to occur in Europe and North America

in addition to Greenland. The phylogenetic tree also features single specimens on long branches, such as clades 6 and 9. It is assumed that they represent additional and largely unsampled components of the *H. setigerum* complex.

No easily interpreted pattern emerges from the plotting of substrata onto the phylogenetic tree. In the clades of comparatively large sampling, many distinct species of both leaf-shedding and leaf-retaining angiosperms and coniferous wood are found.

One aim of this study was to investigate if homothallism characterizes specific clades. All specimens in clade 1B were homothallic, while a representative from another clade in the same area (Greenland) was heterothallic. On the other hand, homothallism and heterothallism are mixed in clade 1A. For most of the clades, however, it has not been possible to investigate the type of thallism because the only available cultures were polysporous. The presence of heterothallic and homothallic specimens in one and the same clade has already been noticed in another corticioid species, *Hypochnicium albostramineum* (Bres.) Hallenb. 1985, without significant genetic divergence (Nilsson & Hallenberg 2003).

The spontaneous interpretation of a nucleotide sequence matrix as diverse as the one in this study would be that its components are very old and diverged from one another early on, or that some (or all) taxa evolved at high rates of evolution; an intricate in-between situation is probably the most likely scenario. That choice notwithstanding, evolution has left comparatively sparse tracks in morphology. While it was suspected that '*H. setigerum*' is a species complex rather than a single species, the capturing of nine or more distinct biological species in a 45-specimen study is surprising. The study thereby accentuates one of the most perplexing problems in contemporary corticioid mycology: how should one handle the many novel species that stem from DNA-based studies and that are supported by the phylogenetic and the biological species concepts (*cfr* Brasier 1997, Petersen & Hughes 1999) but at the same time are impossible or very difficult to tell apart using traditional means? We are reluctant to embrace species erected from DNA analysis alone, but when two or more independent, or but mildly entangled, sources of information flow seamlessly together, notably phylogenetic and intercompatibility attestations, we feel that this is sufficient evidence. That the species potentially cannot be identified other than by very expensive means (like sequencing) is indeed a problem, but a problem of communication rather than of biology.

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