Research article

Acta Botanica Mexicana

Presence of orchid mycorrhizal fungi at two developmental stages under different climatic conditions in *Rhynchostele cervantesii* (Orchidaceae)

Presencia de hongos micorrízicos orquideoides en dos estados de desarrollo bajo diferentes condiciones climáticas en *Rhynchostele cervantesii* (Orchidaceae)

Rosa Elia Magaña-Lemus[1], Irene Ávila-Díaz[2,4], Roberto Garibay-Orijel[3]

Abstract:

Background and Aims: Mycorrhizal symbiosis is important for orchids during early stages of development. This association in subsequent developmental stages is scarcely documented, specifically in epiphytic orchids. The objective of this work was to evaluate the mycorrhization of *Rhynchostele cervantesii* in two developmental stages under contrasting weather conditions.

Methods: The mycorrhization was evaluated *in situ* in 50 adult plants and 50 juvenile plants during wet and dry seasons for two years. We collected one or two roots per plant. The mycorrhization percentage was registered, and digestion state of pelotons as: a) intact, b) digestion process and c) digested. The type of root attachment was registered as in contact with the substrate or aerial, and the root zone as basal, medium and apical.

Key results: The average mycorrhization percentage between juvenile and adult plants was not significantly different, while between seasons, it was higher in wet season 1, followed by wet season 2. The lowest percentages of mycorrhization were registered for dry seasons 1 and 2. Intact peloton average during average wet season (18 pelotons) was significantly higher than in the dry season (0.5 pelotons), while digested pelotons were more abundant in dry season (10.5 pelotons) than in the wet season (0.5 pelotons), with no differences between juvenile and adult plants. Mycorrhization percentage was significantly higher in roots in contact with substrate (32%) than in aerial roots (28%), and higher in basal roots (55%) than in apical roots (2%).

Conclusions: Functional mycorrhization (with degraded pelotons) is present in two orchid developmental stages, suggesting that this association is important during the complete life cycle of *R. cervantesii*. Dry season negatively impacts the mycorrhization percentage and positively the digestion of pelotons. Therefore, we consider that the prevalence of droughts could have an adverse effect on this association.

Key words: developmental categories, mycorrhizal fungi, pelotons.

Resumen:

Antecedentes y Objetivos: La simbiosis micorrízica es importante para las orquídeas durante sus primeras etapas de vida. Esta asociación en estados de desarrollo posteriores está escasamente documentada, especialmente en las orquídeas epífitas. El objetivo de este trabajo fue evaluar la micorrización de *Rhynchostele cervantesii* en dos categorías de desarrollo en condiciones climáticas contrastantes.

Métodos: La micorrización se evaluó *in situ* en 50 plantas adultas y 50 juveniles, durante épocas húmeda y seca por dos años. Se colectaron una o dos raíces por planta. Se registró el porcentaje de micorrización y también el estado de digestión de los pelotones: a) intactos, b) en estado de digestión y c) digeridos. El tipo de raíz se registró como en contacto con el sustrato o aérea; y la zona de la raíz como basal, media y apical.

Resultados clave: El promedio de micorrización entre plantas juveniles y adultas no fue significativamente diferente, mientras que entre estaciones fue mayor en época húmeda 1, seguido por época húmeda 2. Los porcentajes más bajos se registraron en las épocas secas 1 y 2. El número de pelotones promedio intactos en época húmeda (18 pelotones) fue mayor que en época seca (0.5 pelotones), mientras que los digeridos fueron más abundantes en época seca (10.5 pelotones) que en la húmeda (0.5 pelotones), sin diferencias entre plantas juveniles y adultas. El porcentaje de micorrización fue mayor en raíces en contacto con el sustrato (32%) que en aéreas (28%), y mayor en la base de las raíces (55%) que en el ápice (2%).

Conclusiones: La micorrización funcional (con pelotones degradados) en *R. cervantesii* está presente en dos categorías de desarrollo, sugiriendo que esta asociación es importante durante todo su ciclo de vida. Las estaciones secas impactan negativamente el porcentaje de micorrización y positivamente la digestión de los pelotones. Por lo tanto, consideramos que la prevalencia de sequías puede tener un efecto adverso en esta asociación.

Palabras clave: categorías de desarrollo, hongos micorrízicos, pelotones.

[1]Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Instituto de Investigaciones sobre los Recursos Naturales, Programa Institucional de Doctorado en Ciencias Biológicas, Morelia, Michoacán, Mexico.
[2]Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Facultad de Biología, Laboratorio de Biología de la Conservación Vegetal, Morelia, Michoacán, Mexico.
[3]Universidad Nacional Autónoma de México, Instituto de Biología, Cd. Mx., Mexico.
[4]Author for correspondence: irene.avila@umich.mx

Received: February 4, 2025.
Reviewed: July 14, 2025.
Accepted by Marie-Stéphanie Samain: February 4, 2026.
Published Online first: February 13, 2026.
Published: Acta Botanica Mexicana 133(2026).

To cite as: Magaña-Lemus, R. E., I. Ávila-Díaz and R. Garibay-Orijel. 2026. Presence of orchid mycorrhizal fungi at two developmental stages under different climatic conditions in *Rhynchostele cervantesii* (Orchidaceae). Acta Botanica Mexicana 133(2026): e2432. DOI: https://doi.org/10.21829/abm133.2026.2432

e-ISSN: 2448-7589

Introduction

One of the most important characteristics of orchids is the tiny size of their seeds lacking reserve substances. Hence, orchids rely on colonization by compatible mycorrhizal fungi for their germination and seedling development (Selosse et al., 2002; Cozzolino and Widmer, 2005; Otero et al., 2005, 2011; Smith and Read, 2010; Ávila-Díaz et al., 2013; Dearnaley et al., 2016; Khamchatra et al., 2016; Parthibhan et al., 2017; Shubha and Srinivas, 2017; Qin et al., 2019). Orchid mycorrhiza are characterized by a colonization pattern in which hyphae penetrate the cortical cells, forming intracellular hyphal coils, known as pelotons (Hágsater et al., 2005; Selosse et al., 2011; Zettler and Corey, 2018; Yeh et al., 2019). This association has been considered asymmetric, as it appears to be more important for orchids than for the fungi (McCormick and Jacquemyn, 2014; Selosse and Martos, 2014; McCormick et al., 2018). These fungi provide carbon and minerals during the early stages of development (Dearnaley et al., 2016).

Orchid mycorrhizal fungi mainly belong to two major divisions: Basidiomycota and Ascomycota (Martos et al., 2012; Egidi et al., 2018; Novotná et al., 2018). Basidiomycota are grouped within *Rhizoctonia* DC. (Tulasnellaceae, Ceratobasidiaceae, and Serendipitaceae) (Dearnaley, 2007; Jacquemyn et al., 2017). Within the Ascomycota, the order Helotiales is the most frequently reported as putative orchid mycorrhizal fungus (Ávila-Díaz et al., 2013; Khamchatra et al., 2016).

There is a controversy about the importance of this association in subsequent stages of orchid development. Some authors report a greater diversity of fungi in the germination stage, while this diversity decreases in seedlings (Waud et al., 2017). For example, in *Liparis loeselii* (L.) Rich., soil samples, protocorms, seedlings, and roots of adult plants were collected to identify associated mycorrhizal fungi. It was reported that the family Thelephoraceae exhibited high prevalence, accounting for approximately 25 to 65% of the recovered sequences, whereas Sebacinaceae showed a broader distribution but lower relative abundance, representing about 10 to 20% of the identified sequences (Waud et al., 2017). Mycorrhizal fungal communities varied across plant developmental stages: in soil, taxa showed high penetrance, with Inocybaceae being particularly prominent (90% of samples; 11% of sequences) (Waud et al., 2017). Protocorms harbored the highest abundances and penetrance values (75-100%), notably Thelephoraceae (100-64%) and Inocybaceae (100-40%) (Waud et al., 2017). In seedlings and adult plants, a general decline in fungal prevalence was observed, with the exception of Inocybaceae, which reached a penetrance of 100% and a prevalence of 50% in adult roots (Waud et al., 2017). In *Cephalanthera damasonium* (Mill.) Druce and *C. longifolia* (L.) Fritsch, the diversity of mycorrhizal fungi was lower at the seedling stage (one taxon) than during germination (five taxa) and in adult plants (six taxa) (Bidartondo and Read, 2008).

Other authors mention that, in natural conditions, terrestrial and epiphytic juvenile orchids have variable dependence on these fungi, while in adult plants the mycorrhiza become intermittent, and disappears (Bertolini et al., 2014). It has been found that mycorrhizal fungi are not only present in orchid germination but can also remain up to adult stages in terrestrial and epiphytic species (Rasmussen, 2002; Rasmussen and Whigham, 2002; Rasmussen et al., 2015; Dearnaley et al., 2016; Favre-Godal et al., 2020; Rammitsu et al., 2020).

Epiphytic habitat varies due to the existence of a strong water and nutrient limitation as well as changes in physical-chemical characteristics of substrate (phorophyte) (Zotz and Hietz, 2001). Under these conditions, mycorrhization has a significant role in the establishment and survival of epiphytic plants during their life cycle and their spatial distribution (Martos et al., 2012; Ávila-Díaz et al., 2013; McCormick and Jacquemyn, 2014; Cevallos et al., 2017; Rasmussen and Rasmussen, 2018). Indeed, some research on adult orchids demonstrates that most orchids present mycorrhization. Sathiyadash et al. (2012) studied mycorrhization in 31 species (22 epiphytes, 8 terrestrial and 1 both epiphytic and lithophytic) noticing mycorrhization in all species, mainly in the roots in contact with substrate. Root colonization by orchid mycorrhizal fungi varied among growth forms. Epiphytic species generally showed moderate to high colonization, with some species such as *Acampe praemorsa* (Roxb.) Blatt. & McCann being more extensively colonized than others, like *Luisia zeylanica* Lindl. Terrestrial orchids tended to exhibit higher colonization overall, while the lithophyte *Oberonia ensiformis* (Sm.) Lindl. displayed intermediate levels (Sathiyadash et al., 2012).

The presence and intensity of mycorrhization was evaluated in *Laelia autumnalis* (Lex.) Lindl. and *L. furfuracea* Lindl., two epiphytic species, comparing roots in contact with substrate and aerial roots, and comparing the zone of mycorrhizal root (basal, medium and apical) (Jiménez-Peña et al., 2018; García-Sánchez et al., 2024). Both species showed significantly higher mycorrhization in roots in contact with substrate; *L. furfuracea* exhibited 38% mycorrhization in roots in contact with the substrate and 1% in aerial roots (García-Sánchez et al., 2024). *Laelia autumnalis* showed higher mycorrhization in the basal root zone and *L. furfuracea* in the medium root zone (Jiménez-Peña et al., 2018; García-Sánchez et al., 2024).

The number of pelotons in orchid roots can vary among individuals and seasonality plays a key role in mycorrhization percentage (Zettler and Corey, 2018). In the epiphytic species *Epidendrum stamfordianum* Bateman, *Stelis quadrifida* (Lex.) Solano & Soto Arenas, and *Erycina crista-galli* (Rchb.f.) N.H.Williams & M.W.Chase, both the percentage of mycorrhization and that of intact pelotons were higher in the wet season than in the dry season (Bertolini et al., 2014). A major quantity of pelotons in autumn and a higher quantity of digested pelotons in spring was registered in *Gavilea araucana* (Phil.) M.N.Correa, a terrestrial species (Durán et al., 2007).

Understanding the relationships between mycorrhization and orchid life cycle and fitness is the baseline to inform better management practices. For example, it has been reported that mycorrhizal fungi boost the growth and development of *in vitro* orchids, increase the survival and development rates of *ex vitro* orchids, and enhance the development of stems and flowers (Chang, 2008; Wu et al., 2011; Teixeira da Silva et al., 2014).

Rhynchostele cervantesii (La Llave & Lex.) Soto Arenas & Salazar is an endemic orchid from Mexico distributed in the states of Guerrero, Jalisco, Mexico State, Michoacán, Morelos, Oaxaca, and Veracruz (SNIB, 2016). This species is included in NOM-059-SEMARNAT-2010 (SEMARNAT, 2010) as endangered for being a highly valued species for ornamental use worldwide; because of the beauty of its flowers, its populations are under great extraction pressure. Even in some regions of Mexico it is sold during holiday season to decorate churches and nativities (Soto-Arenas y Solano-Gómez, 2007). The only study about the endophyte fungi of *R. cervantesii* is that of Cruz-Higareda et al. (2015), where nine fungal strains were isolated but not identified. Three of them (RC062.4EFIM, RC062.4EFIM, and RCRPTC6.3FIM) promoted germination; however, protocorm development was not successful. There are no studies about *R. cervantesii* mycorrhizal associations, and this information is needed to promote the conservation of this endangered orchid.

Our hypotheses were: a) mycorrhizal fungi are present in both juvenile and adult plants, playing an important role throughout the entire life cycle of *R. cervantesii*; b) the percentage of mycorrhization and the digestion state of pelotons are influenced by seasonality (humidity *vs.* drought), root type (in contact with substrate or aerial), and root zone (basal, medium and apical parts). Therefore, the objective of this research was to understand the importance of *R. cervantesii* mycorrhization by evaluating the mycorrhization in different developmental stages under contrasting weather conditions.

Materials and Methods

Study system and site

Rhynchostele cervantesii is an epiphytic orchid with pseudobulbs that have ovoid to globular shape, dark green with black spots, its flowers are white with concentric brown-reddish lines on the tepal base, occasionally on the labellum (Espejo et al., 2002) (Fig. 1A). This species is distributed in Mexico throughout the forests of the Eastern Sierra Madre, the Trans-Mexican Volcanic Belt and part of the Southern Sierra Madre (SNIB, 2016). In our research site it has been reported mainly as epiphytic both in the humid montane forest (HMF) and the oak-pine forest (OPF). In this zone its main phorophytes are *Ternstroemia lineata*, *Quercus rugosa* and *Styrax argenteus* (Domínguez-Gil, 2015; Cervantes-Uribe, 2018).

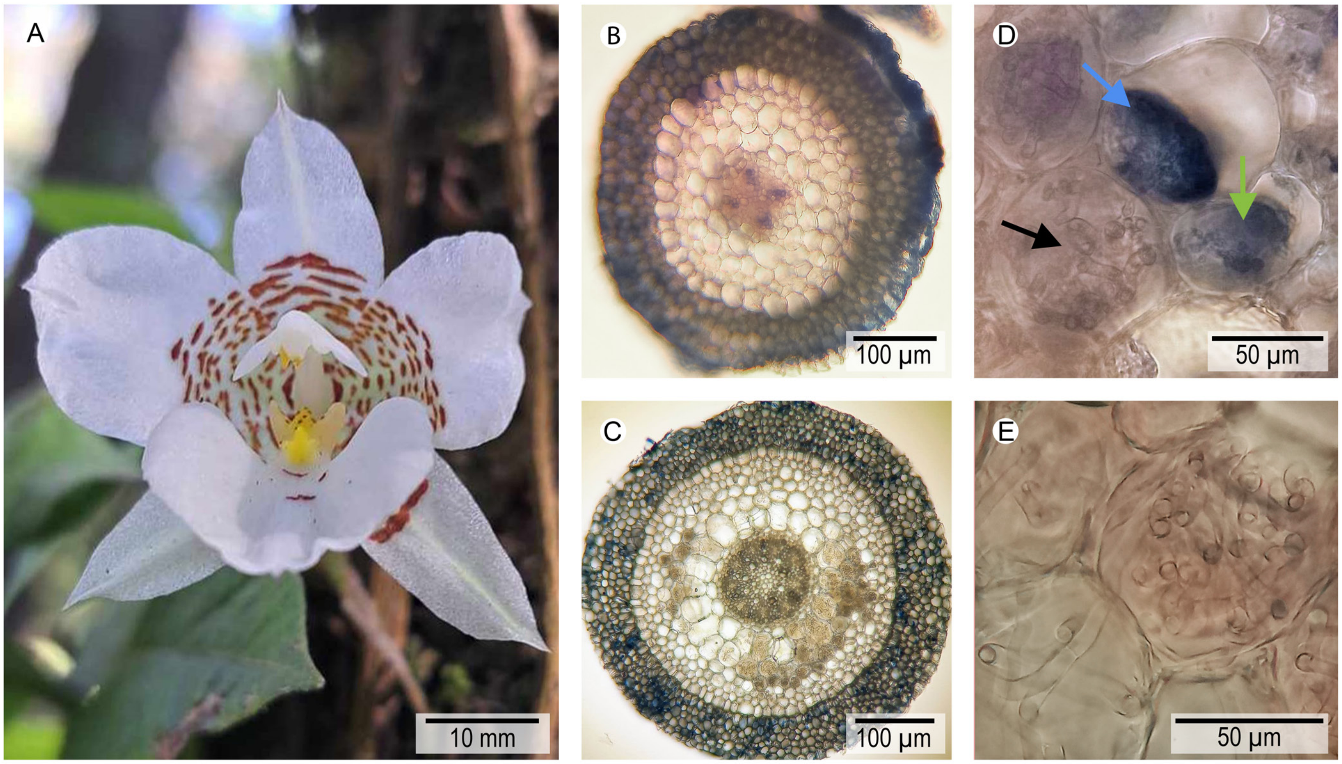

Figure 1: A. flower of *Rhynchostele cervantesii* (La Llave & Lex.) Soto Arenas & Salazar; B. root without mycorrhization at 20×; C. mychorrizated root at 20×; D. black arrow: intact peloton, green arrow: in digestion process peloton, blue arrow: digested peloton at 40×; E. intact peloton at 40×. Photographs: Rosa Elia Magaña Lemus.

The sampling area is found inside the voluntary conservation area of the indigenous community of Santiago Tingambato, on the property of Tenderio, Michoacán, Mexico (Fig. 2). This area covers approximately 360 hectares (INEGI, 2005), is located at 19°30'N and 101°51'W, with an average elevation of 1980 m (INAFED, 2010). Its main vegetation is humid montane forest (HMF), as well oak-pine forest (OPF), pine-oak forest (POF), and pine forest (PF) (Domínguez-Gil, 2015). Our sampling was made in HMF, starting in the summer of 2021 and ending in the spring of 2023.

The municipality of Tingambato has mild weather with rainfall in summer and a historical average annual precipitation of 1100 mm, while the average annual minimum and maximum temperatures are 8 and 27 °C (INAFED, 2010). During the sampling period, the average annual precipitation decreased from 626.3 to 275.9 mm (Fig. 3) (Meteostat, 2024).

Mycorrhization state of *Rhynchostele cervantesii*

Development categories

Field trips were conducted in October-November of 2021 where 100 plants in two development categories (50 adult plants, 50 juvenile plants) were marked and labelled. The juvenile plants had more than one year of development, with visible pseudobulbs, and showed no reproductive activity, whereas adult plants had experienced at least one reproductive event (Tremblay, 2000; Tremblay and Ackerman, 2001; Bayman et al., 2002). It is important to mention that it was also planned to mark seedlings. However, only four seedlings were located and were not included in the analysis.

Contrasting weather conditions

Four samplings were performed in different seasons: wet season 1 (WS1) (October 2021), dry season 1 (DS1) (May

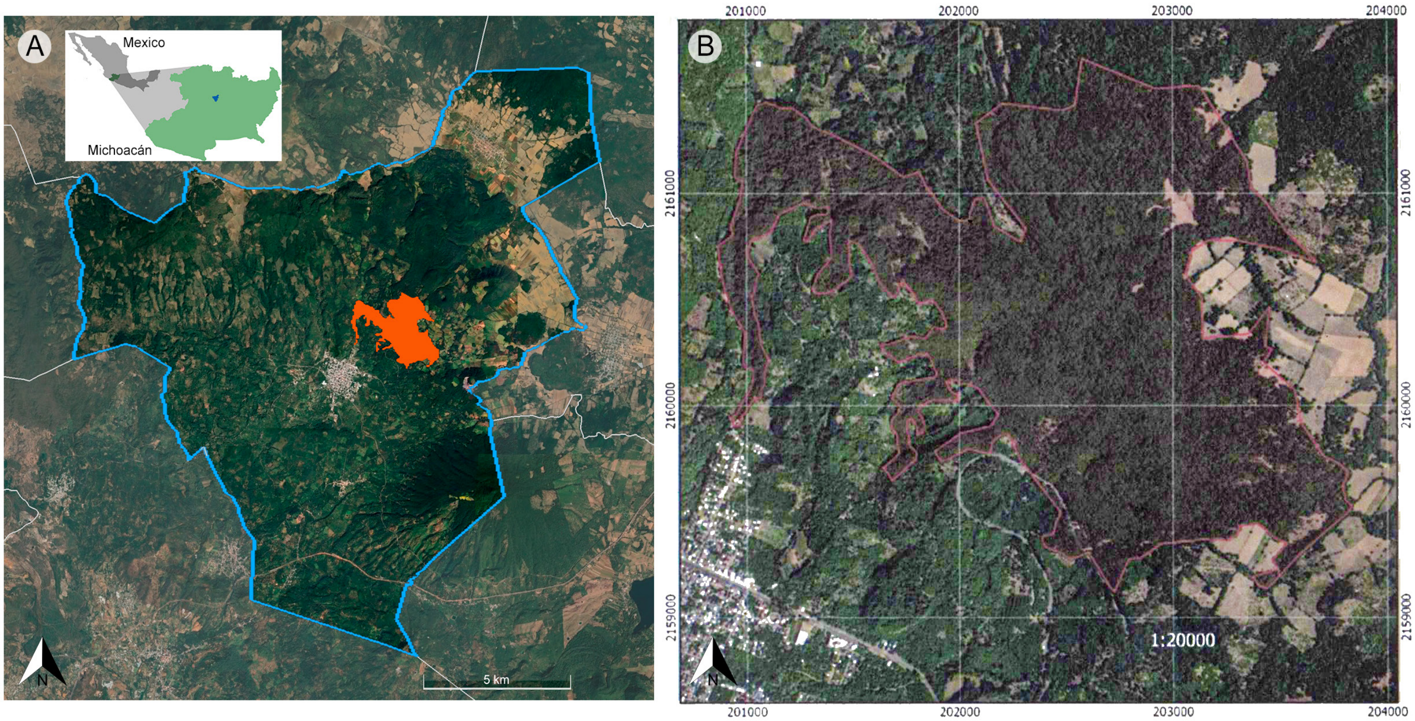

Figure 2: Location of the study site: A. municipality of Tingambato, outlined in blue, in Michoacán, Mexico; B. sampling area found inside the voluntary conservation area of the indigenous community of Santiago Tingambato, on the property of Tenderio, Michoacán, Mexico. Modified from Ávila-Díaz and Chávez Melitón (2022).

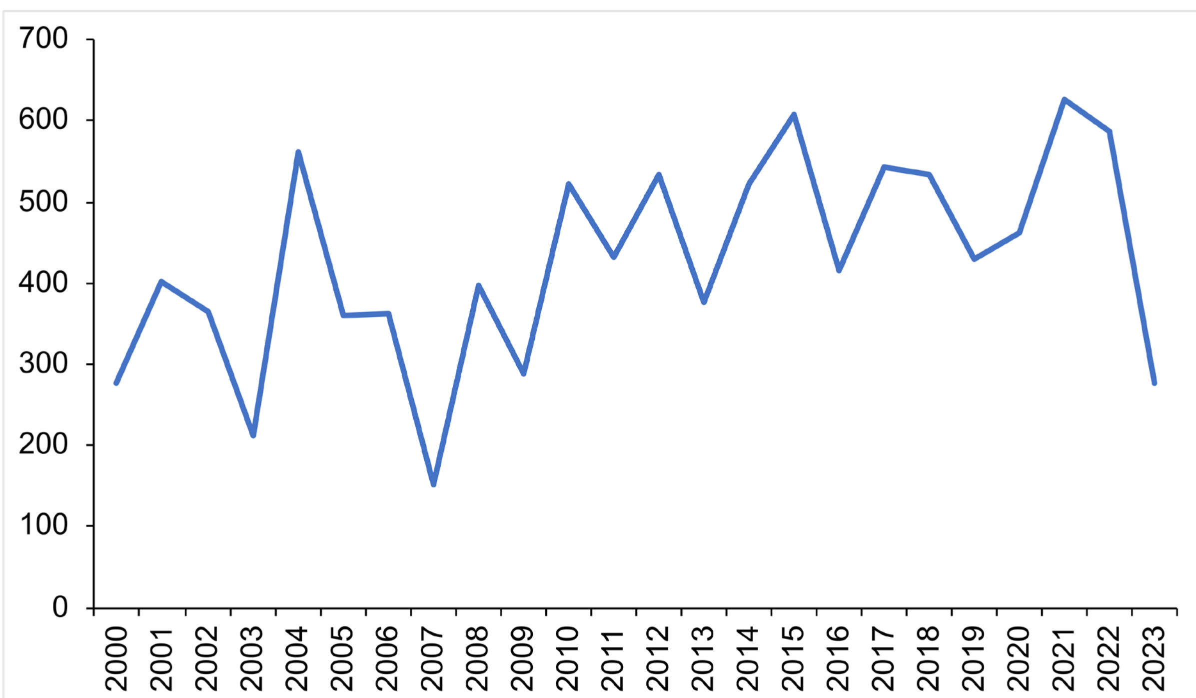

Figure 3: Mean annual precipitation for the period 2000-2023 in the voluntary conservation area of the indigenous community of Santiago Tingambato, on the property of Tenderio, Michoacán, Mexico. Data were sourced from the Zirahuén meteorological station via Meteostat (2024). Coordinates: 19.4533°N, 101.7317°W; elevation approximately 2094 m (Meteostat, 2024).

2022), wet season 2 (WS2) (September 2022), and dry season 2 (DS2) (May 2023). Each time, one root in contact with the substrate was randomly collected per plant, and an aerial root was also collected when present, and placed in a sterilized vial containing previously moisten sterile cotton. The vials were placed in a cooler to avoid direct light and to maintain a temperature of 15-25 °C until they arrived in the laboratory (Zettler et al., 2017, modified).

Mycorrhization percentage and pelotons digestion

The root washing was made with distilled water withdrawing the substrate excess. The mycorrhization percentage was evaluated by dyeing the roots with Trypan Blue (Currah et al., 1997; Ávila-Díaz, 2007). Afterwards, each root was measured and longitudinal sections were made every 5 mm along the root (Fig. 1 B,C). At each section, mycorrhization percentage was recorded based on the area occupied by pelotons. We established the following categories for mycorrhization percentage according to Rasmussen and Whigham (2002): 0%-11%, 12%-24%, 25%-49%, 50%-74%, 75%-99% and 100%. Mycorrhization percentages per plant were calculated by summing the percentages of each section and dividing by the total number of sections. The presence of pelotons was also recorded, and the average was calculated dividing the total number of pelotons by the number of sections made and analyzed roots. In addition, the pelotons' digestion grade was registered with the following categories: intact pelotons (with intact hyphae), pelotons in digestion process (with intact and digested hyphae), and digested pelotons (with hyphae completely digested) (Fig. 1D, E). All parameters were recorded across the four sampling periods (WS1, DS1, WS2, and DS2).

Type of root (aerial or in substrate) and root zone

We also analyzed the effect of substrate on mycorrhization. For this, we categorized roots according to their substrate (phorophyte's bark or aerial) and their root zone (apical, medium, and basal). The number of collected root samples according to orchid development, substrate and season are shown in Table 1.

Statistical analysis

Statistical analyses were made with the program JMP® v. 11 (SAS Institute Inc., 2013). To test our first hypothesis, mycorrhization state was analyzed with the Kruskal-Wallis non-parametric test, with the seasons and the development categories as the variation factor, and the mycorrhization percentage as the response variable. In case of significant differences, the Mann-Whitney pairwise test was carried out to identify groups with differences. To prove our second hypothesis, significant differences were determined by the average number of pelotons per plant and their digestion state with a one-way ANOVA, followed by Tukey's HSD test when appropriate. To evaluate the differences in mycorrhization percentage according to root substrate and root zones a Kruskal-Wallis non-parametrical test was conducted.

Table 1: Number of root samples collected from *Rhynchostele cervantesii* (La Llave & Lex.) Soto Arenas & Salazar, categorized by type of root, weather conditions and orchid development categories. Su=roots in contact with the substrate, Ae=aerial roots, WS1=Wet season 1, DS1=Dry season 1, WS2=Wet season 2, DS2=Dry season 2.

Season	Adults		Juveniles		Total roots	Total cuts
	Su	Ae	Su	Ae		
WS1	67	32	51	35	185	1254
DS1	38	36	26	30	130	705
WS2	50	23	46	24	143	794
DS2	41	32	26	24	123	763
Total	196	123	149	113	581	3516

Results

Mycorrhization percentage and average number of pelotons

Mycorrhization percentage among the different development categories (juvenile and adults) was not significantly different (W=2.793, *df*=1 p=0.095). The mycorrhization percentage between seasons was significantly different (W=23.885, *df*=3, p<0.001) with higher percentages of mycorrhization in WS1 (41% in adult plants and 35% in juvenile plants) followed by WS2 and lastly DS1 and DS2 (32% and 26% in adult plants and 29% y 23% in juveniles) (Fig. 4A).

It is interesting to point out that between WS1 and WS2 a higher mycorrhization percentage was observed in WS1 (W=19.159, *df*=1, p<0.001). Comparing between DS1 and DS2, no significant differences in mycorrhization percentage were registered (Fig. 4A).

Rhynchostele cervantesii showed peloton average ranging between 5 and 9 per cut per plant. In development

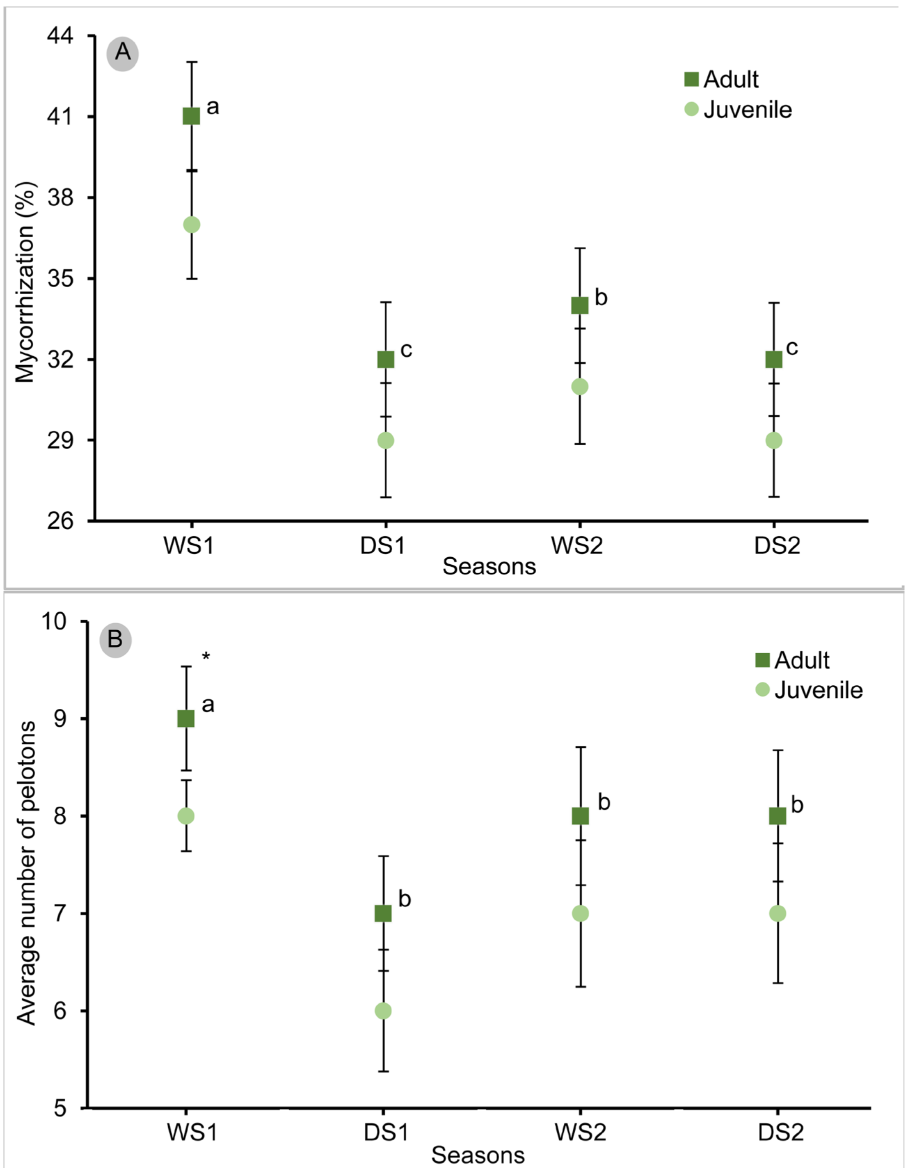

Figure 4: A. Mean percentage of mycorrhizal colonization in *Rhynchostele cervantesii* (La Llave & Lex.) Soto Arenas & Salazar, in different development categories, in wet and dry season; B. average number of pelotons per development categories, in *R. cervantesii* roots. WS1=Wet season 1, DS1=Dry season 1, WS2=Wet season 2, DS2=Dry season 2. Letters (a, b, c) represent significant differences α=0.05 between seasons; the asterisk (*) represents differences in development categories.

categories, in WS1 a higher quantity of pelotons was observed in adults than in juveniles (F1=5.02, p=0.028). The average of pelotons per plant registered in DS1, WS2 and DS2 was not significantly different between development categories (Fig. 4B).

Pelotons digestion state

Without considering development categories (adults and juveniles), the average of intact pelotons was significantly higher in WS1 (F1=139.42, p<0.001) and WS2 (F1=145.31, p<0.001), than in DS1 and DS2. There were no significant differences in the average of intact pelotons between WS1 and WS2 (F1=1.40, p=0.239). Meanwhile, between DS1 and DS2 the average of intact pelotons was significantly different (F1=10.75, p=0.001), being higher in DS1.

The average of digested pelotons differed significantly in different seasons with higher average in DS1 (F1=98.56, p<0.001) and DS2 (F1=107.72, p<0.001) compared to WS1 and WS2. Between WS1 and WS2 there were no significant differences in the average of digested pelotons (F1=1.87, p=0.173). In contrast, significant differences were observed between DS1 and DS2 (F1=29.59, p<0.001) with a higher average in DS2.

Considering the development categories, the average (± standard error) of intact pelotons in adult plants oscillated between 18 (±2.11) in WS2 and zero in DS2. The average of pelotons in digestion process was 11 (±1.06) in DS1 and one (±1.63) in WS2, and the average of digested pelotons varied between 14 (±1.01) in DS2 and zero in WS2 (Fig. 5A). For juvenile plants, the average number of intact pelotons in WS2 was 13 (±3.24) and zero in DS2. The average number of pelotons in digestion process was 10 (±1.15) in WS1 and DS2 and one (±1.73) in WS2. Lastly, the average number of digested pelotons differed in DS2 with 13 (±1.07) and zero in WS2 (Fig. 5B).

In reference to the digestion state of pelotons in different development categories, in WS1 a higher average of intact pelotons was observed in adult plants than in juveniles (F1=6.18, p=0.015). On the other hand, the average number of pelotons in digestion process and digested pelotons was not significantly different between development categories in this season. In DS1, WS2 and DS2 the average of pelotons between adult and juvenile plants was not significantly different in any of the pelotons digestion states.

Type of root (aerial or on substrate)

As for the type of root, the mycorrhization percentage was significantly higher in roots in contact with the substrate than in aerial roots (W=20.447, *df*=1, p<0.001) for all seasons. Mycorrhization percentage in roots in contact with substrate was 35% in DS2 and 26% in WS2. Aerial roots presented mycorrhization percentages of 32% in DS2 and 22% in WS2 (Fig. 6).

Mycorrhization in basal, medium and apical part of the root

The highest mycorrhization percentage was recorded in the basal part of the root (W=730.0067, *df*=2, p <0.001) at 49%, followed by the medium part at 38% and the apical part at 5%. Considering the different seasons, a higher mycorrhization percentage was observed in the basal part of the roots of *R. cervantesii*. In WS1, the mycorrhization percentage was 49%, it was 50% in DS1, 41% in WS2, and 55% in DS2. In contrast, the apical part of the root showed a lower mycorrhization percentage, with 7% in WS1, 5% in DS1, 2% in WS2, and 7% in DS2 (Fig. 7).

A higher average of intact pelotons in the basal part and medium part was recorded (F2=94.36, p<0.001) compared to the apical part of the root. Similarly, the average of pelotons in digestion process (F2=166.75, p<0.001) and digested pelotons (F2=87.36, p<0.001) was higher in the basal part, followed by the medium part and finally the apical root, which has the lowest average.

Discussion

Mycorrhization percentage

The average of mycorrhization percentages of *R. cervantesii* in the different seasons does not exceed 41% considering adult plants and juveniles. This matches the results obtained for *Bletia roezlii* Rchb.f., *B. punctata* La Llave & Lex., and *B. purpurata* A.Rich. & Galeotti, three species of terrestrial orchids, where the mycorrhization percentage did not exceed 40% in any case (Beltrán-Nambo et al., 2010).

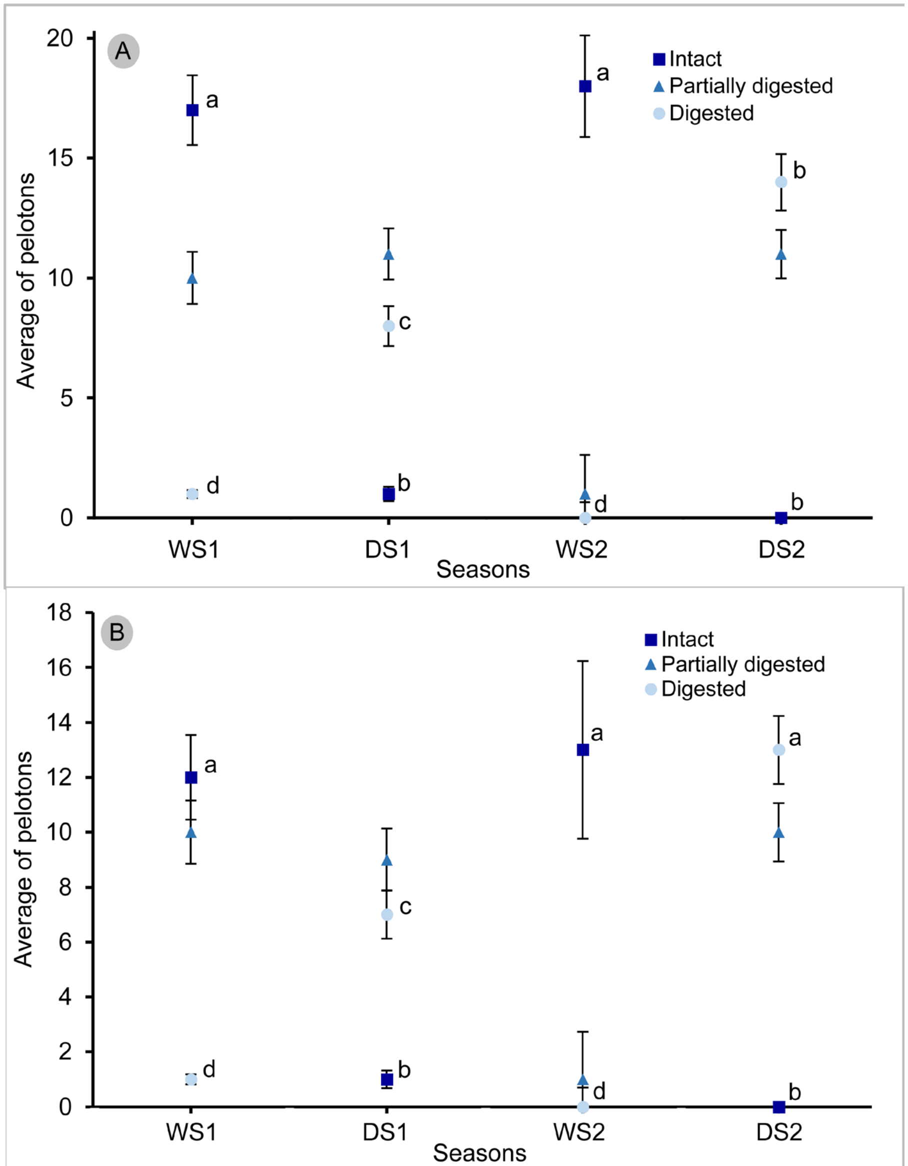

Figure 5: Pelotons average in different digestion states in *Rhynchostele cervantesii* (La Llave & Lex.) Soto Arenas & Salazar roots. A. adult plants; B. juvenile plants. WS1=Wet season 1, DS1=Dry season 1, WS2=Wet season 2 and DS2=Dry season 2. Dark blue squares represent the average of intact pelotons, middle blue triangles represent the average of partially digested process pelotons and light blue circles represent the average of digested pelotons. Letters (a, b, c, d) represent significant differences among peloton digestion stages across seasons (α=0.05). Error bars represent the standard error. N=100.

In contrast, for *Laelia autumnalis*, an epiphytic orchid, mycorrhization percentages of up to 76% were reported (Jiménez-Peña et al., 2018). Meanwhile, mycorrhization percentages of 22 epiphytic species ranged from 34% in *Luisia zeylanica* to 79% in *Acampe praemorsa* (Sathiyadash et al., 2012).

In this research, the mycorrhizal colonization in *R. cervantesii* presents seasonal fluctuations similar as reported for other epiphytic orchids (Pereira et al., 2005; Bertolini et al., 2014). Osorio-Gil et al. (2008) suggested that fluctuation in colonization in *Ionopsis utricularioides* (Sw.) Lindl. may be influenced by factors such as humidity, temperature, pH and substrate, which vary both in space and time, creating small microhabitats that affect distribution of fungal communities surrounding the plant.

For *R. cervantesii*, seasonal fluctuations in colonization could be related to decreases in annual precipitation, which was 626.3 mm in 2021, 586.7 mm in 2022

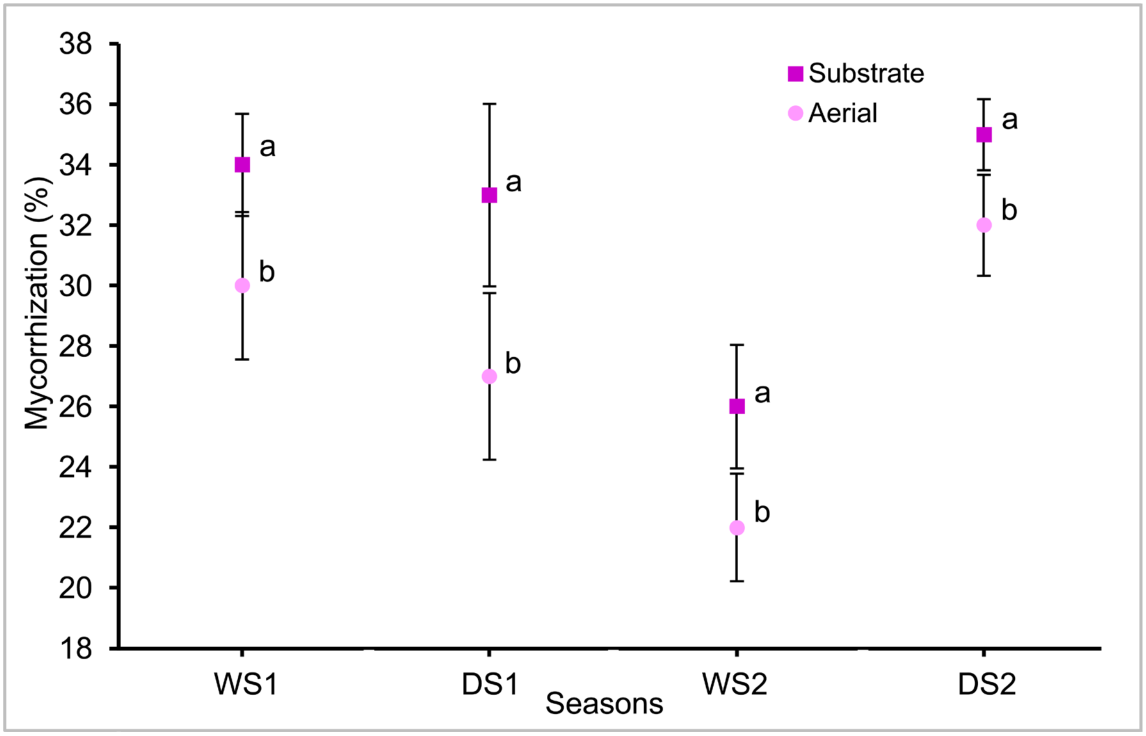

Figure 6: Mean percentage of mycorrhization in *Rhynchostele cervantesii* (La Llave & Lex.) Soto Arenas & Salazar. Dark pink squares represent roots in contact with substrate (Su) and light pink circles represent aerial roots (Ae) in different seasons; α=0.05. Letters (a, b) indicate significant differences between roots in contact with the substrate and aerial roots.

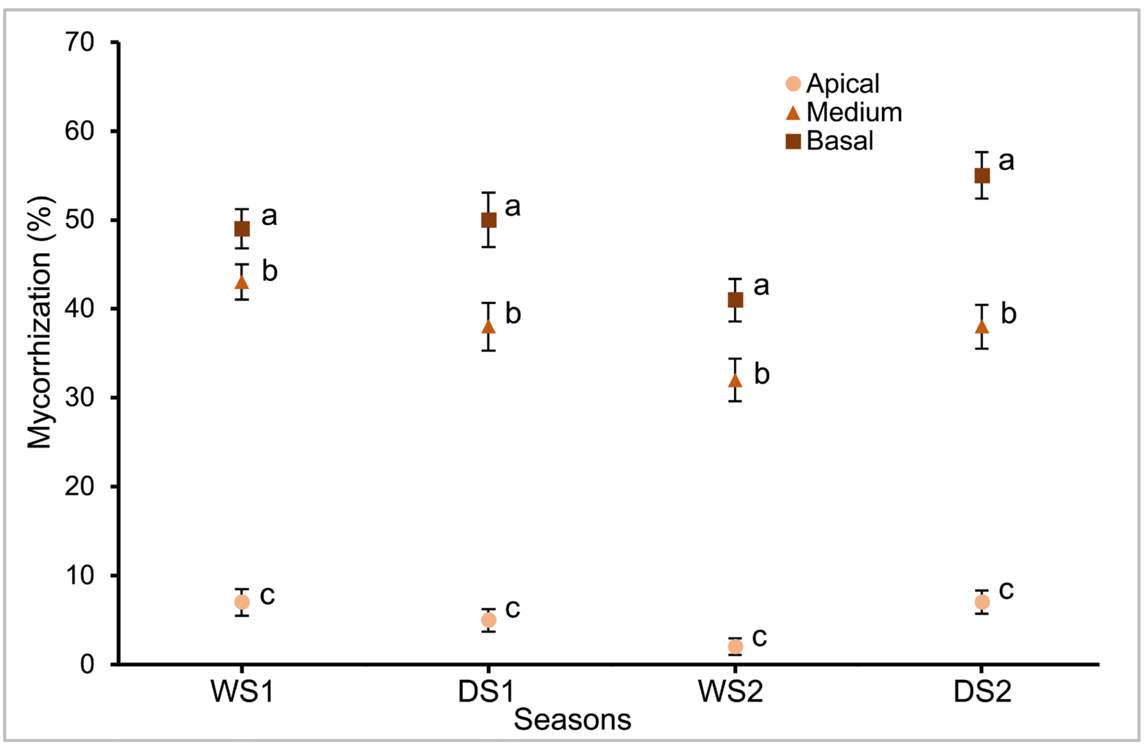

Figure 7: Mean percentage of mycorrhization in different positions of root cuts in *Rhynchostele cervantesii* (La Llave & Lex.) Soto Arenas & Salazar, in different seasons. Apex=apical part, medium=medium part, base=basal part of the root. Light brown squares represent mycorrhization in the apex, medium brown triangles represent mycorrhization in the medium part, dark brown circles represent mycorrhization in the basal part; α=0.05. Letters (a, b, c, d) represent significant differences between basal, apical and middle sections of the root. Error bars represent the standard error.

and 275.9 mm in 2023 (Meteostat, 2024). This reduction in water availability suggests a potential effect of climatic variability on the dynamics of mycorrhizal colonization in this species.

There were no differences in the mycorrhization percentage of *R. cervantesii* between adult and juvenile plants, similar to the results obtained for *Masdevallia coccinea* Linden ex Lindl., another epiphytic orchid, with juveniles at 67.8% and adults at 63.4% of mycorrhization percentages (Ordóñez et al., 2015). Mycorrhizal fungi were present in all development categories in these orchids, suggesting that this relationship is important during their whole life cycle and not only in the first stages of development. These results are consistent with those mentioned by Favre-Godal et al. (2020), who noted that in species such as *Arundina graminifolia* (D. Don) Hochr. and *Dendrobium exile* L.O. Williams, fungi isolated from adult roots do not promote seed germination but are associated with adult plants. This suggests that orchids in adult stages could depend on mycorrhizal association to obtain water and nutrients.

Average number of pelotons

In this research, the average number of pelotons per cut per plant for *R. cervantesii* oscillated between five and nine pelotons, similarly to the average reported for *Vanilla planifolia* Jacks., in Western Ghats (India) with an average of nine (Sathiyadash et al., 2012). In the present study a significantly higher average number of pelotons was observed in adult plants of *R. cervantesii* compared to juveniles in WS1. In the other seasons, there were no significant differences observed. Nonetheless, the same tendency of a higher number of pelotons in adult plants than in juveniles was registered. This contrasts with the results obtained for *Masdevallia coccinea*, with no significant differences between age categories (adults at 53.5% and juveniles at 60.05%), but a tendency of a higher average number of pelotons in the roots of juvenile plants (Ordóñez et al., 2015).

The average number of pelotons per cut per plant, as well as the average percentage of mycorrhization, were higher in WS1 compared to the other seasons. Probably, this was due to higher precipitation in WS1 than in WS2, while in both dry seasons, the humidity in the forest was low. The decrease in precipitation and a slight increase in temperature could be affecting this mycorrhizal relationship. This means that given the expected impact of continuing global warming, the future of *R. cervantesii,* like that of other orchids, will be uncertain.

It is important to note that mycorrhization occurs across all development categories of this epiphytic orchid. This underscores the critical role of mycorrhizal fungi in every stage of development. This reliance is particularly significant considering the inherent instability of water supply and nutrient availability in the epiphytic habitat (Ávila-Díaz et al., 2013; Zhang et al., 2016).

Pelotons in digestion process

Orchids obtain nutrients from mycorrhizal fungi by hyphae digestion (Sathiyadash et al., 2020). In this context, in *R. cervantesii* we found a higher quantity of intact pelotons (18) in wet seasons than in dry seasons (0), while digested pelotons were more abundant in dry seasons. This could be explained because in dry seasons, when conditions are more adverse, plants could be using more mycorrhizal fungi to obtain water and nutrients. Such results coincide with the ones reported for the terrestrial orchid *Gavilea araucana* and the epiphytes *Epidendrum stamfordianum*, *Erycina crista-galli* and *Stelis quadrifida* with higher quantity of intact pelotons in wet seasons compared to dry seasons (in *E. stamfordianum* and *S. quadrifida*, no intact pelotons were observed during the dry season (Durán et al., 2007; Bertolini et al., 2014)). Only *Erycina crista-galli* presented intact pelotons during this period, although in significantly lower quantities than in the wet season (Durán et al., 2007; Bertolini et al., 2014). Sathiyadash et al. (2012), in the Western Ghats (India), also reported a higher percentage of intact pelotons in epiphytic orchids from November to January (wet season), ranging from 12% in *Oberonia ensiformis* to 78% in *Acampe praemorsa*, than digested pelotons, which ranged from 6% in *Gastrochilus acaulis* (Lindl.) Kuntze to 45% in *Oberonia ensiformis*. The average number of digested pelotons indicates that *R. cervantesii* relies on mycorrhizal fungi for nutrition.

Furthermore, the absence of significant differences across development categories suggests that this symbiotic association is important in both juvenile and adult plants of the orchid.

This research determined that there were no significant differences between juvenile and adult plants in the average of pelotons in digestion process in *R. cervantesii* (10 and 11, respectively). These results are similar to those reported for the terrestrial orchid *Masdevallia coccinea* in which there were also no significant differences between juvenile and adult plants regarding pelotons in digestion process in juvenile (6.86%) and adult plants (3.03%). It is important to mention that these results support the hypothesis that mycorrhization plays a relevant role not only in the first development stages, but also in adult stages of epiphytic orchids (Meng et al., 2019; Sathiyadash et al., 2020; Petrolli et al., 2022).

Type of root (aerial or in substrate)

Rhynchostele cervantesii showed higher mycorrhization percentages in roots in contact with substrate (35%) than in aerial roots (22%). Similar results are reported for the epiphytic orchid *Laelia autumnalis,* which showed high colonization rates in roots embedded in organic matter, ranging from 6.94 to 100% (Jiménez-Peña et al., 2018). In *Erycina crista-galli* and *Stelis quadrifida*, both characterized by thin roots entirely in contact with the substrate, mycorrhizal colonization was reported to approach 100% (Bertolini et al., 2014). *Epidendrum stamfordianum*, which possesses thick roots with a significant proportion exposed to the air, exhibited 60% mycorrhizal colonization (Bertolini et al., 2014).

In addition, *Aerides ringens* Lindl., *Bulbophyllum tremulum* Rchb.f., *Epidendrum ibaguense* Kunth*, Pinalia polystachya* (L.) Kuntze (syn. *Eria polystachya* A. Rich.), *Gastrochilus calceolaris* (Rchb. f.) Kuntze, *Luisia birchea* Lindl., *Polystachya concreta* (Jacq.) Garay & H.R. Sweet and 22 other epiphytic species of Western Ghats (India) reported colonization in roots in contact with substrate, whereas in aerial roots no mycorrhization was found (Murugan et al., 2010; Sathiyadash et al., 2012). This is explained by the fact that colonization commonly begins at the apex of root hairs in contact with the substrate, where mycorrhizal fungi are found (Murugan et al., 2010; Sathiyadash et al., 2012).

Mycorrhization in basal, medium and apical part of the root

Higher mycorrhization percentages were found in the basal root of *R. cervantesii*, with an average of 49%. These results are similar to the ones reported for *Laelia autumnalis*, an epiphytic orchid with higher colonization in the basal part of the roots, showing a higher presence of digested pelotons ranging from 8.3% to 90.7% (Jiménez-Peña et al., 2018). Similarly, for *Rodriguezia granadensis* Rchb.f. a higher mycorrhization percentage (70%) was recorded in the basal part of the root, compared to the medium part (60%) and apical part (35%) (Romero-Salazar et al., 2022). In contrast, for *Cephalanthera falcata* (Thunb.) Blume and *C. erecta* (Thunb.) Blume*,* higher colonization percentages (41 and 72% respectively) were recorded in the apical part of the root. Meanwhile, for *Bletia punctata* La Llave & Lex., *Bletia roezlii*, *Bletia purpurata* A.Rich. & Galeotti and *Laelia furfuracea* Lindl. a higher mycorrhization percentage has been reported in the medium part (27.8, 37.0, 28.0, and 50%, respectively) (Matsuda et al., 2009; Beltrán-Nambo et al., 2010; García-Sánchez et al., 2024). This indicates that colonization of mycorrhizal fungi in orchid roots does not have the same pattern in all species. It has been suggested that the distribution pattern in the different root zones could be partly attributed to the stable growth in the basal and medium parts of the root, while the apical part is considered to be a zone of active growth (Bertolini et al., 2014).

Conclusions

In *R. cervantesii* mycorrhizal fungi were present under contrasting weather conditions and across development categories, suggesting that this symbiotic association is important throughout seasons and across two developmental stages. Mycorrhization in *R. cervantesii* was related to precipitation, as the percentage of mycorrhization and the quantity of intact pelotons were higher in wet seasons than in dry seasons. Digested pelotons presented higher values in dry seasons than in wet seasons suggesting that, in adverse conditions, plants could rely more on mycorrhizal fungi to obtain nutrients. Mycorrhization percentages were higher in roots in contact with substrate than in aerial roots, similar to what has been observed in other species. Mycorrhization distribution throughout the roots in *R. cervantesii* presented higher percentages in the basal part, followed by the medium part and finally in the apical part.

Based on the results of this research, including the percentage of mycorrhization and the quantity and type of pelotons in *R. cervantesii*, it can be inferred that the availability of water has an impact on the mycorrhizal association, suggesting that climate change will likely affect this association, with potential implications for the conservation of this orchid.

Author contributions

Conceptualization: IAD; Methodology: IAD, REML; Investigation: REML, IAD; Validation: REML, IAD; Formal analysis: REML; Resources: IAD, REML; Data curation: REML; Visualization: REML; Writing - original draft: REML, IAD, RGO; Writing - review and editing: REML, IAD, RGO; Supervision: IAD; Project administration: IAD.

Funding

This research was funded by the Coordinación de la Investigación Científica (CIC) of Universidad Michoacana de San Nicolás de Hidalgo through the project “Mycorrhizal association and *in vitro* culture of epiphytic orchids in risk, from Tenderio’s land in Indigenous Community of Santiago Tingambato, Michoacán, Mexico”, that IAD is responsible for.

Acknowledgements

To the Indigenous Community of Santiago Tingambato for providing access to carry out this research on the land of Tenderio and their availability to receive environmental education workshops. To M. Sales Figueroa and Jorge de J. Arriaga Cardona for their help in the field. To J. Tonatiuh Gutierrez Zavala for his help in editing some figures. To the Biology Faculty of the Universidad Michoacana de San Nicolás de Hidalgo for the support provided.

Data Availability Statement

The dataset that supports the results of this study was published in SciELO Data and can be accessed at: https://doi.org/10.48331/SCIELODATA.NEZTPY

Literature cited

Ávila-Díaz, I. 2007. Biología de poblaciones de *Laelia speciosa* (HBK) Schltr. (Orchidaceae) para su manejo y conservación. Tesis de doctorado. Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México. México, D.F., México. 162 pp.

Ávila-Díaz, S. and F. Chávez-Melitón. 2022. Certificate of decree of the Voluntary Conservation Area at the site known as “Tenderio”. Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). Cd. Mx. Mexico.

Ávila-Díaz, I., R. Garibay-Orijel, R. E. Magaña-Lemus and K. Oyama. 2013. Molecular evidence reveals fungi associated within the epiphytic orchid *Laelia speciosa* (HBK) SCHLTR. Botanical Sciences 9(4): 523-529. DOI: https://doi.org/10.17129/botsci.429

Bayman, P., E. J. González, J. J. Fumero and R. L. Tremblay. 2002. Are fungi necessary? How fungicides affect growth and survival of the orchid *Lepanthes rupestris* in the field. Journal of Ecology 90(6): 1002-1008. DOI: https://doi.org/10.1046/j.1365-2745.2002.00733.x

Beltrán-Nambo, M. A., Y. Carreón-Abud and M. P. Ortega-Larrocea. 2010. Mycorrhizal colonization patterns of three *Bletia* species in a natural forest reserve in Michoacán, México. International Orchid Symposium 878: 43-52.

Bertolini, V., J. Cruz-Blasi, A. Damon and J. Valle-Mora. 2014. Seasonality and mycorrhizal colonization in three species of epiphytic orchids in southeast Mexico. Acta Botanica Brasilica 28(4): 512-518. DOI: https://doi.org/10.1590/0102-33062014abb3436

Bidartondo, M. I. and D. J. Read. 2008. Fungal specificity bottlenecks during orchid germination and development. Molecular Ecology 17(16): 3707-3716. DOI: https://doi.org/10.1111/j.1365-294X.2008.03848.x

Cervantes-Uribe, J. 2018. Efecto de la perturbación antrópica en la ecología de *Rhynchostele cervantesii* (Orchidaceae) en un Bosque Mesófilo de Montaña Tingambato, Michoacán, México. Tesis de maestría. Facultad de Biología, Universidad

Michoacana de San Nicolás de Hidalgo. Morelia, Michoacán, Mexico. 79 pp.

Cevallos, S., A. Sánchez-Rodríguez, C. Decock, S. Declerck and J. P. Suárez. 2017. Are there keystone mycorrhizal fungi associated to tropical epiphytic orchids? Mycorrhiza 27: 225-232. DOI: https://doi.org/10.1007/s00572-016-0746-8

Chang, D. C. N. 2008. Research and Application of Orchid Mycorrhiza in Taiwan. Acta Horticulturae 766: 299-306. DOI: https://doi.org/10.17660/ActaHortic.2008.766.39

Cozzolino, S. and A. Widmer. 2005. Orchid diversity: an evolutionary consequence of deception? Trends in Ecology & Evolution 20(9): 487-494. DOI: https://doi.org/10.1016/j.tree.2005.06.004

Cruz-Higareda, J. B., B. S. Luna-Rosales and A. Barba-Alvarez. 2015. A novel seed baiting technique for the epiphytic orchid *Rhynchostele cervantesii*, a means to acquire mycorrhizal fungi from protocorms. Lankesteriana 15(1): 67-76.

Currah, R. S., C. D. Zelmer, S. Hambleton and K. A. Richardson. 1997. Fungi from orchid mycorrhizas. In: Arditti, J. and A. M. Pridgeon (eds.). Orchid Biology: Reviews and Perspectives. Kluwer Academic Publishers. Boston, USA. Pp. 117-170.

Dearnaley, J. D. W. 2007. Further advances in orchid mycorrhizal research. Mycorrhiza 17: 475-486. DOI: https://doi.org/10.1007/s00572-007-0138-1

Dearnaley, J. D. W., S. Perotto and M. A. Selosse. 2016. Structure and development of orchid mycorrhizas. In: Martin, M. G. and D. J. Read (eds.). Molecular mycorrhizal symbiosis. Wiley-Blackwell. Oxford, UK. pp. 63-86. DOI: https://doi.org/10.1002/9781118951446.ch5

Domínguez-Gil, I. 2015. Listado y caracterización ecológica de las orquídeas epifitas del predio de Tenderio, de la Comunidad Indígena de Santiago Tingambato, Michoacán, México. Tesis de licenciatura. Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo. Morelia, Michoacán, México. Pp. 53.

Durán, C., M. Rivero and P. Seemann. 2007. Identificación de endomicorrizas en la orquídea nativa *Gavilea araucana* (Phil.) Correa. Agro sur 35(2): 67-69. DOI: https://doi.org/10.4206/agrosur.2007.v35n2-32

Egidi, E., T. W. May and A. E Franks. 2018. Seeking the needle in the haystack: undetectability of mycorrhizal fungi outside of the plant rhizosphere associated with an endangered Australian orchid. Fungal Ecology 33: 13-23. DOI: https://doi.org/10.1016/j.funeco.2018.01.002

Espejo, S. A., C. J. García, F. A. R. López, R. Jiménez-Machorro and L. Sánchez-Saldaña. 2002. Orquídeas del Estado de Morelos. Herbario AMO. Mexico, D.F., México. 158 pp.

Favre-Godal, Q., L. Gourguillon, S. Lordel-Madeleine, K. Gindro and P. Choisy. 2020. Orchids and their mycorrhizal fungi: an insufficiently explored relationship. Mycorrhiza 30: 5-22. DOI: https://doi.org/10.1007/s00572-020-00934-2

García-Sánchez, C., J. Pérez-Moreno, J. J. Almaraz-Suárez, J. Delgadillo-Martínez and V. M. Cetina-Alcalá. 2024. Distribución de la colonización micorrízica en las raíces de la orquídea Neotropical *Laelia furfuracea* Lindl. Caldasia 46(2): 339-349. DOI: https://doi.org/10.15446/caldasia.v46n2.96265

Hágsater, E., M. A. Soto, G. A. Salazar, R. Jiménez, M. A. López and R. L. Dressler. 2005. Las orquídeas de México. Instituto Chinoin, A.C. Cd. Mx., México. 304 pp.

INAFED. 2010. Enciclopedia de los municipios de México: Tingambato, Michoacán. Instituto Nacional para el Federalismo y el Desarrollo (INAFED). http://www.inafed.gob.mx (consulted April, 2024).

INEGI. 2005. Serie III. Mapa de tipos de vegetación. Instituto de Estadística Geografía e Informática (INEGI). Aguascalientes, México. https://www.inegi.org.mx/temas/usosuelo/ (consulted November, 2021).

Jacquemyn, H., M. Waud, R. Brys, F. Lallemand, P. E. Courty, A. Robionek and M. A. Selosse. 2017. Mycorrhizal associations and trophic modes in coexisting orchids: an ecological continuum between auto-and mixotrophy. Frontiers in Plant Science 8: 1497. DOI: https://doi.org/10.3389/fpls.2017.01497

Jiménez-Peña, N., M. Sandoval-Villa, V. H. Volke-Haller, M. E. Pedraza-Santos and E. Fernández-Herrera. 2018. Colonización micorrízica de *Laelia autumnalis* (La Llave & Lex.) Lindl. Ecosistema y Recursos Agropecuarios 5(15): 547-553. DOI: https://doi.org/10.19136/era.a5n15.1756

Khamchatra, N., K., Dixon, K. Chayamarit, S. Apisitwanich and S. Tantiwiwat. 2016. Using *in situ* seed baiting technique to isolate and identify endophytic and mycorrhizal fungi from seeds of a threatened epiphytic orchid, *Dendrobium* *friedericksianum* Rchb.f. (Orchidaceae). Agriculture and Natural Resources 50(1): 8-13. DOI: https://doi.org/10.1016/j.anres.2016.01.002

Martos, F., F. Munoz, T. Pailler, I. Kottke, C. Gonneau and M. A. Selosse. 2012. The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. Molecular Ecology 21(20): 5098-5109. DOI: https://doi.org/10.1111/j.1365-294X.2012.05692.x

Matsuda, Y., A. Amiya and S. Ito. 2009. Colonization patterns of mycorrhizal fungi associated with two rare orchids, *Cephalanthera falcata* and *C. erecta*. Ecological Research 24(5): 1023-1031. DOI: https://doi.org/10.1007/s11284-008-0575-0

McCormick, M. K. and H. Jacquemyn. 2014. What constrains the distribution of orchid populations? New Phytologist 202(2): 392-400. DOI: https://doi.org/10.1111/nph.12639

McCormick, M. K., D. F. Whigham and A. Canchani-Viruet. 2018. Mycorrhizal fungi affect orchid distribution and population dynamics. New Phytologist 219(4): 1207-1215. DOI: https://doi.org/10.1111/nph.15223

Meng, Y. Y., W. L. Zhang, M. A. Selosse and J. Y. Gao. 2019. Are fungi from adult orchid roots the best symbionts at germination? A case study. Mycorrhiza 29(5): 541-547. DOI: https://doi.org/10.1007/s00572-019-00907-0

Meteostat. 2024. Meteostat: Historical weather and climate data. https://dev.meteostat.net/ (consulted November, 2021).

Murugan, T., K. Sathiyadash, V. Muniappan and T. Muthukumar. 2010. The mycorrhizal status of south indian epiphytic orchids. The Journal of the Orchid Society of India 24(1-2): 29-33.

Novotná, A., Á. Benítez, P. Herrera, D. Cruz, E. Filipczyková and J. P. Suárez. 2018. High diversity of root-associated fungi isolated from three epiphytic orchids in southern Ecuador. Mycoscience 59(1): 24-32. DOI: https://doi.org/10.1016/j.myc.2017.07.007

Ordóñez, N. F., J. T. Otero-Ospina and L. A. Díaz. 2015. Interacciones Micorrízicas de *Masdevallia coccinea* Linden ex Lindl. Orquideología 31(2): 123-135.

Osorio-Gil, E. M., J. Forero-Montaña and J. T. Otero. 2008. Variation in mycorrhizal infection of the epiphytic orchid *Ionopsis utriculariodes* (Orchidiaceae) on different substrata. Caribbean Journal of Science 44(1): 130-132. DOI: https://doi.org/10.18475/cjos.v44i1.a15

Otero, J. T., P. Bayman and J. D. Ackerman. 2005. Variation in mycorrhizal performance in the epiphytic orchid *Tolumnia variegata in vitro*: the potential for natural selection. Evolutionary Ecology 19: 29-43. DOI: https://doi.org/10.1007/s10682-004-5441-0

Otero, J. T., P. H. Thrall, M. Clements, J. J. Burdon and J. T. Miller. 2011. Codiversification of orchids (Pterostylidinae) and their associated mycorrhizal fungi. Australian Journal of Botany 59(5): 480-497. DOI: https://doi.org/10.1071/BT11053

Parthibhan, S., M. V. Rao and T. S. Kumar. 2017. Culturable fungal endophytes in shoots of *Dendrobium aqueum* Lindley – An imperiled orchid. Ecological Genetics and Genomics 3-5: 18-24. DOI: https://doi.org/10.1016/j.egg.2017.06.004

Pereira, O. L., M. C. M. Kasuya, A. C. Borges and E. F. Araújo. 2005. Morphological and molecular characterization of mycorrhizal fungi isolated from neotropical orchids in Brazil. Canadian Journal of Botany 83(1): 54-65. DOI: https://doi.org/10.1139/b04-151

Petrolli, R., L. Zinger, B. Perez-Lamarque, G. Collobert, C. Griveau, T. Pailler, M. C. Selosse and F. Martos. 2022. Spatial turnover of fungi and partner choice shape mycorrhizal networks in epiphytic orchids. Journal of Ecology 110(11): 2568-2584. DOI: https://doi.org/10.1111/1365-2745.13986

Qin, J., Z. Wei, G. Zai-Wei and Z. Shi-Bao. 2019. Molecular identifications uncover diverse fungal symbionts of *Pleione* (Orchidaceae). Fungal Ecology 37: 19-29. DOI: https://doi.org/10.1016/j.funeco.2018.10.003

Rammitsu, K., T. Yukawa, Y. Yamashita, S. Isshiki and Y. Ogura-Tsujita. 2020. The mycorrhizal community of the epiphytic orchid *Thrixspermum japonicum* is strongly biased toward a single Ceratobasidiaceae fungus, despite a wide range of fungal partners. American Journal of Botany 107(12): 1654-1662. DOI: https://doi.org/10.1002/ajb2.1575

Rasmussen, H. N. 2002. Recent developments in the study of orchid mycorrhiza. Plant and Soil 244: 149-163. DOI: https://doi.org/10.1023/A:1020246715436

Rasmussen, H. N., K. W. Dixon, J. Jersáková and T. Těšitelová. 2015. Germination and seedling establishment in orchids: a complex of requirements. Annals of Botany 116(3): 391-402. DOI: https://doi.org/10.1093/aob/mcv087

Rasmussen, H. N. and F. N. Rasmussen. 2018. The epiphytic habitat on a living host: reflections on the orchid–tree relationship.

Botanical Journal of the Linnean Society 186(4): 456-472. DOI: https://doi.org/10.1093/botlinnean/box085

Rasmussen, H. N. and D. F. Whigham. 2002. Phenology of roots and mycorrhiza in orchid species differing in phototrophic strategy. New Phytologist 154(3): 797-807. DOI: https://doi.org/10.1046/j.1469-8137.2002.00422.x

Romero-Salazar, N. C., J. M. Galvis-Gratz and J. P. Moreno-López. 2022. Hongos formadores de micorrizas aislados a partir de raíces de la orquídea *Rodriguezia granadensis* (LINDL.) RCHB. F. Revista UDCA Actualidad & Divulgación Científica 25(1): e2086. DOI: http://doi.org/10.31910/rudca.v25.n1.2022.2086

SAS Institute Inc. 2013. JMP® 11 Statistical Discovery Software. SAS Institute Inc. Cary, USA.

Sathiyadash, K., T. Muthukumar, E. Uma and R. R. Pandey. 2012. Mycorrhizal association and morphology in orchids. Journal of Plant Interactions 7(3): 238-247. DOI: http://dx.doi.org/10.1080/17429145.2012.699105

Sathiyadash, K., T. Muthukumar, V. Karthikeyan and K. Rajendran. 2020. Orchid Mycorrhizal Fungi: Structure, Function, and Diversity. In: Khasim, S., S. Hegde, M. González-Arnao, K. Thammasiri (eds.). Orchid Biology: Recent Trends & Challenges. Springer. Singapore, China. Pp. 239-280. DOI: https://doi.org/10.1007/978-981-32-9456-1_13

Selosse, M. A., B. Boullard and D. Richardson. 2011. Noël Bernard (1874-1911): orchids to symbiosis in a dozen years, one century ago. Symbiosis 54(2): 61-68. DOI: https://doi.org/10.1007/s13199-011-0131-5

Selosse, M. A., R. Bauer and B. Moyersoen. 2002. Basal hymenomycetes belonging to the Sebacinaceae are ectomycorrhizal on temperate deciduous trees. New Phytologist 155(1): 183-195. DOI: https://doi.org/10.1046/j.1469-8137.2002.00442.x

Selosse, M. A. and F. Martos. 2014. Do chlorophyllous orchids heterotrophically use mycorrhizal fungal carbon? Trends in Plant Science 19(11): 683-685. DOI: https://doi.org/10.1016/j.tplants.2014.09.005

SEMARNAT. 2010. NORMA Oficial Mexicana NOM-059-SEMARNAT-2010. Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Secretaría del Medio Ambiente y Recursos Naturales. Diario Oficial de la Federación. Cd. Mx., México. http://dof.gob.mx/nota_detalle.php?codigo=5173091&fecha=30/12/2010 (consulted November, 2021).

Shubha, J. and C. Srinivas. 2017. Diversity and extracellular enzymes of endophytic fungi associated with *Cymbidium aloifolium* L. African Journal of Biotechnology 16(48): 2248-2258. DOI: https://doi.org/10.5897/AJB2017.16261

Smith, S. E. and D. J. Read. 2010. Mycorrhizal Symbiosis. 3rd. Academic Press. Cambridge, UK. 1377 pp.

SNIB. 2016. Orquídea Lirio (*Rhynchostele cervantesii*). Naturalista-Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). http://www.naturalista.mx/taxa/206214-Rhynchostele-cervantesii (consulted April, 2022).

Soto-Arenas, M. A. and A. R. Solano-Gómez. 2007. Ficha técnica de *Rhynchostele cervantesii*. In: Soto-Arenas, M. A. (ed.). Información actualizada sobre las especies de orquídeas del PROY-NOM-059-ECOL-2000. Instituto Chinoín & Herbario de la Asociación Mexicana de Orquideología, Mexico. Bases de datos SNIB-CONABIO. Proyecto No. W029. http://www.conabio.gob.mx/conocimiento/ise/fichasnom/Rhynchostelecervantesii00.pdf (consulted February, 2022).

Teixeira da Silva, J. A., G. B. Kerbauy, S. Zeng, Z. Chen and J. Duan. 2014. In vitro flowering of orchids. Critical Reviews in Biotechnology 34(1): 56-76. DOI: https://doi.org/10.3109/07388551.2013.807219

Tremblay, R. L. 2000. Plant longevity in four species of *Lepanthes* (Pleurothallidinae; Orchidaceae). Lindleyana 15(4): 257-266.

Tremblay, R. L. and J. D. Ackerman. 2001. Gene flow and effective population size in *Lepanthes* (Orchidaceae): a case for genetic drift. Biological Journal of the Linnean Society 72(1): 47-62. DOI: https://doi.org/10.1111/j.1095-8312.2001.tb01300.x

Waud, M., R. Brys, W. V. Landuyt, B. Lievens and H. Jacquemyn. 2017. Mycorrhizal specificity does not limit the distribution of an endangered orchid species. Molecular Ecology 26(6): 1687-1701. DOI: https://doi.org/10.1111/mec.14014

Wu, P. H., D. D. Huang and D. C. N. Chang. 2011. Mycorrhizal symbiosis enhances *Phalaenopsis* orchid's growth and resistance to *Erwinia chrysanthemi*. African Journal of

Biotechnology 10(50): 10095-10100. DOI: https://doi.org/10.5897/AJB11.1310

Yeh, C. M., K. Chung, C. K. Liang and W. C. Tsai. 2019. New insights into the symbiotic relationship between orchids and fungi. Applied Sciences 9(3): 585. DOI: https://doi.org/10.3390/app9030585

Zettler, L. W. and L. L. Corey. 2018. Orchid Mycorrhizal Fungi: Isolation and Identification Techniques. In: Lee, Y. I. and E. T. Yeung (eds.). Chapter 2, Orchid Propagation: From Laboratories to Greenhouses—Methods and Protocols. Springer Protocols Handbooks. Humana Press. New York, USA. Pp. 27-58. DOI: https://doi.org/10.1007/978-1-4939-7771-0_2

Zettler, L. W., L. Rajaovelona, K. Yokoya, J. P. Kendon, A. L. Stice, A. E. Wood and W. Sarasan. 2017. Techniques for the collection, transportation, and isolation of orchid endophytes from afar: a case study from Madagascar. Botanical Studies 58: 54. DOI: https://doi.org/10.1186/s40529-017-0209-3

Zhang, W., H. Hu and S. B. Zhang. 2016. Divergent adaptive strategies by two co-occurring epiphytic orchids to water stress: escape or avoidance? Frontiers in Plant Science 588(7): 1-11. DOI: https://doi.org/10.3389/fpls.2016.00588

Zotz, G. and P. Hietz. 2001. The physiological ecology of vascular epiphytes: current knowledge, open questions. Journal of Experimental Botany 52(364): 2067-2078. DOI: https://doi.org/10.1093/jexbot/52.364.2067