REVIEW AND SYNTHESIS

Ecology and evolution of cycad-feeding Lepidoptera

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Abstract

Cycads are an ancient group of tropical gymnosperms that are toxic to most animals – including humans – though the larvae of many moths and butterflies (order: Lepidoptera) feed on cycads with apparent immunity. These insects belong to distinct lineages with varying degrees of specialisation and diverse feeding ecologies, presenting numerous opportunities for comparative studies of chemically mediated eco-evolutionary dynamics. This review presents the first evolutionary evaluation of cycad-feeding among Lepidoptera along with a comprehensive review of their ecology. Our analysis suggests that multiple lineages have independently colonised cycads from angiosperm hosts, yet only a few clades appear to have radiated following their transitions to cycads. Defensive traits are likely important for diversification, as many cycad specialists are warningly coloured and sequester cycad toxins. The butterfly family Lycaenidae appears to be particularly predisposed to cycad-feeding and several cycadivorous lycaenids are warningly coloured and chemically defended. Cycad–herbivore interactions provide a promising but underutilised study system for investigating plant–insect coevolution, convergent and divergent adaptations, and the multi-trophic significance of defensive traits; therefore the review ends by suggesting specific research gaps that would be fruitfully addressed in Lepidoptera and other cycad-feeding insects.

Keywords

acquired chemical defense, aposematism, BMAA, chemical ecology, coevolution, defensive ecology, host transitions, MAM-glycosides, plant–insect interactions, phytotoxin.

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INTRODUCTION

Lepidoptera (butterflies and moths) have long been used to test theories about the evolutionary origins and consequences of ecological traits, and their larval associations with host-plants have served as a scientific cornerstone of research into coevolution and chemical ecology. An extensive literature on the physiological, morphological, behavioural, genetic and ecological mechanisms of plant–butterfly interactions has developed over the last half century, largely in response to Ehrlich's & Raven's seminal 1964 paper describing macroevolutionary patterns of host use among butterflies. These studies have elucidated the biological basis and ecological significance of acquired chemical defence in insects (Brower, 1969), identified key innovations underlying specialisation (Berenbaum et al., 1996) and described chemical communication among plants, herbivores and higher trophic levels (De Moraes et al., 1998; Glinwood et al., 2011). They have identified phytochemical convergence among distantly related plant families (Agrawal et al., 2012) and documented molecular and behavioural convergence among insects in their counteradaptations to plant defences (Birnbaum and Abbot, 2018; Karageorgi et al., 2019). Much of the progress in this field has been borne from studies of agricultural systems and model interactions, such as

monarch butterflies specialised on latex- and cardenolide-producing milkweeds (Brower, 1984; Malcolm and Brower, 1989; Agrawal et al., 2012), *Zygaena* moths that sequester and synthesise cyanogenic glucosides (Zagrobelny and Møller, 2011; Pentzold et al., 2014a), arctiid moths and their pyrrolizidine alkaloid producing hostplants (Rothschild et al., 1979; Boppré, 1990), caterpillars specialised on plants defended by furanocoumarins (Berenbaum and Zangerl, 1993; Berenbaum, 2001), aristolochic acids sequestered by swallowtail butterflies (Rothschild et al., 1972; Nishida and Fukami, 1989; Fordyce, 2000) and pierid larvae feeding on glucosinolate-rich plants in the Brassicales (Braby and Trueman, 2006; Wheat et al., 2007). These systems and others have taught us a great deal about how phytochemicals shape plant–insect interactions over ecological and evolutionary timescales.

But for all its richness and impact, the literature on chemically mediated plant–herbivore interactions has a 'precariously narrow base' (Berenbaum and Zangerl, 2008): the overwhelming majority of research is focused on insects that feed on a handful of angiosperm families, with comparatively little investigation into non-angiospermous diets (but see Braby, 2000; Pierce et al., 2002; Kaliszewska et al., 2015; Cong et al., 2016; Whitaker et al., unpublished data). To achieve a more comprehensive understanding of the generalities and

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Table 1 Larval hostplant records for cycadivorous Lepidoptera. See text for explanation of primary and secondary host use among geometrid moths. Species synonyms are given in Table S2 in Supporting Information. *Non-native plant host

Species	Cycad hosts	Other hosts	Sources
Nymphalidae			
Taenaris Hübner, 1819			
T. butleri (Oberthür, 1880)	*Cycas* (species unknown)		[1, 2]
T. onolaus (Kirsch, 1877)	*Cycas* (species unknown)		[1, 2]
Lycaenidae			
Eumaeus Hübner, 1819			
E. atala (Poey, 1832)	*Zamia integrifolia, Z. vasquezii*, Cycas revoluta*, Encephalartos villosus*, Macrozamia lucida*,* at least 30 other non-native species		[3–5]
E. childrenae (Gray, 1832)	*Dioon edule, D. merolae, Ceratozamia matudae, C. mexicana, C. norstogii, C. robusta, C. chimalapensis, Zamia fischeri, Z. soconuscencis, Cycas revoluta**		[6–9]
E. godartii (Boisduval, 1870)	*Zamia acuminata, Z. fairchildiana, Z. manicata, Z. stevensonii*		[10]
E. minyas (Hübner, [1809])	*Zamia encephalartoides, Z. skinneri*		[11, 12]
E. toxana (Boisduval, 1870)	Unknown		
E. toxea (Godart, [1824])	*Zamia furfuracea, Z. paucijuga, Z. encephalartoides, Z. loddigesii*		[13–15]
Luthrodes Druce, 1895			
L. cleotas (Guérin-Méneville, [1831])	*Cycas* (species unknown)		[2]
L. pandava (Horsfield, [1829])	>85 species of *Cycas*		[16–18]
L. peripatria (Hsu, 1980)	*Cycas taitungensis, Cycas revoluta**		[19]
Theclinesthes Röber, 1891			
T. onycha onycha (Hewitson, 1865)	*Cycas megacarpa, C. ophiolitica, C. media*		[20, 21]
T. onycha capricornia (Sibatani and Grund, 1978)	*Macrozamia spiralis, M. communis, M. pauli-guilielmi*		[19]
Geometridae			
Zerenopsis Felder, 1874			
Z. costimaculata (Prout, 1913)	Primary hosts: *Encephalartos hildebrandtii*	Secondary hosts: unknown in the wild, *Diospyros lycioides* in captivity	[22]
Z. flavimaculata Staude & Sihvonen, 2014	Unknown	Unknown	[22]
Z. geometrina (C. & R. Felder, 1874)	Primary hosts: *Stangeria eriopus, Encephalartos villosus*	Secondary hosts: *Apodytes dimidiata, Mimusops obovata*	[22]
Z. kedar (Druce, 1896)	Unknown	Unknown	[22]
Z. lepida (Walker, 1854)	Primary hosts: *Stangeria eriopus, Encephalartos* (>20 species), *Cycas thouarsii, C. circinalis*, C. revoluta*, Dioon* sp.*	Secondary hosts: *Carissa bispinosa, C. macrocarpa, Diospyros lycioides, D. whyteana, Apodytes dimidiata, Maesa alnifolia, M. lanceolata, Sclerocarya birrea*	[22–24]
Z. meraca (Prout, 1928)	Unknown	Unknown	[22]
Z. moi Staude and Sihvonen, 2014	Primary hosts: *Encephalartos ferox*	Secondary hosts: unknown in the wild, *Diospyros lycioides* in captivity	[22]
Z. tenuis (Butler, 1878)	*Encephalartos hildebrandtii*	*Adansonia digitata*	[2]
Veniliodes Warren, 1894			
V. inflammata Warren, 1894	Primary hosts: *Stangeria eriopus, Encephalartos villosus*	Secondary hosts: *Apodytes dimidiata, Diospyros lycioides*	[24]
V. pantheraria (C. & R. Felder, 1874)	Primary hosts: *Stangeria eriopus, Encephalartos villosus*	Secondary hosts: *Apodytes dimidiata, Diospyros lycioides*	[24, 25]
V. setinata (C. & R. Felder, 1875)	*Stangeria eriopus*		[24]
Callioratis C. & R. Felder, 1874			
C. abraxas Staude, 2001	Primary hosts: *Encephalartos lebomboensis, E. altensteinii, E. villosus*	Secondary hosts: *Apodytes dimidiata, Diospyros whyteana, Carissa* sp.	[24]
C. apicisecta Prout, 1915	*Stangeria eriopus & Encephalartos tegulaneus* in the wild, *E. villosus* in captivity		[24]
C. curlei Staude, 2001	*Stangeria eriopus, Encephalartos friderici-guilielmi*		[24]
C. grandis Prout, 1922	*Encephalartos gratus*		[26]
C. mayeri Staude, 2001	*Encephalartos friderici-guilielmi*		[24]
C. millari Hampson, 1905	Primary hosts: *Stangeria eriopus* in the wild, *Encephalartos villosus* in captivity	Secondary hosts: *Diospyros lycioides* in the wild, *Tropaeolum majus* flowers in captivity	[24]

(continued)

Table 1 (continued)

Species	Cycad hosts	Other hosts	Sources
Erebidae			
Seirarctia echo (Smith, 1797)	*Zamia integrifolia*	*Sabal palmetto, Diospyros* spp.*, *Quercus* spp., *Croton* spp., *Lupinus* spp., many other woody plants, lettuce	[27, 28]
Cosmopterigidae			
Anatrachyntis Meyrick, 1915			
A. badia (Hodges, 1962)	*Zamia integrifolia, Cycas revoluta, C. circinalis*	Dozens of species, including both angiosperms and gymnosperms	[29–31]
A. sp.	*Cycas micronesica*	Unknown	[32–34]
Tineidae			
Dasyses rugosella (Stainton, 1859)	*Cycas micronesica*	Dozens of plant species, mushrooms	[32, 35]
Erechthias sp.	*Cycas micronesica*	Unknown	[32]
Blastobasidae			
Undetermined	*Zamia pumila*	Unknown	[36]

Table References: 1) Parsons, 1984; 2) Parsons, 1999; 3) Koi and Daniels, 2015; 4) Koi and Daniels, 2017; 5) Hammer, 1996; 6) Comstock, 1948; 7) Contreras-Medina *et al.*, 2003; 8) Ramírez-Restrepo *et al.*, 2017; 9) Rodríguez-del Bosque and Rosales-Robles, 2015; 10) Cascante-Marín and Araya, 2012; 11) González, 2004; 12) Clark *et al.*, 1992; 13) Castillo-Guevara and Rico-Gray, 2003; 14) Martínez-Lendech *et al.*, 2007; 15) Ruiz-García *et al.*, 2015; 16) Burkill, 1918; 17) Khew, 2015; 18) Marler *et al.*, 2012; 19) Wu *et al.*, 2010; 20) Forster and Machin, 1994; 21) Wilson, 1993; 22) Staude and Sihvonen, 2014; 23) Donaldson and Basenberg, 1995; 24) Staude, 2001; 25) Staude, 1994; 26) Staude, 2008; 27) Packard, 1890; 28) Wagner, 2005; 29) Hua *et al.*, 2018; 30) Dawidowicz and Rozwaøka, 2017; 31) Bella and Mazzeo, 2006; 32) Marler and Muniappan, 2006; 33) Terry *et al.*, 2009; 34) Marler and Niklas, 2011; 35) Robinson and Nielsen, 1993; 36) Terry *et al.*, 2012.

idiosyncrasies underlying plant–insect interactions, research needs to encompass a broader selection of the rich taxonomic and chemical diversity of plants and their herbivores. To this end, we present a fascinating study system comprised of cycads and their lepidopteran herbivores, which we believe holds great promise for advancing long-standing and new hypotheses in ecology and evolution.

Cycads (order: Cycadales) are a basal, pantropical group of dioecious gymnosperms with a fossil record extending back over 265 million years (Gao and Thomas, 1989). With 75% of the 355 cycad species threatened with extinction, they are the most imperiled plant order in the world (Baillie *et al.*, 2004; Gilbert, 2010; Calonje *et al.*, 2019). Cycads possess an arsenal of distinctive chemical defences that are themselves deserving of review (Schneider *et al.*, 2002; Brenner *et al.*, 2003), the best studied of which are the neurotoxins β-Methylamino-L-alanine and methylazoxymethanol acetate. Yet a number of insects use cycads as larval and adult food plants. The majority of cycad-feeding (cycadivorous) insects belong to a handful of lepidopteran families that exhibit varying degrees of host specialisation and belong to multiple feeding guilds. Some of these species are widespread pests while others are conservation targets. They display a remarkable diversity of defensive strategies and trophic ecologies, suggesting varied adaptations for coping with cycad-specific phytotoxins.

However, the biology of cycadivorous Lepidoptera has never been reviewed, and the majority of relevant studies have concentrated on just a few focal species without examining broader ecological or evolutionary patterns. The aims of this review are therefore to (1) present an authoritative list of cycadivorous Lepidoptera and distinguish verified from unverified records, (2) discuss key ecological and evolutionary implications of cycad feeding in the context of broader plant–Lepidoptera interactions and (3) highlight important data gaps and areas for future study.

LEPIDOPTERAN CYCAD HERBIVORES

Cycadivory occurs in seven Lepidopteran families (Table 1), including the butterfly families Nymphalidae and Lycaenidae. Among nymphalid butterflies, larvae of two species in genus *Taenaris* – *T. onolaus* and *T. butleri* – have been reported to feed on *Cycas* (species unknown) in Papua New Guinea (Parsons, 1984, 1999). *Taenaris* is an Australasian genus of: 25 species (D'Abrera, 1971), most of which feed on monocots in the families Pandanaceae, Arecaceae, Smilacaceae and Asparagaceae (Ackery, 1988). In addition to larval cycad feeding, some adult *Taenaris* butterflies imbibe cycad juices: *T. onolaus* and *T. catops* have been observed visiting fermenting cycad seeds, feeding on exudates from wounded cycad leaves, and even probing the fresh frass of cycadivorous beetle larvae with their proboscs (Parsons, 1984). This behaviour is particularly remarkable in *T. catops*, the larvae of which feed on palms and are not known to be cycadivorous.

Three genera of lycaenid butterflies – *Luthrodes*, *Eumaeus* and *Theclinesthes* – include species that are obligate cycad herbivores. The *Luthrodes*–*Chilades* clade is comprised of two sister genera that have historically been lumped together (typically under name *Chilades*). Here we follow Talavera *et al.* (2013) and treat them as separate genera. Thus, we consider the cycadivorous lycaenid species that are typically referred to in the literature as *Chilades* to be properly placed in *Luthrodes*: *L. pandava*, *L. peripatria* and *L. cleotas*. *Luthrodes pandava* is widespread across southern and southeast Asia and the larvae are often serious pests of *Cycas* (Marler *et al.*, 2012). *Luthrodes cleotas* also occurs in southeast Asia and feeds on *Cycas* (Parsons, 1999), but less is known about its life history. The third species, *L. peripatria*, is endemic to Taiwan and its taxonomic status is unclear: some authors treat it as a full species (Hsu, 1989; Talavera *et al.*, 2013) while others consider it a subspecies of *L. pandava* (Wu *et al.*, 2010;

Ravuiwasa *et al.*, 2011). The larvae of *L. peripatria* historically fed only on *Cycas taitungensis*, also endemic to Taiwan, though it now accepts the ornamental species *Cycas revoluta* (Ravuiwasa *et al.*, 2011) which has been introduced to Taiwan in large numbers since the 1990s (Wu *et al.*, 2010). Non-cycadivorous *Luthrodes* species typically feed on fabaceous host-plants (Talavera *et al.*, 2013).

The neotropical lycaenid genus *Eumaeus* is comprised of six species distributed from Peru to the Caribbean (Lamas, 2004), with *E. atala* extending into southeastern Florida and some (perhaps dubious) records of rare strays of *E. toxea* into southern Texas (Kendall, 1984). All six *Eumaeus* species are obligate cycad herbivores, utilising cycads in the neotropical genera *Zamia*, *Dioon* and *Ceratozamia* (Comstock, 1948; Hammer, 1996; Contreras-Medina *et al.*, 2003; Koi and Daniels, 2015; Ramírez-Restrepo *et al.*, 2017; Koi and Daniels, 2017). Larvae of several *Eumaeus* species have been observed feeding on plants' fresh male and female reproductive cones in addition to stem and leaf tissue (Farrera *et al.*, 2000; González, 2004; Cascante-Marín and Araya, 2012; Rodríguez-del Bosque and Rosales-Robles, 2015; Ruiz-García *et al.*, 2015; Koi and Daniels, 2017), and we know of a single report of *E. childrenae* adults feeding on cycad exudates (Murillo, 1902). Finally, *Theclinesthes* is a mostly Australian genus of six species, of which one species, *T. onycha*, feeds on cycads in the genera *Cycas* and *Macrozamia* in eastern Australia (Braby, 2000). Non-cycadivorous *Theclinesthes* species feed on plants in the families Chenopodaceae, Euphorbiaceae, Amaranthaceae, Fabaceae, Myrtaceae and Sapindaceae (Sibatani and Grund, 1978; Braby, 2000).

Among moths, 23 species from eight genera have been recorded on cycads, but this is likely an underestimation, as many cycad-feeding moths remain poorly collected and understudied. An entire tribe of geometrid moths, the Diptychini, consists of 17 cycadivorous species in three genera (Sihvonen *et al.*, 2015). Colloquially called 'the cycad moths,' these are the best studied of the cycadivorous moths and are the only cycadivorous Lepidoptera known from Africa. The hostplants of all Diptychini larvae are *Encephalartos* and *Stangeria* cycads for the first three instars, but larvae in later instars often switch to angiospermous hostplants (Staude, 1994; Donaldson and Basenberg, 1995; Staude and Sihvonen, 2014). Hostplant species for Diptychini moths are therefore separated into primary (cycad) and secondary (non-cycad) hosts in Table 1.

In addition to these obligate cycad herbivores, a number of facultative cycadivores exist. *Seirarctia echo* (Erebidae) occurs in the southeastern United States where the larvae are highly polyphagous, feeding on leaves of the cycad *Zamia integrifolia* as well as plants in the families Arecaceae, Euphorbiaceae, Fabaceae, Fagaceae and Ebenaceae. In captivity, they have even been reared on lettuce (Asteraceae; Packard, 1890). One undetermined leaf-mining *Erechthias* moth (Tineidae) has been found feeding and pupating in the leaves of *Cycas micronesica* in Guam (Marler and Muniappan, 2006). Larvae of *Dasyses rugosella* (Tineidae) have been observed feeding on dead *Cycas* stems in India, Sri Lanka, Thailand, Indonesia and Guam (Robinson *et al.*, 1994; Marler and Muniappan, 2006). Colloquially called 'yam moths,' *D. rugosella* are best known

as pests of stored yams in West Africa (Iheagwam and Ezike, 1989; Ashamo, 2005), and are broad generalists on decaying vegetable matter (Robinson and Nielsen, 1993). Larvae of *Anatrachyntis badia* (Cosmopterigidae), another highly polyphagous and cosmopolitan moth species, have been found in pollen cones of *Zamia integrifolia* in Florida, USA (Hua *et al.*, 2018) and feeding on leaves of *Cycas revoluta* and *C. circinalis* in Italy (Bella and Mazzeo, 2006). An undetermined *Anatrachyntis* species pollinates *Cycas micronesica* in Guam and feeds on pollen cones as larvae (Terry *et al.*, 2009; Marler and Niklas, 2011). Finally, larvae of an undetermined microlepidopteran in the cosmopolitan and largely detritivorous family Blastobasidae (Watson and Dallwitz, 2003) have been found feeding in copious numbers on pollen cones of *Zamia pumila* in the Caribbean (Terry *et al.*, 2012).

Many records exist for lepidopteran species feeding on cycads which are likely to be erroneous or require further confirmation. We discuss these in the Supporting Information. The species listed in Table 1 have been identified by experts, confirmed by multiple sources, supported with photographic evidence, and in many cases their larvae have been reared in captivity on cycads. However, while we feel that this paper serves as an authoritative list of cycadivory among Lepidoptera, it is likely not an exhaustive account of all cycadivorous species considering that new records of cycad-insect associations are still being reported (e.g. Hua *et al.*, 2018), particularly among cone-feeding microlepidoptera.

DEFENSIVE ECOLOGY

Cycad secondary chemistry

Cycads produce several toxic compounds in their leaves and other tissues, including steryl glycosides, β-Methylamino-L-alanine (BMAA) and methylazoxymethanol acetate (MAM) (Laquer and Spatz, 1968; Morgan and Hoffman, 1983; Spencer *et al.*, 1987; Marler and Shaw, 2010; Kisby *et al.*, 2013). These compounds are toxic to most animals (Whiting, 1963) and are therefore presumed to function as anti-herbivore defences, though MAM is the only compound for which experimental evidence exists for insect deterrence (Bowers and Larin, 1989; Castillo-Guevara and Rico-Gray, 2003; Prado *et al.*, 2014). MAM occurs in cycad tissues in a non-toxic glycosylated form and is known by different names (e.g. cycasin, macrozamin) depending on its sugar moiety. Defensive glycosides are widespread in several angiospermous plant families and include cyanogenic glycosides, cardiac glycosides, iridoid glycosides, salicinoids, glucosinolates and others (Pentzold *et al.*, 2014b). Many of these compounds have convergently evolved in distantly related plant families, whereas cycads are the only plants known to produce MAM. As a two-component chemical defence, MAM's toxicity is activated by β-glucosidase enzymes that cleave the protective sugar moiety from the toxic aglycone (Laquer and Spatz, 1968; Rothschild *et al.*, 1986). MAM then spontaneously degrades into formaldehyde and methyldiazonium, with mutagenic, carcinogenic and neurotoxic effects (Laquer and Spatz, 1968; Morgan and Hoffman, 1983). Numerous non-cycadivorous Lepidoptera ingest and even sequester plant-derived glycosidic defensive

chemicals for their own protection from natural enemies (Nishida, 2002): for example milkweed-feeding butterflies and moths (subfamilies Danainae and Arctiinae respectively) sequester cardenolides (Agrawal *et al.*, 2012); some species in the Nymphalidae, Geometridae, Noctuidae and Arctiinae sequester iridoid glycosides (Bowers, 1991; Dobler *et al.*, 2011); cyanogenic glycosides are sequestered by species in the Heliconiinae, Acraeinae and Zygaenidae (Nahrstedt and Davis, 1986; Engler-Chaouat and Gilbert, 2007); and some Pieridae larvae sequester glucosinolates (Aplin *et al.*, 1975). Early work by Teas and colleagues showed that larvae of the cycadivorous moth, *Seirarctia echo* (subfamily Arctiinae), are able to chemically modify MAM into its glycosylated form and accumulate non-toxic MAM-glycosides in their tissues after feeding on cycad leaves (Teas *et al.*, 1966; Teas, 1967), but the molecular mechanism(s) by which they do so remains unknown. It is possible that other cycadivorous Lepidoptera are capable of similar chemical modifications though this has never been tested.

β-Methylamino-L-alanine (BMAA) is a non-protein amino acid found in cycad tissues, but is also produced by cyanobacteria in aquatic, marine and terrestrial environments (Cox *et al.*, 2005; Metcalf *et al.*, 2015). All cycads engage in endosymbioses with cyanobacteria, which are housed in specialised corraloid roots and are thought to provision plants with fixed nitrogen (and potentially other specialised metabolites) in exchange for carbon and physical protection (Gutiérrez-García *et al.*, 2019; Chang *et al.*, 2019). Given that BMAA is produced by free-living cyanobacteria in other habitats, its biosynthetic source in cycads has been debated (Cox *et al.*, 2003; Marler *et al.*, 2010). As a potent excitotoxin, BMAA interferes with glutamate receptor function and can misincorporate into proteins, and the ingestion of foods containing BMAA has been implicated as a possible cause of amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and other neurodegenerative diseases in humans (Spencer *et al.*, 1987; Cox *et al.*, 2003; Bradley and Mash, 2009; Nunes-Costa *et al.*, 2020). Toxic effects of BMAA have been demonstrated in mammals (Whiting, 1963; Cox *et al.*, 2016; Scott and Downing, 2018), insects (Zhou *et al.*, 2009; Goto *et al.*, 2012; Okle *et al.*, 2013), crustaceans (Metcalf *et al.*, 2015), fish (Purdie *et al.*, 2009; Roy *et al.*, 2017), microbes (Purdie *et al.*, 2009) and plants (Brenner *et al.*, 2000) Hundreds of non-protein amino acids have been identified in other plant families, especially legumes and grasses, and while the functions of these compounds are highly variable and often uncharacterised, many are believed to serve as antiherbivore defences (Bell, 2003; Huang *et al.*, 2011; Vranova *et al.*, 2011). The possible function(s) of BMAA in cycads – defensive and otherwise – have never been experimentally demonstrated, though based on its toxicity to diverse organisms most researchers presume that BMAA serves as a defence against herbivores. Mechanisms of resistance to BMAA have not been investigated for any lepidopteran, though there is evidence that cycadivorous weevils are able to avoid BMAA by consuming only pollen cone parenchyma tissue where BMAA is thought to be sequestered in specialised cells that the weevils excrete in their frass (Norstog *et al.*, 1986; Vovides *et al.*, 1993). In addition to MAM-glycosides

and BMAA, cycads produce steryl glucosides and numerous other chemicals whose roles in plant–herbivore interactions have yet to be sufficiently characterised.

Insect defensive ecology

Cycadivorous Lepidoptera appear to tolerate cycad toxins and several species are brightly coloured, diurnal and gregarious – traits commonly associated with chemically defended Lepidoptera (Figure 1; Bowers, 2003). Indeed, previous studies have shown that some cycadivorous species sequester MAM-glycosides into their larval and adult tissues. Rothschild *et al.* (1986) found that *Eumaeus atala* larvae, pupae and adults contained MAM-glycosides in surprisingly high amounts relative to their hostplants, and Castillo-Guevara and Rico-Gray (2002) detected MAM-glycosides in the eggs, larvae, pupae and adults of *Eumaeus* sp. (probably *toxea*) in Mexico. Nash *et al.* (1992) quantified MAM-glycosides in dried museum specimens of adult butterflies, including some specimens that were over 70 years old. The authors detected MAM-glycosides in *Eumaeus minyas* (male and female), *Luthrodes cleotas* (male and female), *Taenaris butleri* (male and female), *Taenaris catops* (male) and *Taenaris onolaus* (female), but did not detect MAM-glycosides in *Theclinesthes onycha* (either gender), female *Taenaris catops* or male *Taenaris onolaus*. They concluded that MAM-glycosides were not detectable from the latter two because of the advanced age of the museum specimens, but that *Theclinesthes onycha* probably do not sequester MAM-glycosides.

Since several of the species that sequester MAM-glycosides are brightly coloured, their coloration may be considered aposematic. Aposematism and chemical defence are exceedingly rare traits among lycaenid larvae (DeVries, 1977; Rothschild *et al.*, 1986; Fiedler, 1996), which typically rely on crypsis and ant association for protection against natural enemies (Pierce *et al.*, 2002). *Eumaeus* provide a striking exception in that they are gregarious and warningly coloured in all life stages, are known to sequester cycad toxins, and do not form larval associations with ants (Atsatt, 1981); whereas larvae of other cycadivorous lycaenids commonly associate with ants and are cryptically coloured (Wilson, 1993; Eastwood and Fraser, 1999; Tan and Sin Khoon, 2012). Larvae of *Luthrodes cleotas* are cryptically coloured, but adults have much larger orange spots on their hindwings than do their congeners, and it is possible that they are aposematic, particularly given that adults sequester MAM-glycosides (Nash *et al.*, 1992).

Larvae and adults of Dyptichini moths are brightly coloured with gregarious larvae and diurnal adults, but it remains unclear whether they sequester plant toxins at any life stage. Donaldson and Basenberg (1995) suggest that *Z. lepida* sequester MAM-glycosides, but do not provide experimental evidence. *Seirarctia echo* larvae are warningly coloured and covered with protective hairs. This species sequesters MAM-glycosides when feeding on cycads (Teas *et al.*, 1966; Teas, 1967), but it remains unknown how feeding on non-cycad hostplants affects their palatability and predation risk. Finally, *Anatrachyntis* moths and the other microlepidoptera are not aposematic in any lifestage and many species spend
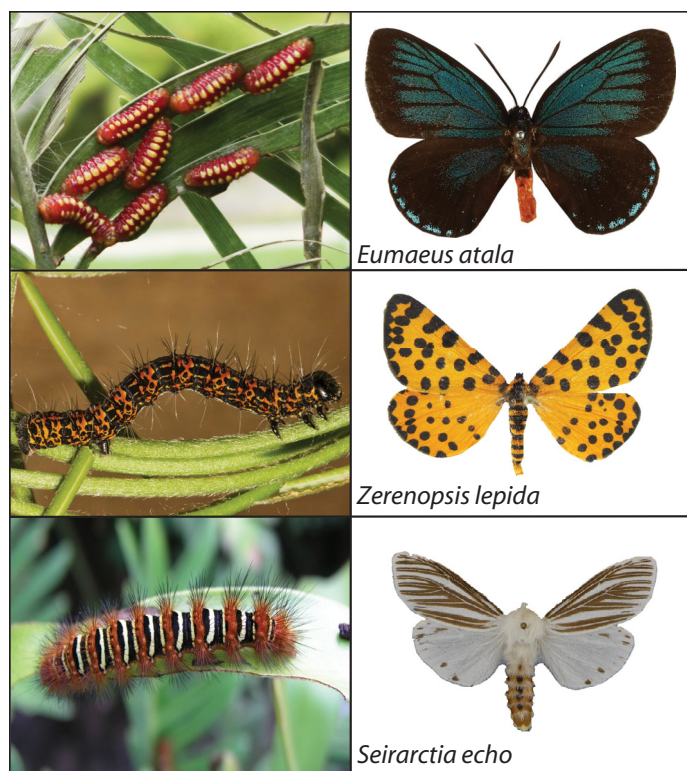
Figure 1 Examples of aposematism among cycadivorous Lepidoptera. Image credits: Left column, top to bottom: Shayla Salzman, Hermann Staude, William Tang; Right column, top to bottom: Melissa Whitaker, Axel Hausmann, BOLD.

their entire development concealed inside plants' pollen cones, where they may avoid some cycad toxins (Norstog and Fawcett, 1989; Vovides *et al.*, 1993). It is completely unknown whether leaf-mining *Erechthias* and detritivorous *Dasyses* larvae encounter cycads' defensive compounds while feeding.

Unfortunately, records of predators and parasitoids are lacking for nearly all cycadivorous species. Natural enemies of Lepidoptera generally include birds, small reptiles, spiders, mantids, reduviid bugs, ants and parasitic wasps and flies, though direct observations of attacks on larvae and adult butterflies are exceedingly rare (Molleman *et al.*, 2010). The best-studied cycadivorous species with regard to defensive ecology is *Eumaeus atala* in southeastern Florida. Both native and non-native ants have been observed consuming *E. atala* eggs and pupae (Smith-Cavros, 2002), but are thought to avoid adult butterflies (Bowers and Larin, 1989). Some assassin and ambush bugs (Reduviidae) will attack *E. atala* larvae (Koi and Hall, 2019) although published records are scarce. Unconfirmed reports exist of native and non-native reptiles attacking *E. atala* larvae and adults. Starlings, peacocks and other non-native birds have been reported to attack caterpillars, though it's possible that only naïve birds will attempt to eat *E. atala*, as adult butterflies were shown to be distasteful to grey jays (Bowers and Farley, 1990). There are no reports of parasitoids using *E. atala* as hosts, a conspicuous absence given that parasitoids are typically significant natural enemies of lepidopteran larvae.

Ruiz-García *et al.* (2015) monitored survival and development of *Eumaeus toxea* butterfly larvae in Oaxaca, Mexico and observed *Dasydactylus* beetles preying on molting *E. toxea* larvae, but did not report finding any parasitoids. In contrast, Manners (2015) reports that 'high levels of parasitism' sometimes occur in *Theclinesthes onycha* butterfly larvae in Australia, and provides photographs of larvae parasitised by braconid wasps (Manners, 2015). Among moths, the only published records of parasitisation come from *Zerenopsis lepida*: Staude & Sihvonen (2014) reared a single parasitoid fly (Tachinidae) from a late instar larva in South Africa, and Sommerer (2014) reared 15 *Z. lepida* larvae and found more than 50 percent had been parasitised by *Charops* sp. (Ichneumonidae) or *Drino* sp. (Tachinidae). Aside from these scattered records we know relatively little about the natural enemies of cycadivorous Lepidoptera in the wild, including the importance of entomopathogenic microbes. Moreover, the effectiveness of aposematism and other defensive strategies against vertebrate, invertebrate and microbial enemies remains an outstanding issue, even among well-studied Lepidoptera.

EVOLUTIONARY ORIGINS OF CYCADIVORY

To evaluate evolutionary origins of cycadivory and relationships among cycadivorous Lepidoptera, cycadivory was mapped on to a phylogenetic tree constructed by combining a Lepidoptera phylogeny (Regier *et al.*, 2013) including butterflies and moths with a heavily sampled butterfly phylogeny (Espeland *et al.*, 2018, Figure 2), which were selected on the basis of their taxon sampling and phylogenetic coverage. Both phylogenies were downloaded as.nex files from published sources and brought into R (version 3.5.1; R Core Team, 2018) where the butterfly clade from Espeland *et al.* (2018) was substituted in place of the less sampled clade from Regier *et al.* (2013) using the R packages ape (Paradis and Schliep, 2018), GEIGER (Harmon *et al.*, 2008) and ggtree (Yu *et al.*, 2017, 2018). In cases where cycadivorous species were not represented as tips on the tree, the represented tip of the closest relative was identified using published phylogenies of families or genera (Wahlberg *et al.*, 2009; Sihvonen *et al.*, 2011; Zahiri *et al.*, 2012; Talavera *et al.*, 2013; Sihvonen *et al.*,2015).

A visual inspection of the Lepidoptera phylogeny suggests that cycadivory has evolved independently in multiple lepidopteran lineages, with several origins likely within single families and potentially even single genera. For example, a poorly resolved phylogenetic hypothesis based on morphological data for *Taenaris* does not place the two cycadivorous species within a monophyletic clade or even closely related to each other (Parsons, 1999), suggesting multiple origins of cycadivory in the genus. Similarly, an unpublished molecular phylogeny that includes some species of *Luthrodes* does not place the two included cycadivorous species as sister clades (Wu, 2009). Conversely, cycadivory appears to be an ancestral trait in *Eumaeus* butterflies (6 species) and Diptychini moths (17 species). Given that both of these clades are warningly coloured and obligately cycadivorous, it seems likely that cycad feeding or defensive traits (or both) have led to limited radiations in these groups. Dated phylogenetic hypotheses for

all genera would be required to understand the general evolutionary significance of cycadivory and why some lineages have diversified while others are represented by just one or two species nested within otherwise non-cycadivorous clades.

Given the Miocene origins of extant cycad species (Nagalingum *et al.*, 2011) and the phylogenetic placement of cycadivorous Lepidoptera, it is likely that transitions to cycadivory among extant cycadivorous Lepidoptera occurred within the last 15–20 million years. Indeed, at least in lycaenid butterflies the evolutionary origins of cycadivory appear to be somewhat

recent. Talavera *et al.* (2013) date the split between *Luthrodes* and its sister genus *Chilades* at 6 MY, but the cycadivorous species of *Luthrodes* included in the analysis are derived, placing the origin(s) of cycadivory in this lineage as even younger. Similarly, an unpublished molecular clock analysis in *Theclinesthes* estimates the origin of the genus at 2–3 MY (Eastwood, 2006). Finally, Espeland *et al.* (2018) place the split between *Eumaeus* and *Calycopis* at 18 MY, making the origin of *Eumaeus* even more recent as this analysis did not include *Eumaeus*'s sister genus *Theorema*.
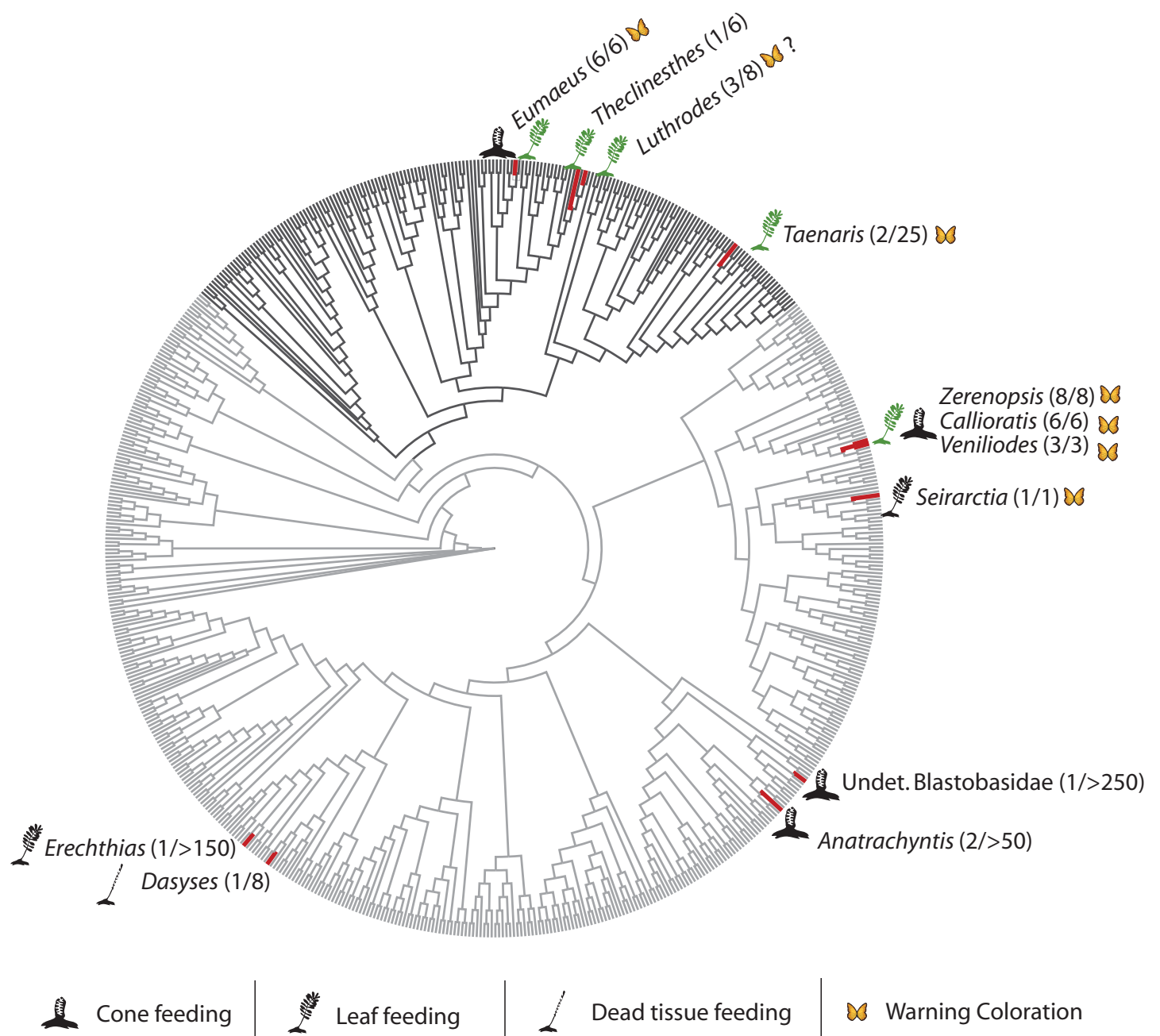

Figure 2 Phylogenetic placement of cycadivorous Lepidoptera. Genera containing cycadivorous species are shown by red tips, with the butterfly clade in black and moths in grey. Warning coloration is indicated symbolically, along with the feeding guild and whether a species is facultatively or obligately cycadivorous (black and green respectively). The number of cycadivorous species and the total number of species in the genus are given in parentheses.

Improved phylogenetic estimates for cycadivorous Lepidoptera would be useful for reconstructing and comparing historical diet evolution among cycadivorous lineages, though research in this area is hindered not only by the unavailability of genus-level phylogenetic reconstructions, but also by incomplete or erroneous hostplant records for many species (see Table S1 in Supporting Information). Some have speculated that monocot-feeding may be an evolutionary precursor to cycadivory because non-cycadivorous *Taenaris* feed on monocots (Schneider *et al.*, 2002), though there is little evidence from other groups to support this as a broad pattern. Among lycaenids, close relatives of cycadivorous species feed on dicots in the families Fabaceae, Amaranthaceae, Proteaceae, Sapindaceae, Myrtaceae and Euphorbiaceae (Dunn and Dunn, 1991; Braby, 2000). Cycadivorous moths and their close relatives exhibit a broad range of hostplant preferences that includes both monocots and dicots. Improved knowledge of the evolutionary histories of cycadivorous lineages would provide a framework for testing hypotheses about evolutionary precursors to cycadivory and host breadth among extant species.

HOSTPLANT USE

Based on the records reported here, cycadivorous Lepidoptera utilise 7 of the 10 recognised cycad genera (Calonje *et al.*, 2019, Figure 3). Absent among accepted hostplant genera are *Lepidozamia*, *Bowenia* and *Microcycas*.

All cycadivorous butterflies appear to be obligate cycad specialists while cycadivorous moths exhibit a broader range of dietary preferences. *Seirarctia echo* is the only confirmed facultative cycad folivore, accepting leaves from a wide variety of hostplants from several plant families. The ecological causes and consequences of feeding on cycad versus non-cycad plants are completely unexplored in this species. Diptychini moths are facultatively polyphagous in their 4th-6th instars, but all species are obligate cycad specialists for the first three instars. Donaldson and Basenberg (1995) found no significant differences in survival rate, developmental duration or pupal mass between 4th instar *Z. lepida* larvae reared on angiosperm versus cycad hosts. Staude & Sihvonen (2014) suggested that some Diptychini moths may not require cycads even in their early stages, as he collected a single final-instar *Z. tenuis* larva feeding on the leaves of a baobab tree (*Adansonia digitata*, Malvaceae) on Misali Island, Tanzania, where no cycads were found. The remaining cycadivorous moth species are either highly polyphagous (e.g. *Dasyses rugosella*) or their host breadth is unknown (e.g. *Erechthias* sp.).

Whereas not all cycadivorous Lepidoptera are specialists of cycads, their larvae are specialised on particular plant tissues and can therefore be categorised into discrete feeding guilds. These guilds include leaf chewers, leaf miners, ovulate cone feeders, pollen cone feeders and detritivores, and the larvae in each of these guilds likely experience qualitative and quantitative differences in exposure to cycad toxins. For example, pollen cone feeders may experience reduced exposure to toxins since at least one cycad toxin, BMAA, appears to be sequestered in specialised cells in the pollen cones that can pass through the guts of other insects intact (Norstog and Fawcett, 1989; Vovides *et al.*, 1993). Detritivorous species feed on decaying cycad pollen cones and stems that may also harbour lower concentrations of toxins. In contrast, *Eumaeus* butterfly larvae feed on both ovulate and pollen cones as well as leaves (González, 2004; Cascante-Marín and Araya, 2012), and some evidence suggests that *Z. lepida* moths also feed on ovulate cones in addition to leaves (Donaldson, 1991). The seeds of some cycad species are known to contain high concentrations of MAM-glycosides and BMAA relative to other plant tissues (Banack and Cox, 2003; Yagi, 2004; Nair and van Staden, 2012) and ovulate cones do not sequester BMAA into specialised cells (Norstog and Fawcett, 1989; Vovides *et al.*, 1993). It is therefore unsurprising that only obligate cycad specialists can utilise ovulate cones, particularly those species which are known to sequester MAM-glycosides.

Among cycad specialists, it appears that larvae can accept diverse cycad species and hostplant breadth is expanding for several species, particularly as exotic cycads are planted as ornamentals in gardens worldwide. The Caribbean species *Eumaeus atala*, historically fed only on Caribbean cycads in the genus *Zamia*, but has been observed laying eggs and feeding on cultivated Central American cycad species that are outside the native range, as well as some species of African, Australian and Asian cycads (Hammer, 1996; Koi and Daniels, 2017; Whitaker *et al.*, in press). The ability to feed on non-native cycads has been observed in other *Eumaeus* species as well (Rodríguez-del Bosque and Rosales-Robles, 2015), and increased hostplant breadth has been reported for *Luthrodes pandava*, a widespread pest that feeds on numerous native and exotic cycads across Asia and the Middle East (Tiple *et al.*, 2009; Wu *et al.*, 2010; Feulner *et al.*, 2014; Fric *et al.*, 2014).

Contemporary host use may challenge the species status of *Luthrodes peripatria*, which some authors consider to be a subspecies of *Luthrodes pandava*. The natural range of *L. pandava* is widespread across southern Asia (excluding Taiwan), whereas *L. peripatria* is endemic to Taiwan and has historically fed on a single cycad species restricted to southeastern Taiwan, *Cycas taitungensis* (Shen *et al.*, 1994). In the past 30 years, *L. pandava* has been introduced to Taiwan along with several exotic *Cycas* species. As both *Luthrodes* species accept native and non-native *Cycas* species as hostplants, expanded hostplant use and range overlap could provide opportunities for interbreeding. Further assessment of the population structure, introgression and species status of *L. pandava* and *L. peripatria* would be fruitful (but see Wu *et al.*, 2010).

Hostplant specialisation may promote divergence in the Australian species *Theclinesthes onycha*, for which two subspecies are recognised, *T. onycha* and *T. onycha capricornia*. *T. o. onycha* feeds only on *Macrozamia* cycads distributed from southern Queensland to New South Wales whereas *T. o. capricornia* feeds only on *Cycas* species in Northeast and central Queensland. They overlap in their distributions in a narrow region in central Queensland, though microhabitat preferences may maintain allopatry even within this contact zone. Patterns of hostplant use and mate choice are not well
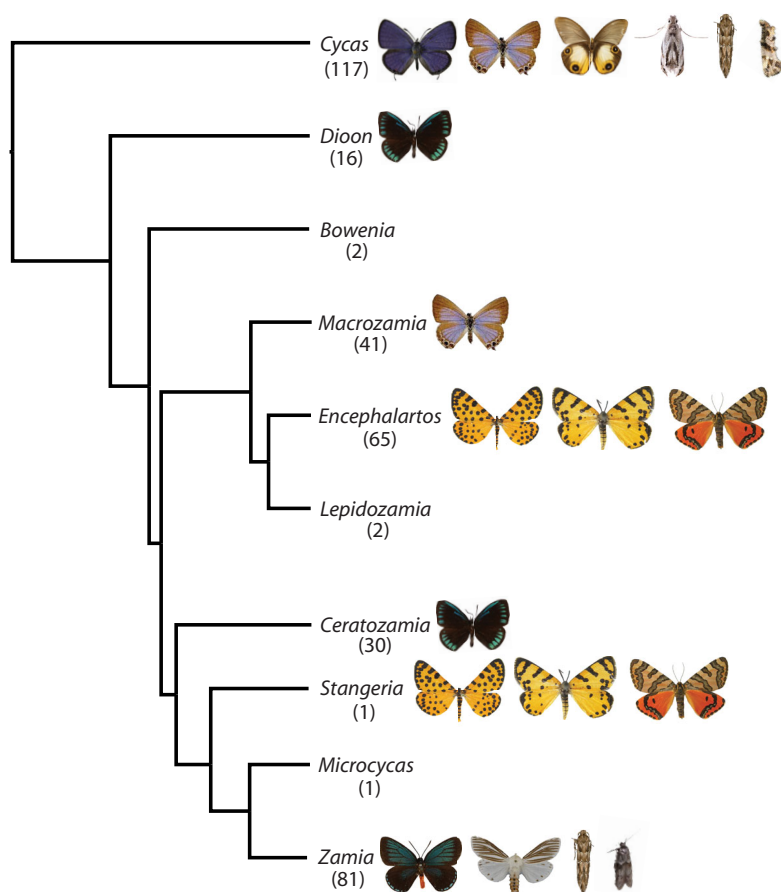
Figure 3 Cycad genera used as lepidopteran hostplants. Cycad phylogenetic tree from Salzman et al. (2020). Number of species in each plant genus is given in parentheses. Each lepidopteran genus is represented by a single species (images not to scale). Image credits: BOLD, CSIRO, Harvard MCZ, Graeme Cocks, Catherine Hua, Shipher Wu, Melissa Whitaker, Axel Hausmann, Marion Friedrich.

described within the contact zone, though Eastwood (2006) found considerable genetic differentiation in the mitochondrial genes of each subspecies, suggesting that there is little to no gene flow between them.

Careful analysis of hostplant use, species relationships and reproductive barriers would also be useful for the two pairs of sympatric species of *Eumaeus* butterflies in Central and South America. *Eumaeus childrenae* and *E. toxea* co-occur in some parts of their ranges in Mexico, where they are easily distinguished based on wing pattern. These species are likely quite diverged and they utilise different cycad genera as hostplants throughout much of their range, though detailed studies of host use in areas of sympatry and allopatry have not been carried out. *Eumaeus toxana* and *E. minyas* both occur in South America and according to published records their ranges overlap in Peru. However, it is difficult to glean even basic natural history information for these two species due to widespread mistakes in species identifications in the published literature. *Eumaeus minyas* is commonly confused with several other *Eumaeus* species, especially *E. toxana* and the isthmus species *E. godartii*, but also *E. toxea* and even *E. atala*. Credible accounts of the distributions and range limits for *E. minyas* and *E. toxana* are needed, with *E. toxana* being particularly under-collected and poorly studied.

Among Caribbean species, the ranges of *Eumaeus atala* and *Seirarctia echo* overlap in southern Florida but there are very few records of both species occurring in the same place, suggesting that there is some displacement at a relatively fine spatial scale. Since *S. echo* is broadly polyphagous, hostplant competition is unlikely to be a sufficient explanation. Furthermore, the range of *E. atala* does not occupy the entire range of its hostplants in Florida, and a better understanding of the factors that determine the range boundaries of these species would be very valuable for the management of local butterfly and cycad populations.

DISCUSSION

Cycadivorous Lepidoptera comprise a 'component community' of distinct lineages with varying degrees of specialisation and diverse feeding ecologies, and therefore present numerous opportunities for comparative studies of eco-evolutionary dynamics (e.g. Farrell, 2001). Because of their distinctive chemical and ecological features, cycads and their herbivores provide a valuable complement to the model systems that dominate plant–insect research. Based on this initial review of the phylogenetic and natural histories of these species, we speculate here on some of the salient questions regarding cycad–Lepidoptera interactions.

Is cycad-feeding adaptive?

Evolutionary transitions to feeding on plants that contain defensive secondary compounds are claimed to promote diversification of Lepidoptera through escape and radiation (e.g. Braby and Trueman, 2006). If cycadivory has similarly promoted diversification in lepidopteran lineages, then it might be considered an adaptive trait. Based on the phylogenetic pattern shown in Figure 2, *Eumaeus* butterflies and Diptychini moths exhibit modest radiations following their transition to cycad-feeding, whereas other cycadivores remain as only one or two species at the tips of otherwise angiosperm-feeding clades. Why have some cycadivorous lineages diversified while others have not?

That the largest clades of cycadivorous Lepidoptera are also aposematic suggests that defensive ecology may play a role in diversification: perhaps it is not cycadivory *per se* that leads to diversification in some lineages, but rather the subsequent evolution of sequestration and aposematism. This explanation is consistent with the cryptic coloration of cycadivorous species that have not radiated, though a few exceptions must be considered. *Luthrodes cleotas* and *Seirarctia echo* are both known to sequester cycad toxins and could be considered warningly coloured; why have these species not diversified? Cycadivorous *Taenaris* species are also warningly coloured but do not appear to have radiated (though even non-cycadivorous *Taenaris* are considered aposematic (Braby, 2000) so this situation may be more complex).

It may be that evolutionary trade-offs or constraints have limited diversification in these groups, that other cycadivorous relatives once existed but have gone extinct, or that cycadivory has evolved too recently for diversification to have yet taken place. This latter possibility cannot be evaluated without better time estimates for the origins of cycadivorous species, but even several hundred thousand years could provide ample time for diversification. It would be interesting to compare the ages of wholly cycadivorous clades (*Eumaeus* butterflies and *Diptychini* moths) to clades in which cycadivorous species have not radiated. Among generalists, cycadivory is not expected to significantly influence speciation rates (at least for detritivorous moths), though *Seirarctia echo* and *Erechthias* sp. may be exceptions given that they likely possess specific adaptations for feeding on cycads' fresh leaf tissue.

Is there evidence of coevolution between cycads and their lepidopteran herbivores?

All cycadivorous Lepidoptera must possess adaptations to circumvent or tolerate cycad-specific defences, and the selective value of cycad defensive traits against herbivores seems clear. But what of the selective influence of cycadivorous Lepidoptera for their host cycads? While there is little debate about the importance of plant defensive traits for herbivore fitness (Janz and Nylin, 1998; Futuyma and Agrawal, 2009), the importance of insect herbivores as selective agents is less clear as most plants seem able to tolerate intermediate levels of herbivory without a significant reduction in fitness (Cornell and Hawkins, 2003). Evidence of reciprocal adaptation between pairs of plants and herbivores has been relatively scarce (Farrell, 1993),

and the step-wise selection scenario initially envisaged by Ehrlich & Raven appears to be extremely asymmetrical: shifts to chemically novel hosts lead to bursts in diversification in many herbivore groups, but escape from herbivores through chemical novelty seems to have had little impact on diversification rates in most plant groups (Farrell, 1998; Wheat *et al.*, 2007).

Still, damage inflicted by folivorous Lepidoptera can be so extreme that just a few generations can decimate a large cycad (Koi, 2017; Whitaker *et al.*, in press). Selective pressures exerted by some specialist herbivores may therefore be especially severe for cycads relative to other plant groups, raising the possibility that some lepidopteran herbivores could select for escalated chemical defences and perhaps influence the diversification of their cycad hosts. Previous work has identified diverse secondary compounds in cycads (Pan *et al.*, 1997; Snyder and Marler, 2011) that appear to be evolving (De Luca *et al.*, 1982), but phylogenetically explicit comparisons of cycad defensive chemistries (toxins, antinutritive compounds and volatile organic compounds) would be required to look for evidence of phytochemical escalation.

Extant cycad species are estimated to have originated within the last: ~12 million years (Nagalingum *et al.*, 2011), similar to the age estimates of most cycad-feeding lepidopterans. However, the phylogenetic distribution of cycadivory in Lepidoptera suggests repeated, independent colonisations of cycads from distantly related angiosperm hosts, and the potential for co-speciation with cycads is reasonably plausible only among *Eumaeus* butterflies and Diptychini moths. Research in this area should therefore focus on assessing coevolution between *Eumaeus* with the new world cycad genera *Zamia*, *Dioon* and *Ceratozamia*, and between the African Diptychini moths with the cycad genera *Encephalartos* and *Stangeria*.

How does cycadivory evolve?

Identifying evolutionary and ecological precursors to cycadivory could help explain the repeated transitions to cycads among Lepidoptera. For example, did the hostplants of ancestral species somehow facilitate shifts to cycad feeding, either through phytochemical similarity or other features? From the data presented here, there is no evidence that cycadivory has evolved from a single, shared host lineage. The ancestors of cycadivorous taxa likely fed on diverse angiosperms including both monocots and dicots, though improved phylogeographic and life history information will be required to infer the most likely ancestral food plants of each cycadivorous lineage. Hypotheses regarding what the ancestors of cycadivorous species ate prior to their transitions to cycads may suggest as yet unknown chemical similarities between cycads and some angiosperm groups. Or, if no chemical similarities are found, it suggests potentially novel adaptations for overcoming cycads' defences.

Lepidoptera are known to employ numerous adaptations for feeding on chemically defended hostplants. These include behavioural adaptations (Dussourd and Denno, 1991), physiological mechanisms (Hartmann *et al.*, 2005), and perhaps even associations with symbiotic gut bacteria (Hammer and Bowers, 2015; Salzman *et al.*, 2018) (though this is perhaps uncommon among Lepidoptera (Whitaker *et al.*, 2016;

Hammer *et al.*, 2017)). Host switching and feeding on select plant tissues can also minimise an insect's exposure to plant defensive compounds. For example Diptychini moths – which we consider to be obligate cycad specialists – commonly switch to feeding on angiospermous plants in late instars and thereby potentially reduce their exposure to cycad defences.

It is presently unknown which adaptations might be required for cycadivory, or how widely specific adaptations are shared across and within feeding guilds, for example among specialised folivores. For example, *Seirarctia echo* are capable of modifying dietary MAM into its non-toxic form (Teas, 1967), but it remains unknown whether other herbivores actively detoxify MAM using a similar mechanism. Moreover, no adaptations have been identified to date that would enable herbivores to cope with BMAA, steryl glycosides, or other defensive compounds, let alone complex phytochemical mixtures. Additionally, herbivores need to locate and discriminate between potential hostplants, and while previous work has described chemical cues used by the insect pollinators of cycads (Terry *et al.*, 2007), no research has investigated chemical communication between cycads and lepidopteran herbivores.

Finally, different lepidopteran lineages may experience different evolutionary constraints in their ability to feed on cycads. Among butterflies, the Nymphalidae appear to be relatively constrained in their ability to colonise new hostplant families (Hamm and Fordyce, 2015), whereas the Lycaenidae exhibit enormous trophic diversity that includes both phytophagous and aphytophagous diets (Pierce *et al.*, 2002). Indeed, Ehrlich and Raven (1964) were able to identify few phylogenetic patterns in lycaenids' host use and were puzzled by their 'bewildering array' of hostplant associations. The only published lycaenid genome demonstrates significant expansion in detoxification and digestion enzymes (Cong *et al.*, 2016), which, if shared broadly across the family, might explain why lycaenid butterflies seem predisposed to trophic innovation, including repeated colonisation of cycads. Yet despite their proclivity for unusual diets, feeding on chemically defended hostplants and sequestering hostplant defensive chemicals is rare among lycaenids (Fiedler, 1996), making the repeated evolution of cycadivory among lycaenids especially exciting.

CONCLUSIONS

Cycadivorous Lepidoptera are remarkably diverse in their defensive strategies, life histories and hostplant relationships, providing numerous opportunities for future research. Their diets span the full range of host specialisation and there is evidence of recent host expansion in some species. Cycad-feeding Lepidoptera include cases of possible incipient speciation and examples of likely introgression, widespread pests as well as locally threatened species, and clades that are relatively understudied in phytochemical ecology research (lycaenid butterflies, Diptychini moths) along with a few familiar standbys (arctiid moths, nymphalid butterflies). Moreover, cycads possess defensive chemistries that are not found in the angiosperm study systems that comprise the bulk of research on plant–insect interactions. Some of these defensive chemicals appear to be influenced by cycads' complex microbial associations and provide opportunities to investigate the effects of plant–microbe interactions on plant–herbivore interactions, as well as the ecological and non-ecological significance of non-protein amino acids, a widespread, but relatively unstudied class of plant metabolites. The diversity in lepidopteran defensive traits, which range from camouflage to aposematism, suggests both convergent and divergent adaptations to these toxins.

Yet despite several decades of research on a handful of focal species, many cycadivorous Lepidoptera remain understudied, undersampled and undescribed. In general, research in this area would benefit from further investigations into cycads' defensive chemistries and insects' adaptations to cycad toxins, systematic surveys of herbivore diversity and host breadth, and studies of predator and parasitoid pressures in natural habitats, along with genus- and tribe-level phylogenies of cycadivorous groups and their sister taxa. We highlight several promising research questions in Box 1. Future studies would do well to consider other insect groups too, as cycadivory has been reported among larvae and adults of non-pollinating beetles (Coleoptera; Marler and Muniappan, 2006); bees (Hymenoptera; DeVries, 1983; Ornduff, 1991; Valencia-Montoya *et al.*, 2017); leaf-mining larvae of an unidentified

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Box 1. Suggested Research Questions

● How widespread is sequestration of cycad defensive compounds among cycadivorous Lepidoptera? Which cycad toxins are sequestered and in which species, life stages and tissues? What adaptations are required for deactivating, transporting and/or storing cycad toxins?

● Do insect-associated gut bacteria contribute to tolerance of cycads' defensive chemicals?

● Who are the natural enemies of cycadivorous Lepidoptera and how effective are lepidopteran defensive traits (e.g. aposematism) against vertebrate predators, invertebrate predators, parasitoids and entomopathogenic microbes?

● Do sequestered phytotoxins provide additional functions beyond defence for specialised Lepidoptera, e.g. sexual pheromones, nutrient storage, biochemical signalling, etc.?

● What is the multi-trophic significance of cycadivory, and how do cycads' phytochemicals affect community structure and nutrient flow within ecosystems?

● Why do some lepidopteran clades appear more likely to evolve cycadivory than others? What evolutionary precursors, constraints and trade-offs might be relevant to the evolution of cycad-feeding among Lepidoptera?

● What role do plant-associated microbes play in the defensive traits of cycads? For example is BMAA produced by endosymbiotic cyanobacteria, by cycads, by both?

● What function(s) does BMAA provide in cycad metabolism and/or defence against herbivores?

● Can specialised insects' adaptations to BMAA-rich diets inform interventions relevant for human medicine and public health?

fly (Diptera; DeVries, 1983); termites (Blattodea; Marler *et al.*, 2011); and phloem-feeding scale insects and mealybugs (Hemiptera; Castillo-Guevara and Rico-Gray, 2003; Marler and Muniappan, 2006). By summarising what is known about the phylogenetic placement of cycadivorous Lepidoptera, along with their hostplant relationships and defensive ecology, we introduce them as a compelling study system with great promise for investigating the causes and consequences of ecological interactions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.