AN INTEGRATED TAXONOMIC APPROACH TO SURVEY ARMILLARIA IN IRAN

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INTRODUCTION

Iran's most valuable forests are located on the coast of the Caspian Sea and cover 1.85 million ha in the northern region of the Alborz mountain range, which is the highest mountain range in the Middle East. Dense forests cover two major provinces, Gilan and Mazandaran; however, less than 10% of Iran is forested. These forests comprise temperate, deciduous, broad-leaved tree species. Conifers are usually absent in Iranian forests, with only a few relics of coniferous species remaining.

Armillaria root disease can cause significant damage in Iranian forests and it is widely distributed throughout these forests. *Armillaria mellea* is a well-known cause of root disease of Caucasian or Persian oak (*Quercus macranthera*) within the Hatam Baigh forest in northwestern Iran (Davari *et al.* 2005). Based on the biological species concept (Korhonen 1978), a previous study of *Armillaria* spp in Iran showed the existence of four intersterility groups, which represented *A. mellea*, *A. cepistipes*, *A. gallica* and *A. borealis* (Asef *et al.* 2003). However, the distribution of *Armillaria* spp in Iran remains largely undocumented a better understanding of Armillaria distribution is needed for disease management and comparisons with other regions. In recent years, the utility of DNA sequence-based identification has been demonstrated for *Armillaria* spp, and translation elongation factor 1α (*tef-1α*) gene sequences have been especially useful for phylogenetic analysis to differentiate closely related *Armillaria* spp (e.g., Maphosa *et al.* 2006; Hasegawa *et al.* 2010; Ross-Davis *et al.* 2012). Previously, no DNA sequence data were available for validating *Armillaria* spp in Iran. The objective of this study is to identify *Armillaria* spp from Iran using integrated taxonomic methods based on basidiocarp morphology, interfertility, and DNA sequences.

MATERIALS AND METHODS

We are basing species identification of Armillaria in Iran on basidiocarp morphology, interfertility (biological species), and phylogenetic analyses of DNA sequences from the *tef-1α* gene. Fresh and dried basidiocarps were used to determine classical morphological characteristics DNA sequences (e.g., *tef-1α*) from a set of European biological tester strain cultures of annulate *Armillaria* spp, obtained from Kari Korhonen (Finland) and Nenad Keca (Serbia), were also included in the analyses. Additional *tef-1α* sequences of North American *Armillaria* spp were obtained from GenBank. Sequences were aligned using the MAFFT software (Katoh & Standley 2013) and a phylogenetic tree was constructed using maximum-likelihood in Garli 2.2 (Bazinet *et al* 2014). Interfertility tests from basidiospore-derived cultures between Iranian collections and European biological species testers are ongoing.

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Figure 1A. *Sampling Armillaria in Iranian forests.*

Figure 1B. *Morphological study of Armillaria.*

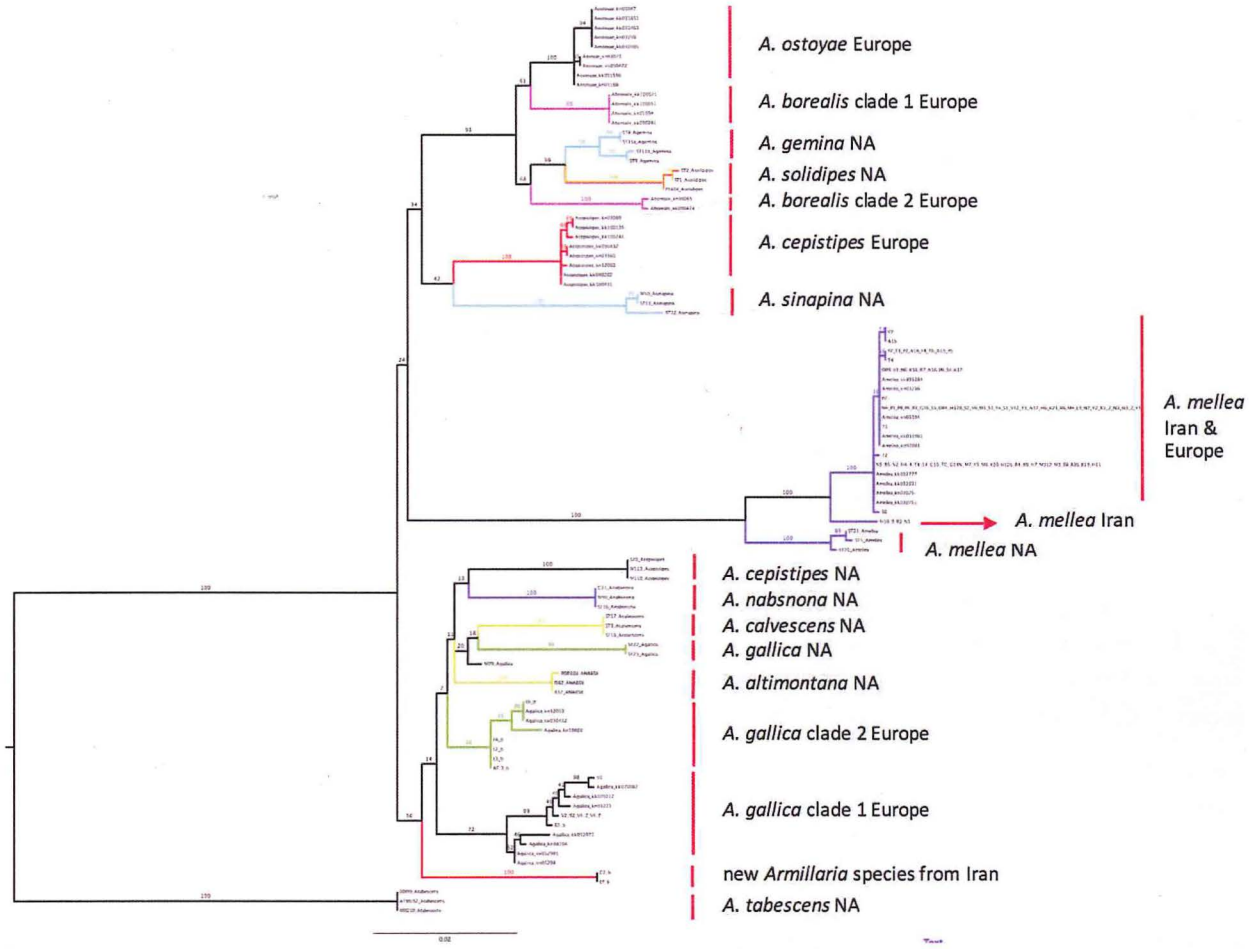

Figure 2. *Preliminary phylogenetic tree of global Armillaria spp constructed in Garli 2.2. (NA = North America).*

RESULTS AND DISCUSSION

In this study, approximately 100 Armillaria basidiocarps were collected from 11 different forest sites in northern Iran (Figure 1A and 1B). Based on morphology and preliminary phylogenetic analyses, most samples are associated with *A. mellea* and *A. gallica* complexes (Figure 2). In Iran, *A. mellea* is associated with wide-spread tree mortality and windthrow, which suggests that *A. mellea* is a primary pathogen in Iranian forests. Interestingly, three *A. mellea* isolates generated *tef-1α* sequences containing 15 different variable sites that separated these isolates into a distinct subclade within the *A. mellea* complex. Although the *A. mellea* complex is widely distributed around the northern hemisphere, phylogenetic analysis shows that North American *A. mellea* and Iranian *A. mellea* reside in distinct clades. Further studies are needed to resolve the taxonomy of the polyphyletic *A. mellea* and *A. gallica* complexes.

A better understanding of the taxonomy and the global distribution of *Armillaria* spp will provide information on the potential invasiveness and potential impacts of climate change on Armillaria root disease.

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