Emarcea castanopsidicola gen. et sp. nov. from Thailand, a new xylariaceous taxon based on morphology and DNA sequences

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Abstract: We describe a unique ascomycete genus occurring on leaf litter of *Castanopsis diversifolia* from monsoonal forests of northern Thailand. *Emarcea castanopsidicola* gen. et sp. nov. is typical of *Xylariales* as ascomata develop beneath a blackened clypeus, ostioles are papillate and asci are unitunicate with a J^+ subapical ring. The ascospores in *Emarcea castanopsidicola* are, however, 1-septate, hyaline and long fusiform, which distinguishes it from other genera in the *Xylariaceae*. In order to substantiate these morphological findings, we analysed three sets of sequence data generated from ribosomal DNA gene (18S, 28S and ITS) under different optimality criteria. We analysed this data to provide further information on the phylogeny and taxonomic position of this new taxon. All phylogenies were essentially similar and there is conclusive molecular evidence to support the establishment of *Emarcea castanopsidicola* within the *Xylariales*. Results indicate that this taxon bears close phylogenetic affinities to *Muscodor* (anamorphic *Xylariaceae*) and *Xylaria* species and therefore this genus is best accommodated in the *Xylariaceae*.

Taxonomic novelties: *Emarcea* Duong, R. Jeewon & K.D. Hyde gen. nov., *Emarcea castanopsidicola* Duong, R. Jeewon & K.D. Hyde sp. nov.
Key words: *Castanopsis,* phylogeny, rDNA, systematics, *Xylariaceae*.

INTRODUCTION

We are studying the microfungi occurring on leaf litter in northern forests of Thailand. This substrate, which has a high fungal biodiversity, has resulted in the description of several new species from *Magnolia liliifera* (e.g. Promputtha *et al*. 2002). We are now studying the fungi on leaf litter of several other hosts including *Castanopsis diversifolia*. Fungal diversity from host species of *Castanopsis* is quite well documented. So far, a total of 175 fungal species from 108 genera have been recorded from *Castanopsis* spp. (EMBL fungal databases 2004). *Anthostomella castanopsis* is the only record from *Xylariaceae*. Preliminary fungal succession studies on *Castanopsis fissa* in Hong Kong have not yielded any xylariaceous fungi (Tang *et al.* unpubl. data) but in this paper, we introduce a new ascomycete in the *Xylariaceae* which was found on *Castanopsis diversifolia.* The aim of this paper is to describe *Emarcea castanopsidicola* as a new taxon and to establish its ordinal and familial placement using morphological characteristics and sequence data.

MATERIALS AND METHODS

Sampling

Dead leaves of *Castanopsis diversifolia* were randomly collected from the forest floor, placed in sterile plastic bags and returned to the laboratory. They were then incubated separately in plastic boxes lined with moistened tissue and examined periodically using a grid method (Paulus & Hyde 2004). Located ascomata were mounted in water to look for asci, ascospores and paraphyses. Melzer's reagent (Dickinson & Lucas 1983) was used to check for iodine reactions and India ink was used to establish if appendages or sheaths occurred on the ascospores. All morphological measurements are in sterile water, with a mean from 25 measurements for each character. Single-spore isolation was carried out from fresh samples, using a hand-made glass needle (Goh 1999). Single-spore cultures grown on artificial medium for 3 weeks were used for further molecular studies.

Molecular methods

DNA extraction was carried out using CTAB lysis buffer and phenol chloroform as outlined by Jeewon *et al.* (2002, 2004). Partial sequences from three different regions of the rDNA molecule (characterised by different rates of evolution) were amplified.

Table 1. Fungi used in the study with their GenBank accession numbers

.Ingroups (18S, 28S)	18S rDNA	28S rDNA	Ingroups (ITS)	ITS, 5.8S
Ambrosiella macrospora		AF282873	*Muscodor albus*	AY555731
Ambrosiella sulfurea	AF348149		*Muscodor albus*	AF324336
Aniptodera chesapeakensis	U46870		*Muscodor albus*	AY527045
Apiosporopsis carpinea	AF277110		*Muscodor albus*	AY527048
Arecophila sp.		AF452039	*Muscodor azulenus*	AY244622
Ascovaginospora stellipala	U85087		*Muscodor* sp. A35	AY034665
Bartalinia robillardoides		AF382366	*Muscodor vitigenus*	AY100022
Cainia graminis		AF452033	*Xylaria arbuscula*	AY183369
Chaetomium globosum	AY545725	AY545729	*Xylaria arbuscula*	AY183369
Clohiesia corticola		AF132329	*Xylaria enteroleuca*	AF163033
Coniochaeta ligniaria	AY198389		*Xylaria hypoxylon*	AJ309350
Cryphonectria havanensis		AF408339	*Xylaria hypoxylon*	AF194027
Cryphonectria parasitica	AF277116		*Xylaria mali*	AF163040
Diaporthe pustulata		AF408358	*Xylaria* sp. F19	AY315404
Discosia sp.		AF382381	*Xylaria* sp. F4	AY315405
Discostroma fuscellum	AF346548		*Emarcea castanopsidicola*	AY603496
Discostroma sp.		AF382380		
Discostroma tricellulare	AF346546		**Outgroup ITS**	
Discula fraxinea	AF277106		*Diatrype flavovirens*	AJ302428
Discula quercina	AF277108		*Cryptosphaeria ligniota*	AJ302418
Emarcea castanopsidicola	AY603494	AY603495		
Halosphaeria appendiculata		U46885	**Outgroup 28S dataset**	
Kretzschmaria clavus		AJ390434	*Dothidea sambuci*	AF382387
Lepteutypa cupressi		AF382379	*Pleospora herbarum*	AF382386
Leucostoma auerswaldii		AF408384		
Lignincola laevis		U46890	**Outgroup 18S dataset**	
Linocarpon pandanicola		AF452041	*Dothidea insculpta*	U42474
Linocarpon sp.		AF452042	*Pleospora betae*	U43466
Lollipopaia minuta	AF301534			
Muscodor albus	AF324337			
Muscodor sp. A3 5	AY034664			
Nais inornata	AF050482			
Neurospora crassa		AF286411		
Nimbospora effusa	U46877			
Nohea umiumi	U46878	U46893		
Ophiodeira monosemeia		U46894		
Ophiostoma africanum		AF221015		
Ophiostoma piliferum		AF221625		
Ophiostoma piliferum	AF136961			
Ophiostoma torulosum	AY497517			
Pestalosphaeria hansenii	AF242846			
Pestalosphaeria sp.		AF452031		
Plagiostoma euphorbiae	AF277114			
Seimatosporium leptospermi		AF382373		
Seynesia erumpens		AF279410		
Sordaria fimicola	AY545724	AY545728		
Thielavia cephalothecoides		AF286413		
Truncatella angustata	AF346560	AF382383		
Umbrinosphaeria caesia		AF261069		
Valsella salicis		AF408389		
Xylaria acuta	AY544719	AY544676		
Xylaria hypoxylon	AY544760	AY544648		

Primer pairs NS1 (5'-GTA GTC ATA TGC TTG TCT C-3') & NS4 (5'-CTT CCG TCA ATT CCT TTA AG-3') as defined by White *et al.* (1990) were used to amplify a region spanning approximately 1200 nucleotides from the small subunit (18S) of the rDNA. LROR (5'- ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3') primer pairs as defined by Vilgalys & Hester (1990) were used to amplify a segment of the large 28S subunit (about 950 nucleotides). In addition, primer pairs ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS 5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') as defined by White *et al.* (1990) were used to generate about 600 nucleotides from the complete ITS (including 5.8S) regions. The amplification conditions were performed in a 50 μL reaction volume as follows: 1×

PCR buffer, 0.2 mM each dNTP, 0.3 µM of each primer; 1.5 mM $MgCl_2$, 0.8 units Taq Polymerase and 10 ng DNA. PCR parameters for all the regions were as follows: Initial denaturation 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 52 °C for 50 s, 72 °C for 1 min, final extension of 72 °C for 10 min. Characterisation of PCR products was done via agarose gel electrophoresis on a 1 % agarose gel containing ethidium bromide as the staining agent. DNA sequencing was performed using primers as mentioned above in an Applied Biosystem 3730 DNA Analyzer at the Genome Research Centre (University of Hong Kong).

Partial sequences generated from the different primers from *Emarcea castanopsidicola* rDNA were assembled using BioEdit (Hall 1999). Once consensus DNA sequences were obtained from the different rDNA regions under investigation, a BLAST search was performed in GenBank. DNA sequences were also submitted in GenBank. Based on the BLAST search results, putative taxa were selected as sister groups for further analyses. In addition, fungal members from *Halosphaeriales*, *Ophiostomatales*, *Sordariales* and the *Xylariales* (*Amphisphaeriaceae*) were also included in the 18S and 28S datasets, while species from *Dothidea* and *Pleospora* were used as outgroups. In the ITS dataset, however, only species from *Xylariaceae* were used as ingroups while *Diatrype flavovirens* and *Cryptosphaeria ligniota* (*Diatrypaceae*) were used as outgroup based on their close taxonomic affinities with *Xylariaceae*. Taxa used and their GenBank accession numbers are shown in Table 1. Multiple alignment was done in BioEdit (Hall 1999) and Clustal X (Thompson *et al*. 1997).

Phylogenetic analyses were conducted in PAUP v. 4.0b10 (Swofford 2002). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimise alignment. Analyses were done under different optimality criteria. Gaps were treated as missing data in all analyses but the characters were also reweighted at different transition transversion ratios to examine the effect of weighting. Maximum Parsimony (MP) analyses were conducted using heuristic searches as implemented in PAUP, with the default options. One thousand pseudo-resamplings were performed, each with 10 replicates of random stepwise addition of taxa, to determine bootstrap support levels. Phenetic and Maximum Likelihood (ML) analyses were also run under a variety of assumptions as described by Jeewon *et al*. (2002, 2003a, b). A strict parsimonious tree generated from a MP analysis was used as starting tree in the ML search. Transition-transversion ratios, shape parameter and base frequencies were initially estimated. Different models of nucleotide substitutions were tested with rates assumed to follow a gamma distribution with no enforcement of a molecular clock. These estimated parameters were used in subsequent ML searches. Descriptive tree statistics (tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], homoplasy index [HI], and Log Likelihood [–Ln L]) were calculated for all trees generated under different optimality criteria. Kishino-Hasegawa tests (Kishino & Hasegawa 1989) and Templeton tests (Templeton 1983), as implemented in PAUP*, were performed in order to determine whether trees were significantly different. Trees were figured in Treeview (Page 1996).

RESULTS

Taxonomy

Emarcea Duong, R. Jeewon & K.D. Hyde, **gen. nov.** MycoBank MB500070.

Etymology: *Emarcea* is derived from MRC (Mushroom Research Centre) where the taxon was described and identified; *castanopsidicola* is from the name of the host *Castanopsis diversifolia.*

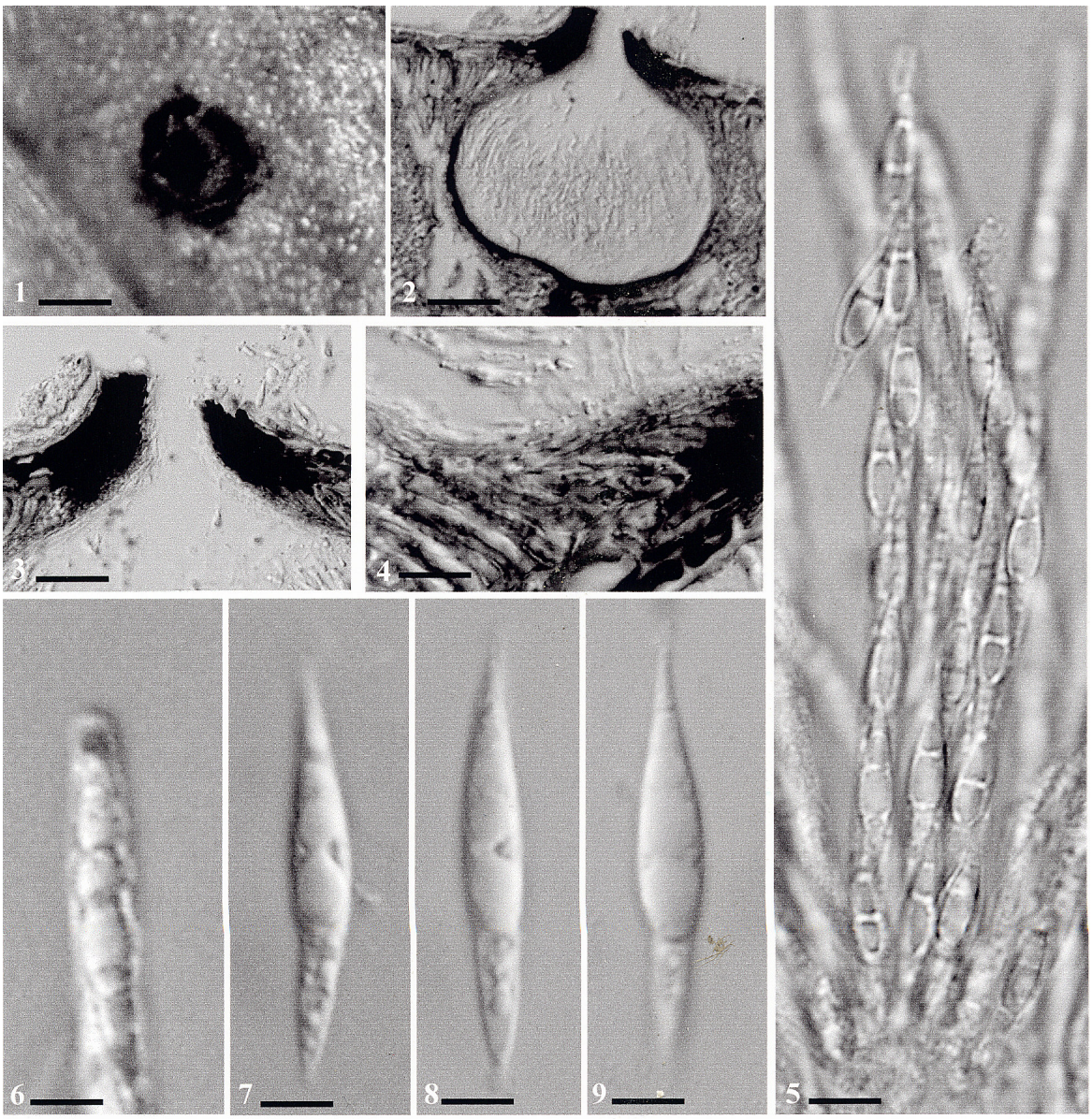
Ascomata sub clypeo immersa, globosa vel subglobosa, coriacea, ostiolo papillato, periphysato. Paraphyses hyalinae septatae. Asci octospori, cylindrici, pedicellati, unitunicati, hyalini, persistentes, rotundati ad apicem, ambitus medius, apparatu apicali J^+. Ascosporae uniseriatae, ellipsoideae, fusiformes, angustatae ad apicem, hyalinae, bicellulares, inaequaliter euseptatae, et aliquando, appendicibus mucilaginosis ad basim praeditae.

Ascomata immersed under a blackened clypeus, subglobose to globose, coriaceous, solitary; ostiole, papillate, periphysate. *Peridium* thin, comprising several layers of flattened ellipsoidal cells, brown outwardly, hyaline inwardly, and often dark brown around at the base. *Paraphyses* hyaline, septate, slightly constricted at septa, tapering at apex, longer than asci. *Asci* 8-spored, unitunicate, cylindrical, pedicellate, persistent, rounded at the apex, with a cylindrical, subapical J^+ ring. *Ascospores* overlapping uniseriate, long fusiform, hyaline, 2-celled; apical cell obclavate, guttulate, tapering to a point; basal cell shorter than the apical cell and usually with mucilage material at the base.

Type species: *Emarcea castanopsidicola* Duong, R. Jeewon & K.D. Hyde, sp. nov.

Emarcea castanopsidicola Duong, R. Jeewon & K.D. Hyde, **sp. nov.** MycoBank MB500071.

Ascomata 196–250 µm alta, 215–280 µm diam, sub clypeo immersa, globosa vel subglobosa, coriacea, ostiolo papillato, periphysato. Peridium e 4–6 stratis cellularum



Figs 1–9. Interference contrast micrographs of *Emarcea castanopsidicola*. 1. Surface view of ascoma immersed as seen on the host. 2. A section of an ascoma with hyaline and septate paraphyses. 3. Ostiole and periphyses. 4. Peridium. 5. Asci containing ascospores. 6. Ascus apical ring (J^+ ring) coloured by Melzer's iodine reagent. 7–9. Ascospores squeezed from ascus. Scale bars: 1–2 = 70 µm; 3 = 25 µm; 4 = 12 µm; 5 = 6 µm; 6–9 = 4 µm.

compositum, extus brunnearum, intus hyalinarum, fuscioribus in regione ostiolari. Paraphyses hyalinae, ramosae vel simplices, septatae, paulo constrictae ad septa, apice attenuato, longiores quam asci. Asci 90–119 × 4–5 µm (in medio 106 × 4.5 µm), octospori, cylindrici, pedicellati, unitunicati, hyalini, persistentes, sursum rotundati, apparatu apicali J^+, 1.5–2.2 × 1–1.5 µm (in medio 1.9 × 1.25 µm). Ascosporae 15.5–20.5 × 3–4.5 µm (in medio 18.5 × 3.5 µm), uniseriatae, ellipsoideae, fusiformes, apice acuminato, hyalinae, bicellulares, inaequaliter euseptatae, aliquando appendicibus mucilaginosis ad basim praeditae.

Ascomata 196–250 µm high, 215–280 µm diam, immersed under a blackened clypeus, globose to subglobose, coriaceous, solitary; ostiole papillate, periphysate. *Peridium* thin (6.5–8.5 µm), comprising 4–6 cell layers, brown outwardly, hyaline inwardly, and often dark brown around the base. *Paraphyses* 5–8 µm wide at base, septate, slightly constricted at septa, tapering at apex, longer than asci. *Asci* 90–119 × 4–5 µm (av. 106 × 4.6 µm, n = 15), 8-spored, unitunicate, cylindrical, pedicellate, persistent, rounded at the apex, with a cylindrical, subapical J^+ ring, 1.5–2.2 high, 1–1.5 µm diam (av. 1.92 × 1.25 µm; n = 25). *Ascospores* 15.5–20.5 × 3–4.5 µm (av. 18.64 × 3.46 µm; n = 25), overlapping uniseriate, long fusiform hyaline, 2-celled, apical cell long obclavate (13–16 × 3–4.5 µm), guttulate, tapering to a point, basal cell cylindrical, tapering to a rounded end (6–8 × 2–2.5 µm), usually with mucilage at the end.

Mode of life: Saprobic on dead leaves of *Castanopsis diversifolia* (*Fagaceae*).

Known distribution: Thailand (Doi Suthep, Chiang Mai).

Holotype: **Thailand**, Chiang Mai Province, Doi Suthep Pui National Park, altitude 1146 m, 18°48.402' North, 98°54.617' East, on dead leaves of *Castanopsis diversifolia* (Fagaceae), 14 Aug. 2003, Duong Minh Lam (**holotype** at Mushroom Research Centre, Chiang Mai, Thailand; **isotypes** in MRC DLA 008, HKUM 17498, CMU H224410, and PDD 78748; ex-type living cultures in CBS and HKUCC 10344.

Additional specimens examined: **Thailand**, Chiang Mai Province, Doi Suthep Pui National Park, altitude 1146 m, 18°48.402' North, 98°54.617' East, on dead leaves of *Castanopsis diversifolia* (*Fagaceae*), 15 Aug. 2003, Duong Minh Lam (Mushroom Research Centre, Chiang Mai, Thailand); *ibid.*, 4 Oct. 2003 (Mushroom Research Centre, Chiang Mai, Thailand); 20 Nov. 2003 (Mushroom Research Centre, Chiang Mai, Thailand); and 25 Jun. 2004 (Mushroom Research Centre, Chiang Mai, Thailand).

DNA analyses

Small subunit (18S) dataset: This DNA matrix consisted of 28 taxa with *Pleospora betae* and *Dothidea insculpta* as outgroups. The dataset was aligned without problems, but ambiguous taxa from other amphisphaeriaceous and xylariaceous genera, which would not properly align, were excluded from the alignment and analyses. The final aligned dataset was 968 characters, out of which 159 were parsimony informative, 58 parsimony uninformative and 751 constant characters. Parsimony analysis treating gaps as missing state and unequal weighting generated two trees, which were similar in topology and not significantly different from each other (based on KH and Templeton tests as implemented in PAUP). Tree length was 410 with a –LnL of 3858.135. Weighted parsimony with a transition transversion of 1.5 to 1 resulted in two trees which are topologically identical to each other except that the *Xylariaceae* clade was more resolved when the dataset was bootstrapped. The strict consensus tree generated from this weighted parsimony was not significantly different from the strict concensus tree generated from the unweighted parsimony (Table 2). A transition transversion (TT) ratio of 1.5 to 1 was used for subsequent analyses, as 1.56 was the estimated value from ML. Figure 10 shows the relationships of *Emarcea castanopsidicola* with other amphisphaeriaceous and xylariacaeous members. Clearly, this new taxon fits in the *Xylariales* and has 60 % bootstrap support. ML analyses of the same dataset under different models of nucleotide substitution resulted in identical tree topologies (results not shown). Treating gaps as fifth state did not affect tree topologies regarding the taxonomic placement of the ingroup under investigation.

Large subunit (28S) dataset: The 34 taxa formed an aligned data matrix of 900 characters in length and consisted of 31.5 % of parsimony informative characters. *Dothidea sambuci* and *Pleospora herbarum* were used as outgroups. Tree indices for different trees obtained have been summarised in Table 2. Unweighted parsimony resulted in 3 trees with a tree length of 1204 and a –LnL of 7723.008, whereas weighted parsimony with a TT ratio of 1.5 : 1 (as estimated from ML analyses) resulted in a single most parsimonious tree with a tree length of 1446 and a –LnL of 7708.490. This tree is shown in Fig. 11 with bootstrap values from 1000 replicates. Bootstrap values support the position of *Emarcea castanopsidicola* in the *Xylariales* (74 %) and it forms a putative monophyletic group with *Xylaria* species and other members from the *Amphisphaeriaceae* and *Cainiaceae* with 99 % bootstrap confidence.

Table 2. Summary of the tree indices, Kishino Hasegawa & Templeton Tests on trees obtained under different criteria.

	TL	CI	RI	RC	HI	No of trees (PIC)	–Ln L	KH/Templeton Tests of strict concensus [a]
18S								
TTr = 1:1	410	0.649	0.782	0.508	0.351	2 (159) *	3858.135	Best tree
TTr = 1.5:1	490.5	0.650	0.789	0.513	0.350	2 (159) *	3858.135	P = 1.00 / P = 1.00
TTr = 2:1	571	0.651	0.793	0.517	0.349	8 (159) *	3885.104	P = 0.0081 / P = 0.0156
28S								
TTr = 1:1	1204	0.515	0.699	0.360	0.485	3 (283) *	7723.008	P = 0.0004 / P = 0.0020
TTr = 1.5:1	1446	0.516	0.700	0.361	0.484	1 (287) *	7708.490	Best tree
TTr = 2:1	1690	0.516	0.699	0.361	0.484	1 (287) *	7711.363	P = 0.6951 / P = 0.8238
ITS								
TTr = 1:1	530	0.745	0.810	0.604	0.255	26 (172) *	3266.192	P = 0.0066 / P = 0.0078
TTr = 1.5:1	635.5	0.741	0.816	0.605	0.259	13 (177) *	3262.711	Best tree
TTr = 2:1	777	0.739	0.820	0.606	0.261	26 (177) *	2277.557	P = 0.0022 / P = 0.0020

TTr = Transition Transversion ratio; TL = tree length; CI = Consistency Index; RI = Retention Index; RC = Rescaled Consistency Index; HI = Homoplasy Index; -Ln L = - Log Likelihood; KH = Kishino and Hasegawa test; PIC= Parsimony informative characters. *Subsequent values for –Ln L, KH and templeton tests were done with the strict consensus trees. [a]Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two tailed test) with significance at $P < 0.05$.

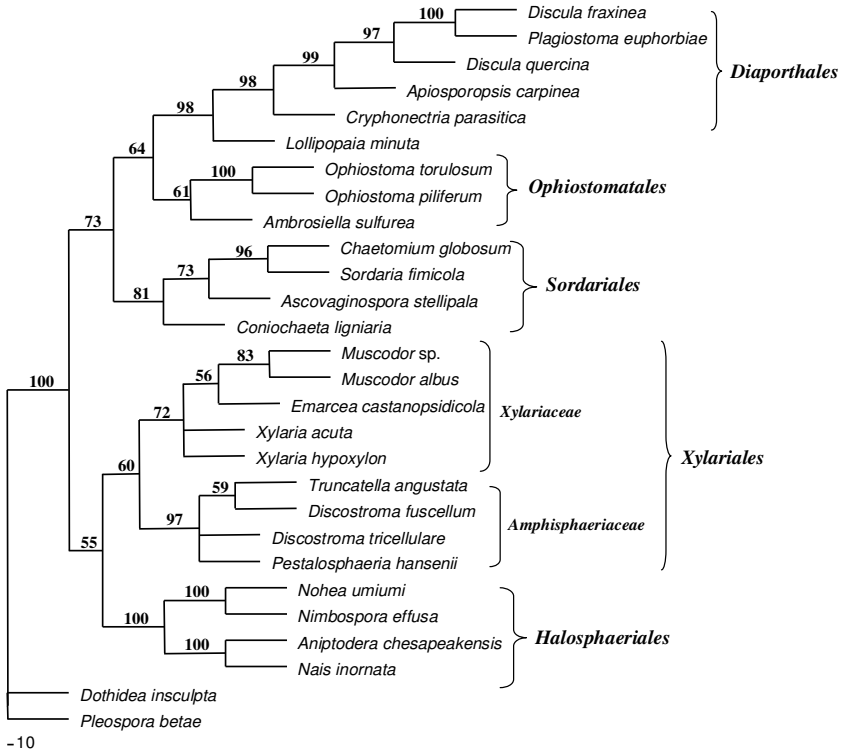


Fig. 10. Phylogenetic tree based on partial 18S DNA sequences. The tree was rooted with *Dothidea insculpta* and *Pleospora betae* and constructed under the Maximum Parsimony criterion with a transition transversion ratio of 1.5 : 1. The number at each branch point represents percentage bootstrap support calculated from 1000 replicates. Branch lengths are proportional to the numbers of nucleotide substitutions and are measured by scale bar (Bar, 10 % sequence divergence).

Trees from unweighted and weighted parsimony were significantly different from each other (Table 2). ML analyses under the HKY model with an estimated TT ratio of 1.6 and estimated shape parameter of 0.3 gave similar tree topologies (results not shown). Estimated base frequencies were as follows: A = 0.205; C = 0.261; G = 0.268; T = 0.263.

ITS (+5.8S) *dataset*: A dataset consisting of 19 taxa from *Xylaria* and *Muscodor* (*Xylariaceae*) and two species from *Diatrypaceae* (*Diatrype flavovirens* and *Cryptosphaeria ligniota*) included as outgroups were used in MP analysis. This dataset contained 600 characters (177 [29.5 %] parsimony-informative; 320 constant and 103 parsimony-uninformative). As shown in Table 2, weighted parsimony gave better tree topologies and *E. castanopsidicola* was found to have close phylogenetic affinities with other *Muscodor* species (results not shown). Other taxa were not included in the analysis as the sequences appeared to be divergent and hence could not be properly aligned.

DISCUSSION

Morphological characters, such as the ascomata being immersed beneath a clypeus, papillate ostioles and unitunicate asci with a J^+ subapical ring, indicate that *E. castanopsidicola* should be placed in *Xylariales* (*sensu* Kirk *et al*. 2001), where it could be included in the *Amphisphaeraceae* or the *Xylariaceae*. Most amphisphaeriaceous species however, have brown two-celled ascospores and *Pestalotiopsis*-like anamorphs (Barr 1994, Kang *et al.* 1998, 1999, Jeewon *et al.* 2003c). On the other hand, most taxa in the *Xylariaceae* have a well-developed stroma and unicellular brown ascospores, invariably with a germ slit and produce mostly hyphomycetous anamorphs in culture (*sensu* Kirk *et al*. 2001). In some genera, however, the stroma is reduced to a clypeus (e.g. *Anthostomella*, *Fasciatispora*) and a sporodochial *Geniculosporium* anamorph has only been determined in a handful of species (Hyde & Goh 1998, Lu & Hyde 2000). *Emarcea* has hyaline bicellular ascospores and did not produce a *Pestalotiopsis* anamorph (or any anamorph) in culture. Morphological characteristics do not clearly indicate whether *Emarcea* should be placed in *Amphisphaeriaceae* or *Xylariaceae* and therefore we decided to use rDNA sequence analysis.

In the *Xylariaceae*, *Emarcea* should be compared with *Anthostomella*. *Anthostomella* species often have ascospores with one large brown cell and one dwarf cell, e.g. *A. clypeata, A. clypeoides, A. foveolaris, A. rostrospora, A. triangularis* and *A. unguiculata* (Lu & Hyde 2000). *Anthostomella* species, however, always have brown ascospores, usually with germ slits, and this has not been observed in mature or old material of *Emarcea*.

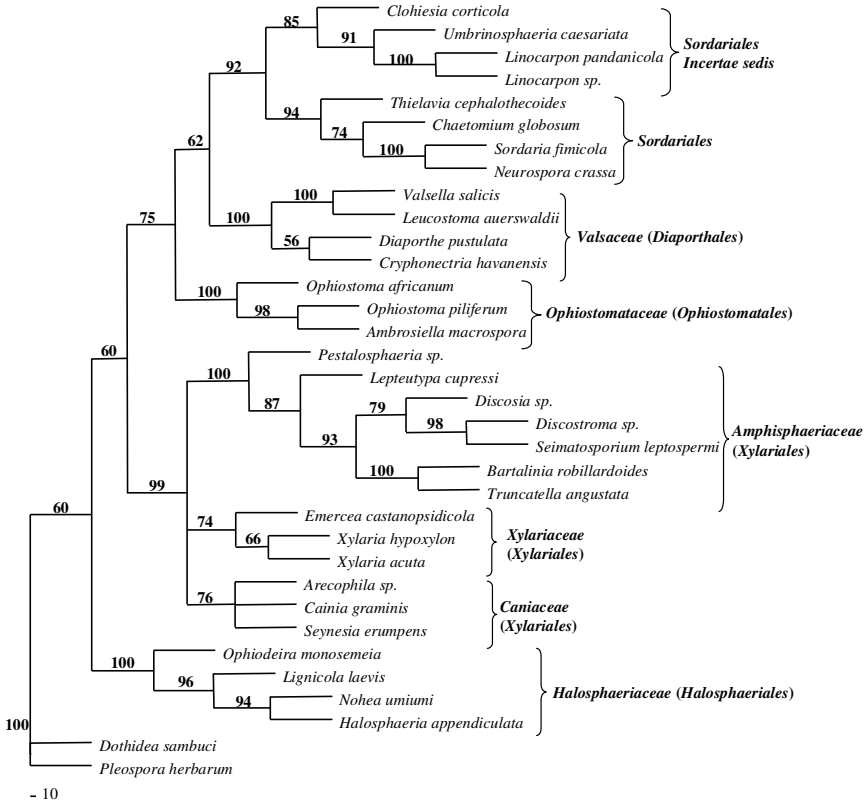


Fig. 11. Evolutionary relatedness of *Emarcea castanopsidicola* based on a Maximum Parsimony analysis of the 28S rDNA partial gene sequence with no molecular clock enforced. This tree is topologically identical to the Maximum Likelihood tree. Designated outgroups are *Pleospora herbarum* and *Dothidea sambuci*. Transition was weighted 1.5 times. Bootstrap support based on 1000 replicates for each clade shown on the branches.

Phylogenies generated using Maximum Parsimony and Maximum Likelihood from the three different datasets (18S, 28S and ITS) support the monophyly of the xylariaceous species under investigation. The small subunit dataset (18S) shows that *Emarcea castanopsidicola* is nested in a clade with *Muscodor* and *Xylaria* species. The *Xylariaceae* clade is connected to the *Amphisphaeriaceae* clade with a bootstrap support of 60 % (Fig. 10). Together these two clades constitute the *Xylariales*. This gene region (18S) evolves rather slowly (White *et al.* 1990) and provides valuable insights into the systematics of *Emarcea castanopsidicola* and its familial placement at the ordinal and familial level. Similar molecular findings were obtained from phylogenies derived from the large sub-unit (28S). *Emarcea castanopsidicola* is phylogenetically related to *Xylaria curta* and *X. hypoxylon* in the *Xylariales* (Fig. 11). The ITS dataset provides further sequence-based evidence to elucidate relationships of *Emarcea castanopsidicola* with other members of the *Xylariaceae*. *Emarcea castanopsidicola* forms a sister group to *Muscodor* species with 100 % bootstrap confidence. This is not unexpected as *Muscodor*, a recently described new anamorphic genus, bears close phylogenetic affinities to *Xylaria* (Worapong *et al.* 2001). Worapong *et al*. (2001) analysed genetic sequences of this endophytic fungus and found that partial 18S rDNA sequences and the entire ITS sequences (including 5.8S) share a high degree of homology with other *Xylaria* species. Phylogenies based on Maximum Parsimony from their study also revealed that *Muscodor* is more closely related to the *Xylariaceae* than to the *Amphisphaeriaceae*. Similar findings are reported here, and given that *Emarcea castanopsidicola* forms a sister group to *Muscodor* in all our analyses, there is no doubt that our new taxon should be accomodated in the *Xylariaceae*.

In this study, we have been quite selective in the ingroups that we used. Several xylariaceous species, however, could not be included (e.g. *Anthostomella*, which appears to have close morphological affinities to *Emarcea castanopsidicola*) because of ambiguous sequence alignment and secondly due to the high degree of polytomies that were encountered in the phylogenetic analyses, especially when the molecular dataset was subjected to bootstrap analyses. Similar results were reported by Smith *et al.* (2003). Morphological observations are therefore still very important in establishing familial placement in this group of fungi. Based on molecular results and morphological examination, there is conclusive evidence to conclude that our new taxon, *Emarcea castanopsidicola,* belongs in the *Xylariaceae*.

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