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The phylogenetic position of the lichenicolous ascomycete *Capronia peltigerae*

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Abstract The genus *Capronia* includes a number of lichenicolous (lichen-inhabiting) species, none of which have previously been characterized in vitro or considered in molecular phylogenetic studies. We cultured *Capronia peltigerae* from *Peltigera rufescens* and report here the growth of this species on a variety of media and its phylogenetic position based on the analyses of nuclear ribosomal RNA, mitochondrial ribosomal RNA, and RNA polymerase II (*RPB*1) gene sequences. This species differs from the majority of *Capronia* studied in axenic culture in lacking a conidial anamorph. Phylogenetic analyses position *C. peltigerae* outside the Herpotrichiellaceae within a robustly supported basal lineage of the Chaetothyriales composed primarily of melanized, rock-inhabiting anamorphic fungi. Our results demonstrate that *Capronia*, as circumscribed currently, is polyphyletic, but they do not resolve the relationship of *C. peltigerae* with members of the Chaetothyriaceae.

Keywords Ascomycota · Chaetothyriaceae · Herpotrichiellaceae · Lichenicolous fungi · Molecular phylogenetics

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Introduction

Capronia Sacc. (Herpotrichiellaceae, Chaetothyriales) encompasses ascomycetes characterized by their very small, typically setose ascomata, aparaphysate centra, fissitunicate asci, and septate, hyaline or pigmented ascospores (Munk 1957; Müller et al. 1987; Barr 1991). Species of *Capronia* produce dark, slow growing colonies in vitro and conidial anamorphs that belong to the genera *Cladophialophora* Borelli, *Exophiala* J.W. Carmichael, *Phialophora* Medlar, and *Rhinocladiella* Nannf. (Schol-Schwarz 1968; Samuels and Müller 1978; Müller et al. 1987; Untereiner 1995, 1997; Untereiner et al. 1995; Okada et al. 1998; Untereiner and Naveau 1999). These anamorph genera comprise a group of fungi known as the black yeasts and include species responsible for important opportunistic infections of vertebrates (de Hoog et al. 2000).

The majority of the nearly 60 species of *Capronia* described to date occur on rotting wood or bark and the decaying stems and leaves of herbaceous plants (Untereiner 2000). Plant-associated *Capronia* are the most frequently cultured members of the genus, and are therefore best represented in molecular phylogenies. Fungicolous *Capronia* are studied less frequently. For example, of the nine species of *Capronia* reported exclusively from the fruit-bodies of other fungi, only five (*C. fungicola*, *C. nigerimma* (R.R. Bloxam) M.E. Barr, *C. dactylotricha* Unter. et al., *C. parasitica*, and *C. spinifera* (Ellis & Everh.) E. Müller et al.) are known in pure culture. The phylogenetic positions of lichenicolous representatives of the genus have not yet been investigated. Thirteen members of the genus are reported to grow obligately on lichens (Etayo and Sancho 2008; Halici et al. 2010) but none of these species have been cultivated in vitro or included in molecular phylogenetic studies.

To explore this latter problem, we studied *Capronia peltigerae* (= *Trichosphaeria peltigerae* Fuckel) the first lichenicolous species assigned to the genus (Hawksworth 1980; Eriksson and Hawksworth 1987). *Capronia peltigerae* is widely distributed geographically. It was described by Fuckel (1874) from the thalli of *Peltigera canina* (L.) Willd. in Switzerland and has since been recorded on species of *Peltigera* Willd. in Alaska and in a number of northern, central, and southern European countries (Martínez and Hafellner 1998; Diederich and Sérusiaux 2000; Kocourková 2000; Zhurbenko and Laursen 2003; Alstrup 2004; Zhurbenko 2004; Schiefelbein and Rätzel 2005; Diederich et al. 2006; Suija et al. 2009; Candan et al. 2010). We obtained mass- and single-ascospore cultures of *C. peltigerae* from freshly collected thalli of *Peltigera rufescens* (Weiss) Humb. and characterized its growth on a variety of media. We also investigated the position of this species within the Ascomycota and its relationship to other *Capronia* based on the analyses of nuclear ribosomal RNA, mitochondrial ribosomal RNA, and RNA polymerase II (*RPB*1) gene sequences.

Materials and methods

Fungal strains and cultural studies

Mass-ascospore (UAMH 11090) and single-ascospore (UAMH 11091) isolates of *Capronia peltigerae* were obtained by streaking the contents of mature ascomata on Modified Leonian's agar (MLA) (Malloch 1981) containing 2.5% agar, chlortetracycline (50 μg/mL), and streptomycin sulfate (50 μg/mL). Germinating ascospores were subcultured to MLA and Potato Carrot agar (PCA) (Gams et al. 1987) containing 1.5% agar. Cultures were maintained on these media at room temperature (20–21 C).

For comparative purposes, mass- and single-ascospore cultures were grown on MLA, 2% malt extract agar (MEA), oatmeal agar (OA) (Tuite 1969), and filtered oatmeal agar (CBSOA) (Gams et al. 1987). Plates were inoculated in triplicate with 3 x 3 mm squares cut from the actively growing edges of colonies on MLA and incubated at room temperature. Colony diameter was measured and descriptions of colony morphology made at 7-day intervals for 28 days. Colour descriptions are based on Kornerup and Wanscher (1978).

Microscopic examinations of cultures and specimens were made from preparations mounted in distilled water.

DNA extraction, amplification and sequencing

The mass-ascospore isolate (UAMH 11090) of *Capronia peltigerae* used for sequencing was grown in 50 mL Modified Leonian's broth (MLA lacking agar) for 5–7 d on a rotary shaker at 100 rpm. Mycelia were collected by centrifugation and stored at −20°C until lyophilized. Total nucleic acids were isolated from ground, lyophilized cultures and purified following Lee and Taylor (1990) or using a modified protocol from Zolan and Pukkila (1986) as detailed in Gueidan et al. (2007). Protocols used for sequencing the large (nucLSU) and small (nucSSU) subunits of the nuclear ribosomal RNA gene and the largest subunit of the RNA polymerase II (*RPB*1) are described in Gueidan et al. 2007. Those used to sequence the internal transcribed spacer (ITS) of the nuclear ribosomal RNA gene and the small subunit of the mitochondrial ribosomal RNA gene (mitSSU) followed Untereiner and Naveau (1999) and Zoller et al. (1999), respectively. Primers used in the amplification and sequencing of the loci used in this study included (ITS) WITS3 (Untereiner et al. 1995), WITS2, WNS9 (Untereiner and Naveau 1999), LR1 (Vilgalys and Hester 1990), (mitSSU) mrSSU1, mrSSU3R (Zoller et al. 1999), (nucLSU) LR0R (Rehner and Samuels 1994), LR3R, LR5, LR7 (Vilgalys and Hester 1990), (nucSSU) nssu131, nssu634, nssu1088R (Kauff and Lutzoni 2002), NS22, NS24 (Gargas and Taylor 1992), SR7R (Spatafora et al. 1995), (*RPB*1) *RPB1*-AF, *RPB1*-G2R (B. Hall, unpublished), *RPB1*-6R1asc, and *RPB1*-DF1asc (Hofstetter et al. 2007).

Taxon sampling and molecular data

To investigate the phylogenetic placement of *Capronia peltigerae*, a total of 57 taxa were sampled across the Chaetothyriales (Table 1) representing the major phylogenetic groups in this order (Gueidan et al. 2008). Two species of Verrucariales (*Placocarpus schaereri* and *Verrucula inconnexaria*) were used as an outgroup.

Alignments and phylogenetic analyses

Sequences were assembled and edited using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI). A manual alignment was performed using MacClade 4.06 (Maddison and Maddison 2003). Ambiguous regions (sensu Lutzoni et al. 2000) and introns were delimited manually and excluded from the alignment. Phylogenetic relationships and confidence were inferred using a Bayesian approach based on a combined nucLSU-nucSSU-mitSSU-*RPB*1 dataset. Additional support values were estimated using a Maximum Likelihood approach with RAxML-VI-HPC (Stamatakis et al. 2005, 2008). These analyses were run on the Cipres portal (http://www.phylo.org/). As a first step, the congruence between gene regions was tested using a 70% reciprocal bootstrap criterion (Mason-Gamer and Kellogg 1996). For each gene region, a non-parametric bootstrap analysis was done with

Table 1 Sources and accession numbers of the isolates examined in this study

Taxon	Source [a]	GenBank Accession Numbers			
		nucLSU	nucSSU	mitSSU	*RPB*1
Capronia fungicola (Samuels & E. Müller) Unter.	CBS 614.96	FJ358224	FJ358292	FJ225722	FJ358356
C. munkii Unter.	CBS 615.96	EF413604	EF413603	FJ225723	EF413605
C. parasitica (Ellis & Everhart) E. Müller et al.	CBS 123.88	FJ358225	FJ358293	FJ225724	FJ358357
C. peltigerae (Fuckel) D. Hawksw.	UAMH 11090	HQ613813	HQ613815	HQ613814	HQ625027
C. pilosella (P. Karsten) E. Müller et al.	MUCL 39967	DQ823099	DQ823106	FJ225725	DQ840554
C. semiimmersa (Cand. & Sulmont) Unter. & F.A. Naveau	MUCL 40572	FJ358226	FJ358294	FJ225726	FJ358358
Capronia sp. WUC15ss1	WUC 15ss1	FJ358227	FJ358295	FJ225727	FJ358359
Capronia sp. WUC26	WUC 26	FJ358228	FJ358296	FJ225728	FJ358360
Capronia sp. WUC102	WUC 102	FJ358229	FJ358297	FJ225729	FJ358361
Capronia sp. WUC236	WUC 236	FJ358230	FJ358298	FJ225730	FJ358362
Capronia sp. WUC315	WUC 315	FJ358231	FJ358299	FJ225731	FJ358363
Ceramothyrium carniolicum (Rehm) Petr.	CBS 175.95	FJ358232	FJ358300	-	FJ358364
Cladophialophora boppii (Borelli) de Hoog et al.	CBS 126.86	FJ358233	FJ358301	FJ225732	FJ358365
Clad. carrionii (Trejos) de Hoog et al.	CBS 160.54	FJ358234	FJ358302	FJ225733	FJ358366
Clad. minourae (Iwatsu) Hasse & de Hoog	CBS 556.83	FJ358235	FJ358303	FJ225734	FJ358367
Clad. modesta McGinnis et al.	CBS 985.96	FJ358236	FJ358304	FJ225735	FJ358368
Coniosporium perforans Sterfl.	CBS 885.95	FJ358237	FJ358305	FJ225736	-
Coniosporium sp.	CBS 268.34	FJ358238	FJ358306	FJ225748	FJ358369
Cyphellophora laciniata G.A. de Vries	CBS 190.61	FJ358239	FJ358307	FJ225737	FJ358370
Exophiala bergeri Hasse & de Hoog	CBS 353.52	FJ358240	FJ358308	FJ225738	FJ358371
E. castellanii Iwatsu et al.	CBS 158.58	FJ358241	FJ358309	FJ225739	FJ358372
E. dermatitidis (Kano) de Hoog	CBS 207.35	DQ823100	DQ823107	FJ225740	DQ840555
E. jeanselmei (Langeron) McGinnis & A.A. Padhye	CBS 507.90	FJ358242	FJ358310	-	FJ358373
E. lecanii-corni (Benedek & G. Specht) Haase & de Hoog	CBS 123.33	FJ358243	FJ358311	FJ225741	FJ358374
E. nigra (Issatsch.) Haase & de Hoog	dH 12296	FJ358244	FJ358312	FJ225742	FJ358375
E. oligosperma Calendron ex de Hoog & Tintelnot	CBS 725.88	FJ358245	FJ358313	FJ225743	FJ358376
E. pisciphila McGinnis & Ajello	CBS 537.73	DQ823101	DQ823108	FJ225744	DQ840556
E. salmonis J.W. Carmichael	CBS 157.67	EF413609	EF413608	FJ225745	EF413610
E. xenobiotica de Hoog et al.	CBS 115831	FJ358246	FJ358314	FJ225746	FJ358377
Fonsecaea monophora (M. Moore & F.P. Almeida) de Hoog et al.	CBS 102243	FJ358247	FJ358315	FJ225747	FJ358378
Phaeococcomyces catenatus (de Hoog & Herm.-Nijh.) de Hoog	CBS 650.76	-	FJ358316	FJ225749	FJ358379
Phialophora europaea de Hoog et al.	CBS 129.96	FJ358248	FJ358317	FJ225750	FJ358380
Phial. verrucosa Medlar	MUCL 9760	EF413615	EF413614	FJ225751	EF413616
Placocarpus schaereri (Fr.) O. Breuss	CG 588	EF643766	EF689850	-	EF689770
Rhinocladiella anceps (Sacc. & Ellis) S. Hughes	CBS 181.65	DQ823102	DQ823109	FJ225752	DQ840557
Sarcinomyces petricola U. Wollenzien & de Hoog	CBS 101157	FJ358249	FJ358318	FJ225753	FJ358381
Verrucula inconnexaria Nav.-Ros. & Cl. Roux.	CG 652	EF643819	EF689890	FJ225716	EF689819
rock isolate A95	A95	FJ358269	FJ358337	-	FJ358401
rock isolate H5	H5	FJ358270	FJ358338	-	FJ358402
rock isolate TRN1	TRN1	FJ358250	FJ358319	FJ225754	FJ358382
rock isolate TRN4	TRN4	FJ358251	FJ358320	FJ225755	FJ358383
rock isolate TRN14	TRN14	-	FJ358321	FJ225756	FJ358384
rock isolate TRN30	TRN30	FJ358252	FJ358322	FJ225757	FJ358385
rock isolate TRN107	TRN107	FJ358253	FJ358323	FJ225758	FJ358386
rock isolate TRN115	TRN 15	FJ358254	FJ358324	FJ225759	FJ358387
rock isolate TRN210	TRN210	FJ358255	FJ358325	FJ225760	FJ358388
rock isolate TRN214	TRN214	FJ358256	-	FJ225761	FJ358389

Table 1 (continued)

Taxon	Source [a]	GenBank Accession Numbers			
		nucLSU	nucSSU	mitSSU	*RPB*1
rock isolate TRN242	TRN242	FJ358257	FJ358326	FJ225762	FJ358390
rock isolate TRN247	TRN247	FJ358258	FJ358327	FJ225763	FJ358391
rock isolate TRN436	TRN436	FJ358259	FJ358328	-	-
rock isolate TRN475	TRN475	FJ358260	FJ358329	FJ225764	FJ358392
rock isolate TRN486	TRN486	FJ358261	FJ358330	FJ225765	FJ358393
rock isolate TRN488	TRN488	FJ358262	-	FJ225766	FJ358394
rock isolate TRN493	TRN493	FJ358263	FJ358331	FJ225767	FJ358395
rock isolate TRN497	TRN497	-	FJ358332	FJ225768	FJ358396
rock isolate TRN506	TRN506	FJ358264	-	FJ225769	FJ358397
rock isolate TRN508	TRN508	FJ358265	FJ358333	FJ225770	FJ358398
rock isolate TRN515	TRN515	FJ358266	FJ358334	FJ225771	FJ358399
rock isolate TRN531	TRN531	FJ358267	FJ358335	FJ225772	FJ358400

[a] Cultures or vouchers are located in the following collections: A, collection of A. Gorbushina, Oldenburg, Germany; CBS, CBS Fungal Biodiversity Center, Utrecht, The Netherlands; CG, collection of C. Gueidan, London, UK; dH, collection of S. de Hoog, Utrecht, The Netherlands; H, collection of A. Gorbushina, Oldenburg, Germany; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; TRN, collection of C. Ruibal, Madrid, Spain; UAMH, University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Canada; WUC, collection of W. Untereiner, Brandon, Canada

RAxML-VI-HPC using 1000 pseudoreplicates and a GTRMIX model of molecular evolution. The resulting groupings and support values were visually compared for conflicts, but, as none were detected, all gene regions were combined for the final analyses. For the Bayesian approach, Modeltest 3.7 (Posada and Crandall 1998) was used to estimate the models of molecular evolution according to an Akaike information criterion for six partitions for which we assumed rate heterogeneity (nucLSU, nucSSU, mitSSU, *RPB*1 first, second and third codon positions). Modeltest selected a GTR+I+G model for each of these partitions. In the combined analysis, this model was therefore used for each of the six partitions. Two analyses of four chains were run for 4,000,000 generations using MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003), and trees were sampled every 500 generations. The trace files were analyzed using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer). All runs converged on the same average likelihood score and topology. A burn-in sample of 4,000 trees was discarded for each run. The remaining 8,000 trees were used to estimate branch lengths with the sumt command in MrBayes, and Posterior Probabilities (PPs) with the majority rule consensus tree command in PAUP* version 4.0b10 (Swofford 1999). The program RAxML-VI-HPC (Stamatakis et al. 2005, 2008) was used for a non-parametric bootstrap analysis with 1000 pseudoreplicates and a GTRMIX model of molecular evolution.

Results

Morphological studies

Ascospores from ascomata on freshly collected (less than 14 d old) thalli of *P. rufescens* germinated within 24 h on MLA to form 1(−2) hyphal germ tubes from each cell. Macroscopically visible colonies were evident on MLA within 96 h.

Colonies 22–27 mm diam on MEA, MLA and OA in 28 d; on CBSOA 30–32 mm in 28 d. Aerial mycelium on CBSOA scant, tufted and lannose-cottony at the centre of the colony, brownish grey to greyish brown (5D2-3), mycelium toward the margin immersed, brown (5F4-6). Aerial mycelium on OA denser, short and cottony, and extending nearly to the margin, brownish grey to greyish brown (5D2-3) in the centre, mycelium toward the margin immersed, dark grey (F1) or brownish grey (4-5F2). Aerial mycelium on MEA and MLA abundant, short, cottony to felty-lannose, appearing powdery, brownish grey to greyish brown (5F2-3) in the centre on MEA and dark grey (E1) to olive grey (3E2) or brownish grey (4E2) in the centre on MLA, mycelium toward the margin immersed, brown (5F5-8) on MEA and olive (3F5-7) to olive brown (4F5-7) on MLA. Colony reverse brownish grey to brown (5F3-4) on CBSOA and MEA, dark grey (F1) to olive grey to olive (3F2-4) on MLA, and dark grey (F1) on OA. Colony margin on all media even and sharply defined.

Hyphae on all media brown, 4.5–6.0 µm wide, thin-walled, and sparingly branched. Conidiogenous cells and conidia absent. Ascomatal initials, thick-walled chlamydospores, and yeast-like cells not observed.

Material examined: LUXEMBOURG, Lorraine district: Between Dudelange and Kayl, Haardt (IFBL: M854.32; UTM: KV.88), on decolourized portions of the upper and lower surfaces of the thalli of *Peltigera rufescens* growing on soil, 22 December 2006, *P. Diederich* 16353 (herbarium Diederich, UAMH 11090 mass-ascospore isolate, UAMH 11091 single-ascospore isolate).

Comments: Our collection of *Capronia peltigerae* (Figs. 1 and 2) corresponds closely to the descriptions of this species given by Fuckel (1874) and Winter (1884). It differs from those provided by Hawksworth (1980) and Marson (2008, http://www.lichenology.info) in possessing ascospores that are less strongly constricted at the septa, but this likely reflects differences in the maturity of collections. Zhurbenko and Laursen (2003) reported smaller ascospores from a collection of *C. peltigerae* on *Peltigera aphthosa* (L.) Willd. from Alaska and also attributed this variation in size to differences in the maturity of specimens. Collections of *C. peltigerae* and *Capronia* cf. *peltigerae* from Turkey

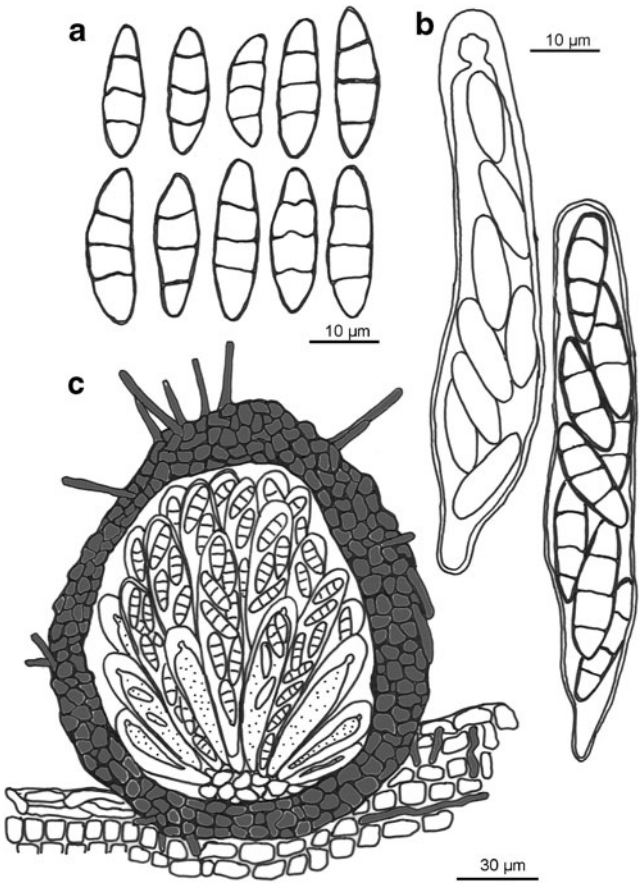

Fig. 1 *Capronia peltigerae* (Diederich 16353) **a** Ascospores in water. **b** Asci in water. **c** Ascoma on thallus of *Peltigera rufescens*

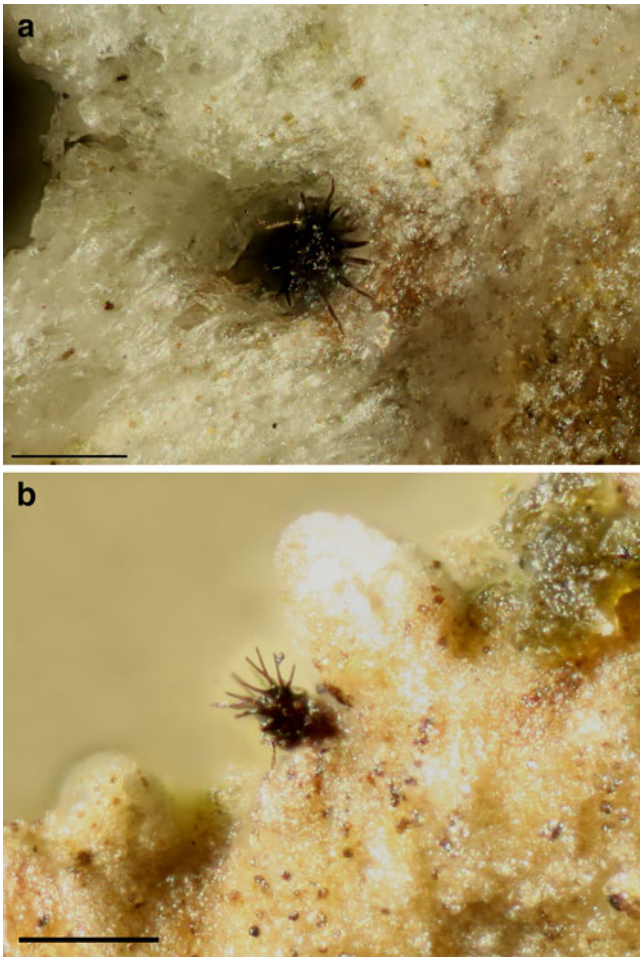

Fig. 2 *Capronia peltigerae* (Diederich 16353) **a**.-**b**. Ascomata on decolourized thallus of *Peltigera rufescens*. Bars=100 µm

(Halici et al. 2010) and Russia (Zhurbenko 2004) described as possessing finely verrucose ascospores that are pale grey-brown or brownish at maturity may represent a different taxon.

Capronia peltigerae differs from the majority of the members of this genus characterized in axenic culture in producing more rapidly growing colonies, exhibiting only filamentous growth (yeast-like cells are not observed on solid or in liquid media), and lacking a conidial anamorph. Cultures of *C. peltigerae* remained sterile even when maintained on CBSOA, MEA, MLA, PCA and OA for 12 months. An anamorph has never been reported on *Peltigera* in association with the ascomata of *C. peltigerae*, nor did we observe one in this study.

Phylogenetic analyses

The phylogeny inferred from the analysis of the combined nucLSU-nucSSU-mitSSU-*RPB*1 sequences (Fig. 3) is consistent with previous analyses of the Chaetothyriales (Badali et al. 2008; Gueidan et al. 2008) and resolves a

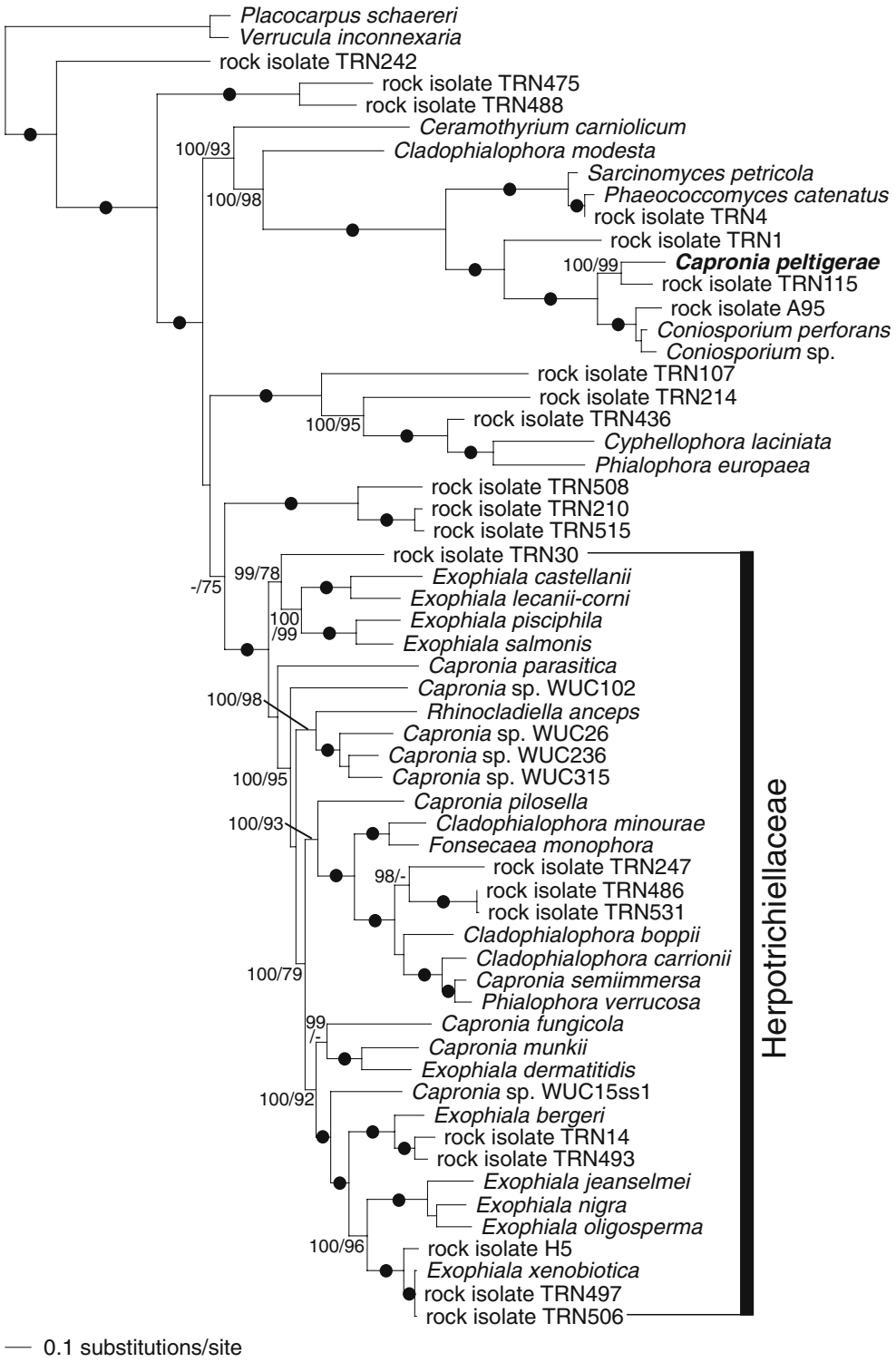

Fig. 3 Phylogenetic placement of *Capronia peltigerae* according to a four-locus phylogeny of the Chaetothyriales obtained from a Bayesian analysis. Black dots on branches represent nodes supported by 100% posterior probabilities (PP) and 100% maximum likelihood (ML) bootstrap. Other values are indicated above or below the branches (PP/ML bootstrap). A dash indicates PP and ML bootstrap values below 95% and 70%, respectively. Nodes without values were not supported in both the Bayesian and the ML analyses

large, well-supported clade corresponding to the Herpotrichiellaceae and a number of smaller, strongly supported, basal lineages comprised primarily of rock-inhabiting fungi and a few clinically important opportunists. The Herpotrichiellaceae encompasses lignicolous and fungicolous *Capronia*, members of the anamorph genera *Exophiala*, *Fonsecaea* Negroni, and *Rhinocladiella*, and the majority of the clinically important *Cladophialophora* and *Phialophora* included in the dataset.

Capronia peltigerae is not inferred as a member of the Herpotrichiellaceae. Rather, it belongs to a lineage that is basal to this family and which includes *Phaeococcomyces*

catenatus, the opportunistic pathogen *Cladophialophoa modesta*, a number of melanised rock-inhabiting fungi, and *Ceramothyrium carniolicum* (Chaetothyriaceae). Within this lineage, *C. peltigerae* is most closely related to TRN115, an unnamed strain from limestone (Ruibal et al. 2005, 2008), rock isolate A95, and members of the rock-inhabiting genus *Coniosporium* Link (*Coniosporium* sp. CBS 268.34 and *Con. perforans*).

Discussion

The generic classification of *Capronia* is in need of revision, but constructing of a comprehensive phylogeny for the genus is hindered by the lack of specimens and cultures. The majority of *Capronia* (including species of the synonymized genera *Berlesiella* Sacc., *Dictyotrichiella* Munk, *Didymotrichiella* Munk, *Herpotrichiella* Petrak, and *Polytrichiella* M.E. Barr) are known only from the type collection (or from three or fewer collections made by the describing author from the type locality), and two thirds of the members of the genus have yet to be cultured (Untereiner 2000). It is hardly surprising, therefore, that the lichenicolous members of the genus have been unrepresented in culture collections and molecular phylogenetic studies. Nearly half of the thirteen lichenicolous species placed in *Capronia* are known only from the type locality, and the few widely distributed lichen-inhabiting members of the genus (i.e., *C. hypotrachynae* Etayo & Diederich, *C. normandinae* R. Sant. & D. Hawksw., *C. peltigerae*) appear to be uncommon or rare in regions where they are documented. Collecting these fungi is further hampered by the fact that they cause no obvious damage to the host thallus (Halici et al. 2010; Hawksworth 1980, 1990) and possess extremely small, inconspicuous ascomata.

Ascomata of *Capronia peltigerae* develop on the bleached or moribund portions of its host (Fig. 2) (Winter 1884; Hawksworth 1980; Kocourková 2000; Zhurbenko 2004; Zhurbenko and Laursen 2003), but it is unclear if *C. peltigerae* is responsible for this discolouration or occurs opportunistically on the aging parts of lichen thalli. If the difficulty of isolating and culturing lichenicolous fungi reflects their host specificity and nutritional requirements (Lawrey and Diederich 2003), then the ease with which *C. peltigerae* was brought into culture and its relatively rapid rate of growth in vitro would indicate that this species obtains fixed carbon from dying or dead thalli of *Peltigera* (i.e., it is saprobic) rather than from a living host. We cultured *C. peltigerae* from freshly collected, air-dried material employing methods used to isolate lignicolous members of the genus (Untereiner et al. 1995), but we did not determine the viability of older ascospores. Because *C. peltigerae* was isolated and grown in vitro without difficulty, we anticipate that culturing other lichen-inhabiting members of the genus should pose few problems, particularly if ascospores from fresh or recently collected, air-dried material are used.

Capronia peltigerae is distinctive among the lichenicolous members of this genus in possessing hyaline ascospores and in occurring on *Peltigera*, a common and geographically widespread genus of terricolous and muscicolous foliose macrolichens (Martínez et al. 2003). It is reported most frequently on *P. canina* and *P. rufescens*, but it is also found on the thalli of *P. aphthosa* (Hafellner 1994; Zhurbenko and Laursen 2003), *P. britannica* (Gyelnik) Holt.-Hartw. & Tønsb. (Martínez and Hafellner 1998), and *P. didactyla* (With.) J.R. Laundon (Miadlikowska and Alstrup 1995). Given its relatively wide host range, the reported variation in the morphology of the ascospores of this species (Zhurbenko and Laursen 2003; Zhurbenko 2004; Halici et al. 2010), it is plausible that collections of *C. peltigerae* may represent more than a single species.

Capronia peltigerae belongs to the Chaetothyriales, but it is not inferred as a member of the Herpotrichiellaceae (Fig. 3). Apart from its lichenicolous habit, *C. peltigerae* differs from the majority of *Capronia* in possessing hyaline ascospores and lacking a conidial anamorph. The type species of the genus, *C. sexdecemspora* (Cooke) Sacc., and species placed formerly in *Polytrichiella*, also possess hyaline ascospores (Cooke 1871; Barr 1972; Müller et al. 1987), and a conidial anamorph was not observed for a species with olive-brown ascospores identified tentatively as *C. pleiospora* (Mouton) Sacc. (Untereiner 2000). However, since other hyaline-spored and lichenicolous *Capronia* have not been cultured or included in molecular phylogenies, and the closest relatives of *C. pleiospora* have yet to be identified, it is not possible to assess if these characters are phylogenetically informative within the Chaetothyriales. And while the results of this study confirm that *Capronia* is polyphyletic, we consider it premature to recognize a new genus for *C. peltigerae* without first investigating the conspecificity of collections identified as this species, determining the phylogenetic position of *C. sexdecemspora*, and ascertaining if other lichenicolous *Capronia* are also extralimital to the Herpotrichiellaceae.

In our phylogeny, *Capronia peltigerae* is one of only two teleomophic fungi included in the largest of four basal lineages in Chaetothyriales. Within this group, *C. peltigerae* is most closely related to the rock isolates TRN115 and A95, *Coniosporium* sp. CBS 268.34, and *Con. perforans.* This relationship is supported by BLAST searches (Zhang et al. 2000) of the ITS of *C. peltigerae* (HQ709322) which reveal that it is most similar to sequences of a variety of uncultured ascomycetes from environmental samples (highest maximum identity 94–98%) and rock-inhabiting species of *Coniosporium*, *Phaeococcomyces* de Hoog, and *Sarcinomyces* Lindner (highest maximum identity 91–93%). *Coniosporium*

encompasses extremotolerant, slow-growing, arthroconidial species isolated frequently from marble monuments (Sterflinger et al. 1997; Sert and Sterflinger 2010) and less commonly from plant surfaces (Hyde et al. 2002) and human skin and nails (Li et al. 2008). Strains of an unnamed species of *Coniosporium* that is closely related to *Con. perforans* have also been isolated from the thalli of saxicolous lichens (Harutyunyan et al. 2008). *Capronia peltigerae* differs from *Coniosporium* in its more rapid growth in pure culture, and in lacking moniliform hyphae, budding cells, and conidia. Although the possibility exists that *C. peltigerae* will be shown to be the sexual state of an endolichenic fungus, there is no cultural or molecular evidence to connect this species to any previously characterized anamorphic, lichen-inhabiting member of the Chaetothyriales.

The lineage that includes *Capronia peltigerae* and rock-inhabiting fungi also contains *Ceramothyrium carniolicum* and may, therefore, correspond to the Chaetothyriaceae. This family encompasses epiphytic and primarily tropical species that produce ascomata beneath a mycelial pellicle that may or may not bear setae (Batista and Ciferri 1962; Barr 1979; Hughes 1976; Pereira-Carvalho et al. 2009). *Capronia peltigerae* resembles some Chaetothyriaceae in possessing hyaline ascospores and cylindrical (i.e., non-moniliform) hyphae, but we cannot confidently assign this species to this family based on the taxa included in our analysis. It is also not possible to ascertain the phylogenetic position of the Chaetothyriaceae within the Chaetothyriales since molecular data are unavailable for the majority of the members of this family, including species of the type genus, *Chaetothyrium* Speg.

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