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Cone insects and putative pollen vectors of the endangered cycad, *Cycas micronesica*

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Abstract–Several sampling methods were used to survey for potential pollinators of the critically endangered *Cycas micronesica* in different forest communities on the islands of Guam and Rota. The most common insects found depended on the method. From direct observations, *Anatrachyntis* sp. (Lepidoptera: Cosmopterygidae) larvae and adults were observed only on male cones. Adult *Carpophilus* sp. beetles (Coleoptera: Nitidulidae) were common on male cones and were occasionally observed on female cones. In bag traps over cones, adult *Anatrachyntis* were consistently trapped and were very abundant on both sexes, and other insects were rarely observed. Sticky collars around cones captured the highest diversity of taxa, mostly Diptera, Hymenoptera and Coleoptera comprising several families within each order, as well as *Anatrachyntis* adults. Two species within the family Phoridae were the most common Diptera and ants were the most common Hymenoptera. The most common Coleoptera were Staphylinidae and Nitidulidae. Similar taxa were trapped on both sexes and from four different habitats on Guam. On female cone sticky traps, ~30% of the pollen grains were associated with *Anatrachyntis* moths or moth scales and less than 5% with other insects; however, over 60% of the pollen was not associated with any insect, suggesting some pollen is wind dispersed. On Rota, 60 km northwest of Guam, *Anatrachyntis* moths and *Carpophilus* beetles were found on cones. In sum, the results suggest both wind and insects as pollen vectors, with *Anatrachyntis* moths the most likely insect vector and, secondarily, nitidulid beetles. To date, no other Lepidoptera has been implicated as a pollinator of any cycad species.

Introduction

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Interest in insect pollination in cycads (Cycadales) has been increasing since studies in the 1980S demonstrated that specialist insects are pollinators of their *Zamia* cycad host, *Rhopalotria mollis* (Coleptera: Curculionoideae) on *Zamia furfuracea* and *R. slossonae* and *Pharaxonotha floridana* (Coleoptera: Erotylidae) on *Z. pumila* (Norstog et al. 1986, Tang 1987) and that wind plays little to no role in vectoring the pollen. These findings were contrary to the long held notion that all gymnosperms are wind pollinated (Chamberlain 1935). Additional research has shown that specialist insects are associated with cones of other cycad genera, and many of these insects have been demonstrated as effective pollinators (see Donaldson 1997, Terry et al. 2005). To date, insects mainly in the orders Coleoptera (primarily within Curculionoidea and Erotylidae) and Thysanoptera (Hall et al. 2004, Kono & Tobe 2007, Mound & Terry 2001, Oberprieler 1995, Stevenson et al. 1998, Tang 2004) have been demonstrated as pollinators, but relatively few species have been studied. All cycads are now believed to have some insect involvement in pollination (Norstog & Nicholls 1997) even in the genus *Cycas* (Cycadaceae, a monogeneric family) whose ovules are enclosed by layers of overlapping sporophylls rather than in a cone as in other extant cycad genera. Herein, for simplicity, we refer to clusters of female megasporophylls as cones. Because cycads are considered the oldest extant lineage of spermatophyte and *Cycas* is considered basal among cycads (Brenner et al. 2003), some of the insect pollination mutualisms may be ancient as well. However, relatively little is known about insects associated with cones of this genus.

In extensive surveys of *Cycas* species in parts of Thailand, Vietnam and China, Tang et al. (1999) discovered beetles (families Curculionidae and Languriidae, now Erotylidae, see Leschen 2003) in male and female cones. In Queensland, Australia, surveys of cones of seven *Cycas* species have reported beetles (families Tenebrionidae, Erotylidae, and Nitidulidae, and Curculionidae) and *Trigona carbonaria* (Hymenoptera) from male cones (Forster et al. 1994, Ornduff 1991). Detailed pollination experiments demonstrated ambophily (both wind and insect vectored pollen) of *Cycas revoluta* on Yonaguni Island (Okinawa, Japan) (Kono & Tobe 2007). Wind effectively vectors pollen to female cones within ~2m of a male cone, and females farther away visited by the pollen bearing beetle, *Carpophilus chalybeus* (Nitidulidae), produced viable seed. In contrast, Keppel (2001) suggested wind as pollen vector for *Cycas seemannii* of South Pacific Islands based on lack of insects; however, Keppel emphasized the need for more systematic surveys. Clearly more studies are needed in this most speciose cycad genus, ~100 out of 300 cycad species world-wide.

Cycas micronesica K.D. Hill is endemic to Guam and the islands immediately north and south of Guam (Rota within the Mariana Islands and Yap and Palau in the Western Caroline Islands). A dominant understory tree and Guam's only native gymnosperm (Fig. 1), *C. micronesica* is a species within the

Figure 1. *Cycas micronesica* in a ravine west of Lamlam site, Guam.

C. rumphii species complex (Hill 1994, Hill 1996) distinguished by a spongy flotation layer within the seed which allows seed dispersal via water currents. Plants of this species complex are found in coastal areas from East Africa and Madagascar, the Andaman and Nicobar Islands, Sri Lanka, coastal SE Asia eastward to the Mariana Islands and western South Pacific islands. On islands that are distant from any mainland cycad, the pollinator-cycad relationship of such cycads may have evolved *de novo*, and a maximum age for an insect - cycad relationship can be established based on the date when any volcanic island formed. Guam's geologic history is complex, with earliest submarine volcanism occurring ~43 Ma during the Eocene, but aerial volcanism and limestone formation much later ~ 21 Ma (Siegrist et al. 1992, Mylroie et al. 2001). Alternatively, if close enough to a mainland, the pollinator may be able to reach the island by flight, wind or on floating vegetation. A third pollination mode would be ambophily, or just wind alone. In this case, the plant could disperse to new sites and still be able to sexually reproduce in the absence of a specific animal vector. Knowledge of the pollination process of *C. micronesica* and related species on islands and the mainland is critical for understanding how these pollination systems arose.

Cycas micronesica has been declared 'endangered' (Marler et al. 2006) due to damage by two invasive cycad-obligate pests, cycad aulacaspis scale (CAS) (*Aulacaspis yasumatsui*) (Homoptera: Diaspididae) and the cycad blue butterfly (*Chilades pandava*) (Lepidoptera: Lycaenidae) (Moore et al. 2005). Eradication of *C. micronesica* from the island is possible due to the combined damage of these invasions and the ongoing cascading responses to the invasions. Some heavily infested areas experienced a 90 percent tree death rate by 2008 (T. Marler, unpublished data). The beetle, *Rhyzobius lophanthae* (Coleoptera: Coccinellidae) has been released for biological control of CAS, and *ex situ* gardens of *C. micronesica* have been established as sources for reintroduction in case of extinction. If this cycad relies on a specific insect pollen vector, then the insect as well as the cycad needs to be conserved. Overall, our objective is to determine the pollination system of *C. micronesica*. The specific goals of this study are to (1) define the taxa associated with cones of both sexes in different habitats on Guam to identify putative pollinators, (2) determine differences in taxa profiles among various trapping techniques, and (3) determine whether *C. micronesica* of Rota, Guam's nearest neighbor, has similar cone visitors as those on Guam.

Methods

STUDY SITES

Insect trapping was accomplished during reproductive growth flushes of June and July 2005 and June 2007. Four native forest areas around Guam were chosen for their geographic location and disparity in habitat factors: Ritidian site northwestern Guam in the Andersen Air Force Base overlay of the Guam

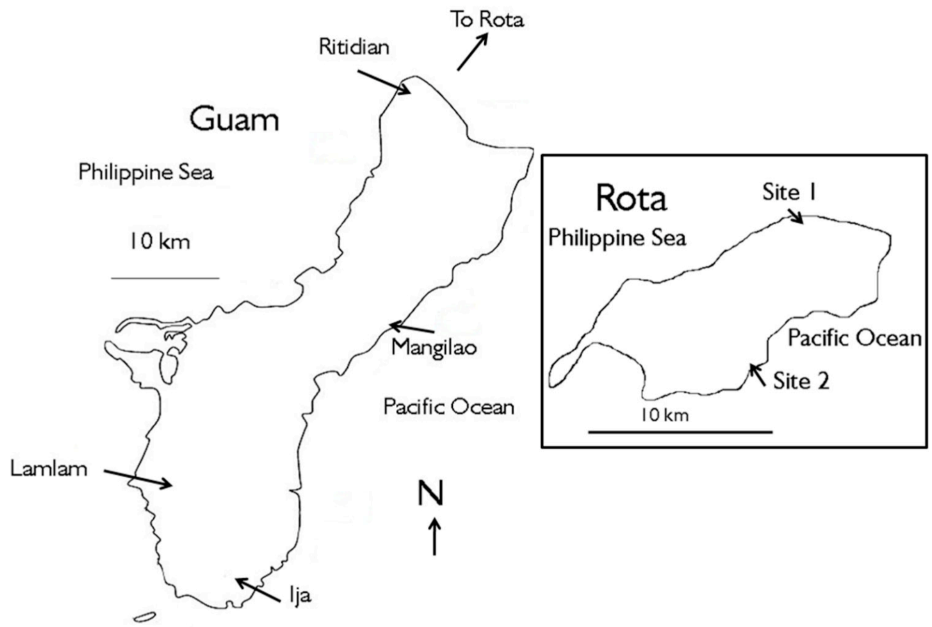

Figure 2. Outline map of the islands of Guam and Rota with sampling sites indicated.

National Wildlife Refuge; Mangilao, northeastern Guam; Ija in a ravine bed formed by Ajayan River in southern Guam; and Lamlam, southwestern Guam (Fig. 2; Table 1). Seed set at Ritidian and Mangilao from the 2007 reproductive flush was 54.5% and 68.5%, respectively (Terry unpubl. data), indicating effective pollination activity at these sites.

Several factors define the geochemistry, water relations, and physiognomy that distinguish each of these sites, and these factors in part define the flora and fauna. Soils: Ritidian, Mangilao, and Lamlam soils are alkaline, highly-drained calcareous sediments of marine derivation and overlie coralline limestone, and Ija soils are acidic, poorly-drained substrates of volcanic origin (Young 1988).

Table 1. Sample site names and locations

Site name	Island	Latitude (E)	Longitude (N)	Elevation Above sea level (m)	Distance from shore (km)
Ritidian	Guam	144º51’24”	13º38’39”	105-135	0.65
Mangilao	Guam	144º50’59”	13º 27’57”	55-65	0.25
Ija	Guam	144º42’31”	13º16’06”	50	2.0
Lamlam	Guam	144º40’17”	13º20’03”	366	2.2
Site 1	Rota	145º14’51”	14º 11’56”	15	0.15
Site 2	Rota	145º13’29”	14º 8’16”	185	0.5

Table 2. Average number of each taxon per sticky trap and taxa richness at each site, and Spearman rank correlations of taxa abundance between sexes

Taxon	Mangilao		Ritidian		Ija		Lamlam	
	F (4) [a]	M (7) [a]	F (7) [a]	M (3) [a]	F(5) [a]	M(1) [a]	F(6) [a]	M(2) [a]
Diptera	10.5	11.7	8.9	6.3	57.4	135.0	68.2	32.0
Hymenoptera	13.8	14.1	6.3	5.3	35.8	12.0	23.2	12.5
Lepidoptera	8.8	8.4	2.4	0	15.4	29.0	7.0	8.0
Coleoptera	3.3	6.7	2.6	3.3	5.8	14.0	8.5	3.5
Arachnid	2.5	3.0	1.1	0.3	3.0	0	1.5	0
Thysanoptera	1.5	0.1	0.4	0	1.8	8.0	2.5	2.5
Acarina	0.8	0.7	2.0	0	3.0	2.0	2.8	0
Orthoptera	0.3	1.0	0	0	0.4	0	0	0.5
Heteroptera [b]	0	0.4	0.4	0	2.2	7.0	4.2	2.0
Curculionoideae	0	0.3	0.6	0	0.2	0	0.2	0
Isoptera	0	0.3	0.3	0	0.2	0	0	0
Blattidae	0	0.1	0	0	0.0	0	0	0
Collembola	5.5	1.9	7.6	3.0	3.6	2.0	2.7	11.0
Unknowns	0.8	3.1	5.3	2.3	3.8	4.0	4.2	5.0
Avg. group richness	8.0	6.7	7.0	4.0	9.2	8.0	8.8	7.0
Total site richness	9	13	11	5	12	8	10	8
R, Spearman rank correlation	0.86		0.80		0.83		0.78	
P	0.0002		0.0009		0.0004		0.0015	

[a] F, Female; M, Male; (*N*), number of cones
[b] Averages exclude CAS, see Table 4)

Table 3. Percentage of each taxa to the total within each insect order on sticky traps, 2005

Insect order	Family/ genus[a]	Mangilao		Ritidian		Ija		Lamlam		Avg	% present[b]
		F (4)[a]	M (7)[a]	F (7)[a]	M (3)[a]	F (5)[a]	M(1)[a]	F (6)[a]	M (2)[a]	%	(F,M)
Diptera	Phoridae[c]	35.5	43.9	67.8	63.9	34.6	90.4	25.7	24.0	48.2	100,92
	Muscidae[d]	17.7	20.4	5.3	19.0	1.6	6.7	12.5	8.9	11.5	59,67
	Ceratopogonidae	7.1	4.9	6.5	3.2	3.8	0.7	1.6	9.4	4.6	38,38
	Drosophilidae[d]	2.4	2.4	0.0	1.0	1.0	0.7	3.0	1.6	1.5	18,31
	Others[d]	7.1	11.0	16.1	3.0	1.7	1.5	18.0	6.3	8.1	n/a
	Undetermined	30.1	17.4	4.3	9.9	57.1	0.0	39.2	49.9	26.0	n/a
Hymenoptera	Formicidae	72.7	67.0	81.6	68.1	54.7	83.0	43.6	55.0	65.7	100, 100
	Chalcidoidea[e]	24.0	31.9	13.6	0.0	27.7	0.0	31.7	40.0	21.1	60,54
	Others	3.3	1.1	4.8	31.9	17.6	17.0	24.6	5.0	13.2	n/a
Lepidoptera	*Anatrachyntis* sp.	97.1	98.3	100	0.0	96.1	100	100	100	98.8	100,77
Coleoptera	Staphylinidae[f]	53.8	44.7	55.6	80.0	48.3	42.9	64.7	42.9	54.1	78,84
	Nitidulidae 1[f]	0.0	0.0	0.0	10.0	0.0	0.0	2.0	0.0	1.5	6, 16
	Nitidulidae 2[f]	15.4	10.6	16.7	10.0	24.1	28.6	11.8	28.6	18.2	45,53
	Others[f]	30.8	44.7	27.8	0.0	27.6	28.6	21.6	28.6	26.2	n/a

[a] Sexes, F, Female; M, Male; (*N*), number of cones
[b] % presence, % of female (F) and male (M) traps with at least one individual
[c] Only two morphotypes are included, other phorids are in 'Other families'
[d] Muscidae, *Atherigona*, Drosophilidae, *Drosophila;* 'Others' include Muscidae, Drosophilidae, Lauxaniidae, Otitidae, Psychodidae, Dolichopodidae, Culicidae
[e] Chalcidoidea, mainly Mymaridae
[f] Staphylinidae,1 type; Nitidulidae, 2 *Carpophilus* types; 'Others', mainly Coccinellidae, Scarabaeidae; Cossoninae

Table 4. Number of cycad aulacasspis scale (CAS) and number within indicated insect order on sticky traps

Site	Cone sex	CAS [a]				Diptera[b]	Hymenoptera	Lepidoptera[c]	Coleoptera
		2005	Range	2007	(*N*) [d]	2007	2007	2007	2007
Mangilao	female	13.6	9.1-23.3	<0.1	(2)	24.5(61)	6	1.5	2
Mangilao	male	5.5	1.5-9.8	<0.1	(10)	46(62)	6.2	2	4
Ritidian	female	29.8	12-99	NA	NA	NA	NA	NA	NA
Ritidian	male	96.5	36-1603	0.1	(6)	26.3(63)	4.1	3.8	6.8

[a] Number of male CAS per cm^2 (10 samples per trap)
[b] Diptera, average (percentage Phoridae)
[c] All moths were *Anatrachyntis* except one
[d] (*N*), number of cones, 2007

Habitat size: The Ritidian and Mangilao sites are located within contiguous *Cycas* forests that surround ~ a third of the northern perimeter of the island. Lamlam and Ija are located within small pockets of forest fragments with adjacent grasslands devoid of *Cycas*. Exposure: The Ritidian and Ija sites are protected from chronic trade winds. The Mangilao site is buffeted by trade winds and aerosol salt sprays but cycads are protected by taller vegetation. The Lamlam site has emergent *Cycas* plants fully exposed to chronic winds.

We sampled cones on Rota, 60 km northwest of Guam, during June 2007. One site was along the north coast within a littoral habitat on excessively drained alkaline sand substrate, and a second site was near a cliff overlooking the southeastern coastline with calcareous soil similar to Guam's Ritidian, Mangilao, and Lamlam sites.

SAMPLING METHODS

Three different techniques, sticky traps, emergence/bag traps, and direct sampling, were used to ensure a thorough collection of insects. Because only a few plants are reproductive within each population, some sites did not have enough cones at appropriate phenology (*i.e.*, male cones with fruity odor at pollen dehiscence and females aromatic with megasporophylls overlapping 10-11 mm diameter, pea-size ovules) to be sampled by all techniques. Voucher samples of the most common insect morphotypes have been deposited in insect museums or with taxonomic specialists.

For sticky trap sampling, we coated a 5 X 30 cm section of a slightly larger clear plastic collar with Tangle Trap® Insect Trap Coating Paste Formula and placed it around a cone. Traps were replaced on cones ~ every four days at Mangilao and Ritidian, and traps were removed after ten days at Ija and Lamlam. The number of traps (cones) per site ranged from one to seven per sex depending upon the available cones (Table 2). In June 2007, traps were placed on cones at Mangilao and Ritidian to compare with 2005 data. Animals were separated into arthropod classes and insect orders, and then counted. Common insects were further classified into families and insect morphotypes, representing a putative genus or species. Species richness indices were calculated at the arthropod and insect order level for each site and cone sex. The relative rankings of these taxa were compared across sites and sexes with a Spearman rank correlation test.

At Mangilao and Ritidian, two male cones in 2005 and four male cones in 2007 were sampled destructively for insects. In 2007, lepidopteran larvae from two male cones were reared to adult stage to determine their identity. Six receptive female cones at Mangilao and Ritidian were examined visually as soon as megasporophylls could be manipulated apart.

To capture insects emerging from /flying to cones, we inverted a clear plastic (30.5 x 44.5 cm) bag over the top of a cone. The bag remained open and the opening was ~ 6 cm above the base of the sporophylls. A wire frame supporting the bag maintained a gap (~6 cm) between the bag and cone, which allowed insects to fly from the outside to the cone, or to exit the cone and fly to the top of the inverted bag. Cone aroma was still detected around the cone base.

Six female and five male cones at Mangilao were observed over several weeks, with most cones assessed daily after the first insects were caught.

In Rota, bag traps were placed over two male and two female cones overnight at one site, and three male cones were dissected at both sites. (Sticky traps placed on cones were destroyed by a typhoon before they could be harvested.)

POLLEN COUNTS

Pollen grains were examined on 10 female sticky traps (at least two per site) that had insects representative of each common morphotype. Any pollen on or immediately adjacent to an insect was counted as associated with that specific insect, and any pollen > 3 mm from insects as wind-blown. We also captured specific insects from a female cone bag trap, washed them in clean 50% ethanol and removed the insects. The fluid was placed in 1.5 ml centrifuge tubes and centrifuged at 12,000 X g for about 30 sec. The supernatant was poured off and examined for pollen, and pollen grains were counted in the remaining ~ 0.1 ml.

Results & Discussion

INSECT TRAPPING

On sticky collar traps the most commonly represented insect orders were Diptera, Hymenoptera, Lepidoptera and Collembola (Tables 2, 3), which were caught on every sticky trap at all sites (except no Lepidoptera on Ritidian male cone traps). Clusters of lepidopteran scales were found without moths, suggesting that some moths escaped.

Indices of arthropod/ insect order richness were similar across sexes and sites, and the relative ranking of abundance of taxonomic groups between sexes within sites was similar (Table 2) as were the rankings between female traps across sites (R=0.76 to 0.93, P= 0.0023 to 0.00001) and between male traps across sites (R=0.51 to 0.84, P= 0.076 to 0.0085). The number of insects trapped on male cones at Ritidian was especially low (Table 2), possibly due to extremely high densities of male CAS saturating traps in 2005 (Table 4). By 2007, two years after the release of the biocontrol beetle for CAS, densities of scale decreased (Table 4), and moths were caught on all traps.

In contrast to sticky traps, the types of insects found by direct sampling and trapped on bagged cones were much more restricted. On all dissected male cones we estimated over a thousand lepidopteran larvae, and small lepidopteran adults escaped rapidly and were not counted. Larvae feed on sporophyll tissue, leaving much frass. In 2007, we captured over 250 adult Lepidoptera of a single morphotype emerged from male cones. In 2007, three of four dissected male cones at both sites had Nitidulidae beetles (avg. 11.3 and 23.3, respectively, adults per cone). Crickets and ants were found occasionally on males and females. Rarely, we observed feeding damage, frass or nitidulid adults on female cones.

The most abundant insect (>99%) captured in bag traps was a small adult Lepidoptera (cumulative averages of 444, SE 387, range 20 - 1606 and 36.3, SE 22.6, range 17-69, moths trapped per male and female cone, respectively). Crickets, ants, small hymenopteran parasitoids, and dipterans (Muscidae, Phoridae) were occasionally found. When cones had no detectable odor, they rarely caught moths, but cones with odor trapped an average of 7.2 and 239 moths per day on female and male cones, respectively.

Sticky traps caught the greatest diversity of insects in terms of "group" richness. Sticky traps capture insects attracted by visual and odor cues over several days, whereas bag traps tend to catch those attracted by odor and that tend to fly up and stay in the trap. Direct observations reveal mainly those present on cones during our daytime observations or using cones as breeding hosts. These results clearly confirm that pioneering research on unstudied pollination systems should employ a variety of trapping methods to ensure capture of a broad range of insect taxa.

INSECT IDENTIFICATIONS

One species of Lepidoptera, *Anatrachyntis* sp. Meyrick (Cosmopterygidae) (~4mm long) [det. Sergij Seniv as reported by Marler and Muniappan (2006)] (Fig. 3) was found consistently on both males and females. Of trapped moths, 98.8% on sticky traps and >99% in bag traps were *Anatrachyntis*. This genus is cosmopolitan and some species are pests of fruits and grains (Saito et al. 1992).

Diptera, Coleoptera and Hymenoptera were abundant on male and female sticky traps at each site. Collembola were also common and are found in leaf litter around the base of cones. Several common morphotypes within Phoridae, Drosophilidae and Muscidae dipteran families were trapped (det. R. Zack, Washington State University; insect vouchers at University of Guam), and all of them may be attracted to the odor of cones as they age. Two phorid types were common, comprising 48% of all flies (Table 3). Phorids are common around decaying matter (Disney 1994). The main drosophilid was identified as *Drosophila*, a genus well represented in Micronesia, and many species are fungivorous (Wheeler & Takada 1964). The common muscid is *Atherigona* possibly *excisa* (Thomson) (Snyder 1965). *Atherigona excisa* is a pantropical pest of many tropical fruits some of which are grown in Guam.

Most (65.7%, Table 3) Hymenoptera were ants. Nests are common at the base of cones. Other common Hymenoptera were tiny parasitoids, mostly fairy flies (Chalcidoidea: Mymaridae). No other morphotypes were common across sites.

The most common beetles were a single type of Staphylinidae and at least two types of *Carpophilus* (Nitidulidae) (Tables 2, 3) on sticky traps. *C. freeman* Dobson 1956 (det. A. Cline, vouchered in the Andrew R. Cline collection, currently housed in the California State Collection of Arthropods, Sacramento, CA) was collected from male cones, and *C. mutilatus* Erichson has been reported previously (Marler & Muniappan 2006). These are part of *C. dimidiatus* species

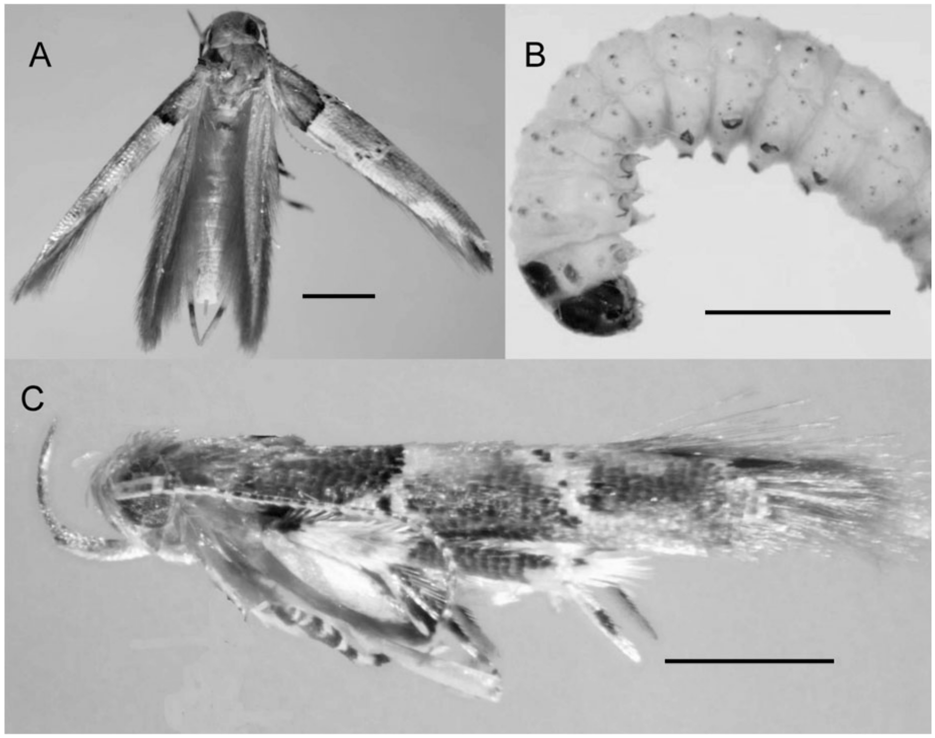

Figure 3. *Anatrachyntis* sp. adult (A) dorsal and (C) lateral view; and (B) last instar larva, lateral view. Scale bar= 1mm.

complex and are not native to Pacific Islands (Ewing & Cline 2005). Staphylinid and nitidulid beetles are common on vegetation and decaying fruit and may be attracted to cone odors as they age.

ROTA

Anatrachyntis moths were the only insect collected in bag traps, 6 and 17 moths on two male cones and 3 moths on one female cone. On dissected male cones we collected *Anatrachyntis* larvae and two species of Nitidulidae, *Carpophilus mutilatus* and *C. dimidiatus* (Fabricius) (det. A. Cline, see above).

POLLEN COUNTS

Cycas micronesica pollen has typical cycad pollen (Deghan & Deghan 1988), *i.e.*, 20 X 28 µm, "coffee-bean" shape with a longitudinal furrow. There was an average of 10.8 grains per cm^2 per female cone sticky trap. Only cycad pollen was observed, and a large proportion (> 50%) of the pollen was remote from the insects (Fig. 4) and was dispersed randomly or in clumps or lines. About 28% of pollen was associated with *Anatrachyntis* moths and clusters of moth scales, and only a small proportion was found near any other taxa (Figs. 4, 5).

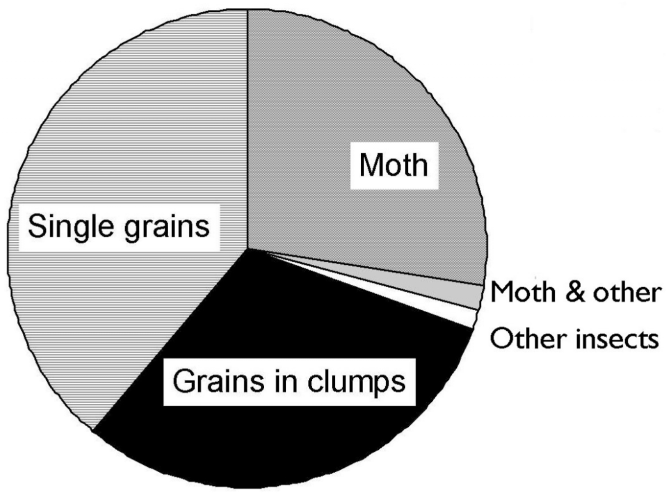

Figure 4. Proportion of pollen grains associated with *Anatrachyntis* moths, other insects, or not associated with any insect or arthropod counted on female cone sticky traps.

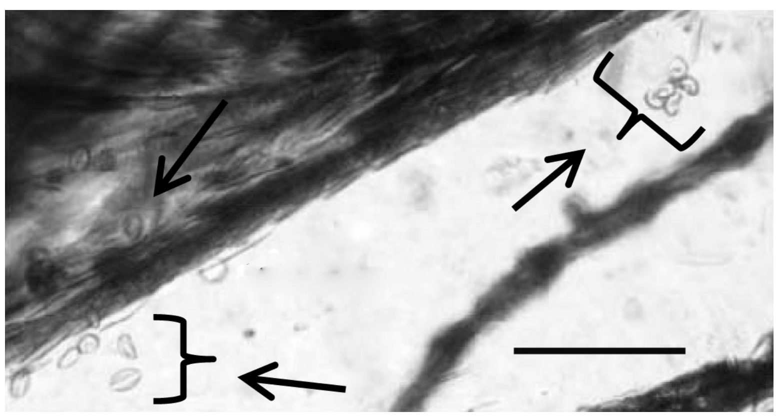

Figure 5. Pollen grains found adjacent to and on the thorax of an adult *Anatrachyntis* moth. Scale bar = 0.2 mm.

Although there may be a false positive association with some insects (if wind-blown pollen was trapped near an insect), the association of pollen with the *Anatrachyntis* moth was fairly consistent, suggesting that it does carry pollen.

From a total of 33 *Anatrachyntis* moths captured in a female cone bag trap overnight, 84 pollen grains were extracted Since the source of the moths is unknown (whether from female or male cones), this may be an underestimate of pollen loads of moths leaving male cones. No male cones were within 20 m of this cone.

SUMMARY

In this survey, we captured a diversity of arthropod visitors to *C. micronesica* cones and we used the information to identify putative pollen vectors and to compare different habitats. Firstly, two of the insect visitors, *Anatrachyntis* moths and several closely related *Carpophilus* nitidulid beetles have been identified as subjects for future pollination studies based on their presence in male and female cones. The moth breeds and depends on male cycad cones, a common life history trait among host-specific pollinators of other cycads (Stevenson et al. 1998). No Lepidoptera to date has been implicated as a pollinator of any other cycad species. Nitidulids are implicated as pollinators of many angiosperms (*e.g.*, Jurgens et al. 2000) and also *Cycas revoluta* (Kono & Tobe 2007). Since these *Carpophilus* species are not native to this region, their involvement with *C. micronesica* is probably recent, but more research on other cycads is needed to analyze any patterns. Secondly, these insects have been identified from cones in Rota, which suggests a similar pollination system as on Guam. Thirdly, in terms of sampling methodology, the sticky trap sampling method captured the most diverse set of taxa. Fourthly, all habitats were similar in taxa caught, and the relative rankings of taxa were similar between sexes and across habitats. The increased density of insects at the more fragmented forest habitats (Ija and Lamlam) may be due to insects concentrating on the few available cones at these sites, although other habitat factors, elevation, exposure, as well as trap design, location, and shading (e.g., Hoback et al. 1999, Wolda 1987) also may affect trap capture. Finally, most of the pollen trapped at female cones was not associated with insects, suggesting that wind also plays a role in vectoring pollen.

Cycads on Guam occur in fragmented forest communities and further fragmentation of cycads is occurring due to the invasive pests that kill trees directly or that make them vulnerable to other perturbations. Pollen-limitation is a significant cause of reduced seed production in fragmented plant populations (Harris & Johnson 2004) and perhaps more particularly in the case of cycads with dependent pollination mutualisms. Further increases in fragmentation may lead to loss of specialist pollinators, further affecting seed set. Therefore, understanding the pollination system of *Cycas micronesica* on Guam is not only of evolutionary interest but also is vital for setting priorities for conservation management of this critically endangered species.

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