Cryptic species in the *Terfezia boudieri* complex

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Abstract: Molecular and physiological tools were employed to study the *Terfezia boudieri* complex. Markers used include the internal transcribed rRNA (ITS) spacer region, sequences from the 5' end of the ribosomal large subunit gene, partial sequence of a chitin synthase, and β-tubulin genes as well as six amplified fragment length polymorphism (AFLP)-based markers. Following initial sequencing of a single PCR-amplified sample for each type, mass analysis relied on RFLP differences between the types. Over 100 fruit bodies, 30 or more specimens for each ITS type, were tested with each of the markers. This analysis divided the isolates into three groups, each correlated to a specific ITS type. Two physiological traits: mycelial proliferation and mycorrhiza formation, consistently showed responses paralleling the ITS types; these data suggest that *T. boudieri* is comprised of three cryptic species.

Zusammenfassung: Das *Terfezia boudieri* Aggregat wurde mit molekularen und physiologischen Methoden untersucht. Die verwendeten Marker waren die ITS rRNA Sequenz, Sequenzen des 5'-Endes des ribosomalen LSU Genes, eine partielle Sequenz einer Chitin-Synthase und β-Tubulin-Gene sowie sechs AFLP Marker. Nach anfänglicher Sequenzierung einer einzigen PCR-amplifizierten Probe für jeden Typ beruhte die Massenanalyse auf RFLP-Unterschieden zwischen den Typen. Über 100 Fruchtkörper, 30 oder mehr Belege für jeden ITS-Typ, wurden mit jedem der molekularen Marker getestet. Die Isolate zerfielen dabei in drei Gruppen, jede mit einem spezifischen ITS-Typ korrelierend. Zwei physiologische Merkmale, Myzelwachstum und Mykorrhizabildung, zeigten konsistent parallele Übereinstimmung mit den ITS-Typen. Diese Daten weisen daher auf drei kryptische Arten in *T. boudieri* hin.

Molecular phylogenetic studies are now routinely employed to reveal the existence of cryptic species within complexes previously believed to be a single homogenic species: Certain morphological species thought to have global distribution have been shown to harbour two or more phylogenetic species, each with geographically restricted distribution (e.g., JAMES & al. 1999, KASUGA & al. 2003, KOUFOPANOU & al. 1997). However, instances of coexistence (sympatry) of cryptic species have lately been reported (PRINGLE & al. 2005, KAUSERUD & al. 2007).

The Israeli Negev is the main truffle-bearing region in Israel, and *Terfezia boudieri* CHATIN is the most abundant desert truffle species in this region (KAGAN-ZUR & ROTH-BEJERANO 2009). An initial analysis of the internal transcribed spacer (ITS) of *Terfezia boudieri* isolates revealed three distinct ITS types within this morphologically uniform species, and emphasized the divergence of type 2 from types 1 and 3 (FERDMAN & al. 2005). Fruit bodies of all types are found in close proximity in the wild (a few meters apart).

Further, extended, molecular and some physiological studies of the three types are reported here.

The molecular markers used included sequences taken from:
1. The 5' end of the ribosomal large subunit gene;
2. A chitin synthase gene partial sequence;
3. β-tubulin partial sequence;
4. Six different amplified fragment length polymorphism (AFLP)-based markers.

All in all, including the ITS spacer ten marker loci were analysed. Following initial sequencing of a single PCR amplified sample for each type and each marker, mass analyses of specimens relied on:

1. The existence or absence of an amplification product.
2. Size differences between the amplified fragments.
3. RFLP (Restriction Fragment Length Polymorphism) differences between the amplification products.

Over 100 fruiting bodies, 30 or more specimens for each ITS type, were tested with each of the markers. The analysis of the markers divided the isolates into three distinct groups, each correlating to a specific ITS type. A total of 16 distinguishing parameters was encountered, where two singled out type 1, eight type 2, and six type 3. TAYLOR & al. (2000) maintained that five consistently differentiating loci are enough to determine the existence of distinct cryptic species. Thus type 2 and type 3 are each distinct from each other and from type 1. The latter may be considered closest to a common ancestor (FERDMAN & al. 2009). A study of physiological traits was also undertaken. Two of the examined traits: mycelial proliferation and mycorrhiza formation, consistently showed responses paralleling the ITS types (FERDMAN & al. 2009); the data obtained suggest that *T. boudieri* is comprised of three sympatric cryptic species.

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