A first record of *Anatrachyntis badia* (Hodges 1962) (Lepidoptera: Cosmopterigidae) on *Zamia integrifolia* (Zamiaceae)

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Adult moths that appeared morphologically similar to species in the genus *Anatrachyntis* (Lepidoptera: Cosmopterigidae) were observed emerging from male cones of *Zamia integrifolia* (Zamiaceae) collected in Coral Gables, Florida, USA, during Dec 2014 at a time when the cones were releasing pollen. These moths have been recorded associating with cycads on only 2 previous occasions: (1) Adults of an unidentified species of *Anatrachyntis* that develop larvae in the pollen cone were reported as pollinators of an Old World cycad, *Cycas micronesica* Hill (Cycadaceae), on Guam (Terry et al. 2009; Marler & Niklas 2011), and larval feeding damage by this species was found to increase lifetime reproductive events of the host plant (Marler 2010a, b); and (2) Bella and Mazzeo (2006) discovered *Anatrachyntis badia* Hodges (Lepidoptera: Cosmopterigidae) larvae feeding on the leaves of cultivated *Cycas revoluta* and *C. circinalis* (Cycadaceae) in Italy. However, no records exist of *Anatrachyntis* occurring on New World cycads, nor have any other Lepidoptera been described to pollinate these endangered plants. Larvae of an unidentified genus of moth in the family Blastobasidae were found once on pollen cones of a species of *Zamia* in the Greater Antilles (Terry et al. 2012a), but their pollination potential remains unknown.

Of the some 50 species of moths in the genus *Anatrachyntis*, research has focused primarily on *A. badia. Anatrachyntis badia* is native to the southern United States (Hodges 1962) but has been introduced across Europe (Dawidowicz & Rozwalka 2017). It is known as a scavenger that feeds on a variety of hosts, including blossoms of coconuts, elm leaves, decaying fruit, and cones of conifers (Hodges 1962) earning it the common name 'Florida pink scavenger.'

Cycad herbivory requires a strategy for dealing with the host plant's secondary metabolites, as all species of cycads are highly and uniquely toxic (Schneider et al. 2002). In most cases, insects that have managed to colonize and feed on cycads have radiated within this relatively open ecological niche. Examples include the leaf-feeding Lepidoptera genus *Eumaeus* (Lycaenidae) (Landolt 1984; Contreras-Medina et al. 2003), and cone-feeding and pollinating Coleoptera genera *Rhopalotria* (Belidae) (O'Brien & Tang 2015), and *Pharaxanotha* (Erotylidae) (Pakaluk 1988). One species of each of these genera can be found in Florida feeding on the native cycad, *Z. integrifolia* (Landolt 1984; Tang 1987; Fawcett & Norstog 1993).

Cycads once were considered strictly wind-pollinated, but it is becoming increasingly evident that most, if not all, rely on insect pollination where the host plant provides a brood site for the pollinator in the expendable male cone tissue (Terry et al. 2012b). Neotropical *Zamia* cycads have symbiotic pollinating relationships with 2 genera of Coleoptera (Stevenson et al. 1998). Larvae and adults of both *Rhopalotria* and *Pharaxanotha* species feed, mate, shelter, and oviposit in male cones (Stevenson et al. 1998; Fawcett & Norstog 1993; Tang 1987). When these pollinators are present, cone fertility approaches 100% but when excluded, fertility drops to nothing (Tang 1987).

The Cycadales is the most threatened plant order (IUCN 2017), and because it relies on insect pollination, identification of another pollinator is beneficial for conservation efforts. One-to-one mutualistic pollinator-plant relationships are fragile; if a pollinator goes extinct, the plant will follow suit and vice versa. Conservationists are currently working to grow these endangered plants ex situ before returning them to the field. However, a major concern is that if the pollinators are not present or have gone extinct in the meantime, these plants might be unable to reproduce following restoration in the wild (Calonje et al. 2011). The identification of a generalist pollinator with multiple host plants like *A. badia* could greatly help conservation efforts for these endangered plants.

Adult moths (Fig. 1) were collected after emerging from dehiscing pollen cones of *Z. integrifolia* collected in Coral Gables, Florida, USA. DNA was extracted from 2 specimens using the DNeasy® Blood & Tissue kit and protocol (Qiagen, Waltham, Massachusetts, USA). PCR of the CO1 gene was performed using Omega 2x master mix (Bio-Tek, Norcross, Georgia, USA) and the primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198: (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). The PCR protocol consisted of a 2 min initial denaturing step at 95 °C, 37 cycles of 30 sec at 95 °C, 1 min at 50 °C, and 1 min 30 sec at 72 °C, and a final 5 min 72 °C extension. The PCR product was sequenced by Eton Bioscience Inc. (Boston, Massachusetts, USA) using Sanger sequencing methods. Sequence reads were assembled and edited using Geneious (Biomatters Limited, version 10.2.3) (Kearse et al. 2012) and searched and identified in the NCBI BLAST database as *Anatrachyntis badia* (100% sequence identity). Adult moths in the series are deposited in the Harvard Museum of Comparative Zoology (Catalog numbers 711075–711094) and sequences are available on Genbank with accession numbers (MG836502-3).

The authors downloaded 109 *Anatrachyntis* CO1 sequences available on the Barcode of Life Database (BOLD) and included them in a phylogenetic analysis. All 113 sequences were aligned using the Geneious alignment algorithm and manually edited. The model of evolution for the CO1 gene alignment was determined using jModelTest (version 2.1.10) (Posada 2008). Both Bayesian information criteria and

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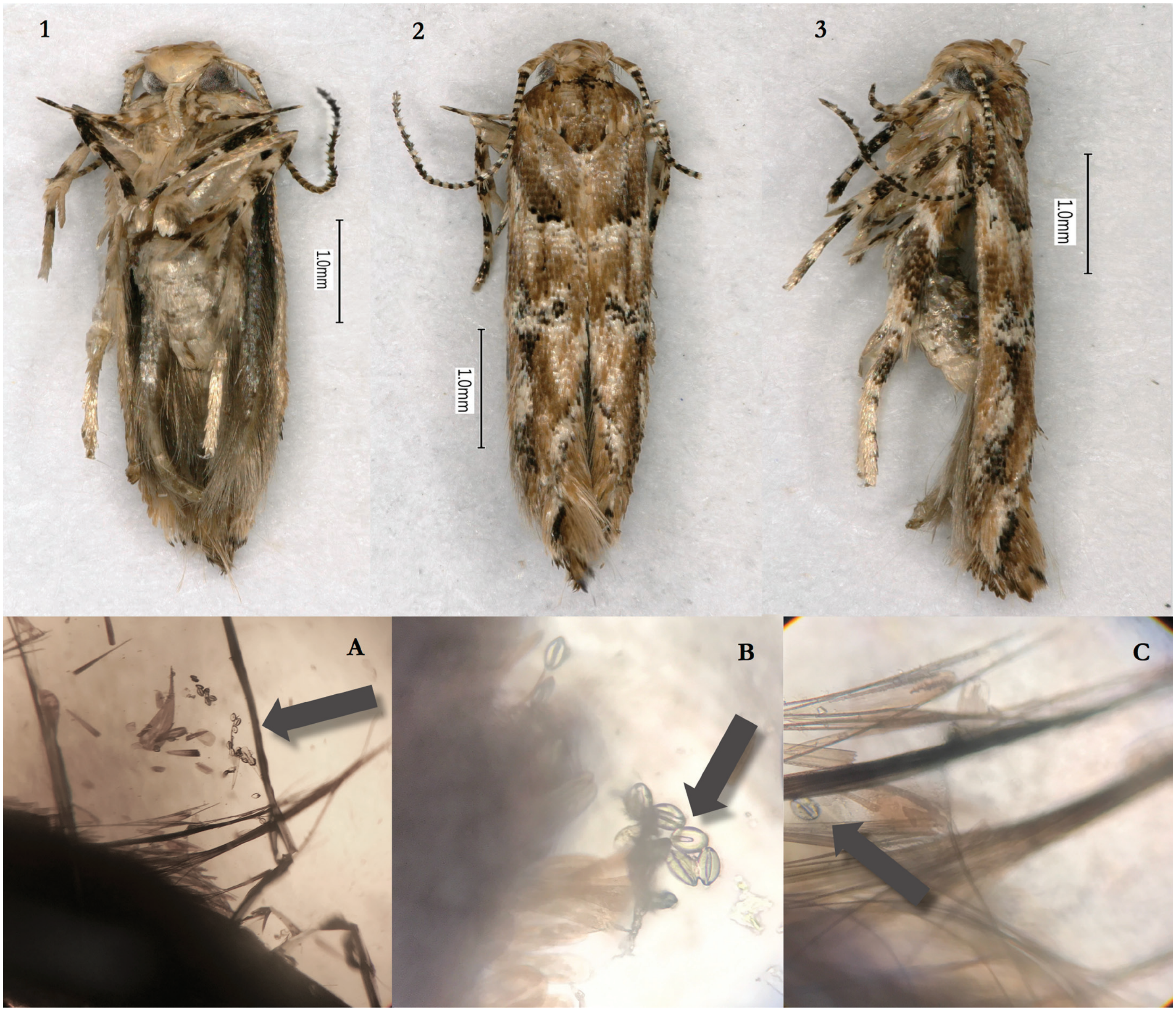

Fig. 1. Pollen found on *Anatrachyntis badia* wing hairs suggest that *A. badia* could be involved in pollination of *Zamia integrifolia*. *Anatrachyntis badia*: dorsal view (1), ventral view (2), lateral habitus view (3). Microscope magnification of wing edges: 4× (A), 40× (B), 40× (C).

the Akaike information criterion determined the model of evolution to be Generalised time-reversible + Gamma (GTR+G) (BIC negative log likelihood 1998.0423). A phylogenetic tree was created using PhyML (Guindon & Gascuel 2003), as implemented in Geneious, and the GTR+G model using the best of nearest neighbor interchange and subtree pruning-regrafting tree topology searches. The maximum likelihood tree had a negative log likelihood of –2020.75868 and placed the samples in a polytomy of *Anatrachyntis badia* BOLD vouchers (Fig. 2).

Light microscopy was used to explore the life history of the *A. badia-Z. integrifolia* relationship. A dissecting microscope was used to look for pupal casings in the pollen cones of the host plant and adult moths were examined under 4×, 10×, and 40× power to look for the presence of pollen on the body (Fig. 1). Microscope analysis showed pollen adhered to the wings, but no specialized mandible structures for pollen transport such as the maxillary tentacles seen in yucca moths (Pellmyr 2003). Pupal casings were found in the pollen cone peduncle (not shown).

This new record of the presence of *Anatrachyntis* on a Neotropical species of cycad suggests that they may be predisposed to feeding on this uniquely toxic host tissue, and that further surveys may find members of this genus associating with species throughout the Cycadales. Additionally, the presence of pollen on the moth body, while not conclusive, suggests the potential for pollination, given that an unidentified *Anatrachyntis* is known to pollinate *Cycas micronesica* (Terry et al. 2009). In many cases, herbivores that feed on cycads also specialize in cycad habitation (e.g., Pakaluk 1988; Contreras-Medina et al. 2003; O'Brien & Tang 2015). Because of cycads' unique toxicity, they can provide 'enemy free space' with low competition for food or brood sites. Developing within the host cycad's dense pollen cone provides physical protection from predators, and consuming toxic tissue may provide chemical protection. The ability to utilize this niche coupled with their global distribution indicates that this genus of moths could be more diversified on cycads than currently appreciated, and could have played an important role in the evolutionary history of the group.

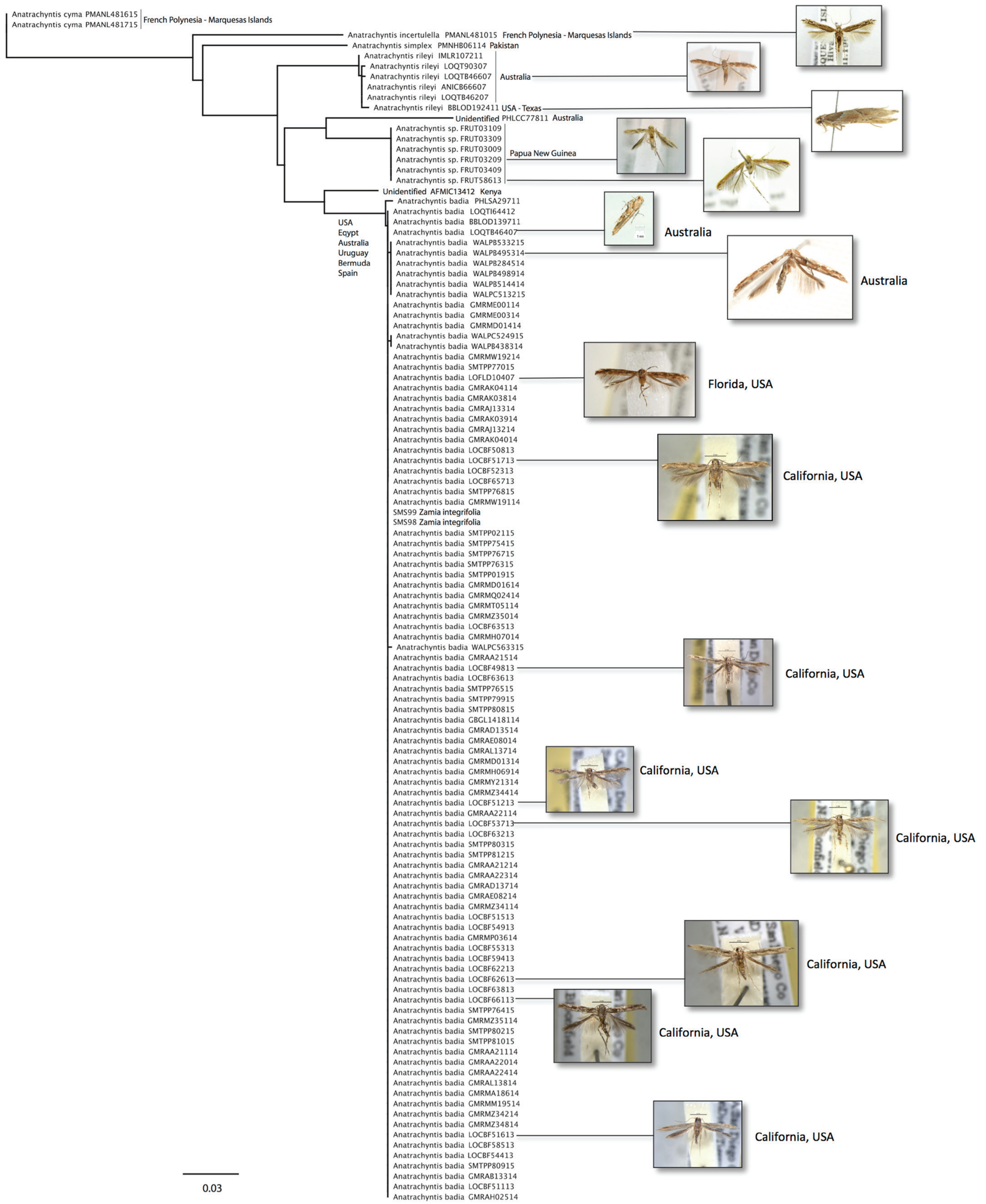

Fig. 2. Moths that emerged from *Zamia integrifolia* are *Anatrachintis badia*. Maximum likelihood phylogenetic tree of genus *Anatrachyntis* including 109 *Anatrachyntis* CO1 sequences from Barcode of Life Database. Representative photographs and localities are included.

Additional populations of cycads should be surveyed and individuals from across the range of *Anatrachyntis* should be included in a genus-wide phylogenetic reconstruction to learn more about the history of their association with cycads. Trapping and exclusion experiments also could be carried out to determine pollination ability and efficiency in this and any additionally identified species pairs.

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Summary

This paper describes the first record of the cosmopolitan moth *Anatrachyntis badia* on the native Florida cycad *Zamia integrifolia*, and discusses its possible pollination benefit for this threatened plant. This new record is interesting due to the highly toxic nature of these endangered plants as well as their strict reliance on insect pollination for reproduction. It suggests that associations between species of *Anatrachyntis* and members of the Cycadales may be more widespread than currently known.

Key Words: Cycadales; pollination

Sumario

Este artículo se informa del primer registro de la polilla cosmopolita, *Anatrachyntis badia,* sobre la cíca nativa de la Florida, *Zamia integrifolia*, y se discute su posible beneficio en polinizar esta planta amenazada. Este nuevo registro es interesante debido a la naturaleza altamente tóxica de estas plantas en peligro de extinción, así como su estricta dependencia de la polinización de insectos para su reproducción. Sugiere que las asociaciones entre las especies de *Anatrachyntis* y los miembros de Cycadales pueden estar más extendidas de lo que se sabe actualmente.

Palabras Clave: Cycadales; polinización

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