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GIANT NECTARIES IN THE PERISTOME THORNS OF THE PITCHER PLANT *NEPENTHES BICALCARATA* HOOKER F. (NEPENTHACEAE): ANATOMY AND FUNCTIONAL ASPECTS

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Abstract. The conspicuous thorns of the pitcher plant *Nepenthes bicalcarata* have been the subject of manifold speculations in the literature. Two theories about their function prevail: (1) the sharply pointed thorns keep predatory animals away from exploiting the pitchers (Burbidge 1880); (2) they play a role in attracting and trapping prey (Clarke 1993). Extrafloral nectaries (EFN) occur in species of *Nepenthes* in all parts of the plant above ground. Unique nectar-secreting structures are found in *Nepenthes bicalcarata* where the upper part of the peristome is enlarged, forming two thornlike structures. Investigations of these thorns revealed that they contain giant nectar glands which open at the apex of the thorn. These nectaries are by far the largest on the plant. Though the extraordinary size of the glandular tissue and amount of secreted nectar strongly support the idea that the thorns play a role in attracting prey, our results do not exclude the additional function of preventing exploitation of pitchers by predatory animals. The combination of a regular ant-plant association with the nectar-feeding *Camponotus schmitzi* Stärke and the extraordinary nectar glands suggests that the thorn nectaries could be preferentially exploited by the ant partner. *Accepted 04 March 1999.*

Key words: Carnivorous plants, extrafloral nectaries, ant-plant interactions, myrmecophytism, Nepenthes bicalcarata *Hooker f.*, Camponotus schmitzi.

INTRODUCTION

The genus *Nepenthes* comprises 82 species (Jebb & Cheek 1997) and is distributed across the eastern Paleotropics. Thirty-one species occur in Borneo, the center of diversity (Phillips & Lamb 1996, Clarke 1997). One of the endemic species is *Nepenthes bicalcarata*, so far recorded from NW Kalimantan, Sarawak, Brunei and SW Sabah from sea-level up to 950 m (Zizka 1991). Its natural habitats are peat swamp forests dominated by *Shorea albida* and occasionally heath forests on white sand soils, where this vine can climb to hights of up to 20 m (Jebb & Cheek 1997, own observations).

Nepenthes bicalcarata is outstanding in the genus in displaying the following characters: (a) two well-developed sharply pointed tooth- or thornlike structures of the peristome, located above the opening of the pitcher; (b) large amounts of nectar secreted from extrafloral nectaries (EFN) (see also Clarke 1992); (c) regular association with ants (mainly *Camponotus schmitzi*) (Schuitemaker & Stärke 1933, Clarke & Kitching 1995); (d) myrmecodomatia, i.e., swollen, hollow tendrils, usually used by *C. schmitzi* as nesting sites.

The relationship of *Nepenthes bicalcarata* and *Camponotus schmitzi* is still not completely understood, and is unique in the combination of carnivory and myrmecophytism. A closer investigation of this interaction undertaken by the authors is intended to reveal information about the benefits to the partners and how myrmecophytism in this carnivorous genus might have evolved. The extrafloral nectaries (EFN) are regarded as a very important feature for the development of the above-mentioned relationships.

Several publications have dealt with the anatomy of the *Nepenthes* peristome (e.g., Macfarlane 1908, Lloyd 1942), but detailed studies of structure and function of the thorns of *N. bicalcarata* have been lacking up to now.

In *N. bicalcarata* the uppermost part of the peristome is prolonged into a pair of characteristic thorns (cf. species name), three to four times longer than the rest of the peristome (Fig. 3). They project downwards from the lid with their points above the middle of the pitcher. Other characteristic features are the form and structure of the tendril. In contrast to other *Nepenthes* species, *N. bicalcarata* displays tendrils swollen and hollow in their apical part which are regularly inhabited by the ant *Camponotus schmitzi* (Schuitemaker & Stärke 1933, Clarke 1997).

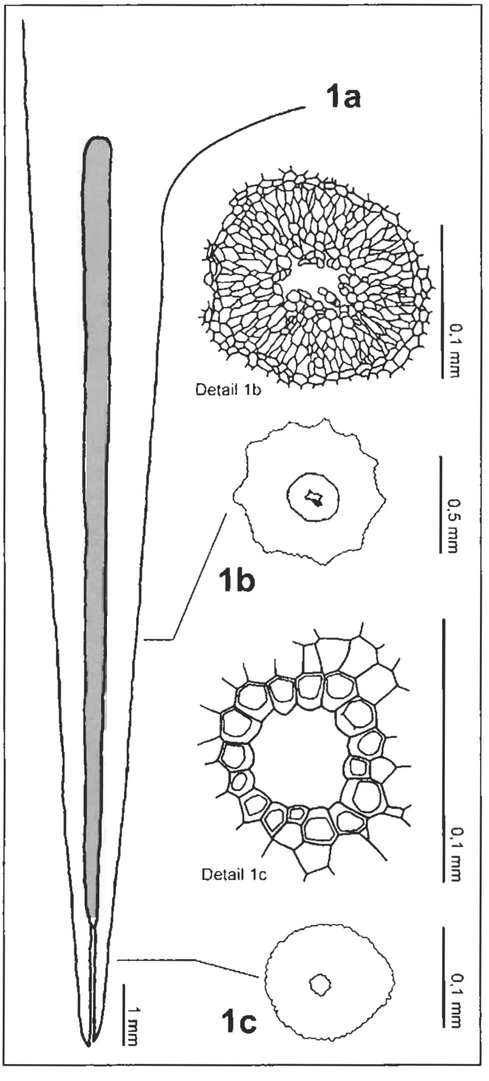

FIG. 1a-c. *Nepenthes bicalcarata*, peristome thorn. 1a: Longitudinal section, reconstruction from cross sections. 1b-c: Cross sections. 1b: Middle region, secretory tissue shaded. Detail of 1b: secretory tissue. 1c: Apical region with nectar duct. Detail of 1c: innermost cell layer of nectar duct.

Various authors have described the extraordinary pitcher morphology and speculated about its biological function.

Burbidge (1880) was the first to mention the relationship with ants (though he described a black ant nesting in dried pitchers instead of the red *C. schmitzi* nesting in the tendrils of the leaf) and to speculate about the function of the two thorns. He came to the conclusion that they prevent tarsiers (*Tarsius bancanus* is the only *Tarsius* species, a small primate, found in Borneo; Payne *et al.* 1985) from exploiting the pitchers without actually observing this behavior. Beccari (1886) agreed with Burbidge and was the first to interpret the hollow tendrils as ant domatia inhabited by a *Camponotus* species.

Dodd (1982) stated that the thorns' function is unclear but supposed them not to play any part in catching prey. Clarke (1993), however, proposed that many insects visit the pitchers to feed on the nectar that accumulates at the tips of the thorns. He observed pitchers that had been torn open and attributed this to monkeys and other small mammals thus obtaining their prey. Clarke concluded that the thorns do not serve to protect the pitcher's prey from marauding mammals but may be part of the prey-catching system.

Vogel (1960, 1961) has shown that in the fly-trap flowers of *Ceropegia sandersonii* (in this genus of the Asclepiadaceae the corolla is elaborated into a pitcher trap) a small peg-shaped structure located above the center of the corolla tube (Uvula or „Gleitzapfen") plays a substantial role in attracting and trapping prey. A similar function for the thorns of *N. bicalcarata* has been discussed, e.g., by Clarke (1993). Nevertheless, our field studies revealed that the thorns are regularly visited by *Camponotus schmitzi* to harvest the nectar. Ants of this species move without problems on the thorns, and if one happens to fall into the fluid it can easily swim to the pitcher wall and so escape. Visits to the thorns by other ant species (see below) are comparatively rare. The function of the thorns has thus remained puzzling.

The aim of this study, which is part of a larger comparative study on extrafloral nectaries in the genus *Nepenthes*, was a reassessment of the thorns' function by a study of their anatomy, nectar secretion and mechanical properties during development.

FIG. 2. *Nepenthes bicalcarata*, thorn apex with opening of nectar duct (SEM).

MATERIAL AND METHODS

The peristome thorns of *N. bicalcarata* were obtained from living greenhouse material cultivated in the 'Palmengarten der Stadt Frankfurt/Main', Germany. We studied nine different plants (accession number 94-12035-4-0).

Objects were fixed in AFE or Bouin, embedded in Paraffin (Art. 7164, Merck) (Gerlach 1984) or Polyethylenglycol (PEG 1500) (Böck 1989) and stained with Astrablue – Safranin solution. Staining time was twice as long as that recommended in Gerlach (1984).

Thorns were studied in transverse and longitudinal section, the sections mounted in Euparal™. Structures observed were documented by photographs and camera lucida drawings.

Micromorphology of the surface and tip of the thorns was studied with SEM (Hitachi S - 4500).

Field observations were made during four trips to Malaysia (1995) and Brunei (1997, 1998).

RESULTS

Mechanical Properties. Texture and induration of the thorns change with the age of the leaf. Fully developed but still closed pitchers, as well as those which have just opened their lid, have soft flexible thorns. A few days after opening, when the pitchers are still soft and flexible, the thorns gradually harden and become as sharp as the point of a needle.

Anatomy. Three principal types of EFN occur in *Nepenthes*. Very small nectaries are found on the lower surface of the lid and the adaxial side of the tepals. A second type is found only in the peristome area, while a third type of nectary is located on all other parts of the plant above ground. *N. bicalcarata* is unique in having single nectaries in each of the two thorns. Investigations of the size of different nectaries (length of the opening in the epidermis, area and length of secretory tissue) reveal that those of the thorns are the largest singular nectaries on the whole plant (Tab. 1). The latter secrete considerable amounts of nectar, often visible as droplets hanging from the tip of the thorns (details on EFN anatomy and on the patterns of nectar secretion in the leaves of *Nepenthes bicalcarata* will be published elsewhere).

The longitudinal section (Fig. 1a) reveals a large cylindrical glandular tissue extending ± from the base of the thorn to the tip. The nectar is released through a narrow channel at the very apex of the thorn. Figure 1c shows a cross section of the thorn with glandular tissue in the center. The thorn nectary (cal-

TABLE 1. Comparison of the size of *Nepenthes bicalcarata* nectaries (approximate volume calculated depending on nectary type for spheroid or cylinder [thorn nectaries] and based on anatomical sections)

Types of nectar glands	Length of glandular tissue [mm]	Estimated radius of glandular tissue [mm]	Estimated volume of EFN [mm^3]
Lid lower surface (n=2)	0.1	0.05 - 0.06	0.00063
All upper parts of the plant except tepals upper surface, lid lower surface, peristome (n=1)	1	0.3	0.19
Ring-like line of nectar glands located at the peristome and opening to the inside of the pitcher (n=1)	0.55	0.045	0.0023
Peristome thorns (n=1)	12.5	0.14	0.77

culated volume) is approximately 1000 times larger than the nectaries of the lower surface of the lid, 300 times larger than the peristome nectaries, and four times larger than the nectaries at the leaf base. The length of the thorns (apex to base) in fully developed pitchers ranges from 17 to 29 mm ($\bar{x}$ = 22 mm, n = 28).

Temporal pattern of nectar secretion. No nectar secretion was found in the early stages of development, when the thorns are still soft. Nectar drops were only observed on the hard thorns of fully developed pitchers. The nectar secretion even persisted when other parts of the pitcher tissue had hardened and died off (age of pitchers: approximately 1 year; Fig. 3).

The nectar secretion of the thorns and the other peristome nectaries coincides with the period when the pitcher is fully developed and able to catch prey.

FIG. 4. *Nepenthes bicalcarata*, uppermost part of an approximately one-year-old lower pitcher. Nectar is accumulating at the thorn apices.

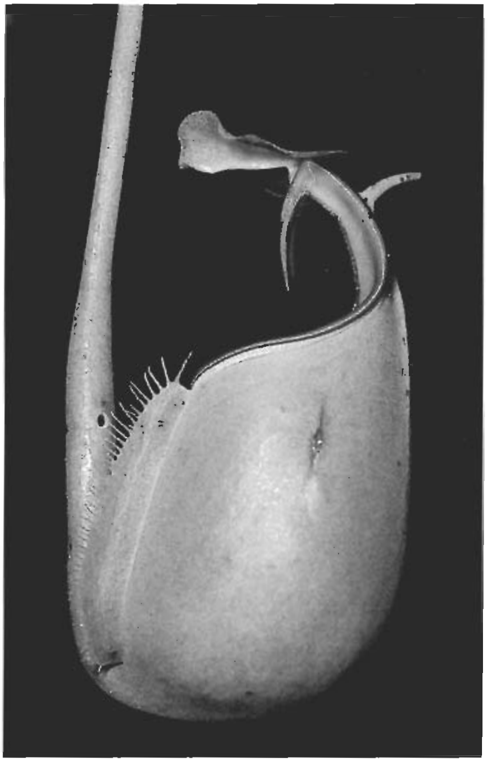

FIG. 3. *Nepenthes bicalcarata*, fully developed pitcher with the two peristome thorns. The ant species *Camponotus schmitzi* lives in the hollow tendril and under the peristome of the leaf.

Most of the other types of nectaries have their maximum nectar secretion in young leaves with developing, non-functional pitchers.

Visiting ants. Our observations revealed that the thorn nectar is regularly harvested by *C. schmitzi* (Fig. 5), while other ant visitors (*Polyrhachis hector*, *Polyrhachis* [*Myrma*] sp., *Crematogaster* spp.) which represent potential prey visited, principally, the other nectaries of the plant and were rarely observed at the thorn nectaries. *C. schmitzi* was never observed feeding on the nectaries of the developing leaves. The nectar produced by the pitcher is therefore considered a major sugar resource for *C. schmitzi* (Fig. 4).

DISCUSSION

The thorns of *Nepenthes bicalcarata* contain a cylindrical, extraordinarily large layer of nectar-secreting tissue. Unlike most other extrafloral nectaries the thorn nectaries do not secrete during the ontogenetic development of the surrounding tissue but release large quantities of nectar continuously during the long life period of the pitcher, a high energetic investment by the plant that presumably would not develop without being to its advantage.

During our 6-month field studies we observed the mutualistic *C. schmitzi* permanently visiting the thorn nectaries, though they are occasionally also visited by other ants, e.g., *Crematogaster* spp., *Polyrhachis* (*Myrma*) sp. (own observations and Charles

Clarke, pers. comm.). Our observations indicate that the thorn nectaries play a central role in feeding the domatia-housed partner ants. This could solve the main dilemma a carnivorous ant plant seems to face: ant partners of myrmecophytic plants usually protect the plant by driving away other visitors. This would be highly counterproductive for a carnivorous plant which aims at catching prey. Offering an extraordinarily rich additional nectar resource, mainly accessible to the ant partner *C. schmitzi*, could stop these ants from protecting the peristome nectaries. These are, in *N. bicalcarata*, without doubt designed to attract and catch insect prey.

So far we have not observed mammals or birds being warded off by the thorns as supposed by Burbidge (1880). However the thorn position at the pitcher entrance, their large size, needle-like shape and hardness indicate that they may function additionally as a mechanical defense, especially against mammalian vertebrates wishing to exploit the arthropods caught by the pitchers or living within them. That these contents are in fact worthwhile being exploited has been demonstrated by observations by Clarke (1993) and ourselves that pitchers are occasionally torn open at the side wall by small mammals. We have actually observed that the domatia in *N. bicalcarata* are opened and the ants exploited, probably by squirrels. It should also be mentioned that *C. schmitzi* workers are always present on the underside of the peristome and thus would also be protected by the thorns.

Our recent studies on myrmecophytic *Macaranga* spp. (Federle *et al.*, submitted) have revealed that rodents found in peat swamp habitats are also able to learn how to open and exploit the domatia contents of these trees.

Thus the occasional opening of the pitchers is not an argument against but in favor of an additional protective function of the *N. bicalcarata* pitcher thorns which might even be advantageous for the ants under the peristome.

Cage experiments with pitchers offered to the squirrels in question (similar to those performed on *Macaranga*; Federle *et al.*, submitted) should resolve this question. They may possibly also provide evidence whether the "poisonous snake mouth" appearance of *N. bicalcarata* pitchers, which looks so dangerous to human observers, also "impresses" and wards off small mammals, as speculated by Dodd (1982).

Finally, it has to be pointed out again that the thorn nectaries are not the only extrafloral nectaries on *N. bicalcarata*. As in other *Nepenthes* species mentioned in this paper, such structures are also found on other sites of the plant, apparently functioning in different contexts, i.e. on young tendrils of leaves and on the inflorescence (see also Vogel 1997 and 1998 for discussion of remarkable nectaries in various systematic groups).

FIG. 5. *Camponotus schmitzi* worker harvesting nectar from the apex of the thorn of *Nepenthes bicalcarata*.

Additional knowledge, not only of nectar secretion patterns, but also of plant surface texture, i.e. slipperiness, as well of differences in the locomotive abilities of different arthropod visitors, will play an important role in understanding the function of these structures.

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