A new species of *Pharaxonotha* (Coleoptera: Erotylidae), probable pollinator of the endangered Cuban cycad, *Microcycas calocoma* (Zamiaceae)

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Abstract: The new species *Pharaxonotha esperanzae* (Coleoptera: Cucujoidea: Erotylidae: Pharaxonothinae) is described. It feeds on the pollen of the endangered Cuban cycad *Microcycas calocoma* (Miq.) A. DC. and breeds in its male cones. Its potential role as a pollinator of *Microcycas* is discussed.

Resumen: Se describe la especie nueva: *Pharaxonotha esperanzae* (Coleoptera: Cucujoidea: Erotylidae: Pharaxonothinae). Ésta se alimenta del polen del cicadal cubano amenazado *Microcycas calocoma* (Miq.) A.DC y se reproduce en su cono masculino. Se discute su papel potencial como agente polinizador.

Key words: *Pharaxonotha,* Erotylidae, Languriidae, new species, Cycadales, pollination.

Palabras claves: *Pharaxonotha,* Erotylidae, Languriidae, especie nueva, Cycadales, polinización.

Introduction

Cycadales (cycads) are the oldest group of insect-pollinated plants (Norstog and Nicholls 1997). Many insects have developed mutualistic interactions that can be host-specific (Donaldson 2003). There are different hypotheses on the antiquity and original association (whether with gymnosperms or angiosperms) of the insects known to pollinate cycads. Some of these interactions apparently have recent origins (Crowson 1991, Oberprieler 1995a, 1995b). Nevertheless, the fossil record, phylogenetic studies and biogeography support the existence of Coleoptera feeding on gymnosperms since the Jurassic, and those associated with cones are considered precursors of important families of phytophagous beetles (Crowson 1991, Farrell 1998). Primitive beetles of the family Erotylidae are among the oldest extant pollinators of cycads. Their biogeography supports the hypothesis that they pollinated cycads before the Cretaceous diversification of angiosperms (Hall *et al*. 2004, Tang 2004). Erotylids are known to pollinate species in six of the eleven cycad genera, but only eight of these genera had been surveyed up to now (Tang 1987, Vovides 1991, Ornduff 1991, Forster *et al*. 1994, Donaldson *et al*. 1995, Tang *et al*. 1999, Wilson 2002, Hall *et al*. 2004, Tang 2004).

Within Erotylidae (which now includes taxa previously in Languriidae) is the genus *Pharaxonotha* Reitter 1875 (= *Planismus* Casey 1890) belonging to the subfamily Pharaxonothinae, which is one of the most primitive in the family (Leschen 2003). Of all insect genera known to pollinate cycads, *Pharaxonotha* is the most important based on the fact it has been associated with three of eleven cycad genera: *Zamia*, *Dioon*, and *Ceratozamia* (Tang 1987, Vovides 1991). This genus of little beetles was taxonomically redefined by Sen Gupta and Crowson (1971). At present, there are 11 described species for the world (Leschen 2003). Pakaluk (1988) described two new species from Costa Rica and presented a key including the four New World species. These are: *P. kirschii* Reitter (= *Thalisella conradti* Gorham), *P. floridana* (Casey) [= *P. zamiae* Blake], P. *clarkorum* Pakaluk, and *P. confusa* Pakaluk.

Pharaxonotha kirschii, the type species, is native to Mexico and Central America, but as a stored product pest, it now has a much wider distribution that includes North America, South America and Europe (Pakaluk 1988). *Pharaxonotha floridana*, from the southeastern United States (Leschen and Skelley 2002), is hosted by *Zamia integrifolia* [= *Z. floridana* (Jones 1993)] and was the first erotylid cited as a mutualistic pollinator of a cycad (Tang 1987). *Pharaxonotha clarkorum* and *P. confusa*, from Costa Rica, feed on pollen and breed in male cones as *P. floridana*, but in *Zamia skinner*i and *Z. fairchildeana*, respectively (Pakaluk 1988).

There are *Pharaxonotha* species yet to be described. All of which are considered mutualistic pollinators and are apparently host specific with other New World Zamiaceae of the genera *Zamia*, *Dioon* and *Ceratozamia* (Vovides 1991, Norstog *et al.* 1992, Cruz 1999, Pérez-Farrera *et al.* 2000, González 2004, P. Skelley and M. Thomas, pers. comm. 2004). The genera *Zamia* and *Dioon* also have mutualistic pollinators among curculionoids in the family Belidae (Tang 1987, Vovides 1991, Norstog *et al.* 1992). The New World Zamiaceae genera: *Chigua* (endemic from Colombia) and *Microcycas* (monotypical endemic from Cuba) have no previously reported insect pollinator (Tang 2004). Prior to this study, there were no reported members of *Pharaxonotha* from the West Indies.

The endemic Cuban cycad, *Microcycas calocoma* (Miq.) A.DC., is a threatened plant listed as Critically Endangered (CR), according to IUCN criteria (Peña *et al.* 1998, Stevenson *et al.* 2003). A very important factor contributing to this status is its scarce reproduction, fundamentally due to limited pollination (Cendrero 1940, Foster and Rodríguez 1942, Peña *et al.* 1997a, 1997b, Vovides *et al.* 1997, Peña *et al.* 1998). This has been attributed to the supposed extirpation or population decline of the yet unknown pollinator in most of its localities (Norstog *et al.* 1986, Peña *et al.* 1997a and b; Vovides *et al.* 1997). Nevertheless, some preserved localities showed a habitual seed production (Risco *et al.* 1984, Peña *et al.* 1988, Peña *et al.* 1997a), which proved the existence of a pollinator. It was believed the unknown pollinator could be a curculionoid of the family Belidae. The discovery and identification of this pollinator was an urgent priority for the development of a conservation strategy (Vovides *et al.* 1997).

The objective of this work is to present the first record for the genus *Pharaxonotha* in Cuba, describe *Pharaxonontha esperanzae* and discuss the potential function of this species as the pollinator of *Microcycas*.

Materials and Methods

Both adult beetles and larvae were collected from male cones of *M. calocoma*, in an area of Parque Nacional Viñales, Viñales Township, Pinar del Río Province, Cuba. This is a well-preserved area where pollination and seed production occurs annually.

Measurements were made with a stereo microscope from 25 adult *Pharaxonotha esperanzae* sp. nov., mounted or taken from alcohol: total body length from anterior head edge to elytral apex; maximum body width; pronotum, length along its midline from anterior to posterior edge, and at its widest point; elytral length from base adjacent to scutellum to its apical edge; head width; dorsal interocular distance; and ventral interocular distance (following Pakaluk 1988). Other measurements taken were antennal length and width of antennomeres VIII and IX.

Genitalia were studied from five specimens. Genitalia were dissected, cleared 1 hour in 10% KOH and preserved in 70% alcohol or slide mounted with euparol. Two specimens were dissected to study mouthparts and wing venation. Drawings were made from photographs taken by digital camera through a microscope, and corrected or detailed after additional observations were made.

Taxonomy

Order Coleoptera: Superfamily Cucujoidea
Family Erotylidae: Subfamily Pharaxonothinae

Pharaxonotha esperanzae, n. sp.

Description. There were no observable external sexual dimorphism. Body oblong, oval (Fig. 1B), length 3.49-4.21mm (x =3.91mm), 2.50-3.06 times maximum width (Table 1), and approximately 4.0 times maximum body height. Body finely pubescent, cuticle between punctures on frons and pronotum minutely granulate, dull; general colour reddish brown, elytra generally uniform in colour, some structures with dark stains of variable intensity and form, generally without fixed pattern. **Head:** Eyes prominent, coarsely faceted (9 facets in longitudinal axis), wide ventrally in relation to dorsal width (Figs. 1B, 1C, and Table 1), with short, inconspicuous setae of variable density. Antennae relatively short, length less than pronotal width and slightly more than head width (Table 1); antennomere relative lengths approximately

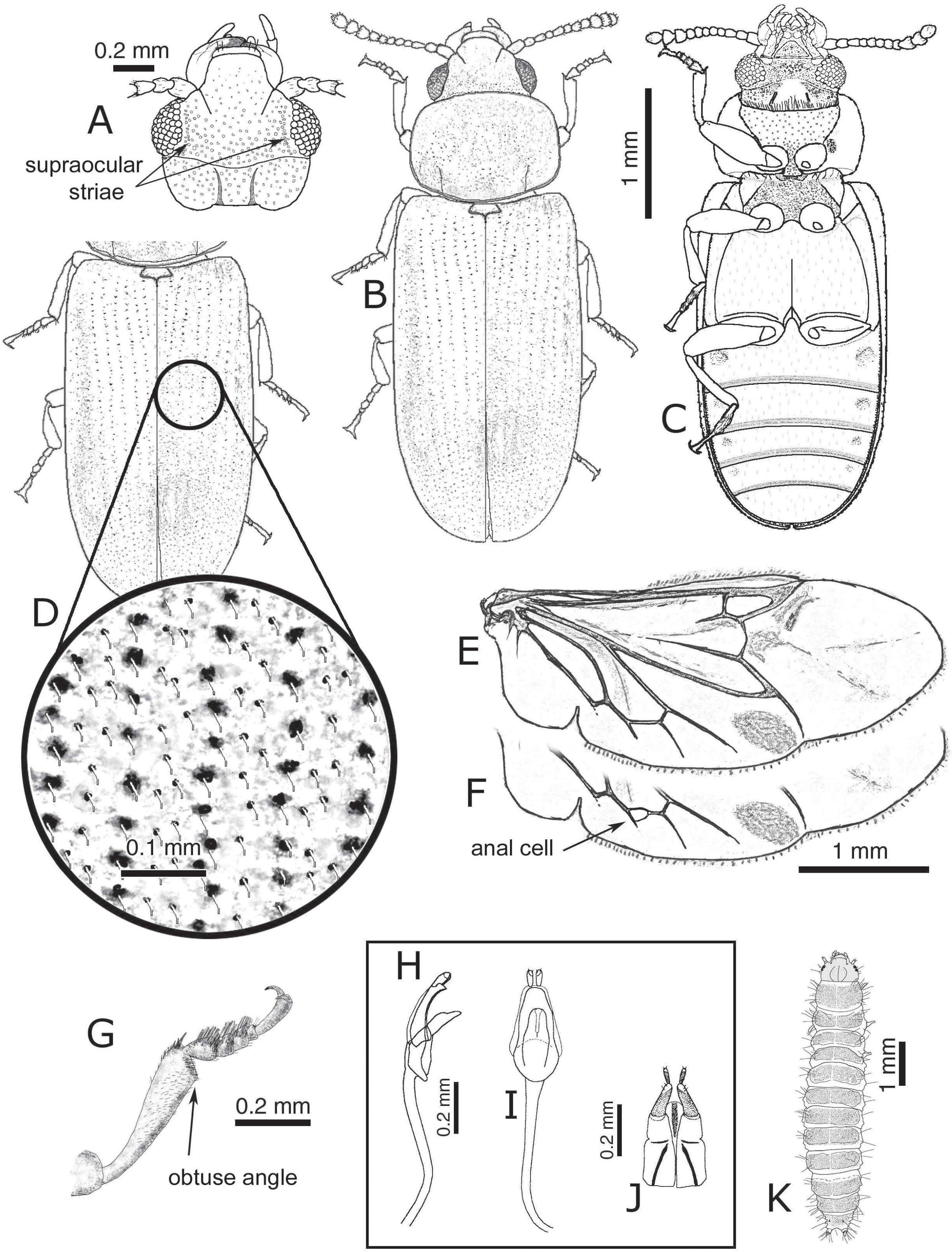

Fig. 1. *Pharaxonotha esperanzae*, n. sp. A) Head, dorsal view; B) Habitus, dorsal view; C) Habitus, ventral view, without left legs; D) Coarse and fine punctations on elytra; E) Posterior wing; F) Posterior wing with anal cell; G) Left protibia with tarsus, posterior view; H) Aedeagus, lateral view; I) Aedeagus, ventral view; J) Ovipositor, ventral view; K) Larva dorsal view.

Table 1. Body sizes and size relations, from 25 adult specimens of *Pharaxonotha esperanzae*, n. sp. x= medial value, SD=Standard deviation.

Sizes (mm)	**x**	**S.D.**	**Rank**
Total length	3.91	0.22	3.49-4.21
Elytral length	2.61	0.12	2.38-2.82
Pronotal length	0.79	0.05	0.64-0.87
Maximum width	1.40	0.06	1.27-1.51
Pronotal width	1.12	0.05	1.03-1.23
Head width	0.83	0.03	0.77-0.87
Size relations			
Maximum length / width	2.79	0.12	2.50-3.06
Head width / dorsal interocular distance	1.53	0.04	1.46-1.64
Head width / ventral interocular distance	2.17	0.08	2.05-2.33
Dorsal / ventral interocular distances	1.42	0.05	1.25-1.50
Antennal length/ head width	1.11	0.05	1.02-1.19
Antennal length / pronotal width	0.83	0.04	0.77-0.92
IX/VIII antennomere widths	1.28	0.09	1.12-1.67
Pronotal length / width	0.70	0.03	0.59-0.76
Elytral length / pronotal length	3.33	0.17	3.00-3.81

18:13:13:8:9:9:9:9:16:15:17, antennal club slightly wider than preceding segments, club three segmented and oval in cross section. Mandibles large, at rest prominent in front of and laterally to labrum (Fig. 1A), right mandible with two apical teeth, left with two or three teeth. Terminal maxillary palpomere visible in dorsal view projecting from under retracted mandible by length or more of antennal scape (Fig. 1A). Apex of labial palp project slightly forward of mandible, and visible from above. Mentum trapezoid, almost triangular, fine and densely punctured, without lateral pockets; submentum no defined. Supraocular striae and transverse line of vertex present (Figs. 1A and 2); punctation on frons coarse and separated about 1 diameter near transverse line on vertex, becoming fine and sparse toward clypeus; clypeus punctures become finer and sparser toward apical margin where they disappear; frontoclypeal suture obsolete at middle; labrum lacking punctures. Occiput with two stridulatory files, dorsal punctures nearly as coarse and dense as in neighbouring frons, punctures extend ventrally as wedges. Eyes edged with ridge ventrally, more prominent on posterior side. Pregula densely punctured, somewhat limited by ventral-lateral ocular ridges. Transverse gular ridge or groove absent, but with transverse unpunctured band that defines posterior limits of pregula and postocular ridges, band narrows laterally and is limited postero-laterally by punctured wedges of occiput; posterior gula smooth, shiny, with very fine transverse striations, narrower anteriorly, laterally limited by occiput-gular sutures; occiput-gular sutures end anteriorly in elongated tentorial pits. **Thorax.** Pronotum transverse (Table 1), convex and nearly oval; anterior angles not developed, curved ventrally, not visible dorasally; posterior angles rounded; punctures similar to larger punctures on frons (Fig. 2); base with pit on each side of midline, each with a short groove extending onto disc. Elytra length 3.00 - 3.81 times pronotal length (Table 1); lacking marginal line at base; with 10 weakly impressed striae, represented by imperfectly aligned rows of punctures slightly coarser than on frons and separated 1 to 3 diameters; scutellary striole with 8 – 12 punctures; interval punctures fine (diameters approximately 1/3 of strial punctures), punctures disorganized in disposition, frequently with two at the same level (Fig. 1D); all elytral punctures with a short seta. Hind wings as in Fig. 1E, anal cell absent or, if present, small (Fig. 1F, observed in only one wing of one specimen). Prosternum finely punctured, punctures denser on process between coxae. Procoxal cavities slightly open externally. Mesosternum coarsely and densely punctured. Mesepimeron turns inward slightly between mesosternum and metasternum. Punctures on metasternum finer and sparser than on prosternum. Protibia (Figs. 1G and 2) slightly expanded and angled to the external apex, angle obtuse. Tarsomeres I-IV slightly elongated, conical shape, I longer than II, II length almost equal to III, IV half length of II, V slightly longer than I+II together. **Abdomen.** Punctures on ventrites fine and

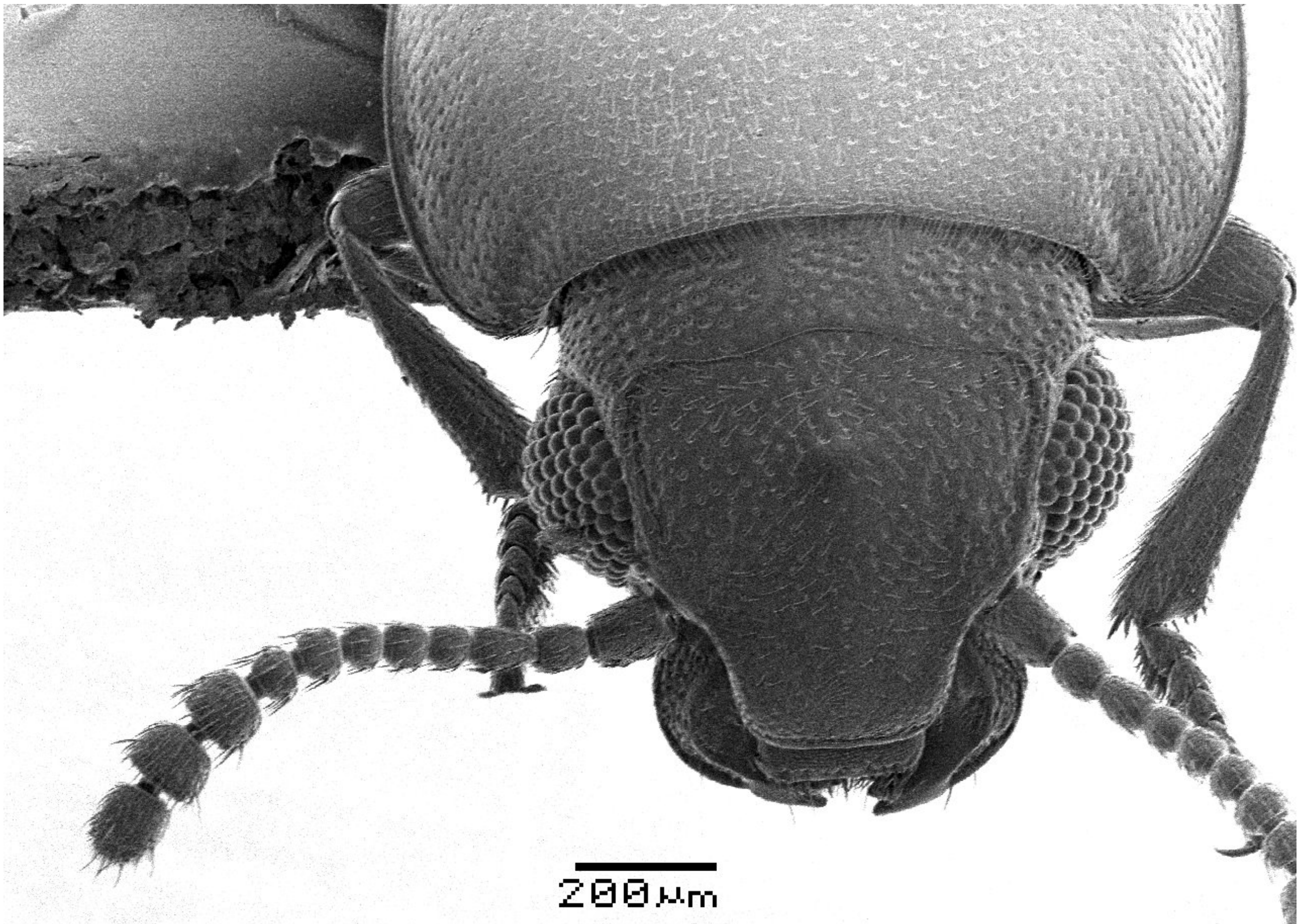

Fig. 2. *Pharaxonotha esperanzae,* n. sp. Scanning Electron Microscope photograph showing dorsal view of head, with partial pronotum and protibiae.

sparse as on metasternum. Aedeagus as in Figs. 1H and 1I. Ovipositor as in Fig. 1J.

Larva. (Fig. 1K) Maximum observed length 7.5 mm. Head pale brown, body segments yellowish, except dorsal bands of dark brown granules. Granules separated by approximately one diameter. Medial line without granules is weakly defined, present on all thoracic and abdominal segments except last one.

Diagnosis. *Pharaxonotha esperanzae* n .sp. differs from other New World species of *Pharaxonotha* by lacking the basal margin on the elytra and possessing supraocular striae. All presently described New World *Pharaxonotha* have a distinct basal margin on the elytra and lack supraocular striae.

Types. Holotype. Male. CUBA, provincia Pinar del Río, municipio Viñales, Parque Nacional Viñales, 3.ix.2003, collectors R. Chaves and J. A. Genaro, in male cone of *Microcycas calocoma*, (deposited in Museo Nacional de Historia Natural de Cuba [MNHNCu]). **Paratypes**. (24 specimens) same locality as holotype: 20.viii.2002, coll. R. Chaves, (N=6: 5 in MNHNCu [sexed 1 male] and 1 in Colección Zoológica del Instituto de Ecología y Sistemática de Cuba [CZACC]); 14.viii.2003, coll. R. Chaves (N=2, in MNHNCu); 16.viii.2003, coll. R. Chaves (N=1, in MNHNCu); 19.viii.2003, coll. R. Chaves (N=5: 4 in MNHNCu [sexed 1 male] and 1 in CZACC); 3.ix.2003, colls. R. Chaves and J. A. Genaro (N=10: 7 in MNHNCu [sexed 6 females and 1 male], 1 in CZACC and 2 in Florida State Collection of Arthropods in Gainesville [FSCA]).

Etymology: The specific name was chosen to honour Prof. Esperanza Peña García, outstanding Cuban researcher in plant conservation, especially in *Microcycas calocoma.* The use of her Spanish name reminds us that the discovery of this species gives hopes of achieving successful conservation of the plant.

Comments: Adult beetles were found feeding on pollen, inside 6 dehiscent male cones of *M. calocoma*. There were 1 to 13 adult beetles per cone. Five cones were just beginning to shed pollen. The sixth cone had already shed its pollen and its microsporophylls were starting to rot. This cone produced five adults and more than 250 larvae of different sizes. These were feeding on pollen, and larger instars could also be found feeding on the parenchyma inside the microsporophylls. This represents the first record for *Pharaxonotha* from Cuba and West Indies.

Discussion

Before this work, Coleoptera had been found as pollinators in three New World Zamiaceae (*Zamia, Dioon* and *Ceratozamia).* These are cucujoids of the family Erotylidae (*Pharaxonotha*, that pollinate all three genera) and curculionoids of the family Belidae (*Rhopalotria* and *Parallocorynus*, that pollinate *Zamia* and *Dioon* respectively) [Norstog *et al.* 1986, Tang 1987, Vovides 1991, Norstog *et al.* 1992, Cruz 1999, Pérez-Farrera *et al.* 2000]. Considering that members of a clade can share morphological characters, ecological and behavioural preferences, Vovides and his collaborators (Vovides *et al*. 1997, Sosa and Ogata 1998) extrapolated the pollination type of *Microcycas*, and suggested that its pollinators could be insects of the same genera or closely related. This reasoning and typical weevil traces found in old male cones, drove them to believe that *Mycrocycas'* pollinator would probably be one of the belid genera found in *Zamia* and *Dioon* (Vovides *et al*. 1997).

In male cones that had passed the moment of dehiscence, with rotting in progress, we found other insects that are still under study. None of them is a member of the family Belidae. Only one curculionoid species, not yet identified, of the subfamily Cryptorhynchinae (Curculionidae), was repeatedly present in those cones. Members of this subfamily have not been recorded as pollinators of cycads, and the morphology of this species does not fit to the general appearance of those that do pollinate (R. Oberprieler, pers. comm. 2002). Based on this, the weevil probably does not serve as a pollinator (C. Lyall, pers. comm. 2002). Besides, this weevil is not present in a proper moment to perform pollination. It is probable that this Cryptorhynchinae is the responsible for the exit holes in the microsporophylls that were observed in some old male cones as mentioned by Vovides and collaborators (1997).

Members of *Pharaxonotha* are the most frequently observed pollinators of New World Zamiaceae (Tang 1987, Pakaluk 1988, Vovides 1991, Norstog *et al.* 1992, Cruz 1999, Pérez-Farrera *et al.* 2000, González 2004). Given that they are known as pollinators in so many New World cycads, it is probable that they are the pollinators for cycads where no pollinator is known. To date, *P. esperanzae* has been the only flying insect found repeatedly on male cones of *Microcycas* at the optimal moment for pollination in an area where natural pollination occurs every year. These facts strongly support the hypothesis that *P. esperanzae* is the pollinator for *Microcycas*.

Nevertheless, to have complete certainty about its role as a pollinator, *P. experanzae* has yet to be found in female cones and demonstrated it carries pollen to micropils in megasporangia. This could be achieved by careful experiments, as those recently done by some researchers of cycad-pollinator interactions (Donaldson 1997, Mound and Terry 2001, Terry 2001, Wilson 2002, Hall *et al*. 2004). Further studies are required to discover if other species are involved in cycad pollination in this area or in other localities with seed production.

The discovery of *P. esperanzae* is an important step forward in identifying factors influencing the natural reproduction of *Microcycas*, and why some populations do not reproduce. The interaction between *P. esperanzae* and *Microcycas* needs to be considered in any management plan to conserve the remaining population of *Microcycas*. This interaction also adds support to the hypothesis that all cycads are insect-pollinated (Wilson 2002, Donaldson 2003). Furthermore, it stresses the major importance of primitive Erotylidae as pollinators of cycads, and especially of *Pharaxonotha*, which is now associated with four of eleven cycad genera.

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