Int. J. Environ. Res., 5(2):509-514, Spring 2011
ISSN: 1735-6865

The phylogenetic placement of *Picoa*, with a first report on *Picoa lefebvrei* (Pat.) Maire *(=Phaeangium lefebvrei*) from Iran

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Received 12 Aug. 2010; Revised 14 Dec. 2010; Accepted 25 Dec. 2010

ABSTRACT: Desert truffles, hypogeous Pezizales (Ascomycota), are difficult to identify due to evolutionary convergence of morphological characters among taxa that share a similar habitat and mode of spore dispersal. In this paper we document the presence of *Picoa lefebvrei* (Pat.) Maire *(=Phaeangium lefebvrei*) in Iran and use phylogenetic analysis of the ITS and 28s rDNA to show that this species belongs to the *Geopora-Tricharina* clade of the Pyronemataceae (Pezizales, Ascomycota). *Picoa lefebvrei* was originally placed in the genus *Phaeangium* because of notable spore ornamentation not present in other species of *Picoa*. However, our analyses of both phylogenetic and morphological data suggest that *P. lefebvrei* is closely related to *Picoa juniperi* Vittad, the type species for the genus *Picoa*.

Key words: Desert truffles, *Phaeangium*, Molecular identification, *Picoa* sp

INTRODUCTION

Paying attention to the bioassays have been remarkably increased during recent years (Cherian and Jayachandran, 2009, Murugan *et al.*, 2009, Rajesh Kannan *et al.*, 2010, Rajashekara Murthy *et al.*, 2010; Gonthier *et al.*, 2010; Adeyemi, 2009; Ma *et al.*, 2010; Resmi *et al.*, 2010; Sahmoune *et al.*, 2009; Shetty and Rajkumar, 2009) Lots of bioassays have been done The Pezizales are a widespread and diverse group of Ascomycota that form either epigeous ascocarps (cup-fungi, morels) or enclosed hypogeous ascocarps (truffles) (Trappe, 1979; Trappe, 1990; Læssøe & Hansen, 2007). Pezizalean fungi inhabit diverse ecosystems worldwide and many lineages form important nutrient-gathering symbioses called ectomycorrhizae (EM) with vascular plants (Trappe, 1979; Díez *et al.*, 2002; Tedersoo *et al*., 2005). In arid and semi-arid ecosystems, hypogeous Pezizales ("truffles") are an important component of EM communities, both as sporocarps and as fungal symbionts on EM roots (Læssøe & Hansen 2007; Smith *et al*. 2007; Morris *et al.*, 2008). In dry habitats of the Mediterranean basin and the Middle East, some of the most common hypogeous fungi are desert truffles in the genera *Tirmania*, *Terfezia* and *Picoa* (including *Phaengium*) (Alsheikh & Trappe, 1983; Díez *et al.*, 2002). Although these genera are occassionally detected with diverse host plants such as *Quercus* (Fagaceae) or *Kobresia* (Cypreraceae) (Díez *et al.*, 2002; Ammarellou & Saremi, 2008), they most often form symbioses with members of the Cistaceae, particularly species of *Helianthemum* and *Cistus* (Alsheikh & Trappe, 1983; Díez *et al.*, 2002; Comandini *et al.*, 2006). These three genera are similar in overall appearance and habit; they form globose to subglobose ascocarps that usually fruit in erumpent mounds at maturity and are either dispersed by the wind or by foraging birds (Alsheikh & Trappe, 1983; Díez *et al.*, 2002). Morphological characters such as spore and peridium morphology, gleba color, and sporocarp odor have been used to differentiate the desert truffles, but they can be difficult to identify to the species level because convergent evolution has

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decreased the number of available characters (Díez *et al.*, 2002). Despite the diversity and extent of arid habitats in Asia, there are few reports of desert truffles outside of the Mediterranean and Arabian Peninsula. We recently collected desert truffles during the mid-winter and early spring (January–March) in the Tarom desert of Zanjan province, Iran. Here we report the presence of *Picoa lefebvrei* (Pat.) Maire *(=Phaeangium lefebvrei*) in Iran and use phylogenetic analysis of the ITS and 28s rDNA to show its relationship among genera of ascomycota. In recent years, molecular phylogenetic research on sequestrate fungi has repeatedly demonstrated that morphology of hypogeous fungi can be misleading (Ferdman *et al*., 2005). More specifically, molecular analyses of the Pezizales and the phylogenetic relations among epi- and hypogeous species have been conducted by ÎDonnell *et al*., 1997; Norman & Egger, 1999; Percudani *et al*., 1999; Roux *et al*.,1999; Becard,1999; Hansen *et al*.,2001; Diez *et al*.,2002; Tedersoo *et al*., 2005; Carriconde *et al*.,2008.

MATERIALS & METHODS

The township of Tarom is located in the northern section of the Zanjan province, Iran. This area receives humid air currents from the north and the climate is subtropical to Mediterranean. During dry years the annual rainfall is ca 150 mm, but in wet years it may be as high as 400 mm (Ammarellou and Saremi, 2008). During years with high autumn rainfall, a good crop of desert truffles can be found in the region (Ammarellou, personal observation). Desert soils are generally low in organic matter (ca 1-2 %) and are granular, chalky, and alkaline(Fig.1).

All morphological characteristics of desert truffles are based on fresh ascocarps, including color, shape, depth of fruiting, texture and anatomical characteristics. For microscopic evaluation, sections of 8 µm thick were

Fig. 1. Geographical location of Tarom in northern Zanjan

prepared for viewing of general morphology. For complementary detection and Final identification specimens of studied truffle were sent to USA laboratories. Specimens of *Picoa lefebvrei* (=*Phaeangium lefebvrei*) and *Picoa juniperi* were obtained from Herbarioi de Real Jardín Botánico, Madrid, Spain (MA) and Harvard University's Farlow Herbarium, Cambridge, MA, USA (FH).

Clean fungal tissue was removed from sporocarps, placed in microcentrifuge tubes, and ground with micropestles. DNA was extracted by a modified CTAB method (Gardes and Bruns 1993). PCR was for the ITS and 28s rDNA performed with two primer combinations according to published protocols: ITS1F/LR3 (Smith *et al*., 2007) and LROR/LR5F (Tedersoo *et al*., 2008). PCR products were visualized on 1.5% agarose gels stained with SYBR Green I (Molecular Probes, Eugene, OR, USA). Successful amplicons were cleaned with ExoSAP-IT (U.S.B. Corporation, Cleveland, OH, USA). Bidirectional sequencing was performed with the above primers using the Big Dye Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI13730xl capillary sequencer (Applied Biosystems). Sequences were edited with Sequencher v.4.1 (Gene Codes Inc., Ann Arbor, MI, USA). ITS and 28s BLAST searches indicated that *Picoa* spp. are most closely related to the Geopora-Tricharina clade of the Pyronemataceae (Pezizales) (Perry *et al.*, 2007). Accordingly, we utilized Pyronemataceae DNA sequences from published studies for our phylogenetic analysis (Perry *et al*., 2007, Perry & Pfister, 2008, Læssøe & Hansen 2007). Sequences were assembled in Mesquite v.1.1 (Maddison and Maddison, 2006), and aligned with the aid of ClustalX (Chenna *et al*., 2003). Parsimony analysis was performed with PAUP* 4.0b10 (Swofford 2001) with the following settings: equal weighting of all characters, gaps treated as missing data and TBR branch-swapping and MulTrees on. We then performed bootstrap analysis using 1000 replicate heuristic searches with 10 random addition sequences, stepwise addition, and TBR branch swapping. For maximum likelihood (ML) analysis, we performed eight runs using Garli v. 0.951 (Zwickl, 2006) with both rates and the appropriate model estimated by the program followed by ML bootstrapping using 500 replicate searches. For all analyses, three species of *Sowerbyella* were selected as outgroup taxa based on their phylogenetic position in Perry *et al*. (2007).

RESULTS & DISCUSSION

A brownish species of Iranian desert truffles grows solitary or assembled of 2-5 ascocarps in the surface soil, is about the size of a Hazel fruit and has white texture (Fig. 2. A,B). This species forms 0.5-1 cm below the soil surface and is brownish inside. The truffles that had been collected from studied areas in Tarom region (Zanjan province) were investigated in laboratories. The microscopic examination showed that each ascus contains 8 ascospores. Measure the diameter of ascus is about 90-100 µm and ascospores is about 30 µm. (Fig.2: C). According to macroscopic and microscopic characteristics and using literature on ascomycetous macrofungi (Breitenbach and Kranzlin, 1984; Philips,1981; Alsheikh and Trappe, 1983; Alsheikh, 1994; Ammarellou & Trappe, 2007) this fungi specimen turns out to be in the genus *Phaeangium*, close to *P. lefevbrei* .

The 28s rDNA alignment consisted of 42 sequences from Pyronemataceae (Fig. 3). After exclusion of ambiguously aligned characters, the alignment was 810 base pairs in length and contained 163 parismony-informative characters. Parsimony analysis generated 48 equally parsimonious trees, each with 703 steps (CI = 0.491, RI = 0.688). Each of the maximum likelihood runs produced trees with similar overall topology and similar likelihood scores (e.g. -ln 4646.77984 – 4646.09659).

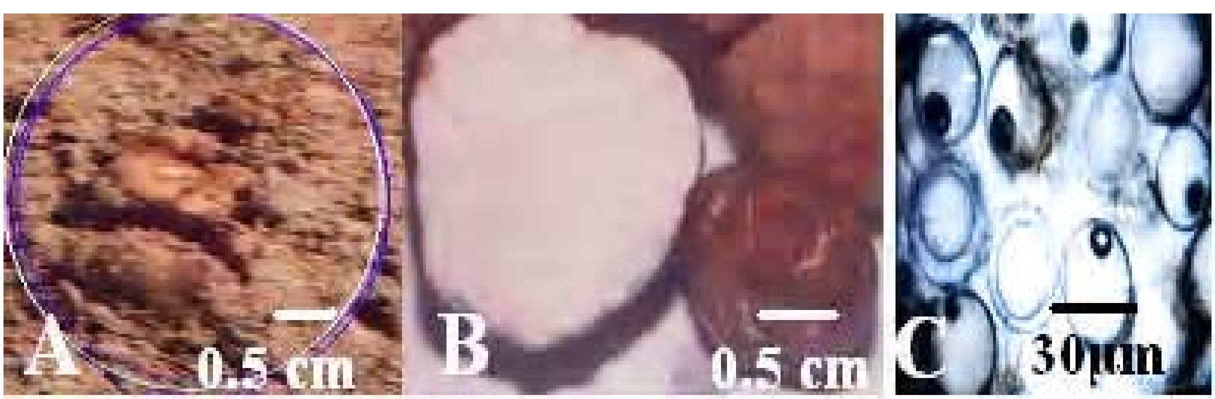

Fig. 2. The appearance of desert truffle *Picoa lefevbrei.* (A).
The morphology of *Picoa lefevbrei*.(B). Every ascus has 8 ascospores and ascus membrane disappeared after maturation. The ascus is about **90-100** µm and ascospores is about **30** µm. (C).

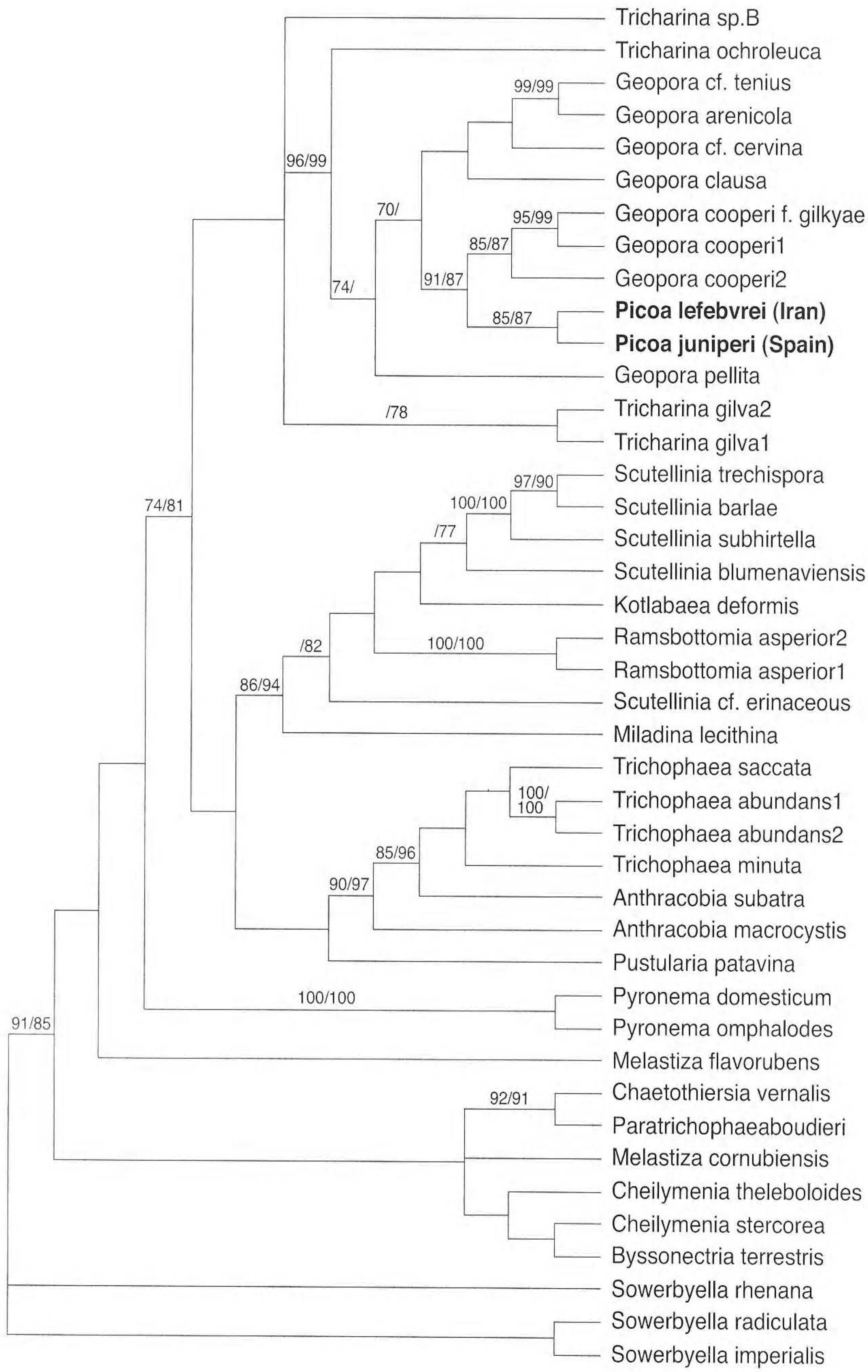

Fig. 3. Phylogenetic relationship of Picoa lefebvrei and Picoa Juniperi based on Maximal Parsimony (MP)

CONCLUSION

Phaengium was erected as a monotypic genus by Patouillard (1894) to accommodate a new truffle species from Tunisia, *P. lefebvrei* . The type collection was characterized by Patouillard as having a smooth, villose, brown surface: a milk-white, homogeneous gleba with no suggestion of veins; and stipitate asci containing two to eight ovoid, smooth, hyaline spores. The desert truffle genus *Phaengium* Patouillard, synonymized with *Picoa* by R. Maire, is resurrected. *Phaengium* has ornamented spores at maturity and a tomentose peridium, whereas *Picoa* has smooth spores and no tomenturn. In this research, *Phaengium* and its single species, *P. Lefebvrei* are redescribed and placed in the family Pyronemiaceae. This species belongs to the *Geopora-Tricharina* clade of the Pyronemataceae (Pezizales, Ascomycota). Our phylogenetic and morphological analyses also confirm that *P. lefebvrei* belongs in the genus *Picoa* because of its close morphological and genetic relationship with the type species, *Picoa juniperi* Vittad.

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