

In the format provided by the authors and unedited.

Obligate plant farming by a specialized ant

Guillaume Chomicki and Susanne Renner

Department of Biology, Systematic Botany and Mycology, Menzinger Str. 67,
University of Munich (LMU), Munich 80638

Corresponding author: guillaume.chomicki@gmail.com (G.C.)

Supplementary Materials and Methods

Supplementary information on collection of material on Fiji and study sites

The study sites in Viti Levu were Colo-i-Suva forest reserve in the south of the island (S 18° 1' 46.808", E 178° 24' 0.4175") and a forest in the vicinity of Navai in the centre of the island (S 17° 37' 49.5979", E 177° 58' 34.9315"); in Vanua Levu, the collection sites were in Waisali forest reserve (S 16° 38' 19.8", E 179° 13' 19.7") and along the Cross Island road before the bifurcation to Nabouwalu and Labasa; in Taveuni, the samples (and herbarium collections) were obtained along the trail to DesVoeux peak and Mt. Manuca on the western side of the island (S 16° 48' 25.8133", E 179° 56' 36.6843") and at the end of Lavena coastal walk, Bouma heritage park, on the eastern side of the island (S 16° 51' 45.4433", E 179° 54' 6.5149"). All collections were made in collaboration with colleagues from the University of South Pacific (Acknowledgements), and vouchers have been deposited in the herbaria of Suva (SUVA) and Munich (M). For DNA extraction, we collected young leaves and dried them in silica gel. *Squamellaria* taxonomy follows Chomicki and Renner (2016).

Host specificity, occupancy rates

Philidris nagasau form large colonies that often occupy several dozen of *Squamellaria* plants (Fig. 1a), and that have several thousands of workers (one large 80 cm-long, queen-bearing domatium had 10,000 workers). We assessed host specificity and host occupancy rates for each species using at least 20 plants. 'Host' here refers to the Hydnophytinae species with domatia suitable as nesting sites for ants. Observations were designed to be as non-invasive as possible. For the six species inhabited by *Philidris nagasau* (*S. imberbis*, *S. wilsonii*, *S. thekii*, *S. grayi*, *S. huxleyana*, *S. major*), we found that all 866 observed individuals (*S. imberbis* [N = 415], *S. wilsonii* [N = 317], *S. thekii* [N = 31], *S. grayi* [N = 38], *S. huxleyana* [N = 45], *S. major* [N = 20]) were *P. nagasau*-inhabited. The *P. nagasau* colonies and polydomy allowed non-invasive observations for *P. nagasau*-inhabited species, since the large colonies span the whole tree and the trails can be observed even from the ground. For the generalist non-*P. nagasau* inhabited *Squamellaria* species, dissection was necessary, and 14 ant species were identified from 100 individuals (collected over three field seasons 2014, 2015 and 2016) (*S. tenuiflora* [N = 20], *S. wilkinsonii*

[N= 60], *S. jebbiana* [S = 20]). For each plant, we recorded whether it was inhabited by ants or not, and determined all domatium-inhabiting species. We analysed statistical differences among species using Welch t-tests, performed in R (RC Team, 2013).

Philidris nagasau colony structure

Since we found that six species of *Squamellaria* were exclusively inhabited by *P. nagasau*, we investigated ant colony size and structure using the two most common *Squamellaria* species, *S. imberbis* (Vanua Levu) and *S. wilsonii* (Taveuni). We dissected a *S. wilsonii* colony of 23 individuals to determine presence of one or more queens, which revealed a single queen in the largest domatium (monogynous), but many alates, with the number of alates correlated with domatium size (Fig. S2b), suggesting dispersal from the queen-bearing domatium. To further confirm monogyny, we dissected two smaller colonies (both of *S. imberbis*, Vanua Levu) of five and eight domatium, respectively, which confirmed monogyny. We monitored ant trails in a colony of 15 *S. imberbis* individuals where all specimens were classified as seedlings, small, medium, large and queen housing (see Fig. S2). We recorded all trails connecting pairs of *Squamellaria* domatia within the system. Monitoring was carried out three times a day for two days. The 25 observed (realised) links have to be contrasted with the 105 possible links (number of links = $15!/2!(15-2)!$). To test whether distance, beyond connectivity to the queen-bearing domatium, was an important factor determining the polydomous network, we recorded the distance between every pair of domatia (either by direct measurement or by estimation when the domatia were out of reach) and noted whether the connection was realised or not. We performed a one-way ANOVA on these data with a Tukey's post-hoc test. Ant monitoring showed that the trails on any tree are hierarchically organized towards the queen-housing plant (Fig. S2) and that distance is an important mediator of link realisation (Fig. S2a; Tukey's test; $P < 0.001$), indicating that continuous visit of all *Squamellaria* (of all sizes) in the colony is centrally controlled.

To evaluate whether the ants inhabiting unspecialized *Squamellaria* were polydomous or monodomous, we searched for trails connecting their colonies, but found none. Instead, we discovered that different ant species inhabited them, proving monodomy.

Squamellaria seedling morphology

To ensure that the unique morphology of *Squamellaria* seedlings was not the result of etiolation, seeds of the specialized *S. thekii* and *S. imberbis* and the unspecialized *S. tenuiflora* and *S. wilkinsonii* were germinated under high light levels, which confirmed that delayed domatium formation is an inherited trait. All nine species were coded as 'hypocotyl foot absent' (0) or 'hypocotyl foot present' based on seedlings observed in the field. Species from the Hydnophytinae genera *Myrmecodia* (*M. tuberosa*) and *Hydnophytum* (*H. formicarum*) have been germinated under the same conditions, and none showed a hypocotyl foot. Moreover, Hydnophytinae taxonomists Camilla R. Huxley and Matthew P. Jebb were consulted and from their extensive field experience no other Hydnophytinae species has an elongated hypocotyl.

Experiments on seed dispersal by ants versus birds

To find out the seed-dispersing vectors of specialized and unspecialized *Squamellaria*, we selected five large flowering and fruiting plants of *S. imberbis* (specialized) and *S. wilkinsonii* (unspecialized) along the cross-island road in Vanua Levu. In each plant, three branches were marked as controls; three branches were enclosed in a bag with holes; in three, all reproductive structures and developing buds were enclosed in Vaseline; and lastly, three were enclosed in Vaseline and additionally in a bag with holes (as a treatment control). A bag with holes should prevent fruit collection by birds but not by ants (confirmed by observation), and Vaseline should prevent ants from removing the fruits. All treatments were applied during ten days in March 2015. We expected that if ants are the main dispersers, the presence of a bag preventing bird access would not significantly affect fruit removal, while conversely, Vaseline treatment preventing ant access would significantly decrease fruit removal. Each stage (buds <0.5 cm in length, buds >0.5 cm, flowers at anthesis, post-anthesis, green fruits, fruits turning red, and mature fruits) was recorded for each shoot at the end of the 10 days, and the results were normalized by the number of shoot metamers (segments between leaf nodes) to ensure comparability across shoots. All corresponding data (N = 3 x 5 replicates) were summarized, and we used R (R core team 2013) to test the difference in means for each treatment relative to the control using generalized linear models (GLM) with a Poisson distribution, followed by an ANOVA of the deviance table. Significance of the p-value are reported as * for P<0.05, ** for P<0.01 and *** for P<0.001. Pairwise analyses using Welch t-tests were similarly significant.

Seed cafeteria experiment

To test whether *P. nagasau* can recognize seeds of specialized *Squamellaria* and differentiate them from those of unspecialized *Squamellaria* (often growing nearby) we placed 20 *S. wilkinsonii* seeds, 20 *S. huxleyana* seeds, and 20 rice grains (as controls) on a large branch, and subsequently monitored seed removal for 6 hours. Replications consisted of blocking by 4 seeds (we placed 5 clusters or blocks with 4 seeds of each three categories), hence with 5 replicates. We analysed statistical difference of each category from the control using a Generalized Linear Model (GLM) with a Poisson distribution followed by an ANOVA of the deviance table. Pairwise analyses of control/obligate or facultative/obligate using Welch t-tests were similarly significant. This first seed removal assay was performed with seeds collected on mature fruits. Because mature fruits are almost impossible to find in specialized (farmed) *Squamellaria*, we collected them from the preceding seed dispersal experiments (in treatments with Vaseline). In order to test whether seeds from immature fruits are also attractive, we performed the same experiment (this time with 30 seeds for each category, and a blocking by 6, so also 5 replicates).

¹⁵N sugar feeding experiments and $\delta^{15}\text{N}$ isotope analyses

Related mature *Myrmecodia* plants are fed by their *Philidris* ants that defecate in specific ‘warted’ chambers (Huxley, 1978), and we therefore tested whether the seedlings of ant-dispersed *Squamellaria* species that are developing their first cavity (domatium), usually at a seedling diameter ~ 2 cm (Fig. 1), were already being fed by ants. To do so, we selected a *Macaranga* tree along the cross-island road in Vanua Levu with a colony of *S. huxleyana* with both mature plants and seedlings. We placed

a solution of 20 mM ^{15}N glycine (enriched at 98% at, Isotec) with 40% (w/v) 1:1:1 mix of sucrose, glucose and fructose in a falcon tube close to the mature plants, and at about 2 meters of the seedlings. A paper wick allowed the ants to reach the solution without drowning in it. We added two millilitres of solution to the falcon tube twice a day during the 10 days of the experiment. On the 11th day, we collected six seedlings of ~2 cm (see Fig. 1f) and microwave-dried them. Five *S. huxleyana* seedling controls of the same stage were collected from a neighboring tree (at about 500 m) and prepared in the same way. Samples were homogenized with a ball mill and ca. 1-3 mg of dry powder was weighted in tin capsules. Isotope-ratio mass spectrometry (IR-MS) analyses were performed at the GeoBiocenter, University of Munich (LMU). Capsules were combusted in an elemental analyser (NC2500, Carlo Erba) in a continuous helium flow at 1080⁰C. The combustion gases passed through a reaction tube filled with chromium and silvered cobaltous oxides, a subsequent reduction tube (560⁰C) filled with copper wires, a water trap filled with magnesium perchlorate, and a gas-chromatography column. The isolated gases N_2 and CO_2 were then analysed in an isotope-ratio mass spectrometer (DeltaPlus, Thermo-Finnigan) to determine the isotope ratios of organic carbon ($\delta^{13}\text{C}_{\text{org}}$) and nitrogen ($\delta^{15}\text{N}$). The total organic carbon (TOC) and total nitrogen (TN) mass percentages were calculated from sample peak areas using the elemental standards atropine, cyclohexanone-2,4-dinitrophenylhydrazone, and peptone for calibration.

Squamellaria host tree association

To evaluate the host tree range of specialized *Squamellaria* species versus that of the unspecialized species and to test for a possible selection of particular tree species by *P. nagasau* workers, we evaluated tree occupancy rate along transects. By comparing research sites on the three islands, we determined that the best study site to compare host tree association of specialized and unspecialized *Squamellaria* species was on Vanua Levu, where *S. imberbis* and *H. wilkinsonii* are abundant and grow in close proximity to each other. The transect started at the track to the Vodafone Tower, near the Cakaudrove-Macuata provincial boundary line, and extended along the cross-island road until Waisali forest reserve and inside Waisali forest reserve. Each tree along the transect with a diameter at breast height (DBH) >5 cm was recorded, and its *Squamellaria* epiphytes (*S. imberbis* and *S. wilkinsonii*) were counted. Data for the 35 host tree species and 253 tree individuals are reported in Table S1. We found high correlations between tree size (log(DBH)) and number of epiphytes per tree (log(plant number)) for the specialized *S. imberbis* (Pearson's correlation coefficient $R=0.58$) and the unspecialized *S. wilkinsonii* ($R=0.55$). Specialized *Squamellaria* further appeared to be concentrated on four tree species (*Macaranga spec. 1*, *Macaranga spec. 2*, *Ficus vitiense* and *Erythrina spec.*), all of which reward the *Squamellaria*-inhabiting ants *Philidris nagasau*. To test for a potential significance of this observation, we determined the occupancy frequencies of rewarding trees versus non-rewarding trees by specialized versus non-specialized *Squamellaria*. Because the occupancy frequencies were too far from a normal distribution and homoscedascity was not verified, we used a non-parametric test. The Kolmogorov-Smirnov test ('ks.test' function in R, null hypothesis (H_0): the samples have the same distribution) confirmed that the difference was statistically significant.

DNA extraction and phylogenetic analyses

For *Squamellaria*, we used our recently generated matrix of eight plastid (trnL-trnF region (trnL intron and trnL-trnF spacer), ndhF, rps16, rpl20-rps12, trnG-

trnS spacer) and nuclear gene regions (ITS region (ITS1, 5.8S, ITS2), ETS, 18S) (Chomicki and Renner, 2016). All accessions of Fijian *Squamellaria* were extracted from silica-dried leaves collected by GC and are all linked to herbarium specimens deposited in the herbaria SUVA and M. A sampling of outgroups (in the tribe Psychotrieae) was selected based on Barrabé et al. (2014). We also generated another matrix using six markers (nuclear ITS and ETS and plastid ndhF, psbA-trnH, trnL intron and trnL-trnF spacer) using a wider sampling of Hydnophytinae to infer the evolution of dispersal type and ant inhabitation, sampling 75 species out of ca. 100, selected based on the current knowledge of their ant occupants. For the ants, we generated a matrix of four nuclear markers (CAD, EF α F1, EF α F2, and LR) and one mitochondrial marker (COI) sampled for 18 ingroup taxa and an additional 4 taxa as outgroups, based on Ward et al. (2010). All primers are shown in Table S2. Because the taxonomy of *Philidris* is poorly understood, and species delimitation is problematic (M. Janda pers. comm. to G.C. and S.S.R. Nov. 2014 and May 2016), we selected samples representing a broad range of host plants and geography. Voucher information is reported in Table S3 (*Squamellaria*) and Table S4 (Hydnophytinae) for plants, and Table S5 for ants. Total genomic DNA was extracted from c. 20 mg of leaf tissues, using a commercial plant DNA extraction kit (NucleoSpin; Macherey–Nagel, Düren, Germany) according to manufacturer protocols. Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (New England Biolabs, Cambridge, MA, USA) and a standard protocol (39 cycles, annealing temperature 56°C). PCR products were purified using the ExoSap clean-up kit (Fermentas, St Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on an ABI 3130 automated sequencer (Applied Biosystems, Perkin-Elmer). Sequences were edited in Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were BLAST-searched in GenBank. Sequence alignment was performed in MAFFT v. 7 in the online server (<http://mafft.cbrc.jp/alignment/>) (Katoh and Standley, 2013) under standard parameters except for the ITS region, which was aligned under Q-INS-i optimization, which incorporates rRNA secondary structure. Minor alignment adjustments were made in Mesquite v. 2.75 (Maddison and Maddison, 2011). In the absence of statistically supported incongruence (i.e., maximum likelihood bootstrap (BS) support >75) between the plastid and nuclear data partitions), we concatenated all markers, yielding an alignment of 9346 bp for the *Squamellaria* matrix, 3055 bp for the Hydnophytinae matrix and 1592 for *Philidris*. Maximum-likelihood (ML) inference relied on RAxML v8.0 (Stamatakis, 2014) and the GTR + Γ substitution model, with empirical nucleotide frequencies and 25 gamma rate categories; bootstrap support was assessed from 100 replicates under the same model. We also conducted Bayesian inference in MrBayes v. 3.2 (Ronquist et al., 2012) under the same substitution model (but with 4 rate categories) and using the program's default two runs and four chains (one cold and three heated), with the uniform default priors. We set a 10×10^6 MCMC chain, sampling trees every 1000th generation. Split frequencies approaching zero indicated convergence. We used the 50% consensus tree to assess posterior probabilities for nodes of interest.

Molecular clock dating

Molecular dating analyses relied on BEAST v. 2 (Bouckaert et al., 2014) and uncorrelated lognormal relaxed clock models. We used the GTR + G substitution model with four rate categories and a Yule tree prior. For both our plant and ant trees, MCMCs were run for 20 million generations, with parameters and trees sampled

every 10,000 generations. We used Tracer v. 1.6 (Rambaut and Drummond, 2007) to check that the effective sample size (ESS) of all parameters was >200 , indicating that runs had converged. After discarding 10% as burn-in, trees were summarized in TreeAnnotator v. 1.8 (part of the BEAST package) using the options ‘maximum clade credibility tree’, which is the tree with the highest product of the posterior probability of all its nodes, ‘mean node height,’ and a posterior probability limit of 0.98. The final tree was visualized in FigTree v. 1.4 (Rambaut, 2012). To calibrate our tree, we constrained the age of the root, i.e., the split between the Pacific clade and the so-called *Psychotria* clade IV of Barrabé et al. (2014), to 22 ± 7 Ma, based on the age of this node estimated by these authors, using a normal prior and a standard deviation of 4 corresponding to the 95% confidence interval of Barrabé et al. (2014). The Hydnophytinae dated tree is shown in Fig. S5 with support values. To calibrate our ant tree, we used a secondary constraint from Ward et al. (2010), specifically the split of *Linepithema humile* from all other taxa to 33 ± 8 Ma, using a normal prior with a standard deviation of 4. Because *Philidris nagasau* contains a clade endemic from the island of Taveuni, which has been dated to 0.8 Ma (Rodda and Kroenke, 1984; Rodda, 1994), we used this age as a geological maximal constraint for the age of this clade, using a uniform prior with a 0.8-0 Ma bound. The *Philidris* dated tree is shown in Fig. S6 with support values.

Ancestral state reconstructions of ant and plant life histories

We inferred the evolutionary history of dispersal type and ant association in the Hydnophytinae and that seed planting, nesting habit and carton nest making in *Philidris* ants (Fig. 2).

The seed dispersal type of the 75 ingroup species plus outgroups (all “0”) was coded “0” for dispersal by frugivorous animals (here birds), “1” for dispersal only by ants (myrmecochory) and birds, “2” for exclusively ant-dispersed (demonstrated for *Squamellaria* in this study and inferred for two species of *Anthorrhiza* based on Huxley and Jebb (1991) and Maeyama and Matsumoto (2000)), or “3” for seeds dispersed by gravity alone (barochory, Barrabé et al., 2014) based on published and unpublished data, especially (i) the clustering of individual epiphytes, with the clustered distribution indicative of ant dispersal and dispersed distribution indicative of bird dispersal, (ii) polydomy versus monodomy of ant colonies (indicated by trails linking distinct specimens), with polydomy indicative of ant dispersal of the seeds, while monodomy points to bird dispersal (Huxley, 1978; personal observations by M.P.H. Jebb and C.R. Huxley-Lambrick, during fieldwork in the 1975-1990 in Papua New Guinea) and observations and experiments made for this study (for the nine species of Fijian *Squamellaria*). The above-described experiments were conducted on *S. wilkinsonii* and *S. huxleyana*. For the seven other Fijian *Squamellaria* species, we use the described traits (clustering and polydomy vs. monodomy) to assign bird versus ant dispersal. Clustering was measured by counting the number of epiphytes per tree and in at least 10 epiphyte-bearing trees for each of the nine species, normalizing the data by the tree diameter at breast height (DBH; see Fig. S1).

Ant association was determined based on Huxley and Jebb (1991a, b), Huxley (1993) and an ongoing revision of *Hydnophytum* from M. Jebb and C.R. Huxley, personal communications to G.C. from M. Janda and observations made by G.C. on the nine Fijian *Squamellaria*.

For the ants, presence of carton nest building, nesting habit and ant dispersal was search for all outgroup species; further information came from consultation with

ant taxonomist P.S. Ward, and for all *Philidris* samples, the information came from G.C., E. Kaufmann and M. Janda who collected the specimens.

To infer ancestral dispersal types, we used the Maximum Clade Credibility (MCC) tree from BEAST, and (i) the stochastic mapping approach implemented in the phytools package (Revell, 2012) and (ii) the ML approach implemented in ape (Paradis et al., 2014). We used the function make.simmap in the phytools package (v. 04-60) (Revell, 2012), which implements the stochastic character mapping approach developed by Bollback (2006). We estimated ancestral states using three models (see thereafter), and then simulated 1,000 character histories on the MCC tree. We summarized the 1,000 simulated character histories using the function densityMap (also in phytools).

We performed the stochastic mapping analyses using three different models: (i) Equal rates (ER) model, wherein all rates of transitions among character states are equal; (ii) Symmetrical rate model (SYM), wherein the backward and forward character state transition rates are equal for each combination of character states, but each distinct state combination can have a distinct rate; (iii) All rates different (ARD), wherein all rates are allowed to vary. The likelihood of each model was compared to select the one fitting our data best, in this case the ARD model. ML ancestral state reconstructions for dispersal by ants are shown in Fig. S7.

GC-TOF-MS determination of metabolic profiles

All samples were field-collected and immediately microwave-dried, a method that preserves metabolites (Popp et al., 1992). Metabolites for gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) were extracted and derivatized following Roessner et al. (2001), Lisec et al. (2006) and Erban et al. (2007). For the extraction, ~ 5 mg plant material (dry weight) was ground in 300 µl cold (-20°C) methanol (80 %) containing 15 µl ribitol (0.1 mg ml⁻¹ in water) and 15 µl ¹³C-sorbitol (0.1 mg ml⁻¹ in water), which were added as internal standards. After incubation at 70°C for 15 min, 30 µl of the extract was dried *in vacuo*. The pellet was resuspended in 10 µl of methoxyaminhydrochloride (20 mg ml⁻¹ in pyridine) and derivatized for 90 min at 37°C. After the addition of 20 µl of BSTFA (*N,O*-Bis[trimethylsilyl]trifluoroacetamide) containing 5 µl retention time standard mixture of linear alkanes (n-decane, n-dodecane, n-pentadecane, n-nonadecane, n-docosane, n-octacosane, n-dotriacontane), the mix was incubated at 37°C for further 45 min. A volume of 1 µl of each sample was injected into a GC-TOF-MS system (Pegasus HT, Leco, St Joseph, USA). Samples were derivatized and injected by an autosampler system (Combi PAL, CTC Analytics AG, Zwingen, Switzerland). Helium acted as carrier gas at a constant flow rate of 1 ml/min. Gas chromatography was performed on an Agilent GC (7890A, Agilent, Santa Clara, USA) using a 30 m VF-5ms column with 10 m EZ-Guard column. The injection temperature of the CIS injector (CIS4, Gerstel, Mühlheim, Germany) increased with a rate of 12°C s⁻¹ from initially 70°C to finally 275°C. Transfer line and ion source were set to 250°C. The initial oven temperature (70°C) was permanently increased to a final temperature of 320°C by 9°C per minute. To avoid solvent contaminations, the solvent delay was set to 340 s. Because of the chemical and physical properties of the different metabolites the mixture was separated on the column over time. Metabolites that passed the column were released into the TOF-MS. The transfer line, connecting the GC and the TOF-MS, was set to 250°C, as was the ion source where the in-coming metabolites got ionized and fractionated by an ion pulse of 70 eV. Mass spectra were recorded at 20 scans per second with an *m/z* 35– 800 scanning range. Chromatograms and mass

spectra were evaluated using ChromaTOF 4.5 and TagFinder 4.1 software (Luedemann et al., 2008). All metabolite profiles are shown in Table S6.

Supplementary references

- Barrabé, L., Maggia, L., Pillon, Y., Rigault, F., Mouly, A., Davis, A. P. & Buerki S. New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Mol. Phylogenet. Evol.* 71, 15-35 (2014).
- Bollback, J. P. SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC bioinformatics* 7, 88 (2006).
- Chomicki, G. & Renner, S. S. Evolutionary relationships and biogeography of the ant-epiphytic genus *Squamellaria* (Rubiaceae: Psychotrieae) and their taxonomic implications. *Plos One* 11, e0151317 (2016).
- Erban A, Schauer N, Fernie AR, Kopka J. Nonsupervised construction and application of mass spectral and retention time index libraries from time-of-flight gas chromatography-mass spectrometry metabolite profiles. *Methods Mol Biol* 358. Metabolomics-Methods and Protocols. Humana Press, 19-38 (2007).
- Huxley, C. R. The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytol* 80, 231-268 (1978).
- Huxley, C.R., Jebb, M.H.P. The tuberous epiphytes of the Rubiaceae 2: the new genus *Anthorrhiza*. *Blumea* 36, 21-41 (1991).
- Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772-780 (2013).
- Lisec, J., Schauer, N., Kopka, J., Willmitzer, L. & Fernie AR. Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nat Protoc* 1, 387-396 (2006).
- Luedemann, A., Strassburg, K., Erban, A. & Kopka, J. TagFinder for the quantitative analysis of gas chromatography - mass spectrometry (GC-MS)-based metabolite profiling experiments. *Bioinformatics* 24, 732-737 (2008).
- Maddison, W. P & Maddison, D. R. Mesquite: a modular system for evolutionary analysis. Version 2.75. Available at: mesquiteproject.org/mesquite/download/download. (2011).
- Maeyama T, Matsumoto T. Genetic relationship of myrmecophyte (*Anthorrhiza caerulea*) individuals within and among territories of the arboreal ant (*Dolichoderus sp.*) detected using random amplified polymorphic DNA markers. *Austral Ecology* 25, 273-282 (2000).
- Pagel, M. & Meade, A. BayesTraits, version 2. Univ. of Reading, Berkshire, UK Available at <http://www.evolution.rdg.ac.uk>. (2013)
- Paradis, E, Claude, J., Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289-290 (2004).
- Popp, M., Lied, W., Meyer, A. J., Richter, A., Schiller, P. & Schwitte, H. Sample preservation for determination of organic compounds, microwave versus freeze-drying. *J Exp Bot* 47, 1469-1473 (1996).
- Rambaut A. FigTree v. 1.4.0. <http://tree.bio.ed.ac.uk/software/figtree>. (2012)
- Rambaut A, Drummond AJ. Tracer – MCMC trace analysis tool version v1.5. URL <http://beast.bio.ed.ac.uk>. (2007).
- Team, R.C., 2013. R: A language and environment for statistical computing.

- Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3:217-223 (2012)
- Ripley, B., Venables, B., Bates, D. M., Hornik, K., Gebhardt, A. R package 'MASS'. <https://cran.r-project.org/web/packages/MASS/MASS.pdf>. (2015).
- Roessner, U., Luedemann, A., Brust, D., Fiehn, O., Linke, T., Willmitzer, L., Fernie, A. R. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *Plant Cell* 13, 11-29 (2001).
- Rodda, P. Geology of Fiji. South Pacific Applied Geoscience Commission (SOPAC) *Technical Bulletin* 8:131-151 (1994).
- Rodda, P. & Kroenke, L. Fiji: a fragmented arc. In: *Cenozoic Tectonic Development of the Southwest Pacific* (ed. L. Kroenke), pp. 87-110. U.N. ESCAP/CCOP/SOPAC (1984).
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. MrBayes 3.2, efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61, 539-542 (2012).
- Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312-1313 (2014).
- Ward, P. S., Brady, S. G., Fisher, B. L. & Schultz, T. R. Phylogeny and biogeography of dolichoderine ants: effects of data partitioning and relict taxa on historical inference. *Syst Biol* 59, 1-21 (2010).

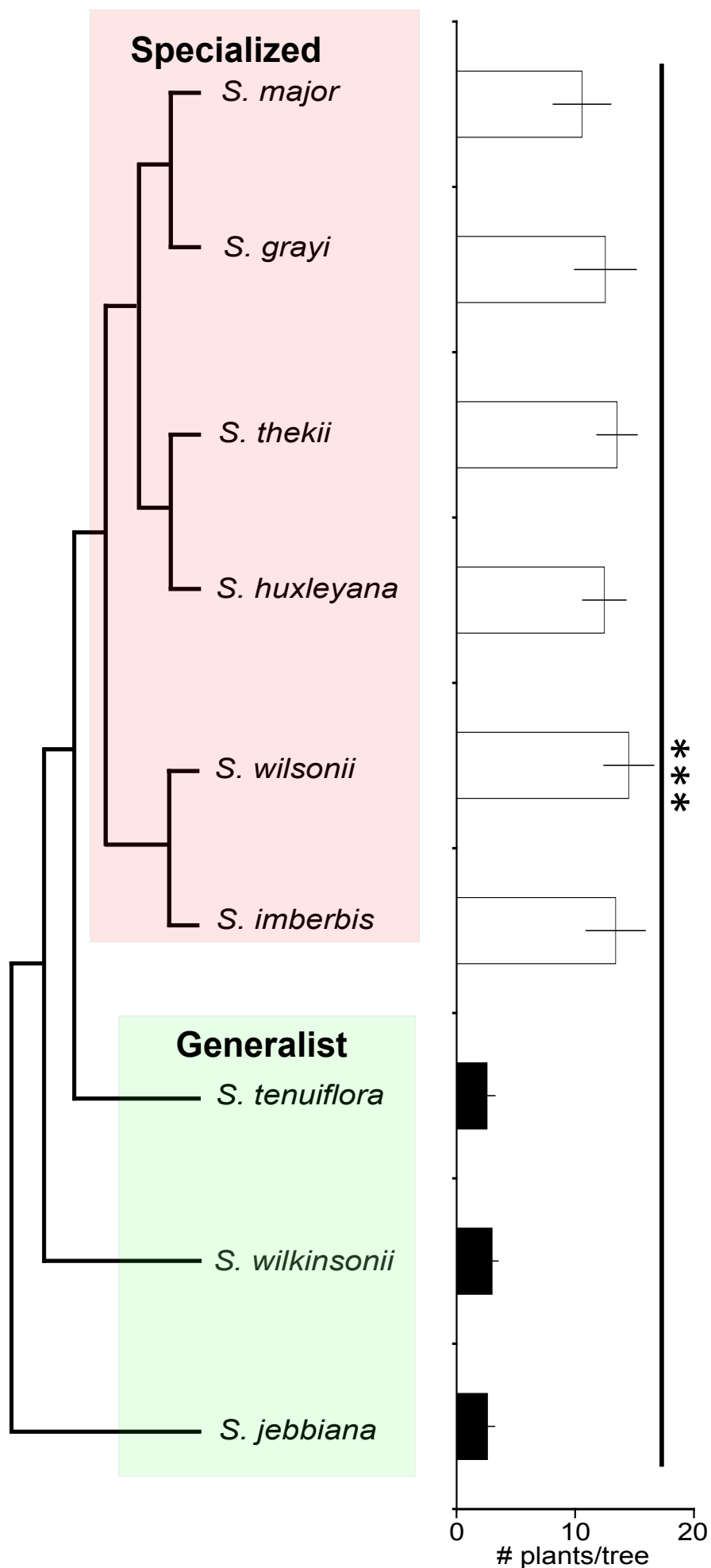


Figure S1. Clustering value (mean number of plants per tree normalized by DBH) in all species of the Fijian Squamellaria clade, shown in black for generalists and in white for specialized ant-plants. Error bars give the standard errors of the clustering value. Stars refer to the significance of the t-tests (see Online Supplementary Materials and Methods).

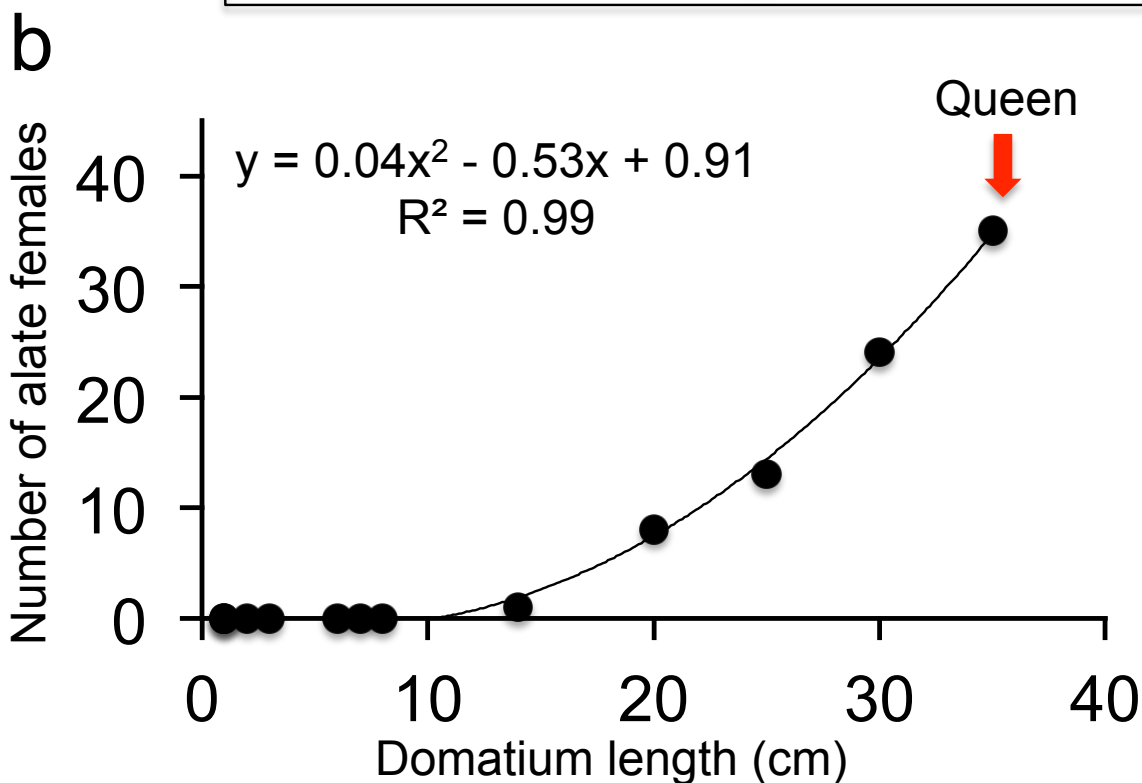
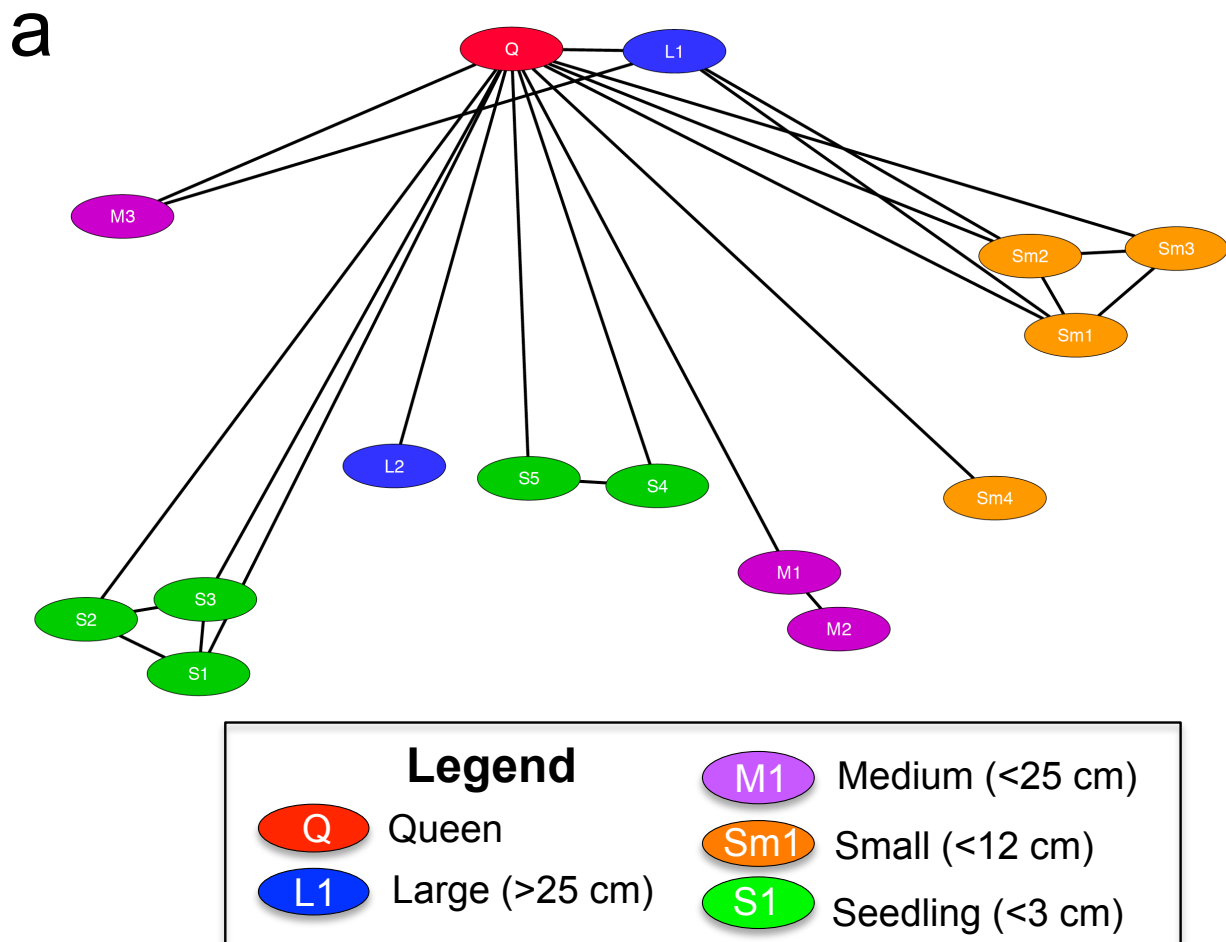


Figure S2. Polydomy of *Philidris nagasau* colonies. (a) detail of a network present on a single *Macaranga spec. 1* tree in Taveuni, all *Squamellaria* specimen are *S. wilsonii*. (b) Relationship between domatium size and alate number.

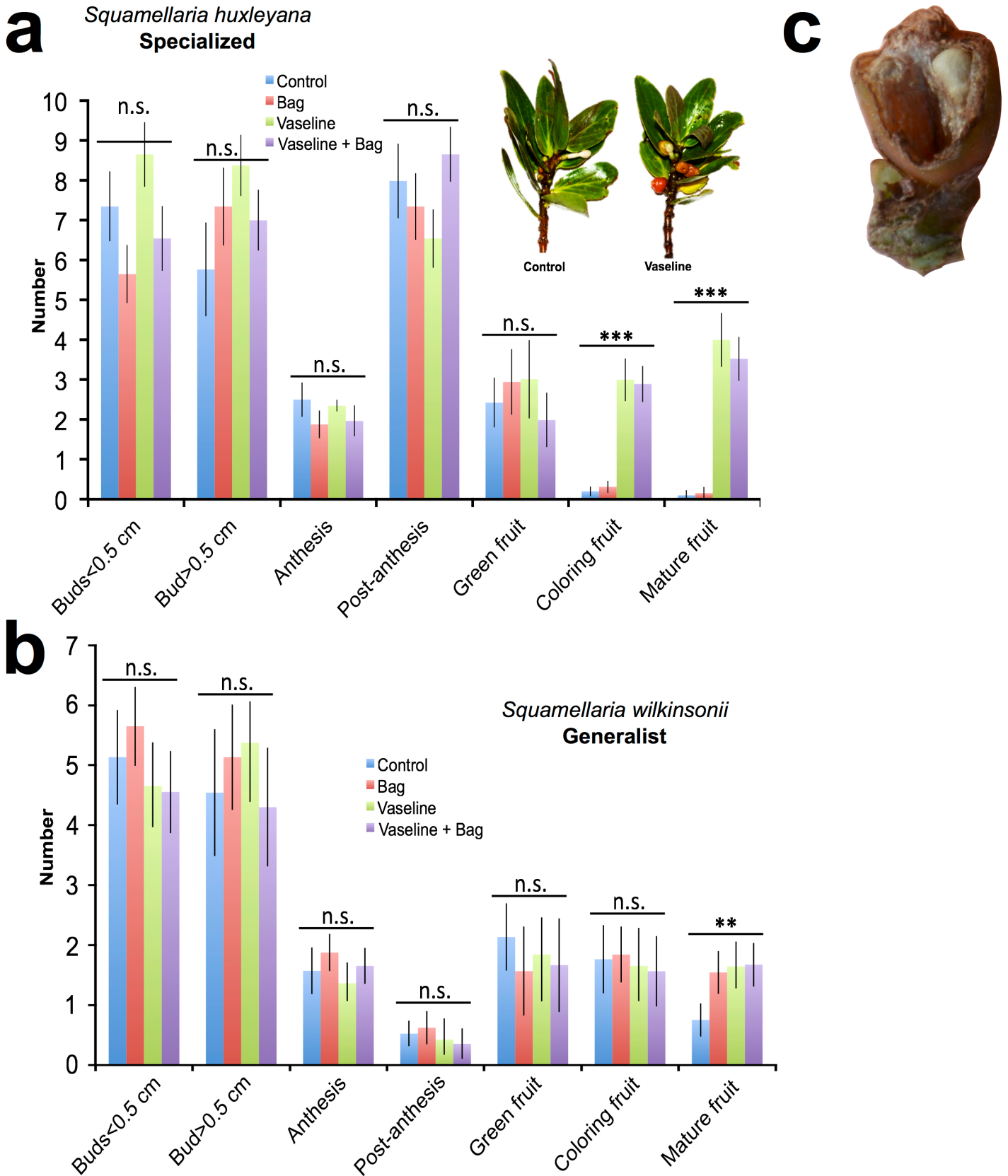


Figure S3. Exclusion experiments to test for bird or ant dispersal in a specialized ant plant (a, *Squamellaria huxleyana*) or a generalist ant plant (b, *Squamellaria wilkinsonii*). Bag-only treatments exclude birds, but not ants which continue to visit the fruits while vaseline and vaseline + bags exclude birds and ants. For each treatment, the sample size was N = 15 (3 branches x 5 plants). The results were normalized by the number of metamer (piece of shoot between two internodes), so that each branch can be compared. In control specialized ant-plants, mature (ripened) fruits are rare. In specialist (a), excluding birds does not affect removal rate, while excluding ants reveal a significant increase in mature fruits. This indicates that *Philidris nagasau* removes *Squamellaria* fruits before they are ripe. Observations showed that *P. nagasau* cut through the fruit flesh and removed seeds one-by-one. In contrast, excluding birds alone resulted in a significant increase in ripened fruits, suggesting that birds are the main dispersing force of facultative ant-plants. (c) *Squamellaria huxleyana* fruit where a single seed has been removed.

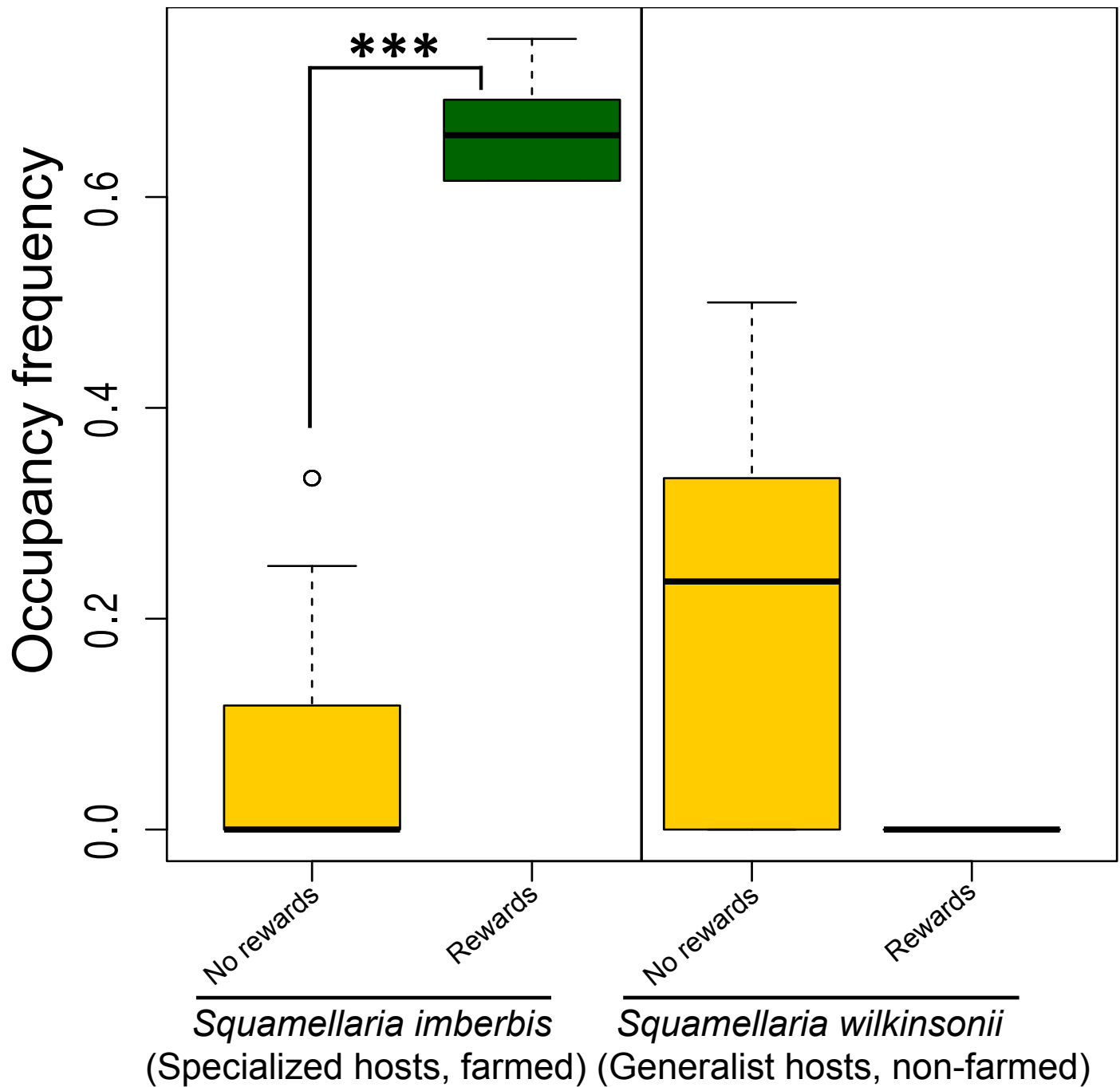


Figure S4. The distribution of specialized *Squamellaria* epiphytes on host trees is biased. Occupancy frequencies by *Squamellaria imberbis* or *S. wilkinsonii* in trees with or without rewards (including extrafloral nectar, floral nectar, and cavities suitable for ant nests). Stars refer to the significance of the Kolmogorov-Smirnov test (*Materials and Methods*).

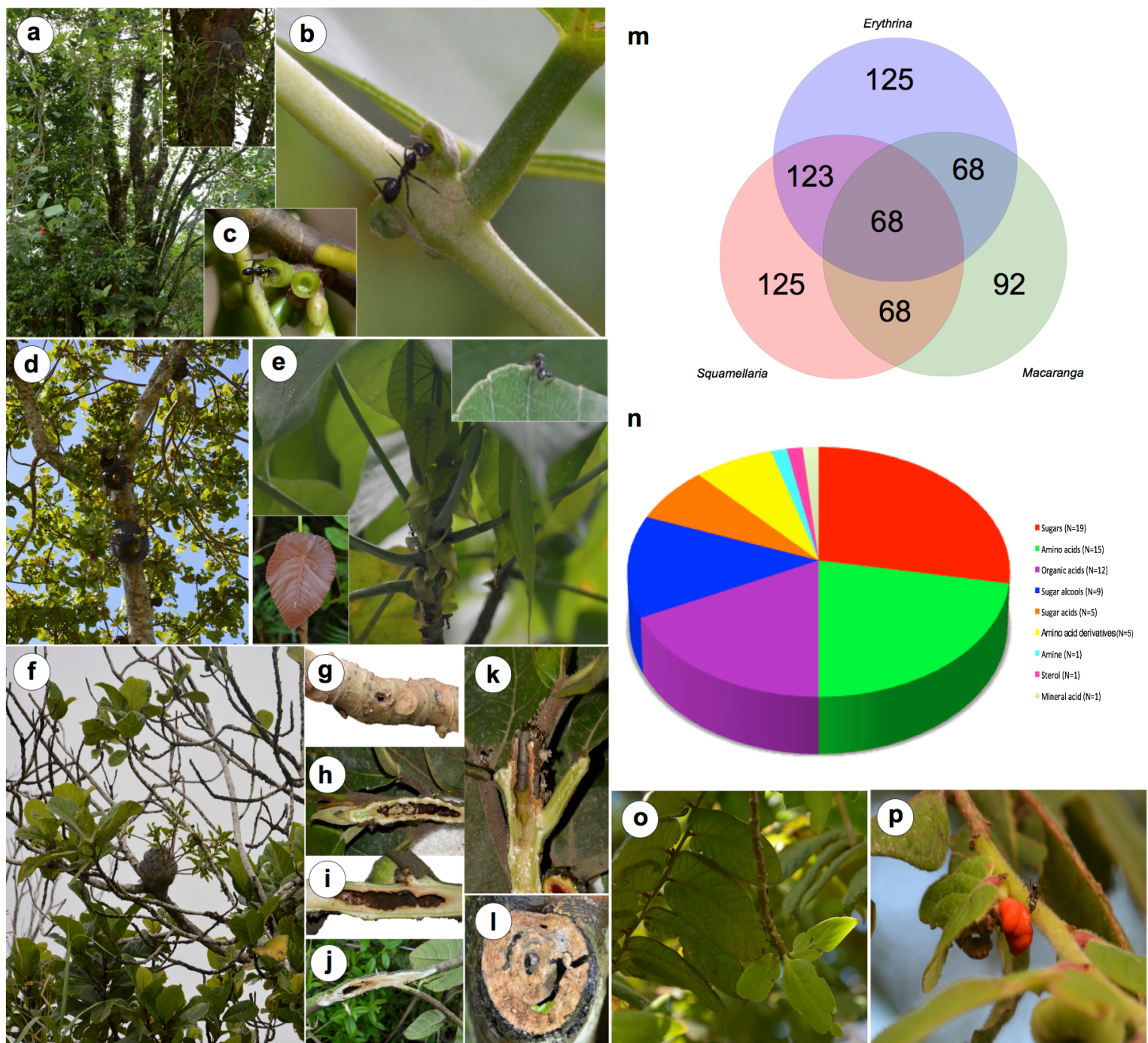


Figure S5. *Philidris nagasau* exploits facultative ant/plant mutualisms. (a-b) *Erythrina*. (a) *Squamellaria imberbis* growing on *Erythrina spec.* (b) *P. nagasau* feeds on *Erythrina* EFNs. (c) *P. nagasau* foraging on *Squamellaria imberbis* post-anthetic nectary. (d-e) *Macaranga*. (d) *Squamellaria major* on *Macaranga spec. 1*. (e) *P. nagasau* feeds on the sugary secretions of *Macaranga* stipules and EFNs. (f-l) *Ficus vitiense*. (f) *S. imberbis* on *Ficus vitiense*. (g) Entrance hole of *F. vitiense* domatium. (h) Nest of *Technomyrmex vitiensis* in a *F. vitiense* stem with no *Squamellaria*. (i) Nest of *Tetramorium insolens* in a *F. vitiense* stem with no *Squamellaria*. (j) *P. nagasau* transiently occupying a *Ficus vitiense* stem. (k) Wood borer eating the soft pith of *F. vitiense* stem, resulting in nest site formation. (l) *S. imberbis* seedlings planted and guarded by *P. nagasau* in a broken hollow branch of *F. vitiense*. (m) Venn diagram showing the overlap of *S. imberbis* post-anthetic rewards, *Erythrina* EFNs and *Macaranga spec. 1* EFNs metabolomes. (n) The 68 metabolites shared by *Squamellaria*, *Macaranga* and *Erythrina*. (o) *P. nagasau* moving from their *Squamellaria huxleyana* to another tree (*Aglaia spec.*) through the canopy. (p) The same *P. nagasau* feeding on *Aglaia* fruits.

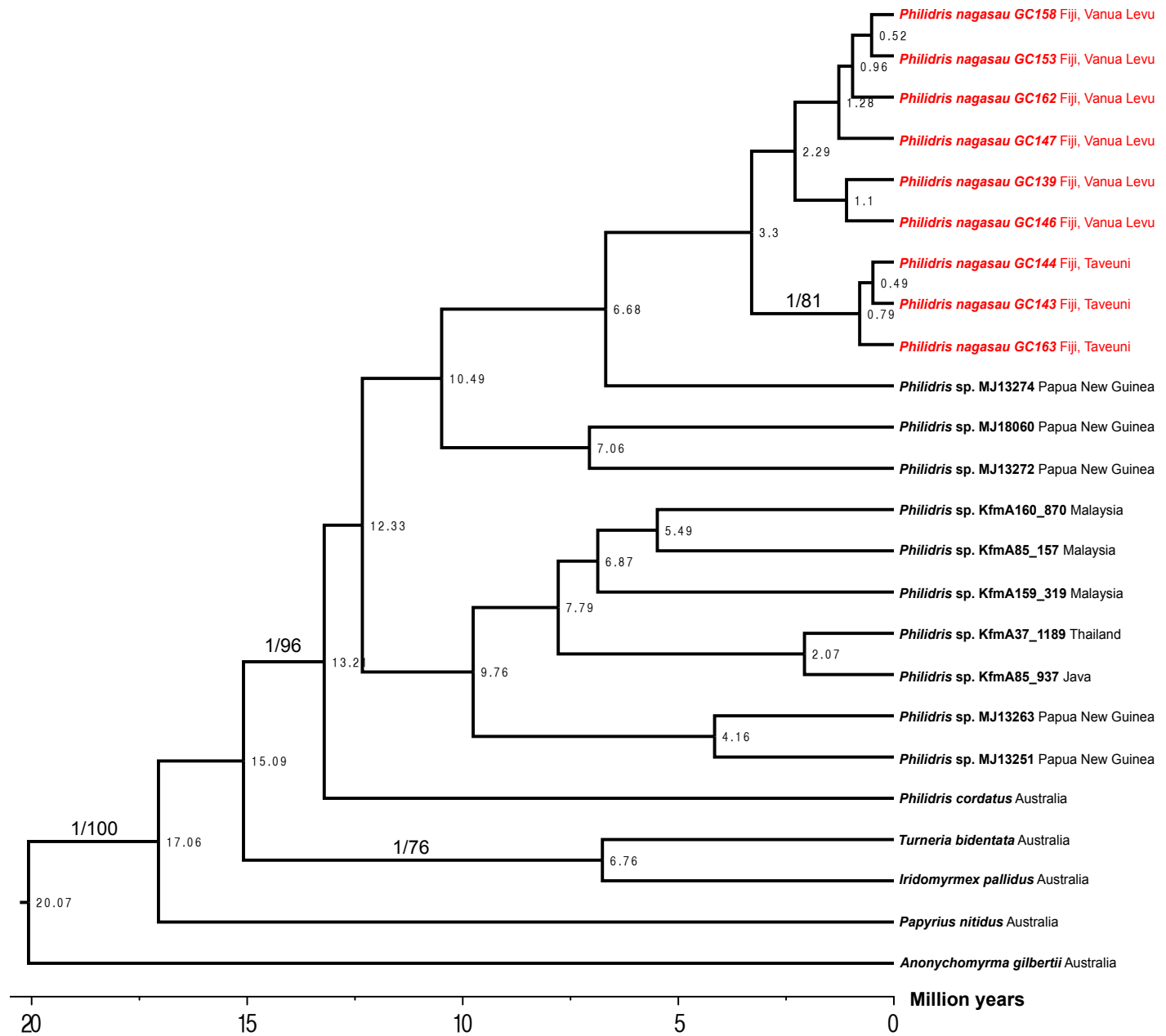


Figure S6. Dated phylogeny for *Philidris*. (*Philidris nagasau* (Fiji) is shown in red), with support values from likelihood (RAxML) and Bayesian analyses (BEAST) shown when greater than 75% (ML Bootstrap) or 0.95 (Bayesian probabilities).

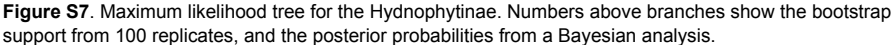


Figure S7. Maximum likelihood tree for the Hydnophytinae. Numbers above branches show the bootstrap support from 100 replicates, and the posterior probabilities from a Bayesian analysis.