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Morphological and behavioral evolutionary trajectories of ambrosia beetle-fungal mutualisms

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Abstract
Multiple bark and ambrosia beetle species, mainly from the subfamily Scolytinae, along with three and two species from the subfamily Platypodinae and the family Bostrichidae, respectively, were collected over one year (June 2023 – June 2024) in the campus of UNESP – Rio Claro, Brazil. Several symbiotic fungi including *Rafaellea* were obtained from these insects. Beetles were collected directly in tree galleries and by light trapping. The first method provided most of the Scolytinae and Bostrichidae species, and the second method provided the three recorded Platypodinae species and all fungal isolates (except for one isolate obtained in the first method). All fungal cultures were obtained from beetles belonging to the tribe Xyleborini (fungi-rearing group). Bark and ambrosia beetles were detected from ca. 10 species of trees, most from mango, jaca, and Ficus trees. *Euwallacea* spp. (Xyleborini) were found on all these trees. Although not an ambrosia beetle, *Hypothemenus* spp. seem to be a common group of bark beetles on campus, affecting dry twigs and dead branches of most trees, whereas Scolytinae were recorded near the bark. In contrast, species from Xyleborini and others borrow galleries in the xylem up to the core. Season differentiation of the groups and species seems uncertain but August-October and February-May seems to be the months with high numbers of species. This is the first report of these beetles for the UNESP campus, providing a first glimpse of the beetle and fungal associations in this location.

Introduction

Ambrosia and bark beetles establish mutualistic associations with fungi mainly by their interactions with the dead wood. These insects are weevils (Curculionidae) belonging to two subfamilies: Scolytinae and Platypodinae (Peris *et al*., 2021). Scolytinae spans more than 6000 species distributed worldwide, however, records do not cover uniformly all continents. Most studied are South Asia and North America. Still, South America remains relatively low covered by the recorded data, considering that potentially harmful fungi are transmitted by beetles in several important plants, such as coffee, *Persea americana*, *Mangifera indica*, *Artocarpus heterophyllus*, *Ficus enormis*, coffee, sugar cane, and others.

Fungal partners, having only passive transition, are expected to be effectively transmitted and protected against random feeders, and beetles farm and feed on them indirectly, consuming nutrients from the hardly decomposable cellulose. Collectively, fungi, wood, and beetles form a specialized and relatively isolated environment (Hulcr *et al*., 2017).

The mutualistic interaction with fungi shows a wide range of specialization virtually in all aspects: from biochemistry to behavior, for fungal transmission and farming. Fungi gain protection and dispersion, and beetles use fungi as an agent for the elaboration of wood into a more suitable form of food (Biedermann; Rohlfs, 2020). In many cases, bark and ambrosia beetles are significant pests of trees by infecting them with fungi (McPherson *et al*., 2012). In turn, these microorganisms contribute to the dead wood decomposition and, consequently, to the minerals, carbon turnover, and land fertilization. With high intraspecific plasticity and under variable ecological factors, one species can be found in both situations. Thus, beetle-fungi interactions can be potentially harmful or beneficial depending on the interaction with the environment and the genetic variability of the interactants (Six, 2020).

As species-rich subfamilies both Scolytinae and Platypodinae include a wide range of social behavior. In Scolytinae obligate eusociality is unknown; however, morphological polymorphism in colonies is present whether evolutionary trajectory to the facultative eusociality (Biedermann, 2020). This as well as other factors causing differences in sociality and development of the ecological forms, associated with wood and tree conditions, could be tested, which probably determines the longevity of the beetles and the size of their communities. Dead trees and wood degradation are somehow associated and generally considered as the following steps: weakness – leafless – dry – partially decomposed/separated branch. The presence of the different species of beetles seems to be specialized with the steps and character of the branch. Interactions of the multiple species of beetles and fungi potentially change the timing of each step of wood degradation and carbon turnover (Hýsek *et al*., 2021).

In our study, we are screening the biodiversity on three ecological levels: trees, beetles, and fungi, which are tightly associated with each other in the trophic network. This study has been conducted in a local plot, which includes elements of the natural biodiversity and introduced trees such as *M. indica* (mango) and *A. heterophyllus* (jackfruit tree). The aims of this proposal were: (i) assess and compare the biodiversity on these different levels, (ii) find potential traits for colonization of introduced plants over native related species, and (iii) assess the association with traits of the beetles with ecological specialization.

Methods

Beetle collection

Our study focused on bark and ambrosia beetle diversity found on the campus of São Paulo State University (UNESP) in Rio Claro, São Paulo, Brazil. Collections were carried out during the whole year from July of 2023 to June of 2024 in different sites. We used two collecting methods: (i) beetles collected from galleries in tree branches, mainly dry, broken, or leafless, by manual opening or using a saw and machete for separation of the big branches, with posterior elaboration by clipper for searching the beetles inside the galleries, and (ii) beetles caught in light traps. Collections from the trees were carried out by crashing dry branches and bark cleaning, then beetles were captured in their galleries.

Fungal isolation

For fungal isolation, the head and pronotum of several specimens of recently collected beetles were taken, crushed, and mixed with 100 mL of the phosphate buffer solution (PBS). Then, the suspension was diluted 10 and 100 times, and surface spread on Potato Dextrose Agar medium (PDA, Neogen® Culture Media, Lansing, MI, USA) supplemented with 100 mg L^{-1} of streptomycin and 250 mg L^{-1} of cycloheximide (Sigma-Aldrich, St. Louis, MO, USA). Plates were incubated at 25 ℃ for 10 to 15 days in darkness (Li *et al*., 2018).

All fungal colonies from the isolation plates were transferred for monosporic isolation on plates with PDA medium. mixed with 100 mL in PBS (O'Donnell *et al*., 2015), with original and diluted ten times concentrations. One of the observed single colonies from each type of fungi was transferred to a separate plate for DNA extraction.

DNA was extracted from the 16 fungal isolates obtained using laboratory protocols (Sampaio *et al.*, 2001, Almeida, 2005). We amplified the LSU (94 °C for 3 min, 30 cycles of 94 °C for 1 min, 50 °C for 45 s, and 72 °C for 1 min) and ß-tubulin genes (95 °C for 3 min, 35 cycles of 95 °C for 30 s, 57 °C for 45 s, and 72 °C for 40s, final extension at 72 °C for 8 min). Amplicons were sequenced by sanger sequencing.

Results and discussion

We obtained twenty Scolytinae beetle species (Tables 1 and 2) out of multiple tree species (Figure 1), including *M. indica* and *A. heterophyllus* with agricultural importance. Three *Euplatypus* specimens (Platypodinae) were found: one specimen in light trapping and two casually in the laboratory and dormitory, with no clear association with trees. In February 2024, in the *Psidium guajava* tree, we found several individuals of Scolytinae (non-Xyleborini), probably belonging in the genus *Dendrocranulus*, like what was found near to *A. heterophyllus* tree. Bostrichidae (not the focus of the present study), *Micrapate bicostula*, with numerous specimens in multiple twigs from the same broken branch, and one specimen of *M. horni* was found in another unidentified tree. In both cases, individuals were burring twigs, occupying their galleries most of their wideness (Figure 2). According to the season of all records and continuous checking of the plants, *M. bicostula* seems active during May and June/July (Liu *et al*., 2024).

Most of the specimens (484 out of 532) and species (19 out of 24) were collected directly from trees. From the dry small branches, we obtained the highest number of beetles species and specimens, mostly tiny specimens in the genus *Hypothenemus* (Figure 1), which do not establish fungal gardens (i.e., associations with fungi). *Amasa truncata* (one specimen) and *Xyleborus ferrugineus* (multiple specimens), belonging to the Xyleborini, were found only in light trapping. *Artocarpus heterophyllus* were inspected in several sites of the campus, during the whole year, mostly intensively from the end of January to the end of May 2024. *Hypothenemus* sp. and *Xyleborus* sp. were found on the same dry, relatively small branch (less than 1 cm wide) with leaves, showing spatial separation: *Hypothenemus* sp. was under the bark (Figure 2), when *Xyleborus* sp. was borrowing galleries deeply in the wood and core of the branch. The internal surface of the galleries was covered by white bloom, probably their fungal gardens. Fungal isolation allowed the recovery of fungi only from *Xyleborus* sp. *Hypothenemus* sp. was mainly found on trees in relatively bad conditions, in the dry small branches, where tend to form masses up to ca. 20 specimens, however, mainly found in small (1-3) numbers in each branch or twig. We found one specimen in the external part of the wood, in the dry fruit branch of the *A. heterophyllus*. There are no records of *Hypothenemus* from wet branches or their parts. *Xyleborus* sp. were found on the trees in all kinds of conditions. Overall, *Xyleborus* sp. was found in the same branch as *Hypothenemus*. Instead of multiple cases coexisting in one branch due to the close disposition of galleries of *Hypothenemus* and Xyleborini species, no direct interactions between them were observed. The species found didn’t show a deep modification of the elytral declivity, however, the species, that construct the shallow tunnels under the bark (bark beetles) showed completely smooth elytral curve, while the species from the deep part of the branch, including the core showed some level of modification.

Most of the species, collected from trees, were recorded from *A. heterophyllus*, *M. indica*, and *F. enormis* (Table 3). A total of 13 species of Scolytinae were recorded in *A. heterophyllus* including 7 from the Xyleborini. *Euwallacea* sp. (Figure 1) was found in two trees with apparently different conditions: (i) good, with few dry and leafless branches, and mediate, middle-size branch, in part with diameter of ca. 1,5 cm, in galleries, surrounding the core, in (ii) the leafless, but not completely dry branches in its internal part. In the young/fruit branch, not completely dry, and softer than old branches, *Euwallacea* sp. was found inside the core. *Xyleborus affinis* was found only once in wood in the same piece of small branch along with cf. *Xyloterinus* sp. and *Coccotrypes* sp. (this one found only once). *Xyleborinus* sp. was found only in *A. heterophyllus*, in the biggest branch (d ca 5 cm), dry and completely leafless, in the basal part, deep inside, but not reaching the core. The gallery system, probably old due to black internal surface, the same found in other cases with empty galleries. *Hypothenemus* sp. found on the branches infested by Xyleborini species wasn’t found in agglomerations with more than ca. 5 exemplars, contrary to the uninfected branches.

One specimen of *Euwallacea* sp., found in *F. enormis*, was detected to choose a piece of wood from the same tree where it was found, along with *A. heterophyllus* and *M. indica* woods, when placed in the same plate in the laboratory. However, on *F. enormis*, *Euwallacea* sp. was found less abundantly, represented by single specimens, than on the *A. heterophyllus*, which indicates the individual adaptation of the beetle to this tree.

On 12 June 24, in one previously separated dry, but with leaves, branch of *F. enormis* tree, in good condition, we found two specimens with barbed declivity by direct observation, belonging to the Xyleborini (probably *Debus*, single record) in the two separated shallow galleries: one gallery was empty, probably indicating saucerful attempt to build gallery system. One specimen was captured in the moment of the gallery start, borrowing the bark in the basal part of the branch, with a diameter around 5 cm, and white substance, appeared during separation by sawing the piece of the branch. The beetle was manually collected for fungal isolation (by splinting the head and pronotum). A few days before, on 3 June 24, two individuals of *Euwallacea* sp. were found in shallow galleries in a smaller leafless branch on the tree, with an intermediate condition, detected by multiple dry branches. However, both specimens died during the next four days in Petri dishes containing original pieces of wood and moistened paper (general condition for keeping beetles).

Apart from *Xyleborus volvulus* (?), two *Euwallacea* spp. and two *Hypothenemus* spp. recorded on *F. enormis* and *A. heterophyllus*, respectively, we found two additional specimens: *Premnobius cavipennis* (one exemplar, Figure 1) and an unidentified beetle from *F. enormis* (another dead specimen of *P. cavipennis* were detected on the dry trunk). This indicates that the colonization of the introduced *A. heterophyllus* was caused not only by the mostly close-to-it tree in Brazil from Moraceae. Black-colored *Euwallacea* sp. 1 was found as a colony comprising around 20 individuals in September 2023 on a *M. indica* tree. Fungal isolation from these specimens failed.

Beetles showed more specialization on the parts of the tree, such as bark, xylem, and core, than on the trees' species, represented by the presence of the same beetle species on the different trees' species, but not under the bark and in xylem. Small and big species of *Hypothenemus* sp. didn't show specialization in the trees or their parts. All their possible species could appear in the same branch. Species with more developed declivity seem to prefer the core of the branch and the xylem in the case of the big branches.

Xylosandrus sp. found in two trees didn't show any difference in occurrence with *Xyloterinus* sp., appearing close to their galleries in intermediate size of the branch. *Xylosandrus* sp. did not arrive to the core, but constructed galleries completely in wood, instead *Hypothenemus* sp. built "channels" between wood and bark in mature, dry branches. *Xylosandrus* sp. could be found in dry twigs on the terminal parts of the branches in short galleries inside the xylem, with no touching the core. This distribution suggests intolerance of the *Hypothenemus* sp. to the trees' fluids.

Among the detected species from different tribes, which rear fungal gardens, only Xyleborini had modifications on the elytral declivity. Instead of the presence of *Xyloterinus* sp. and *Xyleborus* sp. in the same branch, with no visible specialization on its components, *Xyloterinus* sp. seems to be ecologically separated from most of Xyloterini by timing (February-March vs May-June, Table 2) and by presence inside small, up to twig branches. Thus, the presence of the elytral declivity could be one of the adaptations to the construction of the deep galleries in big branches. Any species with modified elytral declivity was found under the bark, but *X. volvulus* and *Xyloterinus* sp. were found inside the small branch and have an elytral shape with no modification (Figure 1). However, *X. volvulus* in all cases was found in association by the gallery system along with *Euwallacea* sp.

Euwallacea sp. and *Xyloterinus* sp. were observed at the same point with no clear spatial divergence in the deepness of the galleries, inside the same mediate branch and inside the same gallery system (Figure 2), according to the multiple similar species in the genus (Wang 2022). However, *Euwallacea* sp. 1 (black) seemed to be more specialized on the core and surrounding parts, and less sensitive to the freshness of the branch, while the smaller species are found more frequently on the smaller branches (d. ca. 2 cm, Figure 2), builds more vertical and less branched galleries from the surface galleries, and seems to be more frequent in *F. enormis*. In *M. indica*, in middle August 2023, we found a colony (more than 20 specimens) of *Euwallacea* sp. 1 (black), and in mid-June found two specimens of *Euwallacea* sp. 2 (brown) in the same tree, in an old gallery system. Presence of *Euwallacea* sp. 2 and the absence of *Euwallacea* sp. 1 open the possibility of the use of the galleries of the *Euwallacea* sp. 1.

In *M. indica*, beetles were obtained from August to mid-December 2023 with moderate rain, and *A. heterophyllus* hosts beetles mainly from February to April 2024 with higher rainfall. On branches with fruits, we did not find any Xyleborini species, but on branches with no fruits, we detected *Coccotrypes* sp., apparently in wood, and on the damaged part of the branches with fruits. Probably, these beetles affect the mature branches or damaged branches and could cause immaturity of the fruit. *Coccotrypes* sp. due to its long life (more than 100 days) in Petri dishes with water and dry branch fragments, seems not harmful, but specialized on the dead branches.

A much narrower species abundance of the beetles in light trap than in the collections from the trees shows selectivity of the light trapping. The abundance of *Hypothenemus* in trees (in May 2024 it could be found on most of the dry twigs of *Artocarpus* and *Ficus*) and its absence in light traps suggest its diurnal activity or lower capacity of flight than Xyleborini species.

According to our observations, the infestation of the branch by Xyleborini, mainly *Euwallacea* sp., starts with a single specimen, from the not completely leafless branch with a significant amount of the liquids in wood, including white gluing substance. Presence of the liquids, instead of representing part of the natural defense of the plant, probably is required for fungal farming.

Hypothemenus, not farming fungi, occurs in dry branches. Parasitoid in the family Bethylidae was found to emerge from only one *Hypothenemus* specimen in *A. heterophyllus*.

All fungi obtained in culture were isolated from specimens belonging to the Scolytinae and captured in light traps. Preliminary identification revealed these specimens to belong to *Xyleborus*. In all cases, heads provided more fungal colonies than pronotum, due to the location of the mycangium on the head, while the pronotum carried only casual conidia from fungal contaminants (Mayers *et al.*, 2022). This is due to the location of the mycangium in *Xyleborus*, in the buccal apparatus, as preoral pockets (Mayers *et al*., 2022). The total number of fungal colonies obtained was not associated with any species of beetles (Table 1). A total of 16 fungal isolates were obtained from all isolations (Table 1), representing between 9 to 12 different morphotypes, likely some are *Rafaellea*

(Figure 3). In one of the beetles, we isolated three morphotypes of mutualistic fungi.

Fungi reared from beetles and isolated on the plates with antifungal and antibacterial additions showed a relatively low abundance of symbionts, and from some beetles' specimens collected by the light trapping the rearing produced empty plates. Growth of the symbiotically specific fungi was observed only from *Xyleborus ferrugineus* and *X. affinis* Rearing of the fungi, collected directly from the wood, in all cases provided growth of different saprotrophic fungi of the dead or damaged trees, apart from the case of the *X. affinis*, found on the *A. heterophyllus*. Symbiotically specific fungi from log-collected beetles were observed only in the case of a single specimen of *X. affinis*, collected from a small branch of the *A. heterophyllus* (DNA extraction ongoing).

The species composition seems seasonally variable, dependent by the rainfall level. During the dry season, flights are lower probably due to the preferences of the wet wood by beetles for the foundation of new colonies, The foundation of the new colonies occurs mainly during springtime, which is confirmed by the detection of the colony of *Euwallacea* sp. in September (*M. indica* tree), which may be observed mainly as the presence of single specimens or their small clusters.

Conclusions and perspectives

Beetles were found in all seasons and in most of the trees on the campus of Rio Claro, mostly on damaged branches of the inspected trees. Fungi in the mycangium of beetles seem to be present very shortly after their infection of wood. During gallery excavation, fungi become mixed with fungal communities of the wood. *Hypothenemus* sp. seems to be the most common species of bark beetles, which didn't show any seasonality. Xyleborini species seem to be more active during fall and spring. Several times multiple species of bark beetles were found in multiple galleries on one branch, when the other branches nearly (observation of *Euwallacea* on 21 of May), apparently with the same condition remain untouched by beetles. This evidence suggests an opportunity to study the fungal community of the dead wood of multiple and economically important trees, in proposing to figure out how it could be affected by the beetle's presence, apart from their fungal symbionts.

Interactions of the different species of beetles in the same branch provide the additional line of research involving the competition of the native and alien species, related to pest detection in cases of the introduced species of beetles (ex. *Euwallacea*) and trees (ex. Mango, Jackfruit) and wood degradation. These topics will be included in the paper in prep, summarizing currently obtained data: (i) interactions and specialization of the beetles on the trees and their parts, (ii) fungal specialization concerning the beetles, with the perspectives in the study of the fungal communities vs beetles' communities in native and changed or damaged environments. Similar topics were mentioned during the Brazilian Congress of Mycology (ex. pests of the avocado tree from Scolytinae).

Publications and other activities

- Rudoy, A; Rodrigues, A. Perspective study on the differences in the fungal community in connection to the Scolytinae beetles' preferences of wood condition of jackfruit tree and their relations. **Paper in prep.**

- Attendance to the 10th Brazilian Congress of Mycology in Belo Horizonte-MG, from 19 to 23 February 2023.
- Outreach: the postdoc carried out in extension activities: (i) explaining about beetles and fungi to elementary school students visiting the laboratory, and (ii) visits of new students to the campus, guided by the author showed collected beetles in original pieces of wood with the galleries and fungal activity.

Supervisor comments

Dr. Andrey Rudoy developed work in my lab from June 1, 2023, to June 30, 2024. He carried out a natural history study on beetles found infecting trees in the campus of UNESP – Rio Claro. His continuous and meticulous observations allowed him to record, for the first time, the beetle populations on campus. As the product of this one-year fellowship, Dr. Rudoy is writing a manuscript that summarizes the discovery report here. He also attended one congress and joined extension activities.

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Table 1. Fungal isolation from beetles by light trapping (gallery indicated specifically).

Beetle_species	**Body part**	**Locality**	**Collecting_date**	**Isolate_ID**	**CFUs**
Xyleborus affinis	Head	UNESP Campus, CEIS	03-Oct-23	ARU001	4
Xyleborus affinis	Pronotum	UNESP Campus, CIES	03-Oct-23	ARU002	1
Xyleborus affinis	Head	UNESP Campus, CIES	03-Oct-23	ARU003	1
Xyleborus affinis	Pronotum	UNESP Campus, CIES	03-Oct-23	ARU003	0
Xyleborus affinis	Head	Rio Claro, SP (Horto edge)	15-Oct-23	ARU004	120
Xyleborus affinis	Pronotum	Rio Claro, SP (Horto edge)	15-Oct-23	ARU005	4
Xyleborus affinis	Head	UNESP Campus, garage	22-Oct-23	ARU006	1(4?)
Xyleborus affinis	Head	UNESP Campus, garage	22-Oct-23	ARU007	3
Xyleborus ferrugineus	Head	UNESP Campus, garage	4-Nov.-23	ARU008	1
Xyleborus affinis	Head	UNESP Campus, garage	4-Nov.-23	ARU009	5
Xyleborus affinis	Head	UNESP Campus, garage	4-Nov.-23	ARU010	4
Xyleborus affinis	Head	UNESP Campus, garage	5-Nov.-24	ARU011	2
Xyleborus affinis	Head	UNESP Campus, CIES	10-Nov-23	ARU12	3
Xyleborus ferrugineus	Head	UNESP Campus, CIES	10-Nov-23	ARU13	8
Xyleborus affinis	Head	UNESP Campus, CIES	10-Nov-23	ARU14	1
Xyleborus affinis	Head	UNESP Campus, CEIS/horto	22-Nov-23	ARU15	8
Xyleborus affinis	Head	UNESP Campus, CEIS (Jack tree)	22-Nov-23	ARU16	1
Xyleborus affinis	Head	UNESP Campus, CEIS (Jack tree)	2-Feb-24	ARU17	1

Table 2. Beetles collected manually

Date	**Plant/method collection**	**Beetle sp/group**	**Number of collected specimens**	**Location**	**Notes**
Date	Plant/method collection	Beetle sp/group	Number of collected specimens	Location	Notes
2023.7.11	*Psidium guajava*	*Micrapate bicostula*,	20	ceis	multiple points in

		bostrichidae			same bush
2023.7.11	*Psidium guajava*	intermediate scolitynae	5	ceis	
2023.7.25	*Psidium guajava*	*Micrapate bicostula*, Bostrichidae	2	ceis	
2023.7.26	*Syzygium jambos*	*Hypothenemus*	4	footboll field	
2023.7.28	*Magnifera indica*	*Hypothenemus*	2	ceis	
2023.7.28	*Percea americana*	*Anisandrus* sp (?)	2	botany	
2023.7.30	*Syzygium jambos*	*Hypothenemus*	2	botany	
2023.8.1	*Syzygium jambos*	*Hypothenemus*	1	footboll field	
2023.8.2	*Syzygium jambos*	*Hypothenemus*	1	footboll field	
2023.8.4	*Ficus enormis*	*Hypothenemus*	3	garage	
2023.8.8	*Persea americana*	*Hypothenemus*	2	garage	
2023.8.11	*Syzygium jambos*	*Hypothenemus*	2	garage	
2023.8.17	*Magnifera indica*	*Euwallacea*, males, females	10, 30	ceis	colony
2023.8.18	*Magnifera indica*	*Hypothenemus*	1	ceis	
2023.8.23	*Magnifera indica*	*Hypothenemus*	2	ceis	
2023.8.25	*Magnifera indica*	*Hypothenemus*	2	ceis	
2023.8.29	*Magnifera indica*	*Hypothenemus*	1	ceis	
2023.8.31	*Magnifera indica*	*Hypothenemus*	3	ceis	
2023.9.1	*Magnifera indica*	*Hypothenemus*	1	ceis	
2023.9.2	*Magnifera indica*	*Hypothenemus*	3	ceis	
2023.9.6	*Magnifera indica*	*Hypothenemus*	1	ceis	

2023.9.6	*Magnifera indica*	small, elitral picks	2	ceis	
2023.9.13	*Magnifera indica*	intermediate	2	football field	
2023.9.16	*Magnifera indica*	big, intermediate	20	lago azul	colony
2023.9.18	fucus enormis	*Hypothenemus*	1	garage	
2023.9.20-22	*Enterolobium contortisiliquum*	intermediate	15	geography	colony
2023.9.23	?	*Hypothenemus*	2	library	
2023.10.03	light trap	*Xyleborus affinis, X. ferrugiensis*	8, 1	ceis	
2023.10.15	light trap	*Xyleborus*	3	ceis, deep bush	
2023.10.16	Ficus enormis	*Hypothenemus*	1	ceis	
2023.10.20	nd	*Hypothenemus*	10	geography/botany	
2023.10.22	light trap	*Xyleborus*	13	garage	
2023.11.4	light trap	*Xyleborus*	2	garage	
2023.11.5	light trap	*Xyleborus*	3	garage	
2023.11.10	light trap	*Xyleborus*	17	ceis	
2023.11.20	light trap	*Xyleborus*	13	small gate	
2023.11.30	light trap	*Xyleborus*	9	near horto	
2024.1.10	*Artocarpus heterophyllus*, small branch (twig)	*Hypothenemus*	10	ceis	
2024.1.25	*Artocarpus heterophyllus*, broken branch	*Xyleborus* sp (*Xyloterinus*?)	1	ceis	
2024.2.2	*Artocarpus*	*Xyleborus*	2	ceis	

	heterophyllus, broken branch				
2024.2.2	*Artocarpus heterophyllus*, small branch	Small, barbed declivity	1	ceis	
2024.2.2	*Artocarpus heterophyllus*, broken branch	*Xyloterinus* (?), brown	1	ceis	
2024.2.2	*Artocarpus heterophyllus*, small branch	*Hypothenemus*	1	ceis	
2024.2.2	*Artocarpus heterophyllus*, fruit branch	brown intermadiate (*Coccotrypes*?)	15	botany	colony
2024.2.3	*Artocarpus heterophyllus*, fruit branch	*Hypothenemus*	1	ceis	
2024.2.5	*Artocarpus heterophyllus*, fruit branch	*Hypothenemus*	10	ceis	colony (?)
2024.2.9	*Artocarpus heterophyllus*, broken branch	*Hypothenemus*	6	ceis	
2024.2.9	*Artocarpus heterophyllus*, broken branch	*Xylebobus* sp	4	ceis	
2024.2.9	*Artocarpus heterophyllus*, brocked branch	*Dendrocranulus*?, black, middle	1	ceis	
2024.2.12	*Artocarpus heterophyllus*, small branch	*Euwallacea* sp, big	1	ceis	

2024.2.15	*Artocarpus heterophyllus*, fruit branch	(*Coccotrypes*?) brown	3	botany	
2024.2.16	*Artocarpus heterophyllus*, small branch (twig)	*Hypothenemus*	2	ceis	
2024.2.26	*Artocarpus heterophyllus*, small branch (twig)	Hypothenemus	5	ceis	
2024.2.26	*Artocarpus heterophyllus*, fruit branch	Brown middle (*Coccotrypes*?)	5	ceis	
2024.2.29	*Artocarpus heterophyllus*, small branch (twig)	*Hypothenemus*	2	ceis	
2024.3.01	*Artocarpus heterophyllus*, small branch (twig, semi dead)	*Xylebobus* sp	2	central	
2024.3.04	*Artocarpus heterophyllus*, small branch (twig, dead)	*Hypothenemus*	7	central	
2024.3.04	*Artocarpus heterophyllus*, big branch (semi dead)	*Euwallacea* sp	1	ceis	
2024.3.05	*Artocarpus heterophyllus*, intermediate branch (semi dead)	*Xyleborus* sp	1	ceis	
2024.3.05	*Artocarpus heterophyllus*,	*Xylosandrus*	5	central	

	intermediate branch (semi dead)				
2024.3.05	*Artocarpus heterophyllus*, intermediate branch (semi dead)	*Hypothenemus*	10	central	colony
2024.3.06	*Artocarpus heterophyllus*, big branch (semi dead)	intermediate, ?	5	ceis	colony (?)
2024.3.06	*Artocarpus heterophyllus*, big branch (semi dead)	*Xylosandrus*	2	central	
2024.3.08	? Small dead branch	intermediate, black ?	1	small gate	
2024.3.08	? Small dead branch	intermediate with declivity, ?	3	small gate	
2024.3.11	*Artocarpus heterophyllus*, small branch (twig, dead)	*Hypothenemus*	2		
2024.3.12	light trap	*Xyleborus*	2	horto	
2024.3.13	*Artocarpus heterophyllus,* small branch		2		
2024.3.14	*Bauhinia* sp	*Hypothenemus*	10	garage	colony
2024.3.18	*Artocarpus heterophyllus* , fruit branch	*Hypothenemus*	5	ceis	
2024.3.19	?	*Hypothenemus*	1	small gate, outside	
2024.3.20	*Artocarpus heterophyllus* , fruit branch	*Hypothenemus* 1 small, 3 intermediates	3	ceis	
2024.3.2	*Artocarpus*	*Xyleborus*, eggs	2	ceis	

0	*heterophyllus* , fruit branch				
2024.3.21	*Artocarpus heterophyllus* , twig	*Xyleborus*	1	football	
2024.3.21	*Artocarpus heterophyllus* , twig	*Hypothenemus*	1	ceis	
2024.3.24	under light	*Xyleborus affinis*	1	ceis	
2024.3.25	*Mangifera indica*	*Hypothenemus*	1	ceis	
2024.3.27	*Artocarpus heterophyllus*	*Hypothenemus*	1	ceis	
2024.3.28	*Artocarpus heterophyllus*	*Hypothenemus*	1	ceis	
2024.3.28	*Ficus enormis*	*Hypothenemus*	1	garage	
2024.4.5	light trap	Xyleborus (2 sp), *Arixyleborus grandis* /Anisandrus (?)	7	ceis	
2024.4.8	*Artocarpus heterophyllus*	*Hypothenemus*	5	ceis	
2024.4.8	Ficus enormis	*Hypothenemus*	1	garage	
2024.4.9	?	Scolytinae (3 spp: *Xyloterinus*, *Xylosandrus*, (*Premnophilus*) sp	30	small gate	colony, pupas, larvas
2024.4.9	*Artocarpus heterophyllus*	*Hypothenemus*	1	ceis	
2024.4.10	*Artocarpus heterophyllus*	*Hypothenemus*	1	ceis	
2024.4.1	*Artocarpus*	*Xylosandrus*	2	ceis	

1	*heterophyllus*				
2024.4.12	*Artocarpus heterophyllus*	*Hypothenemus*	1	ceis	
2024.4.14	light trap	Xyleborus, 2 sp	2	ceis	
2024.4.15	*Artocarpus heterophyllus*	Xyloterinus (?)	1	ceis	
2024.4.16	*Artocarpus heterophyllus*	*Xyloterinus* (?)	2	ceis	
2024.4.16	*Bauhinia* sp	*Hypothenemus*	3	geography	
2024.4.18	*Artocarpus heterophyllus*	*Hypothenemus*	5	ceis	
2024.4.22	*Artocarpus heterophyllus*	*Hypothenemus*	1	ceis	
2024.4.22	*Ficus enormis*	*Hypothenemus* (big)	1	garage	
2024.4.24	*Artocarpus heterophyllus*	*Hypothenemus*	3	ceis	
2024.4.25	*Artocarpus heterophyllus*	Hypothenemus	3	ceis	
2024.4.25	*Artocarpus heterophyllus* (mediate wet branch)	*Euwallacea* sp (?) (big, black)	2	ceis	
2024.4.26	*Artocarpus heterophyllus* (mediate wet branch)	*Xyleborus* sp	1	ceis	
2024.4.26	*Artocarpus heterophyllus* (basal part of big dry branch)	*Xyloborinus*	5	ceis	
2024.4.2	*Artocarpus*	*Hypothenemus*	5	main	

6	*heterophyllus* (fruit branch)			gate	
2024.4.30	*Artocarpus heterophyllus* (basal part of big dry branch)	*Euwallacea* (brown), *Xyleborus* (no picks) (?)	2	central	
2024.5.02	*Artocarpus heterophyllus* (basal part of big dry branch)	*Xyleborus* sp	1	ceis	
2024.5.06	*Ficus enormis* (big branch)	*Euwallacea* sp (big, black)	3	garage	
2024.5.06	*Artocarpus heterophyllus*	*Hypothenemus*	1	ceis	
2024.5.07	*Artocarpus heterophyllus* (fruit branch with unmature fruit)	*Coccotrypes* (?) brown	3	central	
2024.5.09	*Ficus enormis* (small branch)	*Hypothenemus*	1	garage	
2024.5.10	? Twig	*Micrapate horni*, Bostrichidae	1	ceis, parking	
2024.5.13	*Morus alba*	*Hypothenemus* (big)	1	botany	
2024.5.17	?	*Xyleborus*	1	geography	
2024.5.17	?	*Hypothenemus*	1	geography	
2024.5.20	*Psidium guajava*	*Micrapate bicostula*, Bostrichidae	1	ceis	
2024.5.21	?	*Hypothenemus*, big	3	Main Gate	

2024.5.21	*Artocarpus heterophyllus*, fruit branch decomposed	*Euwallacea*, *Hypothenemus* (small)	3	Central	*Euwallacea* inside the core
2024.5.22	*Artocarpus heterophyllus*, fruit/fresh branch	*Coccotrypes* (?) brown	2	botany	
2024.5.23	?	*Hypothenemus*, small	1	botany	
2024.5.23	?	*Hypothenemus*, big	1	garage	
2024.5.23	*Ficus enormis* (small branch)	*Hypothenemus*, big	1	ceis, parking	
2024.5.23	?, dry single branch	*Hypothenemus*, small	2	small gate	
2024.5.25	*Ficus enormis* (big branch)	*Euwallacea* sp (big, black)	1	ecology	
2024.5.27	*Artocarpus heterophyllus*, big branch	*Euwallacea* 2 species (brown, black, 1 male)	6	ceis	
2024.5.28	*Artocarpus heterophyllus* (fruit branch)	*Hypothenemus*, small	4	main gate	
2024.5.29	*Ficus enormis*, big branch	*Premnobius cavipennis*	1	ecology	
2024.5.29	*Artocarpus heterophyllus*, semi dry big branch	*Euwallacea*, 2 species	4	ceis	
2024.6.3	*Artocarpus heterophyllus*, semi dry big branch with juice, 2 shallow galleries	*Euwallacea*	2	ceis	

2024.6.4	?, small leaves	*Hypothenemus*, big	2	library	
2024.6.6	*Ficus enormis*, big, separated dry branch	*Coccotrypes* (?) brown	1	ecology	
2024.6.7	*Ficus enormis* (dry twigs)	*Hypothenemus*, big	5	garage	
2024.6.8	*Artocarpus heterophyllus*, big branch	*Euwallacea* (dead)	5	ceis	
2024.6.8	*Artocarpus heterophyllus*, big branch	*Xyleborus* (dead)	2	ceis	
2024.6.8	*Artocarpus heterophyllus*, big branch	Hypothenemus, big, small	4	ceis	
2024.6.9	Cacao falso	*Euwallacea* (dead)	1	ecology	
2024.6.12	*Artocarpus heterophyllus*, big separated branch, initial gallery	black. Declivity	2	ecology	
2024.6.14	*Artocarpus heterophyllus*, dry intermediate branch,	*Hypothenemus*, big, small	2	central	
2024.6.14	*Artocarpus heterophyllus*, dry intermediate branch,	*Euwallacea*, black, dead	2	central	
2024.6.14	*Ficus enormis*, big, separated dry branch	*Euwallacea*, brown	2	ecology	
2024.6.14	*Ficus enormis*, big, separated	*Xyleborus*, smooth, dead	1	ecology	

	dry branch				
2024.6.14	*Ficus enormis* (dry twigs)	*Hypothenemus*, big	1	garage	
2024.6.20	*Ficus enormis*, big, separated dry branch	*Euwallacea*	1	ecology	
2024.6.20	*Magnifera indica*	*Euwallacea*, brown	2	football	old gallery system
2024.6.23	*Ficus enormis*, big branch	*Euwallacea*, brown	1	ecology	
2024.6.27	*Ficus enormis*, deformed dry branch	*Euwallacea*, *Xyleborus*, dead	2	ecology	with ants
2024.6.27	*Cacao falso*	*Hypothenemus*, big	1	ecology	
2024.6.28	*Magnifera indica*	*Euwallacea*, black	1	football	old gallery system

Table 3. Beetles found on the jackfruit tree.

Species name	Approximate body length	notable traits
Hypothenemus sp1 *	1 mm long, small	
Hypothenemus sp2 *	1,5 mm long, big	
Coccotrypes (?)	1.5 mm	barbed declivity
Dendrocranulus sp. (?)	1.7 mm	black, elongated
Dendroterus sp1 (?)	1.5 mm	found on the fruit branch, brown
Dendroterus sp2 (?)	1.8 mm	found on the fruit branch, brown
Xyloterinus (?)	2.5 mm	brown, smooth elytra
Xyleborus volvulus (?) *	1.5 mm	smooth elytra
Xyleborus affinis	1.7 mm	small elytral barbs
Xylosandrus compactus (?)	1.3 mm	compact body, lateral elytral carinae
Xyleborinus sp	3 mm	elongated, barbed elytral declivity
Euwallacea sp1 *	2.5 mm	brown, flattened elytral pick

Euwallacea sp2 *	2.5 mm	black, round elytral pick

* Found also on *Ficus enormis*

Figure 1. Examples of beetle specimens collected in this study. Dorsal and lateral views. A, B *Premnobius cavipennis*., C, D *Amasa truncata,* E, F *Euplatypus* sp., J, H, *Hypothenemus* sp., J – M *Xyleborus* spp,

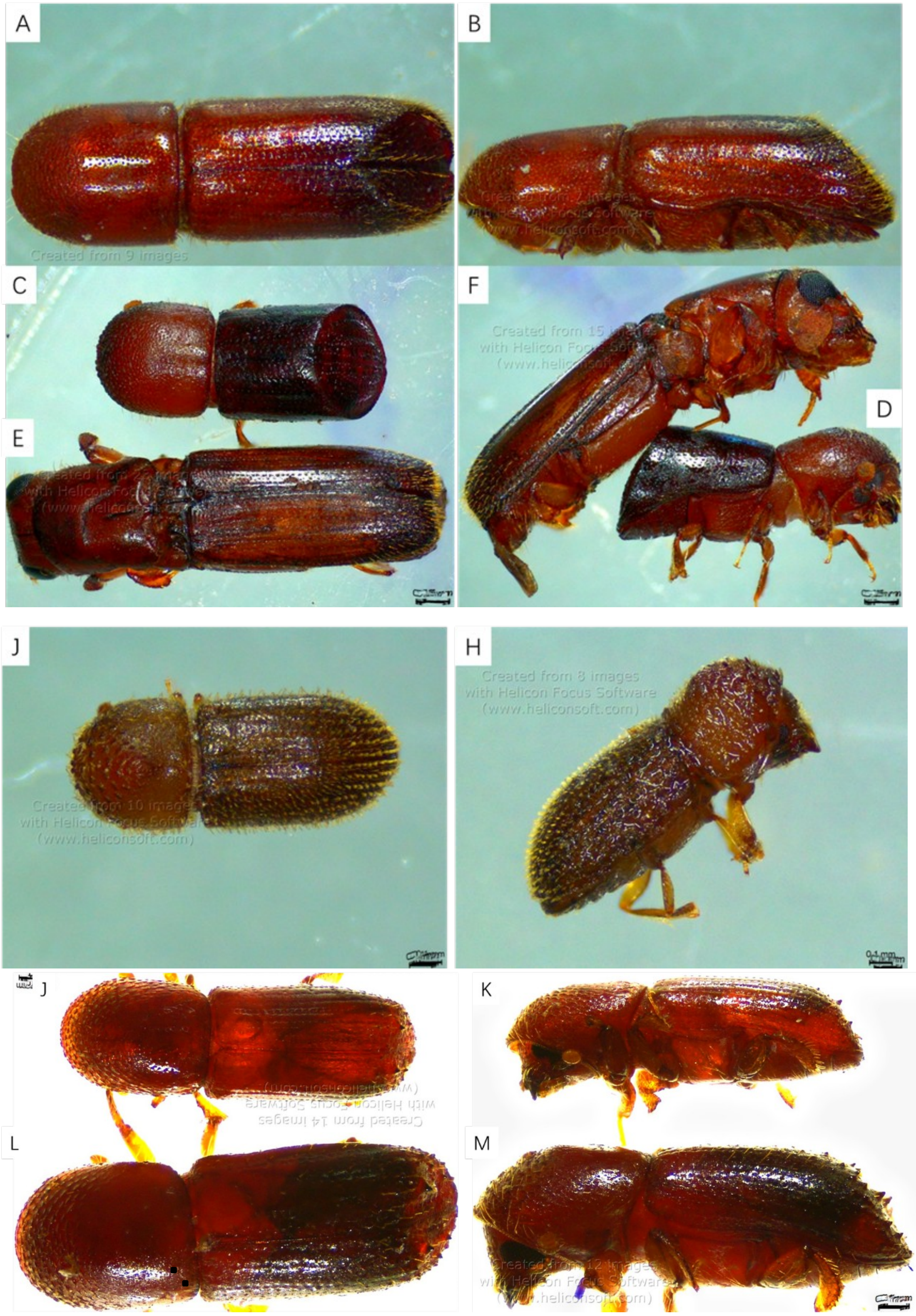

Figure 1 (continue) top to bottom: *Euplatypus* sp, *Anisandrus* (?) sp, *Xylosandrus compactus*, *Xyleborinus* sp, two species of *Euwallacea* with male.

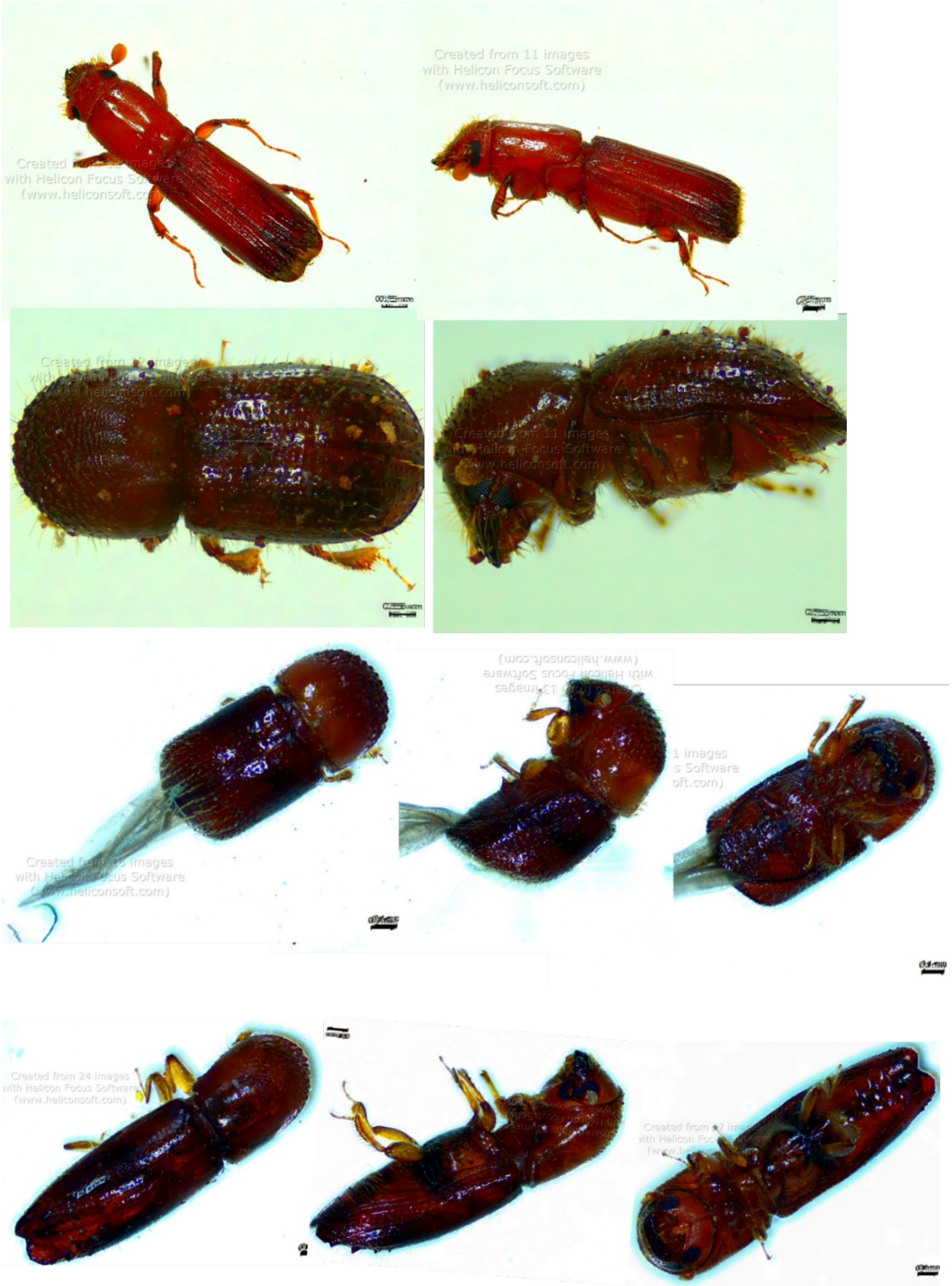

Figure 2. Galleries and damaged wood: (From above to bottom, right to left) 1: *Hypothenemus* with gallery, 2: galley in *Magnifera indica* tree of *Euwallacea* sp, 3: dust, caused by *Hupothenemus*, 4: gallery in *Magnifera indica* of probably Xyleborus sp, 5: *Ficus enormis* with hole of *Hypothenemus* and *Euwallacea,* 6: holes of *Xuleborus* and *Euwallacea* nearly, in *Ficus enormis*, 7: twig, (unknown tree) damaged by multiple species, include *Xylosandrus compactus,* 8: branch of Artocarpus heterophyllus with holes of *Euwallacea,* 9: two species of *Euwallacea* (figure above) competing for one gallery in *Artocarpus heterophyllus*, 10: *Euwallacea* sp in newly bored gallery in *Ficus enormis*, 11: *Micrapate horni* in twig, 12: mass of *Hypothenemus* under the bark of the twig.

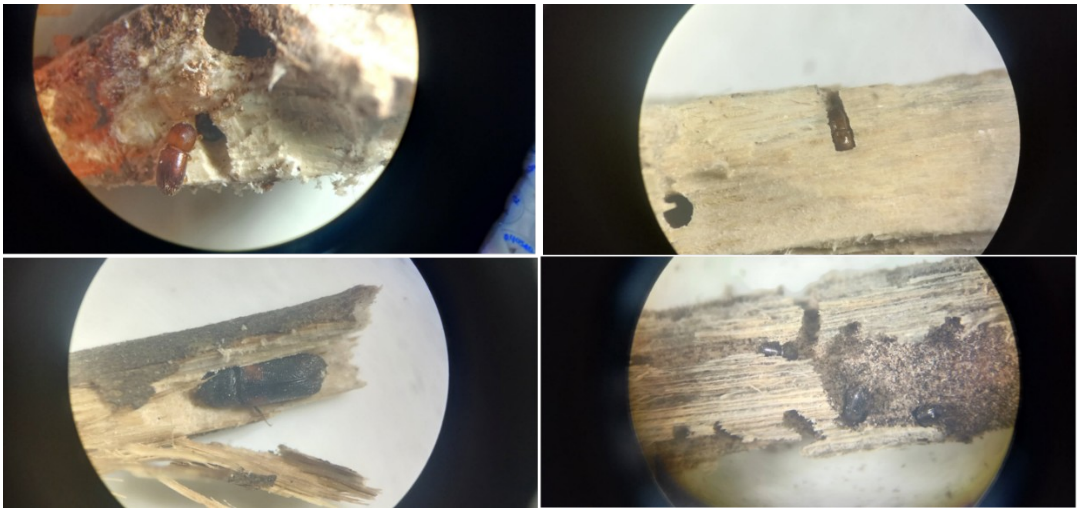

Figure 3. Representative fungal colonies obtained. First row – *Rafaellea* spp., isolated from *X. affinis*, second – other fungal symbionts, probably *Ambrosiella*, third – *Rafaellea* spp. isolated from *X. ferrugineus*.