



Memories of the Agricultural and Livestock Area of the VI CCIUTM, Ecuador

Scolytines (Coleoptera: Curculionidae: Scolytinae) Associated with Cacao Monoculture and Agroforestry Systems

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Abstract | Cacao, *Theobroma cacao* L. has a diverse entomofauna, including the scolytines. During March - August 2021, the scolytinae community was studied in Manabí province, Ecuador. Two farms in agroforestry (AF) and monoculture system (MF) were selected in three areas: Lodana (LAF and LMF), Pachinche (PAF and PMF) and Santa Ana (SAAF and SAMF). Diversity, abundance, and IndVAI were estimated as well as its correlation with climate variables. A total of 8,372 scolytines separated into 14 taxa within nine genera were observed. Scolytine diversity was low in all the agroecosystems, due to high dominance of *Hypothenemus* sp. (87.9%). LAF exhibited the highest scolytinae community, with six indicator taxa (IndVAI: ranging 25.4 to 48.7%). Rainfall and temperature were negatively associated with abundance. Although *Xyleborus ferrugineus* (Fabricius) is the species reported in Ecuador, 13 additional taxa were detected. This research reports for the first time 13 scolytine taxa associated with cacao in Ecuador.

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Introduction

Cacao, *Theobroma cacao* L. (Malvaceae) is a crop of global importance because its beans are the basis for the production of chocolate and other products (Armengot *et al.*, 2020). Its origin is found in the humid forests of South America and from there its cultivation expanded to Central America and later to other parts of the world, and now is grown pantropically (Tscharncke *et al.*, 2023). Worldwide, 5,580,432 t of cacao beans are produced

from 11,535,884 ha (FAO, 2024) of cacao planted areas. Ecuador contributes with 337,149 t of cacao beans harvested from 509,179 ha (MAG, 2024) of cacao planted areas, which represents 6% of world production.

Associated with cacao, there is a diverse entomofauna with different functional roles, i.e., natural enemies (predators and parasitoids), xylophages, xylomycetophages, and phytophages, including several species of scolytines (Coleoptera: Curculionidae:

Scolytinae), among others (Mazón *et al.*, 2018). Scolytines are commonly known as bark beetles, namely those that feed on phloem (phloeophagy) or sapwood and ambrosia beetles, namely those that burrow into sapwood and feed on ectosymbiotic fungi (xylomycetophagy) (Atkinson, 2017). However, other feeding habits can be found in species of scolytines such as xylophagy (wood feeders), myelophagy (pith feeders), spermophagy (seed feeders) and some feed on herbaceous plants (Gerómimo-Torres *et al.*, 2015; Kirkendall *et al.*, 2015).

Although scolytines play an important role in the biological decomposition and nutrient cycling of forests, some species cause significant economic damage to agroecosystems and forests around the world (Rodríguez *et al.*, 2017; Nuriadi *et al.*, 2023). Ambrosia beetles carry and introduce symbiotic fungi, which invade and grow on the wall of tunnels bored into the xylem of their host trees and serve as their sole food source (Kirkendall *et al.*, 2015). Castrejón-Antonio *et al.* (2017) reported that the growth of fungi in the vascular system of living trees could hinder the flow of water and nutrients, which would cause the partial or total death of the plant. Some species are considered cacao pests in South America. For example, in Ecuador *Xyleborus ferrugineus* (Fabricius) is associated with the transmission of *Ceratocystis cacaofunesta* Engelbr. and T.C. Harr. (Microascales: Ceratocystidaceae) (Paladines-Rezabala *et al.*, 2022) and *Xylosandrus compactus* (Eichhoff) has been reported causing damage to nursery plants in Peru (Delgado and Couturier, 2017).

Approximately 6,000 species included in 247 genera of scolytines have been reported in the world, of which 40% are estimated to exist in the Neotropics and 25% in South America (Martínez *et al.*, 2019). Scolytine species have been detected in Ecuador in natural, secondary forests and in balsa plantations of *Ochroma pyramidale* (Cav. ex Lam.) Urb. (Malvaceae) (Martínez *et al.*, 2019; Dole *et al.*, 2021). Smith and Cognato (2021) reported that the neotropical diversity of scolytines is largely undescribed and suggested that the diversity of Ecuadorian scolytines must be three to four times higher than currently known.

Cacao has traditionally been produced as an agroforestry system associated with other crops and timber species (Tscharntke *et al.*, 2023). However, in recent years, cacao planting in a monoculture

system has increased dramatically in order to increase productivity (Lagneaux *et al.*, 2021). This simplification in modern intensive systems have led to the loss of biodiversity in cacao agroecosystems (Lagneaux *et al.*, 2021; Tscharntke *et al.*, 2023). In Ecuador, *Xyleborus ferrugineus* (Fabricius) is the only species of scolytine that has been reported associated with cacao (Paladines-Rezabala *et al.*, 2022). Since Ecuador is part of the center of origin of cacao, it is expected that there should be a greater number of scolytine taxa associated with this crop, which should also be greater in diverse agroecosystems.

Manabí province is the main cacao-producing region in the country, where cacao is planted in both agroforestry and monoculture. However, little is known about the scolytine taxa in agroforestry and monoculture systems in this province and in the country. Thus, the main objective of this work was to identify the scolytine taxa (genus or species) associated with cacao monoculture and agroforestry systems in Manabí province, Ecuador.

Materials and Methods

Location and timing of sampling

During March-August 2021, this research was carried out on farms in the areas of Lodana, Pachinche and Santa Ana de Vuelta Larga (Figure 1), which are towns located in Manabí province, Ecuador.

The study sites are characterized as agricultural systems, with cacao, corn, coffee, banana and rice crops grown at small and medium scales, with traditional production systems, and the crops are shared by a diversity of fruit species (MAGAP, 2012). According to the life zones system, this region is classified as a tropical dry forest (Holdridge, 1967). This area is characterized by having two well-defined climatic seasons, a rainy season from December to May and another dry season from June to November (Rivadeneira *et al.*, 2020). In each area, two farms were selected, one in a cacao monoculture system and the other in a cacao agroforestry system. The selection requirements were an area of one to three hectares in which cacao had been cultivated for four years. Precipitation data were obtained for each area from the meteorological station of the National Institute of Meteorology and Hydrology (INAMHI), located in La Teodomira (code M1208).



Figure 1: Map of the Americas showing the location of Ecuador (blue color). Map of Ecuador indicating Manabi province (light green), as well as the map of the province exhibiting the study sites.

In Lodana, cacao agroforestry is associated with a native tree known as guaba, *Inga edulis* Mart. (Fabaceae), as shade. In Pachinche, the cacao agroforestry system is intercropped with soursop, *Annona muricata* L. (Annonaceae), key lime, *Citrus aurantiifolia* Swingle, mandarin orange, *Citrus reticulata* Blanco, orange, *Citrus sinensis* Osbeck (Rutaceae), coconut, *Cocos nucifera* L. (Arecaceae), mango, *Mangifera indica* L. (Anacardiaceae), banana, *Musa paradisiaca* L. (Musaceae), and avocado, *Persea americana* Miller (Lauraceae). In Santa Ana, cacao was planted with *A. muricata*, *C. aurantiifolia*, *C. sinensis*, araza, *Eugenia stipitata* McVaugh (Myrtaceae), *M. paradisiaca*, *P. americana* and guava, *Psidium guajava* L. (Myrtaceae).

Sampling, counting and identification

In each cacao plantation, seven traps baited with ethanol were placed on cacao plants 1.3 m above the ground. The traps were placed in the center of each plantation to avoid edge effects with an approximate separation of 22 m between traps, covering a 160 m

transect. The trap was developed following the model proposed by [Steininger et al. \(2015\)](#), which consisted of a two-liter plastic bottle with its lid maintained closed, with two openings (25 cm²) near the base to allow the entrance of the attracted scolytines. A puncture hole was made at the base of the bottle and with the help of a rope, which was attached to a branch of the tree at a height of one meter, leaving the bottle with its mouth downwards. A plastic bag containing 96% ethyl alcohol was hung inside the trap. A small perforation was made in the bag so that the alcohol was released slowly to attract the insects. Gel alcohol was placed in the mouth of the bottle to trap the insects. The specimens were collected every fortnight, for which the trap lid was removed, and the individuals were placed in a 100 mL plastic bottle containing ethyl alcohol (70%). A total of 504 samples were taken during the study, obtained from seven traps in 12 samplings (biweekly) in the six agroecosystems.

The collected insects were taken to the Entomology Laboratory of the Faculty of Agricultural Engineering, Technical University of Manabí (TUM), where scolytines were observed under a Motic® SMZ 165 stereoscope (magnification: 10 to 75X). The scolytine specimens were first sorted out according to their morphological characteristics and later they were identified using the taxonomic keys of [Wood \(2007\)](#) and through comparisons with photographs in the [Atkinson \(2023\)](#) catalogs. Specimens of identified scolytines are stored in the Entomology Laboratory of the TUM.

Data analysis

Diversity indices, Shannon Index, Margalef Index, Dominance D Index and Chao 1 Index as well as the number of taxa and abundance were estimated to the six cacao agroecosystems. A non-parametric multivariate permutational analysis (PERMANOVA) was performed to compare agroecosystems using Bray-Curtis distances ($P < 0.05$). An analysis was carried out to associate the abundance of the different taxa of scolytines in the studied agroecosystems using the value of IndVal (%), in order to determine the indicator taxa of the agroecosystems ($P < 0.05$). A Spearman's correlation analysis ($P < 0.05$) was performed between the number of scolytines versus rainfall, temperature and relative humidity for each agroecosystem. The fluctuation of the number of total individuals per agroecosystem was plotted

together with precipitation data. These analyses were performed using the program PAST software version 4.04 (Hammer *et al.*, 2001).

Results and Discussion

General abundance and diversity of scolytines in cacao

A total of 8,372 individuals separated into 14 taxa within nine genera in four tribes were observed associated with the six cacao agroecosystems (Table 1). The tribe Trypophloeini to which *Hypothenemus* belongs to was the most abundant with 87.91% of the total number of captured individuals. *Corthylus* sp. 1 and *Premnobius cavipennis* Eichhoff were the next most abundant taxa with 3.93 and 3.12% of the total number of individuals, respectively. *Xyleborus affinis* Eichhoff and *Xyleborus spinulosus* Blandford showed an abundance of approximately 1% while for the rest of the taxa, the abundance ranged from 0.01 to 0.97%. In the tribes Corthylini and Xyleborini, six taxa were detected in each one, which represents the largest number of taxa compared to the other two tribes. All taxa found in this study had been previously reported

for Ecuador, associated with natural forests and balsa plantations (Martinez *et al.*, 2019).

Diversity indices by agroecosystem

The Shannon index (range: 0.391–0.715) and the Margalef index (range: 1.147–1.600) indicate a low diversity of scolytine taxa associated with a high abundance of *Hypothenemus* sp. in the six agroecosystems studied (range: 374–2849 individuals) (Table 1). In fact, the Dominance D Index determined values close to one (range: 0.710 to 0.861) which corroborates the dominance of *Hypothenemus* sp. over the rest of the taxa (Table 1). We estimate that our sampling efforts of the scolytine taxa were appropriate. According to Chao 1 Index, the observed taxa (S_{obs}) were 100% of those expected in Pachinche monoculture farm (PMF), Pachinche agroforestry farm (PAF) and Lodana agroforestry farm (LAF) while it constituted at least 88% of the species in both systems, agroforestry (SAAF) and monoculture farm (SAMF) in Santa Ana and 81% in Lodana monoculture farm (LMF) (Table 1).

Table 1: Abundance of taxa by tribes detected in the six cacao agroecosystems.

Tribes/ Taxa	LAF	LMF	PAF	PMF	SAAF	SAMF	Total	%
Corthylini								
<i>Corthylus</i> sp. 1	152	59	33	46	15	24	329	3.93
<i>Corthylus</i> sp. 2	2	6	2	43	14	14	81	0.97
<i>Corthylus</i> sp. 3	9	5	8	0	0	1	23	0.27
<i>Corthylus</i> sp. 4	4	4	4	3	0	1	16	0.19
<i>Cryptocarenum</i> sp.	3	0	3	0	0	0	6	0.07
<i>Monarthrum</i> sp.	0	1	0	0	0	0	1	0.01
Ipini								
<i>Premnobius cavipennis</i>	108	20	29	40	30	34	261	3.12
Trypophloeini								
<i>Hypothenemus</i> sp.	2849	1683	831	918	374	705	7360	87.91
Xyleborini								
<i>Coptoborus ochromactonus</i>	5	1	5	0	0	1	12	0.14
<i>Sampsonius</i> sp.	3	1	0	1	0	0	5	0.06
<i>Xyleborus affinis</i>	32	18	16	7	8	27	108	1.29
<i>Xyleborus ferrugineus</i>	25	4	5	7	1	2	44	0.53
<i>Xyleborus spinulosus</i>	36	3	24	17	1	11	92	1.10
<i>Xylosandrus</i> sp.	3	10	6	8	3	4	34	0.41
Total abundance	3,231	1,815	966	1,090	446	824	8,372	100
Taxa (S_{obs})	13	13	12	10	8	11	14	
Shannon index	0.560	0.391	0.689	0.715	0.685	0.672	0.598	
Margalef index	1.485	1.599	1.600	1.287	1.147	1.489	1.439	
Dominance_D index	0.781	0.861	0.743	0.714	0.710	0.736	0.775	
Chao 1 index	13	16	12	10	9	12.5	14	

LAF: Lodana Agroforestry Farm, LMF: Lodana Monoculture Farm, PAF: Pachinche Agroforestry Farm, PMF: Pachinche Monoculture Farm, SAAF: Santa Ana Agroforestry Farm, SAMF: Santa Ana Monoculture Farm.

The low diversity observed in agroforestry systems differs from that found by [Perez-De la Cruz et al. \(2009\)](#) who in cacao associated with different fruit and forest trees found values for the Shannon Index that ranged from 2.29 to 2.45, which is higher than that detected in this study. However, these values are similar to those found by [Mazón et al. \(2013\)](#) who estimated values of 0.89 to 1.29 for the Shannon Index in cacao associated with *I. edulis* as shade tree.

Scolytine communities in the agroecosystems

Although the Shannon and Margalef Index values indicated low diversity, PERMANOVA detected differences among the scolytine communities in the evaluated agroecosystems (Table 2). LAF differed from the rest of the agroecosystems with the highest abundance of scolytines (3,231 individuals) and 13 taxa in total. On the other hand, SAAF exhibited the lowest numbers of scolytines (446 individuals) and taxa (9 taxa). Additionally, the species indicator (IndVal %) (Figure 2) estimated showed that LAF is the agroecosystem that presented the highest number of indicator taxa (six in total) with values ranging from 25.4 to 48.7%, suggesting the preference of these taxa for this agroecosystem.

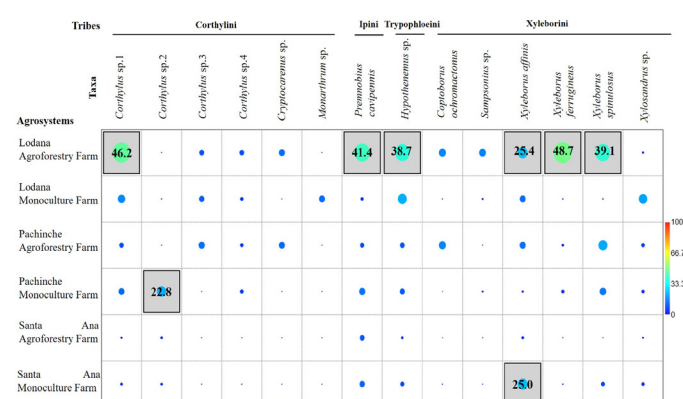


Figure 2: Indicator species analysis (IndVal %) in the evaluated agroecosystems. The values inside the boxes indicate the significance of the IndVal ($P < 0.05$).

Regarding the abundance of *Hypothenemus* sp. found in LAF, these results are similar to those reported in other studies, which point out the higher abundance of this genus in a cacao plantation associated with *I. edulis* trees as shade ([Mazón et al., 2013](#)) and in cacao agroecosystems associated with fruit trees and agroforestry ([Perez-De la Cruz et al., 2009](#)). However, it contrasts with the findings of [Cajaiba et al. \(2018\)](#) who did not observe individuals of the genus *Hypothenemus*, while that *Xyleborus* species were abundant in a cacao monoculture.

The importance of the taxa found in this research is emphasized. [Ramos and Montilla \(2018\)](#) reported *Hypothenemus* sp. as a pest of cacao beans. [Hernandez et al. \(2015\)](#) reported the species *Hypothenemus birmanus* (Eichhoff) and *Hypothenemus interstitialis* (Hopkins) as cacao stem and branch borers. In the tribe Corthylini, four *Corthylus* species (5.36%), one *Cryptocarenum* species and one *Monarthrum* species were observed (Table 1). The genera *Corthylus* and *Monarthrum* are classified as ambrosia beetles, while myelophagy is attributed to the genus *Cryptocarenum* ([Kirkendall et al., 2015](#)). *Premnobius cavipectus* was the only scolytine species detected from the tribe Ipini, accounting for 3.12% of the total species. The genera and species of the tribes Corthylini and Ipini found in the present study had been found in cacao crops in Mexico and Venezuela ([Pérez-De La Cruz et al., 2009; Mazón et al., 2013](#)). In Ecuador, *P. cavipectus* has been reported in both balsa plantations, *O. pyramidale* ([Averos et al., 2021](#)) and in natural forests ([Martinez et al., 2019](#)).

Coptoborus ochromactonus Smith and Cognato, *Sampsonius* sp., three species of *Xyleborus* and *Xylosandrus* sp. were the taxa found in the tribe Xyleborini with low percentages of abundance (3.5%). All of these ambrosia beetle species can occasionally kill healthy trees ([Kirkendall et al., 2015](#)).

Table 2: P-values obtained from Permanova analysis comparing the six agroecosystems.

	Lodana agroforestry farm	Lodana monoculture farm	Pachinche agroforestry farm	Pachinche monoculture farm	Santa ana agroforestry farm
Lodana agroforestry farm					
Lodana monoculture farm	0.04695*				
Pachinche agroforestry farm	0.000999*	0.1558			
Pachinche monoculture farm	0.001998*	0.1888	0.6194		
Santa ana agroforestry farm	0.001998*	0.000999*	0.01099*	0.01099*	
Santa ana monoculture farm	0.002997*	0.03996*	0.5275	0.5974	0.01998*

* Asterisk when significant ($p < 0.05$).

Coptoborus ochromactonus has been found in Ecuador building galleries in young plants of *O. pyramidale* with an incidence of 50% and causing 15% tree mortality (Castro *et al.*, 2017). The genus *Sampsonius* was observed for the first time in Ecuador in 2008 in samples taken from the Amazonian forests (Petrov and Flechtmann, 2013). On agroforestry cacao plantations, *Sampsonius* sp. has been detected with low percentages of abundance in Mexico (Pérez-De La Cruz *et al.*, 2009) and Venezuela (Mazón *et al.*, 2013).

Xyleborus affinis is an ambrosia beetle that stains the wood when building galleries in timber trees; however, the damage of this insect is restricted to sporadic records and its impact has not been evaluated (Rangel *et al.*, 2012). *Xyleborus spinulosus* has a range of hosts circumscribed to the Americas (Castrejón-Antonio *et al.*, 2017) and its presence in agroforestry cacao has been reported in Mexico (Pérez-De La Cruz *et al.*, 2009) and Venezuela (Mazón *et al.*, 2013; Navarro and Liendo, 2010). Although *X. ferrugineus* is the species reported in Ecuador associated with cacao (Paladines-Rezabala *et al.*, 2022), in this study, it reached 0.53% abundance (Table 1). Species of *Xylosandrus* are reported attacking different organs of the cacao plant (Asman *et al.*, 2021; Delgado and Couturier, 2017; Thube *et al.*, 2022). *Xylosandrus compactus* was detected building galleries in stems and branches of nursery plants in Peru (Delgado and Couturier, 2017). *Xylosandrus crassiusculus* (Motschulsky) has been observed causing perforations in cacao pods affected by *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) in India (Thube *et al.*, 2022). In Indonesia, *X. compactus* is reported as a major pest in cacao plantations, associated with phytopathogenic fungi (Asman *et al.*, 2021).

Climate variables and abundance of scolytines by agroecosystem

Abundance of scolytines showed a negative correlation with rainfall and temperature, being highly significant in LAF, LMF, SAAF and SAMF (Figure 3). It is important to note that, in the study areas, temperatures are high in the rainy season, which decrease in the dry season (Rivadeneira *et al.*, 2020). With variations in the number of scolytines according to the locality and system, the highest flight activity was observed during the dry season compared to the rainy season (Figure 4). Regarding the fluctuation of the scolytines in dry and rainy seasons, the present results indicate

that adults exhibit a greater flight activity in the dry season compared to the rainy season.

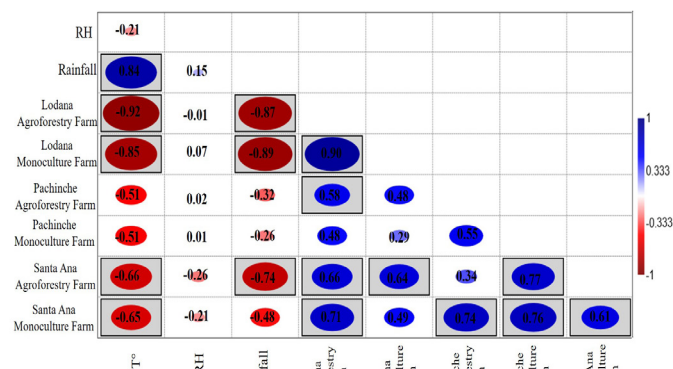


Figure 3: Spearman's correlation analysis between rainfall, temperature (T, °C), relative humidity (RH) and the number of scolytines by studied agroecosystems. The values inside the boxes indicate the significance of correlation ($P < 0.05$).

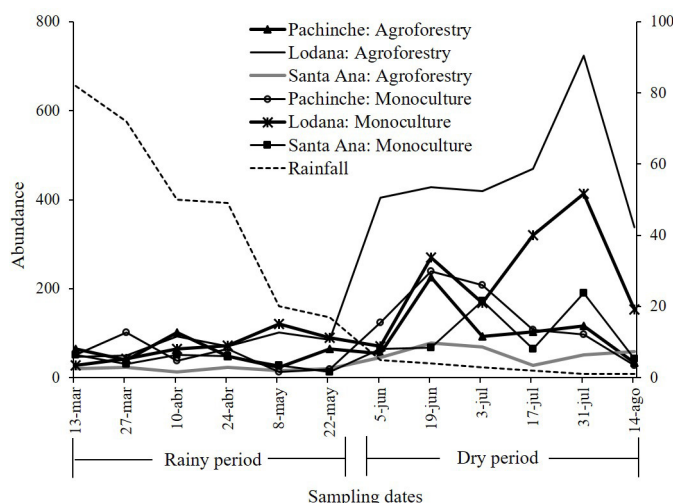


Figure 4: Abundance of scolytines in the six agroecosystems studied. Period March–August 2021, Manabí Province, Ecuador.

Previous studies have shown that some species of scolytines show greater flight activity in times of low rainfall. Navarro and Liendo (2010) collected samples in cacao farms in marine valleys of the coastal mountain range, Venezuela, which life zone is a tropical dry forest; and found a negative and significant association of populations of *X. spinulosus*, *Hypothenemus erectus* LeConte, and *Hypothenemus opacus* Latreille with precipitation. The scolytine taxa present in a plantation of *O. pyramidale* in Ecuador showed a high negative correlation with precipitation (Martínez *et al.*, 2019). However, other studies have obtained contrasting results with those detected in this study. Sittichaya *et al.* (2012) in a study carried out in fruit orchards on the eastern coast of southern Thailand found greater flight activity of xyleborines in the rainy season.

The flight activity of *P. cavipennis* in plantations of *O. pyramidale* located in the provinces of Guayas (tropical dry forest), and Los Ríos (tropical humid forest), Ecuador, did not show a significant association with precipitation (Averos *et al.*, 2021). Nevertheless, a positive and highly significant correlation of scolytines with precipitation was detected in the plantation located in Cotopaxi (premontane very humid forest) (Averos *et al.*, 2021).

A study conducted by De Abreu *et al.* (2012) in a primary forest of the Amazon, Brazil, found no correlation between the abundance of scolytines and precipitation. These researchers indicated that, although previous studies indicate that high rainfall affects the abundance of these insects, there are conflicting results about the effect of rainfall. Hulcr *et al.* (2008) based on research conducted in two primary forests in northern Thailand reported that species with a circumtropical distribution, such as *X. affinis*, *Xyleborinus andrewesi* (Blandford), *Xyleborus perforans* (Wollaston) and *Hypothenemus* spp. are not affected by seasonality.

Given that all agroecosystems are located in the same life zone, the differences detected in the scolytine community of the agroecosystems (agroforestry or monoculture) are likely related to the specific characteristics of each site, such as the type of surrounding vegetation, diversification, agronomic management practices, among others. Rodriguez *et al.* (2017) pointed out that associating native trees in agroforestry systems can favor the survival of scolytines because older and larger trees limit the amount of solar radiation, generating adequate microclimates for the development of these beetles. Hence, the greater abundance of scolytine communities in LAF would be related to the association of cacao with *I. edulis*, a native tree from South America, unlike other cacao agroforestry systems that are associated with fruit trees.

This study represents a scientific contribution to the diversity and abundance of scolytines found in cacao agroecosystems in Ecuador, where until now only the presence of *X. ferrugineus* had been reported. It was not unexpected that in a country that is the center of origin of cacao, at least 14 taxa were found associated with this Neotropical plant under anthropic management. Scolytines play important roles in decomposition, nutrient cycling, and ecosystem dynamics. These

insects are considered secondary pests because most species feed on dying plants or under conditions of nutritional stress, however, several species can attack healthy trees or play an important role in the transmission of phytopathogenic fungi. Fortunately, in Ecuador, little damage caused by this group of beetles have been reported on cacao so far. It is worth noting that the bioecological characteristics of insect pests, including scolytines, would be influenced by agronomic management practices and climate change, which poses important challenges in cacao agroecosystems.

Conclusions and Recommendations

In this study, the diversity of scolytine taxa was low in both agroforestry and monoculture systems and *Hypothenemus* sp. was superior to the other species. In spite of this, we found a higher community of scolytines in LAF and a lower one in SAAF. Another important finding was the detection of at least 14 taxa of scolytines associated with cacao agroecosystems, which until now was limited to *X. ferrugineus*. This represents an important contribution to the knowledge of the diversity of scolytines in Ecuador.

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Novelty Statement

This article represents an important contribution to the knowledge of the diversity of scolytines in Ecuador. In this study, 14 taxa were observed associated with cacao agroecosystems, a country that is considered the center of origin of the crop and where only a single species had been reported.

Author's Contribution

Jessenia Castro-Olaya: Conceptualization, data curation, funding acquisition, investigation, methodology, project administration, writing-original draft.

Dorys T. Chirinos: Data curation, investigation,

formal analysis, methodology, validation, writing-review and editing.

Takumasa Kondo: Methodology, supervision, validation, visualization, writing-review and editing. All co-authors reviewed the final version and approved the manuscript before submission.

Conflict of interest

The authors have declared no conflict of interest.

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