A new species of *Hypocrella*, *H. macrostroma*, and its phylogenetic relationships to other species with large stromata

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Two specimens of a new species of *Hypocrella* with large stromata were collected in Bolivia and Costa Rica. The morphology of the new species, *H. macrostroma* sp. nov., was compared with that of other species with large stromata, i.e. *H. africana*, *H. gaertneriana*, and *H. schizostachyi*. In addition, phylogenetic analyses of partial sequences from three genes, large subunit nuclear ribosomal DNA (LSU), translation elongation factor 1-α (EF1-α), and RNA polymerase II subunit 1 (RPB1), were conducted to determine the relationships of the new species to other species of *Hypocrella/Aschersonia*. Phylogenetic analyses show that *H. macrostroma* belongs to a strongly supported clade that includes *H. africana*, *H. schizostachyi*, and *Aschersonia insperata*, whereas other *Hypocrella* species belong to two sister clades. *Hypocrella macrostroma* is described and illustrated, and a lectotype is designated for *H. gaertneriana*.

INTRODUCTION

Species in the entomopathogenic genus *Hypocrella* (*Clavicipitaceae*, *Hypocreales*, *Ascomycota*) are frequently encountered in the tropics and less often in the subtropics. These fungi are found growing on scale insects (*Coccidae*, *Homoptera*) and whiteflies (*Aleyrodidae*, *Homoptera*) that parasitize living leaves, or rarely branches, of monocotyledonous and dicotyledonous plants. *Hypocrella* species have perithecia immersed in a brightly colored stroma and cylindrical asci that contain four or eight multiseptate ascospores that disarticulate at maturity. Anamorphs of *Hypocrella* are classified in the anamorph genus *Aschersonia*. The latter state is more commonly found in the field, and is sometimes associated with the teleomorph in the same stroma. *Aschersonia* is characterized by pycnidium-like conidiomata, phialides, sometimes paraphyses, and unicellular, fusiform, hyaline conidia that are brightly colored in mass and produced in copious slime.

Approximately 115 names in *Hypocrella* and 79 in *Aschersonia* have been validly published; however, only about 50 and 44 species, respectively, are currently accepted (Petch 1921, Dingley 1954, Mains 1959a, b, Hywel-Jones & Evans 1993). Only about 15 species

have been linked to teleomorphs. Petch (1921) compiled the most complete taxonomic work on *Hypocrella/Aschersonia* to date; he accepted 42 species. Fungal biodiversity surveys in poorly explored geographical regions, detailed morphological examinations, and DNA sequence analyses will probably reveal many undescribed species.

Two specimens of an unidentified species of *Hypocrella* with large stromata (3–22 mm diam) were collected in Costa Rica and Bolivia on stems of living dicot vines. Generally, the stromata of *Hypocrella/Aschersonia* are 2–5 mm diam, however, *H. africana*, *H. gaertneriana*, *H. schizostachyi* (Hywel-Jones & Samuels 1998), and the unidentified *Hypocrella* have markedly larger stromata that measure *ca* 5–30 mm in diameter. These species are probably on scale insects attached to branches of living dicotyledonous plants (*H. africana* and the unidentified *Hypocrella*) or bamboo culms (*H. gaertneriana* and *H. schizostachyi*), whereas the majority of the *Hypocrella/Aschersonia* species occur on scale insects or whiteflies on living leaves. The unidentified species resembles *H. gaertneriana* in the shape and size of the stroma; however, the colour of the stroma and that the unidentified species is on an insect on living dicotyledonous vines suggested that it might be a new species.

H. gaertneriana was described and illustrated by Møller (1901) based on a specimen from Brazil. Specimens were deposited in the Berlin Botanical

Garden and Museum herbarium (B), but, unfortunately, a large part of the herbarium's collection, including Møller's, was destroyed in 1943 (http://www.bgbm.fu-berlin.de/bgbm/research/colls/herb/). *H. gaertneriana* was re-described and illustrated based on collections from Venezuela and French Guiana (Hywel-Jones & Samuels 1998); however, the authors did not designate a neotype. In the present paper, *H. gaertneriana* is lectotypified with the original illustration in Møller (1901).

Other genera in the *Clavicipitaceae*, such as *Ascopolyporus*, *Dussiella*, and *Hyperdermium*, also parasitize scale insects and have relatively large stromata. In all these genera, the stromatal mass greatly exceeds that of the scale insect host. Sullivan *et al.* (2000) suggested that the large size of the stromata results from a kind of secondary plant parasitism: once the fungus has consumed the scale insect body, the fungus may continue to access plant nutrients through the insect's stylet. Other authors have suggested that the mechanism of nutrient acquisition in species of *Hypocrella* with large stromata is through the living scale insect that forms a bridge between the fungus and the plant (Hywel-Jones & Samuels 1998). A similar mechanism has been observed in *Septobasidium* (Couch 1938). More detailed research is needed to elucidate this phenomenon.

The main objectives of the present paper are: (1) to describe a new species of *Hypocrella* with large stromata; and (2) to show its phylogenetic relationships to other species of *Hypocrella*/*Aschersonia*, by using partial DNA sequences of three genes, i.e., large subunit nuclear ribosomal DNA (LSU), translation elongation factor 1-α (EF1-α), and RNA polymerase II subunit 1 (RPB1). We also discuss whether *Hypocrella* species with large stromata should be classified in a separate genus.

MATERIALS AND METHODS

Morphological examination

Dried reference specimens were obtained from US National Fungus Collection (BPI; *H. africana* holotype BPI 635731, and *H. schizostachyi* isotype BPI 635854) and the William and Lynda Steere Herbarium (NY; *H. gaertneriana* GJS 1776). Other specimens (*Hypocrella* sp. J.B. 115 = CUP 67509 and P.C. 605 = CUP 67508, and *H. africana* P.C. 736 = CUP 67510) were collected during recent expeditions to Ghana, Costa Rica and Bolivia; these specimens are deposited at the Cornell University Plant Pathology Herbarium (CUP). Stromata of J.B. 115 and P.C. 605 were rehydrated briefly in distilled water with a trace of Tween® 80 (J. T. Baker Chemical, Phillipsburg, NJ). Then, rehydrated stromata were supported by Tissue-Tek O.C.T. Compound 4583 (Miles, Elkhart, IN) and sectioned at a thickness of *ca* 15 μm with a freezing microtome. The characteristics of the stroma tissue,

perithecia, asci and ascospores were characterized by light microscopy. Colour terminology is from Kornerup & Wanscher (1978).

The only culture available for the unidentified species of *Hypocrella* was obtained from J.B. 115 (ARSEF 7748) by isolating asci containing ascospores and placing them on Difco potato dextrose agar (PDA) with antibiotics. Morphological observations of the colonies and anamorph were based on cultures grown on PDA for four weeks in an incubator at 25 °C with alternating 12 h fluorescent light and 12 h darkness.

Measurements of continuous characters such as spore length were made using the beta 4.0.2 version of Scion Image software (Scion, Frederick, MD). Confidence intervals ($\alpha = 0.05$), minimum and maximum values for 10–30 anamorph and teleomorph measurements (except where indicated) were calculated using Systat 8.0 (SPSS, Chicago, IL).

DNA extraction, PCR, and sequencing

Cultures of *Hypocrella* sp. J.B. 115 and other *Hypocrella*/*Aschersonia* species used in the phylogenetic analyses (Table 1) were grown on potato-dextrose broth in a 6-cm-diam Petri plate for about one week. The mycelial mat was harvested in a laminar flow hood and then dried using clean, absorbent paper towels. DNA was extracted with Ultra Clean™ Plant DNA Isolation Kit (MO BIO Laboratories, Solana Beach, CA). To extract DNA from the herbarium specimen of *Hypocrella* sp. P.C. 605, the surface of the stroma was first cleaned briefly with sterilized distilled water, then rehydrated by placing the stroma in a small Petri plate with sterilized distilled water and letting it stand for a few minutes until the stroma became softer. Subsequently, a very thin layer of the surface of the stroma was shaved off using a scalpel and then discarded. Pieces of the clean inner stroma, including centri, were cut out and then placed in a 1.5-mL Eppendorf tube for immediate DNA extraction with Ultra Clean™ Plant DNA Isolation Kit.

Three partial gene regions were amplified, i.e., large subunit nuclear ribosomal DNA (LSU), translation elongation factor 1-α (EF1-α), and RNA polymerase II subunit (RPB1). The primers used were LSU: LRORf (5′-GTACCCGCTGAACTTAAGC-3′) and LR5r (5′-ATCCTGAGGGAAACTTC-3′) (Vilgalys & Hester 1990); EF1-α: 983f (5′-GCYCCYGGHCAYCGTG-AYTTYAT-3′) (Carbone & Kohn 1999) and 2218r (5′-ATGACACCRACRGCRACRGTYTG-3′) (Rehner 2001); RPB1: cRPB1Af (5′-CAYCCWGGYTTYA-TCAAGAA-3′) and RPB1Cr (5′-CCNGCDATNTC-RTTRTCCATRTA-3′) (Castlebury *et al.* 2004). PCR protocols for LSU and EF1-α are described in Sung *et al.* (2001) and Chaverri & Samuels (2003), respectively. PCR for RPB1 was conducted as follows: (1) 5 min at 95 °, (2) 40 cycles of denaturation at 95 ° for 1 min, annealing at 50 ° for 2 min, and extension at 72 ° for 2 min, and (3) 72 ° for 10 min. The resulting

Table 1. Isolates, geographic origin and GenBank numbers of species used in the phylogenetic analyses.

Species	Isolate/Specimen number	Geographic origin	GenBank accession nos		
			LSU	EF1-α	RPB1
Aschersonia aleyrodis	P.C. 445	Mexico	AY986900*	AY986925*	DQ000326*
A. andropogonis	P.C. 535	Bolivia	AY986901*	AY986926*	DQ000327*
A. basicystis (1)[a]	P.C. 628	Ecuador	AY986902*	AY986927*	DQ000328*
A. basicystis (2)[a]	P.C. 457	Mexico	AY986904*	AY986929*	DQ000330*
A. basicystis (3)[a]	P.C. 374	Costa Rica	AY986903*	AY986928*	DQ000329*
A. blumenaviensis	P.C. 597	Bolivia	AY986905*	AY986930*	DQ000331*
A. cubensis (1)[a]	P.C. 375	Costa Rica	AY986906*	AY986931*	DQ000332*
A. cubensis (2)[a]	P.C. 440	Mexico	AY986907*	AY986932*	DQ000333*
A. incrassata	P.C. 595	Bolivia	AY986909*	AY986934*	DQ000335*
A. insperata	ARSEF 2396	Philippines	AY518374	AY986935*	DQ000336*
A. napoleonae	P.C. 737 (ARSEF 7697)	Ghana	AY986910*	AY986936*	DQ000337*
A. placenta	P.C. 533	Vietnam	AY986911*	AY986937*	DQ000338*
A. 'rhombispora'[b]	P.C. 467	Costa Rica	AY986908*	AY986933*	DQ000334*
A. turbinata	M.C.A. 2432	Guyana	AY986912*	AY986938*	DQ000339*
A. viridans	M.L. 202i (ARSEF 7695)	Puerto Rico	AY986913*	AY986939*	DQ000340*
Aschersonia sp.[c]	P.C. 627	Ecuador	AY986916*	AY986942*	DQ000343*
Aschersonia sp.[c]	P.C. 735 (ARSEF 7699)	Cameroon	AY986914*	AY986940*	DQ000341*
Aschersonia sp.[c]	P.C. 569	Bolivia	AY986915*	AY986941*	DQ000342*
Hypocrella africana	P.C. 736 (ARSEF 7696)	Ghana	AY986917*	AY986943*	DQ000344*
H. castanea	P.C. 613	Bolivia	AY986918*	AY986944*	DQ000345*
H. discoidea (*genus type species*)	#4093, BCC 2097	Thailand	AF327381	AY986945*	DQ000346*
H. macrostroma	P.C. 605 (CUP 67508)	Bolivia	AY986919*	AY986946*	DQ000347*
H. macrostroma	J.B. 115 (CUP 67509), ARSEF 7748	Costa Rica	AY986920*	AY986947*	DQ000348*
H. schizostachyi	CBS 100067	Thailand	AY986921*	AY986948*	DQ000349*
Hypocrella sp.[c]	P.C. 603	Bolivia	AY986923*	AY986950*	DQ000351*
Hypocrella sp.[c]	P.C. 436.2	Mexico	AY986922*	AY986949*	DQ000350*
Balansia henningsiana	GAM 16112	USA	AY489715	AY489610	AY489643
Epichlöe elymi	C. Schardl 760	USA	AY986924*	AY986951*	DQ000352*
Neoclaviceps monostipa	INBio 6-141	Costa Rica	AF245293	AY986983*	DQ000353*

* Sequences produced for this study. The rest were obtained from GenBank.

[a] *Aschersonia basicystis* (teleomorph *H. phyllogena*) and *A. cubensis* (teleomorph *H. epiphylla*) are species complexes that appear to include several cryptic species.

[b] *Aschersonia rhombispora* M. Liu *et al.* (*ined*).

[c] Unidentified species designated here as *Aschersonia* sp. or *Hypocrella* sp. are putative new species.

PCR products were purified with the QIAquick™ PCR Purification Kit (Qiagen, Valencia, CA). Sequencing of forward and reverse strands was performed at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, MD). Sequences were assembled and edited with Sequencher 4.2 (Gene Codes, Madison, WI). Sequences have been deposited in GenBank (Table 1) and the alignment in TreeBASE (study accession number S 1279, http://treebase.bio.buffalo.edu/treebase/).

Phylogenetic analysis

The sequences produced were aligned using Clustal X 1.81 (Thompson *et al.* 1997), and then the alignment was refined by hand. Maximum Parsimony (MP) and Bayesian Inference (BI) were carried out with all sequences. The MP analysis was done in PAUP* version b10 (Swofford 1999) using a heuristic search, with a starting tree obtained via 1000 random stepwise addition sequences, tree-bisection-reconnection as the branch-swapping algorithm, and MULTREES off. Bootstrap values from 1000 replicates were calculated using a 'fast' stepwise addition search. MrBayes 3.0 b4 (Huelsenbeck 2000, Huelsenbeck *et al.* 2001) was used to reconstruct phylogenetic trees using the Bayesian approach (Rannala & Yang 1996, Mau, Newton & Larget 1999). The Bayesian analysis used a different model of evolution for each of the three partitions (LSU, EF1-α, RPB1). The models of DNA substitution were estimated using Modeltest 3.6 (Posada & Crandall 1998). Four chains and 5 M Markov Chain Montecarlo generations were run and the current tree was saved to a file every 100 generations. After confirming that stability had been reached both in terms of likelihood scores and parameter estimation, the first 5000 trees were discarded as 'burn-in'. The remaining 'post-burn-in' trees were pooled and a 50 % majority-rule consensus tree was obtained with PAUP*. *Epichlöe elymi*, *Neoclaviceps monostipa*, and *Balansia henningsiana* were used as outgroup species.

To determine whether the sequences from the three genes should be combined in a single analysis, topological incongruence was examined using a reciprocal 70 % bootstrap (BP) or a 95 % posterior probability (PP) threshold (Mason-Gamer & Kellogg

Table 2. Comparison of *Hypocrella* species with large stromata.

Character	*H. africana*	*H. gaertneriana*	*H. macrostroma*	*H. schizostachyi*
Substrata	On insect on dicot branchlets	On insect on bamboo culms	On insect on dicot vine stems	On insect on bamboo culms
Stroma diameter (mm)	8–17	6–30	3–22	*ca* 12
Stroma colour	Brownish orange to dark brown	White to pale green	Light yellow	Reddish brown
KOH reaction	–	+ (red-orange)	–	–
Asci (µm)	105–140 × 5.5–8	120–178 × 4–6.5	146–189 × 5.5–6.5	113–158 × 6–9
Part-ascospore size (µm)	5–8.5 × 1.5–2	4–8 × 1.5–2	10.5–11.5 × 2.5	4–9.5 × 1.5–2.5

Table 3. Results of phylogenetic analyses of LSU, EF1-α, and RPB1.

	LSU	EF1-α	RPB1	Combined
Total characters included	905	934	755	2594
Number of non-polymorphic sites	701	611	415	1727
Number of unique polymorphic sites, parsimony-uninformative	76	75	53	204
Number of polymorphic sites, parsimony-informative	128 (14%)	248 (27%)	287 (38%)	663 (26%)
Number of equally parsimonious trees	6	10	1	2
Length (steps)	478	1308	1527	3357
Consistency Index	0.548	0.356	0.381	0.390
Retention Index	0.638	0.422	0.559	0.512
Homoplasy Index	0.452	0.644	0.619	0.610

1996, Reeb, Lutzoni & Roux 2004). Bootstrap values were generated using neighbour-joining (NJ) with 1000 replicates and a maximum likelihood distance. Posterior probabilities were calculated using Bayesian analysis in MrBayes. A conflict was assumed to be significant if two different relationships for the same taxa – one being monophyletic and the other non-monophyletic, both with BP ⩾ 70% and PP ⩾ 95% – were observed on each of the LSU, EF1-α, and RPB1 majority-rule consensus trees. The three partitions could be combined if no significant conflicts were detected.

RESULTS

Morphology

The new species of *Hypocrella*, *H. macrostroma*, is distinguished from the other species with large stromata by its substrate, stroma colour, and part-ascospore size (Table 2). It was found on an insect (probably a scale insect based on the type of host of related species) on living dicotyledonous vines, whereas *H. gaertneriana* and *H. schizostachyi* were found on bamboo culms. *H. africana* type material (BPI 635731) was found on scale insects on living dicot twigs, as was the additional specimen examined in this study (P.C. 736). The part-ascospores of *H. macrostroma* are significantly larger than the other species. The stroma of *H. gaertneriana* releases reddish pigment when 3% KOH is added, whereas the other species do not react to KOH. *Hypocrella gaertneriana* and *H. macrostroma* have

the largest stromata; *H. africana* and *H. schizostachyi* have smaller stromata (Table 2). The specimen of *H. africana* (P.C. 736) has smaller stromata (*ca* 5–6 mm diam) than those of the type specimen (8–17 mm) of the proposed new species. All other morphological characteristics, including the substrate, otherwise fit the concept of *H. africana*, which has so far been known only from Africa.

Anamorphs of *Hypocrella* species with large stromata are known only for *H. schizostachyi* and *H. macrostroma*. The anamorph of *H. macrostroma* is a true *Aschersonia* with pycnidia-like conidiomata that produce fusiform conidia from slender phialides. *Hypocrella schizostachyi*, on the other hand, produces a pycnidial anamorph that is not typical of *Aschersonia* (Hywel-Jones & Samuels 1998) in that its conidia are allantoid. The conidiogenous cells, especially the polyphialides of the pycnidial 'B' anamorph, and the shapes of the 'B'-type conidia are more reminiscent of *Hirsutella* than of the typical *Aschersonia* type.

Phylogenetic analyses

Sequencing of three genes resulted in an aligned dataset of 2594 characters, including gaps (insertions and/or deletions): 905 for LSU, 934 for EF1-α, and 755 for RPB1 (Table 3). RPB1 had the majority of the polymorphisms and phylogenetically informative characters (38%), followed by EF1-α with 27% and LSU with 14%. The models of nucleotide substitution suggested by Modeltest 3.6 for each locus were General Time Reversible (GTR + G + I, nst = 6) with
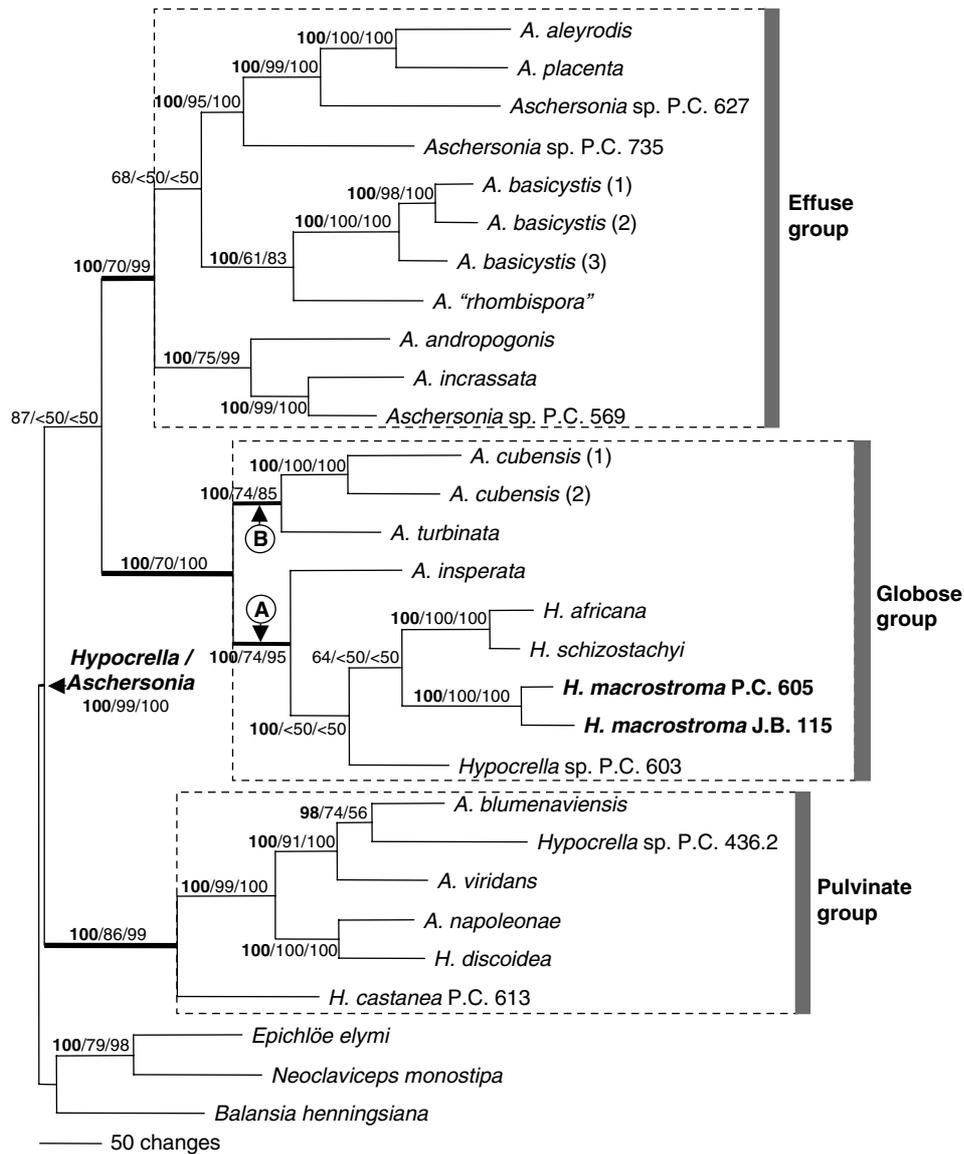
Fig. 1. Relationships of representative *Hypocrella/Aschersonia* species based on Bayesian inference (50% majority rule consensus tree of the combined LSU, EF1-α, and RPB1 data sets). Species with large stromata are indicated by ◆. The three major clades, Effuse, Globose, and Pulvinate, are distinguished by morphological characters described in the text. Branch lengths were calculated and averaged in MrBayes. Numbers at branches indicate posterior probability (%)/MP bootstrap value/NJ bootstrap value.

gamma distributions and invariable sites. The parameters selected for the LSU model were: base frequencies = 0.2283, 0.2716, 0.3201; rates (Rmat) = 0.4772, 2.7390, 0.5495, 0.7659, 6.3667; gamma shape = 0.4985; proportion invariable sites (pinvar) = 0.6212. The parameters for the EF1-α model were: base frequencies = 0.1952, 0.3442, 0.2443; rates (Rmat) = 0.6044, 1.0873, 0.7875, 0.5494, 4.6887; gamma shape = 1.1425; proportion invariable sites (pinvar) = 0.551. The parameters for the RPB1 model were: base frequencies = 0.2335, 0.2826, 0.2611; rates (Rmat) = 1.8006, 3.6967, 0.8486, 0.9245, 6.9621; gamma shape = 0.8631; proportion invariable sites (pinvar) = 0.3752. These models were then used to analyze incongruence among loci. The reciprocal 70% bootstrap and 95% posterior probability thresholds for individual loci

show that the topologies of the three genes are congruent and therefore the partitions were combined.

The combined MP and BI analyses of selected species of *Hypocrella/Aschersonia* reveal three major clades that are supported by high BP and PP and correlated with morphological characters: the 'Effuse' group (BP 70%, PP 100%), the 'Pulvinate' group (BP 86%, PP 100%), and the 'Globose' group (BP 70%, PP 100%) (Fig. 1). All the species in the Effuse group, except *A. basicystis*, have flattened to effuse anamorphic stromata that are formed mainly of loose hyphal tissue and a relatively extensive hypothallus. *Aschersonia basicystis* (teleomorph *H. phyllogena*) in its first stages of development has a stroma composed of loose hyphal tissue that is flattened, and has a wide hypothallus. The hypothallus persists, and the stroma

becomes rounder and more compact as it matures. The Pulvinate group includes species that have pulvinate or cushion-like stromata that are somewhat flattened, and have hypothalli that are small or absent. This group includes the only known species of *Hypocrella/Aschersonia* that change color (reddish or brownish) when mounted in 3% KOH (*H. discoidea*, the type of the genus, *H. gaertneriana*, *A. napoleonae*, and *A. viridans*). The Globose group consists of species that have globose to subglobose, compact and hard stromata that are generally larger than those in the other clades. Within the Globose group, clade 'A', supported by BP 74% and PP 100%, includes species with large stromata that are generally tuberculate and with deep creases giving the appearance of a compound stroma (i.e. *A. insperata*, *H. africana*, *H. macrostroma*, and *H. schizostachyi*), plus *Hypocrella* sp. P.C. 603, which has globose, non-tuberculate, hard and compact stromata that are *ca* 3–4 mm diam. Two specimens of *H. macrostroma* form a monophyletic group supported by 100% BP and 100% PP. Within the Globose group, clade A is 'sister' to clade B. The latter contains the similar species *A. cubensis* and *A. turbinata* (Fig. 1).

TAXONOMY

Hypocrella macrostroma Chaverri & K. T. Hodge, sp. nov. (Figs 2–18)

Anamorph: *Aschersonia* sp.

Stromata globosae ad subglobosae, flava, tuberculata, 3–22 cm diam. Ascosporae multicellulares, ad septum disarticulatae, incolora, partis fusiformi ad subcylindrici, (8.7–)10.5–11.5(–18.0) × (2.2–)2.5–2.7(–3.0) μm. Anamorphosis: *Aschersonia* sp. Holotypus CUP 67508 (=P.C. 605).

Typus: **Bolivia**: *La Paz Department*: Province Franz Tamayo, San José de Uchipiamonas, Madidi National Park, trail to the top of the mountain, along Eslabón River starting at 'Senda RAP' camp, elev. 400–600 m, *ca* 14° 27.802′ S, 67° 56.927′ W, on insects on living vine of dicotyledonous plant, 12 June 2004, *D. De La Quintana*, *M. Sogonov*, *A. Alvarez*, *P. Chaverri* P.C. 605 (CUP 67508 – holotypus).

Stromata large, 3–22 mm diam, yellow, somewhat globose, cerebriform, surface smooth, somewhat glossy, ostiolar openings visible, flush with surface, brownish yellow, stromatic tissue not changing colour in KOH, stromata formed of closely aggregated tubercles, tubercles 1–4 mm diam, stroma outer tissue of *textura angularis* composed of thin-walled cells, stroma internal tissue pale yellow, hard and coriaceous, tissue of *textura epidermoidea* composed of thick-walled cells. *Perithecia* completely immersed in stroma, obpyriform, (377–)389–441(–451) × (137–)138–149(–152) μm. *Asci* cylindrical, (140–)146–189(–197) × (5.2–)5.5–6.5(–7) μm, capitate, cap depth 3–4 μm, 8-spored. *Ascospores* hyaline, smooth, filiform, multiseptate, disarticulating at septa, part-ascospores fusiform to subcylindrical, (8.5–)10.5–11.5(–18) × (2–)2.5(–3) μm (*n* = 51).

The following anamorph description is based on J.B. 115 (ARSEF 7748); the ascospores of P.C. 605 did not germinate. The specimen J.B. 115 included conidiomata in the same stroma as the teleomorph. *Conidiomata* from original substrate, with pycnidium-like depressions in the stroma, lacking well-defined walls, irregular in shape. *Conidia* fusiform, hyaline, smooth, unicellular, (10.5–)11.5–13(–14.5) × (2–)2.5(–3) μm; phialides not observed, probably degraded. *Colony* on PDA at 25 ° after *ca* 4 wk 12–14 mm diam, floccose or fluffy, light yellow, forming irregular pycnidia-like concave depressions or cavities in colony and lacking a differentiated wall; conidial masses oozing from conidiomata in pale yellow, slimy cirri. *Conidiophores* on PDA short, almost indiscernible, mononematous, aggregated into compact hymenium-lined cavities. *Phialides* on PDA slender, tapering towards tip, somewhat irregular not straight, (23.5–)25–27(–27.5) × (2.5–)–3(–3.5) μm; paraphyses not observed. *Conidia* on PDA fusiform, hyaline, (13.5–)15.5–17 (–19) × (2–)2.5(–3) μm.

Habitat: Probably on scale insects on living dicotyledonous vines.

Distribution: Bolivia and Costa Rica.

Paratype: **Costa Rica**: *Heredia*: Sarapiquí, La Selva Biological Station, Oriental Trail at 350 m from the beginning, stromata found on ground, substrate unknown, 26 June 2002, *J. F. Bischoff* (J.B. 115 = CUP 67509, culture ARSEF 7748).

Hypocrella gaertneriana Møller, *Bot. Mitt. Tropen* **9**: 299 (1901).

Typus: Møller, *Bot. Mitt. Tropen*, **9**: taf. III fig. 51 (1901) [based on a specimen from Brazil on bamboo culms] – **lectotypus hic designatus**.

Illustrations: Møller (1901 taf. III fig. 51, taf. IV fig. 62), and Hywel-Jones & Samuels (1998: figs 8–14).

Notes: Specimens of *H. gaertneriana* deposited in (B) were destroyed in a fire in 1943; for that reason we have designated the original illustration as a lectotype above. Because the specimen of *H. gaertneriana* GS 1776 deposited in NY does not have a living culture, we prefer not to designate it as an epitype. However, we consider it a specimen that fits Møller's description.

Additional specimen: **Venezuela**: *Río Negro Department*: Cerro de la Neblina, along Río Mawarinuma, just outside Cañon Grande, near Neblina Base Camp, *ca* 140 m elev., 00° 50′ N, 66° 10′ W, on bamboo culms, April–May 1984, *G. J. Samuels 1776* (NY).

DISCUSSION

Species of *Hypocrella/Aschersonia* with large stromata are uncommon. Hywel-Jones & Samuels (1998) considered these species to be very distinct but did not propose a new genus for them because they lacked sufficient information about their life-cycles. Our
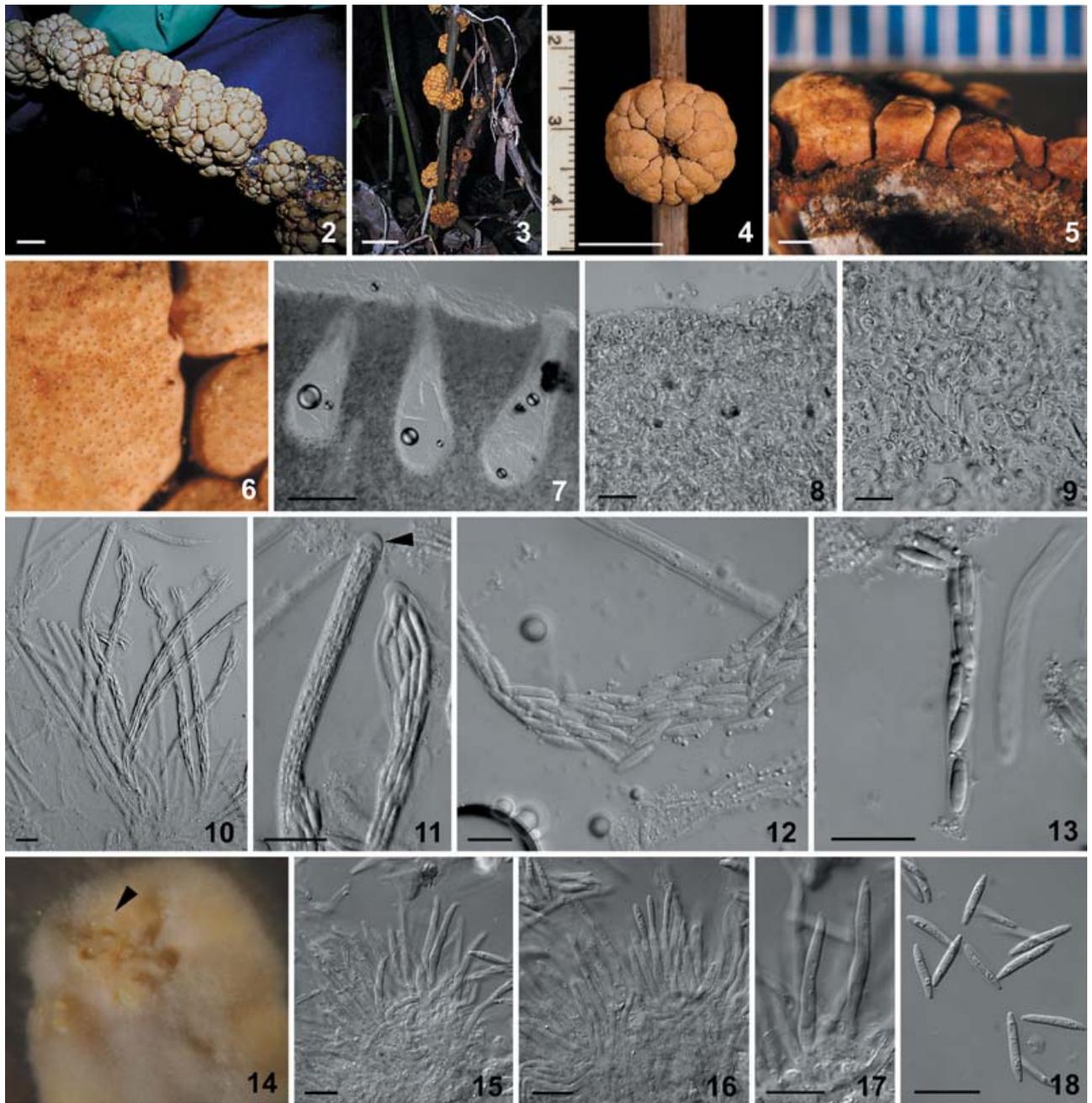
Figs 2–18. *Hypocrella macrostroma*. **Figs 2–3.** Stromata on vine collected in the field site. **Fig. 4.** One stroma. **Fig. 5.** Closer view of smaller stromata. **Fig. 6.** Surface of stroma with view of ostiolar openings. **Fig. 7.** Longitudinal section of stroma showing perithecia. **Fig. 8.** Longitudinal section showing outer tissue of stroma. **Fig. 9.** Longitudinal section showing inner tissue of stroma. **Fig. 10.** Asci. **Fig. 11.** Thickened ascus caps (arrow) and ascospores. **Figs 12–13.** Part-ascospores. **Fig. 14.** Colony on PDA at 25 ° after 4 wk of growth showing oozing conidia (arrow). **Figs 15–17.** Phialides. **Fig. 18.** Conidia on PDA at 25 ° after 4 wk of growth. Figs 1–13: P.C. 605, Figs 14–18: J.B. 115. Bars: Fig. 2 = *ca* 1 cm; Fig. 3 = *ca* 2 cm; Fig. 4 = 1 cm; Fig. 5 = 1 mm; Fig. 7 = 500 μm; and Figs 8–13, 15–18 = 10 μm.

morphological and molecular evidence suggests that species with large stromata are derived from within *Hypocrella*. The anamorph of *H. macrostroma* is clearly an *Aschersonia*; whereas *H. schizostachyi* has an anamorph that deviates somewhat from the typical *Aschersonia* (type species *A. taitensis*.). Phylogenetic analyses of three loci show that the species with large stromata used in this study have a single evolutionary origin and form a monophyletic group that includes two other species with smaller stromata, *A. insperata* and *Hypocrella* sp. P.C. 603. Unfortunately, we cannot unequivocally conclude that *H. gaertneriana* is part of this group because we lack fresh material that can be used for DNA sequencing. However, the morphology of the stroma and the ascospores suggest that *H. gaertneriana* does belong in *Hypocrella*.

Three major groups, referred to as 'Effuse', 'Globose' and 'Pulvinate', are revealed in the phylogenetic

analyses of DNA sequences. Phylogenetically informative morphological characters correlate with each of the groups as defined by DNA sequence data. In general, species in the Effuse group have flat, effuse stromata of loose hyphal tissue, broad hypothalli, and whitish colouration, except in the *A. basicystis* species complex where it is pale yellow to orange. The Globose group includes species that have globose stromata that are generally darker in colour (yellow to brownish), large, compact tissue, hard or coriaceous, moderately to strongly tuberculate, and without hypothalli. The Pulvinate group contains species that have pulvinate or cushion-like stromata, somewhat compact and flattened, yellowish or green, with or without hypothalli, sometimes changing color in KOH.

'The genus for genus concept' symposium at the XVI International Botanical Congress (Saint Louis, MO) in 1999 discussed theoretical concepts and data with the fundamental idea that fungal taxonomy should move towards a 1:1 correlation of teleomorph and anamorph generic concepts and nomenclatural unity. Because of the morphological synapomorphies that characterize the genus *Hypocrella/Aschersonia* and to follow the 'genus for genus' concept (Seifert *et al.* 2000), we do not propose a formal generic or subgeneric revision of the taxonomy to distinguish the Effuse, Globose, and Pulvinate groups. Even though there are apparently distinct phenotypic characteristics that distinguish each of the three major groups, all have the typical characteristics of the genus *Hypocrella*: *Aschersonia s. str.* anamorphs, perithecia embedded in a well-developed stroma, filiform multiseptate ascospores that disarticulate at each septum, and parasitism of scale insects and whiteflies. Sampling of more species is needed to further investigate whether these clades should be considered taxa of subgeneric or generic rank.

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REFERENCES

Carbone, I. & Kohn, L. M. (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.

Castlebury, L. A., Rossman, A. Y., Sung, G.-H., Hyten, A. S. & Spatafora, J. W. (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycological Research* **108**: 1–9.

Chaverri, P. & Samuels, G. J. (2003) *Hypocrea/Trichoderma* (*Ascomycota*, *Hypocreales*, *Hypocreaceae*): species with green ascospores. *Studies in Mycology* **48**: 1–116.

Couch, J. N. (1938) The genus *Septobasidium*. University of North Carolina Press, Chapel Hill, NC.

Dingley, J. M. (1954) The Hypocreales of New Zealand. VI. The genera *Hypocrella*, *Barya*, *Claviceps* and *Podonectria*. *Transactions of the Royal Society of New Zealand* **81**: 489–499.

Huelsenbeck, J. P. (2000) *MrBayes: Bayesian inferences of phylogeny* (*software*). University of Rochester, New York.

Huelsenbeck, J. P., Ronquist, F., Nielsen, E. S. & Bollback, J. P. (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**: 2310–2314.

Hywel-Jones, N. L. & Evans, H. C. (1993) Taxonomy and ecology of *Hypocrella discoidea* and its anamorph, *Aschersonia samoensis*. *Mycological Research* **97**: 871–876.

Hywel-Jones, N. L. & Samuels, G. J. (1998) Three species of *Hypocrella* with large stromata pathogenic on scale insects. *Mycologia* **90**: 36–46.

Kornerup, A. & Wanscher, J. H. (1978) *Handbook of Colour*. Methuen, London.

Mains, E. B. (1959a) Species of *Aschersonia* (*Sphaeropsidales*). *Lloydia* **22**: 215–221.

Mains, E. B. (1959b) Species of *Hypocrella*. *Mycopathologia et Mycologia Applicata* **11**: 311–326.

Mason-Gamer, R. J. & Kellogg, E. A. (1996) Testing for phylogenetic conflict among molecular data sets in the tribe *Triticeae* (*Gramineae*). *Systematic Biology* **45**: 524–545.

Mau, B., Newton, M. & Larget, B. (1999) Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* **55**: 1–12.

Møller, A. (1901) Phycomyceten und Ascomyceten. *Botanischen Mittheilungen aus den Tropen* **9**: 1–319.

Petch, T. (1921) Studies in entomogenous fungi. II. The genera of *Hypocrella* and *Aschersonia*. *Annals of the Royal Botanic Gardens Peradeniya* **7**: 167–278.

Posada, D. & Crandall, K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 917–918.

Rannala, B. & Yang, Z. H. (1996) Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. *Journal of Molecular Evolution* **43**: 304–311.

Reeb, V., Lutzoni, F. & Roux, C. (2004) Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (*Pezizomycotina*, *Fungi*) with special emphasis on lichen-forming *Acarosporaceae* and evolution of polyspory. *Molecular Phylogenetics and Evolution* **32**: 1036–1060.

Rehner, S. A. (2001) *Primers for Elongation Factor 1-alpha* (*EF1-alpha*). http://ocid.nacse.org/research/deephyphae/EF1primer.pdf.

Seifert, K. A., Gams, W., Crous, P. W. & Samuels, G. J. (eds) (2000) Molecules, morphology and classification: towards monophyletic genera in the ascomycetes. *Studies in Mycology* **45**: 1–230.

Sullivan, R. F., Bills, G. F., Hywel-Jones, N. L. & White, J. F. J. (2000) *Hyperdermium*: a new clavicipitalean genus for some tropical epibionts of dicotyledonous plants. *Mycologia* **92**: 908–918.

Sung, G.-H., Spatafora, J. W., Zare, R., Hodge, K. T. & Gams, W. (2001) A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the *Clavicipitaceae*. *Nova Hedwigia* **71**: 311–328.

Swofford, D. (1999) *PAUP*: Phylogenetic analysis using parsimony* (**and other methods*). Version 4. Sinauer Associates, Sunderland, MA.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

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