

FOLIAR DOMATIA, MYCELIAL GALLERIES, AND AMBUSH TRAPS IN SOME NEOTROPICAL MYRMECOPHYTES

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Abstract. The association of the myrmecophilous tree *Hirtella physophora* (Chrysobalanaceae) and the ant *Allomerus octoarticulatus* (Myrmicinae) is described on the basis of early fortuitous field observations made in Central Amazonia and later investigation of fixed material. These are compared with the recently discovered symbiosis of the same host species with *Allomerus decemarticulatus* in French Guiana. Both ant species occupy the leaf domatia of *Hirtella* and build perforated carton galleries along the twigs to ambush insect prey. The main component of the carton walls is a living mycelium, probably sooty molds (Chaetothyriales) as found in French Guiana. Overall traits of the galleries and ant behavior observed in the two localities resemble each other. The course of ontogenesis of the domatium and the early phase of gallery construction are specified. Domatium anatomy and composition of the gallery walls show some differences. *Allomerus octoarticulatus* is less strictly specialized and shows higher flexibility than indicated for its Guianian sister species. Based on own observations and literature data, it inhabits a number of unrelated myrmecophytes with different domatia and growth habit. *Allomerus* galleries are also compared with those of *Azteca brevis* made on *Tetrathylacium macrophyllum* (Flacourtiaceae) in Costa Rica, a case of striking convergence in which the same fungus group is used. Perforations for ambush were also found in the domatia themselves in *Tococa spadiciflora* and *Clidemia killipii* (Melastomataceae), two co-occurring herbaceous myrmecophytes from western Colombia. They share the same ant inhabitant, a species of *Pheidole* (Myrmicinae). The walls of the foliar domatia exhibit numerous eye-shaped pores, behind each of which a soldier ant is posted. Predation of passing insects, not directly observed, is assumed. The domatia that both species produce are superficially similar, but not homologous to those found in their close relationship. Arguments are presented that the pores are cut by the ants. A note on the occurrence of ant-built mycelial carton structures in Cameroon is appended.

Key words: *Allomerus*, carton galleries, Chaetothyriales, Clidemia, foliar myrmecodomatia, fungicultures, *Hirtella*, myrmicinae, *Tococa*.

INTRODUCTION

While purposeful cultivation of Basidiomycetes by leaf-cutter ants (Attni) is long known and well understood, the existence of fungal symbioses between Myrmicine ants, domatia-bearing tropical trees, and Ascomycetes used in nest construction has been

published only in recent years, generating great interest. Around the discoveries by Dejean *et al.* (2001, 2005) of the *Hirtella/Allomerus* association, and the paper by Mayer & Voglmayr (2009) on that of *Tetrathylacium/Azteca*, a considerable body of literature has appeared. Attention has especially been drawn by the involvement of ant-built, perforated mycelial carton galleries established on the host trees in quite a convergent fashion. The pores are used for ambush

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purposes to predate passing insects. Many aspects of these discoveries have in the meantime been explored with modern methods.

I stumbled on the *Hirtella* system traveling in Brazil as a botanist in 1964 and made detailed protocols of my observations, which form part of the present paper. In view of what we know today, this field report might appear out of date; however, when we deal with unexpected findings, a naturally incomplete account lacking statistics and an experimental approach is usually the result, though supplemented by investigations in the laboratory. Considering the fact that the relevant literature data are based on a single habitat and area and mainly on a single ant species, I think it is justified to present an independent report from a quite distant area and involving another species of *Allomerus*. Although basic tenets of the interactions are confirmed, new ecological details are added concerning the ontogeny of domatia, construction of the galleries, and a comparison with those of *Azteca brevis* built on *Tetrathylacium macrophyllum* (Flacourtiaceae), whose description has been recently published. This tree of Central and South America provides caulinar domatia for several ant genera, one of which, *Azteca brevis* (Myrmicinae), builds quite similar mycelial galleries perforated with pores as in *Hirtella*. A second part related to insect capture through gallery pores, deals with the newly found analogous occurrence of functionally similar ambush openings manufactured by *Pheidole* ants in the domatial wall proper of *Tococa* and *Clidemia* species (Melastomataceae) discovered in Colombia. The Discussion deals comparatively with the ecological background of the systems considered and highlights aspects related to herbivory, morphology of domatia, specificity of partners, and nutritional relations. A case of ant-built fungal constructions observed in Cameroon is appended.

MATERIAL AND METHODS.

Samples of *Hirtella physophora* Martius & Zuccarini were collected and investigated on the spot in November 1964 at 50 m altitude in the Reserva Ducke (2°55'S, 59°59'W; *terra firme*) near Manaus, Central Amazonia, and samples deposited in WU Vo 263. For comparison, *Hirtella myrmecophila* Pilg. was collected on 2 December 1964 (WU Vo 221) at the same locality. Both species were inhabited by *Allomerus octoarticulatus*. *Hirtella duckei* Hub. was sampled on 30 October 1964 in riparian forest of the

Serra Cununuri, Território do Amapá, Rio Amaparí, NE Brazil (2°12'S, 51°14'W; WU Vo 136). It contained ants belonging to *Allomerus decemarticulatus*. The host is closely related to *Hirtella physophora*, and it was on this plant that first field notes including galleries and their mycelia were taken.

Samples of the galleries on *Tetrathylacium macrophyllum* were collected on riversides near the Biological Station of La Gamba, Prov. Puntarenas, Costa Rica, by Dr. Veronika Mayer (Vienna). *Tococa spadiciflora* Triana, a perennial rainforest herb, was observed on densely forested premontane slopes in the Anchicaya valley, altitude ca. 800 m, Cauca Department, Colombia, on 3 September 1956 (WU Vo 98) (3°37'N, 76°54'W). At the same time and location, *Clidemia killipii* Gleason (WU Vo 110) was found. Both taxa were occupied by the same undescribed species of *Pheidole* (Myrmicinae, Pheidolini).

Plant material and carton galleries were studied *in vivo* by binocular lens and, when preserved in alcohol, also by light microscope and scanning electron microscope (SEM) in the Vienna Faculty Centre of Biodiversity during 2008-2009. For SEM, samples were sputter-coated with gold in high vacuum mode at 15 kV and photographed using SEM Phillips XL30. Identification of herbarium specimens was provided by Dr. John Wurdack, Smithsonian Institution Washington in 1967, and the ants of their hosts were identified by Walter Kempf † O.F.M., Curitiba, in 1968.

RESULTS

1. The *Hirtella physophora*/*Allomerus octoarticulatus* association

Domatia. The forest trees have a sparse crown 3-5 m tall with entire, lanceolate, alternate leaves measuring ca. 30 x 14 cm (*cf.* Prance 1972). All the lanceolate leaves bear domatia (Figs.1 A, B). These consist of twin separate globular capsules inserted at the base of the lamina. Each domatium, about pea size, with a mean of 4.1 x 5.6 mm, has its own entrance abaxially beside the midrib. The walls are sclerified by a dense net of veins and have a normal palisade layer. Stomata are very sparsely distributed over the inner (0.4 per μm^2) and absent on the outer epidermis. The entire leaf bears stiff, unicellular setae on both sides, with especially dense and long bristles (2-3 mm) on the abaxial ribs and around the domatium. The interior of the cavities is distinguished by 2-3 sessile, secreting nectaries partly on vein ribs,

partly on intercostal fields (Figs.1 B, 2 E). They consist of a flat, discoid layer of columnar glandular cells. Such nectaries, but smaller in diameter, are arranged in groups on the lower side at the base and not far from the tips of the lamina. Floral bracts bear a pair of glands at the basis of a triangular rudimentary lamina.

Here and there, parts of the domatium inner wall were found to be covered with a homogenous layer consisting of coccoid bacteria of 0.2 μm diameter. Fringes of the colony revealed that the bacteria form chains, while the interior structure of the colony is densely net-like (preserved material).

Ontogenesis of the domatia. Originally, the leaf base is auriculate, and each of the two flanks transform to pockets. The process starts early in bud at the primordial leaf length of 2.6 cm. A projection of the margin, already beset with bristles, curls down to the abaxial side; the fold distends proximally forming a cavity and remains open distally, the later entrance. At this earliest stage, the capsule is complete and has a diameter of less than 0.9 mm (Fig. 1 C). Originally, also the interior (abaxial epidermis) bears bristles.

Ant population. *H. physophora* – and *H. myrmecophila* – was found to be colonized solely by *Allomerus octoarticulatus* Mayr in the vicinity of Manaus. The whole colony of a tree included myriads of the agile, brownish-yellow worker ants (2.13 mm) and an uncertain number of the sexual morphs. The ants live in the paired chambers of the domatium. In a single chamber, for instance, 2 alate queens, 16 workers, 38 larvae and 3 prepupae were counted. Scale insects (Coccidae) were very rarely seen. Debris, containing living nematodes (*Diploscapter coronata* Cobb) was deposited near the entrance, and patches of bacterial colonies were found inside a number of domatia. *H. duckei* in Amapá was inhabited by *Allomerus decemarticulatus*.

Galleries. Once completed, these start from the entrance of every domatium pair and join another gallery that runs along the underside of a twig in a basal direction from leaf to leaf until it unifies with the next branch order (Fig.1 E). The galleries often swell dome-like at the junction with the domatia. A single terminal gallery descends the stem irregularly depending on its changing circumference. It was not established whether ant traffic exists onto the forest bottom. The galleries were estimated to extend up to 7.60 m on a treelet. Their smooth walls are ca. 0.2

mm thick (Tab.1) and somewhat brittle, of ash-pale color when dry. All the gallery walls were perforated with more or less evenly distributed circular pits 0.7–0.9 mm wide, separated by an average distance of 4.5 mm. Most openings have a raised collar (Figs. 1 F, 2 A). The inner rim is also protruded, so that the pore becomes a cylindrical tube 0.4 mm in length.

The mycelium. Under the binocular lens, the gallery walls turned out to be composed of a living mycelium. By microscopy and later by SEM it was found that sooty molds are the principal components, making up the bulk of the wall material. This consists of tightly interwoven, rectangularly ramified moniliform hyphae of 8–14 μm in diameter (Fig. 1 G, H) and dark brown in color, except for the paler growing end. The fungal texture is not monospecific. The hyphae form a mixture of several taxonomically related species, which probably belong to the ascomycete order Chaetothyriales. At present, the age of the material precluded cultivation of the fungi. We cannot confirm from morphology alone that they belong to this order, since reproductive structures are absent and DNA data are not yet available. One feature argues for affinity with the order Chaetothyriales: when dry, most hyphae exhibit an uncommon mode of shrinkage. The resulting flattened cell wall planes alternate with those of each subsequent cell by 90°, in accordance with the mode to be found in that order. The hyphae were occasionally supplemented by wood fragments, some single leaves of a liverwort, pieces of filiform cyanobacteria, and shells of thecamoebae (Genus *Quadrula*). It is not known whether these protists represent living inquilines or were simply blown in from outside. Cut setae of the host plant, plenty of which were found in French Guiana (cf. Dejean *et al.* 2001, Ruiz-González *et al.* 2011), were rarely seen to be integrated, save some of them in the gangway roof near the domatium. It is clear that in our samples the fungi alone form the stabilizing element of the construction. No traces of an adhesive were detected.

The fungal network is micro-porous enough to readily absorb rain water and dew. It appears to be drought-tolerant (poikilohydric). The mycelium is saprophytic, epiphytic, and does not invade the host plant. It is evident that the fungus is trimmed since the surface of the galleries and especially the rims of the openings indicate that these are kept free from sprouting hyphae by the ants. We found that pores which had been abandoned by the guards became covered by a mycelial curtain grown from inside the

TABLE 1. Structural comparison of galleries: *Allomerus octoarticulatus* (on *Hirtella*, Central Amazon) and *Azteca brevis* (on *Tetrathylacium*, Costa Rica); aus Mayer & Voglmayr 2009; V. Mayer, pers. comm.)

Feature	<i>Hirtella</i> (<i>Allomerus</i>)	<i>Tetrathylacium</i> (<i>Azteca</i>)
Color outside	Brownish ash-gray	black
Diameter outside (mm)	3 – 4	3 – 4.5
Diameter inside (mm)	2.9 – 3	2.5 – 3.5
Length of galleries (m) – single ductus in continuation	up to 7.6 measured	not assessed
Gallery position on branch	horizontal: lower side vertical (main stem): irregular	horizontal: lower side vertical: irregular
Connection with domatia	yes	yes
Ramification of galleries	twig branching points, domatia	twig branching points
Gangway preparation	prior to tunnel, smoothing floor, cutting bristles away	prior to tunnel, scraping and smoothing of floor
Course of construction I	two parallel rows of \pm equidistant bristles reserved as “bean-poles”, these planted with mycelia	no bristles. Two parallel rows of \pm equidistant piles of pure mycelium planted
Course of construction II	growing mycelia arching between and within rows, then filling the meshes	growing mycelia arching between and within rows, then filling the meshes
Fungus component	probably Chaetothyriales (Ascomyc.), several spp.	confirmed Chaetothyriales at least 5 spp.
Inclusions in gallery wall	fragments of wood, liverworts, blue algae, diatoms, trichomes; shells of thecamoebae <10 %	wood fragments ca. 10 %
Diameter of pores (mm)	0.7 – 0.9	0.9 (\pm 0.2)
Rim of pores	stuffed	stuffed
Distance of pores (mm)	4.5 on average	2.0 (\pm 0.6)
Guild of guards	workers	workers
Prey capture method	seizing of insect legs with mandibles	seizing of insect legs with mandibles
Presence time of guards	permanent, at least daytime	on alarm

wall. Although trimmed, the fungi do not seem to be eaten by the ants. Our microscopic inspection of crushed ant heads did not reveal hyphae fragments in their buccal pockets. The mycelium is apparently nourished by the ants' liquid feces. Nectar from the internal glands may also be applied to maintain the mold.

The gallery construction *in statu nascendi* was studied in alcohol material. After paving an internal runway by removing all hairs, trichomes bordering the path were also cut away, excepting some left untouched at regular distances of ca. 1.4 mm. The mycelium, probably implanted, grows from the hair bases upwards, first forming a pyramidal mantle around the hair. The subsequent process of establishing the tunnel could no longer be directly followed. Hypothetically, the sprouting hyphae distally spin

arcades along the two parallel hair rows, as well as also transversally between the two rows. Hyphae of all frames then join by further growth, forming first a “clathrate” skeleton or lattice, whose meshes finally link together to form the gallery walls. The perforation is probably not cut out by the ants, but certain meshes of the framework are left open and further fashioned to transform to circular pores. Emergent tips of the supporting “bean-pole” bristles were removed by the ants.

Another sort of mold close to *Acrogenotheca ornata* Deighton & Piroz (Ascomycetes-Dotidiales) grew regularly on the setae left intact on the upper flank of the tunnelled ramets: pale, hyaline, cylindrical, elongate-celled, tapering hyphae 6-10 μ m in diameter. They creep along the bristles, forming bunches at the end of a trichome, with hyphae oc-

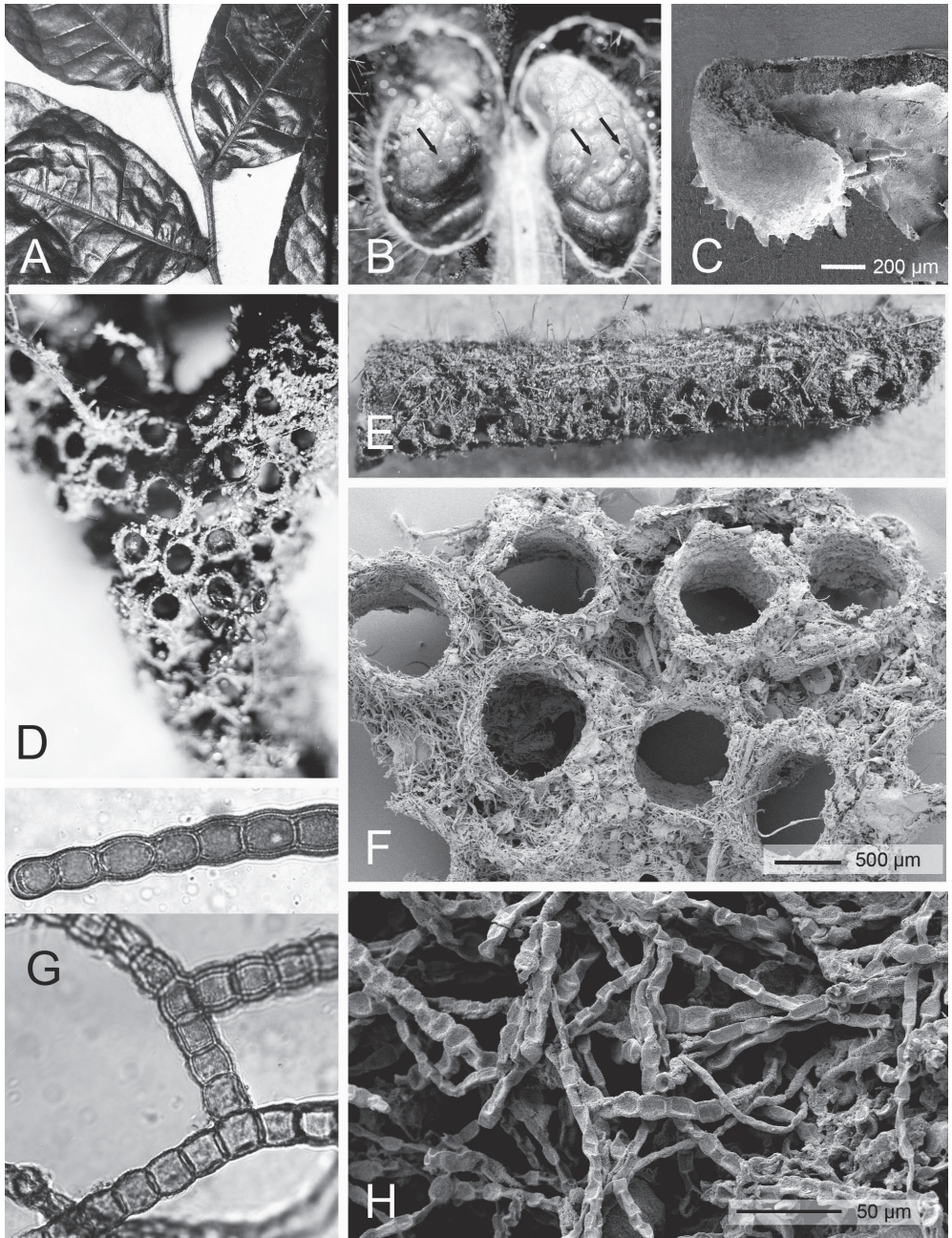


FIG. 1. *Hirtella physophora*. A Twig with foliar domatia. B Domatium, longitudinal section showing extrafloral nectaries (arrows) on inner wall. C Early primordium of right domatium chamber: marginal fold, bending to the underside (viewed from distal). D Part of carton gallery on twig with ambush holes (crowded near domatium), occupied by *Allomerus octoarticulatus* workers. E Ramet with pitted gallery along underside, lateral view. F Ambush holes in mycelial matrix, G A single and a ramified hypha, fungal mycelium of gallery wall (dry condition).

asionally ending in a clathrate club. This fungus was not integrated into the walls. It certainly profits from the ants' excrement. This type was also found on the non-myrmecophytic species *Hirtella* cf. *racemosa*, which also possesses extrafloral nectaries and is associated with ants.

Function of the galleries. Ants were observed to patrol the entire crown of the tree and rushed inside the galleries along the leaf branches. Numerous pores of the galleries were seen to be occupied by a single worker, hiding beneath the opening with mandibles gaping and antennae extended (Figs. 1 D, 2 A). Their head neatly fits into the space which is, however, wide enough to allow the ant to leave the ambush hole. Guards were continuously present in this manner, at least in daytime. One much larger specimen of another ant genus was observed being seized by its legs, but its ultimate fate was not followed. When a pit was touched manually with a bristle, this was immediately grasped. While at that time we considered this behavior as merely defensive, we now regard it

as a predatory strategy. It is clear that the galleries serve equally to protect the workers posted below the pores or rushing through the gangways to reach distant foliage for patrolling.

2. The galleries of *Azteca brevis* compared with those of *Allomerus octoarticulatus*

I was given the opportunity to compare the mycelial gangways laid out along the twigs of *Tetrathylacium macrophyllum* (Flacourtiaceae) with those of *Hirtella*. These are constructed by *Azteca brevis* (Myrmicinae), whose colonies also serve them for ambush, as described by Mayer & Voglmayr (2009). Their colonies dwell in the cauline domatia of this plant. The substance of the galleries is likewise a dense network of living, sterile chaetothyrialean hyphae, a mixture of several related species, among them iridescent hyphae. Table 1 compares the corresponding features of the galleries of the two host species, and they are almost identical down to the smallest details, demonstrating a striking convergence. This raises the question of the mutual contributions to the architec-

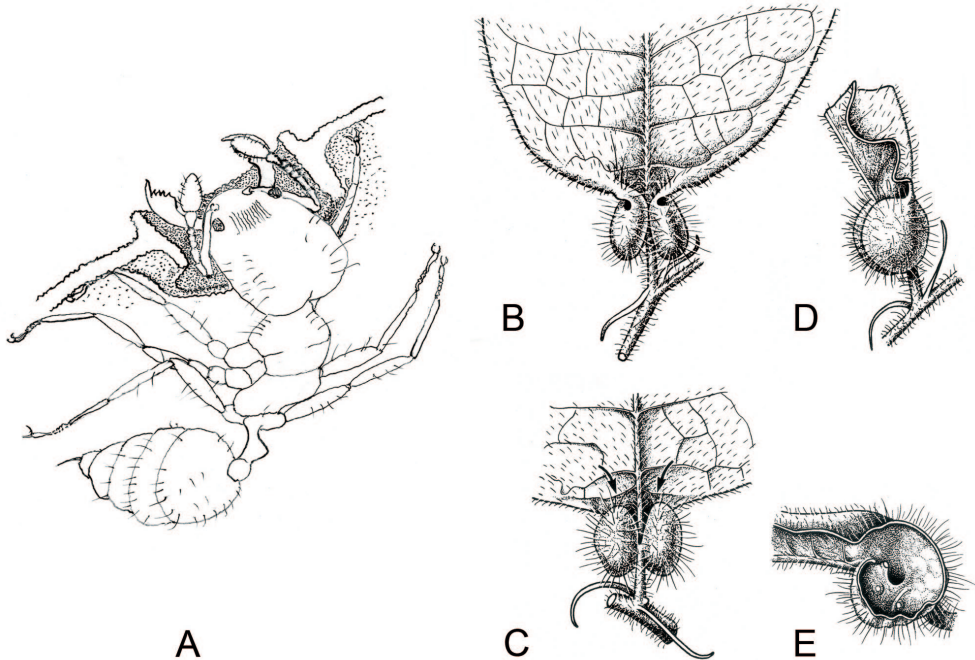


FIG. 2. A *Allomerus octoarticulatus* worker, luring for prey underneath ambush hole (cut open). B – E Leaf domatia of *Hirtella physophona*. B Adaxial view, C Abaxial view (arrow: entrances of vesicles). D Lateral view. E Longitudinal section through one vesicle, showing nectaries.

tural design by ants and fungi. Since these fungi grow as a simple discoid layer when cultivated on agar in the absence of ants, the latter appear to play the governing role. Yet the congruity of the two galleries also points to some innate potential of the (related) fungi to contribute their part in shaping them, possibly elicited by the ants. For example, the smooth surfaces of the galleries are not only due to extrinsic influences, but external hyphae tend to curve inwards towards the mycelium body.

3. The association of *Tococa spadiceiflora* and *Clidemia killipii* with *Pheidole* sp.

The plants and their domatia. Both plant species are understory herbs ca. 1 m in height, with opposite, sessile, broadly lanceolate leaves of 24 x 12.5 cm, and terminal inflorescences in *T. spadiceiflora*, and leaves of 16 x 13 cm and (pseud-)axillary inflorescences in *C. killipii*. The domatia of both species, inserted at the leaf base, are ovoid, about 28 mm long, 17.5 mm broad and 6 mm thick (Fig. 3 A, B). The interior is separated by a longitudinal septum. Unlike the

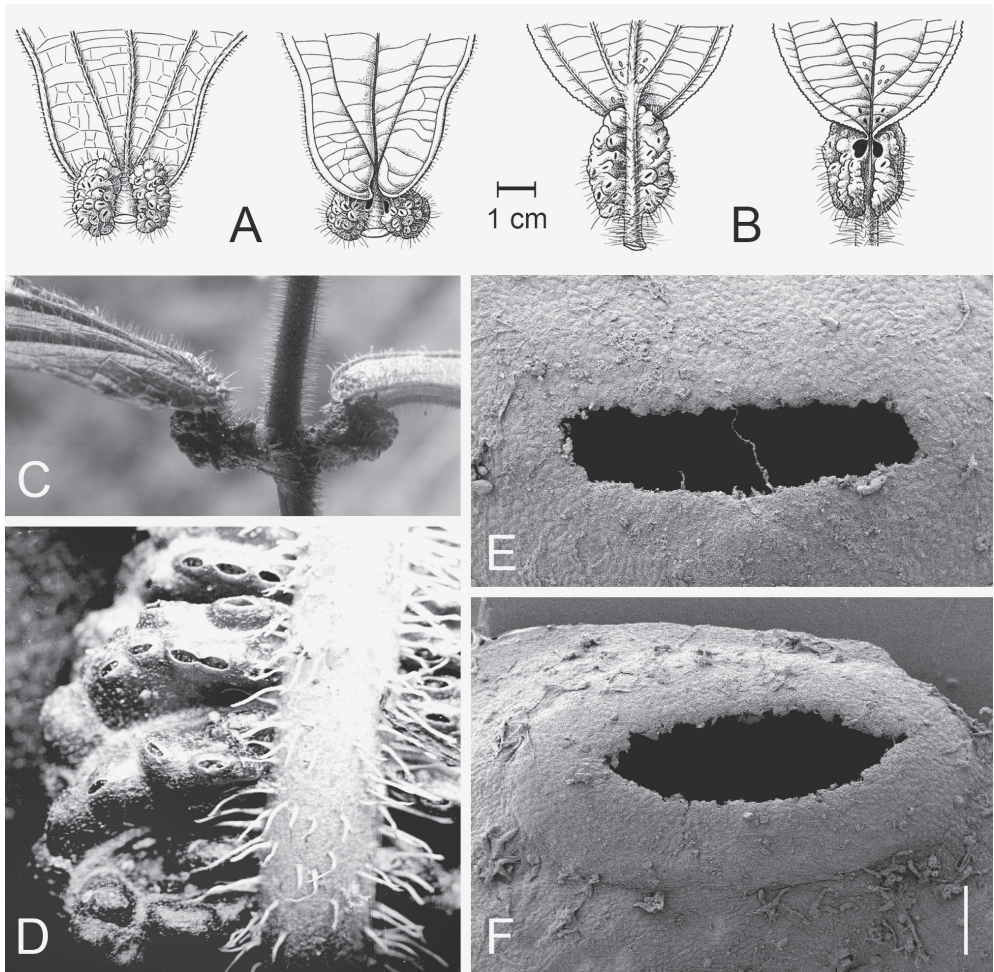


FIG. 3. Leaf domatia of *Clidemia killipii* (A) und *Tococa spadiceiflora* (B) with ambush holes (left abaxial, right adaxial view, respectively). C – F *Tococa spadiceiflora*: Leaf pair, laterally. D Part of domatium, abaxial side with ambush holes occupied by *Pheidole spec.* E, F. Ambush holes. Scale bar 200 μ m.

smooth vesicles of most other ant-housing Melastomataceae (hereafter melastomes), the surface of the entire domatium is deeply corrugated, resembling in form a brain or a walnut kernel. They are deep maroon in color and covered with bristles. Their morphology differs fundamentally from the ascidiate mode predominant in *Tococa* and is also unique among the myrmecophilous species of *Clidemia*.

In the more common (bi-)ascidiate type, the domatia have their entrances on the underside, in the angles between the midrib and the two opposite main lateral veins. In this type, both angles become extended by forming two bullate adaxially prominent ascidia with the basal lamina, or, in other species, running down the petiole as channels and only becoming inflated where they end to form the domatium chambers proper (cf. Schumann 1888, Bitailon 1982).

The domatia of *Tococa spadiciflora* and *Clidemia killipii* on the other hand, have a non-ascidiate structure. The entrances are adaxial (Fig.3 B). As the inspection of growing leaves as small as 4.2-13 mm with domatium primordia shows, these are still smooth. The lamina is first panduriform basally; its two basal wings then curl upwards, forming a concave chamber adaxially on each side of the midrib. The two wings are also partly fused with the cryptic petiole, but have the distal opening framed by the laminal margins, that allows access to the two compartments. The domatium finally becomes separated from the main lamina by a constriction. In both of our species, the interior of the chambers is compressed and labyrinthic, corresponding to the bulges seen from the outside.

Tococa spadiciflora and *Clidemia killipii* were colonized by the same undescribed species of *Pheidole* (Myrmicinae – Pheidolini). Sexual morphs (4.7 mm), workers (1.4 mm), and soldiers (2 mm) were present, as well as full-grown larvae. Coccids also belonged to the inhabitants and were probably tended by the ants. Detritus, interwoven by living septate hyphae of unknown affinity, was deposited inside the domatia. It contained, apart from living nematodes and tiny millipedes, insect fragments, among them the head of a ponerine ant – hinting at a possible ambush prey.

No galleries are built. In both melastomes, however, the domatium walls themselves are regularly perforated and apparently have the same function as the pores in *Hirtella*. The openings are not circular but represent almost uniformly eye-shaped perfora-

tions, usually situated at the summit of the domatium bulges or of several longitudinal folds (Figs.3 D-F). They are about 1.3 mm broad and 0.2-0.5 mm wide and bordered by a paler rim. They numbered between 50 and 126 per domatium in both species, appearing on both lower and upper surfaces, being more frequent in the basal portions. Some of these pores even occur outside the formicarium in the intercostal fields of the basalmost part of the lamina. The origin of the pores still poses a problem (see Discussion).

Many of the domatium pores in the two species were guarded day and night each by a soldier ant. The animals held their heads such that their gaping mandibles and extended antennae fit across the eye-shaped hole, the body being firmly attached to the inner wall of the pore, a position being retained by some even after fixation. When a hair was manually introduced toward the slits, it was seized by the mandibles under initial antennating. Although we did not witness the natural event of predation, i.e. grasping the legs of foreign insects that step over the domatia, the analogy to the ambush behavior as treated in *Hirtella* is convincing.

DISCUSSION

The associations in the systems investigated comprise heterogeneous three-fold symbioses, involving three living components: plant, ants, and imperfect fungi in *Hirtella*, and plant, ants and coccids in the melastomes. The host plants (Chrysobalanaceae and Melastomataceae respectively) are unrelated, and their inhabitants, *Allomerus* and *Pheidole*, housed in foliar domatia, are related (Myrmicine ants, tribe Solenopsidini and Pheidolini respectively). The third comparable myrmecophyte, *Tetrathylacium* (Flacourtiaceae), with cauline domatia, houses as a main guest an *Azteca* species (Myrmicinae, subfam. Dolichoderinae) (Mayer & Voglmayr 2009).

Obviously, all three symbioses have independently evolved in parallel. The most striking overall parallel, however, is that the ants involved practise an ambush strategy, though instrumentally different; in *Hirtella* and *Tetrathylacium* the ants build fungal galleries as a platform for pores, while in the melastomes the domatium walls themselves bear them.

The system of *Hirtella* and *Allomerus*

The findings of Dejean *et al.* (2001, 2005), obtained in the system *Hirtella physophora* / *Allomerus decemarticulatus* in French Guiana, including mycelial galler-

ies and ant behavior, fairly match and confirm our early observations made in Central Amazonia (a distance of 1260 km away) on the same host plant species but colonized by another ant, *Allomerus octoarticulatus*. *Allomerus* is an Amazon genus with eight species (Fernandez 2007). Since a single host individual investigated in French Guiana harbored *A. octoarticulatus*, the inhabitant of *H. physophora* in Central Amazonia (and of *H. myrmecophila*, co-occurring with the former, own obs.), the two ant species occasionally share their preferences. Minor deviations refer to the palisade layer in the domatium walls, which was missing in the paler domatia in French Guiana (Leroy *et al.* 2008) but normally developed in our specimens; cut setae were there included in the network of gallery mycelia, but rarely so in Central Amazonia. While we originally considered the behavior of ant guards in the galleries as merely defensive, we are now convinced, in line with Dejean *et al.* above, that it is a predatory strategy.

Within the Neotropical genus *Hirtella*, seven of ca. 90 species are known to possess foliar domatia (*H. physophora*, *H. myrmecophila*, *H. vesicula*, *H. dorvallii*, *H. guainiae*, *H. duckei* and *H. revillae*), all members of the section Myrmecophila (Prance & Lovejoy 1996) are mainly rainforest understory trees of Amazonia. The foliar domatia of *H. physophora*, described by Bitaillon (1982) and Leroy *et al.* (2008, 2011), develop early in leaf ontogenesis as we demonstrated, by curling of the basal lamina margin downward towards the abaxial flank. Their morphology is paralleled by the domatia of species of the Rubiaceae *Remijia* and *Hoffmannia* (Windsor & Jolivet 1996). *Hirtella physophora* possesses a number of floral nectaries on the domatium inner wall, and additionally on the underside of the leaf lamina. Also the domatia of *H. myrmecophila*, a tree of different habit, co-occurring with the latter species in the Ducke Reserve and also harboring *Allomerus octoarticulatus*, do have such nectaries in their interior – a fact denied by Romero & Izzo (2004). Similar nectaries, either sessile or stalked, also occur on the leaves, bracts, and inflorescences of numerous *Hirtella* species which lack ant domatia (e.g. *H. racemosa*).

The galleries. Recently, molecular analyses yielded the identification of the gallery fungi in Costa-Rican *Tetrathylacium* (Voglmayr *et al.* 2011) and in the *Allomerus decemarticulatus*-populated *Hirtella physophora* of French Guiana (Leroy *et al.* 2011, Ruiz-González *et al.* 2011). They reveal and confirm that the fungi of both symbioses belong to genera of the

tribe Chaetothyriales (Ascomycetes). This tribe of sooty molds appears closely connected ecologically with symbiotic associations with ants throughout the tropics (Ruiz-González *et al.* 2011, Voglmayr *et al.* 2011). In *Tetrathylacium* as well as in *Hirtella physophora*, including our specimens, the mycelia are not monospecific but involve several closely related, mostly still undescribed species or intermixed strains. The main *Hirtella* symbiont from French Guiana turns out to be *Trimmatostroma cordae*. The high genetical variety found among these gallery components presents us with the challenge of carrying out a molecular analysis of our geographically so distant, mid-Amazonian material for comparison.

After clearing a path by removing the hairs, the equidistant trichomes left standing as “bean poles” on either side of the pad were implanted with the fungus and form the initial pillars of the mycelial wall. According to Ruiz-González *et al.* (2011), the implantation derives from a mycelial pellet, deposited by a founding queen inside a domatium. So gallery construction starts from each occupied domatium. The findings of Dejean *et al.* (2005) in French Guiana showed that cut bristles were the main stabilizing element, a scaffold of the gallery walls, grown through by the hyphae. This differs markedly from the wall composition observed by us. Except for the “bean pole” bristles left *in situ*, cut trichomes appear in all checked probes only sparingly within the mycelial mass, which by itself functions as a supporting element of the construction. In *Tetrathylacium* there are no hairs; nevertheless its *Azteca* ants build quite similar galleries without them.

As in *Tetrathylacium*, the small, regularly distributed pores are a constant element of the galleries in *Hirtella*. They are created by the ants, but not actively cut out. They originate from mycelial open meshes in an early phase of construction. The pores were noticed by some previous observers when describing galleries as “honey-combed”, as in *Cordia* spp. (Boraginaceae), *Duroia saccifera* (Rubiaceae), and *Pourouma* (Moraceae) (Benson 1985), but their creator was not established. Perforated galleries are also found in *Triplaris* (Polygonaceae). Dejean *et al.* (2005) recognized the pores as devices of the ambush behavior of *Allomerus*: ants posture themselves below the pores, one worker individual per pore, awaiting passing insects with spread mandibles to seize them as prey. Occupation of the holes occurs after an alarm in *Azteca*, while we found *Allomerus* permanently present at least during daytime. Following Romero

& Izzo (2004), in *Hirtella myrmecophila* helpers are recruited from the domatia for jointly killing and dismembering the retained prey.

Less sophisticated ant ambush methods were observed by Morais (1994) in *Azteca* cf. *lanuginosa*: a line of workers hides below leaf margins to catch insects passing on the upper leaf surface. The ponerine ant *Ectatomma* ambushes bees (Schatz & Wcislo 1999). Analogies to this approach exist among solitary arthropods living in earthen or woven tubes, such as beetles (cicindelid larvae) and among spiders of the families Atypidae (*Atypus*), Nemesiidae (*Nemesis*), Lycosidae (*Arctosa*), and Segestriidae (*Segestria*) (Schaefer, pers.comm.). An important difference between ants and those other arthropods is the group action, which requires recruitment and communication.

The systems of *Tococa spadiceiflora* and *Clidemia killipii*

These herbs belonging to the tribe Miconieae and also elements of the (subandine) Hylaea, have foliar ant domatia superficially similar to those of their congeners, but both diverge from them by their morphology, which is unique among the melastomes. While the leaf domatia elsewhere in this relationship arise by deepening and ascidiate expansion of the basal, abaxial angles between the midrib and the two opposite lateral costae, in our plants two marginal portions of the primordial leaf base roll upward and extend to blisters. This non-homology remained hardly noticed until now. Benson (1985) remarks for *Tococa spadiceiflora* that “nothing is known about the mode of development”, but he was right in suggesting a marginal upward curling. In view of this peculiarity, it is surprising that *vide* Michelangeli’s (2000) cladistic analysis, *Tococa spadiceiflora* is nested deeply within the genus and does not represent a separate major clade; morphology suggests a much wider separation. The same must be postulated for *Clidemia killipii*, which has not yet been phylogenetically analyzed. It merits mention that Goldenberg *et al.* (2008), discussing the phylogeny of Miconieae, consider *Tococa spadiceiflora* to be a species of *Conostegia* rather than of *Tococa*.

If compared with *Hirtella*, the domatia of these melastomes have in common that they develop by inrolling of the basal laminal margin, but this happens in the opposite direction, from the abaxial to the adaxial flanks.

The multiple, eye-shaped openings present in the domatia walls of *Tococa spadiceiflora* recur, curiously

enough, in those of neighboring *Clidemia killipii*, occupied by the same species of *Pheidole* ants. We surmise a connection with ambush tactics, which is strongly suggested by a similar posture and behavior of the guard ants – in this case soldiers – below the pores. This seems to be a new, analogous type of ambush. Being unprepared, I failed to pursue the development of the pores. Benson (1985) writes: “Since the density of pores varies with plant species, I presume that they are innate plant structures, acquired by different plants in response to selection pressures exerted by their shared *Pheidole*... these orifices are not made by the ants, because they were present on young plants in the absence of ants”. Some details we found favor Benson’s opinion: (1) The rim of many pores is framed by slightly swollen tissue (a reaction rare in plant wounds); (2) the pores are linearly arranged along the domatial ridges; (3) the pores do not cross leaf nerves; (4) pores are occasionally obstructed by the walls of adjoining bulges and are thus not functional. We presume, however, that the pores are ant-made. Details that contradict Benson, or at least appear ambiguous, include the following: (1) eight collected formicaria of young *C. killipii* plants between 4.2 and 12.7 mm in size showed no traces of preformed openings or cell arrangements heralding a subsequent controlled destruction of tissue; (2) the margin of the slits suggests a disruptive origin – they are bordered with necrotic, tanned cell fragments and indented, as if pierced by the animals; (3) the debris deposited inside fully grown domatia chambers of *T. spadiceiflora* contained not a few pieces of leaves, some of them (but not all) resembling the shape putatively cut out of the pores (“negatives”). This observation also suggests that the pieces had been cut from the inside. Remarkably, neither Triana (1871) nor Gleason (1941) mentions the presence of pores in their respective diagnoses. Thus some doubts remain, and direct observation, of course, could resolve the open questions. Revisiting the locality is problematic, however, because partisans have been present in the area for many years.

Further comparative aspects

Mutual specificity. There is no exclusive specificity in the associations under discussion. The current literature often emphasises the *Hirtella/Allomerus* symbiosis as standing out by a high adaptive specificity of its partners. *Hirtella physophora*, however, may be alternatively occupied by colonies of seven species of several

genera. On the other hand, the main guest of *H. physophora*, *Allomerus octoarticulatus*, also dwells in the caulinar domatia of *Cordia nodosa* and *C. alliodora* (Beccari 1884-1886, Schumann 1888, Wheeler & Bequaert 1929, Opler & Janzen 1989, Jolivet 1996, Longino 1996, own obs.), if they are not occupied by *Azteca* (Yu & Pierce 1998, Edwards *et al.* 2006) or *Myrmelachista* spp.. *Allomerus* even constructs the same type of perforated galleries in *C. nodosa* as in *Hirtella* (own obs.). Other hosts of *A. octoarticulatus* are *Duroia saccifera* (Rubiaceae) (own obs.), *Pourouma* (Moraceae) (Benson 1985: "galleries honey-combed"), *Tococa macrosperma* (Michelangeli 2003), *T. acuminata* and *T. longisepala* (own obs.). Regarding *Tetrathylacium* (Mayer & Voglmayr 2009), there is, apart from its main inhabitant, *Azteca brevis*, a variety of other ant genera, which neither construct galleries nor use ambush (Tennant 1989, Schmidt 2001).

In the Anchicayá valley, *Tococa spadiceiflora* and *Clidemia killipii* were occupied by *Pheidole* sp., but these species do not exclusively house this ant species. In the Colombian Choco, where that *Tococa* species was studied by Alvarez *et al.* (2001), it was – apart from *Pheidole* (presumably the same species as ours) – occupied by a minority (2 % each) of *Brachymyrmex heeri* (Plagiopidini), *Wasmannia aureopunctata* (Attini), and *Azteca* sp.. Interestingly, the authors do not mention any perforation of the domatia. Coccids were also tended there.

Nutrition. In addition to the usual ways of photosynthesis of carbohydrates and mineral absorption by the roots, the h o s t p l a n t s possibly gain some nutrients by absorption from debris deposited inside the domatia. The transfer is mediated by certain other Chaetothyrialean fungi, which are specifically restricted to the inner domatium walls (Leroy *et al.* 2011, Voglmayr *et al.* 2011). This remains to be proved in our plants. I also found mycelial patches similar to those identified in *Hirtella* (Ruiz-González *et al.* 2011) in the domatia of the melastomes. The internal debris included fragments of insects. Regarding a n t nutrition, *Hirtella* provides nectar in- and outside the domatia as a source of carbohydrates for the worker ants. Such a source is missing in the melastomes. Coccids living in the latter group may be exploited for honeydew in place of nectar, while coccids were rare in *Hirtella*. The main source of N for the ants are insects obtained by ambush preying or from the leafage they patrol (Dejean *et al.* 2001). *Allomerus octoarticulatus* also descends to the leaf litter for chasing on the forest floor, at least they do

so when dwelling in *Cordia nodosa* (Bailey 1924). There were no indications that the g a l l e r y fungus is eaten or harvested by the ants. The fungi grow superficially on the plant, are saprophytes, not plant parasites, and are presumed to thrive on the ants' liquid excrement. In addition, carbohydrates from the domatial nectaries regurgitated by the inhabitants may also contribute. Remarkably, the gallery fungus dies if deprived of the ant colony (Orivel *et al.* 2011), because it no longer profits from the ant excrement.

Plant protection. Another aspect of these systems is mutual protection. The plants offer, in analogous ways, shelter in the form of cauline or foliar domatia housing the ant populations or parts of them, and by ant-made gallery constructions that protect individuals traveling to and fro in the tunnels. Attempts to solve the question as to whether the host plants profit from protection by the ants against herbivores produced different results in our cases. Fowler (1993), who determined the degree of foliar damage caused by herbivores in Central Amazonia in a series of myrmecophytes, among them *Hirtella physophora*, found that leaf damage was not significantly lower in them than in conspecifics with ants removed. But in recent exclusion experiments, Grangier *et al.* (2008) showed unequivocally that protection is effective in this plant. During one tree life, however, several ant colonies may relieve one another, which eventually causes intermission of occupation (Orivel 2011). My own herbarium specimens of *Hirtella* and the melastomes are far from being free of insect injury. On the other hand, similar investigations with experimental exclusion of ants performed by Alvarez *et al.* (2001) clearly show in *Tococa spadiceiflora* colonized by, among others, *Pheidole* sp., that resident ants provide effective defense against herbivory (see also Kattan *et al.* 2008 concerning *Clidemia killipii*).

APPENDIX

An accidental observation corroborating the occurrence of ant-cultivated fungal carton walls also in Africa may be appended. It was made in secondary rain forest near Victoria, Cameroon, on February 1978. On the underside of several of the large peltate leaves of an arboreal *Macaranga* species (Euphorbiaceae), we found blackish, somewhat fragile shelters constructed by ants (*Crematogaster* sp., Myrmicinae). The wall material consisted of undefinable organic matter including tangled plant hairs spun around,

and held together, by a fungus mycelium, a synnema of tawny, moniliform hyphae of an ascomycete very similar to those of *Hirtella*. The walls covered the basal, palmate forkings of the main leaf ribs (Fig. 4), closely covering crowded herds of large, pale green, wax-secreting mealybugs (*Gigantococcus theobromae* Newstead or near relative; Margarodidae). The ants attending them were seen licking their sugary excretions, while others carried the coccids in their jaws. Starting from the central establishment, galleries extended alongside the ribs, also covering mealybugs. Here, windows were irregularly installed. As there was no breeding site of the ants, the complex constituted one of several annexes of a main nest, which could not be located. South Asian members of *Macaranga* are known to be myrmecophytes, but such a status remains unknown in our case. Mayer & Voglmayr (2009) cite chaetothyrialean carton galleries in Cameroon built by *Crematogaster* (without naming the host plant).

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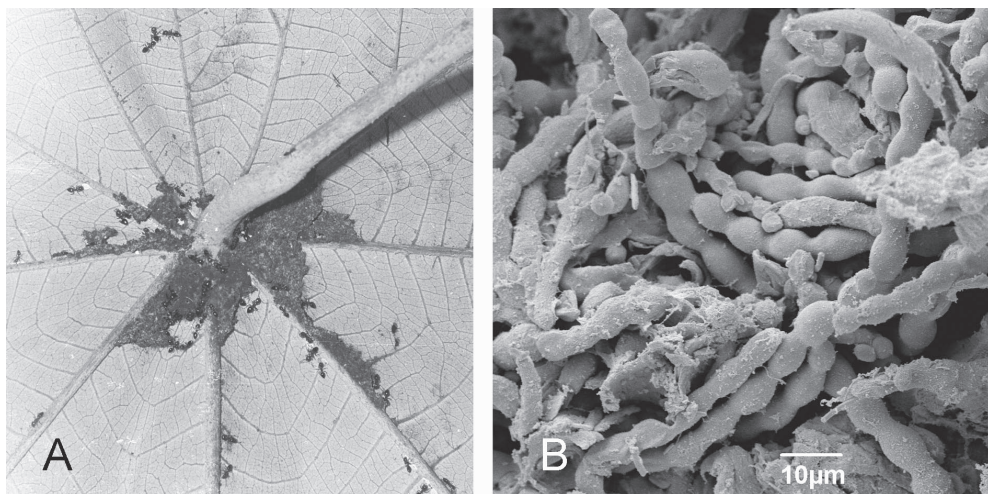


FIG. 4. A. Mycelial galleries sheltering mealybugs, built by *Crematogaster* spec. (Myrmicinae) on the underside of a *Macaranga*-leaf in Cameroon. B Mycelium of the nest wall (alcohol - preserved), SEM photograph..

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