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Molecular phylogeny and evolution of the genus *Neoerysiphe* (*Erysiphaceae*, *Ascomycota*)

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ABSTRACT

The genus *Neoerysiphe* belongs to the tribe *Golovinomyceteae* of the *Erysiphaceae* together with the genera *Arthrocladiella* and *Golovinomyces*. This is a relatively small genus, comprising only six species, and having *ca* 300 species from six plant families as hosts. To investigate the molecular phylogeny and evolution of the genus, we determined the nucleotide sequences of the rDNA ITS regions and the divergent domains D1 and D2 of the 28S rDNA. The 30 ITS sequences from *Neoerysiphe* are divided into three monophyletic groups that are represented by their host families. Groups 1 and 3 consist of *N. galeopsidis* from *Lamiaceae* and *N. galii* from *Rubiaceae*, respectively, and the genetic diversity within each group is extremely low. Group 2 is represented by *N. cumminsiana* from *Asteraceae*. This group also includes *Oidium baccharidis*, *O. maquii*, and *Oidium* spp. from *Galinsoga* (*Asteraceae*) and *Aloysia* (*Verbenaceae*), and is further divided into four subgroups. *N. galeopsidis* is distributed worldwide, but is especially common in western Eurasia from Central Asia to Europe. *N. galii* is also common in western Eurasia. In contrast, the specimens of group 2 were all collected in the New World, except for one specimen that was collected in Japan; this may indicate a close relationship of group 2 with the New World. Molecular clock calibration demonstrated that *Neoerysiphe* split from other genera of the *Erysiphaceae ca* 35–45 M years ago (Mya), and that the three groups of *Neoerysiphe* diverged between 10 and 15 Mya, in the Miocene. *Aloysia citriodora* is a new host for the *Erysiphaceae* and the fungus on this plant is described as *O. aloysiae* sp. nov.

Introduction

The *Erysiphaceae* are a group of obligately biotrophic fungi that cause powdery mildew disease on about 10K angiosperm species (Amano 1986), and consists of 15–16 genera and *ca* 650 species (Braun & Takamatsu 2000; Braun *et al.* 2002; Takamatsu *et al.* 2005a, b; Liberato *et al.* 2006). The host range of this fungal group is strictly restricted to angiosperms and the fungi have never been reported to infect ferns or gymnosperms (Amano 1986). Molecular phylogenetic analyses demonstrated that the *Erysiphaceae* form a distinct monophyletic group (Mori *et al.* 2000b; Lutzoni *et al.* 2004; Takamatsu 2004;

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Wang et al. 2007). Thus, the *Erysiphaceae* are derived from a single ancestral taxon that may have acquired parasitism to plants just once. Molecular clock calibration suggested that the fungi originated in the late Cretaceous (Mori et al. 2000b; Takamatsu & Matsuda 2004), which is consistent with the hypothesis of Heluta (1992) and the fact that their host range is restricted to angiosperms. Molecular clock analysis also demonstrated that the first explosive radiation occurred within a short period near the Cretaceous/Tertiary boundary, resulting in the divergence of the five tribes of the *Erysiphaceae*, i.e. the tribes *Blumerieae*, *Cystotheceae*, *Erysipheae*, *Golovinomyceteae*, and *Phyllactinieae* (Takamatsu & Matsuda 2004). Both tree-parasitic and herb-parasitic fungi are included in three of the five tribes, i.e. tribes *Cystotheceae*, *Erysipheae*, and *Phyllactinieae*. Tree-parasitic fungi usually take basal positions in the respective tribes concerned and herb-parasitic fungi have derived positions. These results, as well as the fact that the most basal genera of the *Erysiphaceae*, i.e. *Parauncinula* and *Caespitotheca*, infect trees, suggest that tree parasitism is ancestral in the *Erysiphaceae* (Mori et al. 2000a). Host jumps from trees to herbs may have occurred multiple times during the Tertiary (Takamatsu 2004). Accompanying the host jumps, convergence of the morphology of chasmothecial appendages has occurred, resulting in a simple, mycelioid type, probably due to adaptation of their life cycles to herbs (Mori et al. 2000a; Takamatsu 2004). These multiple-time host expansions from trees to herbs may be evolutionary key events for the respective tribes of the *Erysiphaceae*.

Previously, the genus *Neoerysiphe* was classified as section *Galeopsidis* within the genus *Erysiphe* s. lat. Because molecular phylogenetic analyses revealed that *Erysiphe* s. lat. is polyphyletic (Takamatsu et al. 1998; Saenz & Taylor 1999), the sections *Galeopsidis*, *Erysiphe*, and *Golovinomyces* have been raised up to generic rank (Heluta 1988; Braun 1999). The genus *Neoerysiphe* belongs to the tribe *Golovinomyceteae* together with the genera *Arthrocladiella* and *Golovinomyces*. These three genera share a common anamorph type characterized by having catenate conidia without distinct fibrosin bodies. *Arthrocladiella* is a monotypic genus containing a single species with *Lycium* (*Solanaceae*) as the host genus (Braun 1987, 1995). In contrast, *Golovinomyces*, composed of 27 species and seven varieties, is a large genus having 2283 host species covering 58 plant families (Amano 1986; Braun 1987, 1995). *Neoerysiphe* is a relatively small genus, comprising six species and having *ca* 300 species from six plant families as hosts (Amano 1986; Braun 1999; Bahcecioglu et al. 2006). The host range of the tribe *Golovinomyceteae* is mostly restricted to herbs, except for *Lycium*, the host genus of *Arthrocladiella*.

Matsuda & Takamatsu (2003) conducted a comprehensive phylogenetic analysis of *Golovinomyces* to investigate the genetic diversity and host expansion process that occurred in this genus. These authors suggested that the ancestor of *Golovinomyces* acquired parasitism to the *Asteraceae* first, diverged with the tribes of the plant family, and then jumped to other plant families from the tribe *Lactuceae* of the *Asteraceae*. Further analyses, integrating specimens from South America, demonstrated that the ancestor of *Golovinomyces* first infected the *Asteraceae* after the migration of *Asteraceae* to the Northern Hemisphere from South America (Takamatsu et al. 2006). This suggests a Northern Hemisphere origin of *Golovinomyces*.

In contrast, there is no comprehensive phylogenetic study of *Neoerysiphe* to date. A comparative phylogenetic analysis of *Neoerysiphe* together with *Golovinomyces* is expected to reveal the host expansion route in herbaceous plants that occurred in the tribe *Golovinomyceteae* and the *Erysiphaceae*. In this study, nucleotide sequences of the rDNA ITS regions and the divergent domains D1 and D2 of the 28S rDNA of *Neoerysiphe* were determined and used for phylogenetic analyses. The aims of this study were to investigate the phylogenetic placement of *Neoerysiphe* within the *Erysiphaceae*, the phylogenetic structure within *Neoerysiphe*, and to consider evolutionary relationships of *Neoerysiphe* with their hosts.

Materials and methods

Morphological study

The morphological characteristics were examined using dried herbarium material. The lactofuchsin boiling method (Shin 2000) was used to restore the shrivelled structures of dried specimens. Observations and drawings were made using a light microscope equipped with a micrometer and drawing camera at ×400 and ×1000 magnifications.

DNA extraction and amplification

Sources of powdery mildew specimens used for the molecular analyses and the database accession numbers of their DNA sequences are listed in Table 1. Whole-cell DNA was isolated from chasmothecia or mycelia using the chelex method (Walsh et al. 1991; Hirata & Takamatsu 1996). The ITS region, including 5.8S rDNA, and the 5′ end of 28S rDNA, including the variable domains D1 and D2, were separately amplified by two sequential PCR reactions using partially nested primer sets. PCR reactions were conducted with TaKaRa Taq DNA polymerase (TaKaRa, Tokyo) in a TP-400 thermal cycler (TaKaRa) under the following thermal cycling conditions: an initial denaturing step for 2 min at 95 °C, thermocycling for 30 cycles where each cycle consisted of 30 s at 95 °C, followed by 30 s at 52 °C for annealing, and 30 s at 72 °C for extension, and a final extension cycle of 7 min at 72 °C. A negative control that lacked template DNA was included for each set of reactions. The PCR product was subjected to electrophoresis in a 1.5 % agarose gel in TAE buffer, and the DNA product of each amplification was then excised from the ethidium bromide-stained gel and purified using the JETSORB Kit (Genomed, Oeynhausen) as per the manufacturer's protocol. Nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in a DNA sequencer CEQ2000XL (Beckman Coulter, Fullerton, CA). The sequence reactions were conducted using the CEQ Dye Terminator Cycle Sequencing Kit (Beckman Coulter) according to the manufacturer's instructions.

For amplification of the ITS region, primers ITS5 (White et al. 1990) and P3 (Kusaba & Tsuge 1995) were used for the first amplification. One microlitre of the first reaction mixture was used for the second amplification with the partially nested