Orchidioid fungi of the form-genus *Rhizoctonia* associated with the roots of *Chloraea cuneata* Lindl. from Araucanía, Chile

Hongos orquidioides del género-forma *Rhizoctonia* asociados a las raíces de *Chloraea cuneata* Lindl. de la Araucanía, Chile

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**RESUMEN**

*Chloraea cuneata* es una orquídea endémica de Chile en Peligro Crítico de Extinción. Se aislaron e identificaron hongos orquidioides a partir de pelotones ubicados en células corticales de sus raíces pertenecientes al género-forma *Rhizoctonia*, teleomorfo *Thanatephorus* (multinucleado), los que podrían ser usados en iniciativas de conservación.

Orchidaceae is one of the largest flowering plant Families in the world (Jones 1993, Dressler 1993, Heywood et al. 2007, Reina-Rodríguez et al. 2010), with an almost cosmopolite distribution (Novoa et al. 2006, Heywood et al. 2007). Orchidaceae in Chile are represented by 7 terrestrial genera: *Aa*, *Bipinnula*, *Brachystele*, *Chloraea*, *Codonorchis*, *Gavilea*, and *Habenaria* (Novoa et al. 2006). Chile has a high orchid endemism, with approximately half of the 54 Chilean species endemic to the country (Novoa et al. 2006). Many of these species are considered endangered or even critically endangered (Van Nieuwenhuizen 1998). However, the poor taxonomic and biological knowledge of the group makes it difficult to determine the conservation status of many taxa (Novoa 2008). *Chloraea cuneata* Lindl. (Fig. 1a) is an endemic and Critically Endangered orchid species from Chile (Novoa et al. 2006, Elórgtegui & Novoa 2009, MMA 2011). This species is a terrestrial and non-parasitic orchid species from Chile (Novoa et al. 2006, Elórgtegui & Novoa 2009, MMA 2011). This species is a terrestrial and non-parasitic orchid (Correa 1969, Elórgtegui & Novoa 2009) found in Chile only in the Araucanía district (~39°S) with few known populations (Romero 2012).

Most terrestrial orchids depend on fungal partners to germinate and grow (Pereira et al. 2005, Murguia & Lee 2007, Smith & Read 2008, Steinfort et al. 2010, Valadares et al. 2012). This is due to the lack of nutritious reserves in their seeds (Smith & Read 2008). In the soil, orchids commonly associate with Basidiomycota (McLaughlin & Spatafora 2014) fungi from the form-genus *Rhizoctonia* (Otero et al. 2002, Durán et al. 2007, Otero & Bayman 2009, Steinfort et al. 2010, Pereira et al. 2014). This association is vital for plant success (Otero & Bayman 2009) and can persist in the adult stage, where the cortical cells of the roots usually show hyphae clusters known as pelotons (Pereira et al. 2014). Some *Chloraea* species that co-occur with *C. cuneata* in the Araucanía district have been found to associate with orchidioid fungi belonging to the Tulasnellaceae (Pereira et al. 2014). These fungi show the typical traits of the form-genus *Rhizoctonia*; namely hyphae constriction in the septum, right-angle branching of hyphae, and presence of monilioid cells (García et al. 2006, Pereira et al. 2014).

The present study aims to isolate and identify possible fungal partners of *C. cuneata*. This could have relevant consequences for future conservation and propagation programs for this Critically Endangered species since reintroduction should include the inoculation of the substrates with the associated fungi.

Three tuberous roots from 2 individuals of *C. cuneata* we collected (Fig. 1a-b). The population was located at Nahuelbuta National Park (Araucanía Region) and consisted only in those 2 individuals. Root segments were cut in a laminar flux chamber and were externally sterilized by seriated ethanol baths (Otero et al. 2002, Otero & Bayman 2009). Roots segments with evident hyphae pelotons inside
cortical cells were cultured in Water-Agar (WA) and later re-cultured in Potato, Dextrose, Agar (PDA). All culture media were autoclaved at 121±1 ºC for 20 min. The isolated material was incubated in the dark in a stove at 24 ±1 ºC for 10 days. Pure fungal isolates were successfully obtained from each individual (from one root each). After 10 days, the microscopic traits of the fungal isolates were observed using an optic microscope (Primo Star, Carl Zeiss, Germany) attached to a digital camera (Canon EOS Rebel). Hyphae size was measured and nuclei number per cell in young hyphae was recorded in each isolate using image software (AxioVision rel. 4.8). The presence of monilioid cells and other possible morphological traits typical of Rhizoctonia were registered. The possible teleomorph was assigned based on the number of nuclei per cell (Currah et al. 1997, Corrêa et al. 2011, Pereira et al. 2014).

The roots of C. cuneata (Fig. 1b) showed presence of hyphae in their cortical cells. Pelotons (hyphae clusters) were evident inside those cells (Fig. 1c). From those pelotons, two fungal strains were isolated (Fig. 1d) both showing Rhizoctonia-like traits (Fig. 1e-i, Table I). The two isolates were coded as CCANG1 and CCANG2. Based on nuclei number per young hyphae, the isolates correspond to the Thanatephorus teleomorph (Table I). Both isolates showed similar hyphae traits (Table I, t-test p>0.05) and presence of monilioid cells (Fig. 1i). There were no statistical differences between isolates in the measured fungal traits (t test p>0.05, n = 30 measurements per isolate, Table I).

Chloraea cuneata associates with orchidioid fungi of the form-genus Rhizoctonia. In particular, individuals from Araucanía Region were found to have their roots colonized by fungi that belong to the Thanatephorus teleomorph (Table I). Previous studies on C. collicensis and C. gavilu have observed orchidioid fungi from the Tulasnellaceae, a family of Rhizoctonia-like fungi (Pereira et al. 2104). Other Chilean orchid species, however, have been found to associate with other Basidiomycota (Steinford et al. 2010). Many orchidioid fungi show Rhizoctonia-like traits (García et al. 2006, Pereira et al.
Rhizoctonia fungi associated with C. cuneata roots: Atala. C. et al.

Table I. Morphological traits of hyphae of two fungal strains isolated from the roots of Chloraea cuneata. Average values ± SE of two independent isolates showing the same morphological traits are shown. Values correspond to the average of 30 measurements per isolate. For nuclei number/cell average values and range (inside brackets) are shown.

| ISOLATE   | HYphaE WIDETh (MM) | HYphaE LENGuTH (MM) | NuCLEI NuMBER/CEll           | MONiLiOd CEll  | POSSibiLE teLEOMORPh |
|-----------|--------------------|--------------------|-------------------------------|----------------|-----------------------|
| CCANG1    | 57.01 ± 9.13       | 115.64 ± 23.61     | Multinucleated 1.9 (1-3)      | +             | Thanatephorus         |
| CCANG2    | 54.55 ± 13.08      | 119.97 ± 24.07     | Multinucleated 1.5 (1-3)      | +             | Thanatephorus         |

2014), but there is also evidence for non-Rhizoctonia fungi involved in orchid-fungi symbiosis (Darnaley 2007). It is possible that other fungal species can associate with C. cuneata in the field that can not be isolated by the methods used in this work. Also, the fungal partner may change with plant ontogeny and/or with environmental conditions (McCormick et al. 2006). In our lab we are currently testing the effect of the isolated fungi on seed germination. This will allow us to confirm if these fungi can colonize the seeds and induce their germination or if it is only found as a fungal partner in the adult (late association). Seemingly, this is the first report of orchidioid fungi (Rhizoctonia-like traits) present in the roots of C. cuneata.

Chloraea cuneata is a Critically Endangered species, with a very narrow distribution (MMA 2011, Romero 2012). Association with fungal partners can increase germination and growth of orchids (Pereira et al. 2005, Murguia & Lee 2007, Smith & Read 2008, Bidartondo & Read 2008, Valadares et al. 2012) as well as survival under stress (Smith & Read 2008). It is possible that the distribution and abundance of C. cuneata can be limited by the presence and abundance of specific orchidioid fungi in the soil (Ramsay & Dixon 2003, Otero & Bayman 2009). Thus, conservation initiatives could use the isolated fungi identified in our study in order to germinate seeds and inoculate adult plants for ex situ conservation and re-introduction programs. Pure cultures of the fungi are currently held at our laboratory for such initiatives.

ACKNOWLEDGEMENTS

We would like to thank CONAF Araucanía Region for field support. Funding was provided by Project PIA-iniciación 037.480/2013 (PUCV) and Project DI-iniciación 037.371/2014 (PUCV).

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