

Obligate plant farming by a specialized ant

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Many epiphytic plants have associated with ants to gain nutrients. Here, we report a novel type of ant-plant symbiosis in Fiji where one ant species actively and exclusively plants the seeds and fertilizes the seedlings of six species of *Squamellaria* (Rubiaceae). Comparison with related facultative ant plants suggests that such farming plays a key role in mutualism stability by mitigating the critical re-establishment step.

Farming mutualisms, wherein an organism promotes the growth of another on which it depends for food, have evolved in many lineages in the tree of life, including amoeba¹, crabs² and sloths³. The most complex forms of farming evolved in several insect groups—most notably ants—that convergently cultivate fungi⁴. Despite the diversity of ant-plant mutualisms and even though plants are obvious ‘crops’, no farming mutualisms involving ants and plants have been reported. Most ant-plant symbioses involve plants with preformed cavities (domatia) that house ants in return for protection or extra nutrients⁵. Typically, the symbiosis needs to be re-established at each generation. Symbioses persisting for several generations are found in ant gardens. These are associations of epiphytes planted by ant workers inside carton nests⁶ made of organic material, such as plant parts, ant secretions and vertebrate faeces. Different from ground-dwelling ant-plant symbioses that are mostly defence mutualisms, epiphytic ant-plants usually are nutritional mutualisms^{5,7}. Such trophic mutualisms, which involve ants that provide detritus or faeces to their host plants’ domatia, have evolved many times^{8–10}, probably because epiphytes are usually more nutrient stressed than ground-living plants, especially during seedling establishment¹¹. Here we show that epiphytes in the genus *Squamellaria* (Rubiaceae) are obligately farmed by the ant species *Philidris nagasau* (Dolichoderinae), which actively plants and fertilizes its hosts. The interactions are confined to Fiji¹².

All investigated domatia ($n = 866$) of six species of *Squamellaria* (*S. grayi*, *S. huxleyana*, *S. imberbis*, *S. major*, *S. thekii* and *S. wilsonii*, sharing a single recent common ancestor) were occupied by *P. nagasau*, either by transient workers or by permanent colonies. Five of these species also offer nutritious sugar rewards to the ants¹³. Conversely, *Philidris nagasau* is only known from these *Squamellaria* species, indicating that the symbiosis is obligate for both partners. By contrast, only 70–80% of the domatia of *S. jebbiana*, *S. tenuiflora* and *S. wilkinsonii* were ant-occupied ($N = 100$ individuals; Figs 1a and 2a), and were occupied by 14 generalist ant species that can also nest outside plants.

To test whether any *Squamellaria* species are ant dispersed, we first recorded the spatial distribution of *P. nagasau*-inhabited *Squamellaria*, which was over three times more clustered than that of the non-*P. nagasau*-inhabited species (Supplementary Fig. 1; *t*-tests, all $P < 0.001$), suggesting a difference in seed dispersal or establishment or, alternatively, differential seed predation or herbivory. *Philidris nagasau*-inhabited *Squamellaria* clusters sometimes comprised more than 25 individuals (Fig. 1a), all linked by ant trails, implying that the ant species is polydomous, with the offspring of one queen living in several domatia (monogynous,

Supplementary Information). The trail system sometimes spans across several trees with touching branches. In contrast, all 14 generalist ant species nesting in *S. jebbiana*, *S. tenuiflora* and *S. wilkinsonii* examined so far are monodomous (the queen and all her offspring live in a single nest). In the specialists, the pattern of trails linking was centralized towards the queen-bearing domatium and distance appeared important in determining network structure (Supplementary Fig. 2; Methods).

To assess whether *P. nagasau* ants disperse their hosts, we monitored several ant colonies. We observed that *P. nagasau* inserted the seeds of its plant hosts in cracks in tree bark (Fig. 1b) and that workers constantly patrol these planting sites. To test whether *P. nagasau* can recognize its hosts’ seeds and prefers them to seeds of the closely related facultative *Squamellaria* species, we conducted a cafeteria experiment, which showed that when ants face a choice between the seeds of an obligate and of a facultative species of *Squamellaria* (here, *S. huxleyana* vs. *S. wilkinsonii*; Fig. 1c), they only collect the seeds of the specialized species (generalized linear model (GLM), $\chi^2_{1,13} = 6.69$, $P < 0.01$). In neotropical ant gardens, the seeds of *Peperomia macrostachya* are recognized by *Camponotus femoratus* using chemical cues¹⁴, but this ant species also plants other unrelated epiphytes³. The absence of fatty outgrowths (elaiosomes) on the seeds in *Squamellaria* combined with the results of our seed removal assays suggests the existence of specific chemical cues (limited to the six obligate *Squamellaria* species).

We noticed that *P. nagasau*-inhabited *Squamellaria* lacked ripe fruits, suggesting that birds or *P. nagasau* workers remove them before full maturity. To verify that seeds from unripe fruits are attractive to *P. nagasau*, we performed a new seed removal assay, which showed similar results (Fig. 1c). We tested for ant versus bird dispersal in *Squamellaria* by exclusion experiments that prevented either birds (bag only) or ants and birds (Vaseline and bag) from accessing fruits and seeds (Supplementary Information). Bird exclusion (bag only) did not affect fruit removal of *P. nagasau*-inhabited *Squamellaria* (*S. huxleyana*), but the treatment that excluded ants and birds (Vaseline and bag) decreased fruit removal significantly (Supplementary Fig. 3a; GLM, $\chi^2_{1,17} = 13.32$, $P < 0.001$). Exclusion experiments in one of the non-*P. nagasau*-inhabited species (*S. wilkinsonii*) yielded no differences between the two treatments (control vs. Vaseline or bag, GLM, $\chi^2_{1,17} = 7.23$, $P < 0.01$; Vaseline vs. bag, NS (colour-changing fruits) and NS (mature fruits); Supplementary Fig. 3b). Subsequent observations confirmed that *P. nagasau* removed seeds from immature fruits one by one by cutting the fruit flesh (Supplementary Fig. 3c). These data confirm that *P. nagasau*-inhabited (specialized) *Squamellaria* species are dispersed by their ant symbionts—an essential step in a farming mutualism—whereas *Squamellaria* species occupied by unspecialized (only facultatively plant nesting) ant species are bird dispersed.

The second essential step required for a farming mutualism is a cultivation behaviour that improves the growth conditions before crop maturity. We observed that each *Squamellaria* seedling was

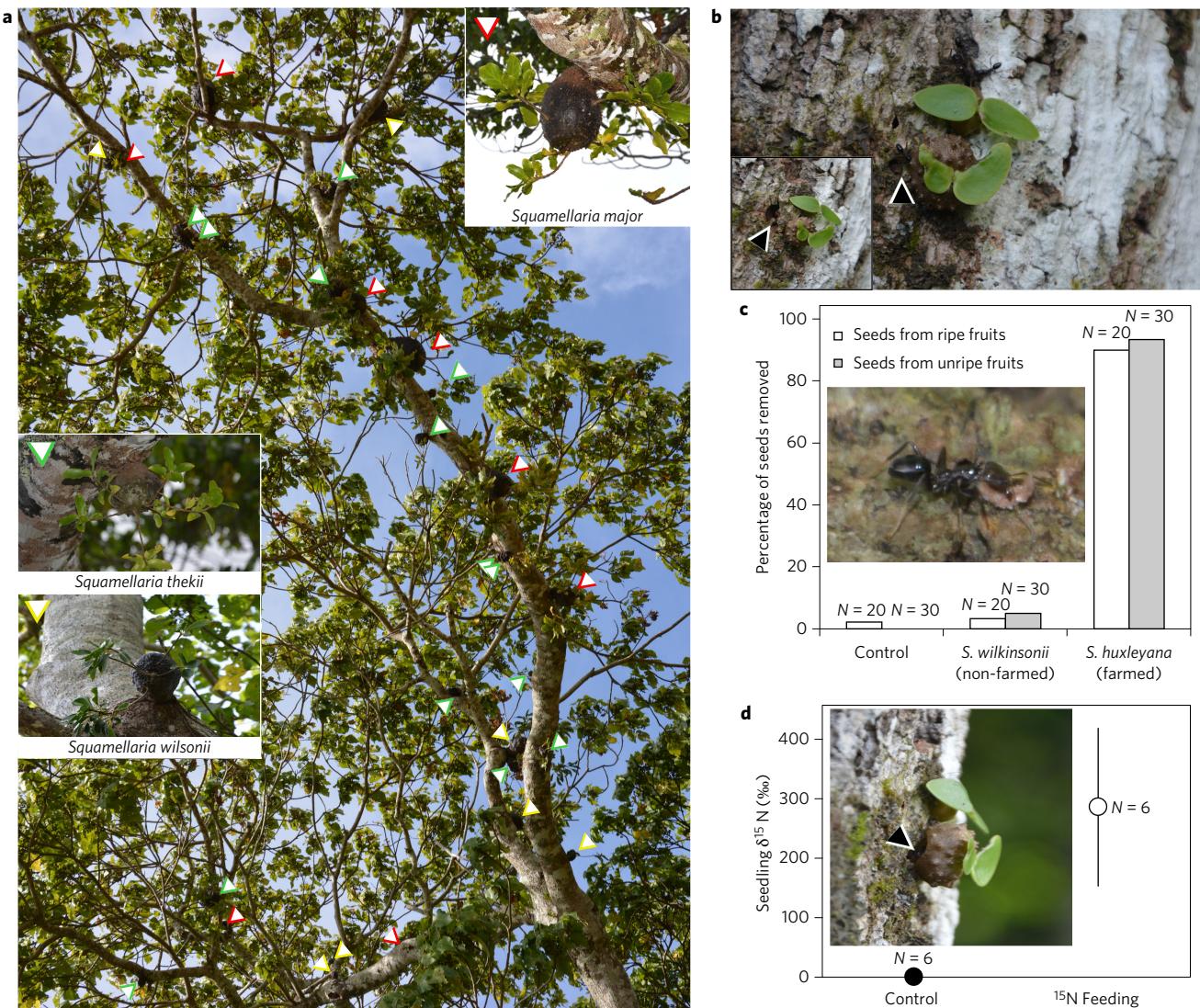


Figure 1 | *Philidris nagasau* farms *Squamellaria*. **a**, Cluster of 28 *Squamellaria* individuals inhabited by a single (polydomous) colony on a *Macaranga* branch, DesVoeux peak, Taveuni. **b**, *S. huxleyana* seed inserted in a bark crack with other seedlings. Arrowheads point to seeds. **c**, *P. nagasau* seed removal assay comparing seeds from ripe or unripe fruits of a non-farmed *Squamellaria* species with a farmed *Squamellaria* species, with rice grains as a control, all placed on the surface of a host tree. The photograph shows a *P. nagasau* worker transporting a *S. huxleyana* seed. **d**, Seedlings of specialized *Squamellaria* species, too young to have a domatium, are fertilized by *P. nagasau*. $\delta^{15}\text{N}$ values for control seedlings (no feeding) and feeding experiments where a ^{15}N source was placed at 2 metres from the seedlings (Supplementary Information).

transiently entered by *P. nagasau* workers as soon as it had formed its first cavity, which occurs in ~2 cm tall seedlings (Fig. 1d, inset); typically, three to ten workers coming from the queen-bearing domatium were constantly shuttling in and out of the tiny seedling domatium. Our ^{15}N labelling experiment (Methods) revealed a ca. 300-fold increase in $\delta^{15}\text{N}$ compared with the control (285.93 ± 133.32 vs. 0.32 ± 0.59). Taken together, these findings demonstrate that the workers fertilize the seedlings actively, probably by defecating into their tiny domatia (although defecation could not be observed directly). To our knowledge, the *P. nagasau*/ *Squamellaria* system is the first instance of obligate farming of plants by ants, potentially as sophisticated as the attine ants–fungi mutualism.

The difference in seed dispersal mechanisms between facultative and obligate *Squamellaria* species raises the question whether the two groups vary in the range of host trees on which they grow. We addressed this in a forest transect where *S. imberbis* (only and always inhabited by *P. nagasau*) and *S. wilkinsonii* (inhabited by several generalist ant species) grow sympatrically (Supplementary

Table 1). *S. imberbis* occurred on only 34% of the 35 tree species, whereas *S. wilkinsonii* occurred on 69%. Four tree species (*Ficus vitiense*, *Erythrina* spp., *Macaranga* sp. 1, *Macaranga* sp. 2) were the most frequent hosts of *S. imberbis* (Supplementary Fig. 4; Kolmogorov-Smirnov test, $P < 0.001$), suggesting a preference of the seed-planting workers for these species. *Squamellaria wilkinsonii* were not found on these species. Of the four species, *Erythrina* and *Macaranga* have extrafloral nectaries on the petiole and rachis or on the leaf blade (Supplementary Fig. 5), and we observed *P. nagasau* workers foraging on these nectaries. *F. vitiense* does not offer nectar but has sugary fruits as well as soft, easily hollowed pits (Supplementary Fig. 5g-k) transiently occupied by *P. nagasau* workers (Supplementary Fig. 5j). Gas chromatography time-of-flight mass spectrometry analyses (Methods) showed that the chemical composition of the rewards of *Macaranga* and *Erythrina* largely overlaps that of *P. nagasau*-inhabited *Squamellaria* nectar in sugars, amino acids and organic acids (Supplementary Fig. 5m,n). The potential host tree selection by *P. nagasau* workers is limited to neighbouring trees, but choosing the most suitable trees to

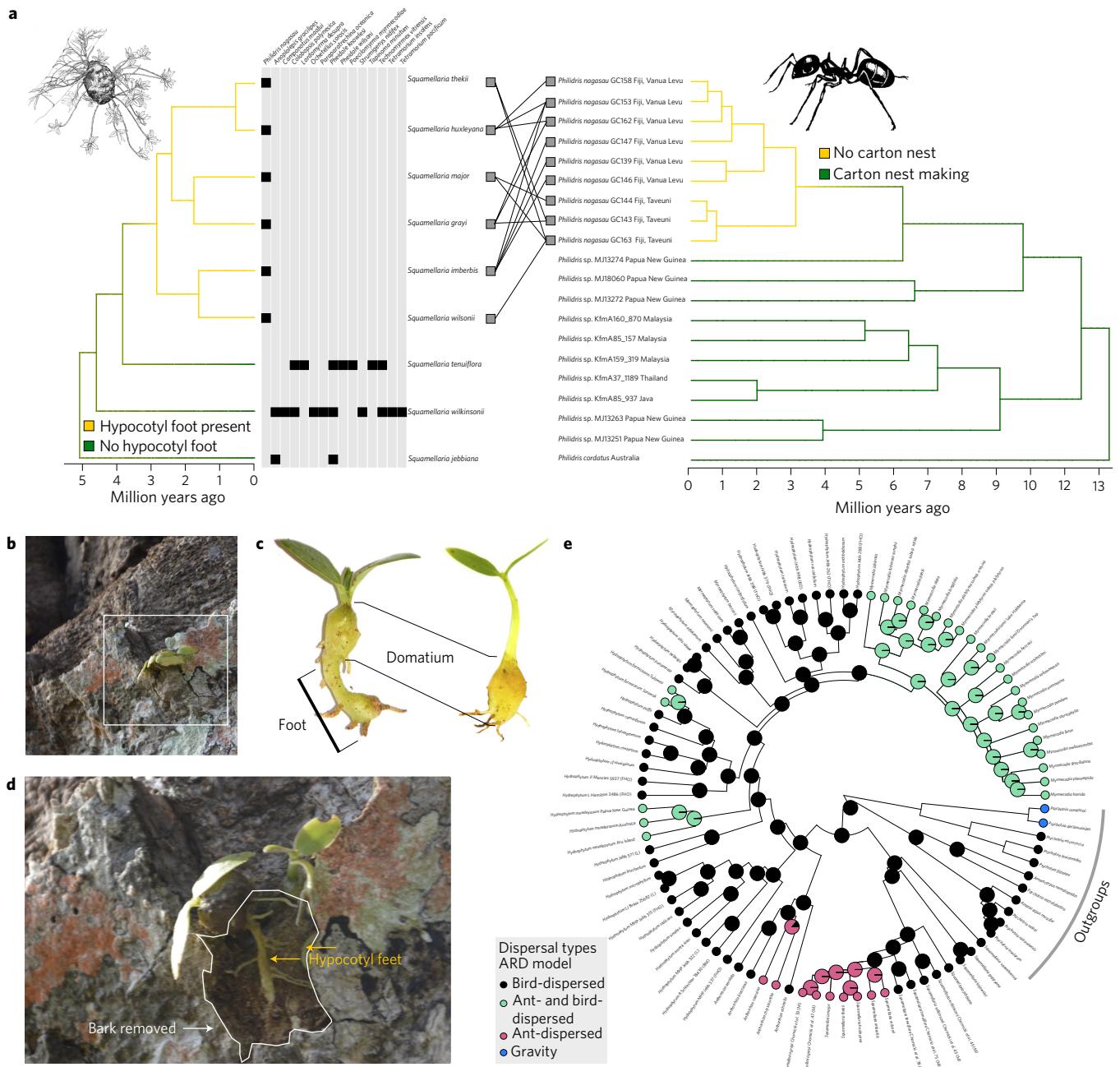


Figure 2 | Coevolution of *Philidris nagasau* and *Squamellaria*, and multiple ‘domestication’ events in epiphytic ant plants. a, Dated phylogenies for Fijian *Squamellaria* and *Philidris*, and ancestral state reconstruction for hypocotyl foot presence or absence in *Squamellaria* and carton nest-making ability in *Philidris* reveal the correlated gain of the hypocotyl foot together with the loss of carton nest building (implying seed planting under the bark) approximately 3 million years ago. The *S. huxleyana* thumbnail was drawn by Yasumin S. Lerner. **b**, Young seedlings of *S. thekii* emerging from under the bark. **c**, *S. imberbis* seedling with a hypocotyl foot (farmed, left) and *S. wilkinsonii* without (non-farmed, right). **d**, Removing the bark from **b** reveals the hypocotyl feet. ARD, all rates different. **e**, A phylogenetic reconstruction of dispersal types in epiphytic Hydnophytinae reveals several potential ‘domestication’ events.

establish their ‘farms’ maximizes colony fitness and has also been reported in Neotropical and Southeast Asian ant gardens, typically involving trees with food rewards or chemical defences^{2,3}.

A phylogenetic framework indicates that facultative mutualism with opportunistic ants is the ancestral condition, but obligate symbioses with *P. nagasau* arose in the common ancestor of the six species¹³ (Fig. 2a). Typically, ant garden ants plant seeds inside carton nests^{6,15}. The puzzling lack of carton nest building in the *P. nagasau/Squamellaria* mutualism led us to investigate the life history of *Philidris* ants. We generated a phylogeny for this group and reconstructed ancestral states for carton nesting (Fig. 2a and

Supplementary Fig. 6). Our analyses show that carton nest making has been lost in *P. nagasau*, but the remaining *Philidris* species all make carton nests.

Seedlings of *P. nagasau*-inhabited *Squamellaria* have a unique morphology with an elongation of the hypocotyl (hypocotyl foot) before domatium formation, allowing them to escape the bark cracks (Fig. 2b-d). This type of seedling growth characterizes the *P. nagasau*-inhabited clade (Fig. 2a), but is absent in ‘facultative’ (occupied by generalist ants) *Squamellaria* species (Fig. 2a,c) and is, as far as we know, unique in Hydnophytinae. This strongly suggests trait coevolution whereby seedlings first escape their

sunken germination sites thanks to the elongated hypocotyl foot (Fig. 2a–d) and only then develop a domatium, in response to the loss of carton nest making in *P. nagasau*. As for the *Philidris* ants, we generated a phylogeny for the Hydnophytinae (Supplementary Fig. 7), the group to which *Squamellaria* belongs, to trace the evolution of seed dispersal mechanisms. Our results show that dispersal by ants evolved five times (Fig. 2e), suggesting that other complex farming mutualisms may exist in this group largely concentrated in New Guinea and remain to be studied.

This unique farming mutualism involves one ant species cultivating six plant species, sometimes several (up to three) in the same colony (Fig. 1a). Four species are rare (*S. grayi*, *S. major*, *S. thekii*, *S. huxleyana*¹²), whereas two sister species (*S. imberbis* in Vanua Levu and *S. wilsonii* in Taveuni) are common. In both islands, *P. nagasau* forms large monocultures consisting of plant siblings, a trait found in the most complex farming mutualisms^{4,16}. Based on molecular-clock dating, the *Philidris nagasau*/ *Squamellaria* system is some 3 million years old (Fig. 2a). Further work on this system will determine the chemical signalling that is likely to govern the key steps of plant farming by ants.

Methods

All material and methods are described in detail in the Supplementary Information.

Collection of material in Fiji and at study sites. In September 2014, March 2015 and July–August 2016 the first author conducted fieldwork on Viti Levu, Vanua Levu and Taveuni and collected all species of Fijian *Squamellaria*, including three new species (*S. jebiana*, *S. grayi* and *S. huxleyana*; Chomicki and Renner¹²). Except for a few cases, *Squamellaria* plants were accessed by tree climbing, using a rope secured by a partner on the ground. This technique allowed long stays in the canopy with minimal disturbance of the ant colony. See the Supplementary Information for details on the field sites and *Squamellaria* host tree association.

Experiments on seed dispersal by ant versus birds and seed cafeteria experiment. To find out the seed-dispersing vectors of specialized and unspecialized *Squamellaria*, we excluded either birds only (bags) or birds and ants (Vaseline) on specialized and generalist *Squamellaria* species. Sample sizes and statistical analyses are described in the Supplementary Information. Seed cafeteria was performed by providing seeds from specialized vs. generalist (plus control) to *P. nagasau*, followed by six-hour monitoring.

¹⁵N sugar-feeding experiments and $\delta^{15}\text{N}$ isotope analyses. We tested whether *P. nagasau* workers fertilizes seedling by providing sugar solutions with ¹⁵N glycine for 10 days, and subsequently analysing samples (with appropriate control) via isotope ratio mass spectrometry. See the Supplementary Information for details.

DNA extraction, phylogenetic analyses, molecular clock dating and ancestral state reconstructions. We used our recent eight-gene matrix for *Squamellaria*¹², and generated a six-gene phylogeny for the larger Hydnophytinae clade to which *Squamellaria* belongs and a five-gene matrix for *Philidris*. All procedures are described in detail in the Supplementary Information.

Gas chromatography time-of-flight mass spectrometry determination of metabolic profiles. All procedures are described in detail in the Supplementary Information.

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Author contributions

G.C. designed the study, conducted the experiment and analysed the data. G.C. and S.S.R. wrote the manuscript. S.S.R. and G.C. contributed reagents.

Additional information

Supplementary information is available for this paper. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to G.C.

Competing interests

The authors declare no competing financial interests.