A morphologically ill-founded powdery mildew species, *Pleochaeta indica*, is recognized as a phylogenetic species based on the analysis of the nuclear ribosomal DNA sequences

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Abstract

Morphological characteristics of a powdery mildew fungus found on *Celtis australis* in the Indian Himalayas coincided with those of *Pleochaeta indica*, described from this tree species in India, as well with those of *P. shiraiana*, known to infect *C. australis* and other plant species in Asia. This suggested that the original description of *P. indica* based on morphological patterns was not well founded and this taxon could be reduced to synonymy with *P. shiraiana*. However, phylogenetic analyses of the rDNA 28S and ITS sequences determined in some Indian *Pleochaeta* specimens from *C. australis* showed that this fungus is closely related, but not identical to *P. shiraiana* infecting *C. sinensis* in Japan which served as the basis of the original description of *P. shiraiana*. Molecular clock analyses of the ITS region and that of the 28S rDNA indicated that the split between the Japanese *P. shiraiana* infecting *C. sinensis* and *Pleochaeta* sp. infecting *C. australis* in India may have occurred 8.5–2.0 million years ago in the Pliocene and may have coincided with the production of the Himalayan mountains and the global cooling of the Earth during the late Tertiary. Thus, *P. indica* is recognized in this study as a distinct phylogenetic species, although our morphological study showed that its description as a morphological species was not well founded. This is a striking example for a cryptic species which is genetically different from close relatives but cannot be distinguished from them based on morphology.
INTRODUCTION

Powdery mildew fungi belonging to the genera Pleochaeta, Leveillula and Phyllactinia are characterized by a partly endophytic mycelium consisting of hyphae that enter the host plant tissues through stomata and produce haustoria in the mesophyll cells of the plants (Braun 1987). The ectophytic part of the mycelia of these fungi is found mostly on the lower surfaces of the leaves. As all the three partly endo-parasitic genera of the Erysiphales (Pleochaeta, Leveillula and Phyllactinia) form one single monophyletic group within the Erysiphales (Mori et al. 2000a), it was suggested that endo-parasitism had evolved from ecto-parasitism only once during evolution (Mori et al. 2000a, Takamatsu 2004). This might have been the result of a xerophytic adaptation as suggested by Blumer (1933) and Braun (1987) in earlier works (see Takamatsu 2004).

Pleochaeta is the most ancestral genus of this group (Mori et al. 2000a, Takamatsu 2004). Therefore, its study could help to understand the evolution of endo-parasitism within the Erysiphales. Only five species of Pleochaeta are described in Braun (1987). Two of these, P. shiraiana and P. salicicola, are known from Asia, while P. polychaeta, P. turbinata (syn. Queirozia turbinata), and P. prosopidis are all found in North and South America. More recently, another species, P. indica, was described in India as a pathogen of Celtis australis (Ahmad et al. 1995). However, none of the six Pleochaeta species have been studied in detail so far, except the morphology of their anamorph stage, Streptopodium (Liberato et al. 2004). In particular, little is known on the molecular phylogeny of Pleochaeta spp. as there are only a very few DNA sequences published for this genus (Hirata and Takamatsu 1996, Mori et al. 2000a). In contrast, other, more widespread powdery mildew genera have already been intensively studied with molecular tools (e.g., Hirata et al. 2000, Matsuda and Takamatsu 2003, Wyand and Brown 2003, Hirose et al. 2005).
In July 2004, symptoms of a powdery mildew infection were observed on the adaxial parts of the leaves of a few *C. australis* trees in Kullu, Himachal Pradesh state (HP), India. By late August, the disease became widespread in the region and a microscopic examination of the causal agent identified it as a *Streptopodium* sp. which is the anamorph stage of *Pleochaeta* spp. (Braun 1987, Liberato et al. 2004). The ascomata of the pathogen were also found on the adaxial parts of the leaves later in the season. As *P. indica* was described in the same region of India, and from the same host plant (Ahmad et al. 1995), the fungus was tentatively identified as belonging to this species and a comprehensive study was carried out to (i) compare the morphological characteristics of its anamorph and teleomorph with the morphological patterns of other *Pleochaeta* species, (ii) determine the nucleotide sequences of both the internal transcribed spacer (ITS) region and the D1 and D2 domains of the 28S gene of the nuclear ribosomal DNA (rDNA) in this fungus, and (iii) reveal its phylogenetic relationships with other powdery mildew fungi based on the analysis of ITS and partial 28S rDNA sequences.

**MATERIALS AND METHODS**

**Fungal materials**

*Celtis australis* leaves infected with powdery mildew (Figs. 1-3) were collected in and around Kullu, HP, India, many times in summer and autumn 2004. Herbarium materials were deposited at Mie University Mycological Herbarium (MUMH), Tsu, Japan and at the Herbarium of Martin Luther University, Halle, Germany (HAL) under the accession numbers MUMH 3208 and HAL 1888 F and HAL 1889 F, respectively.
**Light microscopy of fresh and herbarium materials**

Fresh powdery mildew mycelia scraped off the leaves before and after the production of the ascomata were examined under a light microscope in India to examine the morphological patterns of both the anamorph and the teleomorph stages. Also in India, fresh conidia were germinated on microscope slides to describe the morphology of the germ tubes. For this purpose, slides with fresh conidia were placed in plates containing wet filter paper, incubated for 48-72 hours at room temperature, and then examined under a light microscope. In addition, herbarium materials were sent to Hungary, Japan and Germany for further studies. These were re-hydrated in boiling lactic acid, as described by Shin and La (1993), before morphological studies.

**DNA extraction, PCR amplification and DNA sequencing**

In Japan, whole-cell DNA was extracted from mycelia of the anamorph stage found on leaves collected in India on 9 August 2004 (herbarium accession no. MUMH 3208). DNA isolation was performed using the chelex method (Walsh *et al.* 1991) as described in Hirata and Takamatsu (1996). The rDNA ITS region, and the 5’ end of the 28S rDNA, including D1 and D2 regions, were separately amplified two times by polymerase chain reaction (PCR) using nested primer sets, and then sequenced using direct sequencing, as described in Matsuda and Takamatsu (2003) for other powdery mildews.

In Hungary, DNA was extracted from ascomata coming from different leaf samples collected in India between 25 August – 10 September 2004 (herbarium accession nos. HAL 1888 F and HAL 1889 F). DNA extraction was performed using a Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Several whole-cell DNA samples were prepared, each coming from 20-25 ascomata collected from the same leaf. The ITS region was
amplified using the ITS1F/ITS4 primer pair (Gardes and Bruns 1993), and then sequenced in a total of three samples, as described in Szentiványi et al. (2005) for *Ampelomyces*.

**Molecular phylogenetic analyses**

The sequences were initially aligned using the Clustal X package (Thompson et al. 1997). The alignments were then improved visually with a word processing program with colour-coded nucleotides and deposited in TreeBASE (http://www.treebase.org/) under the accession number SN2597. Phylogenetic trees were obtained from the data by the maximum-parsimony (MP) method using the heuristic search option of PAUP* 4.0b8 (Swofford 2001). This search was repeated 100 times with different random starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. Transversions and transitions were treated as equal weight. All sites were treated as unordered, with gaps treated as missing data. The branch-swapping algorithm was the **tree bisection and reconstruction (TBR) method**, the ‘save multiple trees’ (MulTrees) option was in effect, and zero-length branches were collapsed.

The strength of the internal branches from the resulting trees was tested by bootstrap analysis (Felsenstein 1985) using 1000 replications. Molecular clocks for rDNA ITS and partial 28S sequences (Takamatsu and Matsuda 2004) were used to estimate the time when different *Pleochaeta* taxa had diverged from each other during the evolution of the Erysiphales.
RESULTS

Microscopic observations

In July 2004, when the first symptoms appeared, only a few small powdery mildew colonies were found on the adaxial surfaces of *C. australis* leaves (Fig. 1). Later, these colonies spread on the leaves, coalesced, and finally the whole adaxial leaf surfaces were covered by mycelia (Fig. 2).

Microscopic examination of the pathogen at the beginning of the epidemic revealed that it is a powdery mildew anamorph belonging to the genus *Streptopodium*. The 180-245 µm long, 3-7 celled conidiophores (Fig. 4) arose from an ectophytic mycelium and produced two types of conidia. The 45-68 x 10-15 µm large primary conidia were lanceolate and apically pointed (Fig. 6) while the 45-78 x 8-18 µm large secondary ones were ellipsoid-cylindrical (Fig. 7). The foot cells of the conidiophores were spirally twisted (Fig. 4). Basal septa of the foot cells were displaced at a distance of 8-15 µm from the point of branching. Hyphal appressoria were lobed, opposite or spread along the hyphae. Thus, the morphological patterns of the powdery mildew anamorph found by us on *C. australis* were in agreement with both the original description of *Streptopodium* (Zheng and Chen 1978) and the recently proposed amended circumscription of this anamorph genus (Liberato *et al.* 2004) focusing on the conidiogenesis and shape of conidia, as already suggested by Gorter and Eicker (1983) and Braun (1987).

Both primary and secondary conidia produced one or two germ tubes, arising from the ends of conidia, or sub-terminally, terminating in lobed to multi-lobed, or sometimes simple, apexes (Figs. 6-8). The length of the germ tubes was highly variable...
after conidia have germinated on microscope slides. The first germ tubes, one or two for each conidium, appeared 5-6 hours after incubation. Some of them remained curt, shorter than the conidial length (Figs. 6, 7), or approx. as long as the conidia (Fig. 8), even 48-72 hours after incubation, while others continued their growth and produced branched and septate hyphae, sometimes 2-2.5 times as long as the conidial length, on microscope slides. The production of branched and septate primary mycelia on a substrate other than the host plant surface is an uncommon phenomenon in powdery mildew fungi.

Mature ascomata appeared in the powdery mildew mycelia at the end of August (Fig. 3). Their microscopic examination revealed that the teleomorph belonged to the genus *Pleochaeta*. They were 194-311 μm diameter and bore numerous (>150) hyaline appendages which were shorter than the ascomal diameter and terminated in uncinate-circinate tips (Fig. 5). Ascomata contained 23-31 stalked asci, 57-100 x 25-36 μm, with 2-4 ascospores measuring 17-30 x 13-20 μm (Figs. 9, 10). Yellowish oil droplets were present both inside and outside the ascospores in the asci and did not disappear when herbarium materials were re-hydrated in boiling lactic acid on microscope slides (Figs. 9, 10).

Intracellular pycnidia of *Ampelomyces*, common mycoparasites of powdery mildews worldwide (Kiss *et al.* 2004), were found in abundance in both conidiophores and immature ascomata of the fungus, especially on heavily infected *Celtis* leaves.

The morphological characteristics of the powdery mildew pathogen found on *C. australis* in India corresponded to those described for *P. indica* by Ahmad *et al.* (1995) as well as to those reported for *P. shiraiana* by Homma (1937), Braun (1987) and Nomura (1997) because these two species cannot be clearly distinguished from each other based on either morphological or host range data (see the Discussion). In order to
get more insights on the identity of this powdery mildew fungus, we have compared its rDNA ITS and partial 28S sequences with those of other closely related fungi during molecular phylogenetic analyses.

**Molecular phylogenetic studies**

**28S rDNA analysis**

We determined a 1402 bp long sequence comprising 5'-end of the 18S rDNA, 5.8S rDNA, the domains D1 and D2 of the 28S rDNA, and the two ITS regions of the *Pleochaeta* specimen MUMH3208. This was deposited in DDBJ under the accession number AB243757. The sequence of the domains D1 and D2 of the 28S rDNA was 99.6% similar to that of *P. shiraiana* collected from *C. sinensis* var. *japonica* in Japan (AB022403, Mori et al. 2000a) and 94.7% with that of *P. turbinata* (syn. *Queirozia turbinata*) collected from *Platycyamus regnellii* in Brazil (AB218773, Liberato et al. 2006).

In order to clarify the phylogenetic relationships of the *Pleochaeta* sp. collected from *C. australis* in India, nucleotide sequences of the D1 and D2 domains of 28S rDNA from 40 taxa covering all five tribes of the Erysiphaceae, and, in addition, the genera *Parauncinula* (Takamatsu et al. 2005a), *Caespitotheca* (Takamatsu et al. 2005b) and *Oidium* subgenus *Microdium* (To-anun et al. 2004), were retrieved from DNA database and used for the phylogenetic analyses. The alignment data matrix consisted of 42 taxa and 825 characters, in which 254 sites were variable and 174 sites were phylogenetically informative for parsimony analysis. The partial 28S rDNA sequence of *Byssosascus striatosporus* (U17912) was used as an outgroup taxon based on Mori et al. (2000a,b).
Twelve equally most parsimonious trees with 814 steps were generated by the MP analysis which differed in only minor branching orders of terminal taxa. A tree having the highest likelihood scores among the 12 trees is shown in Fig. 11. The five tribes, viz. the Erysipheae, Golovinomycetceae, Phyllactinieae, Cystotheceae and Blumerieae, recognized in the Erysiphales (Cook et al. 1997, Braun 1999, Braun and Takamatsu 2000, Mori et al. 2000a) were again supported, although the tribe Golovinomycetceae was paraphyletic. Oidium subgenus Microidium, proposed by Tanun et al. (2004) as a new subgenus of the mitosporic powdery mildew genus Oidium, formed an independent clade which was clearly separated from the other five tribes. The basal position, and, thus, the presumed ancestral origin of two recently proposed genera, Parauncinula and Caespitotheca (Takamatsu et al. 2005a,b), was also supported by this analysis. The Pleochaeta sp. from India grouped with P. shiraiana collected in Japan with high bootstrap support (99%). Then, this group further clustered with P. turbinata with low bootstrap support (54%) to form a clade of the genus Pleochaeta.

**ITS analysis**

We have determined the rDNA ITS sequences in a total of four Indian Pleochaeta specimens. In addition to the ITS sequence determined in the herbarium specimen MUMH 3208, and deposited under the accession number AB243757, three other ITS sequences were determined from DNA samples extracted from ascomata collected from three different leaves coming from herbarium materials deposited under the accession numbers HAL 1888 F and HAL 1889 F. DNA database accession numbers of these sequences are DQ374647, DQ374648 and DQ374649, respectively. All the four ITS sequences obtained were identical and were 548 bp long: 223 bp in ITS1 region,
171 bp in ITS2 region and 154 bp in the 5.8S rDNA. Only three more ITS sequences are available in public DNA databases for *Pleochaeta*, two for *P. shiraiana* collected from *C. sinensis* in Japan (D84380 and D84381, Hirata and Takamatsu 1996) and one for *P. turbinata* collected from *Platycyamus regnellii* in Brazil (AB218773, Liberato et al. 2006). The ITS regions, including the 5.8S rDNA, were 534 bp long in *P. shiraiana* and 575 bp long in *P. turbinata*. These values differ from the ITS sequence determined by us in the four Indian *Pleochaeta* specimens in 14 bp and 27 bp, respectively. The Indian *Pleochaeta* ITS sequence was 93.3% similar to that of *P. shiraiana* and only 75.1% similar to that of *P. turbinata*. We did not use the *Pleochaeta* ITS sequences for a comprehensive phylogenetic analysis of many taxa of the Erysiphales, because they were too variable to align them unambiguously with ITS sequences of other powdery mildew taxa. Thus, these *Pleochaeta* sequences were used in a simple analysis that included the ITS sequences of *Phyllactinia kakicola* (AB000937), *Ph. moricola* (U84385) and *Leveillula taurica* (AB000940) as outgroup taxa. The total length of the alignment was 655 characters, of which 142 characters were removed from subsequent analyses because of ambiguous alignment. Of the 513 remaining characters, 171 sites were variable and 103 sites were phylogenetically informative for parsimony analysis. The parsimony analysis generated two equally most parsimonious trees of 233 steps with identical topology and slightly different branch length. A tree with higher likelihood value is shown in Fig. 12. Although *Pleochaeta* sp. from India groups with *P. shiraiana* with a bootstrap support of 100%, the two taxa formed two distinct clades. They further grouped with *P. turbinata* with 100% bootstrap support.

**Molecular clock analyses**
In order to estimate the divergence time of the *Pleochaeta* spp. included in our analysis, we used the molecular clocks of ITS and the 28S rDNA (Takamatsu and Matsuda, 2004). The genetic distances between *P. shiraiana* collected in Japan and the Indian *Pleochaeta* sp., calculated by the Kimura 2-parameter criterion (Kimura 1980), were 0.04281 for ITS and 0.00261 for 28S rDNA. Based on the molecular clock of the ITS region (2.52 x 10^{-9} substitutions per site per year) and that of the 28S rDNA (6.5 x 10^{-10} substitutions per site per year), provided by Takamatsu and Matsuda (2004), the split between the Japanese *P. shiraiana* infecting *C. sinensis* and *Pleochaeta* sp. infecting *C. australis* in India may have occurred 8.5–2.0 million years (myr) ago in the Pliocene.

The genetic distance between *P. turbinata* and the *P. shiraiana/Pleochaeta* sp. clade is 0.04809. Based on the molecular clock of the 28S rDNA, the split between *P. turbinata* and the *P. shiraiana/Pleochaeta* sp. clade may have occurred 37 myr ago.

**DISCUSSION**

The species concept in *Pleochaeta* is not well defined, which is not surprising for a little studied genus. For example, *P. indica*, described on *C. australis* in Northern India, was distinguished from *P. shiraiana* based on differences in both the size of ascomata, asci and ascospores, and the number of asci in ascomata (Ahmad *et al.* 1995). *Pleochaeta shiraiana* was also reported to occur on *C. australis*, as well as on other *Celtis* spp., in India and in other parts of Asia and South Africa (Kimbrough and Korf 1963, Gorter & Eicker 1983, Amano 1986, Zheng and Chen 1978, Braun 1987). According to Ahmad *et al.* (1995), ascomata are 240-285(-300) µm in *P. indica* and contain 10-30(-35) asci,
which they considered to be smaller than those in *P. shiraiana*. However, these values, similar to the sizes of ascospores, do not differ, in fact, from those indicated for *P. shiraiana* by Homma (1937) (ascomata 196-322 µm diameter with 20-40 asci), Braun (1987) (ascomata 200-400 µm diameter with 20-60 asci) and Nomura (1997) (ascomata 190-315 µm diameter with 20-40 asci). Also, the size of ascomata, asci and ascospores, and the number of asci per ascoma in *P. indica* (Ahmad et al. 1995) are not very different from what we have determined for the powdery mildew pathogen found on *C. australis* in Himachal Pradesh state in northern India. In fact, *P. indica* was described from *C. australis* in the same region of northern India, from Uttar Pradesh (UP) state (Ahmad et al. 1995). Thus, it is likely that the *Pleochaeta* sp. found by us on *C. australis* in the Indian Himalayas represents the same taxon as the specimens collected by Ahmad et al. (1995) and described as *P. indica*.

Unfortunately, our efforts to receive the holotype of *P. indica* from Herbarium Cryptogamae Indiae Orientalis (HCIO), Indian Agricultural Research Institute, New Delhi, or any other materials examined by Ahmad et al. (1995) failed. Thus, we could not compare either the morphology or the rDNA sequences of any authentic *P. indica* specimens with those of the *Pleochaeta* sp. described in this study. However, it is clear that *P. indica* cannot be distinguished from *P. shiraiana* based on the morphological data reported by Ahmad et al. (1995) as these are almost alike in all aspects to those given by Homma (1937), Kimbrough & Korf (1963), Braun (1987) and Nomura (1997) for *P. shiraiana*. Thus, the description of *P. indica* is questionable even in the absence of the holotype or any other authentic collections. Also, it is likely that the fungus found by us in HP, India, belongs to the same fungal taxon as the specimens described as *P. indica* because their host plants are identical and their places of collection are close to each other. This fungus can be identified as either *P. indica* or *P. shiraiana* when its
morphology is compared with published data. In consequence, based on morphological data, *P. indica* seems to be an erroneously described fungal species and it could be reduced to synonymy with *P. shiraiana*, which is the oldest valid name for this taxon (Kimbrough and Korf 1963).

However, the low sequence similarity (93.3%) between the ITS sequence of *P. shiraiana*, determined in two specimens collected from *C. sinensis* var. *japonica* in Japan (Hirata and Takamatsu 1996), and that of the *Pleochaeta* sp. found by us in India, is an interesting result, and might suggest that the *Pleochaeta* population infecting *C. australis* in the Indian Himalayas had split up with the *P. shiraiana* population found on *C. sinensis* in Japan a long time ago. Molecular clock of the ITS and 28S rDNA regions showed that the split between the Indian and the Japanese *Pleochaeta* populations might had occurred 8.5-2.0 myr ago in the Pliocene. This result suggests that the split between the two *Pleochaeta* populations coincided with the formation of the Himalayan mountains and the global cooling of the Earth during the late Tertiary. This would mean that the two *Pleochaeta* populations could be considered as genetically isolated lineages as, most probably, gene flow did not occur between these two populations for millions of years.

The host plant species of the Japanese *P. shiraiana* population and that of the Indian *Pleochaeta* population are different. This might have also contributed to their isolation. Indeed, Giraud *et al.* (2006) have recently shown theoretically that in many plant pathogens specialization to different host plant species may be sufficient to cause genetic isolation without other mechanisms of reproductive isolation. Giraud *et al.* (2006) have therefore argued that the use of the Phylogenetic Species Concept (PSC) is more appropriate than that of the common Morphological Species Concept (MSC) or the Biological Species Concept (BSC) for such fungal plant parasites. In fact, many
morphological species, described and routinely identified based on morphological criteria only, consist, in fact, of several genetically isolated lineages that are often specialized to different host plant species (Le Gac and Giraud, unpublished). *Par excellence*, many powdery mildew species with wide host ranges, described based on the MSC, consist of phylogenetically distinct lineages that infect only a few host plant species or sometimes only a few varieties of a single host plant species. This was shown in *Podosphaera* section *Sphaerotheca* (Hirata *et al.* 2000), *Golovinomyces* (Matsuda and Takamatsu 2003), *Blumeria graminis* (Wyand and Brown 2003) and *Sawadaea* (Hirose *et al.* 2005). Consequently, the molecular differences between the Japanese and Indian *Pleochaeta* pathogens could be explained based on both isolation by distance, suggested by molecular clocks of the ITS and 28S sequences, and isolation by host, suggested by the difference in the host plant species of the two taxa. However, host specialization of *Pleochaeta* spp. has never been studied. Thus, we do not know whether the Japanese and Indian *Pleochaeta* pathogens infect host plants other than those on which they were found.

The search for well-founded, useful species concepts in fungi is a very complex and complicated problem. DNA sequence analyses, above all those of rDNA ITS data, could be useful tools for more precise circumscriptions of most species. These molecular approaches have, however, their own limitations similar to the morphological methods. Apparent morphological differences between closely allied species, together with ITS similarities below 98–99%, and, in addition, obvious ecological distinctions, i.e. different hosts in plant pathogenic fungi, clearly indicate separate species. The most complicated and taxonomically challenging dilemmas are raised by morphologically indistinguishable, but genetically obviously different taxa, such as *P. shiraiana* and *P. indica*. Such taxa are often considered cryptic or functional species.
The taxonomic value of the molecular differences found between the two fungal populations of *Pleochaeta* cannot be evaluated until more ITS, 28S and other DNA sequences are available for species of this genus worldwide. In this study, we have analyzed all available ITS and 28S sequences, in addition to those determined by us, but these data are not sufficient for a meaningful evaluation of the molecular differences among *Pleochaeta* specimens. Our analyses have shown that the Indian and Japanese *Pleochaeta* species are closely related to each other. In fact, the closest relative of the Indian *Pleochaeta* sp. is *P. shiraiana* from Japan based on the analyses of both 28S and ITS sequence data (Figs. 11, 12). However, until more detailed works, we suggest to retain the name *P. indica* for the powdery mildew pathogen infecting *C. australis* in northern India because all our phylogenetic analyses, including the molecular clocks for ITS and 28S sequences, have shown that it had long diverged from the Japanese *Pleochaeta* infecting *C. sinensis* var. *japonica* which served as the basis of the description of *P. shiraiana* (Kimbrough and Korf 1963). Thus, *P. indica* is recognized in this study as a distinct phylogenetic species, although our morphological study has shown that its description as a morphological species (Ahmad *et al.* 1995) was not well founded. This is a striking example for a cryptic species which is genetically different from close relatives but cannot be distinguished from them based on morphology.

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FIGURE LEGEND

Figs. 1-10. *Pleochaeta indica* on *Celtis australis*. Figs. 1-2. Mycelia of the anamorph, a *Streptopodium* sp., on the adaxial leaf surface. Fig. 3. Ascomata of the fungus on the adaxial leaf surface. Fig. 4. A conidiophore of the anamorph producing a primary conidium. Fig. 5. Appendages of an ascoma. Fig. 6. A germinating primary conidium. Fig. 7. A germinating secondary conidium producing a short germ tube with a multi-lobed apex. Fig. 8. A germinating secondary conidium developing a longer germ tube with a lobed apex. Fig. 9. An ascus with two ascospores. Note the yellowish oil droplets apparent both inside and outside the ascospores. Fig. 10. An ascus with four ascospores. Bars equal 50 µm in figs. 4 and 5 and 10 µm in figs. 6-10.

Fig. 11. Phylogenetic analysis based on the 5' end of the 28S rDNA sequences for *Pleochaeta* sp. on *Celtis australis* and 40 taxa of the *Erysiphales* covering all known tribes, and an outgroup taxon. The tree is a phylogram of one of the 12 most parsimonious trees with 814 steps, which was found using a heuristic search employing
100 times random stepwise addition option of PAUP* treated gaps as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. The bootstrap values of 1000 replications are shown above or below nodes.

Fig. 12. Phylogenetic analysis based on the nucleotide sequences of the ITS region for Pleochaeta sp. on Celtis australis and two other Pleochaeta species, and three outgroup taxa. The tree is a phylogram of one of the two most parsimonious trees with 233 steps, which was found using a heuristic search employing 100 times random stepwise addition option of PAUP* treated gaps as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. The bootstrap values of 1000 replications are shown above or below nodes.
28S rDNA
42 taxa; 825 characters
814 steps
Cl = 0.4496
RI = 0.6809
RC = 0.3062
ITS
10 taxa; 513 characters
233 steps
CI = 0.9099
RI = 0.8852
RC = 0.8055

Pleochaeta sp. A1 (HAL1888 F)
Pleochaeta sp. A2 (HAL1888 F)
Pleochaeta sp. A3 (HAL1889 F)
Pleochaeta sp. M1 (MUMH3208)
Pleochaeta shiraiana D84380
Pleochaeta shiraiana D84381
Pleochaeta turbinata AB218773
Phyllactinia kakicola AB000937
Phyllactinia moricola D84385
Leveillula taurica AB000940

10 changes