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ORIGINAL ARTICLE

Fingerprinting by amplified fragment length polymorphism (AFLP) and barcoding by three plastidic markers in the genus *Wolffiella* Hegelm

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Abstract

Amplified fragment length polymorphism (AFLP) fingerprinting and three different plastidic DNA regions (*rpl*16, *rps*16, *atp*F-*atp*H) were used to investigate species identity in the genus *Wolffiella*. For this purpose, clones (67 in total) belonging to all ten species were selected. Almost all the species were represented by more than one clone. The fingerprinting technique, AFLP, clearly distinguished the species, *W. caudata*, *W. gladiata*, *W. neotropica*, *W. rotunda*, and *W. welwitschii*. Apart from confirming the molecular identity of these five species, the plastidic markers could delineate two additional species, *W. hyalina* and *W. denticulata,* although the conclusion concerning the latter is restricted by the availability of only one clone. The efficiency of the plastid-derived markers in identifying the number of species-specific clusters followed the sequence *rps*16 > *rpl*16 > *atp*F-*atp*H. The species *W. lingulata*, *W. oblonga*, and *W. repanda* could not be identified by any of the molecular methods presented here, but could be strictly defined on a morphological basis. In several clones, high amounts of genetic admixtures between different species were detected. Further, simulation studies demonstrated that these clones are genetic hybrids. This might be one of the obstacles in molecular identification of species in the genus *Wolffiella*.

Keywords AFLP fingerprinting · cpDNA · Duckweed · Lemnaceae · Structure · *Wolffiella*

Introduction

Duckweeds (Lemnaceae) represent the smallest and the fastest growing angiosperms (Landolt 1986; Sree et al. 2015; Ziegler et al. 2015) that are divided into five monophyletic genera (Les et al. 2002; Appenroth et al. 2013; Sree et al. 2016). High level of reduction in morphological and anatomical structures renders difficulty in morphological identification of all the 37 species of Lemnaceae even to highly specialised experts of duckweed research. Despite this, Landolt (1986) developed the most comprehensive taxonomic description of this plant family based on morphological markers, which is widely accepted to date. In contrast to the Angiosperm Phylogeny III definition according to which duckweeds belong to a subfamily (Lemnoideae) of Araceae, we consider their taxonomic level as a plant family, i.e. Lemnaceae. This consideration is possible and is in agreement with the general taxonomic rules as already discussed in detail in our previous publications (Appenroth et al. 2013, 2015; Sree et al. 2016). Keeping in mind the difficulty in handling morphological markers, molecular taxonomy of Lemnaceae is of great importance. This requirement is supported by the existence of duckweed clone collections providing access to hundreds of identified accessions.

Among duckweeds, the genus *Wolffiella* Hegelm. has not yet been well investigated concerning its molecular taxonomy. The genus was introduced by Hegelmaier (1895), composing six species *Wolffiella lingulata*, *W. denticulata*, *W. rotunda*, *W. gladiata*, *W. oblonga*,

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