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**Article** *in* Asia life sciences · January 2016



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ASIA LIFE SCIENCES 25(1): 1-22, 2016
The Asian International Journal of Life Sciences

# Potential pollinators of nipa palm (*Nypa fruticans* Wurmb.)

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The potential pollinators of *Nypa fruticans* Wurmb. were studied from February to August 2013 after determining the natural pollinating strategy of the palm in Hagonoy, Davao del Sur, Mindanao, Philippines. Observations showed that all six replicates of both the emasculated inflorescence (percent fruit set, 42.17±33.16) and the untouched (control) inflorescence (percent fruit set, 55.17±27.75) had fruit sets significantly different over the bagged inflorescence (percent fruit set, 11.17±27.35) which fruit set developed only in 3 out of 6 replicates. The emasculated inflorescence reveals the important role of pollinating vectors, especially insects during the female anthesis. Potential insect pollinators hovering over tagged inflorescences were collected from three different sites in Talomo, Davao City, Carmen, Davao del Norte and Hagonoy, Davao del Sur at three periodic sampling, 9:00 AM, 12:00 PM and 3:00 PM for 14 days, the entire duration of male phase. Three species of coleopterans were identified, namely: *Epuraea* sp. 1, and *Epuraea* sp. 2 under the family Nitidulidae and one under the family Staphylinidae. They were observed in abundance inside the bracts of the male and female inflorescences. Three dipteran species were also identified, namely: *Drosophila* sp. 1 and *Drosophila* sp. 2 under the family Drosophilidae and one under the family Agromyzidae. The hymenopterans reckoned were the stingless bee, *Tetragonula* sp. and the Asian honeybee, *Apis cerana* under the family Apidae. Temporal distribution showed that *Epuraea* sp. 1 and *Drosophila* sp. 1 were consistently abundant at 9AM in all areas. The amount of pollen grains carried by or adhering to the body and appendages of the insects revealed that hymenopterans carried more pollen grains, but are known to carry them into their hives after foraging. Thus, the good amount of pollen grains recorded adhering to the bodies and appendages of *Drosophila* sp. 1 and *Epuraea* sp. 1 and their respective frequencies within the site establish their apparently considerable role in the pollination of the *N. fruticans* inflorescences. The identification of insect species is also helpful in generating a comprehensive inventory of potential *N. fruticans* pollinators.

***Keywords:*** *Nypa fruticans,* nipa palm, inflorescence, anthesis, pollination, pollen load, Coleoptera, Diptera, Hymenoptera

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Received 21 June 2015; Accepted 10 October 2015

Printed in the Philippines

## INTRODUCTION

*Nypa fructicans* Wurmb., locally known as 'nipa' in the Philippines, is adapted to saline environment (Joshi et al. 2006, Teo et al. 2010) helping this palm to produce sap with higher sugar content (Rasco 2011). It is estimated that *Nypa* can produce 6,480 to 10,224 $L.ha^{-1}yr^{1}$ of sap which is significantly higher than sugarcane that yields 3,350 to 6,700 $L.ha^{-1}yr^{1}$. This high yield of sap from the palm's fully developed inflorescence stalk is fermented to become alcohol, and in effect biofuel (Hamilton & Murphy 1988, Fong 1992, Päiväke 1996, Rasco 2011).

The inflorescence stalk serving as a conduit of toddy where it is tapped relies on pollination. However, information on the pollination in *N. fruticans* has not been fully studied. Literature accounts on insects serving pollinating agents of this palm are limited as well.

Anthophiles are animals which feed on flowers and are known to bring about pollination (Inouye, 1980, Kevan & Baker 1983, 1998). Most species of anthophiles under the Class Insecta include bees and wasps, many ants (Hymenoptera), true flies (Diptera), moths and butterflies (Lepidoptera), and some families of beetles (Coleoptera) (Arita & Martinez 1990).

These insect species are important agents of pollination particularly among cross-pollinating plants such as *Nypa*. Since this is a protygynous palm, the female flowers open ahead of the male ones. According to Mantiquilla et al. (2013), the male inflorescence (staminate rachilla) opens a day after pistillate head anthesis, thus giving more significance to the role of insect pollinators.

Among the various spectra of insect species that visit the palm inflorescences, coleopterans are the dominant visitors especially when the male anthesis occurs where fragrance emitted (Azuma et al. 2002) and there is thermogenesis (Barfod et al. 2011). Beetles constitute the most important group of pollinators in palms, followed by bees (Hymenoptera) and flies (Diptera) (Barfod et al. 2011).

A preliminary study conducted by the University of the Philippines Mindanao on possible pollination of *Nypa* inflorescence involved a variety of insects. Caballero (2012) observed five different insect orders hovering over its inflorescence, namely Coleoptera, Diptera, Lepidoptera, Hymenoptera and Hemiptera. Only families of *Staphilinoidea* and *Curculionidae* from the Order Coleoptera, Family *Drosophilidae* from the Order Diptera and Family *Apidae* from the Order Hymenoptera were observed to carry pollen grains, thus considered as potential pollinators.

The pollen grain of *N. fruticans* is identified as zonasulcate. This means that the aperture of the pollen is perpendicular to the equatorial region. It has also broad and solid bases and a finely perforate exine (Muller 1964, Erdtman 1969, Thanikaimoni 1987, Chumchim & Khunwasi 2011). Moore et al. (1991) also established the shape of the *Nypa* pollen, which he characterized as non-angular. It may exhibit either circular or elliptical shape.

Given the limited information on *Nypa* pollination, the study was to generally collect and identify potential pollinators of the palm. Specifically, the objectives of the study were as follows: (1) determine the natural pollination strategy of *N. fruticans* in support of the role of entomophily; (2) characterize and identify the collected potential pollinating insects; (3) establish an inventory of potential pollinating insects belonging to Orders Coleoptera, Diptera and Hymenoptera within *Nypa* stands; (4) examine and quantify the pollen grains attached to the bodies and appendages of the potential insect pollinators and (5) determine the temporal distribution patterns of the specific potential pollinating insects.

## MATERIALS AND METHODS

***Sampling sites.*** The field and laboratory activities were conducted from February to August 2013. The field collections include the *Nypa* stands of the following areas: (1) Talomo, Davao City; (2) Carmen, Davao del Norte and (3) Leling, Hagonoy, Davao del Sur (all in Mindanao, Philippines).

*N. fruticans* grows in different environmental conditions with brackish or mineral-rich waters in the Davao region (Mohammad Isa 2011). The sampling site in Brgy. Ising, Carmen, Davao del Norte is described as a floodplain where a river runs through it. The stands in Shimric Beach Resort, Talomo, Davao City are found in an estuary, i.e. at the mouth of the river near the coast. The stands in Leling, Hagonoy, Davao del Sur are found in a riparian environment but drier than those of the other two sampling sites. All the stands go through seasonal inundation which makes the soil moist.

Sorting, classification and identification of insects as well as pollen counts were conducted at the Dr. Severo T. Bastian, Jr. Plant Science Laboratory of the College of Science and Mathematics, University of the Philippines Mindanao, Mintal, Davao City.

***Natural pollination strategy of Nypa.*** The field set-up was conducted in Leling, Hagonoy, Davao del Sur. It was aimed to determine the incidence and extent self-pollination in *Nypa*. It also sought to ascertain the role of other possible agents such as wind (physical) or insects (biological) in *Nypa* pollination as mentioned in literature accounts.

For this experiment, 18 randomly selected pre-anthesis inflorescences were tagged for the following three treatments replicated 6 times:

T1 – inflorescence emasculated before the female flower opens;

T2 – inflorescence bagged upon inflorescence emergence to exclude arthropods and pollen coming from other sources, and bags removed only after stigmatic receptivity and

T3 – inflorescence left undisturbed;

The inflorescence was observed up to the period of fruit set. T1 was to resolve whether other agents affect pollination of *Nypa* if fruit set ensues. The bagged experiment (T2) was to answer whether self-pollination is a natural phenomenon in *Nypa* if fruit set occurs thereafter. T3 served as Control for the entire experiment.

***Collection of insects.*** Five palms having an inflorescence were randomly selected per sampling site. Each palm was considered a replicate. Insects were collected during the male phase that overlaps with the female anthesis within a period of 14 days.

Flying insects were caught randomly using two lids of a Petri dish and creeping individuals were scraped off from the staminate rachilla using a blood collection tube (13 x 75 mm). The capture of insects was done three times to serve as a subsample per inflorescence: 9:00 AM, 12:00 PM and 3:00 PM, to determine the temporal distribution patterns of specific insects. One species per Petri dish or blood collection tubes was captured regardless of the number of individuals for ease of pollen counting. Average pollen count was calculated if there are more than one individual of a certain insect species in each container. Clean Petri dishes were used for each sampling.

***Sorting and labelling of collected insects.*** Sorting and labelling of collected insects were done after 24 hr from capture to ensure that the insect is immobilized for ease of transfer into blood collection tubes containing at least 2 mL 70% ethyl alcohol (Duporte 1977). The Petri dishes from which the insects were first stored were cleaned with 70% ethyl alcohol to ensure complete removal of all pollen grains from the collected insect.

Grouping of samples was based on the period of collection, inflorescence number and area. Each collection was labelled accordingly using a small strip of paper, approximately 0.5 x 2 cm, with the aforementioned information (Raros & Reyes 1980, Forrest 2010).

***Identification of insects.*** Identification of the potential pollinators was carried out in the laboratory based on actual observations, field guide by Borror and White (1970) and dichotomous keys used by Raros and Reyes (1980). Mounted specimens with 40x magnification on a dissecting microscope (Raros & Reyes 1980) were photographed using a digital camera. The body length, body width and wing venation of five randomly selected individuals of the same species from every site were also measured for its characterization. Individual count was also conducted to produce an inventory of the potential pollinators.

The identified insects were stored in a 3 mL blood collection tubes containing 95% alcohol (Raros & Reyes 1980) labelled with their respective order, family, genus, species (if possible), date collected, locality, period of the day and palm number (Forrest 2010, Raros & Reyes 1980). These specimens served as guide to identify succeeding collections. Confirmation of the identification of the insects was solicited from an entomologist and from the Museum of Natural History, University of the Philippines Los Banos, College, Laguna.

***Pollen count.*** Collected insects were examined under a compound microscope for the presence of *Nypa* pollen grains attached on their body. The samples in every container were kept in 3 mL 70% ethanol solution. A haemocytometer was used for the counting of pollen grains wherein 0.3 mL of the suspension using a micropipette was placed onto the chamber of the haemocytometer and observed under light microscope with 40x magnification for the average of five chambers (Caballero 2012).

The number of pollen grains the individual carried per 3 mL ethanol solution was determined with the following formula (Mather & Roberts 1998):

$$\frac{\text{Average count per species x 10 x }(10^4)}{3(\text{volume of solution})}$$

where:

average pollen count per species = average values per replicate divided by three replicates divided by the total number of individuals

A high pollen count value indicates that an insect species is an effective pollinator because it was able to successfully use its body as a mechanical vector for pollen transfer if it eventually visits female flowers.

***Data analysis.*** Data collected on the determination of natural pollination strategy of *Nypa* was analyzed using a one-way ANOVA in completely randomized design (CRD). Data was log transformed for proper statistical analysis. Duncan's Multiple Range Test (DMRT) for comparison of treatment means was used through DSAASTAT program.

## RESULTS AND DISCUSSION

***Natural pollination strategy of Nypa.*** All inflorescences in Treatment 3 attained fruit set indicating occurrence of pollination (Table 1). Interestingly, all inflorescences in Treatment 1 also achieved fruit set, indicative of pollination by external agents. However, three out of six inflorescences in Treatment 2 did not develop fruit set suggesting absence of pollination in the former.

The Treatment 1 set-up revealed that *Nypa* depends on cross-pollination. According to Mantiquilla et al. (2013), the male flower of *Nypa* opens a day after the emergence of the female flower. This sequential inflorescence development, called protogyny, permitted the emasculation of the male flower at a perfect timing for the experimental treatment. With the absence of staminate rachilla, the pollination of the female flower could have been brought about only by pollen transfer from different palms through either physical or biological agents.

However, wind as a possible agent of pollination is deemed a remote possibility. The following obstacles make it difficult for wind to lodge pollen by chance on receptive female inflorescence: (a) the thickness of the palms in the patches of *Nypa* in the area; (b) the long distances of the randomly selected inflorescence under study spread over an estimated 10-hectare area; (c) the low-lying nature of the inflorescence under the thick foliage canopy of the palms and (d) the direction of wind currents which may not be in the path leading to female inflorescence. Adding to these perceived deterrents, is the synchrony in time of pollen availability and stigma receptivity of female flowers in which only biological agents could discern.

Table 1. Status of the inflorescence 30 days from treatment application.

| Treatment | Pollination Occurrence [Y (Yes) / N (No)] | | | | | |
|---|---|---|---|---|---|---|
| | Inflorescence | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 (emasculated inflorescence) | Y | Y | Y | Y | Y | Y |
| 2 (bagged inflorescence) | N | N | N | Y | Y | Y |
| 3 (control - no treatment) | Y | Y | Y | Y | Y | Y |

As such, alate insects that are always seen in abundance among inflorescence possess receptors that allow them to home in on pollens that are ready for picking and the receptive female flowers that are oozing with nectar.

Treatment 2 has revealed that self-pollination was not an obligate event in *Nypa*. Indicative signs of this observation were the absence of elongation in the individual florets and the deficiency in growth of the inflorescence diameter, which theoretically was supposed to have increased after a month into the developmental phase. Furthermore, the underdeveloped florets were falling off easily from the cluster, suggesting even more that self-pollination has not occurred. The three inconsistencies in this treatment were surmised to be attributed to the loosening of the rope used to tie down the net bags with the inflorescence inside. The loosened rope may have allowed the entry of creeping insects and arthropods which could have pollinated some of the inflorescence inside the bag.

However, it was also observed that slim chances of self-pollination may occur in the same palm within 48 hr from pistillate head emergence as long as the staminate rachilla are open and contain viable pollen. Not all female flowers may be fertilized through gravity or light air currents by pollen coming from the same inflorescence within the 48-hr period since bracts still cover the other female flowers situated at the basal portion of the pistillate head. Therefore, the suggested two possible ways for fertilization to come about even after the 48-hr time frame are: (1) by entomophilous insects carrying pollen from other palms and (2) by beetles that forage on the same inflorescence and coming in contact with receptive stigma carrying still viable pollen in their bodies or appendages.

Observation on the experimental inflorescence was extended up to 60 days. The number of seeds were counted and tallied from the tagged inflorescences (Table 2). Compared to the previous data in Table 1, there were some major differences recorded. In the case of the Treatment 1 set-up, the all-pollinated status of the six replicates was reduced to four. The two replicates, specifically Replicate 3 and Replicate 6, had stopped developing resulting to fruit abortion. Not discounting other factors, it is theorized that only a few of the florets in the inflorescence were pollinated hence their loose attachment to the core of the flower head. This uneven pollination of the pistillate head may have resulted to a decrease in the hold of the florets to each other at their proximal end, permitting the gradual spread of halted growth all over the inflorescence. This may have also been the probable cause of the failure of infructescence development of Replicates 4 and 6 of Treatment 2.

Seed counts between the control and Treatment 1 did not vary but both were significantly higher than the number of seeds yielded by Treatment 2 (Table 2). The 0 value of the third replicate in Treatment 3 may have been caused by anthropogenic factors. Since the site was continuously exploited for *Nypa* products, the tagged fruit head may have been accidentally cut off.

Table 2. Seed count per fruit head after 60 days.*

| Treatment | Replicate | | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| 1 (emasculated inflorescence) | 58 | 62 | 0 | 59 | 74 | 0 | 42.1667 ± 33.1567[a] |
| 2 (bagged inflorescence) | 0 | 0 | 0 | 0 | 67 | 0 | 11.1667 ± 27.3526[b] |
| 3 (control - no treatment) | 74 | 67 | 0 | 63 | 56 | 71 | 55.1667 ± 27.7159[a] |

*Treatment means having the same letter are not significant at α = 0.05 using DMRT.

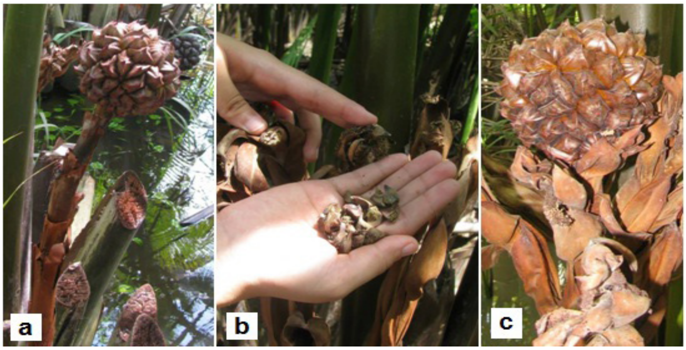


Figure 1. After 60 days, (a) T1 showed fruit set of a pollinated inflorescence; (b) T2 showed unpollinated detached florets from the whole inflorescence and (c) T3 showed normal development of untouched inflorescence to fruit set.

A similar study was done by Clarke and Myerscough (1991) which also aimed to identify the degree of influence of insect pollinators on the overall growth and development of *Avicennia marina* mangroves in Australia. In their experiment, a set of flowers of the mangrove species were bagged with nylon mesh, while flowers in the other set were left untouched. All flowers that were left open developed mature fruits while those that were bagged for months, produced significantly fewer mature fruits. However, *A. marina* is a protandrous species, that is, male flowers mature first than the female flowers. According to Primack et al. (1981), protandry among mangrove species promotes cross-pollination and that insect pollinators facilitate it. Moreover, this evidence may explain the analogous result yielded by natural pollination strategy experiment on the protygynous *N. fruticans.*

## POTENTIAL POLLINATORS OF *NYPA*

***Order Coleoptera.*** The beetles were found abundant during hot weather and their activities intensified during male phase where staminate rachillae emerged and dehisced pollen. In this study, specimens identified include two from the Family Nitidulidae and one from the family Staphylinidae.

*Epuraea* sp. 1 (Figure 2) is an orange-brown beetle which is oval in shape, has capitate antennae, mandibular mouthparts, cursorial legs and short elytra which expose the terminal abdominal segment. The average size of this beetle measures 2.04 x 0.98 mm.

In the field, the beetle is commonly observed on the male inflorescence especially at the onset of anthesis. It was also seen inside the bracts of the male and female inflorescence.

*Epuraea* sp. 2 (Figure 3) has brown to black, elongated-oval shaped body, capitate antennae, mandibular mouthparts and cursorial legs. Its elytra are much shortened, compared to *Epuraea* sp. 1, exposing 3 abdominal segments. However, this beetle is slightly bigger at an average size of 3.31 x 1.20 mm.

In the field, the beetle is also commonly seen on the staminate rachillae and inside their bracts but a few days after male anthesis (Figure 5). It rarely visited the female inflorescence, and they appeared to feed on available nectar (Watson & Dallwitz 2003, Gullan & Cranston 2010). Compared with *Epuraea* sp. 1, it was less abundant and relatively a slow creeper.

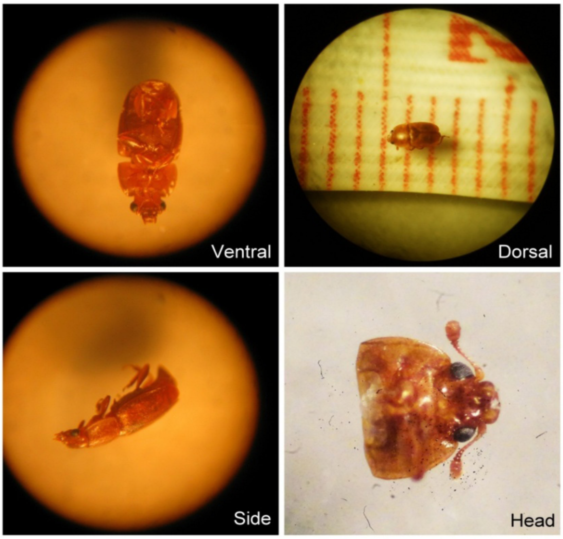


Figure 2. *Epuraea* sp. 1 (Coleoptera: Nitidulidae) (40x).


Figure 3. *Epuraea* sp. 2 (Coleoptera: Nitidulidae) (40x).

The adults were observed to inhabit the interior of the bracts of the female and the male inflorescences as they provide them shelter and protection against predators (Sakai 2002, Barfod et al. 2011). There were white larvae, likely of nitidulids, found inside the staminate rachillae, and a larger colored larvae including some adult beetles inside the female bracts. Their presence together with the adult beetles caused damage not only in the bracts of the *Nypa* inflorescence prior to its anthesis but also to the staminate rachillae and female flowers during their respective antheses.

The adult insects are strong fliers and measure from 0.9 to 15 mm in length. Their antennae are usually eleven-segmented with a club composed of 3 flattened distal segments (Parsons 1943, Raros & Reyes 1980). The elytra cover the entire dorsal and sometimes shortened exposing two or three abdominal segments. The prosternum has a process produced between the front coxae. They have 5-segmented tarsi and segmentation is 5-5-5. The adult nitidulids also possess olfaction capabilities (Okumura & Savage 1974).

Only one species under the Family Staphilinidae was collected (Figure 4) with a black body and filiform antennae having orange-brown to black gradient. The legs are cursorial also orange-brown in color and elytra that are very much shortened exposing 5 abdominal segments. Samples of the specimen when viewed laterally show their abdomen lifted up looking like an obtuse angle. From the sample specimens collected, the average size of the beetle is 1.67 x 0.59 mm.

The staphylinids were observed creeping on the side of the staminate rachillae that is not directly exposed to the sun and under the bracts. They were also rarely seen on the female head that seems to be preying on other invertebrates including fly larvae which is previously reported by Henderson (1986) to be present on the female inflorescence or may be eating remaining stigmatic secretions of unpollinated florets (Labarca & Loewus 1973). As observed, they crept and were not strong flyers as they hovered over the same inflorescence.

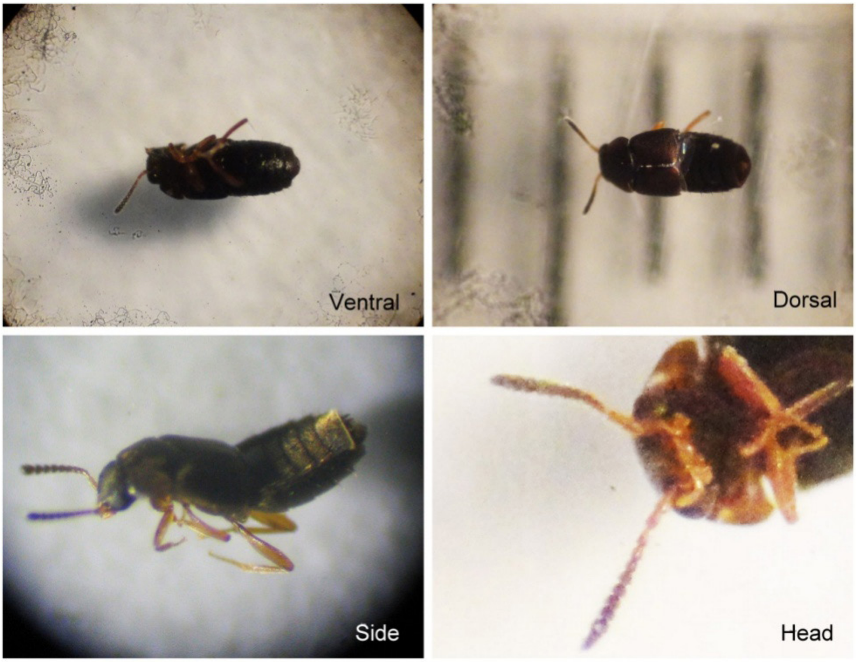


Figure 4. Staphylinid (Coleoptera: Staphylinidae) observed on nipa inflorescence (40x).

There were other coleopterans found on the inflorescence but considerably lower in numbers relative to the other beetles collected. They were identified to be under the families Nitidulidae and Curculionidae.

The nitidulid from this group was identified as *Haptorcus* sp. looking similar to *Epuraea* sp. 1 except that it has a constricted part right after the prothorax which makes it distinct. The curculionids long narrow snout or rostrum and clubbed and elbowed antennae (Schneider 1999) distinguish them from other beetles (Hilty 2013). On African oil palms (*Elaeis guineensis*), some curculionids are known to feed on pollen grains and complete their whole life cycle on the male inflorescence (Sambathkumar & Ranjith 2011). Aside from being known as primary pollinators of oil palm (Sambathkumar & Ranjith 2011, Tuo et al. 2011), curculionids have been also known as pests of some palm species (Weissling & Giblin-Davis 1997).

***Order Diptera.*** Three dipterans were reckoned from the collection namely: *Drosophila* sp. 1 and *Drosophila* sp. 2 from the family Drosophilidae and one under the family Agromyzidae. Of the three species, *Drosophila* sp. 1 (Figures 6 & 7) was the most abundant caught in all the three sampling sites. Its high visibility during male anthesis strongly suggests its relevance in promoting pollination by shuttling between male and female flowers. Moreover, the presence of *Drosophila* sp. 1 was still extended even with the staminate rachillae dried off and devoid of pollen grains albeit decreased in number.


Figure 5. Adult *Epuraea* sp. 2 (A) creeping on staminate (male) rachilla (B) with some staphylinids inside a bract of a male inflorescence.

*Drosophila* sp. 2 (Figures 8 & 9) was second in abundance where its number eventually declined with time ending up in the female flowers after foraging the staminate rachillae. The Agromyzid (Figures 10 & 11), outnumbered the two drosophilids in Hagonoy area only. It moved from one palm to another promoting a greater distance of outcrossing during pollination.

The other prominent group of insects found in the *Nypa* sampling sites were hymenopterans (Figures 12 & 14).

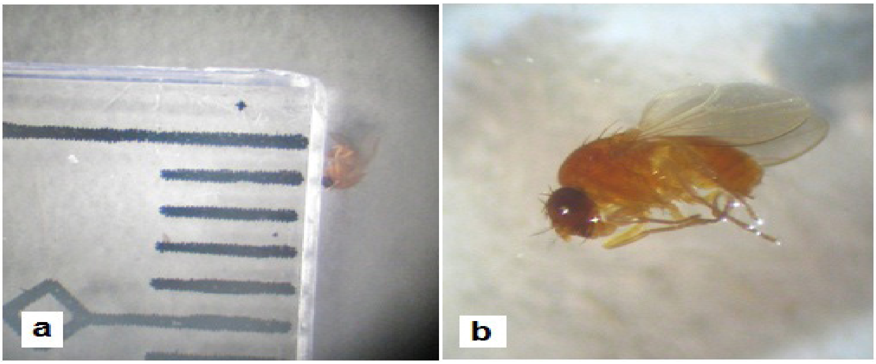


Figure 6. (a) *Drosophila* sp. 1 from Order Diptera measuring 1 mm in body length. (b) shows the yellow-orange color of the entire insect body.

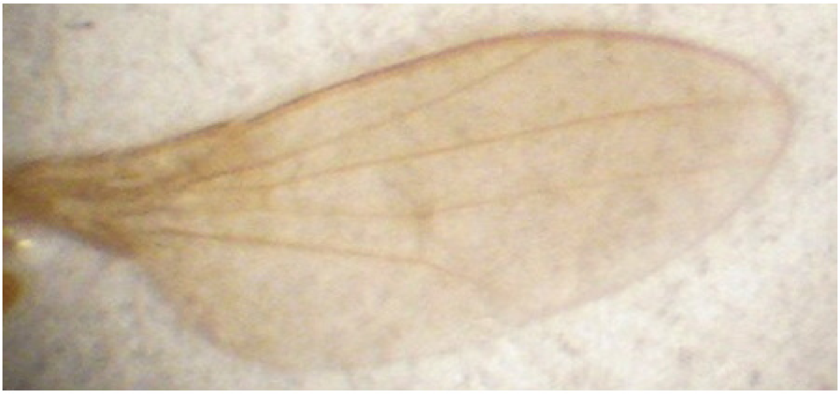

Figure 7. Wing morphology of *Drosophila* sp. 1.

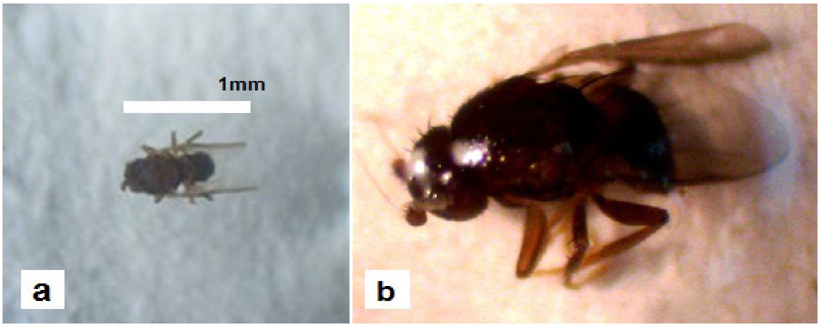


Figure 8.(a) *Drosophila* sp. 2 from Order Diptera measuring 1 mm in body length. (b) shows the black color of the entire insect body.

Figure 9. Wing morphology of *Drosophila* sp. 2.

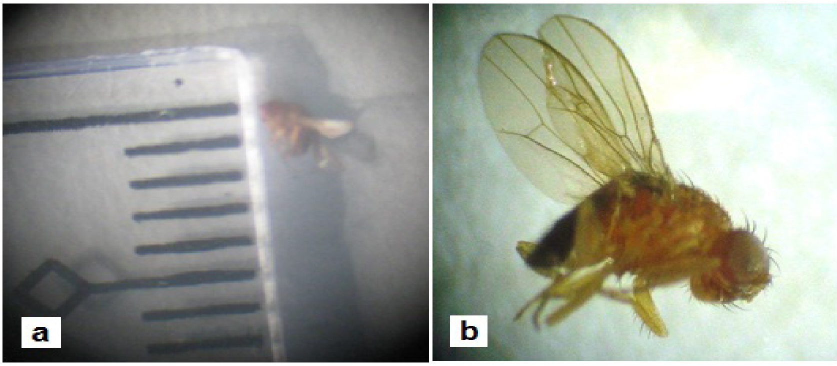


Figure 10. (a) The Agromyzid from Order Diptera measuring 1.75 mm in body length. (b) shows the yellow-orange and black color combination of the entire insect body.

Figure 11. Wing morphology of species the Agromyzid.

***Order Hymenoptera.*** The stingless bee (*Tetragonula* sp.) was constantly present during sampling periods 9:00 AM and 12:00 PM period in Talomo and Carmen, but very rarely observed on *Nypa* inflorescence in Hagonoy. The insect was noted to carry abundant pollen grains on their legs which likely lodge them on female flowers after feeding off from male flowers. The other species of bee recorded was *Apis cerana* which are considerably larger than the stingless bee.

Other insects frequently found near the *Nypa* inflorescence at the sampling sites, but are not regarded as pollinators were braconids of Order Hymenoptera and earwigs of the Order Dermaptera. These insects are identified as predatory insects.

***Temporal distribution patterns.*** Three groups of insects were observed consistently present during male anthesis, namely: *Epuraea* sp. 1, *Drosophila* sp. 1 and the staphylinid beetle. It was in only in Carmen, Davao del Norte where *Epuraea* sp. 2 slightly outnumbered the staphylinid (Figure 16).

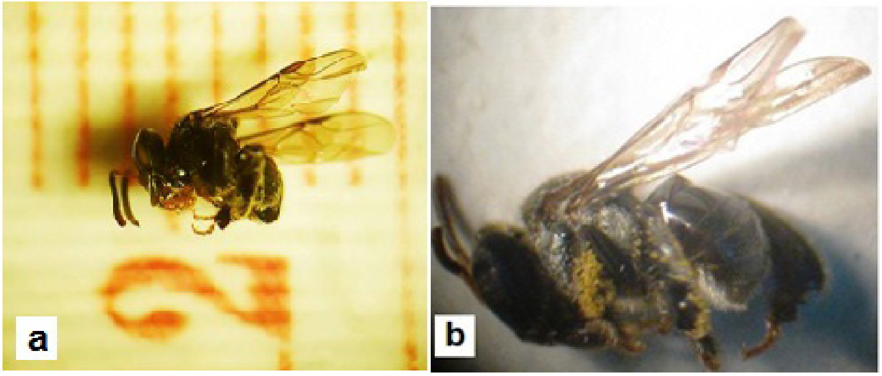


Figure 12.(a) *Tetragonula* sp. of Order Hymenoptera measuring 4 mm in body length. (b) showing the pollen grains stuck to the corbiculum of the insect's leg.

Figure 13. Wing morphology of *Tetragonula* sp.

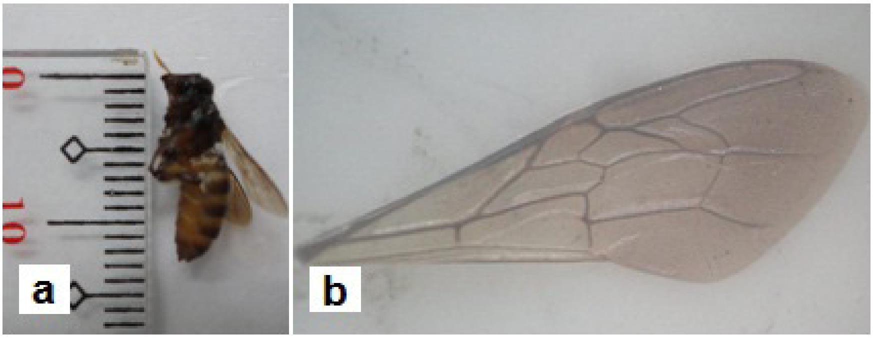


Figure 14. (a) *Apis cerana* under the Order Hymenoptera measuring 10.1 mm in body length. (b) Its wing morphology.

While the staphylinid increased, both *Epuraea* sp. 1 and *Epuraea* sp. 2 decreased in number during the late afternoon in areas near the coast such as Talomo, Davao City and Hagonoy, Davao del Sur in the estuaries (Figures 15 & 17).

However, in an inland environment, while the nitidulids decreased at the middle of the day and increased again by late afternoon. The temporal distribution of the three beetles was most probably affected by the changes in the temperature in the environment. Metabolic rates of the insects, including the beetles, tend to increase when the environment temperature rises since they are cold-blooded, so when the tide is low, the beetles were less active (Museum of Archaeology and Ethnology 2010).

*Drosophila* sp. 1 stood out as the most abundant among the drosophiloid insects in the three sampling sites. It was the most consistent flower visitor throughout the day, since its number did not markedly lessen throughout the day. It even surpassed the number of *Epuraea* sp. 1 in Carmen, Davao del Norte in the latter part of the day (Figure 16). *Drosophila* sp. 2 and the agromyzid were even rarely seen during male phase as observed in three areas. This trend was also noticed among the hymenopterans *Tetragonula* sp. and *A. cerana* that consistently decreased in number even to the point of their absence in the latter part of the day.

The continuous appearance of the two drosophiloid species and the agromyzid during male anthesis is thought to be brought about by sapromyiophily, a pollination system by deception. Entomophilous plants have developed several attractants, such as colors and odors in order to increase interactions with insect pollinators (Proctor et al. 1996). *Nypa'*s floral scent contains 25 chemical compounds which includes; fatty acid derivatives, terpenoids, carotenoid derivatives, benzenoids and unknown compounds which gives the inflorescence a distinct odor that could have enticed over the said insect species (Azuma et al. 2002).

The hymenopterans on the other hand are known to forage early in the day at about 9:00 to 11:30 AM (Himalayan Time) when the temperature ranged between 15.5 and 21°C and stops later in the day. Observations from all three sampling sites where *Tetragonula* sp. and *A. cerana* decreased in number as each collection hour passed, concur with Verma et al. (1986).

***Pollen Count.****Nypa* pollen is identified through its unique morphology which is circular with spiky projections (Figure 18). Pollen counts among the hymenopterans were found remarkably higher compared to dipterans and coleopterans sampled in all areas (Figures 19, 20 & 21). While their number was fewer during inflorescence anthesis, the species *A. cerana* carried the most pollen followed by *Tetragonula* sp. These pollen loads can be ascribed by the presence of corbicula in the tibia of their legs which act as pollen baskets (Britton 2012). As the bees forage on the flower, pollen grains are brushed into the basket enabling the insect to "carry" it to their hives or other flowers. The amount of pollen grains carried by an individual insect, *A. cerana* and *Tetragonula* sp. is suggestive of qualifying them to making the list of pollinators of *Nypa* if they are also found to alight on female flowers during

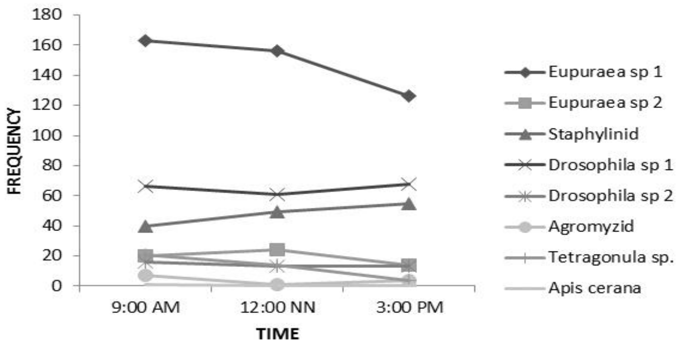


Figure 15. Temporal distribution of the five species at selected time periods in Talomo, Davao City.

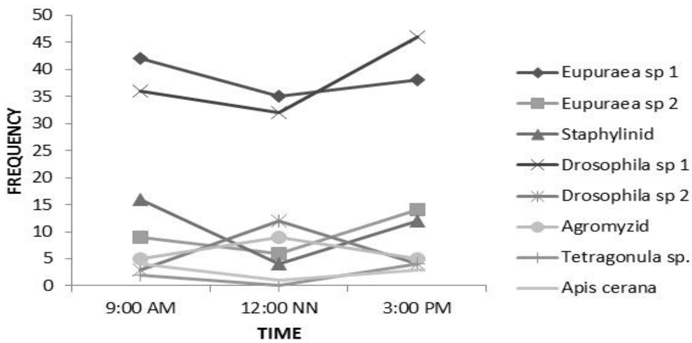


Figure 16. Temporal distribution of the five species at selected time periods in Carmen, Davao del Norte.

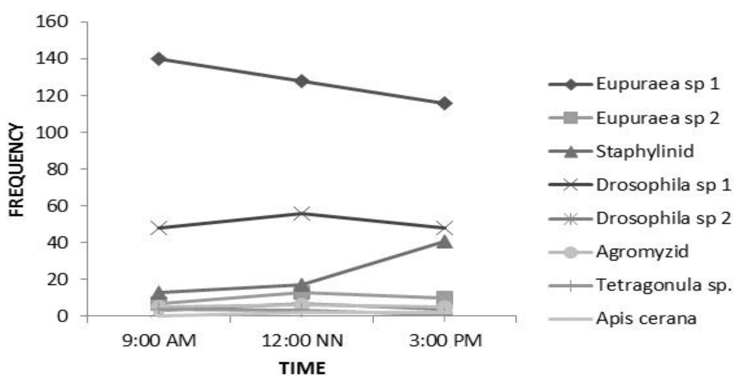


Fig. 17. Temporal distribution of the five species at selected time periods in Hagonoy, Davao del Sur.

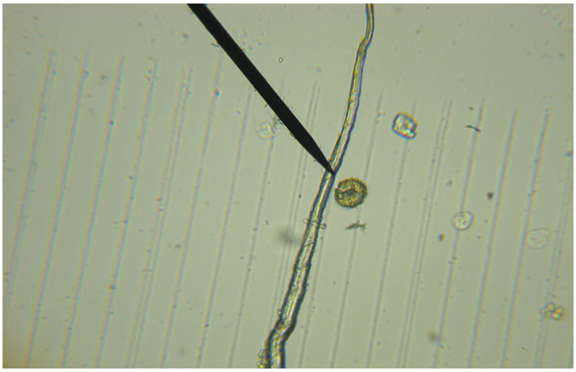

Figure 18. *Nypa* pollen on a haemocytometer under light microscopy (100x).

The three drosophilid species, on the other hand, had less pollen counts compared to the bees. This is overtly attributable to their small size, which sets a limit to their pollen-carrying capacity. However, they are continuously present within the inflorescence area, thus providing enough evidence that these drosophilids are pollinators of *N. fruticans.* They could possibly be even more efficient pollinators than the bees as the latter are naturally designed to deposit their pollen loads to their hives after foraging. Furthermore, as the dipterans are more frequently seen in high numbers on female flowers than other insect species lend support to the report of Hamilton and Murphy (1988) that the drosophilids may be the most effective pollinators of *N. fruticans.*

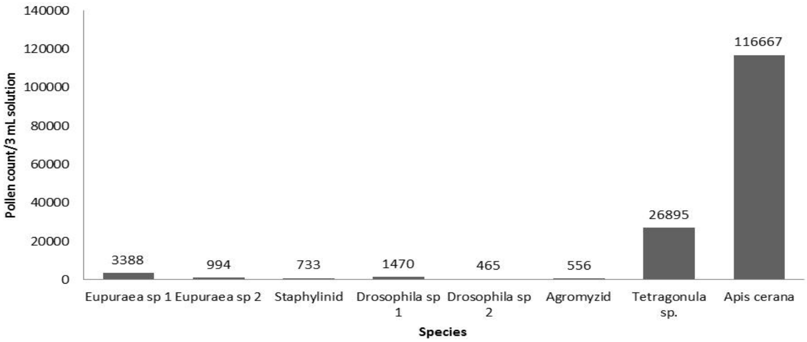


Figure 19. Pollen count from collected specimens in Talomo, Davao City.

Like the drosophilids, the three commonly collected coleopterans had low pollen load albeit highly visible during inflorescence opening. *Epuraea* sp. 1 and *Epuraea* sp. 2 had relatively strong associations with staminate rachillae compared to Staphylinid (Figures 19 & 21). The latter was observed to increase pollen capacity only with the decrease of the load of the nitidulids as shown in Carmen (Figure 20) likely attributed to competition. According to Krakos et al. (2010), coleopterous insects are considered too small to carry effective pollen load and have a short flying range. They were recorded as plant pollinators but were not recognized as the primary pollinator. Hence, regardless of pollen load, the beetles' capability to carry a good amount of pollen is a condition necessary for pollination as they forage and move around amongst inflorescence.

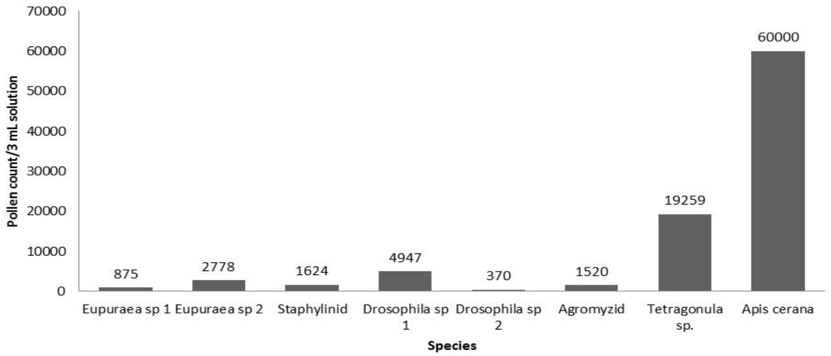


Figure 20. Pollen count from collected specimens in Carmen, Davao del Norte.

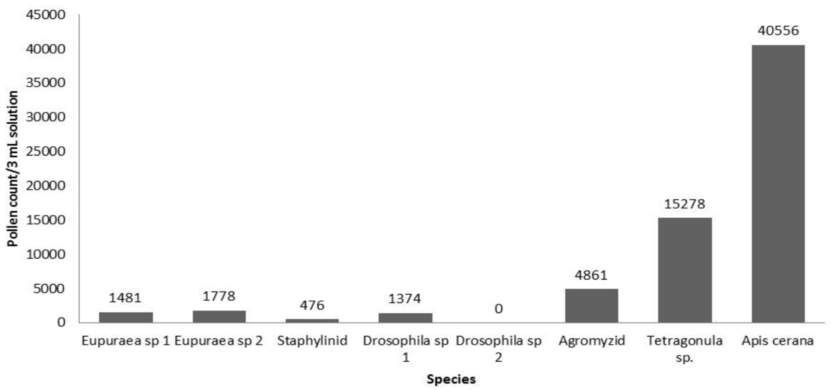


Figure 21. Pollen count from collected specimens in Hagonoy, Davao Del Sur.

## SUMMARY AND CONCLUSION

*Nypa fruticans* has numerous ecological and economic benefits. Its sugar-rich sap tapped from its inflorescence stalk holds a promise as source of biofuel in the future. Hence, understanding its natural propagation through insect pollination is essential.

The study was conducted from February to August 2013 by first setting up the natural pollinating strategy experiment. It involved 18 palms with inflorescence assigned to three treatments replicated 6 times, namely: inflorescence emasculation, inflorescence bagging and inflorescence undisturbed serving as Control. Apart from the Control (percent fruit set, 55.17±27.75), all emasculated inflorescence (percent fruit set, 42.17±33.16) developed into infructescence while the bagged inflorescence (percent fruit set, 11.17±27.35) had only 3 out of 6 replicates developed into fruit heads. The results indicated that open pollination in *Nypa* exists and that the availability of pollen from other inflorescence or palms at stigma receptivity periods is important to ensure pollination.

Insects were collected from the inflorescence during a 14-day period, a time frame within which female anthesis occurs. The collection of insects was done daily at the following periods: 9:00 AM, 12:00 PM and 3:00 PM at 3 replicates per period. A Petri dish was used to capture flying individuals randomly, and a blood collection tube for the creeping individuals, scraping them off from the staminate rachillae. Individuals were identified using morphological characterization and dichotomous key.

There were three commonly collected species of coleopterans from the three sampling sites. Two species were identified under Family Nitidulidae, namely: *Epuraea* sp. 1 and *Epuraea* sp. 2 and one species under the Family Staphylinidae. The two other minor coleopterans identified was *Haptorcus* sp. belonging to the Family Nitidulidae and a snout beetle under Family Curculionidae.

Three species from Order Diptera and two from Order Hymenoptera were identified as potential pollinators of *N. fruticans.* The three species from the Order Diptera were identified as *Drosophila* sp. 1 and *Drosophila* sp. 2 under the Family Drosophilidae and one species belonging to the Family Agromyzidae, while the hymenopterans were identified as *Tetragonula* sp. and *Apis cerana*.

These insects were considered as pollinators for three reasons. Firstly, *Nypa* is a self-incompatible protygynous palm. It needs the help and activity of pollinating agents, such as insects to induce fertilization. This was confirmed by the natural pollinating strategy in the aforementioned experiment conducted on the inflorescence. The results indicated that open pollination in *Nypa* exists and that the availability of pollen from other inflorescence or palms at stigma receptivity periods is important to ensure pollination.

Secondly, the insects of interest of this study were always noted to be present during the male anthesis (peak of pollinating activity), but differed in abundance during the three collection hours, signifying their physical contribution to the process of pollination leading to fruit set. Lastly, the insects were observed to carry significant amount pollen upon investigation during the pollen count, indicating their roles as vectors for pollen transfer from the male to the female flower. These findings indicate the endowment of the identified insect species, especially the drosophiloids, as pollinators of *N. fruticans*.

For future research, it is highly recommended to conduct similar studies in different *Nypa* sampling sites with varied environmental conditions to know if other physical parameters affect the presence and pollinating behavior of the identified insect pollinators. Moreover, other insect orders that can be potential *Nypa* pollinators should be investigated.

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***Asia Life Sciences*** is a recipient of the Journal Accreditation Award of the Commission on Higher Education (CHED), Republic of the Philippines (2010-2016).

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