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Low Sampling Effort and High Genetic Isolation Contribute to Underdocumented Diversity in Philippine Fig Wasps

Lillian Jennifer V. Rodriguez[1]*, **Astrid Cruaud**[2], and **Jean-Yves Rasplus**[2]

[1]Institute of Biology, University of the Philippines Diliman
Quezon City 1101 Philippines
[2]CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro
Univ Montpellier, Montpellier 34000 France

One of the unique attributes of tropical forests is the diversity of fig-fig wasp interactions. In the Philippines, there is a good estimate of the number of fig species, but none for the pollinating fig wasps. According to our review of the literature, there are only 10 described species of *Ceratosolen* in the Philippines, eight of which are endemic to the country (80% endemicity) out of a global count of 72 species worldwide. However, most historical collections were only from Luzon island. In this study, because of an increased sampling effort across 10 islands – coupled with morphological comparisons with described species and analysis of genomic data – we identified 29 as yet undescribed *Ceratosolen* species (which represents an increase of nearly 300% for the Philippine fig wasp fauna). This shows that the major factors contributing to under-documented diversity in Philippine *Ceratosolen* are low sampling effort and high genetic isolation between lineages. More importantly, this is probably not limited to fig wasps and we suspect under-estimated diversity in most Philippine faunal lineages. Building a strong taxonomic foundation is key to the establishment of good conservation practices. It is time to start investing in Philippine biodiversity research before we lose our natural treasures.

Keywords: Agaonidae, *Ficus*, geographic variation, Mindanao, Visayas

INTRODUCTION

One of the unique attributes of tropical forests is the diversity of fig-fig wasp interactions. In the Philippines alone, there are more than 100 species of fig trees (Moraceae: *Ficus*), mostly called *balete* (in *Tagalog*) or *dalakit* (in *Bisaya*) (Berg and Corner 2005). These fig trees are pollinated by a specific set of wasps belonging to the family Agaonidae (Hymenoptera: Chalcidoidea). The specificity of these interactions has led to the radiation of many fig wasp lineages (Cruaud *et al*. 2012).

*Corresponding Authors: lvrodriguez@up.edu.ph

In 1906, Brown published a catalog of Philippine Hymenoptera in the first-ever volume of the Philippine Journal of Science (PJS). It included three fig wasp species from *Ficus nota*: *Kradibia brownii*, *Philotrypesis bakeri* (=*Sycoryctes philippinensis*), and *P. ficicola*. After this historical publication, two more highly-cited papers were published in the PJS describing new species of fig wasps from the Philippines: Baker (1913) and Grandi (1927). In the 1960s, J.T. Wiebes received a grant to study figs and fig wasps in the Philippines. He made important collections from different islands (mostly Luzon, but also Mindanao, Basilan, and Negros). Ninety-three years after Grandi's contribution, we are given here

the opportunity to publish in the same journal that historically launched Philippine fig wasp research.

After more than a century of fig wasp collections, where are we now in terms of describing our species? Have we established a good taxonomic foundation for Philippine fig wasps? Do we have a good estimate of Philippine fig wasp diversity? With this study, we aimed to analyze patterns of fig wasp discovery and diversity in the Philippines. We looked into historical records, collected wasps from fig trees in different islands, performed morphological identifications and comparisons of species, and – for a subset of them – sequenced and analyzed genomic markers to get a better idea of how many species of fig wasps occur in the Philippines. The objective is to learn lessons from the past to look for keys to the future.

MATERIALS AND METHODS

Study Group

We centered our study on the fig wasp genus *Ceratosolen* that pollinates *Ficus* species belonging to the subgenus *Sycomorus*. We chose this genus because it is present throughout the Old-World tropics, where a lot of the historical expeditions were conducted. *Sycomorus* species (with 18 species occurring in the Philippines) are globally easy to find and to sample with figs producing wasps nearly all year-round. Within the Philippines, *Ceratosolen* and *Sycomorus* figs are widely distributed, which allowed us to look into the geographical and genetic structuring of sampled *Ceratosolen* species.

Synthesis of Philippine *Ceratosolen* Taxonomy

To look at historical patterns of discovery and taxonomy of Philippine *Ceratosolen*, we synthesized data from historical publications dating back to 1885. The most significant publications related to this topic were Ashmead (1904a, b), Brown (1906), Baker (1913), Grandi (1927, 1928, 1941, 1952, 1955, 1963), and Wiebes (1963, 1991, 1994).

Fig Wasp Collection

To elucidate current diversity patterns of Philippine *Ceratosolen*, we sampled wasps from 36 localities in the Philippines, representing 10 distinct islands (Figure 1e). The sampling protocol is described in Rodriguez *et al*. (2017b).

Fig Wasp Genetic Analysis

To compare historical taxonomic records with species found throughout the archipelago, we focused on a species complex that we have been carefully studying for many years – the *Ceratosolen bisulcatus* species complex. We captured ultra-conserved genomic elements (UCEs) from wasps in this species complex. UCE analysis allows the recovery of hundreds to thousands of loci that can be used to reconstruct phylogenetic relationships at multiple evolutionary scales (Faircloth *et al*. 2012). Protocols on DNA extraction, UCE library preparation, and sequencing – as well as the bioinformatics pipeline for data analysis – are detailed in Cruaud *et al*. (2019).

A phylogenetic tree was built using 19 samples and the 1,007 UCEs for which at least 10 samples out of the 19 (*i.e.* 50%) had a sequence. Indeed, it is technically impossible to capture all UCEs targeted by the probes (N = 1,432) for all samples and we needed to accept a certain amount of missing data in the analysis. Alignment was done with mafft (Katoh and Standley 2013) using default parameters and cleaned with Gblock (Talavera and Castresana 2007) using relaxed parameters (−t = d −b2 = b1 −b3 = 10 −b4 = 2 −b5 = h). The total concatenated alignment was 620,183 bp. We used RAxML (Stamatakis 2014) without data partitioning and 100 rapid bootstrap replicates for phylogenetic inference.

RESULTS AND DISCUSSION

Synthesis of Philippine *Ceratosolen* Taxonomy

The first *Ceratosolen* species from the Philippines was collected from Los Baños, Laguna, and described by Baker (1913) from *Ficus nota*: *Ceratosolen notus* (Appendix Table I). Fourteen years later, three more *Ceratosolen* species (*C. bakeri*, *C. pygmaeus*, *C. jucundus*) were described by Grandi (1927). These additional species were all collected from Mt. Makiling (Figure 1a). By 1928, the Philippines hosted four described species of *Ceratosolen* compared to the 26 species known at the global level (Grandi 1928) (Figure 2). In the subsequent global catalogs published by Grandi (1941, 1952, 1955), the number of *Ceratosolen* species in the world steadily increased due to an increased collection effort in the African continent (Figure 2). However, it was not until 1963 where there was a spike in the global number of species (from 32 to 55) (Grandi 1963). This increase was mostly due to the work of Wiebes (1963) in the Indo-Australian region, including the Philippines. Consequently, Wiebes (1963) added three more taxa to the Philippine tally: one new species (*C. corneri*), one new subspecies (*C. constrictus gracilis*), and one new record (*C. appendiculatus*). More importantly, Wiebes was also the first taxonomist to collect samples from localities outside Los Baños, Laguna (Figure 1b). This meant that for 50 years, the Philippine *Ceratosolen* fauna was known only from Laguna. About three decades later, Wiebes

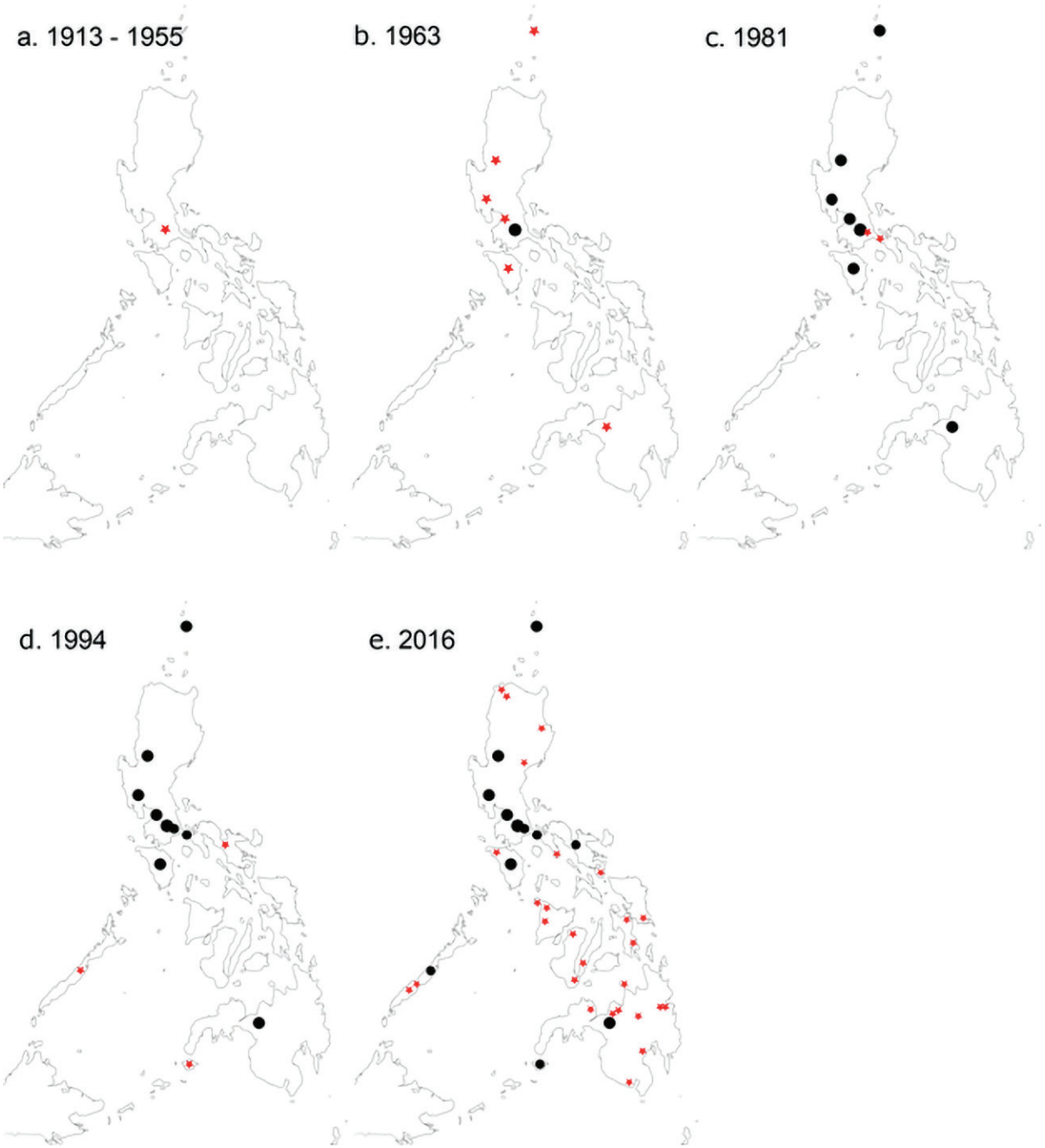

Figure 1. Map of the Philippines showing the sampling localities of Philippine *Ceratosolen*. Red stars represent new sampling localities for the specific period indicated. Black circles represent sampling localities from previous periods. Collections are from a) Baker (1913) and Grandi (1927, 1941, 1952, 1955), b) Wiebes (1963), c) Wiebes (1991), d) Wiebes (1994), and (e) Rodriguez *et al*. (in prep.).

(1991, 1994) described two new Philippine species (*C. ramirezi* and *C. cornutus*). Finally, Davis and Engel (2008) described one new species from Los Baños that appears to be a synonym of *C. pygmaeus*. This brought the Philippine fauna of *Ceratosolen* to 10 species out of the 72 species described in the world (14 %). Eight species are endemic to the Philippines (80% endemicity). Nonetheless, of the 10 *Ceratosolen* species cited from the Philippines, two were wrongly identified (*C. appendiculatus* and *C. bisulcatus*). Indeed, *C. appendiculatus* and *C. bisulcatus* do not occur in the archipelago and the two misidentified species are in fact new to science. After examination of morphological characters of all described species worldwide, including close relatives to Philippine species, we can state that the

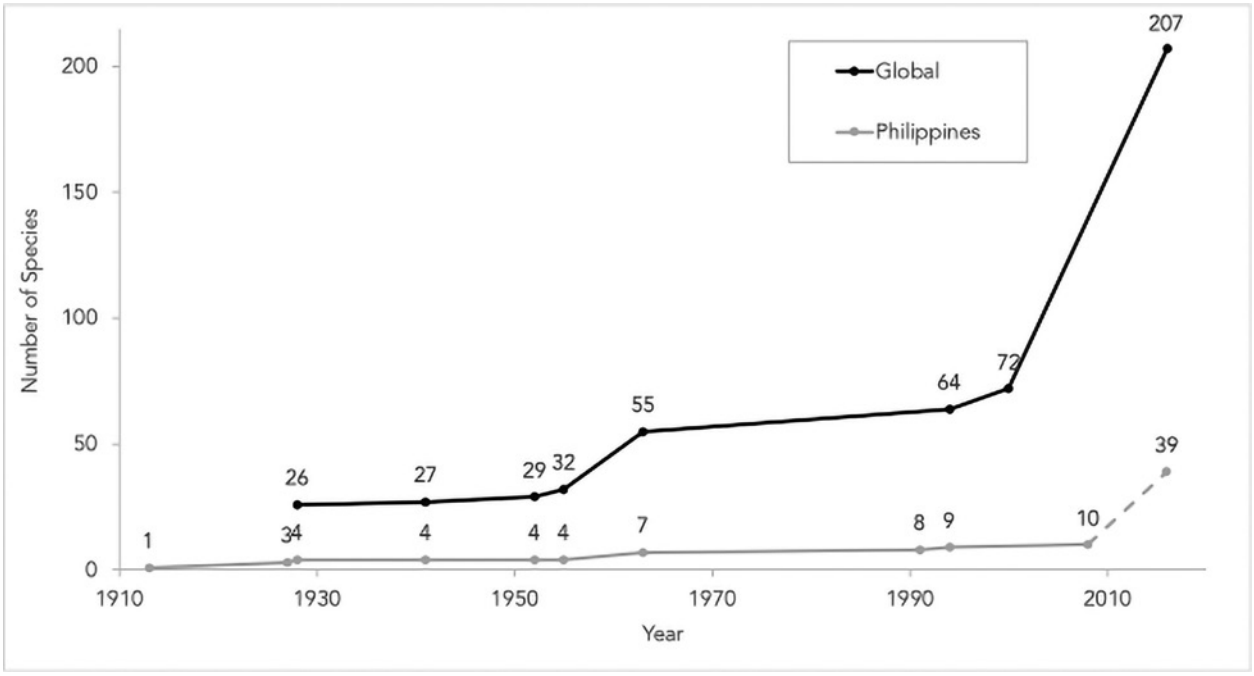

Figure 2. Summary of *Ceratosolen* diversity from 1913–2016. Black points represent global species tally; grey points represent number of Philippine species. The broken line indicates the increase in Philippine *Ceratosolen* upon description and publication of new samples.

nine species currently listed in previous literature for the Philippines are valid (*C. polyodontos* being a synonym of *C. pygmaeus*).

Despite the already-high endemicity, these historical data show the underrepresentation of sampling in the Philippine islands, most especially in the Visayas area (Figure 1). We addressed this in 2016 by collecting from more localities in Visayas and Mindanao (Figure 1e). Preliminary morphological and molecular identification of our samples resulted in 29 undescribed *Ceratosolen* species in the Philippines (Rodriguez *et al*., in prep.), all of them endemic to the archipelago (Figure 2; Appendix Table I). This rise in the number of *Ceratosolen* species shows that the status of the taxonomy and diversity of this genus is highly dependent on sampling effort. Furthermore, this was also unequivocally demonstrated in Philippine small mammals by Heaney and colleagues (2016). Because of similar patterns in such different animals as Philippine fig wasps and small mammals, we suspect underestimated and undocumented diversity in most Philippine faunal lineages.

Genetic Analysis of a *Ceratosolen* Species Complex

In Rodriguez *et al*. (2017b), we showed the first genetic evidence that the pollinator of *Ficus septica* throughout its range, *Ceratosolen bisulcatus*, was a species complex. UCE analysis provides a more accurate circumscription of the different lineages in this species complex because we are now using more than 1000 loci compared to the three loci that we used previously. UCE phylogenetic analysis shows that there are three lineages pollinating *F. septica* in the Philippines (Figure 3): 1) *C. jucundus*, which is present throughout the Philippines; 2) a new *Ceratosolen* species known only from Mindanao; and 3) new *Ceratosolen* species sampled from Luzon and Negros islands. Historically, Wiebes (1994) considered this complex as a single species (*C. bisulcatus*), whereas our analysis points to six Indo-Australian clades. *Ceratosolen bisulcatus* appears to occur only in Java and Sumatra, and is clearly different from the species occurring in the Philippines. Furthermore, the *C. jucundus* lineage requires more analysis as it could be a complex of cryptic species. This lineage showed limited genetic structuring based on UCEs (Figure 3), while it was highly structured when mitochondrial markers were analyzed (Rodriguez *et al*. 2017b). These show that, in addition to the underestimation due to limited sampling effort, Philippine *Ceratosolen* lineages are also underestimated because of genetic diversification. Radiation and diversification of Philippine lineages is mostly attributed to geographic isolation between islands (Jansa *et al*. 2006; Jones and Kennedy 2008).

Updated Host Catalog

In 1966, Wiebes published a provisional *Ficus* host catalog. Since then, there have been no published updates. In Appendix Table I, we present an updated

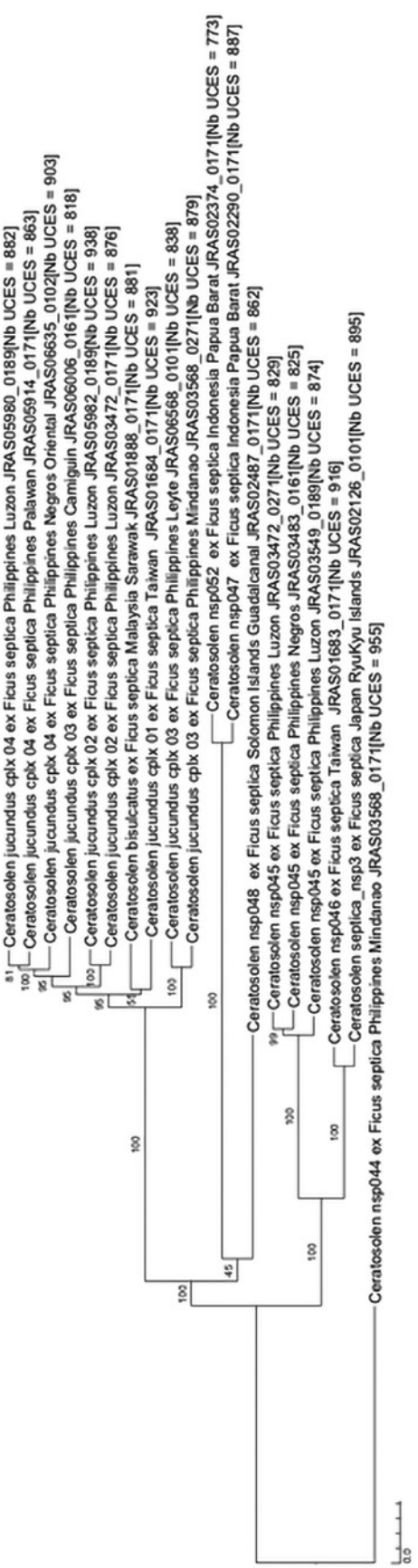

Figure 3. Maximum likelihood tree of the *Ceratosolen bisulcatus* species complex. Loci used to build the tree were UCEs (number of UCE per taxon is indicated). Six distinct Indo-Australian clades comprise the species complex.

catalog showing the *Ficus* subg. *Sycomorus* species occurring in the Philippines, their published *Ceratosolen* pollinators, and their pollinators in the Philippines. Before our sampling, 10 *Ficus* subg. *Sycomorus* fig species (out of 18 species, 55%) had no published pollinator species: *F. carpenteriana*, *F. cassidyana*, *F. fistulosa*, *F. laevicarpa*, *F. lepicarpa*, *F. linearifolia*, *F. multistipularis*, *F. satterthwaitei*, *F. sulcata*, and *F. variegata*. The pollinator of *F. variegata* was incorrectly identified as *C. appendiculatus* (Wiebes 1963). Following our dense sampling, only *F. lepicarpa* remains with no sampled pollinator in the Philippines. While only *F. septica* was previously documented to be pollinated by more than one species, nine species of figs appear now to be pollinated by more than one pollinator species.

IMPLICATIONS AND CONCLUSIONS

Our study shows that there are two main factors contributing to the under-documented diversity in Philippine *Ceratosolen* – low sampling effort and high genetic isolation between lineages. Increased sampling effort, coupled with appropriate morphological and genetic analyses, resulted in the identification of more *Ceratosolen* species (with an increase of at least 300%). Furthermore, our study focused on widespread *Ceratosolen* and *Sycomorus* figs. If we documented quadrupled diversity in this widespread group, how much more of an increase will we see in geographically-restricted and more genetically-isolated fig-wasp systems? Looking at the bigger picture, this is most probably not limited to fig wasps. Vertebrates are a much better-studied group in the Philippines and yet under-documented diversity has also been recently reported in small mammals (Heaney *et al*. 2016). Ensuring a future for our many yet "unknown" species entails investing heavily in Philippine biodiversity research.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

NOTES ON APPENDICES

The complete appendices section of the study is accessible at http://philjournsci.dost.gov.ph/

DATA ACCESSIBILITY

Demultiplexed reads are available as an NCBI Sequence Read Archive (BioProject ID: PRJNA682415).

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APPENDIX

Table I. Catalog of Philippine *Ficus* subg. *Sycomorus* host and their published *Ceratosolen* pollinator across their range and in the Philippines. Species in boldface are endemic to the Philippines. Publications citing the occurrence of the respective species in the Philippines are indicated inside parentheses. For all host species, except *F. lepicarpa*, we were able to sample additional pollinators. These are reported in this table, although these species have not yet been formally described.

Host *Ficus*	**Published *Ceratosolen* pollinator**	**Philippine pollinators sampled in this study**
F. benguetensis	***C. cornutus*** (Wiebes 1994)	***C. cornutus*** plus **3 undescribed species**
F. botryocarpa ssp. *botryocarpa*	***C. corneri*** (Wiebes 1963)	***C. corneri***
F. botryocarpa ssp. *subalbidoramea*	None	***C. corneri*** (shared with ssp. *botryocarpa* and *F. linearifolia*)
F. carpenteriana	None	1 undescribed species
F. cassidyana	None	2 undescribed species
F. cuneiformis (ex *F. cuneata*)	***C. crassitarsus gracilis*** (Wiebes 1963)	***C. gracilis*** (valid species)
F. fistulosa	None	2 undescribed species
F. laevicarpa	None	1 undescribed species
F. lepicarpa	None	Not yet sampled
F. linearifolia	None	***C. corneri*** (Rodriguez *et al.* 2017a)
F. minahassae	***C. pygmaeus*** (Grandi 1927) [= ***C. polyodontos*** Davis and Engel 2008]	***C. pygmaeus*** plus **1 undescribed species**
F. multistipularis	None	Possibly 2 undescribed species
F. nota	***C. notus*** (Baker 1913)	***C. notus*** plus **3 undescribed species**
F. pseudopalma	***C. bakeri*** (Grandi 1927)	***C. bakeri*** **plus 2 undescribed species**
F. rivularis	***C. ramirezi*** (Wiebes 1991)	***C. ramirezi***
F. satterthwaitei	None	3 undescribed species
F. septica	*C. bisulcatus* (Machado *et al*. 2001, mis-identified) *C. jucundus* (Grandi 1927; Rodriguez *et al*. 2017b)	*C. jucundus* plus **4 undescribed species**
F. sulcata	None	1 undescribed species
F. variegata var. *variegata*	*C. appendiculatus* (Wiebes 1963, mis-identified)	**3 undescribed species** (one shared with other subspecies)
F. variegata var. *garciae*	*C. appendiculatus* (Wiebes 1963, mis-identified)	**1 undescribed species** (shared with other subspecies)
F. variegata var. *sycomoroides*	*C. appendiculatus* (Wiebes 1963, mis-identified)	**2 undescribed species** (one shared with other subspecies)