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REGULAR PAPER

Reproduction of *Oncidium poikilostalix* (Orchidaceae), potentially invading coffee plantations in Soconusco, Chiapas, México

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Context – *Oncidium poikilostalix* (Kraenzl.) M.W.Chase & N.H.Williams (Orchidaceae) was first reported in Mexico in 2008 and grows on coffee bushes in shaded plantations in Soconusco region in southeast Mexico.
Goal – To study the reproductive characteristics and the endophytic fungi with which this orchid is associated to evaluate current conservation status, its possible influence on other epiphytic orchids sharing the same coffee agroecosystem and identify the morphological and adaptive response that have permitted successful colonization of this human landscape.
Methods – We determined flower production and percent fruit set, percent loss of seeds due to mechanical or biological damage and total seed rain in two populations, during two years (2008–2009). We characterized morphologically the seed of *O. poikilostalix* and isolated the fungi associated with its roots.
Results – Rapid growth and development of the populations were observed at various levels: recruitment and survival levels were high, with many more adult plants and flowers found in both populations in the second year. Combined percent pollination rose from 1.82% in 2008 to 3.37% in 2009, resulting in the production of 3,387,468 seeds in 2008, rising to 10,001,096 in 2009. We isolated and identified to genus level, thirteen taxa of fungi, including various strains of *Rhizoctonia* sp.
Conclusions – *O. poikilostalix* interacts with a variety of mycorrhizal symbionts in new habitats. *O. poikilostalix* is a vigorous and weedy species which should be monitored to prevent it from displacing native, rare and slower growing species, such as *Oncidium guatemalenoides* M.W.Chase & N.H.Williams, growing in coffee plantations in southeast México.

Key words – Reproduction, *Oncidium poikilostalix*, Orchidaceae, coffee plantations, flowering, pollination, fruit set, seed rain, mycorrhizal fungi, invasive species.

INTRODUCTION

México has a rich and diverse orchid flora with approximately 1,150 species distributed within 146 genera (Espejo & López-Ferrari 2001, Hágsater et al. 2005) and estimates suggest that the final figure may reach 1,400 species (Hágsater et al. 2005). At least 213 species (18.52%) have been found in the extensive coffee plantations mainly located in the states of Chiapas, Veracruz and Oaxaca where orchid-rich cloud forest has been replaced by coffee (Espejo et al. 2005). Recent studies suggest that many more orchid species have found refuge in coffee plantations as their natural habitats have become depleted and fragmented and that these plantations function as biological corridors between the few remaining patches of natural forest ecosystems (Damon 2011).

The epiphytic orchid *Oncidium poikilostalix* (Kraenzl.) M.W.Chase & N.H.Williams (fig. 1A–C) is native to Guatemala and Costa Rica (Behor & Tinschert 1998), and was reported for the first time in Mexico in 2008 (Solano et al. 2011). Small populations were found growing in coffee plantations at approximately 1,400 m a.s.l. in two sites within the Tacaná-Boquerón Biological Corridor (Soconusco Region, Chiapas) (Solano et al. 2011), which is one of the three most orchid species-rich areas in Mexico (Soto-Arenas 1994, Salazar & Hágsater 1997, Soto-Arenas 2001) and is connected to the Mesoamerican Biological Corridor.

Figure 1 – *Oncidium poikilostalix*: A, flowers; B, plant; C, plant with fruits.

Although these plantations have a predominance of introduced, monospecific shade trees ["Chalum"; *Inga micheliana* Harms (Mimosaceae)] with few remaining native tree species, the otherwise favourable environmental conditions and low impact management of the coffee make for suitable conditions especially for smaller orchid species such as *O. poikilostalix*, *Erycina crista-galli* (Rchb.f.) N.H.Williams & M.W.Chase, *Leochilus labiatus* (Sw.) Kuntze, *L. oncidioides* Knowles & Westc., *L. scriptus* (Sw.) Rchb.f., *Notylia barkeri* Lindl., and *Ornithocephalus tripterus* Schltr., adapted to growing on coffee bushes (Hágsater et al. 2005, Damon & Valle-Mora 2008, Pérez-Hernández 2009, García-González et al. 2011).

Generally orchids depend upon specific animals for pollination, which may limit the possibilities of colonizing new areas (Ackerman & Montalvo 1990, Johnson & Brown 2004, Hágsater et al. 2005). The principal pollinators of orchids are bees and flies, but many orchids are pollinated by wasps, butterflies, moths and humming birds (Ollerton 1999, Johnson & Brown 2004, Hágsater et al. 2005). The literature and personal observations suggest that *O. poikilostalix* offers an oil reward to its pollinators (Pupulin et al. 2008), but there is no mention in the literature of which species pollinate this orchid. Bees that specialize in the collection of oils can be found in four families (Melittidae, Ctenoplectridae, Anthophoridae and Apidae) and fifteen genera (Buchmann 1987) and the oil is used for nest construction and/or to provision larval cells.

The number of adult or reproductive plants in a population is important, especially for orchids (Mondragón 2009) which typically have a tendency towards very low fruit set, in which case the longevity of the plant and the production of very large numbers of seeds per fruit can be seen as compensatory factors.

The total population of individuals of *O. poikilostalix* in the two study sites was 1,359 in 2008, of which 30.46% were adults, and there were more plants in 2009, of which 37.06% were adults (García-González et al. 2011). The microsites preferred by this orchid are the branches of the coffee bushes (55.18% of the plants found on coffee phorophytes) and rarely the trunks of the shade trees (García-González et al. 2011).

Every orchid capsule may contain thousands or even millions of seeds, of which the vast majority fail to become mature plants. In most cases it is probably due to the seed falling upon an inhospitable substrate, or, if it falls on a suit-

able substrate, then failing to concur with a suitable mycorrhizal fungal symbiont. The dependence of orchids upon a particular type of endomycorrhizal fungi, especially during the first stages of life (Popoff 2008, Kennedy 2009) is an important component of the biology of these plants (Arditti 1992, Gravendeel et al. 2004, Mujica et al. 2010) and may limit and determine distribution patterns (Clements 1987, Mujica et al. 2010) as well as the rates of recruitment of new individuals into a population (Mujica et al. 2010).

Considering that *O. poikilostalix* is a species new to Mexico, presently with a limited distribution, we chose to study the reproductive characteristics of this orchid and the endophytic fungi with which it is associated, to evaluate its possible influence on other epiphytic orchids sharing the same coffee agroecosystem and identify the morphological and adaptive response that have permitted successful colonization of this human landscape.

MATERIALS AND METHODS

Study sites

The study was carried out in six plots, each measuring 625 m² (25 × 25 m; 0.0625 ha), in two coffee plantations in the region of Soconusco in the extreme southeast tip of México bordering Guatemala. Fracción Montecristo (FM), plots 1, 2, 3: 15°5'31.5"N 92°9'57.9"W; Benito Juárez El Plan (BJ), plots 4, 5, 6: 15°5'15.4"N 92°8'54.7"E.

The coffee plantations FM and BJ are approximately 15–20 years old, situated at 1,410 m and 1,440 m a.s.l., respectively, and separated by a distance of 2.5 km. The plantations are composed of arabica coffee bushes (*Coffea arabica* L.; Rubiaceae), shade trees, the majority of which are "chalum" (*Inga micheliana*), and occasional fruit and timber trees, some of which are exotic, and may include *Cedrela mexicana* Roem. (Meliaceae), *Citrus* sp. (Rutaceae), *Nectandra* sp. (Lauraceae), *Inga lauriana* (Sw.) Willd. (Mimosaceae), *Trema micrantha* (L.) Blume (Ulmaceae) and *Vernonia deppeana* Less. (Asteraceae). No agrochemical products are applied to either plantation, weeds are controlled twice a year with machete and both coffee bushes and shade trees are pruned annually before the onset of the rains in May. Unlike many coffee producers in the region, the farmers do not eliminate mosses and other epiphytes that tend to grow on the trunks and thicker branches of the coffee bushes.

Flowering, fructification and pollination rate

In each of the six plots, all flowers and all seed capsules (fruits) were counted in 2008 and 2009. From those data, the percentage of pollination was calculated: (number of capsule obtained × 100) / total flowers produced.

Two sites in FM with abundant individuals of *O. poikilostalix* were chosen for the observation of pollinators, visiting adult plants (sexually mature plants, García-González et al. 2011). In one site flowers were more exposed and in the other they were hidden within the surrounding vegetation. These two sites were monitored for pollinator visits, by direct observation, on alternate days, for a total of fourteen days (7 days each site), from 7.00 to 12.00 h.

Seed rain

To determine an average number of seeds per seed capsule, two small, two medium and two large capsules were used. In the laboratory, the total seed content was extracted from each capsule and individually weighed. Then, 200 seeds from each capsule were taken and also individually weighed. From that data, the approximate number of seeds per capsule was calculated (200 × TWS: W200S; where TWS is the Total Weight of Seeds and W200S is the Weight of 200 Seeds) and an average was calculated from the sum of the six capsules. From there the total seed rain per plot was calculated for years 2008 and 2009. The capsules showing signs of mechanical or biological damage were counted separately, to obtain the total loss of seeds per plot in both years.

Morphological characterization of the seeds

Dry seeds of *O. poikilostalix* were characterized using a scanning electron microscope (TOPCON, model SM-510, with 10kv acceleration), for general taxonomic interest as a reproductive structure, and to determine whether they have structures that facilitate adherence to the relatively smooth bark of the branches of the coffee bushes. We used eighteen seeds, which were dispersed upon double sided carbon tape and then covered with silver to a depth of approximately 20 nm, using a gold-palladium metal depositor (DENTON VACCUM, model DESK II). Photographs of the images obtained were taken using the image processor Orión 6.5.

Detection, isolation and identification of mycorrhizal fungi in orchid roots

Three juvenile plants of *O. poikilostalix* were selected at random in each of the two study sites, including all microsites, except branch forks.

Transverse sections measuring 1 cm were made in the roots, which were then cut and open longitudinally. The roots, covered by a solution of 10% KOH, were submitted to a pressure of 10 psi for 10 min in a pressure cooker and then washed in running tap water. Alkaline hydrogen peroxide was added for 20 min with agitation and the roots were then washed again in running water. A 1% solution of HCL was added to the roots for 4 min and then poured off.

Using different root samples each time, the colorants Tryptan Blue 0.5% or Fuchsin Acid 1% were added so as to cover the samples and were left to stain the roots for 10min at a pressure of 10 psi. The excess colorant was poured off and the roots were washed with running water and then covered with a solution of acetoglycerol (40% distilled water 40%; 60% acetic acid and glycerol 2: 2.5) (Kormanik & Mcgraw 1982).

Finally, the root sections were mounted and sealed on glass microscope slides for observation.

To obtain the root samples, sections (9 cm^2, 3 × 3 cm) of surface bark were excised from areas of phorophytes where well-differentiated juvenile plants of *O. poikilostalix*, measuring less than 2 cm, were present (García-González et al. 2011). In the case of samples of bark from twigs, an equivalent surface area was collected. Samples were taken in both

Table 1 – Summary of reproduction data for *Oncidium poikilostalix* adults for two study periods, 2008 and 2009, for Site 1, Fracción Montecristo (FM) and Site 2, Benito Juárez El Plan (BJ) and per type of phorophyte (coffee or shade tree).
Number of: adult individuals, adult plants without flowers, flowers, fruits, damaged fruits, seeds lost due to damaged seed capsule, adult orchids lost in 2009 and the number of orchids that passed from juvenile to adult stage in 2009.

	FM		BJ	
	Coffee	Tree	Coffee	Tree
2008				
Adult orchids	317	21	64	12
Non-flowering adult orchids	12	2	6	2
Flowers	7047	580	970	283
Fruits	129	1	32	0
Damaged fruits	9	0	6	0
Seeds	2 765 280	23 044	599 144	0
Lost seeds	207 396	0	138 264	0
2009				
Lost adult orchids	30	0	2	12
Orchids passing to adult stage	108	6	14	-
Adult orchids	397	27	76	-
Non-flowering adult orchids	2	0	11	-
Flowers	10 078	1563	1431	-
Fruits	289	13	139	-
Damaged fruits	3	0	4	-
Seeds	6 590 584	299 572	3 110 940	-
Lost seeds	69 132	0	92 176	-

sites, FM and BJ and 2 samples were taken from each microsite except branch forks, with one additional sample taken from the trunk of a shade tree in FM, giving a total of thirteen samples.

In the laboratory, using a laminar flow cabinet, fresh, untreated root samples were divided into three sections (apical, central and basal) and were disinfected using chlorine bleach at 2% for 1 min, washed with sterile distilled water and then washed again with 3% hydrogen peroxide for 1 min before being rinsed with sterile distilled water. Each section was then sown onto PDA (Potato Dextrose Agar) in Petri dishes and then incubated at 30°C until fungal colonies appeared (Molina & Palmer 1982).

Two methods were used for the identification of fungi using morphological characteristics.

Samples taken directly from root samples – Samples of the growing tips of the fungi, extending out of the root sections and into the PDA, were taken and mounted in a drop of Lactophenol Blue onto glass microscope slides for observation, and for resowing onto fresh PDA. The procedure was repeated until pure cultures had been obtained, and from those, microcultures were prepared, which facilitate a morphological analysis of the fungal structures and the identification of each sample (Noyd 2000). Mounted samples for reference were prepared by taking a small portion of mycelium from each fungal isolate using a dissecting needle, placing it into a drop of Lactophenol Blue on a glass microscope slide, covering with a coverslip, and fixing with transparent lacquer (Hernández & Ortigoza 1994).

Microcultures – To be able to observe intact fungal structures under the microscope, microcultures were developed as an aid to identification. A small portion of mycelia from each sample was sown onto a cube of PDA (0.5 cm, 3 mm depth), applying a dot of fungus to each lateral face. The PDA cube was then placed onto a microscope slide with a coverslip. This was then placed into a Petri dish with 10% glycerol with V shaped glass rod to avoid the slide coming into contact with the glycerol.

The microculture was incubated for 48 hrs at 28°C (Hernández & Ortigoza 1994). Once hyphal growth was observed, the PDA cube was removed. Both microscope slide and coverslip were used to visualize fungal structures using a stereo microscope.

Endophytic fungi were identified by studying the morphological characteristics of the colonies, in terms of appearance and development. The characteristics of the fungal hyphae were also studied, including thickness (using a light microscope at 40×) and the dimension of moniliod cells and/or spores. For the correct interpretation of these morphological characteristics, we referred to Tu & Kimbrough (1978), Moore (1987), Zelmer et al. (1996) and Pereira et al. (2005). It is noteworthy that in almost all cases, orchid mycorrhizal isolates have binucleate hyphae and do not sporulate *in vitro* (Mosquera-Espinosa et al. 2010).

RESULTS

Flowering, fructification and pollination rate

In the region, *O. poikilostalix* flowers from July to December, peaking in August and September. In both sites, due to the distribution of the orchids, most flowers were observed on branches of the coffee bushes and the trunks of the shade trees.

An unidentified chinche (Order Hemiptera, Family Miridae) was observed feeding upon the youngest buds of *O. poikilostalix*, although it was never seen to be numerous or widespread.

Towards the end of the experimental period, we observed that a minority of plants of *O. poikilostalix* proved to be capable of a second flowering on the same spike, within the same year, in which the buds develop directly from the scars left from the first set of flowers, and even where fruits were present. We were not able to study this phenomenon in depth, and superficially no pattern, in relation to the size or position of the plant on the phorophyte, was observed and these second flowers were identical to the first ones.

A small proportion of adult individuals did not produce flowers during the period of observation. Globally, in 2008, in FM and BJ, 4.14% and 10.52% respectively of adult plants did not flower (table 1). In 2009 this fell to 0.47% in FM, but rose to 14.47% in BJ (table 1).

Furthermore, several adult plants were lost between the flowering periods of 2008 and 2009, due to management practices, such as the pruning of branches. To counteract these negative tendencies, many juvenile plants reached the adult stage and contributed to flowering in 2009 (table 1). Globally in 2009 there were a greater number of flowers.

To date, there is no mention in the literature of the pollinator(s) of *O. poikilostalix* and despite spending hours observing the flowers in both sites, the only insect seen to visit the flowers was the small bee *Trigona fulviventris* Guérin, and only two visits were observed, of 1 min and 1.5 min duration, at the site with more exposed orchids. However, as these insects were not seen to remove pollinaria during the visits, it is impossible to confirm that it is the pollinator of *O. poikilostalix*. The size of *T. fulviventris* coincides with that of the flower and the bees moved close to column while exploring the base of the lip of the flowers. The individual flowers had a life span of approximately twenty days.

Taking into account both types of phorophytes, percentage of pollination in 2008 was 1.7% in FM and 2.93% in BJ, rising to 2.86% and 9.71%, respectively, in 2009. There were far more orchid plants on coffee bushes, percentage of pollination was much higher for plants on coffee bush phorophytes and only one fruit was set on plants on shade tree phorophytes, that being in FM in 2008 (0.17% pollination).

Fruits of *O. poikilostalix* were observed from May onwards and they opened at the beginning of the rainy season in May one year later. The fruit set was significantly higher in 2009 ($\chi^2 = 129.090$; df = 1; P = 6.48119e-30; Chi-squared).

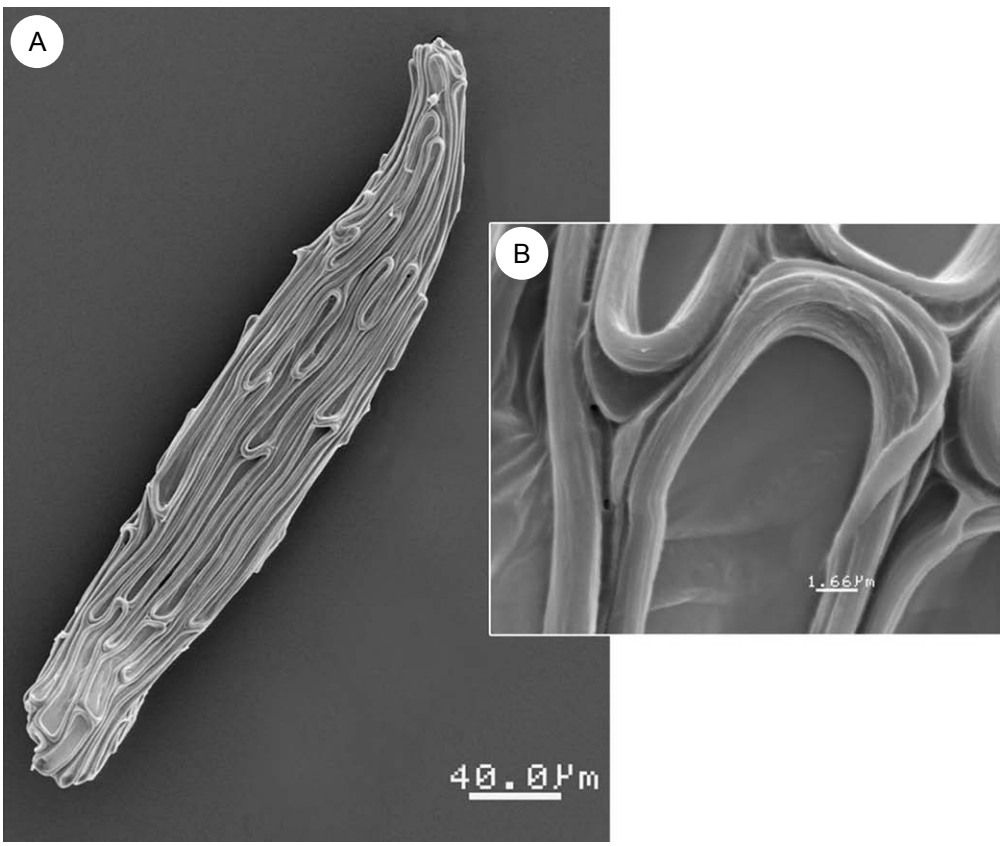

Figure 2 – Scanning electron micrographs: A, seed of *Oncidium poikilostalix*; B, portion of a cell from the surface.

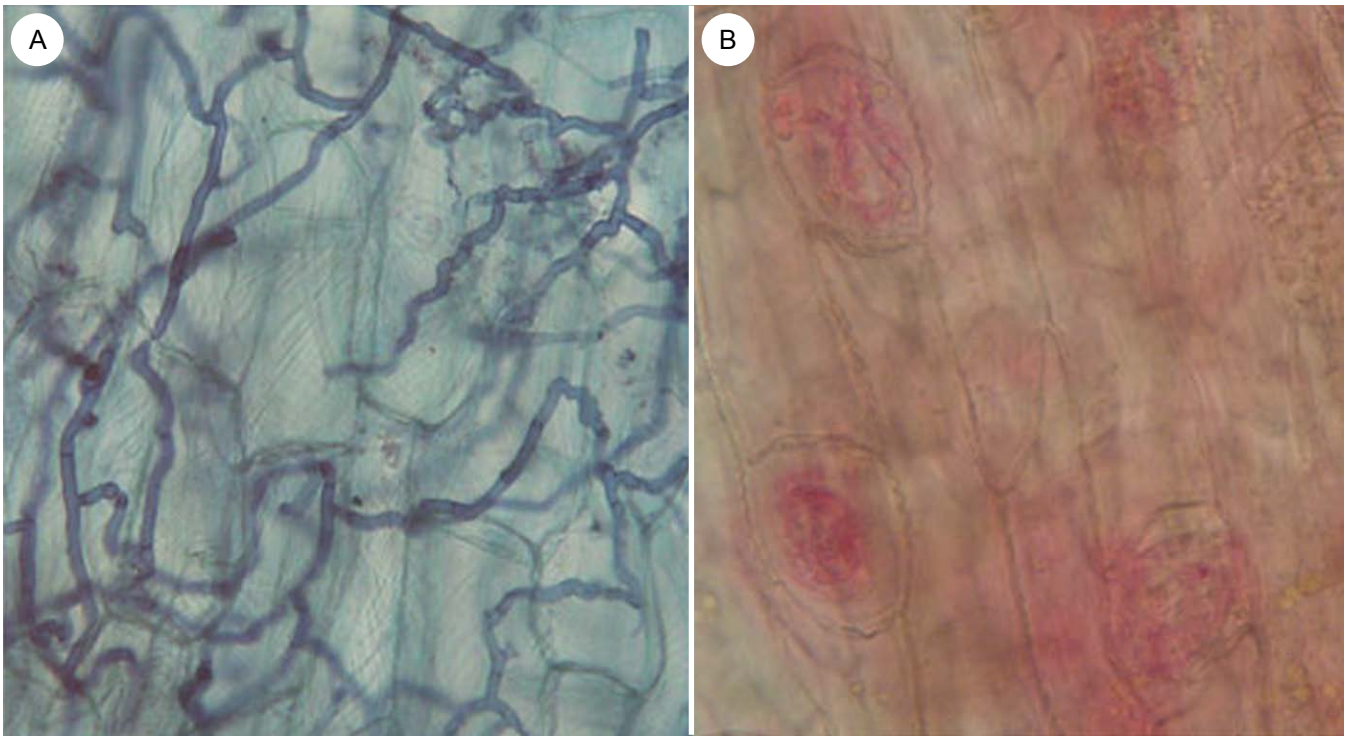

Figure 3 – Root section stained with Trypan Blue (A) and with Fuchsin Acid (B): A, hyphae of the endophytic fungi; B, pelotons formed from balls of hyphae.

A small proportion of fruits were damaged in both sites, and solely on coffee bush phorophytes (table 1), with a higher proportion of damage in BJ (18.75% of damaged fruit) as compared to FM (6.92% of damaged fruit) in 2008 and reduced damage in both sites in 2009, which coincided with an increased production of fruits in that year (table 1).

Seed rain

The average number of seeds produced per fruit for *O. poikilostalix* was estimated at 23,044 in the two years sampled.

In 2008 in FM, healthy fruits produced 2,788,324 seeds and 207,396 seeds were lost due to damaged fruit, whereas in BJ, 599,144 seeds were produced and 138,264 were lost due to damage. In 2009, in FM, 6,890,156 seeds were produced and 69,132 lost and for BJ, 3,110,940 seeds were produced and 92,176 were lost (table 1).

Total seed rain per hectare for *O. poikilostalix* was estimated at 14,871,061 in 2008 and 36,747,498 in 2009 in FM, and 3,195,434 in 2008 and 16,591,680 in 2009 in BJ.

Morphological characterization of the seed

The seeds of *O. poikilostalix* are fusiform with a romo mycropyle and wide calazal (fig. 2A), average length of 384.84 ± 27.4229 µm and average width 58.68 ± 8.2937 µm.

The surface cells of the seeds are elongated, with an average length of 104.23 ± 50.2158 µm and average width of 6.62 ± 2.2262 µm. The anticlinal walls are elevated, wavy, without ornamentation and slightly curved towards the centre at the extremes, so as to form tiny hooks. The periclinal walls are smooth (fig. 2B).

Detection, isolation and identification of mycorrhizal fungi in orchid roots

Fungal hyphae were observed within the cortical cells of the root samples (fig. 3A & B) and in many cases the classic rolls or pelotons were observed (fig. 3B). Fungi were found in all sections of the roots, except the growing tip, or apex. Thirteen fungal genera were identified morphologically (table 2) but it was not possible to detect which of these were specifically involved in a symbiotic relationship with *O. poikilostalix*.

The most frequent genera found were *Trichoderma* and *Rhizoctonia*, which were found in all microsites (except branch forks which were not included because of the infrequent presence of *O. poikilostalix*). *Rhizoctonia* was not found in BJ and was the only fungal genera found in the shade tree samples (table 2).

DISCUSSION

The adult stage is the most important life stage of orchids, particularly for long-lived, rare, widely dispersed and infrequently pollinated species for which recruitment of new individuals is scarce (Zotz 1998, Winkler & Hietz 2001, García-Soriano 2003, Mondragón 2009). It is precisely the low reproductive success, and small proportion of reproducing individuals that has probably led to genetic drift being the most plausible explication for the great diversity found within the orchid family (Tremblay et al. 2005). Within that

context, the loss of adult individuals due to natural phenomena or human intervention may be devastating for many orchid species. However our data show that populations of *O. poikilostalix* in the two sites studied contain a relatively high percentage of adult plants, and suggest that *O. poikilostalix* has an unusually high capacity for recovery, with abundant seed production and rapid maturation of new plants.

Annual seed production is highly variable, depending upon multiple biotic and abiotic factors that may vary widely between years and between sites. We compared the seed production of *O. poikilostalix* with two other miniature orchids: *Notylia barkeri* Lindl., a frequent weedier species, and *Erycina crista-galli* (Rchb.f.) N.H.Williams & M.W.Chase, a protected species with a more limited and patchy distribution. All three species occupy a similar niche, although *E. crista-galli* is more likely to be confined to the thinner twigs of the coffee bushes. Comparing the average seed production per capsule, in another coffee plantation in the Soconusco area, *N. barkeri* produced 68,643 (2005–2006) and 52,215 (2007–2008) seeds per capsule on average whereas *E. crista-galli* produced 17,709 (2005–2006) and 2,430 (2007–2008) (Damon & Valle-Mora 2008, Pérez-Hernández 2009). *O. poikilostalix*, with an average number of 23,044 seeds per capsule falls half-way between these two extremes. Similarly, comparing seed rain per hectare for these three miniature orchid species, *N. barkeri* produced far greater quantities of seeds, at 29,919,977 (2005–2006) and 60,516,250 (2007–2008) and *E. crista-galli* the lowest quantities at 2,547,105 (2005–2006) and 1,127,437 (2007–2008); *O. poikilostalix* again fell between the two extremes with 14,871,061 (2008) and 36,747,498 (2009) in FM, and 3,195,434 (2008) and 16,591,680 (2009) in BJ.

The management of coffee plantations directly affects orchids epiphytic on coffee bushes and shade trees, however, in the study area, for economic and cultural reasons, management is limited and affects minimally these orchid populations (García-González et al. 2011).

Table 2 – Fungi associated with *Oncidium poikilostalix*.
Per microsite, type of phorophyte and plot.

Fungus	Microsite	Phorophyte	Plot
Rhizoctonia sp.	Branch	Coffee	1
	Trunk	Tree	2
	Twig	Coffee	2
Trichoderma sp.	Branch	Coffee	1
	Trunk	Coffee	2
	Branch	Coffee	4
	Branch	Coffee	6
Aspergillus sp.	Twig	Coffee	2
	Branch	Coffee	4
Epicocum sp.	Trunk	Coffee	1
Alternaria sp.	Twig	Coffee	2
Fusarium sp.	Twig	Coffee	2
Aspergillus sp.	Twig	Coffee	2
Rhizopus sp.	Trunk	Coffee	1

Many of miniature species of orchids, such as *O. poikilostalix*, are twig epiphytes, and tend to have a shorter life cycle, as a response to the ephemeral habitat they occupy and the scarcity of resources (Gravendeel et al. 2004, Mondragón et al. 2007). Some species, such as *N. barkeri*, produce abundant progeny similar to the behavior of r-strategy weedy species (Damon & Valle Mora 2008) and our preliminary results suggest that *O. poikilostalix* behaves in a similar way.

Despite the loss of various adult plants in the interim period between 2008 and 2009, and a moderate proportion of adult plants which do not produce flowers in any given year, the number of flowers increased significantly in the second year of this study, as did seed set.

For Orchidaceae, there are very few reports of a second batch of flowers being produced on the same flower spike (R. Solano; Instituto Politécnico Nacional (IPN) y Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), México, pers. comm.) and this probably gives advantage to *O. poikilostalix*, increasing the possibility of being pollinated, as well as increasing the efficient use of scarce resources and the investment made in the production of flower structures.

It is likely that in the study region *O. poikilostalix* is being pollinated by *T. fulviventris*, a common, generalist pollinator which collects oils from flowers, although we never observed pollinarium removal. *Oncidium guatemalenoides* M.W.Chase & N.H.Williams is pollinated by *Trigona fulviventris* subsp. *fulviventris* (F.L. Archila, The Archila Family Experimental Orchid Station, Guatemala, pers. comm.), this being a very similar species to *O. poikilostalix*. Nonetheless, *T. fulviventris* is also known to be a robber of nectar and/or pollen, interfering with or competing with legitimate pollinators (Reyes-Novelo 2004).

The loss of a small proportion of the fruits produced by *O. poikilostalix* can be considered as a natural process derived from unthreatening, low levels of herbivory. Abortion of fruits may also occur due to resource limitation (Ackerman & Montalvo 1990).

The hooks that form the curvature of the anticlinal walls, at the extreme edges of the cells probably play an important role in securing the seed to the substrate, i.e. possible phorophytes, which for *O. poikilostalix* in these sites, were coffee bushes, which have a thin and relatively smooth bark.

The genera of fungi found in association with juvenile plants of *O. poikilostalix*, would appear to be frequent in the coffee growing region of Soconusco and Lara (2006) isolated and identified the same genera, except for *Epicocum*, associated with other miniature orchid species in coffee plantations. *Rhizoctonia* is the genera most cited in relation to orchids (Milligan & Williams 1988, Peakall & Beattie 1996, Bayman et al. 1997), especially *Rhizoctonia solani* (Milligan & Williams 1988, Bayman et al. 1997). The strains of *Rhizoctonia* isolated in FM were found on the branch and twig microsites of coffee bushes where the majority of individuals of *O. pokilostalix* were growing, and was the only genus isolated from samples taken from the most heavily populated shade tree in FM, which included 35 juvenile plants (table 2).

Although located in an area with a well-studied orchid flora (Espejo et al. 2005, Hágsater et al. 2005, Damon 2011), the two populations of this species had not previously been observed, suggesting recent entry into Mexico. Despite this, *O. poikilostalix* appears to be highly resistant and flexible and displays characteristics of a weedy species, such as: rapid growth, precocious maturation, production of a large number of propagules and mechanisms for wide dispersal of seeds (Silván & Campos 2001, GV & RBU 2003, Schüttler & Karez 2008). However, it does not reach the same levels of seed production per hectare as *N. barkeri* (Damon & Valle-Mora 2008), a notably frequent and weedy species in the region. Another characteristic is that it is developing within an agroecosystem with little competition, which is a characteristic of some invasive species (GV & RBU 2003).

The study area evidently supports population expansion of *O. poikilostalix* with suitable pollinators and mycorrhizal fungi which facilitate seed germination and development of the young plants, respectively.

The study area is the same in which, historically, the similar *O. guatemalenoides* is found (Soto-Arenas & Solano-Gómez 2007), however by contrast, this species is infrequent and slow growing. We have observed *O. poikilostalix* individuals growing next to and in some cases, virtually on top of the scarce individuals of *O. guatemalenoides* and they may eventually competitively displace them, however, to confirm this, the site should be monitored for several additional years.

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