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Theissenia reconsidered, including molecular phylogeny of the type species *T. pyrenocrata* and a new genus *Durotheca* (*Xylariaceae, Ascomycota*)

Thomas Læssøe[1], Prasert Srikitikulchai[2], J. Jennifer D. Luangsa-ard[2], and Marc Stadler[3,*]

[1]Department of Biology, University of Copenhagen, Universitetsparken 15, Copenhagen, DK-2100 Ø, Denmark
[2]National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Phahonyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand
[3]Helmholtz Centre for Infection Research GmbH, Dept. Microbial Drugs, Inhoffenstraße 7, 38124 Braunschweig, Germany; corresponding author e-mail: marc.stadler@helmholtz-hzi.de

Abstract: The genus *Durotheca* is introduced with *D. depressa* sp. nov., as type. *Hypoxylon comedens* is transferred to *Durotheca*, based on its morphology with further evidence from molecular phylogenetic studies; a combined β-tubulin and α-actin gene dataset. *Theissenia cinerea* is synonymized with *D. comedens,* and the type of *Theissenia*, *T. pyrenocrata,* is shown to occupy a basal, rather distant position in a monotypic clade in relation to sequenced taxa of *Durotheca*. This clade has an unresolved position in relation to the two informal subfamilies "*Xylarioideae*" and "*Hypoxyloideae*" within the *Xylariaceae*. New distributional data for *D. comedens* and *T. pyrenocrata* are presented, with the former found to be widespread in South-East Asia and the latter is reported as new from western Amazonia (Ecuador). One further species described in *Theissenia*, *T. rogersii,* is transferred to *Durotheca,* whilst *T. eurima* is accepted in *Theissenia*.

Key words:
α-actin
ß-tubulin
Biodiversity
SEM
Thailand

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INTRODUCTION

The genus *Theissenia* was introduced by Maublanc (1914) for *Ustulina pyrenocrata.* Læssøe (1994) accepted this genus within *Xylariaceae*, and Ju *et al.* (2003) recognized three species in their monographic treatment. Subsequently, another species was added and a phylogenetic analysis based on DNA sequences from two of the accepted taxa, but not the type species *T. pyrenocrata*, was provided (Ju *et al*. 2007). The inclusion in *Xylariaceae* was confirmed, and data were presented to show affinities within the subfamily "*Hypoxyloideae*"[1], a position never previously proposed. Ju *et al.* (2003) had previously accepted, *ad interim*, a placement within the *Xylariaceae* based on a *Nodulisporium*-morph found in cultures of *T. cinerea*, but noted that the aleurisporous asexual morph found in *T. eurima* was not as expected for such a position. Furthermore, a similar asexual morph had been observed in *T. pyrenocrata*. Ju *et al*. (2003) also noted the extreme variability displayed among the four recognized species, such as the absence/presence of germ slits, surface ornamentation, and variability in ascospore wall thickness and asexual morphs. In our continuing studies on the biodiversity of Thai *Xylariaceae*, we have repeatedly encountered a fungus that we identified as *Hypoxylon comedens* based on a comparison with type material. Læssøe *et al.* (1989) excluded *H. comedens* from both *Camillea* and *Hypoxylon*, but were unable to suggest a revised placement. Also, the new material, including cultures, did not provide sufficient information to suggest a placement with confidence, not least due to our cultures failing to produce an asexual morph. Ju *et al*. (2003) shed some light on the situation, and we recognized that our fungus was a member of *Theissenia* in the sense of these authors.

Hypoxylon comedens was originally described from the Malaysian state Sarawak on Borneo (Cesati 1879), and has since been reported from China (Tai 1979, Zhuang 2001). Material determined as "*Hypoxylon* cf. *comedens*" was also reported from Mexico (San Martín González & Rogers 1993). Martin (1969) also published on a presumed *H. comedens* from Mexico, including data on the asexual state, but the material he used is evidently a species of *Camillea*. The cardinal features that excludes *H. comedens* from *Hypoxylon*, and its segregate genera, are the combination of the highly carbonized and large perithecia seated directly on the substrate, the clavate, deliquescing asci, and the peculiar spore shape and pale pigmentation. Furthermore, most collections yield no extractable pigments (with KOH) unlike most members of *Hypoxylon*.

Here we report on further morphological and molecular studies on material referable to *H. comedens s.l.* or *Theissenia*

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[1] This subfamily name does not appear to have been validly published, but is nevertheless widely used.

from Thailand and other parts of South-East Asia, and most importantly, on sequenced material of the type species of *Theissenia* from Ecuador.

MATERIAL AND METHODS

Sampling and culturing

Herbarium and genetic resource collection acronyms follow Thiers (2010). Field collected stromata of *Hypoxlon comedens s.lat.* and other xylariaceous species were taken to sites where isolation work could be carried out within a few hours. Within 2–3 d, ascospores germinated, and the resulting cultures were transferred to fresh plates (in Thailand) and later transferred to the collections at BCC. In Ecuador no culture work was carried out, and attempts to culture *Theissenia pyrenocrata* from dried material failed. All dried voucher collections are held at BBH, with cultures deposited in BCC (Thai material), or C and QCNE (Ecuadorian material).

Growth for DNA extraction

Cultures of *Xylariaceae* were grown on Potato Dextrose Agar (PDA) Petri plates. These plates were incubated at room temperature in darkness for 3–4 wk. A few small blocks of PDA with sterile or sporulating mycelium of each sample were taken from a plate and placed in 50 mL Sabouraud Dextrose Broth (Sigma; SDB) in 250 mL Erlenmeyer flasks, and incubated at 25 °C in darkness for 4 wk. The mycelial mass on SDB was then harvested over a sterile Whatman filter paper and washed with sterile, distilled water.

DNA extraction

Total DNA of each mycelial sample, or in the case of *Theissenia pyrenocrata* from perithecial contents, was extracted using Cetyltrimethyl-ammonium bromide (CTAB) following the procedure described in Mackill & Bonman (1995), with minor modifications (to adapt the procedure to the study of fungal material): Lyophilized mycelium (40–50 mg) was placed into a microcentrifuge tube and ground to powder. This mycelial powder was suspended in 700 µL of extraction buffer (NaCl 0.7 M; Tris-HCl 50 mM pH 8.0; EDTA 2 mM pH 8.0, 1 % CTAB) preheated to 65 °C. The suspension was thoroughly mixed and incubated for 1 h at 65 °C. After the suspension had cooled, 500 µL of chloroform/isoamyl alcohol (24:1 v/v) was added. The supernatant was gently mixed until an emulsion was obtained and centrifuged at 10 000 rpm for 20 min. The aqueous phase was transferred to a new sterile tube. A 10 % CTAB solution was added at one tenth of the volume of the aqueous phase and mixed. The supernatant was transferred to a new tube after a spin-down of 20 min. 700 µL of precipitation buffer (CTAB 1 %; Tris-HCl 50 mM pH 8.0; EDTA 10 mM pH 8.0) was then added to the supernatant, left at room temperature for 5–10 min and centrifuged. The aqueous phase was discarded and 300 µL of TEHS buffer (NaCl 1M; Tris-HCl 10 mM pH 8.0; EDTA 1 mM pH 8.0) was added to the pellet to remove the CTAB from the DNA. The pellet was treated with ribonuclease A, incubated at 37 °C for 30 min, followed by addition of 750 µL of cold absolute ethanol and centrifuged at 10 000 rpm for 20 min. The supernatant was discarded and the pellet was washed in 500 µL 70 % (v/v) ethanol and air-dried at room temperature. The DNA pellet was then dissolved in 50 µL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0).

PCR and sequencing

PCR amplification was done in a 50 µL volume consisting of 1× PCR buffer, 200 µM of each of the four dNTPs, 2.5 mM $MgCl_2$, 1 U Taq DNA polymerase (Promega, Madison, Wisconsin) and 0.5 µM of each primer. Amplification of the partial β-tubulin gene and α-actin were done using the primer pairs T1/T22 (O'Donnell & Cigelnik 1997) and ACT-512F/ACT-783R (Carbone & Kohn 1999), respectively. Amplifications were performed using a MJ Research DNA Engine ALD1244 thermal cycler following the procedure described in Ju *et al*. (2007). PCR products were purified using a QIAquick PCR purification Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Purified PCR products were sent to Macrogen (Korea) for sequencing.

Sequence analysis

Each DNA sequence was checked for ambiguous bases, assembled using BioEdit v. 6.0.7 (Hall 2004), and submitted to GenBank (Table 1). Proofed sequences were then aligned using ClustalW (Larkin *et al*. 2007) incorporated in BioEdit v. 6.0.7 and alignments were refined by directed examination. Parsimony and Bayesian analyses were first carried out for each gene on individual datasets. Potential conflicts were assessed by comparing individual parsimony bootstrap trees. In case two different relationships for the same set of taxa were both supported by bootstrap values greater than 70 % from different genes, it was assumed that the incongruence was signicant (Wiens 1998). Parsimony (PAUP v. 4.0b10, Swofford 2002) and Bayesian (MrBayes v. 3.0, Huelsenbeck & Ronquist 2001) phylogenetic analyses were performed on the combined data set of the β-tubulin and α-actin genes. The maximum parsimony analysis was performed using the heuristic search, starting with trees obtained via 1000 random stepwise addition sequences, and tree-bisection-reconnection as the branch-swapping algorithm. All characters were given equal weights and gaps were treated as missing data. No topological constraints were enforced and the 'Multrees' option was in effect. Relative support for the branches was obtained from bootstrap proportions (BP) using 1000 heuristic searches using the aforementioned parsimony settings and 10 random sequence additions per bootstrap replicate. Prior to conducting the Bayesian analysis MrModeltest v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model. After the best nucleotide substitution model was determined for each gene partition and combined dataset, Bayesian analysis was conducted using MCMC using a GTR+I+G model. Four default chains were sampled every 100 generations and run for a total of 2 M generations. Bayesian posterior probabilities (PP) were calculated on the posterior distribution of trees excluding the initial set of burn-in trees.

SEM and HPLC

Scanning electron microscopy (SEM) was carried out using a conventional procedure as described in Stadler *et al.* (2002). Analytical HPLC of stromatal methanol extracts was

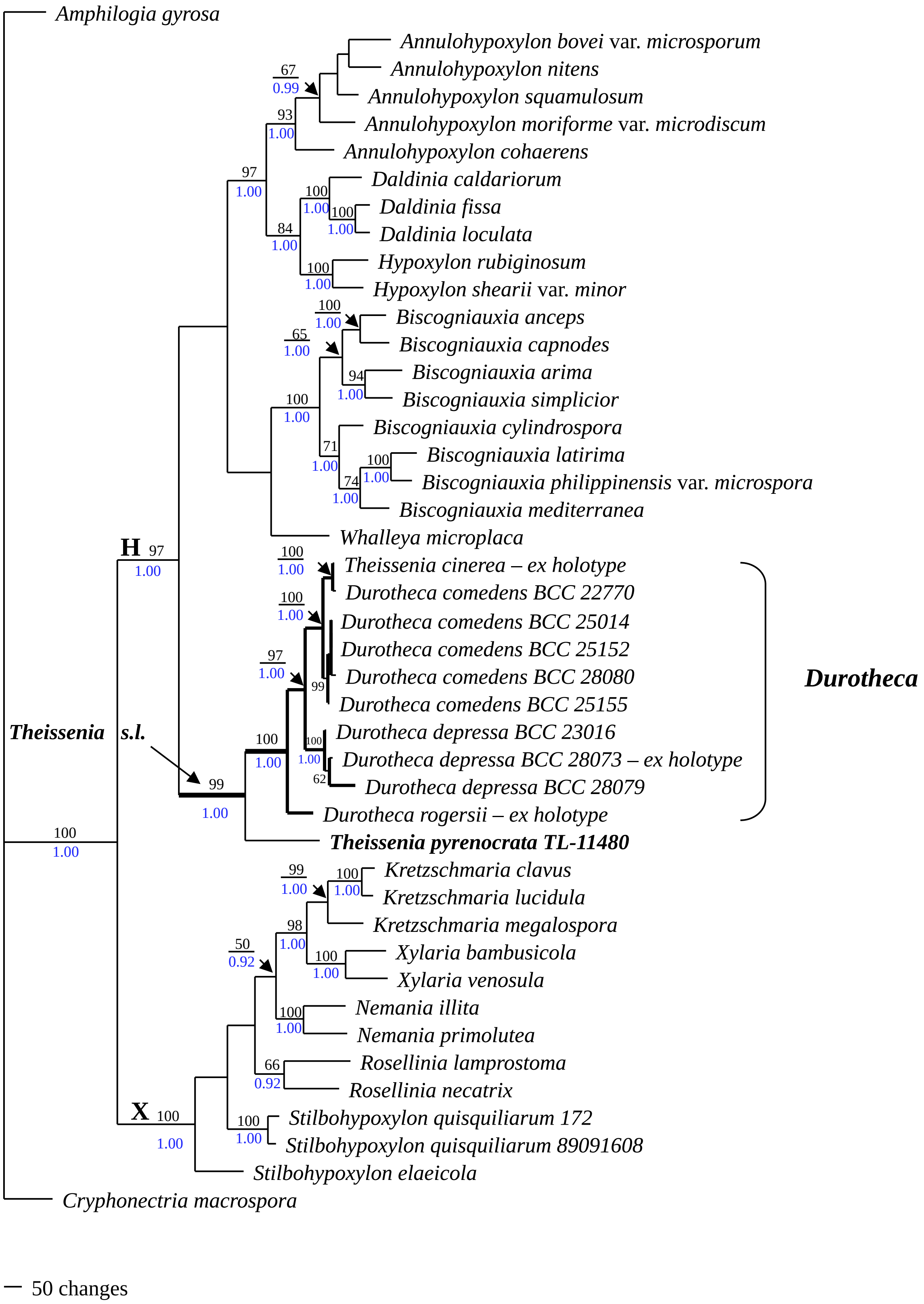

Fig. 1. Phylogenetic relationships of *Theissenia* and *Durotheca* species within *Xylariaceae* generated from a combined β-tubulin and α-actin gene dataset. Numbers above each branch represent bootstrap values and those below the branch are posterior probabilities.

performed using the standardized method, comprising diode array detection as described by Hellwig *et al.* (2005) and mass spectrometric detection in the positive and negative electrospray mode, using a comprehensive library of reference compounds (Bitzer *et al*. 2007). The HPLC reference library included, among numerous other pure natural products, lepraric acid (Læssøe *et al.* 2010) and various metabolites of the *Xylariaceae* as authentic standards, allowing for their unambiguous detection in crude extracts by comparison of their retention times, diode array spectra and mass spectra.

Table 1. List of specimens used for the molecular phylogenetic study (Fig. 1).

Taxon	Original code	Culture acc. no.	Origin	Locality/Collecting data (or origin in case of reference sequences retrieved from GenBank)	GenBank Acc. no.	
					Alpha-Actin	**Beta-Tubulin**
Ootheca comedens	XY00290	BCC22770	Thailand	Phu Hin Rong Kla National Park, Phitsanulok; BBH18116	GQ160478	GQ160486
Ootheca comedens	XY00513	BCC25014	Thailand	Khao Nan National Park, Nakhon Si Thammarat; BBH25875	GQ160479	GQ160487
Ootheca comedens	XY00531	BCC25152	Thailand	Khao Nan National Park, Nakhon Si Thammarat; BBH25876	GQ160480	GQ160488
Ootheca comedens	XY00534	BCC25155	Thailand	Khao Ban That Wildlife Sanctuary Wildlife Sanctuary, Trang; BBH25877	GQ160481	GQ160489
Ootheca comedens	XY00638	BCC28080	Thailand	Kaeng Krachan National Park, Phetchaburi; BBH19755	GQ160482	GQ160490
Ootheca depressa	XY00402	BCC23016	Thailand	Doi Inthanon National Park, Chiang Mai; BBH18222	GQ160483	GQ160491
Ootheca depressa	XY00619	BCC28073	Thailand	Doi Inthanon National Park, Chiang Mai; BBH19737	GQ160484	GQ160492
Ootheca depressa	XY00636	BCC28079	Thailand	Doi Inthanon National Park, Chiang Mai (no specimen)	GQ160485	GQ160493
Theissenia pyrenocrata	TL-11480	none	Ecuador	Orellana, TL-11480 (QCNE, C)	GQ247716	GQ247717
Amphilogia gyrosa	none	BCRC34145	Taiwan	Ju & Hsieh 91123101 (HAST) (Ju *et al.* 2007)	EF025600	EF025615
Annulohypoxylon bovei var. *microsporum*	none	BCRC34012	Taiwan	Ju & Hsieh 90081914 (HAST) (Hsieh *et al.* 2005)	AY951765	AY951654
Annulohypoxylon cohaerens	none	BCRC34013	France	Fournier JF-03041 (Hsieh *et al*. 2005)	AY951766	AY951655
Annulohypoxylon moriforme var. *microdiscus*	none	BCRC34018	Taiwan	Ju & Hsieh 90080807 (HAST) (Hsieh *et al.* 2005)	AY951769	AY951660
Annulohypoxylon nitens	none	BCRC34021	Taiwan	Guu 91022108 (HAST) (Hsieh *et al.* 2005)	AY951772	AY951663
Annulohypoxylon squamulosum	none	BCRC34022	Taiwan	**Holotype** (HAST), see Ju *et al*. (2004) as *Hypoxylon squamulosum*. and Hsieh *et al.* (2005)	AY951774	AY951665
Biscogniauxia anceps	none	BCRC34029	France	Candoussau (Rogers *et al.* 1996, (Hsieh *et al.* 2005)	AY951783	AY951671
Biscogniauxia arima	none	BCRC34030	Mexico	**Isotype** (Ju *et al.*. 1998, Hsieh *et al.* 2005)	AY951784	AY951672
Biscogniauxia capnodes	none	BCRC34032	Taiwan	Ju 77031509 (Ju *et al.* 1998, (Hsieh *et al.* 2005)	AY951787	AY951675
Biscogniauxia cylindrispora	none	BCRC33717	Taiwan	**Holotype** (Ju & Rogers 2001, (Hsieh *et al.* 2005)	AY951791	AY951679
Biscogniauxia latirima	none	BCRC34036	Taiwan	Ju & Hsieh 90080703 (HAST) (Hsieh *et al.* 2005)	AY951795	AY951683
Biscogniauxia mediterranea	none	BCRC34037	France	Candoussau 366 (Ju *et al.* 1998, Hsieh *et al.* 2005)	AY951796	AY951684
Biscogniauxia philippinensis var. *microspora*	none	BCRC33720	Taiwan	Ju 89041101 (HAST) (Ju & Rogers 2001, Hsieh *et al.* 2005)	AY951797	AY951685
Biscogniauxia simplicior	none	BCRC34038	France	Candoussau 5354A (Ju *et al.* 1998, Hsieh *et al*. 2005)	AY951798	AY951686
Cryphonectria macrospora	none	BCRC34146	Taiwan	Ju & Hsieh 94031513 (HAST) (Ju *et al*. 2007)	EF025587	EF025618
Daldinia caldariorum	none	BCRC34042	Taiwan	Chen 957 (HAST) (Hsieh *et al.* 2005)	AY951802	AY951690
Daldinia vernicosa	none	BCRC34048	Germany	Wollweber 2899 (Ju *et al*. 1999 and Bitzer *et al*. 2008; as *D. fissa*); now deposited in KR 0026318	AY951809	AY951697
Daldinia loculata	none	KC1525 (Kew)	UK	K[M] 24541 (Stadler *et al.* 2001, Hsieh *et al.* 2005)	AY951810	AY951698
Hypoxylon rubiginosum	none	BCRC34116	UK	J.D. Rogers (Ju & Rogers 1996, (Hsieh *et al.* 2005))	AY951862	AY951751

Table 1. (Continued).

Taxon	Original code	Culture acc. no.	Origin	Locality/Collecting data (or origin in case of reference sequences retrieved from GenBank)	GenBank Acc. no.	
					Alpha-Actin	Beta-Tubulin
Hypoxylon shearii var. *minor*	none	BCRC34093	Mexico	**Isotype** (WSP) (San Martin *et al.* 1999, (Hsieh *et al.* 2005))	AY951864	AY951753
Kretzschmaria clavus	none	BCRC34147	French Guiana	Huhndorf 803 (WSP) (Rogers & Ju 1998, Hsieh *et al*. 2009)	EF025596	EF025611
Kretzschmaria lucidula	none	BCRC34148	French Guiana	Huhndorf 677 (Rogers & Ju 1998, Hsieh *et al*. 2009)	EF025595	EF025610
Kretzschmaria megalospora	none	N / A	Malaysia	M. Whalley FH 64-97 (JDR) (Hsieh *et al*. 2009)	EF025594	EF025609
Nemania illita	none	BCRC34150	USA	Missouri, Columbus, S.J. Tsai (JDR) (Hsieh *et al*. 2009)	EF025593	EF025608
Nemania primolutea	none	BCRC34151	Taiwan	**Holotype** (WSP) (Ju *et al*. 2005, Hsieh *et al*. 2009)	EF025592	EF025607
Rosellinia lamprostoma	none	BCRC34152	Taiwan	Ju & Hsieh 89112602 (HAST) (Hsieh *et al*. 2009)	EF025589	EF025604
Rosellinia necatrix	none	BCRC34153	Taiwan	Ju & Hsieh 89062904 (HAST) (Hsieh *et al*. 2009)	EF025588	EF025603
Stilbohypoxylon elaeicola	none	BCRC34154	French Guiana	Huhndorf 928 (Rogers and Ju 1997, as *S. moelleri*; Hsieh *et al*. 2009)	EF025601	EF025616
Stilbohypoxylon quisquiliarum	none	BCRC34155	French Guiana	Huhndorf 940 (Rogers & Ju 1997) (Hsieh *et al*. 2009)	EF025590	EF025605
Stilbohypoxylon quisquiliarum	none	BCRC34156	Taiwan	Ju & Hsieh 89091608 (HAST) (Hsieh *et al*. 2009)	EF025591	EF025606
Theissenia (Ootheca) cinerea	none	BCRC34157	Taiwan	**Holotype** (HAST) (Ju *et al.* 2003)	EF025598	EF025613
Theissenia (Ootheca) rogersii	none	BCRC34158	Taiwan	**Holotype** (HAST) (Ju *et al.* 2007)	EF025597	EF025612
Whalleya microplaca	none	BCRC34159	Taiwan	Ju & Hsieh 91111215 (HAST) (Hsieh *et al*. 2009)	EF025599	EF025614
Xylaria bambusicola	none	BCRC34102	Taiwan	**Holotype** (WSP) (Ju & Rogers 1999, Hsieh *et al*. 2009))	AY951873	AY951762
Xylaria venosula	none	BCRC34160	USA	Hawaii, Ju & Hsieh 94080508 (HAST) (Hsieh *et al*. 2009)	EF025602	EF025617

RESULTS AND DISCUSSION

Phylogenetic analysis

Fifty-four strains were used in the analysis, 17 of which were Thai material sequenced in this study. From the 17 strains, eight represented isolations from *Hypoxylon comedens s.l.*, five strains from *Xylaria,* and one strain each from *Annulohypoxylon, Biscogniauxia, Hypoxylon,* and *Kretzschmaria*. The remaining 38 sequences across *Xylariaceae* used were taken from GenBank. Two species ancestral to *Xylariales*, *Cryphonectria macrospora* and *Amphilogia*, were used as outgroup taxa. All 17 strains were sequenced for the α-actin and the β-tubulin gene (Table 1) for comparison with the data in Ju *et al*. (2007). After initially examining individual trees for α-actin (247 parsimony-informative characters; CI = 0.390, RI = 0.650, RC = 0.253, HI = 0.610) and β-tubulin (1165 parsimony-informative characters; CI = 0.384, RI = 0.588, RC = 0.226, HI = 0.616) these were combined based on the similar topologies of the individual trees.

Of the 2528 characters in the combined alignment, 1412 characters were parsimony informative. Maximum parsimony analyses yielded four most parsimonious trees that had similar topologies except for the terminal branches. One of the four trees generated from maximum parsimony (CI = 0.383, RI = 0.597, RC = 0.229, HI = 0.617) is shown in Fig. 1. The result of MrModeltest selected the General Time Reversible (GTR) model with proportion in invariable sites (I) and gamma distribution (G) (GTR+I+G; Tamura & Nei 1993). This model was then used in MrBayes. Four MCMC chains were run in MrBayes for 2 M generations, sampling every 100 generations. From the 20 K trees obtained the first 2 K trees were discarded as 'burn-in'. The remaining 18 K trees were pooled and a consensus tree was created. The Bayesian analysis gave a similar result to the maximum parsimony analysis and the PP results were shown as numbers below the branches of the tree (Fig. 1).

The eleven *H. comedens s.l.* and *Theissenia* sequences all fall in a well-supported clade without other elements. The relationship to other groups within *Xylariaceae* is less clear, but the clade is definitely outside the subfamily '*Xylarioideae'* that constitute a highly supported cluster. Moreover, the *H. comedens s.l.* material falls in two well-supported sister clades, with one clade further divided in two based on a limited number of substitutions. *T. pyrenocrata* falls in a well-supported, rather distant basal position. *Theissenia rogersii* constitutes a sister group to the combined *H. comedens s.l.* and *T. cinerea* clades. On molecular phylogenetic evidence in combination with morphological evidence, we thus recognize two genera and four species in the *H. comedens/Theissenia* complex, with a further possible separation in the *H. comedens* complex.

Scanning electron microscopy (SEM)

The images obtained from spores of Thai *Hypoxylon comedens* material (Fig. 5) confirm the results in Læssøe *et al*. (1989), i.e. that the spores are completely smooth as they also appear to be in KOH mounts at 1200× (including the type material). The possible germ slit observed in that study could not be found in these better preserved ascospores, Since definite germ sites have not been observed by LM, we can conclude that this species lacks obvious germ sites. Occasionally, some of the ascospores, when mounted in 10 % KOH, appeared to have germ slit-like features, but this may have been due to an artefact created by creasing or folding when the spores collapse (or longitudinal ruptures may occur before germination). In any case, even meticulous observations of ascospores of these materials have not revealed a germ slit, as generally observed in many other xylariaceous species. Nevertheless, an ascospore showing a very faint germ slit-like structure, that could just be about to germinate, was observed in the type of *D. depressa* (see below)

HPLC analyses

Young as well as mature stromata of specimen BBH 15200, identified as *Hypoyxlon comedens,* were studied for extrolites by HPLC. As previously reported for *Biscogniauxia* species and various other members of '*Xylarioideae'* (Stadler *et al.* 2001, Stadler & Hellwig 2005), none of the characteristic compounds usually encountered in species of *Hypoxylon* and its immediate allies were found. Not even very young stromata contained binaphthalene BNT, azaphilones, cytochalasins, and other products that occur in various species of *Daldinia* and *Hypoxylon*, as well as in their cleistocarpous relatives, *Pyrenomyxa*, *Phylacia*, and *Rhopalostroma* (Stadler *et al*. 2004, 2005; 2010a,b). These results, in conjunction with morphological features and that the species is devoid of visible and extractable stromatal pigments, indicate that the closest chemical relationships of *H. comedens* within *Xylariaceae* are with *Biscogniauxia* and *Camillea*, and that the above-mentioned taxa containing pigments are more distantly related. It should, nevertheless, be noted that there are no strong chemotaxonomical syndromes connecting *H. comedens* with species of *Biscogniauxia* and *Camillea*. We observed some minor components, especially in the young, freshly collected stromata, that were apparently absent in the latter genera as well, but could not be safely assigned to any of the known *Xylariaceae* metabolites. Recently, Læssøe *et al.* (2010) examined some peculiar taxa assigned to *Xylariaceae* that deviate from the mainstream of the family in having conspicuous green or blue stromatal surfaces, i.e. *H. aeruginosum* and representatives of the genus *Chlorostroma*. Aside from specimens growing fungicolously on stromata of *Hypoxylon*, the above taxa did not yield any known compounds of the `*Hypoxyloideae*´, but the substituted chromone, lepraric acid, which had hitherto only been found in lichenized ascomycetes, and derivatives thereof, were detected as major stromatal components of both *Chlorostroma* and *H. aeruginosum*. Due to these findings, our attention was directed toward such compounds, also in other *Xylariaceae* that we examined during our ongoing study using the well-established HPLC profiling technique. One of the recently collected specimens of *H. comedens* (XY01706/BBH26963) yielded particularly high amounts of yellowish pigments in KOH and was also studied by HPLC. Surprisingly, it yielded lepraric acid, too. The amounts of the compound present in the stromata were estimated to be at least ten times lower in *H. comedens* than in *Chlorostroma* and *H. aeruginosum*, but its identity with lepraric acid (or an isomer thereof) was established by matching DAD and mass spectra. The compound was not detectable at all in mature stromata, suggesting that its biosynthesis only occurs in the initial stages of stromatal formation and ceases as the stromata become mature and carbonaceous. However, traces of lepraric acid were also found in BBH15200 (the one studied by SEM). No molecular data and no DNA suitable for PCE were so far obtained for *Chlorostroma* and *H. aeruginosum.* The ascospores of these fungi do not easily germinate, and their stromata are very rarely observed and collected. Therefore, the significance of these findings remains to be confirmed by means of molecular phylogeny, and by studying their conidiogenous structures (in those species that produce them).

MORPHOLOGY AND TAXONOMY

Durotheca Læssøe, Srikitikulchai, Luangsa-ard & M. Stadler, **gen. nov**.
MycoBank MB803610

Etymology: Indicative of the highly carbonized perithecia without surrounding tissue, easily seen on the underside of detached stromata.

Description: Stromata more or less erumpent through bark or wood, bipartite in nature, initially covered in white pruina, highly carbonaceous including encasement of large, globose to cylindrical perithecia without or with an indistinct basal columella; crust without extractable pigments or with yellow pigmentation. *Paraphyses* filiform, attenuating towards the apex, distantly septate, without obvious contents. *Asci* more or less clavate, thin-walled without apical apparatus, deliquescent early, the spores in a tight cluster. A*scospores* moderate to very thick-walled, pale to medium brown at maturity, ellipsoid-oblong to allantoid, with or without a germ-slit. *Asexual morph*, where known nodulisporium-like. Lignicolous, terrestrial.

Type species: *Durotheca depressa* Læssøe & Srikitikulchai 2013.

Durotheca depressa Læssøe & Srikitikulchai, **sp. nov**.
MycoBank MB803611
(Figs 2–3)

Etymology: Based on the deeply seated ostioles.

Diagnosis: Differs from *Durotheca comedens* in narrow stromata with deeply seated ostioles in crater like depressions.

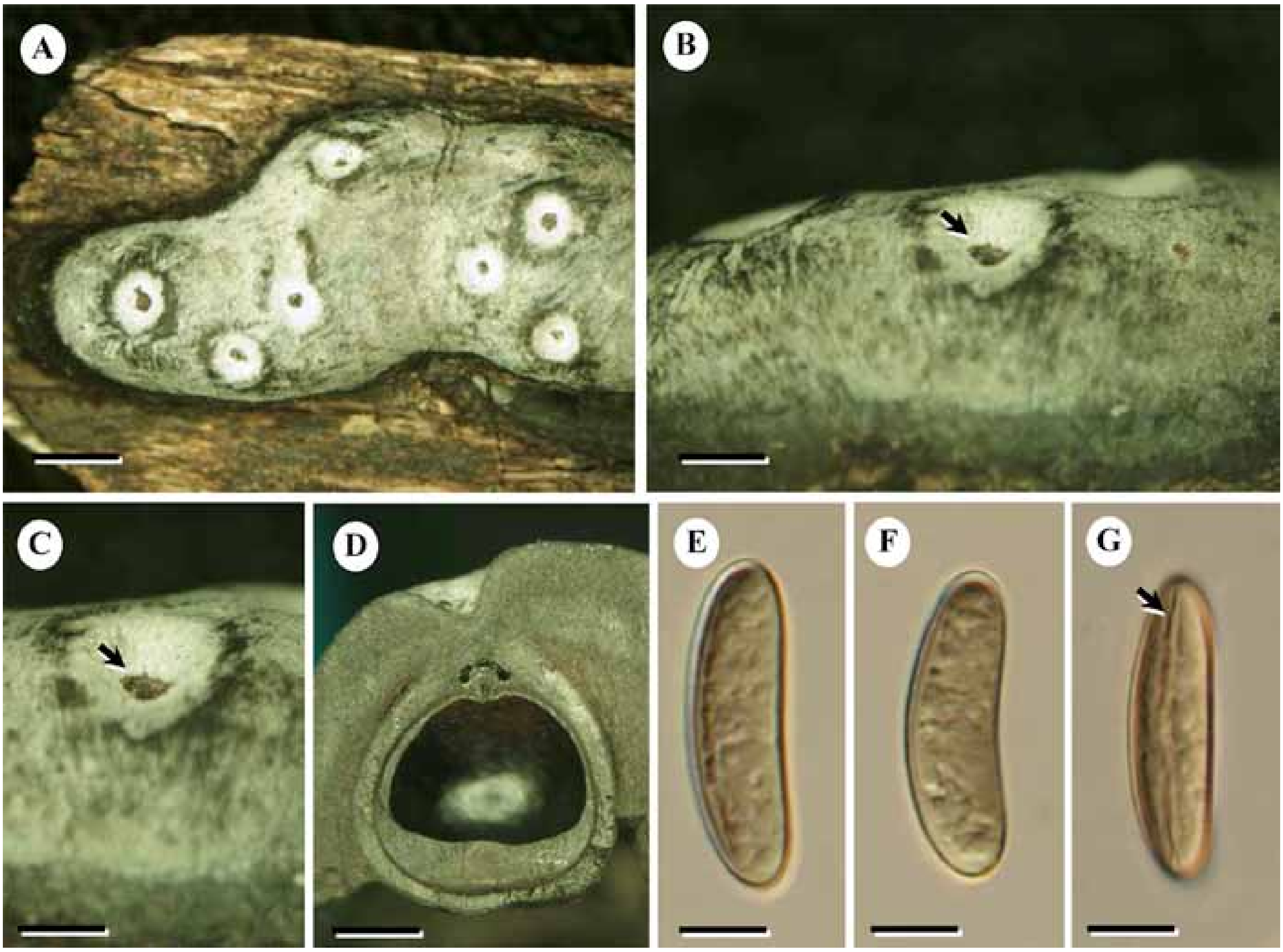

Fig. 2. *Durotheca depressa* (BCC23016). **A.** Stromata. **B.** Stromata with ostioles, arrow = ostioles. **C.** Deep ostiole: arrow = deep ostiole. **D.** Perithecia. **E–G.** Ascospore: arrow = germination slit. Bars: A = 1.5 mm; B = 0.7 mm; C = 0.5 mm; D = 1.0 mm; E–G = 6.0 µm.

Type: **Thailand**: *Prov. Chiang Mai*: Doi Inthanon National Park, Pun Churee study trail, on indet. angiosperm wood, 9 May 2006, *P. Srikitikulchai XY00402* (BBH 18222 – **holotype**; BBC 23016 – culture ex-holotype).

Description: *Stromata* seen from above very narrow and often undulating, effused-pulvinate, with beveled margins, 0.5–6 cm long, 0.5–2 cm broad, up to 2 mm thick; at first chalky white creamy owing to the presence of a thin pruina with mature surface light grey, plane with umbilicate ostioles deep in crater-like depressions; crust highly carbonaceous extending downward to encase each perithecium; tissue between perithecia scarce, fibrous and soft, extending into interstices of overlying carbonaceous stroma; tissue beneath perithecia thin and fibrous to almost absent. *Perithecia* globose-ovoid, 2.5 mm diam, with conspicuous basal columella. *Paraphyses* not observed. *Asci* deliquescing, not observed. *Ascospores* light brown to brown (absent pigmentation in KOH), unicellular, oblong to allantoid in side view, smooth, wall thick, (19–)20–24 (–26) × 8–11 µm (av. 21.9 × 9.3 µm, n = 10), with straight, inconspicuous germ slit spore length; perispore non-dehiscent in 10 % KOH.

Cultures: No conidiogenous structures were produced in cultures derived from the type and paratypes. The morphology of the cultures resembled those of *Durotheca rogersii* (Ju *et al*. 2003). The mycelia were initially whitish, melanising with age, the reverse attained a brownish colour with age and even the bramble like structures described by Ju *et al.* (2003) were evident in ageing cultures.

Host: Unidentified, huge log (possibly *Dipterocarpaceae*).

Distribution: Only known from a single site at the Doi Inthanon Mountain in northern Thailand.

Additional material (from the same log): **Thailand**: *Prov. Chiang Mai*: Doi Inthanon National Park, Pun Churee study trail, alt. 1679 m, on indet. angiosperm wood, 28 May 2006, *P. Srikitikulchai XY00619* (BBH 19737, BCC 28073).

Notes: This new species has been repeatedly collected from the same very big log and is so far only known from this material at mid-elevation at the Doi Inthanon Mountain. *Durotheca comedens* has been collected on an adjacent trail so the two species co-exist at this site. Already in the field the peculiar features of *D. depressa* were noted

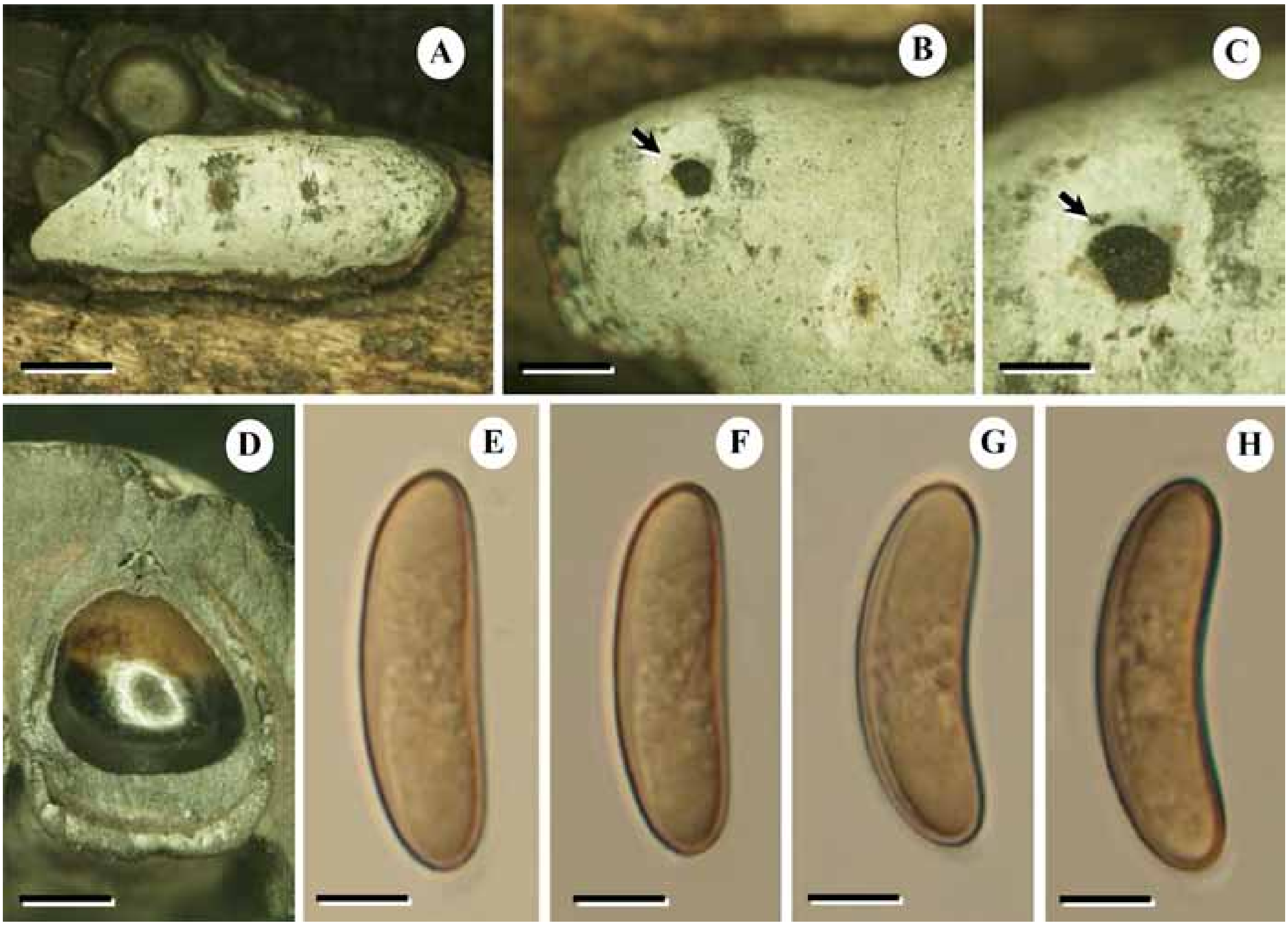

Fig 3. *Durotheca depressa* (BCC28073). **A.** Stromata. **B.** Stromata with ostioles: arrow = ostioles. **C.** Deep ostiole: arrow= deep ostiole. **D.** Perithecia. **E–H.** Ascospores. Bars: A = 1.5 mm; B = 1.0 mm; C = 0.5 mm; D = 1.0 mm; E–H = 5.0 µm.

(i.e. the narrow, undulating stromata with ostioles in deep depressions), and the phylogenetic data corroborates the distinction. We have chosen this taxon as type of *Durotheca* since we consider it of value to have DNA sequences from type material.

Durotheca comedens (Ces.) Læssøe & Srikitikulchai, **comb. nov.**

MycoBank MB803613

(Figs 4–5)

Basionym: *Hypoxylon comedens* Ces., *Atti Accad. Sci. fis. mat. Napoli* **8**: 19 (1879).

Synonyms: *Nummularia comedens* (Ces.) Cooke, *Grevillea* **11** (no. 60): 126 (1883).

Nummulariola comedens (Ces.) P. Martin, Jl S. Afr. Bot. 35: 318 (1969) [basionym as “*Nummularia comedens* Ces.”]. *Type*: **Malaysia**: *Borneo*: Sarawak, [*O. Beccari*] *218* (K - several, incl. one presumed to be in Cesati handwriting –isotypes).

Theissenia cinerea Y.M. Ju *et al.*, *Mycologia* **95**: 111 (2003). *Type*: **Taiwan**: Pingtung Co., Heng-chun, Ken-ting, on wood stump, 16 July 2001, *Hsieh & Ju 90071615* (HAST – holotype).

Description: *Stromata* erumpent, often sunk rather deep in the decorticated wood, possibly reflecting repeated sporulation in the same position, from above rather variable in outline, from almost circular to very elongate and somewhat irregular, applanate or slightly convex, with abrupt, bevelled dark margins; initially covered by a black, outer, dehiscent layer, exposing a thin white, fairly fugacious, pruinose layer on top of the black, highly carbonised upper stroma, with ostioles in dark pits. *Perithecia* globose, highly carbonized, densely packed below the crust with hardly any surrounding tissue, or sometimes with a small amount of fibrous tissue below some of them; the base of the perithecia convex to concave, evident in remnants left on the wood when stromata are dislodged, 2.5 mm in diam. *Paraphyses* as in *Hypoxylon*/*Xylaria* (not filled with lipids as in most *Camillea* species) with distant septation and gradually tapering upwards.

Asci clavate-pedicellate, very early deliquescent and thin-walled, 8-spored. *Ascospores* in a densely packed cluster, young spores appearing very thick-walled with a central granular part, older spores with pale yellow-brown walls (in water, olivaceous in KOH), suballantoid to allantoid in side view and oblong in front view, few to many guttulate, 15–23 (–26) x (5.5–)6–9(–11) µm (av. 16.1–22.4 x 5.9–7 µm, *n* = 90).

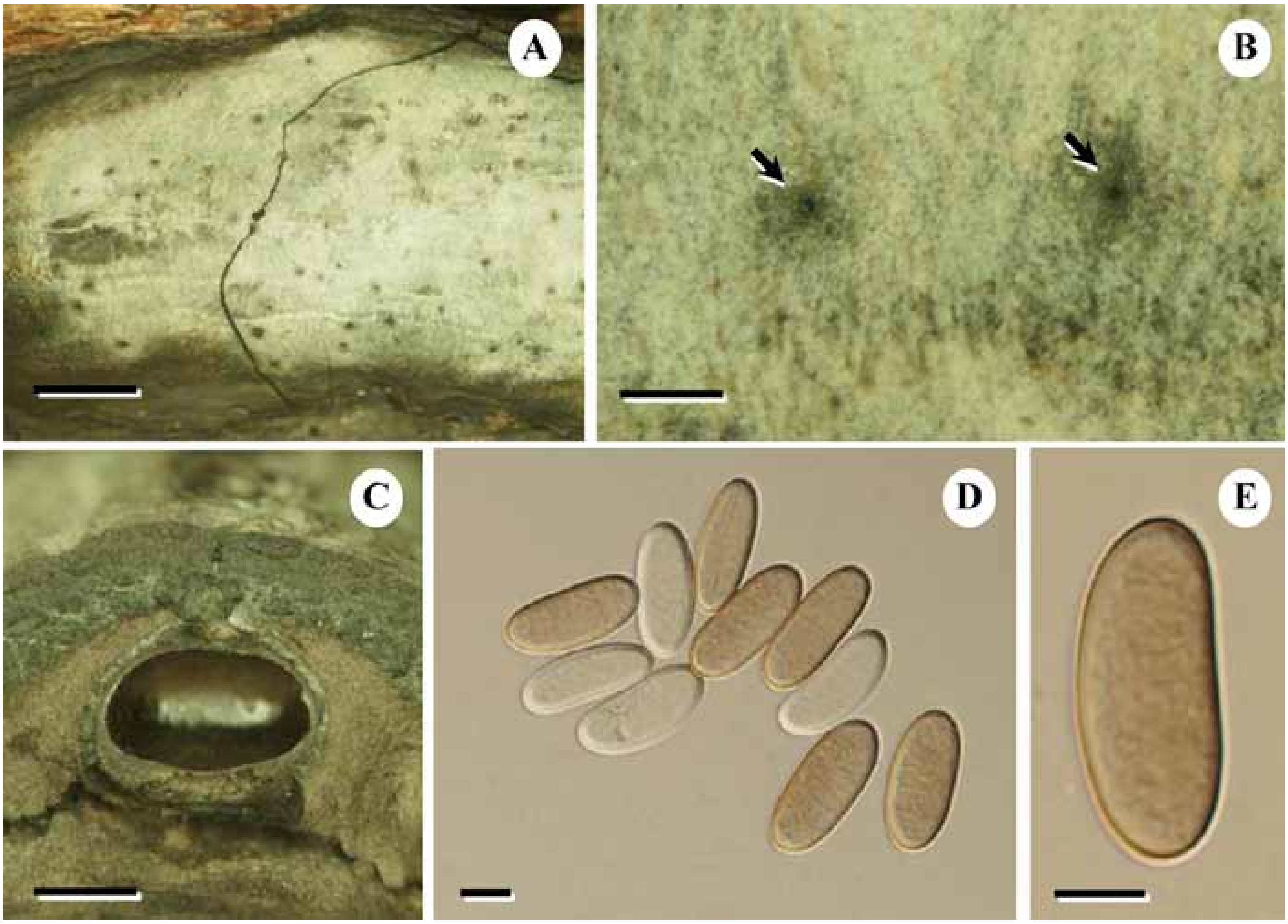

Fig. 4. *Durotheca comedens* BBH15200. **A.** Stromata. **B.** Stromata with ostioles, arrow: ostioles. **C.** Perithecia. **D–E.** Ascospores. Bars: A = 1.0 mm; B = 0.7 mm; C = 1.0 mm; D = 5.0 µm; E = 5.0 µm.

*Conidiogenous structures***:** None were found in cultures from the Thai material, but Ju *et al*. (2003) reported a nodulisporium-like state with long, slender conidia in the Taiwanese material of *T. cinerea*.

Host: Stromata appear to be restricted to large, fallen, decorticated dicotyledoneous logs in "wet tropical forests" at low altitudes. No specific hosts have been identified but members of *Dipterocarpaceae* are likely candidates.

Distribution: Apparently restricted to South-East Asia, where it appears to be widespread, although unrecorded in many places within the region. Tai (1979) and Zhuang (2002) reported it from China. Ju & Rogers (1999) did not include this species in their detailed account of Taiwanese *Xylariaceae*. Material reported as *Hypoxylon* cf. *comedens* from Mexico by San Martín González & Rogers (1993) should be re-evaluated as it may represent a species of *Theissenia*.

Specimens examined: **China**: *Yunnan*:, Xichou, 18 May 1959, *Wang Quing-zhi 194* (HMAS 33628(S). – **Malaysia**: *Malay Peninsula*: State of Perak, Maxwell's Hill, alt. 3800 ft, on dead trunk, growing where the bark is removed, 23 Mar 1924, *J. H. Burkill 13193* (K); *Borneo*: Sarawak, Gunong Mulu NP, 4th Division, Baram District, between Melinau Gorge and *ca* 2 km upstream on S side of Sungei Melinau, alt. *ca* 150-170 m, on leaning decorticate trunk in alluvial forest, no date, *B. J. Coppins 5168* (E, C); Sabah, Danum Valley, Field Centre, West Trail/ Rhino Ridge Trail, on old, decorticated trunk in lowland dipterocarp rain forest, alt. 150-200 m, 3 Feb 1999, *T. Læssøe & J. Omar, TL-6118* [old, weathered material] (C, UMS). – **Thailand**: [* indicates that specimens are included in the phylogenetic analysis, Fig. 1] *Prov. Chaiyaphum*: Phu Khiao Wildlife Sanctuary, Ban Chak Kha, on indet. wood, 23 Oct 2007*, P. Srikitikulchai XY00854* (BBH 22419). *Prov. Chiang Mai*: Doi Inthanon National Park, Pa Mek – Pa Tonnam Lamthan Nature Trail, on indet. angiosperm wood, 26 Nov 2008, *P. Srikitikulchai XY001464* (BBH 25163 (BCC 34524)). *Prov. Kamphaeng Phet*: Khlong Lan National Park, indet. dicot. wood, 7 Nov 2007, *P. Srikitikulchai XY00771, XY00834, -835, -836 & -837* (BBH 22341 (BCC 28439), 22400 (BCC 28746), 22401 (BCC 28747), 22402 (BCC 28748) [22401 and 22402 from the same log], 22403 (BCC 28749)). *Prov. Nakhon Si Thammarat*: Khao Nan National Park, Sunantha Waterfall, on indet. dicot. wood, 20 Feb 2007, *P. Srikitikulchai XY00513 & XY00531** (BBH 25875 (BCC 25014), *25876* (BCC 25152)); *ibid*., Pa Pra nature trail, on indet. dicot. wood, 30 Oct 2008, *P. Srikitikulchai XY01412* (BBH 25466 (BCC 33654)). *Prov. Phattalung*: Khao Puu-Khao Ya National Park, a mixture of young white stromata and very old grey to black stromata, on blackened, very thick, hard-wooded, but very wet, decorticated, dicot log in calcareous lowland, wet evergreen forest, 22 Feb 2006, *T. Læssøe & P. Srikitikulchai XY00212* (BBH 15200 (BCC 21319)); *ibid*., Khao Ban That Wildlife Sanctuary, Khao Chet Yot, 19 Mar 2007,

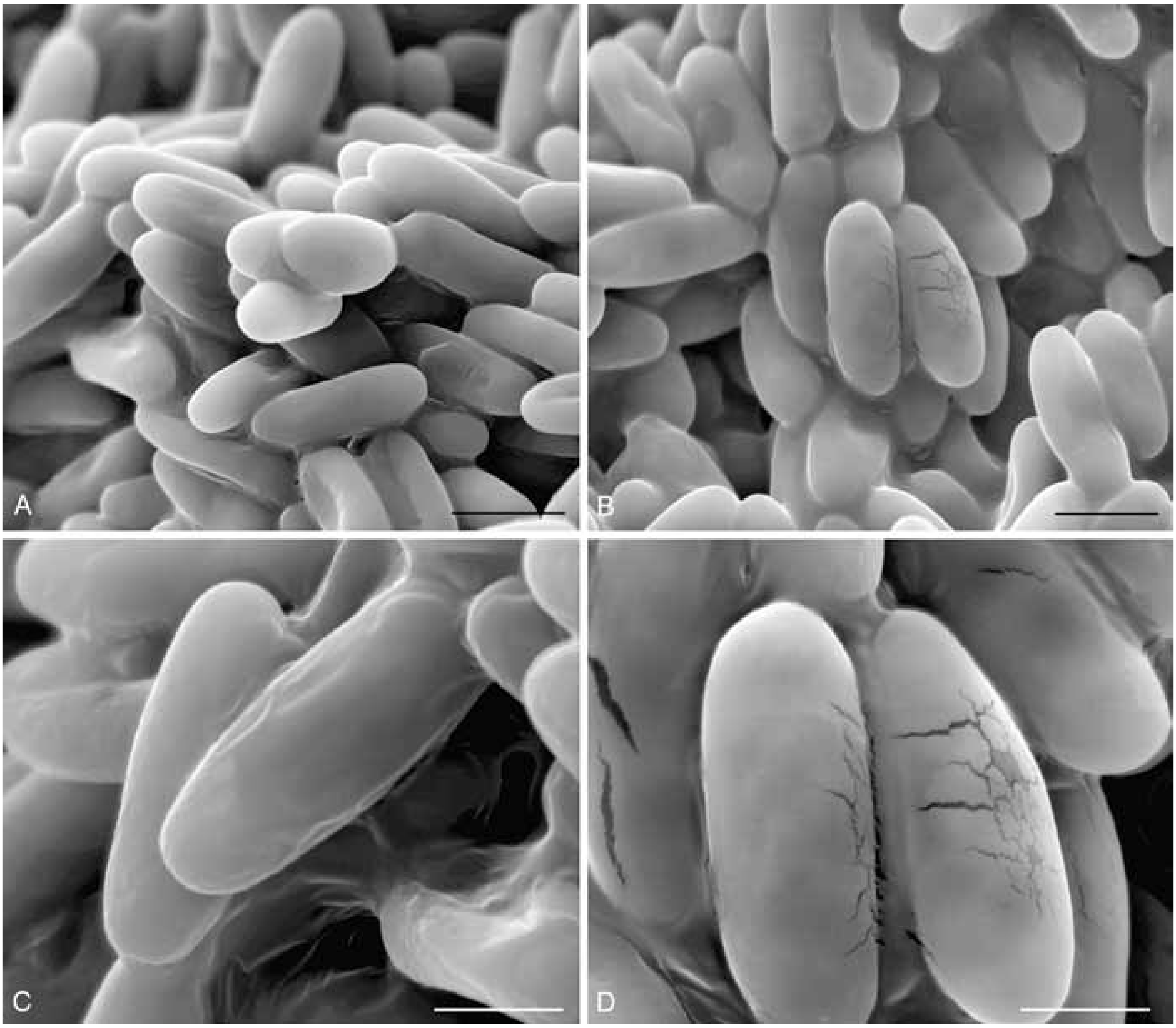

Fig. 5. SEM of mature ascospores of *O. comedens* (BBH 15200). **A, B.** 2000x. **C, D.** 5000x. Fig. 5D shows disruptions on the surface of the spores in the center, and the spore to the left has been ripped open. Bars: A = 10.0 µm; B = 10.0 µm; C = 5.0 µm; D = 5.0 µm.

P. Srikitikulchai XY00866 (BBH 22430,(BCC 28891)). *Prov. Phet Buri*: Kaeng Krachan National Park, Paneontung, indet. dicot. wood, 28 June 2007, *P. Srikitikulchai XY00638** (BBH 19755 (BCC 28080)); *ibid*., Ban Krang, indet. dicot. wood, 26 July 2007, *P. Srikitikulchai XY00535* (BBH19738). *Prov. Phitsanulok*,: Phu Hin Rong Kla National Park, on indet. dicot. wood, 8 Sep 2006, *P. Srikitikulchai XY00290**, *XY00291* (BBH 1816 & 18117). *Prov. Surat Thani.* Khao Sok National Park, Sanyang Roi nature trail, 14 Oct 2008, on indet. dicot. wood, *P. Srikitikulchai XY01415* (BBH 25469 (BCC 33657)). *Prov. Trang*: Khao Ban That Wildlife Sanctuary, indet. wood, 17 Mar 2007, *P. Srikitikulchai XY00534**, *XY00535* (BBH 25877 (BCC 25155), *BBH 25878* (BCC 25156)).

Notes: We found the material from a wide geographical area to be morphologically identical, including the isotypes of *Hypoxylon comedens*, the type of *Theissenia cinerea,* and material from peninsular Malaysia, the Bornean part of Malaysia, Thailand, and China. The only deviating material is described above as *Durotheca depressa*. Ju *et al.* (2003) stated that the perispores of *D. comedens* (as *T. cinerea*) ascospores were dehiscent in 10 % KOH and they also provide a picture to support this statement. In all the material we studied of *D. comedens*, and likewise in the type of *T. cinerea*, dehiscence was neither observed upon addition of KOH to water mounts, nor when perithecial contents were mounted directly in 10 % KOH. A similar phenomenon, i.e. the occurrence of material with dehiscent and indehiscent perispores in different specimens assigned to the same species, was also attributed to other *Xylariaceae* in the past (*cf*. *Daldinia fissa,* Ju *et al*. 1997). As we did not find any other deviating criterion to distinguish *D. comedens* and *T. cinerea*, we regard these species names as synonyms.

Durotheca rogersii (Y.M. Ju & H.M. Hsieh) Srikitikulchai & Læssøe, **comb. nov.**
MycoBank MB803632
Basionym: *Theissenia rogersii* Y.M. Ju & H.M. Hsieh, in Ju *et al*., *Mycologia* **99**: 613 (2007).

Notes: *Durotheca rogersii* is placed here based on the description and molecular data provided by Ju *et al.* (2007), which leave no doubt on the affinities of this species to *Durotheca*.

Notes on *Theissenia pyrenocrata*

Theissen (1908) described this new species, from southern Brazil (Rio Grande do Sul), as *Ustulina pyrenocrata*. Maublanc (1914) coined a new generic name for it, and reported it from further north in São Paulo State, and Ju *et al*. (2003) even from northeastern Brazil. Dennis (1964) and Ju *et al*. (2003) reported it from Africa (`Zaire´, now Democratic Republic of Congo), while Miller (1961) and Ju *et al*. (2003) confirmed its presence in Sri Lanka based on the type of *Nummularia porosa* that Dennis (1964) considered a likely additional *Theissenia* species with smaller spores. Here we add a record from western South America that agrees in all morphological characters with those reported in the cited references. It grew on a very large, unidentified hardwood log in black water, inundated lowland rainforest in the eastern part of Ecuador, and was used for sequence analysis. Despite the wide distribution, very few records are known of this rather conspicuous and characteristic species. Ju *et al*. (2003) discovered the striate-furrowed nature of the ascospores that had been overlooked by previous workers.

Specimens examined: **Ecuador**: *Prov. Orellana*: along small black water tributary to Río Tiputini near Tiputini Field Station, alt. 190-270 m, 16 July 2004, *T. Læssøe, J.H. Petersen, A. Alsgård Jensen* TL-11480 (C, QCNE).

Notes on *Theissenia eurima*

Theissenia eurima was described from Brazil by Ju *et al*. (2003). We accept this taxon in *Theissenia* at present, since it apparently produces an asexual morph equivalent to that of *T. pyrenocrata*, both taxa occur in South America, and since we have no other morphological or molecular data to suggest another position. There are several other examples of xylariaceous genera that encompass species with and without germ slits, *Nemania* being an obvious well-known example, aside from the new genus established here.

Key to taxa in the *Theissenia-Durotheca* clade (Fig. 1)

1 Ascospores striate, almost cylindrical with one side slightly flattened, without germ slit .... **T. pyrenocrata**
Ascospores smooth, with or without germ slit, ellipsoid to slightly allantoid 2

2 (1) Ascospores with short germ slit; known from Amazonian Brazil .... **T. eurima**
Ascospores with very faint germ slit, or without obvious germ slit; known from SE Asia ***Durotheca*** 3

3 (2) Ascospores broadly ellipsoid, wall very thick, 25–36 µm long; perithecia cylindrical .... **D. rogersii**
Ascospores ellipsoid-cylindrical to allantoid, usually less than 25 µm long, wall moderately thickened; perithecia subglobose 4

4 (3) Stromata with variable outline, not narrowly linear, ostioles in shallow depressions .... **D. comedens**
Stromata more or less linear with ostioles in crater-like depressions .... **D. depressa**

DISCUSSION

As already noted, the clade with *Theissenia pyrenocrata* and taxa placed in *Durotheca* here (Fig. 1) has a rather unresolved position within *Xylariaceae.* However, the clade has very limited affinities to the '*Xylarioideae*' subclade, whilst it is difficult to speculate on affinities to the '*Hypoxyloideae'*, but such relationships cannot be ruled out at present. The highly carbonized, very thick, and layered ascomatal wall more or less seated directly on the substrate is a common feature of all currently recognised members of *Theissenia* and *Durotheca*. The two genera are also separated on several stromatal characters and, possibly, in the type of asexual morph. The molecular phylogenetic data show two very distinct groups with *T. pyrenocrata* in a well-supported basal position. Further phylogenetic analyses would probably benefit from an expanded taxon sampling and from the inclusion of other genes. Ju *et al*. (2007), in their analysis, placed *Theissenia s.lat*. within the `*Hypoxyloideae*´ in a clade containing species with a bipartite stromatal development.

The chemotaxonomic data so far available on these fungi are rudimentary at best, since the cultures have not been studied for metabolites, and the surprising detection of lepraric acids in young stromata of some representatives merely provides a hint as to their possible affinities to other *Xylariaceae*. Due to the study by Bitzer *et al.* (2008), a rather comprehensive overview of chemical traits in cultures of the hypoxylid clade have become available, but *Biscogniauxia*, *Camillea*, as well as the xylarioid *Xylariaceae,* were underrepresented in this work. In addition, no cultures and no molecular data on *Hypoxylon aeruginosum* and *Chlorostroma* species have so far been available, and, therefore, it is at present difficult to assess whether the production of lepraric acid derivatives has a common history in the taxa with green and blue coloured stromatal surfaces and *Durotheca*. Interestingly, these substituted chromones seem to be very rare even in lichenised ascomycetes, where they have hitherto only been found in members of the rather distantly related genera *Lepraria* and *Roccella*, aside from the above mentioned *Xylariaceae* (*cf*. Huneck & Yoshimura 1996, Læssøe *et al.* 2009). Notably the previous studies on lichen chemotaxonomy mostly relied on thin layer chromatography, rather than the much more sophisticated and sensitive HPLC-MS technique, and the data presented here are actually based on studies of several thousands of *Xylariaceae* specimens. On the other hand, the absence of the typical pigments of *Hypoxylon* and allies in all the above taxa, as well as in *Biscogniauxia* and *Camillea* may support the molecular phylogeny. It is too early to draw final conclusions on the affinities of basal groups of *Xylariaceae* as inferred from molecular phylogenetic studies. However, studies based on rDNA and other DNA sequence data (Pelaez *et al.* 2008, Tang *et al*. 2009) have also suggested that *Biscogniauxia* and *Camillea* might be basal to both the xylarioid and the hypoxyloid lineages. Unfortunately, these studies, as well as other phylogenetic work cited above, have dealt with different isolates, different genes, and, to some extent, even different species concepts. These issues mean that results cannot be directly compared. Possibly, *Durotheca*, *Theissenia*, and even *Chlorostroma* and *H. aeruginosum* may represent hitherto unknown lineages that separated quite early from the ancestors of mainstream *Xylariaceae*. The availability of living cultures of *Durotheca* and *Theissenia* will now facilitate further testing of such hypotheses.

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