**Chaenothecopsis khayensis**, a new resinicolous calicioid fungus on African mahogany

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**Abstract:** The new species *Chaenothecopsis khayensis* (Ascomycota, Mycocaliciaceae) is described from Ghana, western Africa, on the resin of *Khaya anthotheca* and *K. ivorensis*. The species is distinctive in forming asci without crossers and in possessing ascospores that are faintly longitudinally striate. Analysis of large subunit rDNA gene sequences positioned this species within a clade corresponding to the Mycocaliciales and identified its closest relative as *Sphinctrina leucopoda*. *Chaenothecopsis khayensis* occurs commonly on resin exuding from trees damaged by the larvae of the mahogany shoot borer (*Hypsipyla* sp.), and we discuss the possible ecological relationship between the fungus and these moths.

**Key words:** exudate, insect dispersal, Mycocaliciales, resinicolous fungi

**INTRODUCTION**

Resin, consisting of phenolic and terpenoid compounds, is known to be an efficient repellent of parasitic fungi. However some fungi also are known to grow exclusively on resin substrates. Most of these fungi are hyphomycetous ascomycetes, but several species in the Mycocaliciaceae (Ascomycota) also produce their stalked ascocarps on resin.

Within the Mycocaliciaceae at least three species of *Mycocalicium* Vain. and 12 species of *Chaenothecopsis* Vain., occur exclusively on plant exudates. Most of these fungi grow on the resin and/or resin-soaked wood and bark of a single tree species or genus. Research has concentrated primarily on boreal and temperate regions, and most of the host plants have been conifers, primarily species of *Abies* Mill., *Larix* Mill., *Picea* A. Dietr., and *Tsuga* Carrière (Tibell and Titov 1995, Titov 2006). Only four species have been described from angiosperm exudates: *Mycocalicium chaudhari* Tewari & Pant from *Mangifera indica* L. in India (Tewari and Pant 1966), *Mycocalicium viscinicola* Funk & Kujt from *Tristerix longibracteatus* (Desr.) Barlow & Wiens in Equador and Peru (Funk and Kujt 1982), *Chaenothecopsis schefflerae* Samuels & Buchanan from *Schefflera digitata* J.R. Forst. & G. Forst in New Zeland (Samuels and Buchanan 1983), and *Chaenothecopsis tristis* (Körber) Titov from *Acer* L. and *Tilia* L. species in Europe (Titov 1999). However our recent findings suggested that many additional taxa have specialized to live on exudates of subtropical and tropical broadleaf trees.

Both the phylogenetic affinities of the Mycocaliciaceae and generic relationships within the family are still insufficiently understood. In a study based on ITS1–5.8S–ITS2 and LSU rDNA sequences Tibell and Vinuesa (2005) found that the Mycocaliciaceae was strongly supported but monophyletic only when the Sphinctrinaceae was included. While species in this extended family were divided into two clades that did not correspond to *Mycocalicium* and *Chaenothecopsis* in the traditional sense (Schmidt 1970), morphological characteristics such as ascospore septation were consistent with the major groups inferred in this analysis (Tibell and Vinuesa 2005).

In this paper we describe a new resinicolous species of *Chaenothecopsis* from tropical Africa. Because this taxon possessed structural features not described for members of the Mycocaliciaceae, or even of Mycocaliciales, we confirm its systematic position with molecular methods. The host plants of the novel fungus, African mahoganies (*Khaya ivorensis* A. Chev and *Khaya anthotheca* (Welw.) C. DC.), are among the most valuable tropical timber tree species of Africa. Continued supply of these trees are threatened by the overexploitation of natural forests and the difficulties in mahogany plantations due to mahogany shoot borers, especially *Hypsipyla robusta* Moore. The larvae of these moths destroy shoots, retard growth, reduce the economic value of the timber and cause abundant resin flows that seem to provide an optimal substrate for the newly found fungus. Accordingly a possible ecological relationship between the mahogany shoot borer and the new fungus is discussed briefly.

**MATERIALS AND METHODS**

**Sampling and observation.**—Field work in Ghana was conducted May 2004. Our first study site was in the southwestern part of the Subri Forest Reserve, near the...
from each reserve) were sampled from Pra Anum (Forest Reserve. An additional 60 mahogany trees (20 trees from the canopy by a climber, also were inspected. wounds and cankers. Some branches of each tree, brought for mycocalicioid fungi, with attention given to possible (not shown). The basal 2 m of each trunk were inspected and environmental parameters were recorded for each tree forests and young trees in plantation forests. Morphological numerous additional trees, including saplings in natural anthotheca ivorensis F. K. Morphological features of fungal speci- Laboratory work.—Morphological features of fungal speci-men s were observed and measured in water under a light microscope (Leica DMLS) with a 100× oil-immersion objective. Thin sections of ascocarps on resin were made with a freezing microtome (Leica CM 3050 S). To observe specific microscopic structures several reagents were used: Lugol’s, Melzer’s (MLZ), 10% potassium hydroxide (KOH), 10% nitric acid, cresyl violet and Congo red.

DNA was extracted from dry fungal specimens with the NucleoSpin Plant Kit (Macherey-Nagel, Düren) according to the manufacturer’s instructions, except that specimens were incubated 12–15 h. The nuclear large subunit ribosomal (LSU) RNA gene was amplified with primers LR0R (Rehner and Samuels 1994) and LR3 (Vilgalys and Hester 1990) as described by Niskanen et al. (2006). PCR product purification and sequencing were performed by Macrogen Inc. (Seoul, South Korea).

The phylogenetic position of the new fungus was inferred from the analysis of similar LSU gene sequences (Table I) obtained from GenBank with BLAST queries. Outgroups were chosen based on the results of Tibell and Vinuesa (2005). Sequences were aligned with Clustal W 1.8 (Thompson et al. 1994), and the alignment was corrected manually. Bayesian analyses were conducted with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Program default parameters were used to run four chains 2,000,000 generations. Trees were sampled every 100th generation. Parsimony analyses were performed with programs DNA-Penny and Seqboot (both set to default parameters) found in PHYLIP 3.61 (Felsenstein 2005).

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Chaenothecopsis khayensis Rikkenen & Tuovila sp. nov.

Supra exudatum Khaya anthotheca et Khaya ivorensis. Apothechia parva, (300–)350–650(–700) μm alta, olivacea vel nigra, epruinosa. Stipes rectus, per occasionem ramosus. Capitulum obovoides vel obconicum, (100–)120–240(–260) μm diam. Epithecium et excipulum olivaceo-viridia, bene evoluta. Excipulum et stipes hyphis periclinaler ordinatis, viridibus. Hypothecium hyalinum ad viride. Supra excipul um et summam partem stipis rete sparsum hyphis horizontaliter et verticaliter ordinatis 2 μm latis. Asci cylindrici, (46.0–)49.0–66.0(–70.0) μm alta, (3.0–)3.5–5.5 μm, 8- spori. Ascosporae asceptatae, ellipsoidales ad cylindricales,
olivaceo-cinereae, cum ornamento distincto, minuto, (6.1–) 6.9–8.8(–9.0) μm x (2.0–) 2.7–3.9(–4.2) μm.


Some specimens of *Chaenothecopsis khayensis* were found on resin that had exuded from large cuts on mahogany trunks (Fig. 3a). Because *Khaya* bark is widely used for medical purposes in Ghana many of the few remaining trees around human settlements are severely scarred. However most specimens of *C. khayensis* from natural habitats were found on the resin exuding from smaller cankers near the growing tips of canopy branches and from the stem apices of young trees. These cankers were caused by mahogany shoot borers and/or other wood-boring insects. The fungus clearly thrives in areas where *Hypsipyla* damage is severe. For example *C. khayensis* was abundant on the cankers of damaged trees in a young experimental plantation of African mahoganies in the Bobiri Forest that had been severely damaged by insects (Fig. 3b).

*Chaenothecopsis khayensis* grows on exudate and on exudate-impregnated wood and bark. The spores seem to germinate rapidly because germinating spores often were seen on ascocarp surfaces and atop fresh resin. Also small fungal primordia often were seen growing on recently accumulated and hardened resin. In thin sections the hyphae of *C. khayensis* were observed both on the surface and deep within the resin. Such hyphae appeared to be surrounded by narrow cavities, suggesting that *C. khayensis* degrades the resin and uses the exudate as a source of nutrition.

In all specimens of *C. khayensis* examined the resinous substrate also supported abundant growth of a dark brown hyphomycetous fungus; only fresh resin surfaces were without this fungus. We did not culture the fungus, and all attempts to identify the hyphomycete by direct DNA extraction and sequencing failed. Thus the phylogenetic relationship of this hyphomycete to *C. khayensis* could not be established.
DISCUSSION

Taxonomy.—Chaenothecopsis khayensis is distinguished from nearly all other species of its genus by forming asci without crosiers. Furthermore ascospores have a unique and clearly discernible type of ornamentation and no other species of Chaenothecopsis has been reported from the exudate of Khaya species or other tropical members of the Meliaceae.

While all species in the Mycocaliciales, including Chaenothecopsis, generally have been thought to have crosiers (e.g. Tibell 1999, Tibell and Wedin 2000), the original descriptions of these taxa do not actually mention their presence (Vainio 1927, Schmidt 1970). Inasmuch as C. khayensis does not produce crosiers we investigated the relationship of this species to other members of the Mycocaliciales based on the analysis of partial LSU sequences. The resulting phylogeny (Fig. 4) was consistent with the phylogeny of Tibell and Vinuesa (2005), provided strong support for inclusion of C. khayensis in the Mycocaliciales (posterior probability and jackknife values of 1.00 and 0.88 respectively) and identified its closest relative as Sphinctrina leucopoda.

Tibell and Vinuesa (2005) found that Sphinctrina Fr. was nested within genus Chaenothecopsis. Because C. khayensis does not share the morphological characters that distinguish species of Sphinctrina (e.g. a thick gelatinous coat around semimature ascospores) (Löfgren and Tibell 1979) we placed the new fungus in genus Chaenothecopsis.

Only two other Chaenothecopsis species have been described growing on angiosperm exudate. Chaenothecopsis tristis (Körber) Titov grows on exudate and bark of Acer and Tilia species. The ascospores of this species are one-septate and smooth (Titov 1999). Chaenothecopsis schefflerae, which grows on exudate and bark of Schefflera digitata, resembles C. khayensis more closely than any other species of the genus (Samuels and Buchanan 1983). Both fungi grow on angiosperm exudate, have green or greenish ascocarps and greenish ascospores with punctate ornamentation. Samuels and Buchanan (1983) reported that crosiers were not seen in C. schefflerae, so it is likely that it also lacks these structures. Additional features recorded in the fine line drawings of C. schefflerae also support a close relationship. Chaenothecopsis schefflerae differs from C. khayensis in possessing paraphyses surrounded by a gelatinous sheath.

The resinicolous species Chaenothecopsis montana Rikkinen, C. oregana Rikkinen, C. resinicola Tibell & Titov and C. tsugae Rikkinen all have nonseptate spores, but unlike C. khayensis they grow on
coniferous resin in boreal or temperate regions (Rikkinen 1999, 2003; Titov and Tibell 1993). In addition C. montana has densely branched paraphyses, C. oregana has a distinctive (pink) reaction in KOH and C. tsugae has pruina on the stalk surface. Spore size also distinguishes these species from C. khayensis.

Given that the generic delimitation of Chaenothecopsis and Mycocalicium remains problematic, two resinicolous Mycocalicium species also must be discussed here. Mycocalicium chaudhari had been found growing on exudate of Mangifera indica in India. It is characterized by short asci without thickened apices. The ascospores are light brown, smooth and more globose than in C. khayensis (Tewari and Pant 1966). Mycocalicium viscinicola grows on the exudate of mistletoe seedlings at high altitude in South America. The ascospores of this species are brown and much larger (7–11 x 5–6 μm) than those of C. khayensis, and the ascus apex is not penetrated by a canal (Funk and Kujt 1982).

Ecology and distribution.—Chaenothecopsis khayensis is the first species of resinicolous Mycocaliciaceae from tropical Africa. Most of the previously known species have been found from temperate and boreal forests, usually growing on resin of conifers. However, considering our limited current understanding of tropical ascomycetes (Hawksworth 2001, Hyde 2001) and the fact that many tropical angiosperms produce large amounts of exudate (Langenheim 2003), it is quite likely that many additional resinicolous Chaenothecopsis species will be found in future studies.

Despite the fact that Chaenothecopsis khayensis grows on both Khaya ivorensis and K. anthotheca, no morphological differences were found among the specimens collected. The climatic tolerance of the fungus is relatively wide because it has been collected from both moist evergreen forests of southern Ghana and from moist and dry semideciduous monsoon forests farther north. One could expect that the species also can grow on other Khaya species in tropical Africa.

It is possible that the activity of mahogany shoot borers and other insects attract Chaenothecopsis khayensis to fresh resin substrates that result from the activities of stem-boring insects. It also is possible that the fungus plays some role in the biology of Hylisipyla (i.e. moth larvae feed on fungal mycelia or on fungal-infected wood). While fungal hyphae and spores are observed in thin sections of the frass of Hylisipyla larvae (data not shown), it is not clear whether these hyphae and spores belong to C. khayensis or other lignicolous fungi. It also has not been determined that the larvae intentionally ingest fungal mycelia or whether hyphae are consumed when the larvae feed on plant tissue. Because of their potential importance for the development of new methods to control Hylisipyla species these and many other questions should be explored in future studies.

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