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# Pollination of endangered Cuban cycad *Microcycas calocoma* (Miq.) A.DC.

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Observations indicate that natural regeneration in the field of the cycad *Microcycas calocoma* (Miq.) A.DC. is extremely low, and has been so since early this century. It appears that populations of its insect pollinator are practically extinct, but some pollinator activity appears to be present in the largest population of *Microcycas*. Recommendations for identification and captive breeding of the pollinator are suggested as a complementary conservation strategy to a *Microcycas* propagation programme already established at the Cuban National Botanical Garden.



ADDITIONAL KEY WORDS:—cycad conservation – endangered species – pollination biology.

CONTENTS





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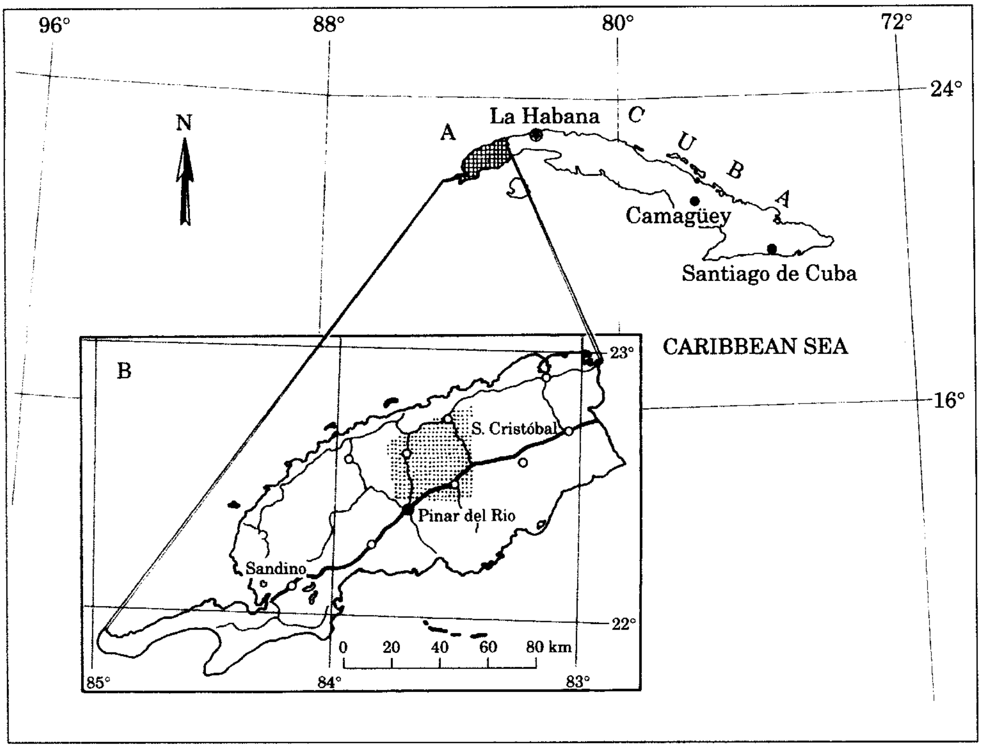


Figure 1. Map of Cuba. A, location of Pinar del Rio province B (inset), *Mycrocycas* populations indicated by dotted hatching.

## INTRODUCTION

*Microcycas calocoma* (Miq.) A.DC. is an endemic and endangered monotypic genus of cyads, listed in the *IUCN Plant Red Data Book* (Lucas & Synge, 1978). Workers of the National Botanical Garden of Cuba have developed conservation projects for this species based on propagation and embryo tissue culture (Peña-García & Grillo, 1982; Peña-García, Díaz-Canals & Grillo-Mensa, 1986). However, field preservation of *M. calocoma* is still needed because of poor natural regeneration and so little is known of its pollination biology. Natural regeneration has been reported low since early this century by Caldwell (1907) and later by Foster & San Pedro (1942). This cycad has recently been decreed a national treasure of Cuba by the Cuban Government (Peña-García & López-García, 1995).

### *Distribution*

Populations of *Microcycas calocoma* are restricted to the Province of Pinar del Rio (Fig. 1). This province is one of the most ancient zones of the island and contains approximately 38% of its endemic flora (Bisse, Sánchez & Ranking, 1984). Historically, this area, as well as the entire island, has been highly disturbed by agricultural expansion. By 1959, the deciduous tropical forest cover had been reduced to 14% of its original cover (Silva-Taboada, 1992). At present, populations of *M. calocoma* are restricted to ravines, slopes, or tops of hills either in open grasslands or remnants of the tropical forest that have been restricted by agricultural expansion. Distribution and natural regeneration does not appear dissimilar to that reported by Caldwell (1907) and Foster & San Pedro (1942), though several new populations have been discovered recently by Peña-García, Chaves-Guevara & Pimentel-Pimentel (1988) and Peña-García *et al.* (1997).

### *An endangered species*

*Microcycas calocoma* has been cited by Caldwell (1907) as rare. Foster & San Pedro (1942) explored 11 localities, with an estimation of approximately 500 individuals. However, establishment of seedlings between 8 months and 10 years old over several consecutive years had been reported in only one population (Foster & San Pedro, 1942); some natural regeneration has been observed recently in other localities by Peña-García (pers. comm.)

Efforts to understand the biology of this species have been related to evaluation of populations, seed production, morphology, vascular anatomy, spermatogenesis and general characteristics of its habitat (Caldwell, 1907; Dorety, 1909; Reynolds, 1924; Chrysler, 1926; Downie, 1928; Foster & San Pedro, 1942; Del Risco, Morell & Samek, 1984; Peña-García *et al.*, 1986; 1988; Norstog, 1990; Peña-García & López-García, 1995); little or no work has been done to elucidate its reproductive biology in the field. Pollination and natural regeneration from seed appears to be practically nil, with very few exceptions (Foster & San Pedro, 1942; Del Risco *et al.*, 1984). However, this species has shown high seed viability and germination percentage if pollinated by hand (Niklas & Nostog, 1984; Peña-García *et al.*, 1986; Peñã-García, López-García & Pérez-Montesinos, 1997). This study is an attempt to understand the pollination biology of this cycad by field observations and inference from the reproductive biology of the better studied sister genus *Zamia*. This will enable guide lines to be set for future research and set out conservation strategies without experimenting unnecessarily with the already endangered populations of the species (Brooks, Mayden & McLennan, 1992a, b).

## MATERIAL AND METHODS

Sixteen *Microcycas* populations were previously monitored by one of us (E. Peña-García) (Table 1). During 1995 we visited three of these populations (Fig. 1, Table 1) as well as one visited by Foster & San Pedro (1942) in an attempt to determine the pollination system. In every locality cone material was collected for examination of pollinator presence and number of individuals and sex were recorded. Detailed description of the populations and localities can be found in Peña-García *et al.* (1988) and in Osborne & Milanés-Santana (1995). Crane's (1988) phylogenetic hypothesis for the living cycads was used to extrapolate cone morphology and pollination from the sister genus *Zamia*.

## RESULTS

### *Populations*

Populations of *M. calocoma* occur in small patches varying from 60 $m^2$ to 21 168 $m^2$ in a total area of approximately 222.35 ha. In this area 528 individuals were located,

TABLE 1. *Microcycas* populations studied at Pinar del Rio. DF = tropical deciduous forest; P = pasture land; PF = induced pine-forest; S = savannah; A–Q population localities

| *Microcycas* populations | | Altitude (m) | Habitat | Area ($m^2$) |
|---|---|---|---|---|
| A | Hoyada de las Catalinas I | 230 | DF | 2400 |
| B | Hoyada de las Catalinas II | 235 | DF | 4489 |
| C | Hoyada de las Catalinas III | 340 | DF | 15 600 |
| D | San Juan de Sagua | 40 | DF | 3916 |
| E | Cinco Palmas | 50 | P | 60 |
| F | Curva del Peligro | 200 | PF | 5000 |
| G | Santo Tomás | 250 | DF | 25 000 |
| H | Moncada | 200 | DF | 50 000 |
| I | Arroyo Sabanetón | 70 | P | 4500 |
| J | Camino Cruz de San Joaquín | 70 | P | 2500 |
| K | Maceo | 300 | PF | 10 000 |
| L | Fornegruera Mil Cumbres | 380 | DF | 21 168 |
| M | Galalón I | 400 | P | 4400 |
| N | Galalón II | 400 | DF | 1848 |
| O | Galalón III | 380 | DF | 1848 |
| P | Galalón IV | 400 | S | 1232 |
| Q | Loma Granadinos | 330 | DF | 15 000 |

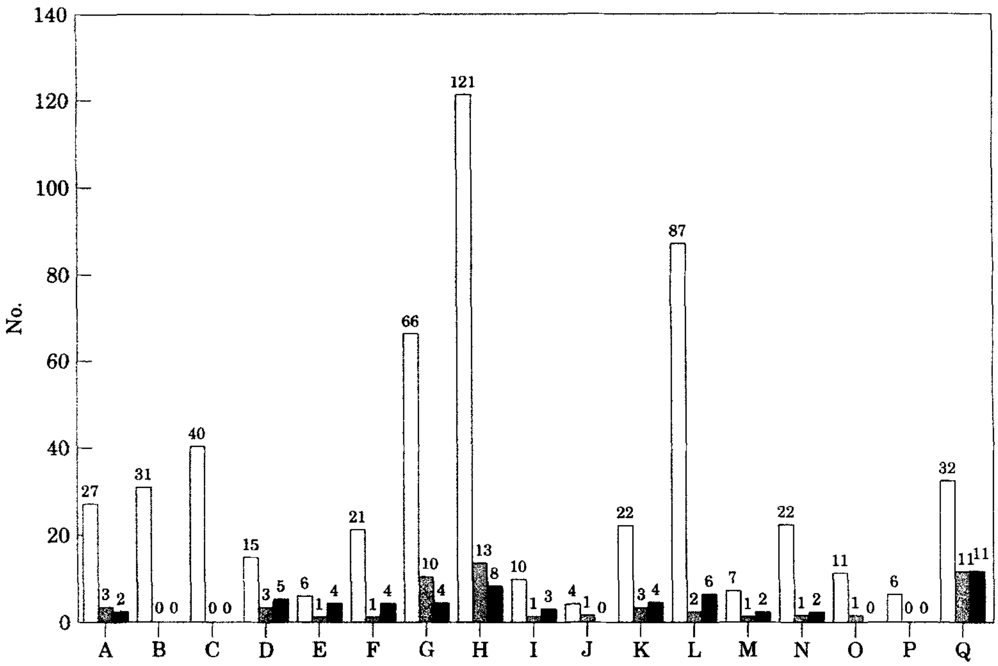


Figure 2. Numbers of *Microcycas calocoma* individuals in the populations studied from 1988 to 1991 at Pinar del Rio. (□) total plants; (▦) male and (■) female cone bearing plants (letters designate population localities, see Table 1).

107 plants being fertile (52 males and 55 females). The number of individuals per population varied from 4 to 121 with Moncada having the largest population (Fig. 2). Vegetation types are tropical deciduous forest, planted pine forests, induced pasture and savannah (Table 1).

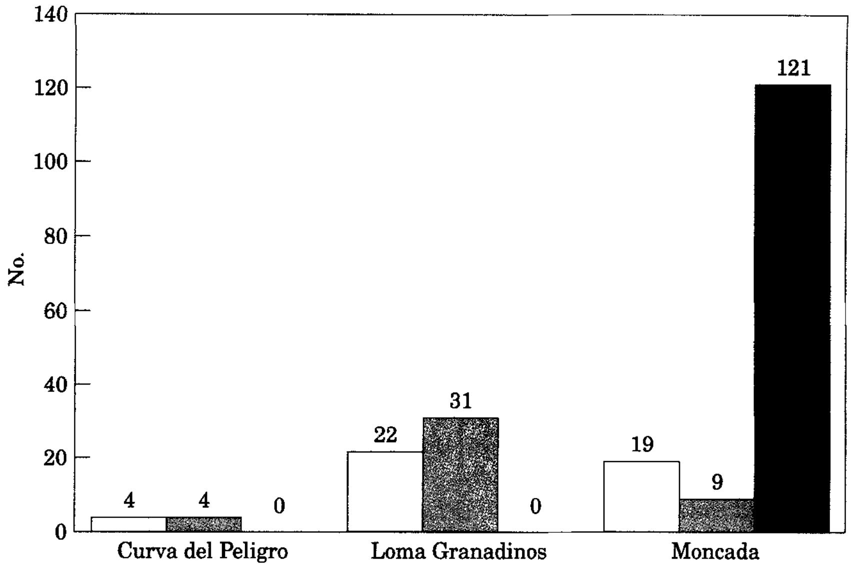


Figure 3. Numbers of female plants (□), cones (▩) and seeds (■) in the three populations of *Microcycas* investigated during 1995 at Pinar del Rio.

### *Seed germination*

In the three localities visited there was no evidence in the field of seed germination, except in Moncada where only two seedlings were found but no older juveniles. Forty-four female cones were found, but only one of these bore seeds (Fig. 3) with a germination percentage of 40% in the laboratory. No predation of seed (i.e. remains of seed sclerotesta) was noted.

In Moncada, sporophyll remains from two male cones from the previous year were collected. Observations in the laboratory on remains of these cones revealed presence of galleries and exit holes with a similar pattern to those found in microsporophylls of *Zamia furfuracea* and *Z. pumila* made by their weevil pollinators (Norstog, Stevenson & Niklas, 1986; Tang, 1987a; Norstog & Fawcett, 1989) (see Figure 3).

## DISCUSSION

### *Regeneration*

The low rates of natural pollination and the lack of fertile cones observed in the 17 localities contrasts with the high percentages of fertilization and germination when artificial pollination is conducted. Only two seedlings were found at Moncada, the largest population (Fig. 3). Low regeneration rates were probably aggravated also by the low number of individuals of *Microcycas calocoma* attributed to habitat disturbance since colonial times and fragmentation of the original vegetation (Borhidi, 1991). Habitat disturbance through cattle grazing and the associated burning of grasslands spreading fire into natural forest could also be factors leading to the decimation of pollinator populations. Seed and juvenile plant predation by rodents (hutias) has been reported and could also be responsible for low seedling survival (Akeroyd & Wyse Jackson, 1995).

## *Pollination in cycads*

Wind as a sole source of pollination in cycads has now been largely discredited (Norstog 1980; Norstog, Stevenson & Niklas, 1986; Norstog, 1987; Crowson, 1989, 1991; Norstog, Fawcett & Vovides, 1992) and experimental evidence has demonstratged pollination in *Zamia* and *Ceratozamia* by insects (Tang, 1987a; Norstog & Fawcett, 1989; Sánchez-Rotonda, 1993). Pollen and female cone morphology as well as heat and odour production does not relate to wind pollination (Niklas & Nostog, 1984; Tang, 1987b). Evidence from the fossil record also supports insect pollination of cycadophytes (Delevoryas, 1968; Crepet, 1974) and the Curculionidae genera so far found associated with living cycad cones (Norstog, 1987; Vovides, 1991; Forster *et al.*, 1994) are considered primitive (Crowson, 1989, 1991).

## *Inference of* Microcycas *pollination*

There is a consensus that extant cycads constitute a monophyletic group (Crane, 1985; Doyle & Donoghue, 1986; Crane 1988; Stevenson, 1990) with *Zamia* and *Ceratozamia* in the same clade as *Microcycas.* We infer insect pollination of *M. calocoma* by citing Brooks & McLennan (1993), who claim that members of the same clade should share a number of ecological, morphological and behavioural characters. Additional support for our inference has been provided by *a posteriori* observations of insect galleries and exit holes in *Microcycas* microsporophylls in the field, which are similar in size and position to those shown by *Zamia* (Fig. 4). The relationship of cycads and beetles throughout the cycad clade is presented (Fig. 5) with the *Microcycas*, *Zamia* and *Ceratozamia* clade enlarged with cone and beetle illustrations. A similar phylogenetic inference with respect to the putative weevil pollinator of *Microcycas* can be drawn from *a posteriori* field observations which demonstrated that an endangered and poorly known species of darter fish, *Etheostoma wapiti*, have encountered ecological problems affecting oviposition similar to those of a well known species of the same clade (Brooks *et al.*, 1992a, b).

We therefore suggest that the apparent scarcity of an insect pollinator, probably related to historic habitat disturbance, could be an important cause of low recruitment, in spite of frequent cone and ovule production observed in the *Microcycas* populations. An example of this has been witnessed in the Everglades of Florida where regular burning of the pine-palmetto forest reduces the amount of humus or debris on the forest floor thus destroying the over-wintering diapause phase of the pollinator larvae *Rhopalotria slossoni* Schaef. of *Zamia pumila* L. (Norstog *et al.*, 1992; Fawcett & Norstog, 1993). Diapause larvae in male-cone debris pupate and emerge over several seasons, thus maintaining a presence of pollinator populations when the cycad female cones are receptive (Fawcett & Norstog, 1993; Espinosa, 1996). This makes frequent fires an important factor in larvae deaths.

The type species in the snout weevil genus *Rhopalotria* was described from a Cuban *Zamia* (Muñiz & Barrera, 1969). The weevil *Rhopalotria mollis* Sharp pollinates the

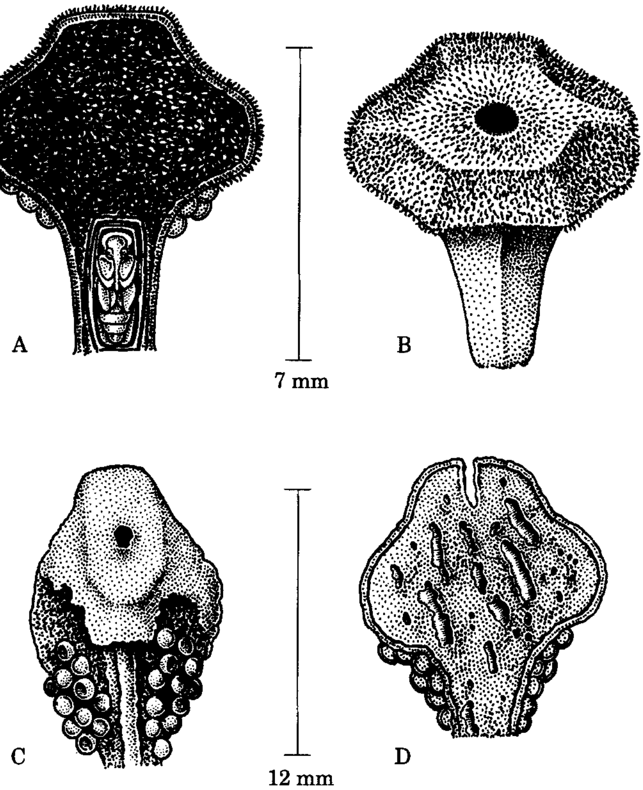


Figure 4. A, longitudinal section of *Zamia* microsporophyll showing insect pupa, and interior of sporophyll eaten away. B, insect exit hole in microsporophyll of *Zamia*. C, insect exit hole in microsporophyll of *Microcycas*. D, longitudinal section of *Microcycas* microsporophyll showing insect galleries.

Mexican *Zamia furfuracea* L.fil. (Norstog & Fawcett, 1989) and *R. bicolor* Voss appears to pollinate *Dioon califanoi* De Luca, Sabato & Vázq. Torres (Vovides, 1991; Espinosa, 1996). We therefore believe that a species of *Rhopalotria* or the related *Parallocorynus* is the probable pollinator of *Microcycas*.

Because of its monotypic status, the extinction of *Microcycas* implies the extinction of a whole genus. Artificial propagation of this cycad is laudable but its future survival *in situ* will depend on efforts to understand its pollination biology. Cycads as a group are cladistically basal to the seed plants and there are only 185 known species world wide (Jones, 1993). Therefore conservation efforts in this group as a whole should be a priority.

## CONCLUSION

The extinction of *Microcycas* would be tantamount to the loss, not only of a national treasure but, according to Norstog, of a living 'Rosetta Stone' of spermatophyte evolution. Location and identification of the insect pollinators of *Microcycas calocoma* is now an urgent priority. The Moncada population is one of the few sites

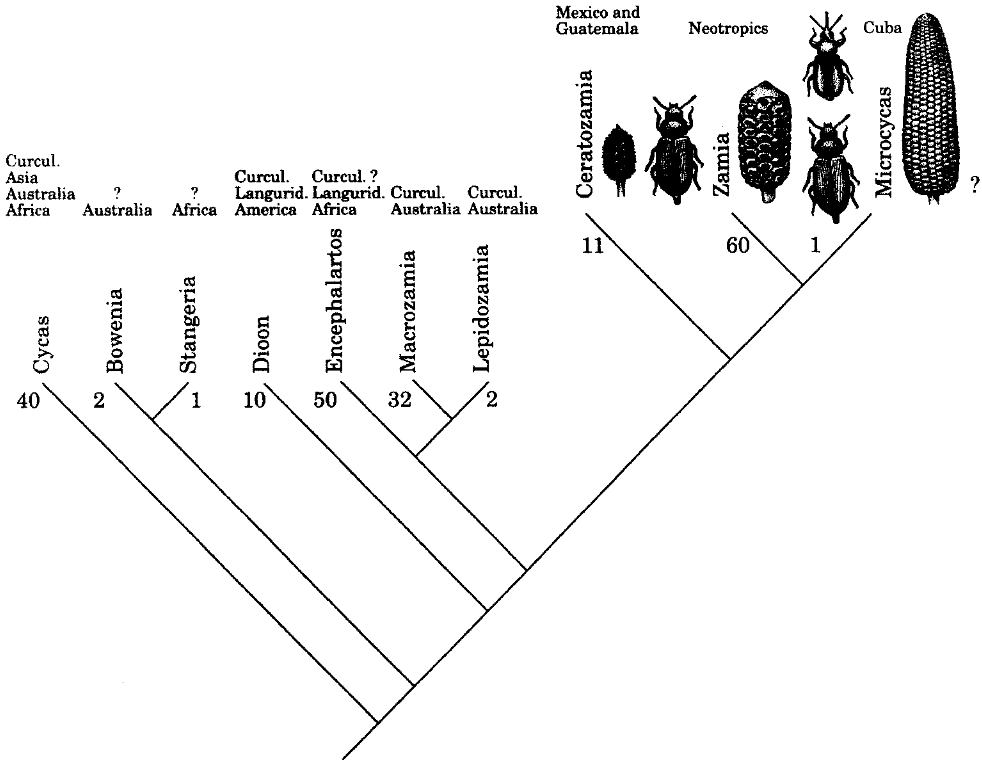


Figure 5. Cladogram of the extant cycad genera, modified after Crane (1988). We added information on distribution and associated insects on Old World cycads from Forster *et al.* (1994). Numerals below cycad genera indicate number of species per genus. Question mark indicates pollinator unknown. Cone and insect illustrations not to scale. Pollinator insects: *Ceratozamia*, languriid beetles; *Zamia*, snout weevils and languriid beetles; *Microcycas*, (probably) snout weevil.

where they are still extant but these populations are obviously low or becoming extinct. If this population is to be preserved, Moncada must be made a restricted sanctuary as soon as possible, and reproduction of the insects increased by captive breeding. This, alongside artificial propagation of *Microcycas* by the Cuban National Botanical Garden, should eventually contribute to the long-term survival of a national treasure.

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