

The importance of petiole structure on inhabitability by ants in *Piper* sect. *Macrostachys* (Piperaceae)

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Several Central American species of *Piper* sect. *Macrostachys* have obligate associations with ants, in which the ant partner derives food and shelter from modified plant structures and, in turn, protects the plant against fungal infection and herbivory. In addition to these obligate ant-plants (i.e. myrmecophytes), several other species in *Piper* have resident ants only sometimes (facultative), and still other plant species never have resident ants. Sheathing petioles of sect. *Macrostachys* form the domatia in which ants nest. Myrmecophytes in sect. *Macrostachys* have tightly closed petiole sheaths with bases that clasp the stem. These sheathing petioles appear to be the single most important plant character in the association between ants and species of sect. *Macrostachys*. We examined the structure and variation of petioles in these species, and our results indicate that minor modifications in a small number of petiolar characters make the difference between petioles that are suitable for habitation by ants and those that are not. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 153, 181–191.

ADDITIONAL KEYWORDS: ant–plant mutualisms – domatia – myrmecophytes – pearl bodies.

INTRODUCTION

Close associations between ants and plants are common throughout the tropics. Although plants benefit from ants in short-term associations, which are often nectary mediated (for example, Stephenson, 1982; Koptur, Rico-Gray & Palacios-Rios, 1998; de la Fuente & Marquis, 1999), potential benefits to plants are greater when ants are permanent residents within the plant (for example, Janzen, 1966; Huxley, 1978; Rico-Gray, 1993). This type of association has evolved in over 40 plant families (Davidson & McKey, 1993), and strikingly similar plant structures that ants exploit for food or shelter have evolved repeatedly in distantly related plants. Saccate leaf bases in which ants nest, for example, have evolved in Melastomataceae (Benson, 1985), Sterculiaceae (Bequaert, 1922), and Piperaceae (de Candolle, 1916), and swollen, hollow internodes are found in Fabaceae (McKey, 1984), Rubiaceae, and Passifloraceae (Bequaert, 1922). The majority of the literature has focused on how ants that nest in plants benefit their hosts by deterring herbi-

vores (reviewed in Beattie, 1985; Davidson & McKey, 1993; Jolivet, 1996) and by reducing competition by vines and other vegetation (Janzen, 1966). However, ant mutualists can also benefit plants by reducing fungal infection (Letourneau, 1998; Heil, Fiala & Maschwitz, 2001; Solano & Dejean, 2004) and by providing nutrients to the host plant (Rico-Gray *et al.*, 1989; Treseder, Davidson & Ehleringer, 1995; Sagers, Ginger & Evans, 2000). To describe these associations, we have adopted the term ‘myrmecophyte’, as it was used by Bequaert (1922) to include any plant in which ants continuously nest within ‘normal cavities’ or ‘special swellings or myrmecodomatia’; resident ants in these associations may or may not derive food from host plant-produced substances.

Piper L. sect. *Macrostachys* (Miq.) C.DC. is a monophyletic (Jaramillo & Callejas, 2004; Tepe, Vincent & Watson, 2004), neotropical section that contains a range of associations with ants including: (1) obligate myrmecophytes that are associated with a single ant species, *Pheidole bicornis* Forel (Risch *et al.*, 1977; Risch & Rickson, 1981; Risch, 1982; Letourneau, 1983, 1998); (2) facultative myrmecophytes in which a wide range of arbicolous ants are found nesting in the plants (Tepe *et al.*, 2004); and (3) non-myrmecophytes

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that do not support ant colonies. In the obligate and facultative myrmecophytes of sect. *Macrostachys*, domatia (hollow plant structures in which ants nest) are formed by the appressed margins of the petioles. Pearl bodies, epidermal cells filled with lipids, proteins, and carbohydrates (Risch *et al.*, 1977; O'Dowd, 1982; Fischer *et al.*, 2002), are produced in abundance inside the domatia of obligate myrmecophytes (Risch & Rickson, 1981; Rickson & Risch, 1984), and appear to make up the majority, if not the entirety, of the ant's diet (Fischer *et al.*, 2002, 2003). Furthermore, in several species of obligate myrmecophytes, ants excavate the stem pith to create cauline domatia, thereby increasing the volume of the inhabitable plant body several fold (Tepe, Vincent & Watson, 2007).

All species of sect. *Macrostachys* have sheathing petioles with the potential to form domatia; yet, of the 50 or more species in the section, only five are obligate myrmecophytes (Risch *et al.*, 1977; Letourneau, 1998; Tepe *et al.*, 2004) and only two have been observed to be facultative myrmecophytes (Tepe *et al.*, 2004). A number of studies have described petiole morphology for several Old and New World species of *Piper* (Debray, 1886; Chibber, 1912; Hoffstadt, 1916; Rousseau, 1927), but none have included members of sect. *Macrostachys*. In this study, we survey species of sect. *Macrostachys* encountered in the field for occupation by ants, explore and compare their petiole morphology to clarify which traits characterize petioles that are used as domatia and those that are not, and consider the role of petiole morphology (including pearl bodies) in the evolution of ant–plant associations in sect. *Macrostachys*.

MATERIAL AND METHODS

Fieldwork was conducted at 17 locations in Costa Rica during June–August 2000 and May–June 2001 and at 13 locations in Panama during May–June 2003. All plant collections are vouchered and deposited at the W.S. Turrell Herbarium at Miami University (MU). Duplicates from Costa Rica are held at the Museo Nacional de Costa Rica (CR) and the Universidad de Costa Rica (USJ); duplicates of the Panamanian collections were deposited at the University of Panama (PMA) and the herbarium at the Smithsonian Tropical Research Institute (STRI). In total, petioles from 111 individuals from 85 populations of 21 species were examined in this study (Table 1).

Unless stated otherwise, all anatomical sections are of the third youngest petiole and are from half the distance from the petiole base and the end of the sheathing margins. The third youngest petiole was chosen because most plants had at least three leaves per branch, the third leaf was always fully expanded on the plants observed, and the petioles of obligate

myrmecophytes were always occupied by ants at this stage.

All plants were surveyed for the presence of resident ants. If present, ant vouchers were either made in the field or recovered from the petioles of pressed plants and stored in 70% ethanol for identification. Generic identifications were made using Hölldobler & Wilson (1990). Ant vouchers are deposited in the Museum of Comparative Zoology, Harvard University.

In addition, 1106 herbarium sheets (MO, NY, U, and US) of members of sect. *Macrostachys* were examined. Data that can be unambiguously determined from herbarium specimens are whether the petiole margins were persistent at the time of collection (but not the degree of petiole closure), the presence of pearl bodies in petioles of obligate myrmecophytes, and the presence of obligate ant remains in petioles. In our experience, all colonies of facultative ants abandon the plants between collection and removal from the plant dryer, whereas *Ph. bicornis* colonies remain in the plant and, as a result, are preserved in the petioles of herbarium specimens.

The anatomy of the petioles was examined using standard hand or Vibratome Series-1000 (Vibratome, St. Louis, MO) sectioning and light microscopy techniques. Sections were stained with toluidine blue, safranin-fast green, or cresyl violet (Dizeo, 1980; D'Ambrogio, 1986), examined using standard light (Olympus AX70 and Nikon E-600) and dissecting (Olympus SZX12 and Nikon SMZ-2T) microscopy, and photographed with a Nikon 5F or a SPOT digital camera.

RESULTS

ANT OCCUPATION

Of the individual specimens surveyed in the field, occupation of the five obligate myrmecophytic *Piper* species by *Ph. bicornis* was high: *P. calcariformis*, 100% occupied; *P. cenocladum*, 92% ($N = 47$); *P. fimbriulatum*, 88% ($N = 8$); *P. obliquum*, 78% ($N = 9$); and *P. sagittifolium*, 83% ($N = 6$). All of these species were inhabited by *Ph. bicornis*, with the exception of one individual of *P. obliquum* in which *Ph. bicornis* inhabited all branches except one; the exception to this was a branch inhabited by *Crematogaster* sp. The *Crematogaster* colony inhabited part of the branch that was distal to an unusually long and slender internode that was not hollow. No conflict between the ant species was observed, and no difference in health or level of herbivory was observed between branches occupied by either species. Pearl body production was typically high in the petioles occupied by *Ph. bicornis*, but low (compared with descriptions by Risch & Rickson, 1981) in those occupied by *Crematogaster* sp.

Table 1. Accessions that were studied anatomically. Collectors are Eric J. Tepe (MU) and Armando Estrada (CR), and MOBOT numbers are Missouri Botanical Garden accession numbers for plants cultivated in the garden. All vouchers are deposited at MU. Duplicate sets of Costa Rican collections are held at CR and USJ and sets of Panamanian collections are held at PMA and STRI

Species	Collection/accession number	Source
<i>Piper arboreum</i> Aubl.	EJT 377	Costa Rica: Puntarenas
<i>P. arboreum</i> Aubl.	EJT 1039, 1040	Panama: Panamá
<i>P. arboreum</i> Aubl.	EJT 620	French Guiana: Cayenne
<i>P. archeri</i> Trel. & Yunck.	EJT 1000	Panama: Chiriquí
<i>P. auritifolium</i> Trel.	EJT 94, 102, 103, 427	Costa Rica: Heredia
<i>P. auritifolium</i> Trel.	EJT 178, 179	Costa Rica: Cartago
<i>P. biseriatum</i> C.DC.	EJT 95, 438	Costa Rica: Heredia
<i>P. biseriatum</i> C.DC.	EJT 140, 141	Costa Rica: Guanacaste
<i>P. biseriatum</i> C.DC.	EJT 183	Costa Rica: Cartago
<i>P. biseriatum</i> C.DC.	A. Estrada 2398, EJT 468	Costa Rica: Alajuela
<i>P. calcariformis</i> Tebbs	A. Estrada 2397	Costa Rica: Alajuela
<i>P. calcariformis</i> Tebbs	EJT 1009	Panama: Chiriquí
<i>P. campanum</i> Yunck.	EJT 1033	Panama: Panamá
<i>P. campanum</i> Yunck.	EJT 1048	Panama: Coclé
<i>P. cenocladum</i> C.DC.	EJT 92, 99, 105, 428 430, 431, 435, 436, 440	Costa Rica: Heredia
<i>P. cenocladum</i> C.DC.	EJT 144	Costa Rica: Guanacaste
<i>P. cenocladum</i> C.DC.	EJT 180, 185	Costa Rica: Cartago
<i>P. cenocladum</i> C.DC.	EJT 393	Costa Rica: Alajuela
<i>P. cordulatum</i> C.DC.	EJT 975	Panama: Chiriquí
<i>P. cordulatum</i> C.DC.	EJT 1011	Panama: Colón
<i>P. cordulatum</i> C.DC.	EJT 1016	Panama: Panamá
<i>P. daguanum</i> C.DC.	EJT 1044	Panama: Panamá
<i>P. fimbriulatum</i> C.DC.	EJT 115, 119, 321, 343, 352	Costa Rica: Puntarenas
<i>P. fimbriulatum</i> C.DC.	EJT 971	Panama: Panamá
<i>P. gibbosum</i> C.DC.	EJT 168, 170	Costa Rica: San José
<i>P. gibbosum</i> C.DC.	EJT 411	Costa Rica: Cartago
<i>P. hebetifolium</i> W.C.Burger	EJT 448, 454	Costa Rica: Alajuela
<i>P. imperiale</i> (Miq.) C.DC.	EJT 97, 100, 106, 107, 419, 432	Costa Rica: Heredia
<i>P. imperiale</i> (Miq.) C.DC.	EJT 169, 182	Costa Rica: San José
<i>P. imperiale</i> (Miq.) C.DC.	EJT 401	Costa Rica: Alajuela
<i>P. imperiale</i> (Miq.) C.DC.	EJT 473	Costa Rica: Puntarenas
<i>P. imperiale</i> (Miq.) C.DC.	EJT 989	Panama: Veraguas
<i>P. imperiale</i> (Miq.) C.DC.	EJT 1055	Panama: Chiriquí
<i>P. longispicum</i> C.DC.	EJT 410	Costa Rica: Cartago
<i>P. marsupiatum</i> Trel. & Yunck.	MOBOT # 931716	Ecuador
<i>P. maxonii</i> C.DC.	EJT 370	Costa Rica: Puntarenas
<i>P. melanocladum</i> C.DC.	EJT 134, 426	Costa Rica: Heredia
<i>P. obliquum</i> Ruiz & Pav.	EJT 114, 345, 351, 385, 386	Costa Rica: Puntarenas
<i>P. obliquum</i> Ruiz & Pav.	EJT 173	Costa Rica: San José
<i>P. obliquum</i> Ruiz & Pav.	EJT 974, 1022	Panama: Panamá
<i>P. obliquum</i> Ruiz & Pav.	EJT 1008, 1067	Panama: Chiriquí
<i>P. obtusilimum</i> C.DC.	MOBOT # 930887	Ecuador
<i>P. sagittifolium</i> C.DC.	EJT 116, 126, 320, 326, 327	Costa Rica: Puntarenas
	MOBOT # 931714	Unknown
<i>P. tuberculatum</i> Jacq.	EJT 1061	Panama: Panamá

Piper biseriatum and *P. imperiale* are considered to be facultative myrmecophytes based on occupation by a number of unrelated ant species. Five of 12 individuals of *P. biseriatum* (41.7%) and three of 13 individuals of *P. imperiale* (23.1%) surveyed were occupied by ant colonies of *Crematogaster* spp., *Solenopsis* spp., *Wasmannia* spp., and other species of *Pheidole*; the remaining individuals were uninhabited. These ant colonies were found to inhabit from one to all petioles of the occupied plants. Finally, numerous species of ant were found foraging on the remaining species of sect. *Macrostachys*, but no nests were detected in these species.

PETIOLE MORPHOLOGY

The base of the sheathing petioles of sect. *Macrostachys* nearly encircles the stem and encloses the apical growth (the apical meristem and all leaves, nodes, and internodes distal to the youngest, fully expanded petiole and associated node; Fig. 1). After the apical growth emerges, the shape of the petiole cavity varies by species (Fig. 2A–E).

Petioles of obligate myrmecophytes that can be inhabited by ants are characterized by chambers formed by closed sheaths and by margin bases clasping the stem (Fig. 2F vs. 2G; Table 2). The margins of the sheathing petioles are persistent throughout the life of the leaf and are uniformly tightly appressed (Fig. 2E). The margin bases also clasp the stem, resulting in completely closed cavities at all nodes (Fig. 2F). These characters vary little in obligate myrmecophytes in which petioles at all nodes, including branching nodes, form domatia.

The width of the petiole chamber (the smallest dimension) varies dramatically between species. However, that of the obligate myrmecophytes is more restricted in size range than that of the facultative species or non-myrmecophytic species (Fig. 3). In all species of obligate myrmecophytes, except *P. calcariformis*, ants regularly excavate the stem creating additional domatia, and ants chew an entrance hole through the stem from within the petiole chamber resulting in continuity between the petiolar and cauline domatia (Fig. 2H). We have never observed ants occupying cauline domatia to the exclusion of the petioles.

In the majority of the remaining species, the margins of the sheathing petioles partially senesce, to a greater or lesser degree, soon after emergence (Fig. 2A–C; Table 2). Once the apical growth has emerged, the persistent portions of the margins stay in an open position in some species, resulting in an open 'U'-shaped cavity (Fig. 2A, B), but, in others, the margins return to their positions prior to emergence of the apical growth and form a more or less closed, hol-



Figure 1. Young leaf and apical growth emerging from sheathing petiole of *Piper imperiale*. The membranous margins of the petiole remain green until the next leaf emerges, or longer, and arboreal ants may use the resulting chamber as a nesting site. Elevated spots on stems and petioles are tubercles.

low chamber (Fig. 2C–E). In addition, the bases of the petiole margins in some species tightly clasp the stem, whereas, in others, they are more lax.

As in obligate myrmecophytes, petiole chambers constitute the domatia of the facultative myrmecophytes (Fig. 2C). Overall petiole size and shape, the degree of chamber closure, and the longevity of the chamber (determined by how long the margins remain green after the emergence of apical growth) are quite variable (Table 2; Fig. 3). The degrees of chamber closure and stem clasping are variable both within and between individual plants of facultative myrmecophytes. Petioles that do not form domatia have weakly closed or open sheaths and do not clasp the stem (Fig. 2G); these petioles are frequently found to be filled with water and debris. Within an individual, some petioles form tightly closed, sheltering cavities, whereas the sheaths of others are only weakly closed or the margin bases do not clasp the stem (Fig. 2F). Petioles at branching nodes are more likely to be open

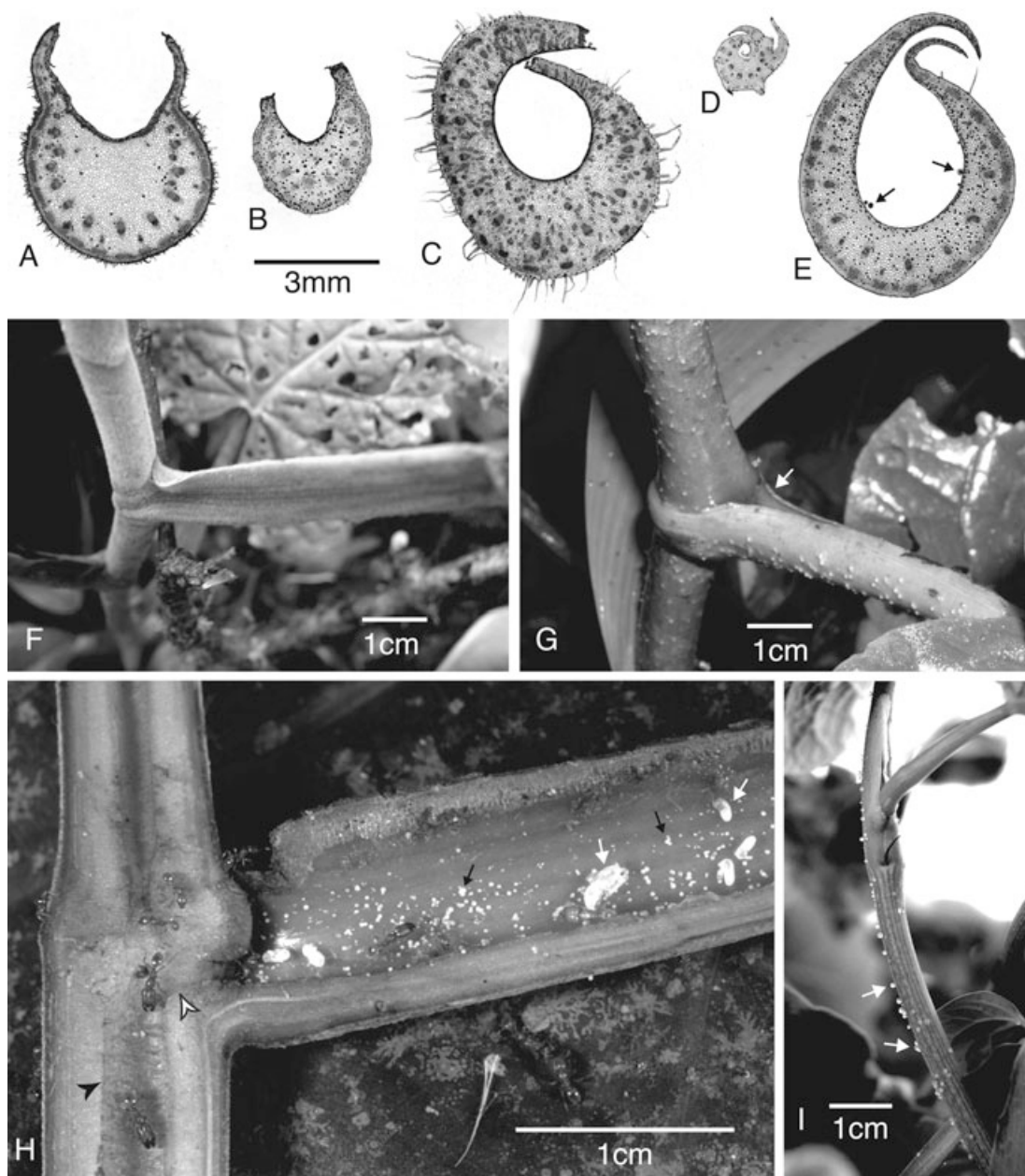


Figure 2. A–E, Cross-sections of petioles of *Piper* sect. *Macrostachys*. A, *P. campanum*. B, *P. gibbosum*. Margins of the petioles of *P. gibbosum* and *P. campanum* are deciduous or partly deciduous, resulting in open ‘U’-shaped petioles. C, *P. imperiale*, a facultative myrmecophyte. Although the petiolar margins of *P. imperiale* are partially abscised, the remaining margins curl, resulting in a closed chamber that can be used as a nesting site by ants. D, *P. tuberculatum*. E, *P. obliquum*, an obligate myrmecophyte. Arrows indicate pearl bodies. F, G, Petiole–stem junction of species of *Piper* sect. *Macrostachys*. F, *P. obliquum*, an obligate myrmecophyte. Note that petiolar margins are appressed along the length of the petiole and to the stem, resulting in a completely closed chamber. G, *P. imperiale*, an occasional facultative myrmecophyte, but this petiole is not inhabitable. Notice gap between petiolar margins and between the petiole and stem (arrow). Chamber fills with debris and rain water. H, Longitudinal section through stem and petiole of *P. cenocladum*, an obligate myrmecophyte occupied by *Pheidole bicornis*. Note excavated domatium in stem (black arrowhead) and passage between petiolar and cauline domatia (white arrowhead). Small white spots inside petiole chamber are pearl bodies (black arrows); larger white structures are ant larvae and pupae (white arrows), and two castes of workers are visible. Photograph by Dr Greg Dimijian. I, Stem and petiole of *P. auritum* Kunth (non-myrmecophyte, not a member of *Piper* sect. *Macrostachys*) showing pearl body production on young stem and petiole (arrows). These pearl bodies may function as generalist ant attractants in the absence of extrafloral nectaries in *Piper*.

Table 2. Petiole characters and dimensions of specialized, generalized, and non-myrmecophytes. Measurements are in millimetres (standard deviation)

Species	<i>N</i>	Petiole sheath closed?	Petiolar margins deciduous?	Petiole sheath clasping stem?	Petiole chamber length	Petiole chamber width	Petiole chamber height
Specialized myrmecophytes							
<i>P. calcariformis</i>	2	Yes	No	Yes	35.0 (6.65)	3.6 (0)	5.15 (0.49)
<i>P. cenocladum</i>	13	Yes	No	Yes	51.23 (15.81)	3.23 (0.31)	5.28 (0.88)
<i>P. fimbriulatum</i>	6	Yes	No	Yes	47.86 (14.60)	2.56 (0.63)	4.47 (1.14)
<i>P. obliquum</i>	10	Yes	No	Yes	47.93 (10.40)	2.69 (0.53)	4.57 (0.85)
<i>P. sagittifolium</i>	6	Yes	No	Yes	39.8 (<i>N</i> = 1)	2.82 (0.69)	4.03 (0.75)
Generalized myrmecophytes							
<i>P. biseriatum</i>	7	Yes	No	Occasionally	37.54 (10.82)	2.68 (0.56)	3.88 (0.68)
<i>P. imperiale</i>	12	Yes	Partially	Occasionally	55.2 (24.15)	3.94 (0.79)	5.3 (1.17), 'U'
Non-myrmecophytes							
<i>P. arboreum</i>	4	Open 'U'	Yes	No	12.75 (2.29)	1.10 (0.34)	1.05 (0.07), 'U'
<i>P. gigas</i>	1	Yes	No	No	51.9	2.5	4.9
<i>P. auritifolium</i>	6	Open 'U'	Yes	No	34.52 (9.68)	2.1 (0.56)	'U'
<i>P. campanum</i>	2	Open 'U'	Partially	No	53.9 (16.40)	3.4 (0.28)	4.6 (0), 'U'
<i>P. cordulatum</i>	3	Open 'U'	Yes	No	11.83 (3.39)	0.82 (0.20)	1.5, 'U' (<i>N</i> = 1)
<i>P. daguanum</i>	1	Open 'U'	No	No	12.9	2.5	'U'
<i>P. gibbosum</i>	3	Open 'U'	Yes	No	33.4 (<i>N</i> = 1)	1.85 (0.07)	'U'
<i>P. hebetifolium</i>	2	Weakly closed	No	No	22.7 (4.67)	2.25 (0.07)	4.6, 'U' (<i>N</i> = 1)
<i>P. longispicum</i>	1	Yes	No	Occasionally	n/a	3.45 (0.21)	4.7 (0.56)
<i>P. marsupiatum</i>	1	Open 'U'	No	No	21.8	2.6	4.0
<i>P. maxonii</i>	1	Weakly closed	No	No	26.8	1.7	3.2
<i>P. melanocladum</i>	2	Weakly closed	Partially	No	25.75 (5.89)	1.7 (0.14)	3.4 (0.99)
<i>P. obtusilimbium</i>	1	Open 'U'	Partially	No	24.1	3.5	'U'
<i>P. tuberculatum</i>	1	Yes	No	Yes	7.5	0.23	0.25

n/a, material not available to measure.

and filled with water and debris than those at non-branching nodes. All facultative myrmecophytes observed lack cauline domatia.

The petioles of non-myrmecophytic species are either very small (Fig. 2D), or are open along some part of the sheath or do not clasp the stem, thereby allowing the chamber to become flooded with water and filled with debris (Table 2; Fig. 2A, B, G). As apical growth emerges in several species of non-myrmecophytes (i.e. *P. gibbosum*), margins tear from the petiole (Fig. 2B; Table 2). This trait appears to be variable, as it was observed in some petioles of a single individual, but not in others. No colonies were found nesting in the petioles of any species listed as non-myrmecophytic (Table 2).

In some species, there is evidence of organ differentiation between the petiole proper and the margins (i.e. Fig. 2A vs. 2E). The petiole margins of species with high degrees of differentiation are thin and membranous, and a clear boundary is evident between the petiole and margin (Figs 1, 2A). These margins are typically early deciduous, and all species observed with such differentiation are non-myrmecophytes. The

distinction between petiole and margin is less distinct in other species (Fig. 2D, E).

DISCUSSION

Occupation of four of the obligate myrmecophytes (i.e. *P. cenocladum*, *P. fimbriulatum*, *P. obliquum*, and *P. sagittifolium*) by *Ph. Bicornis*, observed in this study, is comparable to the observations of previous studies (Letourneau, 1998; Letourneau & Dyer, 1998; Dyer & Letourneau, 1999). For the fifth obligate myrmecophyte, *P. calcariformis*, neither the protologue (Tebbs, 1987) nor any description of the species refers to the presence of resident ants; however, all plants encountered in the field (six individuals in three populations) and 11 of the 12 herbarium specimens examined, including the holotype, were occupied by ants. The ant species living in *P. calcariformis*, like all other known obligate *Piper* ant-plants, is *Ph. bicornis* (S. Cover, pers. comm.).

The occupancy rates of facultative myrmecophytes are reported here for the first time. These myrmecophytic associations are interpreted to be facultative in

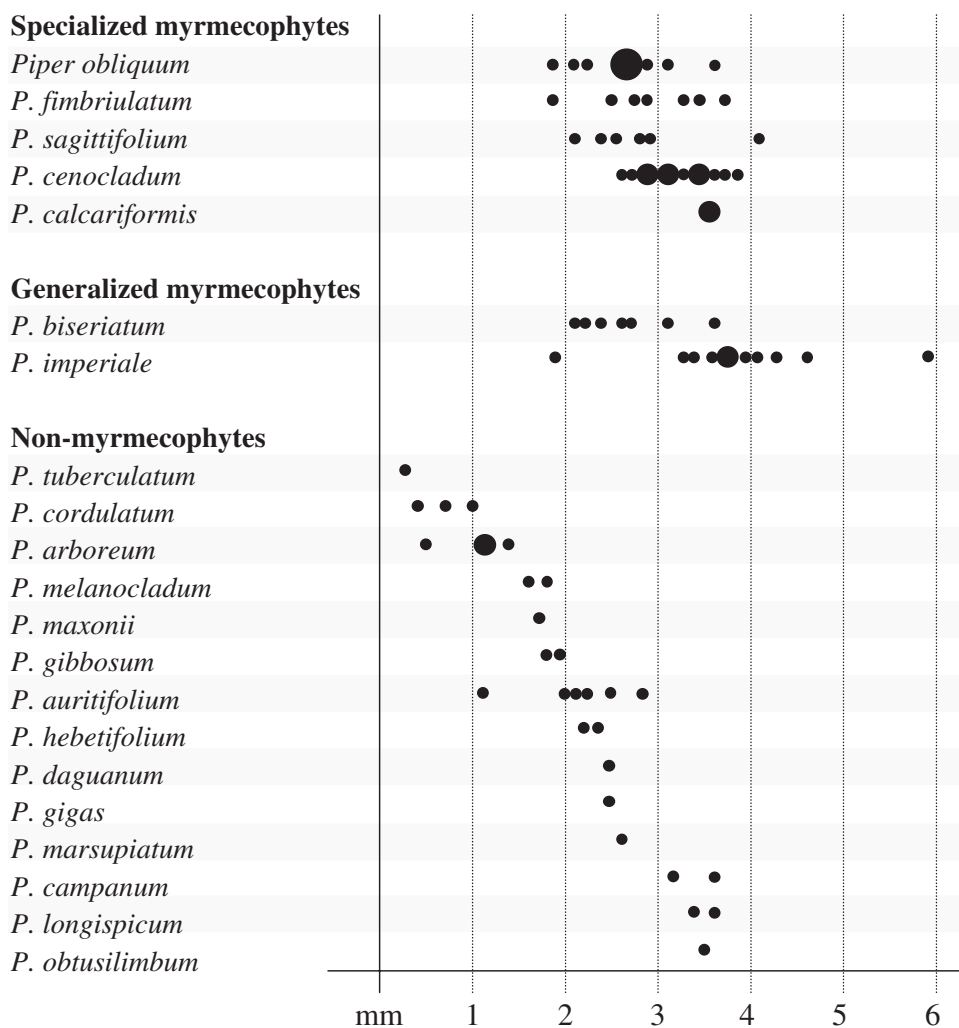


Figure 3. Range of petiole chamber widths for specialized, generalized, and non-myrmecophytes. Increasing size of spots indicates increasing number of superimposed points.

nature because ant nests were found in approximately 25% of the individuals surveyed (five of 21 collections of *P. biseriatum* and three of 13 collections of *P. imperiale*), ants were of numerous species, but never *Ph. bicornis*, and no pearl body production was observed inside the petiolar domatia of any of the occupied plants. Species of sect. *Macrostachys* typically grow as widely spaced individual plants or small clusters of individuals, and large populations are rarely encountered. Consequently, we have only encountered one or a few individuals of some species, and have not yet seen some South American species in the field. On the basis of the results presented here and of the examination of petioles from herbarium specimens (from which the degree of petiole closure cannot be determined), we predict that facultative myrmecophytism is much more common in sect. *Macrostachys* than is reported here.

PETIOLE MORPHOLOGY

The structure of the petiole is key in myrmecophytic associations between ants and species of sect. *Macrostachys*. Apical growth of most neotropical *Piper* species is protected by a deciduous prophyll (an appendage distinct from the sheathing petiole; Burger, 1972), but, in sect. *Macrostachys*, the prophyll is reduced and the apical growth is protected by the sheathing petiole. When the new growth emerges from the petiole, a large, empty chamber is left behind. For this reason, the morphology of sect. *Macrostachys*, amongst New World *Piper*, is particularly suited to supporting ant colonies.

The petioles of non-myrmecophytes are uninhabitable for several reasons. The margins of the petioles of some species become dry after the emergence of apical growth, resulting in an open 'U'-shaped cavity. In

other species, the margins remain green and fleshy, but do not come into contact with each other. In other species, the margins may come into contact, but are not pressed against the stem and thus do not form a weatherproof cavity. Even a small gap at the stem–petiole junction can result in the petiole becoming flooded with water. Ants were found exclusively in petioles that were well closed throughout the length of the cavities and at the petiole–stem junction.

In general, petiole chambers are taller than wide (see Fig. 2E), and thus the chamber width is the dimension that limits colonization by ants (Moog, Drude & Maschwitz, 1998). The dimensions of the petiolar cavities are relatively similar in all of the obligate ant-plants, and the width of the chambers averages 3.0 mm, with the smallest occupied petiole measuring 2.4 mm in width (Fig. 3). Similarly, the average diameter of the ant-excavated stem cavities is 2.7 mm (E. J. Tepe, unpubl. data). The petiole chambers of facultative myrmecophytes are more variable in width and range, from sizes comparable with those of obligate myrmecophytes to much larger (Fig. 3). The chambers of non-myrmecophytes are either very small (Fig. 3) or are open, forming a ‘U’-shaped cavity. Either condition is unsuitable for occupation by *Ph. bicornis*. The minimum chamber dimension tolerated by *Ph. bicornis* is unknown, but must certainly be greater than the head width of major workers (> 1 mm; Longino & Cover, 2004).

PEARL BODIES

Pearl bodies were not investigated in this study, but appear to be important to the association between *Ph. bicornis* and *Piper* species. Abundant pearl bodies are produced on the adaxial surface of the petiole (i.e. inside the domatium; Fig. 2E, H) when the ant partner *Ph. bicornis* is in residence (Risch & Rickson, 1981); however, similar structures are found on the surfaces of leaves, petioles, stems, and inflorescences of a number of *Piper* species worldwide (Fig. 2I; Meyen, 1837; Penzig, 1892; Nestler, 1893; Gastreich & Gentry, 2004; E.J. Tepe, pers. obs.). The nature of pearl body contents from non-myrmecophytes is unknown, but the presence of proteins, lipids, and carbohydrates (*P. aduncum*; Tepe *et al.*, 2004) suggests that they may be important in the attraction of generalist ants to *Piper* plants. Several authors have suggested that pearl bodies produced openly on the plant body could attract foraging ants as well as other natural enemies of herbivores, and that plants would benefit from the presence of the ants (Penzig, 1892; O’Dowd, 1982; Gastreich & Gentry, 2004). This hypothesis is corroborated by the observations of generalist ants collecting pearl bodies whilst foraging on plants (Fiala *et al.*, 1994; Gastreich & Gentry, 2004) and of reduced levels

of herbivore damage in non-myrmecophytes that produce pearl bodies (Fiala & Maschwitz, 1992b). If this is the case, pearl bodies, together with the characteristic large, sheathing petioles, may have been instrumental in the evolution of myrmecophytism in *Piper* sect. *Macrostachys*. Additional studies are needed to test whether these pearl bodies actually attract ants in *Piper* and whether any benefit is conferred to the plants.

ORIGIN AND EVOLUTION OF ASSOCIATIONS

Petiolar domatia are evidently essential for the development of close evolutionary relationships between ants and *Piper* species, as no examples of myrmecophytic associations exist in sect. *Macrostachys* in which plants produce pearl bodies in the absence of a suitable nesting site. Similar results were found in the Asian euphorb *Macaranga*, a genus that also includes species with different degrees of association with ants (Fiala & Maschwitz, 1992a). The evolution of myrmecophytism in plants with domatia, in the absence of food production (nectar or food bodies), appears to be relatively common (McKey, 1989). Only *Macaranga* species with hollow stems in which the ants could nest developed obligate, species-specific associations with ant mutualists, even though all species studied had extrafloral nectaries and produced food bodies (Fiala & Maschwitz, 1992a, b; Fiala *et al.*, 1994). In other words, food bodies appear to strengthen the association between ants and plants, but are secondary in importance to nesting space (i.e. domatia), and no case of a close species-specific association has been reported in *Piper* in which ants derive food, but not nesting space, from the plant. It is probable that, as shelter provides ant mutualists protection from predators, plants should derive more benefit from more stable populations of ants than in systems with pearl bodies only (Gastreich & Gentry, 2004).

Myrmecophytes have evolved repeatedly in plant groups which possess structures that, with little or no modification by ants, are usable as nesting sites (McKey, 1989). Large, deeply sheathing petioles are characteristic of most species of sect. *Macrostachys*, as is the potential for them to form domatia. Because these petioles do not require further modification by ants, myrmecophytic associations with arboricolous ants are expected to be frequent and widespread (Byrne, 1994; Alonso, 1998). For these reasons, we believe that facultative myrmecophytes in sect. *Macrostachys* are more widespread geographically and taxonomically than reported here. Hypothetically, ants nesting opportunistically in petioles should provide some level of sustained benefit to the plant, which could lead to the selection for petioles more suitable for occupation. The potential for selection is present

and several studies have demonstrated the benefit of these ants to facultative myrmecophytes (Fiala *et al.*, 1994; Maschwitz, Fiala & Linsenmair, 1994; Maschwitz *et al.*, 1996; Alonso, 1998; Di Giusto *et al.*, 2001).

As pointed out by McKey (1988), ant mutualists are not inherited and must be acquired anew in each generation of plants. Primary domatia, i.e. the petioles of *Piper* that do not need to be modified by ants before occupation, may facilitate colonization by founding queens. Furthermore, although not produced in abundance in the absence of *Ph. bicornis*, pearl bodies are present in small numbers in unoccupied petioles (Risch & Rickson, 1981), and these few pearl bodies may be sufficient to sustain the queen during the first stages of colonization.

Little is known about the founding of ant colonies and the occupation of young plants. Presumably, a foundress can enter a petiole chamber as soon as the apical growth has emerged from the first petiole. A population of *P. cenocladum* with numerous young plants was encountered at Parque Nacional Guanacaste (Guanacaste Prov., Costa Rica). Individuals 50 cm in height and with an average of five leaves (including leaf scars) were occupied at a rate of more than 50%.

The relationship between ecology, leaf size (and associated stem and petiole size), and myrmecophytism has been documented (Davidson & McKey, 1993; Brouat & McKey, 2001). This same pattern is apparent in sect. *Macrostachys*, in which most species in the section are large-leaved shrubs of exceedingly humid habitats. The petiole margins of smaller leaved *Piper* from other sections are often much reduced, resulting in nearly terete petioles. It is possible that the margins in sect. *Macrostachys* contribute to the support function of the petiole, much like the flanges of an I-beam, to support the large leaves (Ennos, Spatz & Speck, 2000). Phylogenetic inertia has provided *Piper* with sheathing petioles (Takhtajan, 1997), and the original function of petiole morphology in sect. *Macrostachys*, leaf support aside, is undoubtedly the protection of apical growth. However, the retention of margins and their return to a tightly closed position after the emergence of apical growth are the only characters required to form petiolar domatia in those species with petioles large enough to house ant colonies. In summary, minor modifications in petiole morphology and in patterns of pearl body production are responsible for the shift from non-myrmecophytes to facultative myrmecophytes and obligate myrmecophytes, or vice versa.

Although petiolar domatium structure and the location of pearl body production are strikingly similar in the five species of obligate myrmecophytes, preliminary phylogenetic analyses have indicated that obli-

gate mutualisms have evolved independently two to four times in sect. *Macrostachys* (Tepe *et al.*, 2004). Thus, it appears that the same conditions have evolved in parallel in at least two lineages, albeit from similar starting material and through minor modifications. Facultative myrmecophytes have evolved independently at least twice, and only once in a clade that also includes an obligate myrmecophyte (Tepe *et al.*, 2004).

CONCLUSIONS

Whether a given species of *Piper* sect. *Macrostachys* can support resident ant colonies is closely linked to a small number of plant traits, and, when ants are present, the nature of the association (i.e. obligate or facultative) is contingent on variations of these same traits. These critical plant characters include persistence of the petiolar margin (or enough of the margin to form a closed chamber), a tendency for the petiole to form a closed chamber (i.e. for the margins to come into contact with each other and to become pressed against the stem), and abundant pearl body production inside the petiole chamber. The first two characters are found in all myrmecophytes, whereas the third is restricted to and appears to be crucial for the development of obligate associations. Further studies are required to better understand the nature of the associations between facultative myrmecophytes and their resident ants, and to test how, if at all, facultative myrmecophytes benefit from these associations. Answers to these questions would help to evaluate whether ants in facultative associations in *Piper* can act as selective agents, driving the evolution of the more specialized obligate myrmecophytes.

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