ARPHA Preprints

PENSOFT

PREPRINT

Author-formatted, not peer-reviewed document posted on 09/05/2022

DOI: https://doi.org/10.3897/arphapreprints.e86124

Seed predation and potential seed dispersers of the endemic, narrowly distributed *Ceratozamia norstogii* (Zamiaceae)

Héctor Gómez-Domínguez, Jessica Hernández-Tapia, Andrés Ortiz-Rodriguez

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 09/05/2022*. DOI: https://doi.org/10.3897/arphapreprints.e86124

Seed predation and potential seed dispersers of the endemic, narrowly distributed *Ceratozamia norstogii* (Zamiaceae)

Héctor Gómez-Domínguez[‡], Jessica E Hernández-Tapia[§], Andrés E. Ortiz-Rodriguez[§]

‡ Senda sustentable, AC, Berriozábal, Chiapas, Mexico
§ Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, Mexico

Corresponding author: Andrés E. Ortiz-Rodriguez (andres.ortiz@ib.unam.mx)

Abstract

Reproductive ecology in *Ceratozamia* has been little studied. In particular, very little is known about seed dispersal. Here we report the observation of seed predators and potential seed dispersers of the endemic to Mexico and narrowly distributed *Ceratozamia norstogii* (Zamiaceae). Camera traps were installed in front plants of *Ceratozamia norstogii* and also strobili phenology until their maturity and disintegration was determined. The female strobili of *Ceratozamia norstogii* has a development of ten months, from the time it emerges until it disintegrates. We identified four stages of strobili development: 1) emergent strobili, 2) young strobili, 3) adult strobili, and 4) mature strobili. Our results support an animal-dispersal hypothesis in *Ceratozamia*. Three mammals (a mouse, a southern spotted skunk and a kinkajou) were observed consuming or removing seeds of *Ceratozamia norstogii*. Removal and consumption of mature seeds by frugivorous occur at night. The most frequent visitor was the mouse, followed by the southern spotted skunk and the kinkajou. Significant differences (GLM, $P<0.05$) in visitor frequency and time for interaction were found between the kinkajou and the rest of the frugivores, but not between the mouse and the skunk. At local scales, seed dispersal by small mammals could be related to the high-density populations of several *Ceratozamia* species, and at larger scales, related to events of allopatry.

Keywords

Cycads, mammals-seed dispersal, Mexico, reproductive ecology, skunk

Introduction

Ceratozamia (Zamiaceae), with ~35 species is one of the most diverse gymnosperms in Mexico (Martínez-Domínguez et al. 2021). All species of *Ceratozamia* (except *Ceratozamia robusta*) are endemic to Mexico, so its diversification can be attributed to the evolutionary history of this country. In recent years the taxonomy and distribution of *Ceratozamia* species have been widely studied and many aspects of their evolutionary history are now understood (Vovides 2000, Nicolalde-Morejón et al. 2014). Interestingly, it is poorly known about their reproductive ecology (Martínez-Domínguez et al. 2018 and references there), in particular, it is poorly known about seed dispersal in this group of plants.

Some authors suggest that cycad species in general can be dispersed by rodents (González-Christen 1990), birds (Eckenwalder 1980, Velazco-García et al. 2016) and small and medium-sized mammals (Velazco-García et al. 2016, Pérez-Farrera and Vovides 2004 , Hall and Walter 2013). Something interesting once the seeds of the cycads have toxic substances for animals (Moretti et al. 1983). In the particular case of the genus *Ceratozamia*, it is believed that gravity plays a very important role in seed dispersal (Martínez-Domínguez et al. 2021), but some observations of peccaries consuming seeds of *Ceratozamia matudae* Lundell and *C. mirandae* Vovides, Pérez-Farr. & Iglesias (Pérez-Farrera and Vovides 2004, Pérez-Farrera et al. 2000, Pérez-Farrera et al. 2006), and seeds inside burrows (Martínez-Domínguez et al. 2018), suggests that this generalization for the genus is wrong. In addition, the presence of mucilage and a hard seed coat in *Ceratozamia* seeds supports an animal-dispersal hypothesis (Hall and Walter 2013, Yang et al. 2012, Martínez-Domínguez et al. 2018).

Here we report the observation of seed predators and potential seed dispersers of the endemic to Mexico and narrowly distributed *Ceratozamia norstogii* D.W. Stev. (Zamiaceae). Specifically, we show the results of ten months of observation on strobili of this species. Our results aim to contribute to the knowledge about the reproductive ecology of this important group of plants, where most of its species are endangered (https://www.iucnredlist.org/search?query=Ceratozamia&searchType=species).

Methods

Study system

Ceratozamia norstogii is a species endemic to Mexico restricted to the Pine-oak forests and cloud forests in southern Mexico, in the states of Chiapas and Oaxaca. Individuals of this species have underground trunks 12 to 130 cm long, with a crown of 15 or more long-pinnate fronds at the tip, the fronds between 60 and 140 cm in length (Pérez-Farrera et al. 2001, Fig. 1). In this species of *Ceratozamia*, the very narrow leaflets are spirally distributed along the rachis and have small spines on the margin that decrease in frequency towards their base, being a distinct characteristic of the species (Stevenson

1982). *Ceratozamia norstogii* has two strobili, the male or microstrobilus, and the female or megastrobilus. The male strobili are conical, thinner towards the tip, pale-yellow or cream when mature, it can measure from 25 to 36 cm in length and from 3.8 to 5.1 cm in diameter (Pérez-Farrera et al. 2001). The female strobili are cylindrical about the same width at the base as at the tip, dark-brown when mature, it can measure 21-37 cm long and 9.1-13.1 cm in diameter, with more than 100 angulars to ovoid, arylated seeds, 2.4-2.9 cm long and 1.5-2.1 cm in diameter. Interestingly, when mature, the aril on the seeds changes color from yellow to brown and releases a sweet, pungent odor (Pérez-Farrera et al. 2001). Frugivores have not been observed consuming the seeds and the importance of these attributes is not clear. Overexploitation and land-use change threaten the populations of *Ceratozamia norstogii* [Endangered under criteria A2abd; B1ab (iii, iv, v)]. Evolutionary and ecological information that helps its conservation is necessary and fundamental to improve its conservation plans.

Data collecting

For ten months (October 2020 to July 2021) six camera traps (Bushnell prime 24 megapixles low glow) were installed in front plants of *Ceratozamia norstogii* with the objective of determining the strobili phenology until their maturity and disintegration and identifying predators and potential seed dispersers. We measured and recorded the changes in size and coloration of the strobili at each stage of their development and selected a mature female strobili to see foraging activity, including frequency of visits and time for interaction. The observations were carried out within the "La Sepultura" Biosphere Reserve in Chiapas, Mexico (the exact location of the population is not declared considering the vulnerability of this species).

Data analyses

We used a poisson regression approach for handling the count data. We performed two generalized linear models (glm) using the frequency of visits per night and the time of interaction per night as response variables and each frugivore as a factor. For each analysis, we compared foraging activity of frugivores through a chi-square test using the “nagelkerke” function of “rcompanion” package (Mangiafico 2022) with R software (R Core Team, 2020). A higher frequency of visits with prolonged times of activity on the seeds (e.g. eating the seeds or removing the seed coat) was considered as evidence of potential seed dispersers. The graphs were made with the ggplot2 package (Wickham 2016).

Results

Phenology

The female strobili of *Ceratozamia norstogii* has a development of ten months, from the time it emerges until it disintegrates. In the population studied here, this period includes the months between October 2020 and July 2021. We were able to identify four stages of

strobili development (Fig. 2): **A** Emergent strobili, an erect strobili, 80 to 110 by 30 to 40 mm, with an overall reddish-brown coloration, but with cream-colored spine tips and a short, 50 to 60 mm, straight peduncle, duration two months (Oct.-Nov.); **B** Young strobili, a slightly inclined strobili, 100 to 120 by 35 to 52 mm, with a much longer peduncle, 90 to 110 mm, but retaining the coloration of the previous stage, duration three months (Dec.-Feb.); **C** Adult strobili, a totally decumbent strobili, 200 to 240 by 60 to 80 mm, with a longer peduncle, 100 to 120 mm, and with a dark green color, lasting two months (Mar.-May); **D** mature strobili, an adult strobilus, 220 to 240 by 60 to 80 mm, exuding along its surface a yellow-amber mucilage, lasting two months (Jun.-Jul.) and **E** disintegrated strobili, a mature adult strobilus, attacked to disintegration by several frugivorous visitors.

Diversity of visitors

The camera traps captured seven visitors to the female strobili of *Ceratozamia norstogii* (Table 1). The highest number of observations was recorded during the night hours (nocturnal visitors) and less frequently during sunny hours (diurnal visitors). Observations were recorded both at young stages of the strobilus and at mature stages of its development. During sunny hours, three bird species were recorded using the strobili as a perch, two on an immature strobilus and the other on the remains of a disintegrated strobilus. Also in daylight, a badger stopped for a moment to smell the strobili and then continued its way. During the night, the visitors were small and medium-sized mammals (Fig. 3), which interacted directly with the female strobilus. A mouse was frequently observed feeding on the strobilus exudate and carrying seeds. A kinkajou was observed visiting the strobilus and consuming seeds. And, a southern spotted skunk was very active and was observed consuming seeds on several occasions (Table 1, Fig. 3).

Frugivores foraging activity and potential seed dispersers

Three mammals (a mouse, a southern spotted skunk and a kinkajou) were observed consuming or removing seeds of *Ceratozamia norstogii* (Table 1, Fig. 3). Removal and consumption of mature seeds by frugivorous occur at night (after eight at night). Frugivore activity increases between midnight and three in the morning (Fig. 4) and it is during the second week of July that frugivore activity was most frequent (Fig. 5). The mouse of the genus *Peromiscus* was observed frequently during a large part of the strobili maturity period (eleven nights, 40 visits in total, time per interaction=6.1 ± 3.1 seconds), followed by the southern spotted skunk (four nights, fourteen visits, time per interaction = 6.8 ± 3.4 seconds) and the kinkajou (one night, two visits, mean time per interaction =10 seconds) (Fig. 4, Fig. 5). Interestingly, significant differences (GLM, $P<0.05$) in visitor frequency were found between the kinkajou and the rest of the frugivores (Fig. 6), but not between the mouse and the skunk. Similarly, when the time for interaction between frugivores was compared (Fig. 6).

 Author-formatted, not peer-reviewed document posted on 09/05/2022. DOI: https://doi.org/10.3897/arphapreprints.e86124

Discussion

Our results support the animal-dispersal hypothesis in *Ceratozamia*. As has been observed, mice play a very important role in seed dispersal in cycads (González-Christen 1990) and *Ceratozamia norstogii* is no exception. The mouse of the genus *Pteromiscus* was a frequent visitor to the mature strobili of this species and was observed biting and carrying seeds for several nights and for a long time. As has been observed in other cycads (Hall and Walter 2013, Monteza-Moreno et al. 2022), seed removal is carried out by a very low diversity of small mammals, almost specifically. In this study, the observation of the spotted skunk and the kinkajou consuming seeds of *Ceratozamia norstogii* is reported for the first time with no other frugivores species observed. Interestingly, all strobili visitors are nocturnal animals, and the removal and dispersal of *Ceratozamia norstogii* seeds occurs at night. Nocturnal seed dispersal has been observed in other cycad species (Hall and Walter 2013, Monteza-Moreno et al. 2022), so it is likely that nocturnal dispersal is common among cycad species.

The southern spotted skunk and the kinkajou are small mammals that feed mainly on insects, but as omnivores they can also feed on other smaller animals, carrion, fruits, and seeds (Kinlaw 1995). The kinkajou is a mammal considered arboreal, so its participation in the removal of *Ceratozamia norstogii* seeds with strobili at ground level is a very interesting finding. In general, skunk species and the kinkajou are not recognized as important seed dispersers. However, our results show that the role of the skunk as a potential seed disperser in *Ceratozamia norstogii* is not different from that of the mouse. For the kinkajou, on the other hand, it seems a more occasional activity. It is likely that the seeds of *Ceratozamia norstogii* represent a very important seasonal food resource for skunks and kinkajou. Therefore, removal experiments will be necessary to assess the effectiveness of these two mammals as seed dispersers of *Ceratozamia norstogii*. Also, realize observation in other populations, more time duration of the study, and count the number and distance of seed disperses. The above is to improve our knowledge about phenology and seed dispersal of Mexican cycads.

It has been suggested that seed dispersal by small mammals encourages new plants to grow within a few meters of the original plants, and therefore, at local scales, cycads occur in high-density stands (Hall and Walter 2013). In parallel, short-distance dispersal promotes allopatric speciation as the main mechanism of evolution in this group of plants. In this sense, it is likely that dispersal by small mammals has played a very important role in the most recent evolutionary history of cycads in Mexico.

Conservation implications

Knowing the main seed dispersal vectors in cycads broadens the knowledge about the organisms that it is important to conserve. Knowing the mechanisms and modes of

dispersal of cycads also provides us with better tools to plan strategies for their conservation such as germination or propagation work.

Acknowledgements

We are particularly grateful to Yuyini Licona Vera, and all reviewers for providing many useful comments on the manuscript.

Author contributions

HG-D & AE Ortiz-Rodriguez conceived and designed the study. HG-D performed the fieldwork. HG-D, JHT & AE Ortiz-Rodriguez processed and analysed the data. AE Ortiz-Rodriguez wrote the manuscript. All authors commented the manuscript and approved its final version.

Conflicts of interest

The authors declare that they have no competing interests.

References

- Eckenwalder JE (1980) Dispersal of the West Indian cycad *Zamia pumila* L. Biotropica 12: 79-80. https://doi.org/10.2307/2387779
- González-Christen A (1990) Algunas interacciones entre *Dioon edule* (Zamiaceae) y *Peromycus mexicanus* (Rodentia: Cricentidae). La Ciencias y el Hombre 5: 77-92.
- Hall JA, Walter GH (2013) Seed dispersa lof the Australian cycad *Macrozamia miquelii* (Zamiaceae): Are cycads megafuana-dispersed “grove forming” plants? American Journal of Botany 100: 1127-1136. https://doi.org/10.3732/ajb.1200115
- Kinlaw A (1995) *Spilogale putorius*. Mammalian Species 511: 1-7.
- Mangiafico S (2022) rcompanion: Functions to support extension education program evaluation. version 2.4.13. R package. URL: https://CRAN.R project.org/package=rcompanion
- Martínez-Domínguez L, Nicolalde-Morejón F, Vergara-Silva F, Stevenson D, et al. (2018) Taxonomic review of *Ceratozamia* (Zamiaceae) in the Sierra Madre Oriental, Mexico. PhytoKeys 100: 91-124. https://doi.org/10.3897/phytokeys.100.23152
- Martínez-Domínguez L, Nicolalde-Morejón F, Stevenson D, Santiago-Jiménez Q, et al. (2021) The need for multidisciplinary conservation: A case study of *Ceratozamia* (Zamiaceae, Cycadales) in eastern Mexico. Oryx 55 (6): 947-956. https://doi.org/10.1017/S0030605320000204
- Monteza-Moreno CM, Rodriguez-Castro L, Castillo-Caballero PL, Toribio E, et al. (2022) Arboreal camera trapping sheds light on seeddispersal of the world’s only epiphytic gymnosperm: *Zamia pseudoparasitica*. Ecology and Evolution 12: e8769. https://doi.org/10.1002/ece3.8769

- Moretti A, Sabato S, Gigliano S (1983) Taxonomic significance of methylazoxymethanol glycosides in the cycads. Phytochemistry 22: 115-117. https://doi.org/10.1016/S0031-9422(00)80069-2
- Nicolalde-Morejón F, González-Astorga J, Vergara-Silva F, Stevenson DW, et al. (2014) Biodiversidad de Zamiaceae en México. Revista Mexicana de Biodiversidad 85: S114-S125. https://doi.org/10.7550/rmb.38114.
- Pérez-Farrera MA, Quintana-Ascencio PF, Salvatierra-Izaba B, Vovides AP (2000) Population dynamics of *Ceratozamia matudai* (Zamiaceae) in El Triunfo Biosfere Reserve, Chiapas, México. Journal of the Torrey Botanical Society 127: 291-299. https://doi.org/10.2307/3088647
- Pérez-Farrera MA, Vovides AP, Iglesias C (2001) The cycad *Ceratozamia norstogii* D. W. Stev. (Zamiaceae) from southern México: new information on distribution, habitat and vegetative morphology. Botanical Journal of the Linnean Society 137: 71-76. https://doi.org/10.1111/j.1095-8339.2001.tb01106.x
- Pérez-Farrera MA, Vovides AP (2004) Spatial distribution, population structure and fecundity of *Ceratozamia mirandae* Lundell (Zamiaceae) in El Triunfo Biosfere Reserve, Chiapas, Mexico. Botanical Review 70: 299-311. https://doi.org/10.1663/0006-8101(2004)070[0299:SDPSAF]2.0.CO;2
- Pérez-Farrera MA, Vovides AP, Octavio-Aguilar P, González-Astorga J, et al. (2006) Demography of the cycad *Ceratozamia mirandae* (Zamiaceae) under disturbed and undisturbed conditions in a Biosfere Reserve of Mexico. Plant Ecology 187: 97-108. https://doi.org/10.1007/s11258-006-9135-2
- Stevenson D (1982) A new species of Ceratozamia (Zamiaceae) from Chiapas, Mexico. Brittonia 34: 181-184. https://doi.org/10.2307/2806372
- Velazco-García MV, Valdez-Hernández J, Ramírez-Herrera C, Hernández-Hernández ML, et al. (2016) Estructura, heterogeneidad de estadios y patrón de dispersión espacial de *Dioon holmgrenii* (Zamiaceae). Botanical Sciences 94: 75-87. [In Spanish]. https://doi.org/10.17129/botsci.258.
- Vovides AP (2000) Mexico: segundo lugar mundial en diversidad de cícadas. Biodiversitas 6 (31): 6-10. https://doi.org/10.7550/rmb.38114
- Wickham H (2016) ggplot2: Elegant graphics for data analysis. 2nd Edition. Springer-Verlag, New York. https://doi.org/10.1007/978-0-387-98141-3
- Yang X., Baskin JM, Baskin CC, Huang Z (2012) More than just a coating: ecological importance, taxonomic occurrence and phylogentic relationships of seed coat mucilage. Perpectives in Plant Ecology, Evolution and Systematics 14: 434-442. https://doi.org/10.1016/j.ppees.2012.09.002

Author-formatted, not peer-reviewed document posted on 09/05/2022. DOI:
https://doi.org/10.3897/arphapreprints.e86124

Figure 1.

Ceratozamia norstogii in the study area.

Author-formatted, not peer-reviewed document posted on 09/05/2022. DOI:
https://doi.org/10.3897/arphapreprints.e86124

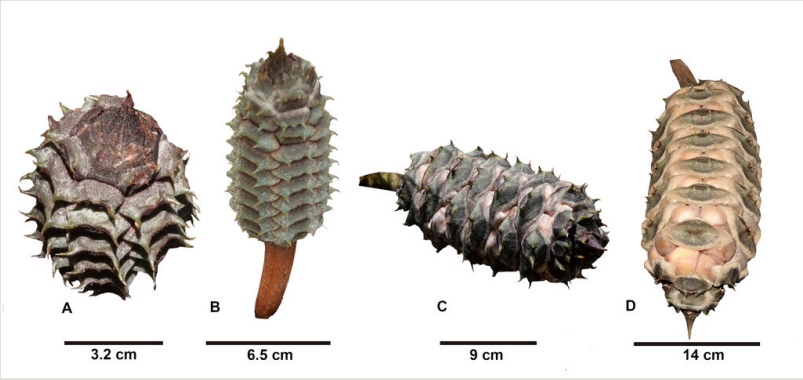

Figure 2.

Female strobili maturation. **A** Emergent strobili. This stage is characteristic of the strobilus erect position on the plant, and its reddish-brown coloration. Occurs from October to November. **B** Young strobili. In this stage, the position of storbilus is slightly inclined and has the same coloration that when emerging. Occurs from December to February. **C** Adult strobili. In this stage, the strobilus is totally decumbent and turns into a dark green coloration. Occurs from March to May. **D** Mature strobili. In this stage, the strobilus has the same appearance as adult strobili, but the seeds are visuals. Occurs from June to July.

Author-formatted, not peer-reviewed document posted on 09/05/2022. DOI:
https://doi.org/10.3897/arphapreprints.e86124

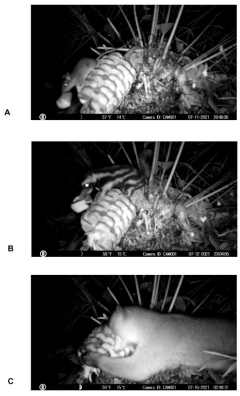

Figure 3.

Strobilus visitors in *Ceratozamia norstogii*. **A** *Pteromiscus* sp. (Mouse) collecting seeds **B** *Spilogale angustifrons* (a southern spotted skunk) biting a strobilus and collecting seeds **C** *Potus flavus* (Kinkajou) collecting seeds.

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 09/05/2022*. DOI: https://doi.org/10.3897/arphapreprints.e86124

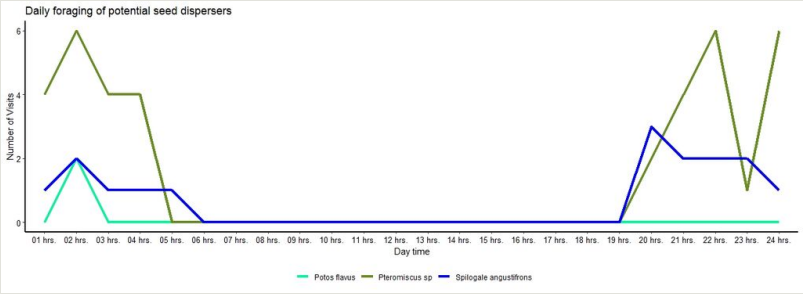

Figure 4.

Nocturnal foraging activity of potential seed dispersers.

Author-formatted, not peer-reviewed document posted on 09/05/2022. DOI:
https://doi.org/10.3897/arphapreprints.e86124

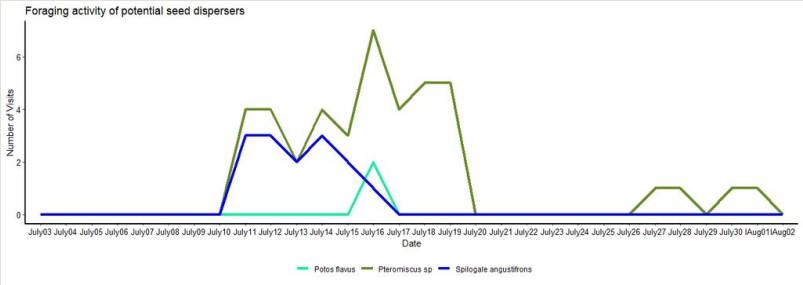

Figure 5.

Foraging activity and potential seed dispersers.

Author-formatted, not peer-reviewed document posted on 09/05/2022. DOI:
https://doi.org/10.3897/arphapreprints.e86124

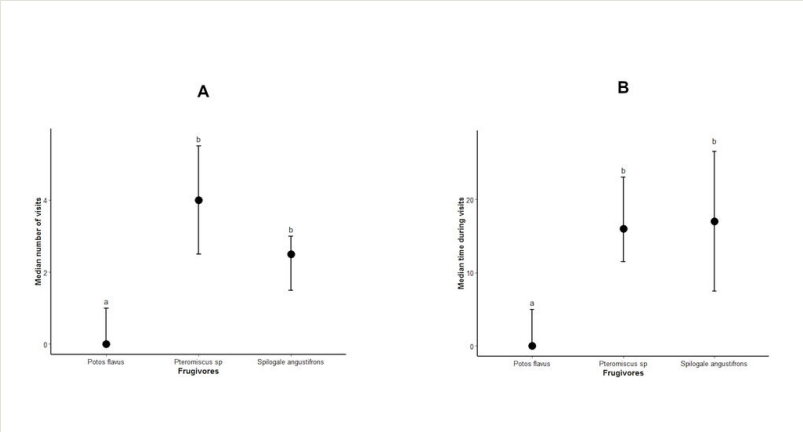

Figure 6.

Number of visits and visits duration per frugivore. **A** The mean values of visits per frugivores. *Pteromiscus* sp. is the frugivore more constant, followed by *Spirogale angustifrons* and the last *Potos flavus*. **B** The mean values of visits duration. In contrast with the number of visits, the *Spirogale angustifrons* present a major visit duration, followed by *Pteromiscus* sp. and *Potos flavus*.

Author-formatted, not peer-reviewed document posted on 09/05/2022. DOI:
https://doi.org/10.3897/arphapreprints.e86124

Table 1.

Strobilus visitors in *Ceratozamia norstogii*. The activity period includes diurnal and nocturnal. The diurnal visit covers from five in the morning until eight at night. The nocturnal visit covers from eight at night until five in the morning. The general behavior of each visitor is reported.

Species	**Period of activity**	**General behavior**
Momotus mexicanus	Diurnal	Perched
Basileuterus lachymosa	Diurnal	Perched
Spilogale angustifrons	Nocturnal	Bite and take some seeds, walk around
Pteromiscus sp.	Nocturnal	Bite and take some seeds, walk around
Nassua narica	Diurnal	Sniffs the strobili and walks away
Potus flavus	Nocturnal	Bite and take some seeds, walk around