WHY A CARNIVOROUS PLANT COOPERATES WITH AN ANT – SELECTIVE DEFENSE AGAINST PITCHER-DESTROYING WEEVILS IN THE MYRMECOPHYTIC PITCHER PLANT *NEPENTHES BICALCARATA* HOOK. F.

Marlis A. Merbach[1,3], Georg Zizka[3], Brigitte Fiala[2], Dennis Merbach[1], Webber E. Booth[4] & Ulrich Maschwitz[1]

[1] Department of Biosciences, Institute for Ecology, Evolution and Diversity,
J.W. Goethe-University, D-60323 Frankfurt
[2] Department of Animal Ecology and Tropical Biology, University of Würzburg, Zoologie III,
Biozentrum, Am Hubland, D- 97074 Würzburg
[3] Department for Botany and Molecular Evolution, Research Institute Senckenberg and
J.W. Goethe-University, Senckenberganlage 25, D-60325 Frankfurt am Main
[4] 2 Peacock Gardens Drive, Kerikeri, New Zeland

Abstract. Nepenthes bicalcarata Hook. f., (Nepenthaceae) is the only myrmecophytic carnivorous pitcher plant. Its ant partner *Camponotus schmitzi* Stärke (Formicinae) is obligately associated with the plant, while the plant is not obligately dependent on the ant. A protective function as is common in other myrmecophytic systems was previously unknown. We examined herbivore damage on *N. bicalcarata* in Brunei Darussalam, Borneo, and tested a possible protective function of the inhabiting ants. Our surveys revealed two types of damage: areal leaf damage, which played no major role, and very small but deep holes which destroyed developing pitchers and even whole leaves and the vegetative tip. A weevil (*Alcidodes* sp., Curculionidae) was identified as originator of the damage and main threat to the plant. We examined the behavior of *C. schmitzi* workers confronted with *Alcidodes* sp. and also their reaction to injured host-plant material. In an ant exclusion experiment we analyzed the effects of the ants on the drilling damage. All results showed that *C. schmitzi* can effectively protect its host plant against damage caused by *Alcidodes* sp. The ants acted selectively, not attacking other visitors and potential prey, a behavior unique in myrmecophytic interactions. *Accepted 20 February 2007.*

Key words: *Ant-plant interaction*, *Borneo*, Camponotus schmitzi, *carnivory*, *herbivory*.

INTRODUCTION

The insect-plant relationships of the carnivorous Palaeotropical pitcher plant genus *Nepenthes* have been a subject of intense interest for more than 200 years (e.g., Linnaeus 1737). This genus represents a classical example of carnivorous plants believed to attract their prey insects mainly by nectar, produced by numerous extrafloral nectar glands (EFN) spread all over the plant but mostly concentrated on the pitchers (e.g., Merbach *et al.* 2001). In general, EFN in plants function as attractants for ants that provide protection against herbivores (Davidson & McKey 1993, Jolivet 1996). No study on *Nepenthes* has yet focused on herbivore damage. The main attention has been on carnivory, attraction, and composition of prey

(Juniper *et al.* 1989, Moran 1996, Moran *et al.* 1999, Tan 1997) or the ecology of the pitcher's phytotelmata (e.g., Clarke & Kitching 1993, 1995, and historical overview in Juniper *et al.* 1989).

Only recently have a few authors suggested that the extrafloral nectar of *Nepenthes* might additionally attract ants as a herbivore defense. This has been discussed especially for *Nepenthes bicalcarata*, the only myrmecophytic species in the large pitcher plant genus that lives in a tight symbiosis with a partner ant. However, no experimental study has been presented as yet to support this hypothesis (Joel 1988, Hölldobler & Wilson 1990, Zizka 1990, 1991). Our former studies (Merbach *et al.* 2001) indicated that EFN most likely function as far more than just prey attraction. Secretion of nectar can be observed especially in young developing leaves which are not involved in trapping.
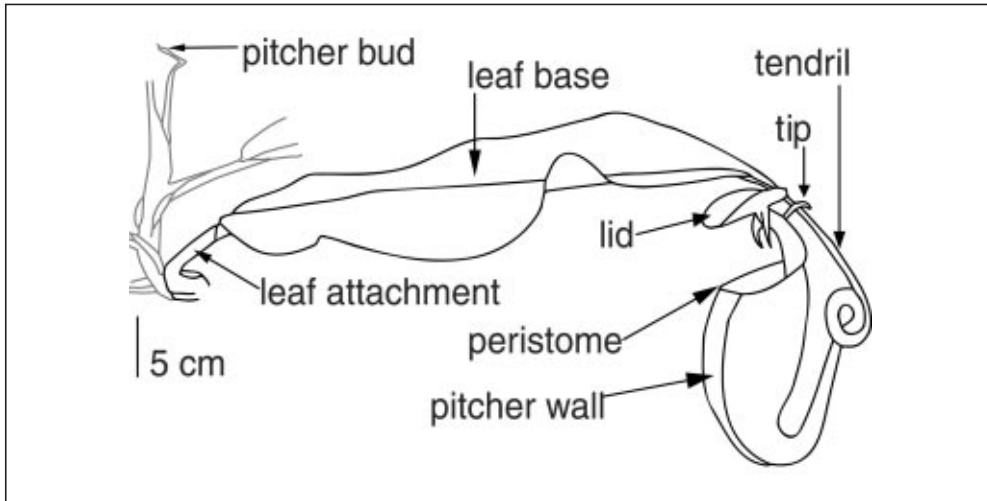
e-mail: marlis@merbach.net

FIG. 1. Upper leaf of *N. bicalcarata*. The basal, blade-like part is morphologically the leaf base. The often curled tendril corresponds to the petiole, the pitcher – the catching organ – to the leaf blade.

Nepenthes bicalcarata, besides being carnivorous, has developed a unique and highly unusual myrmecophytic system. *Camponotus schmitzi* obligately colonizes *N. bicalcarata*, while the plant is not obligately dependent on the ant (Schuitemaker & Stärke 1933, Clarke 1992, Clarke & Kitching 1995). Most of the workers usually rest under the pitcher's peristome, while the brood is raised in domatia located in the self-hollowing pitcher tendrils. *Camponotus schmitzi* workers as a rule do not leave the host plant (own unpublished observations by M. Merbach). The ants obtain sugar mostly from the peristome nectaries, especially from the giant peristome thorn nectaries which are found only in this plant species (Merbach *et al.* 1999). Their only source of protein appears to be prey from the pitcher fluid and mosquito larvae caught by skilfully diving into the pitcher's fluid (own observations; Clarke & Kitching 1995). Besides this host-specific ant species various other host-unspecific ants have been observed visiting the nectaries.

Generally in ant-plant associations the ants defend their resources and thus protect their plant partners against herbivores (see overviews e.g. in Huxley 1986, Bronstein 1998, Davidson & McKey 1993). The protective value of the ants may vary depending on the visiting or colonizing ant species involved, as well as on other factors such as the colony size, etc. The benefits of having inhabitants and visitors to the plants (other than prey), especially in terms of protective function, have not yet been investigated for *N. bicalcarata*. Therefore the first aim of our study was to determine the herbivory damage in *N. bicalcarata* and to identify the relevant herbivores. During this we found a specific curculionid beetle to be the key herbivore of this plant species. Thus we subsequently investigated experimentally whether and how the *C. schmitzi* partner ants protect their host plant against this specific phytophagous enemy.

METHODS

Study site. The research was carried out during four field expeditions (covering a total of seven months) in Brunei Darussalam, Borneo in 1997, 1998, and 2000. The principal habitats of *N. bicalcarata* are peat swamp forests dominated by *Shorea albida* (Dipterocarpaceae) (corresponding to the Alan bunga forest type, Whitmore 1985), peat swamp fragments, and open degraded heath forests on white sand.

Plant, ant, and weevil specimens and localities are deposited in the Senckenberg Museum (Herbarium Senckenbergianum and Entomological Collection), Frankfurt. Localities are documented with the specimens but not published here for conservation reasons.

Survey of damage. *N. bicalcarata* plants were examined for leaf area and pitcher damage. Different patterns of damage were observed and photographically doc-

umented. Animals which were found feeding on *N. bicalcarata* were collected. All of the plant's leaves were examined for leaf area damage in two different locations, one in a peat swamp forest and one in an open degraded area. The damage was estimated and classified according to < 5 %, ~ 25 %, ~ 50 %, ~ 75 %, and > 90 % leaf area loss, separately for 'leaf attachment', 'leaf base', 'tendril', 'pitcher wall', 'lid' and 'peristome' (see Fig. 1). The damage caused by the weevil *Alcidodes* sp. to *N. bicalcarata* plants was documented separately from the leaf-area damage patterns. A distinction was made between destruction of the pitcher bud and destruction of the whole leaf, which often resulted in a loss of the whole shoot apex normally protected by the leaf base.

In a defined area of peat swamp forest (app. 2000 m²), all fully developed *N. bicalcarata* plants which were within reach and larger than approximately 30 cm stem height were checked (total of 117 plants with 1605 leaves). Plants colonized by *C. schmitzi* (85 plants with 1134 leaves) were compared with uncolonized plants (32 plants with 471 leaves, using the Chi-square test with Statistica 6.0). The majority of these plants were inhabited, thus resulting in unequal group sample sizes.

Protective role of the partner ants. In order to standardize the observations and experiments, only large colonies of *C. schmitzi* were investigated. The colony size was estimated by counting the number of workers under the peristome of the occupied pitchers. This was possible by using a dentist's mirror, which was inserted into the opening of the pitcher and thus allowed counting the workers under the peristome. This observation did not cause any apparent disturbance. Afterwards, the colony was left undisturbed for at least one day before the experiments were started. We defined as large colonies those that occupied at least five pitchers and had at least ten workers present under the peristome of a minimum of three pitchers.

Confrontation with injured plant tissue. To test whether *C. schmitzi* responds to plant damage, especially that of its host plant, freshly crushed material of the young leaves of *N. bicalcarata*, *N. gracilis*, or an undetermined Poaceae species was placed on the lid of occupied pitchers in large *C. schmitzi* colonies (4 colonies, 3–4 pitchers per colony, 15 tested pitchers). The colonies were located at the edge of a peat swamp forest fragment. Each of the 15 colonized pitchers of locality 1 was tested once during daytime and twice at night.

The undisturbed activity of the ants (number of ants on the pitcher or peristome) was observed for 3 minutes, with the number of active *C. schmitzi* recorded every 30 seconds. The plant material was then placed on the lid and the activity of the ants recorded every 30 seconds for a further 6 minutes. Care was taken that crushing of plant tissue was done with freshly washed hands so that no odor from previously handled material was transferred. The inhabitants of each pitcher were confronted with the three different types of plant material. The tests were conducted consecutively with a minimum interval of one hour and within 4 hours for each plant. The sequence of plant material types was always determined randomly.

For analysis using the t-test with dependent samples (Statistica 6.0), all activities in the undisturbed 3 minutes pre-investigation were compared with the first 3 minutes of the experiment.

Confrontation with Alcidodes. Specimens of *Alcidodes* sp. were collected from *N. bicalcarata* and *N. ampullaria* plants in peat swamp forest. They were kept in terraria together with fresh young *N. bicalcarata* leaf material. Shortly before beginning the experiment, fine cotton thread was knotted on the thorax between pronotum and elytres of each weevil so that they were still able to fly but could not escape. They were then dropped and started flying in circles until they touched the plant and landed. The weevils were used only once for each experiment and then replaced by fresh individuals. Nine colonies of *C. schmitzi* from peat swamp forests and open degraded areas on white sand were tested for their behavioral response to the weevils.

Long-term ant exclusion experiment. In addition to the survey of plant damage, an ant-exclusion experiment was conducted at the same site. Plants were assigned to four groups: C⁺O⁺: colonized by *C. schmitzi* but also visited by other ant species (39 plants with 160 leaves), C⁺o⁻: colonized by *C. schmitzi* but not visited by other ants (26 plants with 146 leaves), c⁻O⁺: only with other ant visitors (37 plants with 166 leaves), c⁻o⁻: no ants (30 plants with 133 leaves). To avoid contact with the ground, all stems and lower pitchers were tied up with plastic bands on young trees or onto poles fixed on the ground. An insect glue (Tangle-trap®, Tanglefoot Corp., Grand Rapids, Mich., USA) was applied to the plant stems and all plastic bands of group C⁺o⁻ and c⁻o⁻ to prevent access of other foraging ant species. In group C⁺o⁻, the *C. schmitzi* colony was left on the plant. In group c⁻O⁺, unoccupied plants were used or the *C. schmitzi* colonies were killed with Spruzid®, Neudorf, Germany by
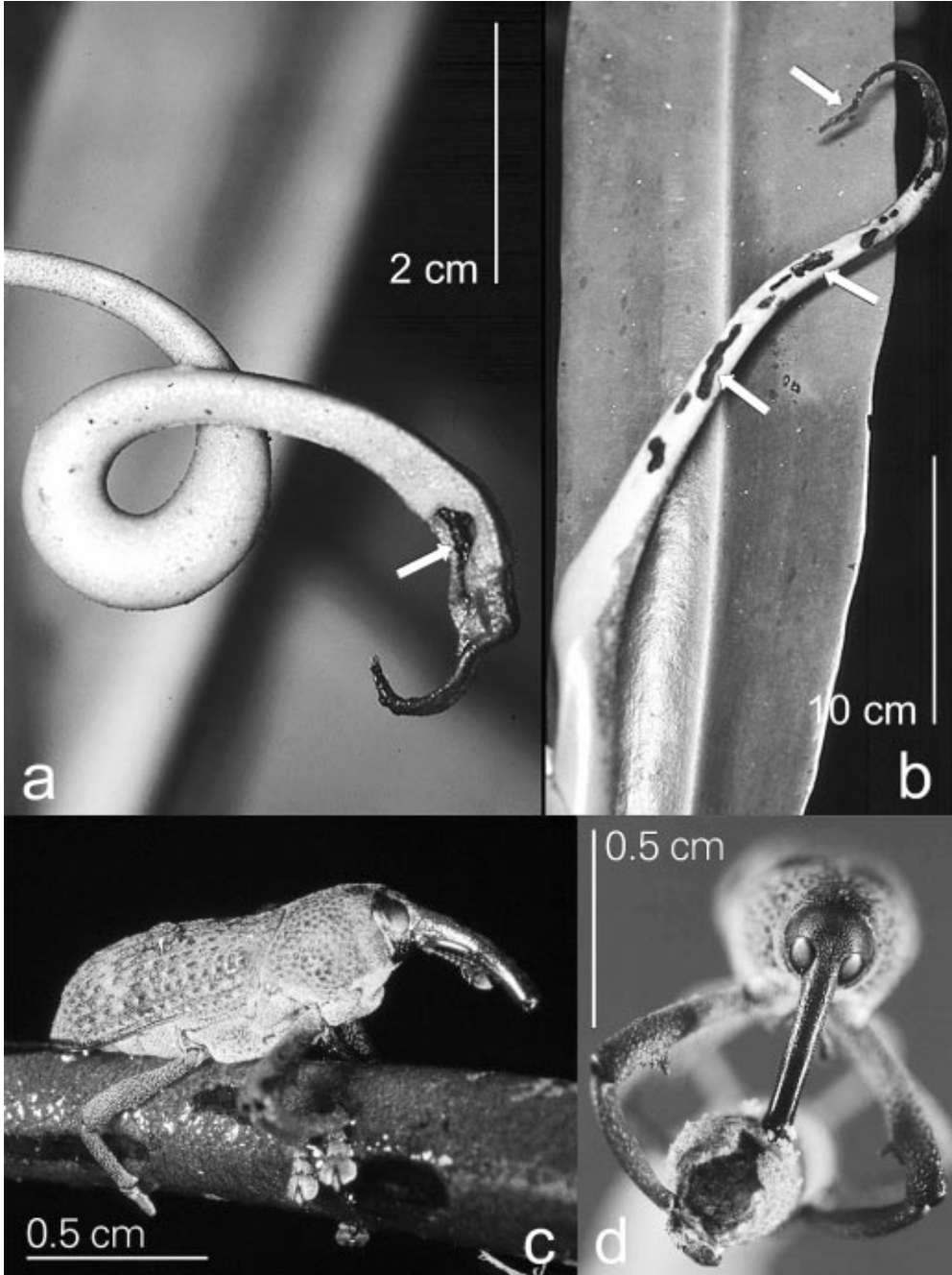
FIG. 2. a+b: Damage caused by *Alcidodes* sp. (see arrows): a) destruction of a pitcher bud (D_P). b) destruction of the whole young leaf (D_L). c+d: *Alcidodes* sp. sitting on a tendril of *N. bicalcarata*. The weevil was observed to cause severe damage to developing leaf parts, especially the young pitchers of *Nepenthes* spp. c) side view, d) front view.

spraying under the peristome and into the domatia. Previous experiments had shown that Spruzid is not harmful to the plants. Spruzid is based on pyrethrum, a natural daylight-sensitive insecticide that decomposes within hours.

The plants were checked every four or five days for a period of 79 days. Defective isolation was corrected by coating with Tangle-trap. Plant groups C$^+$o$^-$ and c$^-$o$^-$ were searched for ants, which if present were removed immediately. Also twigs which had fallen onto the plant were removed to avoid contact with the ground or surrounding plants. All leaves which developed during the experiment were documented and damage was categorized in the same way as described above. As areal damage played no role, the categories were undamaged, pitcher bud damage (D_P), and whole leaf damage (D_L).

Statistic analysis was made using the Chi-square test with significance assumed if $p < 0.05$ (χ^2), calculated with Statistica 6.0.

Influence of potential chemical repellents. It was tested if the presence of *C. schmitzi* might have a repellent effect on the weevil *Alcidodes* sp., for instance by leaving traces of any pheromones or alarm substances on the plant surface.

Plant material of *N. bicalcarata* was freshly collected in the field and tested within about two hours. Five weevils were put into a terrarium together with the apex of the plant, with two young leaves including the young stem. One terrarium contained material from occupied or from unoccupied plants only. Each experiment was repeated eight times with fresh plant material and freshly caught weevils.

The weevils usually walked over the plant parts and then started to drill holes. After 11 hours the number of holes per occupied/unoccupied *N. bicalcarata* material was counted.

RESULTS

Survey of damage and identification of herbivores. We first observed *Alcidodes* sp. feeding on the pitcher buds of young leaves of *N. bicalcarata* in July 1998. Since then they have been frequently observed on the youngest leaves or actually boring holes into the youngest plant parts.

We found two distinct types of damage: a) areas missing from parts of the leaf (areal damage), and b) very small drilled holes.

a) In the peat swamp forest (13 plants with 117 leaves examined) substantial areal damage never oc-
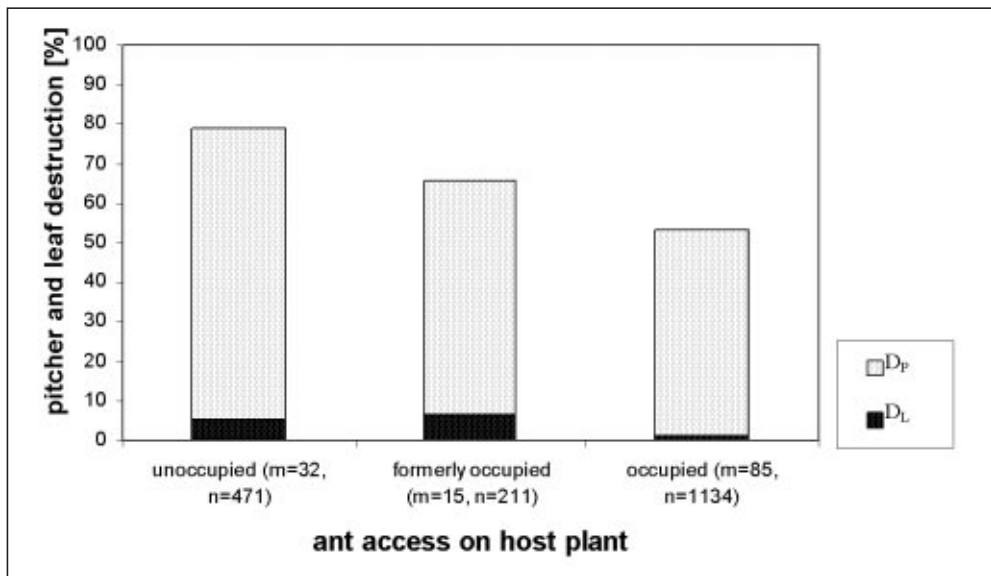

FIG. 3. Survey of damage types in occupied, unoccupied, and formerly occupied *N. bicalcarata* plants in a *Shorea albida* forest [Relative abundance of destroyed pitcher buds (damage type D_P) and destruction of whole leaves (damage type D_L) sometimes including the young stem (m = number of plants, n = number of leaves)].
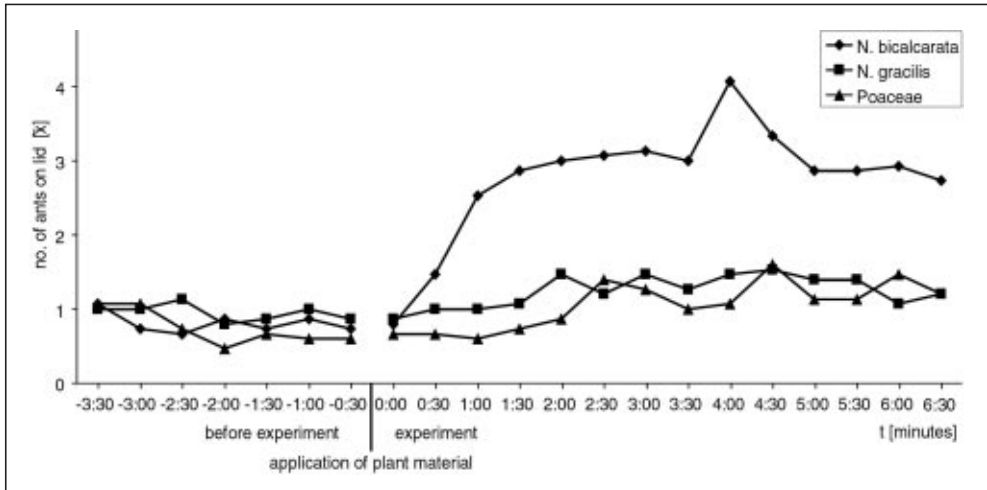
FIG. 4. Number of *C. schmitzi* workers on the pitcher before and after confrontation with crushed plant material (average numbers of 15 observed pitchers).

curred on pitchers and was only found at the leaf base (damage pattern AL,). It seemed to be mainly caused by unidentified caterpillars. The overall damage was very low. Only 15 leaves (12.8%) showed minimal damage (< 5%), one leaf showed about 25%, one about 50% areal loss. In the open degraded area (9 plants with 92 leaves examined) no damage at all occurred. Thus areal damage obviously plays no major role in *N. bicalcarata*.

b) In contrast to areal damage, the puncture damage (Fig. 2a+b) caused by the weevil *Alcidodes* sp. (Fig. 2c+d) was considerable. We frequently observed *Alcidodes* sp. feeding on the pitcher buds of young leaves. They drilled approximately 0.5 mm-diameter-sized holes into pitcher buds. These drilled pitcher buds dried and did not develop into functional traps. They often even fell off the developing leaf resulting in a fully developed leaf without pitcher.

We distinguished two types of drilling damage: single holes drilled in the pitcher buds (Fig. 2a), which appeared when the weevils were present for short time intervals on the pitcher bud. In this case they often just destroyed the pitcher bud but not the whole leaf. We called this damage type 'destruction of pitcher, type D_P'. The other type of damage appeared after the weevils had been present for a long time undisturbed on the plant. In this case perforations could result in the destruction of the youngest leaf as a whole, often including the vegetation-point hidden within the de-

veloping leaf base (Fig. 2b). We called this damage type 'destruction of youngest leaf, type D_L'.

A comparison of plants occupied by *C. schmitzi* and unoccupied plants revealed a clear difference in the intensity of damage caused by *Alcidodes* sp. (Fig. 3). Drilling damage D_P and D_L was lowest in occupied and highest in unoccupied plants. In unoccupied plants, 73.5% (346 of 471) of the pitchers were damaged and not developed (D_P), and destruction of the youngest leaf (D_L) was found in 4.3% (20 of 471). The frequencies of both damage types were significantly lower in occupied plants: only 52.0% (590 of 1134) of the pitchers (D_P), and 1.4% (16 of 1134) of whole leaves (D_L) were totally destroyed (D_P: N = 1605, $\chi^2_{df=1}$ = 62.89, p < 0.0001; D_L: N = 1605, $\chi^2_{df=1}$ = 12.2, p = 0.0005).

Formerly occupied plants showed the typical entrance holes bored by *C. schmitzi* workers in the domatia but no ants could be found under the peristome. These plants were intermediate in the degree of damage of the pitchers (D_P: 59.2%) and highest in terms of destruction of whole leaves (D_L: 6.6%). Undamaged leaves were most frequently observed in occupied plants (42.1%), less in formerly occupied (22.7%), and least in unoccupied plants (17.5%).

Confrontation of the partner ants with injured plant tissue. The activity of *C. schmitzi* significantly increased when crushed *N. bicalcarata* plant material was pres-

ented (t = −4.696, df = 105, p = 0.000008) (Fig. 4). This was never observed with crushed grass material (t = −1.347, df = 105, p = 0.181). In a small number of trials with *N. gracilis* an increase in ant activity occurred but no significant difference was found (t = −1.513, df = 105; p = 0.133).

Confrontation of the partner ants with Alcidodes *sp.* In a further experiment, *C. schmitzi* colonies were confronted with *Alcidodes* sp. on occupied pitchers or on the youngest leaf of their host plant. In most experiments, the ants attacked the weevils. This happened when the weevil landed on the plant but not when it was cautiously placed there. The landing of the beetle caused a "pop" sound clearly audible to the human ear and vibrations which seemed to alarm the ants. Simply tapping on the plant did not result in the same response.

Of a total of 104 experiments with weevils placed onto the youngest leaves, 72 attacks (69.2%) were recorded. In 109 experiments with *Alcidodes* sp. placed onto occupied pitchers, 65 attacks (59.6 %) were observed. No significant difference between these numbers was found (N = 213, $\chi^2_{df=1}$ = 2.14, p = 0.144). In a number of observations (32 of 104 [30.8 %] on the youngest leaf, 44 of 109 [40.4 %] on the pitcher) the ants did not detect the weevil and remained inactive.

The attack started with a recruitment by the ants. One worker detected the weevil, went under the pitcher's peristom and a short time later a number of ants (Fig. 5) attacked the weevil. If the beetle did not drop from the plant (which is its usual reaction to disturbance) attacks lasted without decrease of intensity as long as the experiment continued; for up to 1 hour,
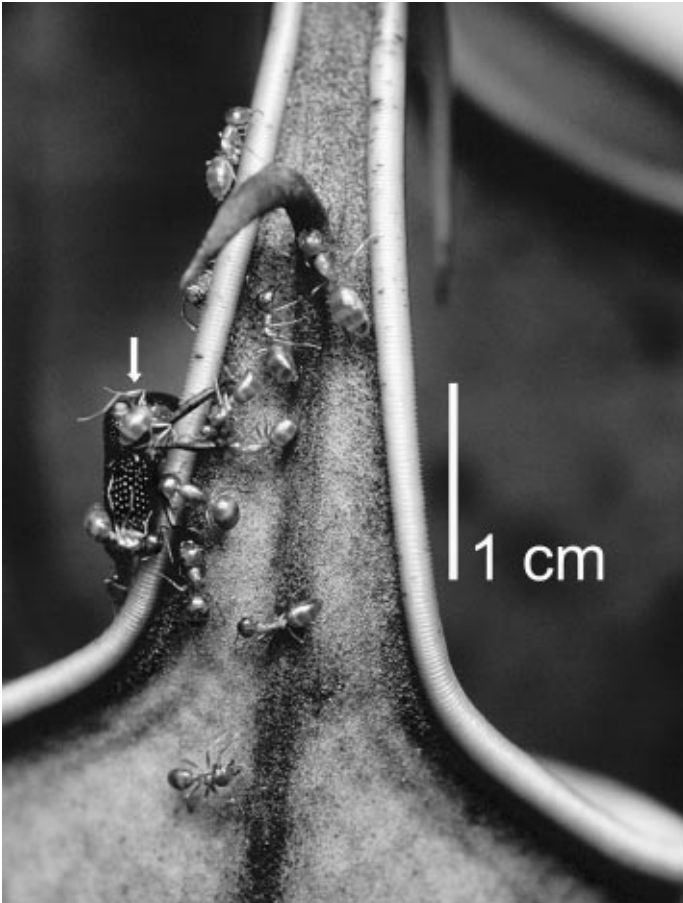

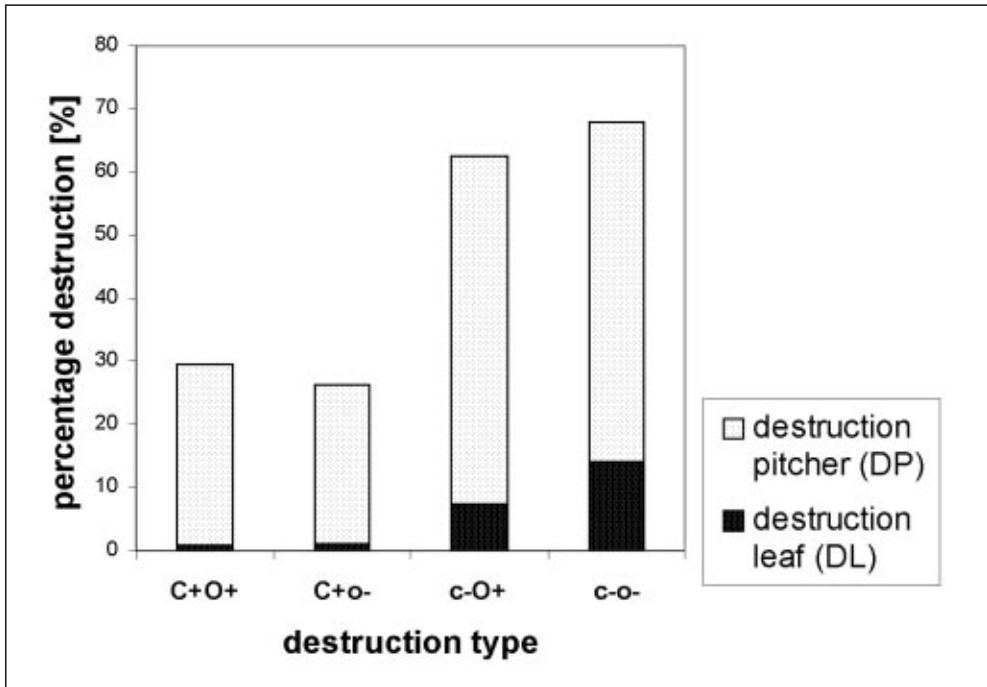FIG. 5. *Alcidodes* sp. on an aerial pitcher of *N. bicalcarata* being attacked by *C. schmitzi* workers.

FIG. 6. Ant exclusion experiment. The protective role of the partner ants was tested on four plant groups: occupied plants together with other visiting ant species (group C+O+), occupied plants without other visiting ant species (group C+o-), unoccupied plants but with other visiting ant species (group c-O+), and unoccupied plants without other visiting ant species (group c-o-). The figure compares destruction of pitchers (D_P) and destruction of whole leaves (D_L), the latter sometimes including the vegetative tip of the plant.

which was observed twice. In one case the weevil was even carried into the pitcher.

Long-term ant exclusion experiment. In an ant exclusion experiment the damage caused by *Alcidodes* sp. was compared between plants with and without ant access (Fig. 6). We compared the groups C+O+ (n [leaves]: 119), C+o- (n: 103), c-O+ (n: 96), c-o- (n: 93) for pitcher loss ($D_P + D_L$). The experimental groups inhabited by *C. schmitzi* (C+O+ and C+o-) had highly significantly less damage compared with both uninhabited experimental groups (c-O+ and c-o-) [C+O+/c-O+: N = 215, $\chi^2_{df=1}$ = 23.59, p < 0.0001; C+O+/c-o-: N = 212, $\chi^2_{df=1}$ = 30.85, p < 0.0001; C+o-/c-O+: N = 199, $\chi^2_{df=1}$ = 26.59, p < 0.0001; C+o-/c-o-: N = 196, $\chi^2_{df=1}$ = 33.94, p < 0.0001].

No significant difference was found between groups with and without other ants: c-O+ and c-o- (N = 189, $\chi^2_{df=1}$ = 0.57, p = 0.45), C+O+ and C+o- (N

= 222, $\chi^2_{df=1}$ = 0.28, p = 0.60). All *N. bicalcarata* plants were regularly visited by a wide range of ant species (e.g., *Polyrhachis* spp., *Crematogaster* spp., *Diacamma* spp.) in order to collect nectar from the extrafloral nectaries. The results show no significant protective effect of these ant species. This does not however exclude protection by single aggressive ant species as the experimental design did not differentiate between ant species except *C. schmitzi*.

Influence of potential chemical repellents. We attempted to determine if *Alcidodes* sp. had a preference for feeding on unoccupied or occupied *N. bicalcarata* plants. *C. schmitzi* workers rub their gaster frequently over the plant surface while walking over it. We do not know yet if they lay trails or if pheromones, repellents, or alarm substances are involved. However, the weevils showed no preference for any plant group and drilled about the same numbers of holes in ma-

terial from eight occupied (mean value 15.8 ±12.7) and unoccupied plants (mean value 14.5 ±10.2). So there seems to be no effect of chemical repellents.

DISCUSSION

Up to now the unique and enigmatic relationship between *N. bicalcarata* and its partner ant *C. schmitzi* has been mainly investigated under the aspect of the plant's carnivory. Thus hypotheses about possible benefits for the plant partner have been focused on the ant's effect on pitcher function, as well as on the ecology of the pitchers as phytotelma habitats (Clarke 1992, Clarke & Kitching 1993, 1995). Protection against herbivores has not been observed, and Clarke & Kitching (1995) have explicitly stated that the ants showed no aggressive behavior at all against visiting insects thus excluding any defensive function. However, some authors (Hölldobler & Wilson 1990, Zizka 1990) have suspected here a protective role, as is typical for myrmecophytic interactions (overview in, e.g., Davidson & McKey 1993, Jolivet 1996), but no observations or experimental data were presented to support this idea. As the benefit of the aquatic pitcher infauna to the plant is speculative (Kitching & Schofield 1986), and the effect of ant partners on pitcher lifetime is questionable, as discussed below, it was not even clear if the plant receives any benefit from the ants.

Our results clearly show a very sophisticated, yet in the overall effect of its main features fairly familiar, myrmecophytic pattern. The *C. schmitzi* partner ant plays a major protective role for the plant partner, *N. bicalcarata*, and receives food and nesting space in return. However, since the plant partner is a carnivorous plant, the balance between the partners' interests is still more complicated than in other cases of tight ant-plant relationships. Although we only rarely observed areal leaf damage in *N. bicalcarata*, damage by herbivores nevertheless appears to be a very important factor for the plants. The drilling by the weevil *Alcidodes* sp. was so widespread in the investigated plants without occurrence of *C. schmitzi* that it at least prevented a large number of pitchers, as well as a considerable amount of whole leaves, from developing.

For a carnivorous plant, damage to nutrient-catching organs in a low-nutrient habitat such as a peat swamp forest is certainly a much more severe loss than a simple reduction of its photosynthesis rate caused by areal leaf damage. This is of special importance for

Nepenthes species with a comparatively low pitcher production rate and high pitcher longevity (up to more than a year, Clarke 1997). The organism responsible for the loss of the pitchers was found to be a weevil, *Alcidodes* sp., found mainly on the tips of the youngest developing leaves of *Nepenthes*, which were effectively destroyed. It was most often observed on *N. bicalcarata*, frequently also on *N. ampullaria* Jack. and occasionally on *N. mirabilis* (Lour.) Druce var. *echinostoma* (Hook. f.) and *N. rafflesiana* Jack. Similar drilling damage was found in *N. albomarginata* T. Lobb ex Lindl. (unpublished own data). The weevil was found in peat swamp forests and, less frequently, in surrounding degraded areas, but never on plants other than *Nepenthes*.

Our comparative survey of damage revealed significantly fewer destroyed pitcher buds on *N. bicalcarata* plants inhabited by *C. schmitzi* than on uninhabited plants. Both plant groups were about equally often visited by non-specific ants and other nectar visitors (mostly insects). Our ant exclusion experiment showed the same pattern: plants occupied by *C. schmitzi* had significantly less drilling damage than unoccupied plants, whereas no protective effect of visits by other ants was evident. Why *N. bicalcarata* in general did not suffer from major areal leaf damage has still to be examined, but as there was no significant difference between colonized and uncolonized plants this seems not attributable to defense by ants.

In contrast to Clarke & Kitching (1995) we were able to demonstrate aggressive behavior under two special circumstances: *C. schmitzi* attacked *Alcidodes* sp., and the workers showed aggressive behavior when confronted with freshly damaged leaf parts of *N. bicalcarata*. Reaction to host plant damage has been observed for other ant associates of myrmecophytic plants, such as *Crematogaster* (*Decacrema*) spp. (Fiala & Maschwitz 1990) and *Camponotus* sp. (Federle *et al.* 1998) on *Macaranga*, as well as for several other Neotropical ant species (overview in Agrawal & Rutter 1998).

In our experiments the effectiveness of *C. schmitzi* against *Alcidodes* sp. was impressive: Of a total of 213 experiments, in which the attack of *Alcidodes* sp. by *C. schmitzi* was tested, 64.3 % resulted in an attack against the weevil. All beetles discovered by the ants were immediately attacked.

A defense against a specific herbivore has been documented in only a few cases in myrmecophilic

nectar-producing plants: against weevils of the genus *Cyrtotrachelus* that exclusively attack young shoots of the giant bamboo *Gigantochloa scortechinii* in South-East Asia (Stein, unpublished data), and against the pyralid moth *Filodes fulvidorsalis* attacking the climber *Thunbergia grandiflora* (Fiala *et al*. 1996). However in these cases, while the plants need the ants for protection against one specific enemy, the ants do not discriminate between different enemies, thereby also attacking the major pests of their host plants. The *N. bicalcarata – C. schmitzi* association goes beyond this in a sophisticated way: the ants recognize the plant's main enemy and do not attack other visitors like opportunistic ants. This is unique in ant-plant relationships.

C. schmitzi does not clean the surface of its host plant. On leaf and pitcher surfaces epiphyllic organisms such as mosses, algae, lichens, and fungi grow undisturbed by the ants, which do not damage the phorophyte either. This is plausible as its *Nepenthes* host plant is a climber, being dependent on contact with other plants.

These findings reflect the antagonistic interests in a very special and complex symbiosis. Unlike other specialized ant-plant systems, the carnivorous strategy of the plant partner here limits the possibilities of the ants to defend their home and feeding grounds. Driving away all visitors would result in reducing or eliminating the nutritive pitcher function. Similar conflicts of interest have been reported between plant-ants and pollinators (e.g., Jaffé 2003). This kind of conflict may also occur in the present relationship.

C. schmitzi sometimes even castrates *N. bicalcarata*'s inflorescences (own unpublished observations), a behavior also observed in other ant-plant relationships (Gaume *et al*. 2005, Yu *et al*. 1998). It is therefore advantageous for *N. bicalcarata* if aggressiveness and colony size of its specific partner ant are limited and as far as possible separated from potential prey. Indeed *C. schmitzi* does not impede the trapping mechanism of the plant, since it does not attack and chase away any other visitors, being potential prey.

Obviously, the *C. schmitzi* colonies contribute to the successful development of the pitchers and thus to the development of the plant and the gaining of nutrients by carnivory. We assume this makes up the costs of the ant colony to the plant (although we cannot estimate the cost/benefit relation). The ant receives in return an easily accessible permanent food source of both proteins (from the pitcher prey) and carbohydrates (from the nectaries). This conflict of interests is also reflected in the plant morphology. Besides the domatia being inhabited by the ants, the two peristome thorns, with their giant nectaries unique to *N. bicalcarata* can be explained as specific adaptations to their partner ant (Merbach *et al*. 1999). The ants mainly feed there instead of on the "prey-targeted" peristome nectarines, or take up peristome nectar, which is essential for pitcher function, not only as bait but also to maintain the slipperiness of the pitcher entrance (Bohn & Federle 2004).

Clarke and Kitching (1993,1995), seeing this ant-plant association mainly from the viewpoint of food webs in plant phytotelmata, have presented another explanation for the development of this system, focusing on the pitcher's function. They suggested that ants help to digest large prey items and thus prevent putrefaction (i.e., development of high amounts of poisonous ammonia), which in their opinion could impair or even destroy the pitcher tissue and the infauna food web.

Given the hypothesis that weevils are a major threat to *Nepenthes* in the peat swamp forest habitats, *N. bicalcarata* is the only species dealing with that situation by having protective ant partners. Among the other sympatrically occurring *Nepenthes* species, *N. gracilis* may be too small to attract *Alcidodes* sp.. In *N. rafflesiana* growing in peat swamp forests we found surprisingly few upper pitchers compared with plants in other habitats. *N. ampullaria* may follow another strategy unique in this *Nepenthes* species: usually no upper pitchers and only very few lower pitchers on exposed leaf positions are produced. Most pitchers are growing directly on the ground or very close to the stem, thus not as likely a target for a flying weevil. The pitcher buds of the upper leaves are damaged by *Alcidodes* sp. in the same way as in uncolonized *N. bicalcarata* plants (own observations) but they usually do not develop into functional traps anyway.

Clarke (1997) has observed *C. schmitzi* behavior focused on the inside of the pitchers. He states that the ants do not show any aggressive behavior towards other visiting insects and thus rules out any protective function against herbivores. In his interpretation, the ants function as a kind of digestion aid preventing putrefaction following death of the infauna and so avoiding pitcher damage. The finding of a major benefit of the partner ants to *N. bicalcarata* through

herbivore protection does not conflict in principle with the putrefaction avoidance hypothesis of Clarke (1997). Nevertheless to us this hypothesis seems rather unlikely. Our observations on *N. albomarginata* (Merbach *et al*. 2002) showed that even extremely large amounts of decomposing and putrefying prey – this plant in a unique way captures termites thousands at a time – do not effect nutrient absorption or pitcher longevity in this species. Mass captures are even a strategy here, not an accident. Additional experiments with large crickets placed in *N. bicalcarata* pitchers (details to be published elsewhere) showed putrefaction to be only a temporary phenomenon which in general did no damage to the pitchers. Capturing a single prey individual, large enough to cause putrefaction and small enough to be handled by the ants, seems to be an extremely rare and more hypothetic event. Most of the few cases of putrescent pitcher content in living pitchers that we found in *N. bicalcarata* were caused by mass trapping of small ants and were not prevented by *C. schmitzi*. It is surely in the ants' interest to keep the pitcher fluid, their "hunting ground", clean as they rely on fresh prey, but it is not necessarily in the interest of the plant. A positive effect of other infaunal organisms on the pitcher has been proposed by Kitching & Schofield (1986). We assume that any help of the ants with digestion plays a negligible role in the mutual benefit balance, if any.

Ant-plant mutualism in *Nepenthes* need not be restricted to *N. bicalcarata*. As we have published elsewhere (Merbach *et al*. 2001) most pitcher plant species checked attract ants with their nectaries, a general function of EFN which is also true in this plant genus. General nectar secretion patterns are quite similar to those of *N. bicalcarata*. They do, however, catch only a tiny percentage of the ant visitors (Merbach *et al*. 2001), which thus continue to visit the nectaries, recruit further ants, and perhaps even protect their *Nepenthes* plant more or less effectively against herbivores, depending on ant species and number. Closer examination of this aspect of *Nepenthes* ecology should follow.

ACKNOWLEDGMENTS

REFERENCES

Agrawal, A.A., & M.T. Rutter. 1998. Dynamic anti-herbivore defense in ant-plants: The role of induced responses. Oikos 83: 227–236 .

Bohn, H., & W. Federle. 2004. Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. PNAS 101 (39): 14138–14143.

Bronstein, J.L. 1998. The contribution of ant-plant protection studies to our understanding of mutualism. Biotropica 30 (2): 150–161.

Clarke, C.M. 1992. The ecology of metazoan communities in *Nepenthes* pitcher plants in Borneo, with special reference to the community of *Nepenthes bicalcarata* (Hook. f.). PhD thesis, University of New England (Monash University).

Clarke, C.M. 1997. *Nepenthes* of Borneo. Natural History Publications (Borneo), Kota Kinabalu, Sabah, Malaysia.

Clarke, C.M., & R.L. Kitching. 1993. The metazoan food webs from six Bornean *Nepenthes* species. Ecol. Entomol. 18: 7–16.

Clarke, C.M., & R.L. Kitching. 1995. Swimming ants and pitcher plants: a unique ant-plant interaction from Borneo. J. Trop. Ecol. 11: 589–602.

Davidson, D.W., & D. McKey. 1993. The evolutionary ecology of symbiotic ant-plant relationships. J. Hym. Res. 2(1): 13–83.

Federle, W., Maschwitz, U., & B. Fiala. 1998. The two-partner ant-plant system of *Camponotus* (*Colobopsis*) sp. 1 and *Macaranga puncticulata* (Euphorbiacaea): natural history of the exceptional ant partner. Insectes Soc. 45: 1–16.

Fiala, B., & U. Maschwitz. 1990. Studies on the South East Asian ant-plant associaton *Crematogaster borneensis* / *Macaranga*: adaptations of the ant partner. Insectes Soc. 37 (3): 212–231.

Fiala, B., Krebs, S.A., Barlow, H.S., & U. Maschwitz. 1996. Interactions between the climber *Thunbergia grandiflora*, its pollinator *Xylocopa latipes* and the ant *Dolichoderus thoracicus*: the "nectar-thief hypothesis" refuted? Malayan Nat. J. 50: 1–14.

Gaume, L., Zacharias, M., & R.-M. Borges. 2005. Ant-plant conflicts and a novel case of castration parasitism in a myrmecophyte. Evol. Ecol. Res. 7(3): 435–452.

Hölldobler, B., & E.O. Wilson. 1990. The ants. Springer Verlag, Berlin.

Huxley, C.R. 1986. Evolution of benevolent ant-plant relationships. Pp. 257–282 *in* Juniper, BE., & T.R.E. Southwood (eds.). Insects and the Plant Surface. Edward Arnold, London.

Jaffé, K., Hernández, J.V., Goitía, W., Osio, A., Osborn, F.R., Cerda, H., Arab, A., Rincones, J., Gajardo, R., Caraballo, L., Andara, C., & H. López, 2003. Flower ecology in the neotropics: a flower-ant love-hate relationship. Pp. 213–219 *in* Basset, Y., Novotny, V., Miller, S.E. & R.L. Kitching (eds.). Arthropods of Tropical Forests: Spatio-Temporal Dynamics and Resource Use in the Canopy, Cambridge University Press.

Joel, D.M. 1988. Mimicry and mutualism in carnivorous pitcher plants (Sarraceniaceae, Nepenthaceae, Cephalotaceae, Bromeliaceae). Biol. J. Linn. Soc., 35: 185–197

Jolivet, P. 1996. Ants and plants. An example of coevolution (enlarged edition). Backhuys Publ., Leiden.

Juniper, B.E., Robins, R.J. & D.M. Joel. 1989. The Carnivorous Plants. Academic Press, Oxford.

Kitching, R.L., & C. Schofield. 1986. Every pitcher tells a story. New Scientist 109: 48–50.

Linnaeus, C. 1737. *Nepenthes*. Hortus Cliffortianus. Amsterdam.

Merbach, M.A., Zizka, G., Fiala, B., Merbach, D., & U. Maschwitz. 1999. Giant nectaries in the peristome thorns of the pitcher plant *Nepenthes bicalcarata* Hook f. (Nepenthaceae): Anatomy and functional aspects. Ecotropica 5: 45–50.

Merbach, M.A., Zizka, G., Fiala, B., Maschwitz, U., & W.E. Booth. 2001. Pattern of nectar secretion in five *Nepenthes* species from Brunei Darussalam, Northwest Borneo, and implications for ant-plant relationships. Flora 196: 153–160.

Merbach, M.A., Merbach, D., Booth, W.E., Maschwitz, U., Zizka, G., & B. Fiala. 2002. Mass march of termites into the deadly trap. Nature 415: 36–37.

Moran, J.A. 1996. Pitcher dimorphism, prey composition and the mechanisms of prey attraction in the pitcher plant *Nepenthes rafflesiana* in Borneo. J. Ecol. 84: 515–525.

Moran, J.A., Booth, W.E., & J.K. Charles. 1999. Aspects of pitcher morphology and spectral characteristics of six Bornean *Nepenthes* pitcher plant species: Implications for prey capture. Ann. Bot. 83 (5): 521–528.

Schuitemaker, J.P., & A. Stärke. 1933. Contribution a l'étude de la faune Nepenthicole. Art. III. Un nouveau *Camponotus* de Borneo, habitant les tiges creuses de *Nepenthes*, recolté par J. P. Schuitemaker et décrit par A. Stärke, den Dolder. Natuurhist. Maandbl. 22 (3): 29–31.

Tan, H.T.W. 1997. A guide to the carnivorous plants of Singapore. Singapore Science Center, Singapore.

Whitmore, T.C. 1985. Tropical rain forests of the Far East. Oxford University Press, Oxford.

Yu, D., Pierce, W., & E. Naomi. 1998. A castration parasite of an ant-plant mutualism. Proc. R. Soc. Lond. B 265: 375–382.

Zizka, G. 1990. Pflanzen und Ameisen. Palmengarten Sonderheft 15: 112–116.

Zizka, G. 1991. Über die Kannenpflanze *Nepenthes bicalcarata*. JD Hooker. Palmengarten. 91(3): 39–44.