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A NEW CASE OF ANTS NESTING IN *NEPENTHES* PITCHER PLANTS

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INTRODUCTION

Carnivorous pitcher plants in the genus *Nepenthes* are known to capture large numbers of formicine ants as prey (e.g. Moran 1996; Adam 1997; Di Giusto *et al.* 2008) which they trap and digest in fluid-filled pitchers. The pitcher rim, or peristome, as well as the inner pitcher wall are slippery and designed to capture and retain arthropod prey (e.g. Gaume *et al.* 2002; Bohn & Federle 2004; Bauer & Federle 2009). In most *Nepenthes* species, the inner pitcher wall is segregated into two distinct zones: an upper waxy zone covered with slippery, waxy crystals (Juniper & Burras 1962) and a lower secretory zone much of which is filled by fluid (Lloyd 1942; Juniper *et al.* 1989; Clarke & Wong 1997).

Despite its arthropod-capturing habits, one pitcher plant species, *Nepenthes bicalcarata* Hook. f., is known to be a myrmecophyte, harboring the ant *Camponotus schmitzi* Stärcke in domatia within its tendrils (Clarke & Kitching 1995; Merbach *et al.* 2007; Bonhomme *et al.* 2011; Thornham *et al.* 2012). Other long-term associations between pitcher plants and ants are likely, as many ants regularly visit pitcher plants to harvest the nectar from extrafloral nectaries. For example, *Polyrhachis pruinosa* Mayr is commonly found feeding on the inner side of the pitcher lid of *N. bicalcarata* in northwestern Borneo (TUG pers. obs.). Likewise, at Varirata Range in Papua New Guinea, numerous workers of *Polyrhachis semiobscura* Donisthorpe were observed foraging around the rims of the pitchers of a species of unidentified *Nepenthes*. Despite the large number of workers and their rather busy activity around the peristome, none were found trapped in the fluid at the bottom of the pitchers (RJK pers. obs.). However, apart from *C. schmitzi*, no ants are known to enter the pitcher with impunity.

Here we report on observations of the recently described *Polyrhachis nepenthicola* Kohout (Kohout 2013) nesting within the pitchers of *Nepenthes stenophylla* Mast in northern Sarawak on the island of Borneo. *Polyrhachis* ants have diverse nesting habits ranging from subterranean to arboreal, including cavities within rocks and living or dead plants (Robson & Kohout 2007, 2008) but none have been observed to nest inside the pitchers of living pitcher plants.

METHODS

The study site was located at Paya Maga, Lawas, Sarawak (4°27'10.27'' N, 115°33'34.1''E) and at 1810m a.s.l. The area is characterized by sub-montane, mixed-dipterocarp rainforest, though considerable disturbance was found along the abandoned dirt logging road used to access the area. *N. stenophylla* were found on 10th and 11th of October 2010 adjacent to the dirt road in secondary, bushy vegetation in an area of approximately 2 ha. Upon inspection of the pitcher contents, nests of *Polyrhachis* ants were found inside. Seven *N. stenophylla* plants with 22

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pitchers were examined. Ants inside the pitchers were identified as a new species and described as *Polyrhachis* (*Myrmothrinax*) *nepenthicola* Kohout, 2013. Only accessible aerial pitchers were inspected. No other species of *Nepenthes* was growing at the site. One nest was collected on 11th October 2010 and preserved together with its inhabitants in 75% ethanol. Observations of ant behavior were made on two days, with four hours of observations during daytime (10:40 - 12:40 h and 15:15 - 17:15 h) and two hours at nighttime (19:30 - 21:30 h). A Mann-Whitney U-test was performed using SPSS software (SPSS, Chicago, IL, USA). Descriptive statistics are given as means ± standard deviation.

RESULTS AND DISCUSSION

Nests of *P. nepenthicola* were observed in 10 of the 22 pitchers examined. All nests were attached to the inner pitcher wall within the waxy zone and thus well above the fluid level (Figure 1a). The collected nest was oval in shape, 44.9 mm long, 41.4 mm wide, and 2-3 mm thick, and consisted of just one chamber with a single entry from below. It had a carton-like texture and was made of small pieces of coarse plant material and fiber, as described for other *Polyrhachis* species (e.g. Robson & Kohout 2007, 2008). The nest did not appear to include larval silk. It comprised 21 workers, 1 dealate queen, and 8 males. Worker ants were seen to enter and exit through small holes in the pitcher wall (Figure 1b). These holes did not give direct access to the nest. Ants always passed into the pitcher and then entered the nest by a separate opening from inside the pitcher. Holes were 2-3 times wider than the head capsule of the ants passing through. No ant attempted to enter or exit over the curled lip of the peristome. However, at night, two ants were seen to briefly walk on the waxy surface without slipping, but the wax crystal surface may have been locally damaged. Ants were not seen to walk on the waxy layer during daytime observations.

Pitchers containing nests had significantly more holes on average (3.60 ± 3.53) than pitchers that did not contain nests (1.17 ± 1.75; Mann-Whitney U-test; U=26.0 N_1=10, N_2=12; p=0.025; Figure 2) suggesting that ants might bite holes in the pitcher wall to gain access to the inside of the pitcher. In support of this notion, holes were not randomly distributed along the length of the pitcher; 31 out of 39 holes were located a few mm below the waxy zone, giving ants immediate access to the non-slippery pitcher interior. Alternatively, since non-inhabited

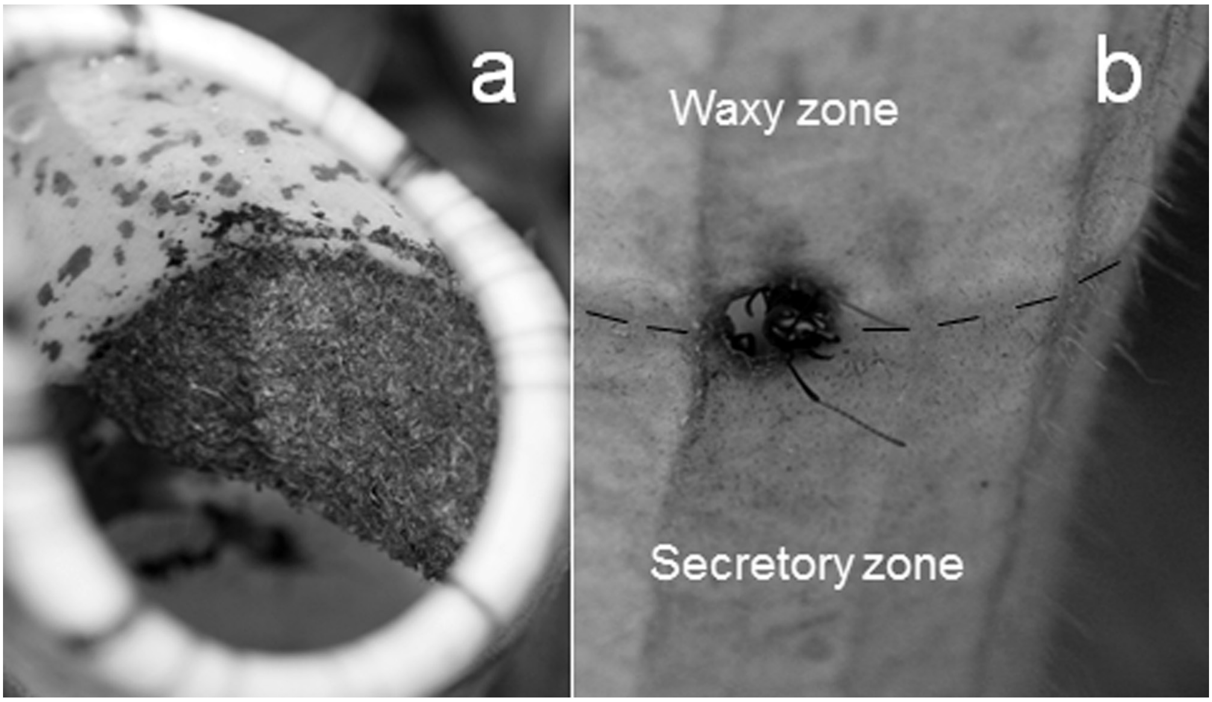

FIG. 1. Ant-pitcher plant association. (a) Nest of *Polyrhachis nepenthicola* inside a pitcher of *Nepenthes stenophylla*; (b) pitcher viewed from the outside with worker ant exiting pitcher through a small hole at the border (dotted line) between the waxy zone and the secretory zone.

pitchers also have holes in their pitchers, ants could simply be choosing to colonize pitchers with more holes, possibly older pitchers. However, the preponderance of holes just below the waxy zone suggests otherwise.

It remains unclear if *P. nepenthicola* uses other nesting sites apart from pitcher plants. *N. stenophylla* and other pitcher plant species with large pitchers such as *N. fusca* or *N. vogelii* (Cheek & Jebb 2001) appear to be well suited for ants as there is enough space for nest building above the pitcher fluid. Although two ants were seen to briefly walk on the waxy surface of the inner wall of *N. stenophylla* pitchers, this surface is generally slippery because the wax crystals are too small for insect claws to lock onto and the crystals readily become dislodged (Juniper & Burras 1962; Gorb *et al.* 2005). It thus seems practical for *P. nepenthicola* to enter the pitchers below the waxy surface. In addition, entry to the pitcher is restricted by the curled lip of the peristome. Thus, the presence of holes beneath the waxy zone clearly facilitates access to the pitcher interior, but we can only speculate as to the origin of the holes. Although we did not see ants creating the holes we think that the distribution of holes suggests that they may be involved in producing them.

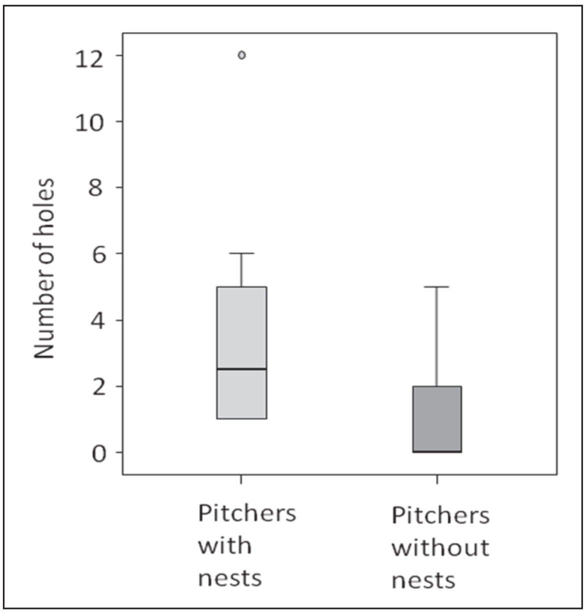

FIG. 2. Number of holes found in pitchers containing nests versus pitchers without nests. Box plots show the median number with interquartile range and 10th and 90th percentile. The two distributions are significantly different at $p<0.05$.

It is too early to make firm conclusions concerning the nature of this ant-plant interaction. Is the ant a parasite, a commensal, or a mutualist? The benefits provided to the ant would appear to be a suitable above-ground nesting site that in addition might secure reduced intrusion by conspecifics or predatory ants. The benefit to the pitcher plant, if any, is even more uncertain. Some ants may get washed into the digestive fluid in heavy rains. For example, *N. gracilis* uses the impact of rain drops to flick insects from the underside of the pitcher lid into the pitcher (Bauer *et al.* 2012). In Southeast Asia, several species of *Polyrhachis* are known to restrict their nesting sites to bamboo (Dorow & Maschwitz 1990; Schellerich-Kaaden *et al.* 1997) or ginger (Dorow *et al.* 1990) without a known benefit accruing to the host plant. It seems likely that *P. nepenthicola* forms a similar non-mutualistic relationship with *N. stenophylla* as the pitcher plant does not provide any specialized nesting space in the form of domatia.

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