

# The beetle *Lilioceris nigripes* (Fabricius) (Coleoptera: Chrysomelidae: Criocerinae) feeding on cycads in north-east Australia

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## Abstract

Field studies and laboratory trials show the Golden Leaf Beetle *Lilioceris nigripes* (Fabricius) (Criocerinae) feeds on the foliage of species in two families of cycads. The cycads consumed are species of *Cycas* L. (Cycadales: Cycadaceae) that form a continuum along 1000 kilometers of coastal Queensland, and the northern of two species of *Bowenia* Hook. ex Hook. (Cycadales: Zamiaceae) in the same areas but separated by the Burdekin Gap. *Lilioceris nigripes* browsed the foliage of *Cycas ophiolitica*, *C. media* subsp. *banksii*, *C. cairnsiana* and *C. platyphylla*, activity being restricted to young foliage produced in flushes prior to onset of the wet season or after fires when previous foliage had been burned away. *Lilioceris nigripes* also fed on leaflets of both nominate and putative *B. spectabilis* in north-east Queensland but not on the foliage of *B. serrulata* in central Queensland despite feeding on *C. ophiolitica* growing two kilometres away. Laboratory trials were conducted of *L. nigripes* feeding on different species of cycads without other food sources being available. *Lilioceris nigripes* previously feeding on nominate *B. spectabilis* did not eat the foliage of *B. serrulata* but did eat that of *C. ophiolitica*. Beetles collected from *C. ophiolitica* did not eat the foliage of *B. serrulata* but ate that of *B. spectabilis*. Adult and larvae *L. nigripes* collected from nominate *B. spectabilis* ate the foliage of *C. media* subsp. *banksii* and the reverse. The data presented in this paper extend the record of *L. nigripes* feeding on species and genera of cycads, and records of the chrysomelid genus *Lilioceris* (Criocerinae) piercing leaves and feeding on the sap in them.

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## Introduction

The shining leaf beetles, subfamily Criocerinae, are pan-tropical in distribution and the currently oldest known fossil material is from the Eocene (33.9 – 55.8 Mya) (Santiago-Blay 1994; Gómez-Zurita *et al.* 2007; Nie *et al.* 2020). Leaf beetles of the genus *Lilioceris* are known to feed on the foliage of species of both families of cycads, namely Cycadaceae and Zamiaceae (Schmitt 1988). Jolivet & Hawkeswood (1995) note records of *L. clarkii* Baly on an indeterminate *Cycas* in New Guinea,

*L. consenta* Lacordaire on an indeterminate *Cycas* in Vietnam and Golden Leaf Beetle *L. nigripes* (Fabricius) on *Bowenia spectabilis* Hook. ex Hook. f. in Australia. Wilson (1993) describes *L. nigripes* feeding on *C. ophiolitica* K.D. Hill in central Queensland and Forster & Machin (1994) summarize records of it feeding on species of *Macrozamia* (Zamiaceae) in Queensland and New South Wales. Shepard (1997) records an

indeterminate *Lilioceris* species browsing on *C. siamensis* Miquel in South-east Asia.

Records of *Lilioceris* species feeding on species of two families of cycads are of interest because criocerines are considered monophagous or narrowly phytophagous (Jolivet 1988). The association is also of interest because cycads contain a suite of compounds, e.g. methylazoxymethanol (MAM) glycosides (De Luca *et al.* 1980; Moretti *et al.* 1983; Siniscalco 1990), non-protein amino acids ( $\beta$ -methylamino-L-alanine (BMAA)) (Vega & Bell 1967; Charlton *et al.* 1992; Castillo-Guevara & Rico-Grey 2003) and flavonoides (Dossaji *et al.* 1975; Meurer-Grimes & Stevenson 1994) that dissuade most predators and pathogens. *Cycas* species also contain *cycasterone*, a phytoecdysone that disrupts developmental sequences in some insects (Harborne 1988), but it is not known if it is present in other cycad genera. In addition, cycads also contain thick mucilage that acts as a deterrent to feeding by most phytophagous insects (Futuyma 2000).

Futuyma (2000) observed 'many traditional statements about the host associations of major taxa of insects are valid descriptions of [plant] evolutionary lineages' and 'some of the associations of insect lineages with plant lineages are astonishingly old'. This suggests studies of specialised plant-insect associations can provide insights into the phylogenetics and systematics of host plants (Mitter *et al.* 1994); for example Herre *et al.* (1996) and Dixon *et al.* (2001) correlated molecular phylogenies of figs and their pollinator wasps.

This study describes the association of *Lilioceris nigripes* (Coleoptera: Chrysomelidae: Criocerinae) and species of *Cycas* (Cycadales: Cycadaceae) and *Bowenia* (Cycadales: Zamiaceae) in Queensland, Australia. In addition, it describes how *L. nigripes* distinguishes between species of *Bowenia*, and assists in resolving the status of morphologically different populations of the genus in north Queensland.

## Methods

Laboratory trials examining the association of *L. nigripes* and cycads were conducted on *Bowenia serrulata* and *Cycas ophiolitica* in coastal central Queensland, *B. spectabilis* at Tarzali and Tinaroo, and *C. media* subsp. *banksii* at Tinaroo in north-east Queensland. Opportunistic observations were

made of the association of *L. nigripes* with *C. cairnsiana* and *C. platyphylla* in north-east Queensland. The central and north-east Queensland study areas and two species of *Bowenia* are separated by 800 km including the Burdekin Gap, a lowland area of drier, warmer, more open woodland and savannah habitat dividing the adjacent closed forest habitat, that established in the mid Miocene, 12-15 Mya (Sluiter & Kershaw 1982; Kemp 1987; Truswell 1993; Bryant & Krosch 2016). However, *C. media* subsp. *media* K.D. Hill and *C. cupida* P.I. Forster, provide a continuous distribution of *Cycas* species between the two study areas (Fig. 1). Records of the Australian National Insect Collection (ANIC) in Canberra and University of Queensland Insect Collection (UQIC) in Brisbane show collections of *L. nigripes* from woodland and forest from Darwin to northern New South Wales and overlapping the distribution range of cycad species.

## Field studies

The presence and activities of *L. nigripes* on the foliage of each cycad species was recorded. Forty plants of *B. serrulata* at Byfield in central Queensland were identified with aluminium tags numbered 1 – 40 and tied with copper wire to the plants, and inspected once a month in 1991-94, bimonthly in 1995 and 1996, and in March and November of 1997 and 1998. In addition, a population of *C. ophiolitica* at Keppel Sands in central Queensland was monitored for the presence and activity of *L. nigripes* at the same intervals. Populations of *B. spectabilis* and *C. cairnsiana*, *C. platyphylla* and *C. media* subsp. *banksii* in north Queensland were surveyed for *L. nigripes* in September and November of 1994 and 1995 and October 1998 and 1999. Specimens of *L. nigripes* were collected from the plants (Collection: GWWIn46, 25 Feb 2008) and staff of the CSIRO Division of Entomology, Canberra, provided identifications of them.

## Laboratory studies

Two trials were conducted of *L. nigripes* feeding preferences on different species of cycads without other food sources being available. The first was to investigate (a) previous observations (Wilson 2003) that *L. nigripes* feeds on *B. spectabilis* but not on *B. serrulata* despite its presence in the range of both species, and (b) ascertain if *L. nigripes* distinguishes between morphologically-different

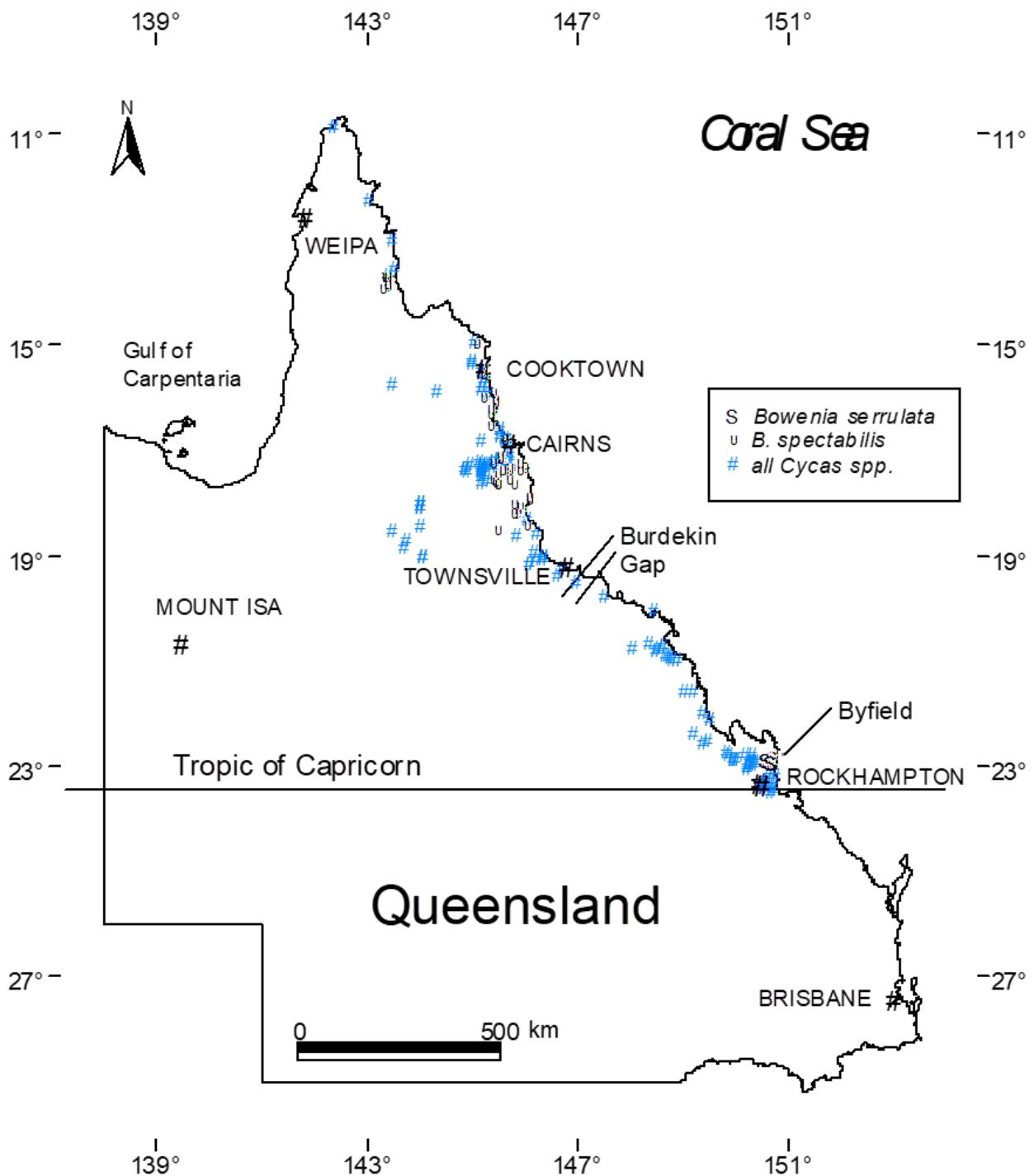


Figure 1. Location of study sites, the Burdekin Gap, and distribution of the cycads discussed in this paper.

*Bowenia* in north-east Queensland suggested in Jones (2002) as being two species. In this and other papers and reports, e.g. Wilson (2002, 2003), I refer to these as nominate and putative *B. spectabilis*. The second trial was to test the hypothesis that *Lilioceris* first adapted to feeding on *Cycas* or a predecessor and then adapted to the more recently evolved and more toxic *Bowenia*.

Trial 1: Ten adult *L. nigripes* collected from nominate *B. spectabilis* growing at Tarzali in north-

east Queensland were transported to Rockhampton. Five specimens were placed in each of two 0.5 l beakers closed with fine gauze and offered leaflets of *B. serrulata* and *B. spectabilis* respectively for five days. In addition, five *L. nigripes* collected from *C. ophiolitica* were held in each of two containers, and offered leaflets of *B. serrulata* and of *B. spectabilis* respectively, for five days.

Trial 2: Ten adult and ten larvae of *L. nigripes* were collected from each of nominate and putative *B. spectabilis* growing at Tarzali and Tinaroo respectively and *C. media* subsp. *banksii* growing at Tinaroo. Five individuals were placed in each of two 0.5 l beakers and for five days offered the foliage of the opposite taxon to that from which they were collected.

## Results

### Field observations

*Lilioceris nigripes* browsed the foliage of the four species of *Cycas*, i.e. *C. ophiolitica*, *C. media* subsp. *banksii*, *C. cairnsiana* and *C. platyphylla*. The feeding activity was restricted to young foliage produced in flushes prior to onset of the wet season or after fires when previous foliage had been burned away. The larvae fed on immature leaflets after they lost their tomentose covering and used the frass to form a faecal shield; the Purple Meat Ant *Iridomyrmex purpureus* (Smith) fed on the frass of *L. nigripes* feeding on *C. ophiolitica*. Adult *L. nigripes* were observed to cut away distal portions of leaflets of *C. ophiolitica*, including those covered with fine trichomes, and to pierce the epidermis and feed on the flow of mucilage that resulted; *I. purpureus* was also observed to feed on this exudate.

*Lilioceris nigripes* were observed feeding on leaflets of both nominate and putative *B. spectabilis* in north-east Queensland. Larvae fed on glabrous juvenile leaflets but not on tomentose emerging or mature leaflets and were most frequently found on

the underside of immature leaflets (Fig. 2 left). They used the frass from feeding to form a faecal shield. Adult beetles browsed both juvenile and mature leaflets and, on most occasions removed all but the lower third of them (Fig. 2 right).

*Lilioceris nigripes* was not observed on the foliage of *B. serrulata* in central Queensland at any time during the study despite feeding on *C. ophiolitica* growing two kilometres away. Less than 1% of leaflets on *B. serrulata* surveyed were attacked by insects; larvae of the longicorn beetle *Sybra centurio* Pascoe (Coleoptera: Cerambycidae) and the moth *Cryptoptila immersana* (Walker) (Lepidoptera: Tortricidae) browsed the leaflets and the larvae of the former pupated in the petiole of leaves.

The bright orange-yellow coloured adults of *L. nigripes* were particularly obvious on the new foliage of all cycads in the study and when approached the beetles initially moved to the underside of leaflets and when further stressed took flight and flew up and away. No predation of either larvae or adult *L. nigripes* was observed during the study.

### Experimental results

Trial 1: *Lilioceris nigripes* previously feeding on nominate *B. spectabilis* did not eat the foliage of *B. serrulata* but did eat that of *C. ophiolitica* (Table 1). Beetles collected from *C. ophiolitica* did not eat the foliage of *B. serrulata* but ate that of *B. spectabilis* (Table 2).



Figure 2. Images of *Lilioceris nigripes* larvae on the cycad *Bowenia spectabilis*: left – on the underside of immature leaflets; and right – with faecal shields feeding on leaflets.

**Table 1. Feeding over five days by *Lilioceris nigripes* collected from nominate *Bowenia spectabilis* on glabrous juvenile foliage of *B. serrulata* and *Cycas ophiolitica*.**

The amount of foliage eaten by *L. nigripes* in the previous 24 hours was scored on a scale of 1 – 4 where 1 = none eaten, 2 = sample bites only, 3 = 5-50% eaten and 4 = >50% eaten.

Day of trial	<i>B. serrulata</i>	<i>C. ophiolitica</i>
1	2	3
2	1	4
3	1	4
4	1	4
5	1	4

**Table 2. Feeding over five days by *Lilioceris nigripes* collected from *Cycas ophiolitica* on glabrous juvenile foliage of *Bowenia serrulata* and nominate *B. spectabilis*.**

The amount of foliage eaten by *L. nigripes* in the previous 24 hours was scored on a scale of 1 – 4 where 1 = none eaten, 2 = sample bites only, 3 = 5-50% eaten and 4 = >50% eaten.

Day of trial	<i>B. serrulata</i>	<i>B. spectabilis</i>
1	2	2
2	1	2
3	1	2
4	1	2
5	1	2

Trial 2: Adult and larvae *Lilioceris nigripes* collected from nominate *B. spectabilis* ate the foliage of *C. media* subsp. *banksii* and the reverse. *Lilioceris nigripes* previously feeding on *C. ophiolitica* did not eat the foliage of *B. serrulata* but ate that of *B. spectabilis* (Table 3).

## Discussion

The data presented here extend the record of *Lilioceris nigripes* feeding on species and genera of cycads in different families. The results reported here and the widespread co-occurrence of *Lilioceris* and *Cycas* support the hypothesis that *Lilioceris* initially evolved to feed on *Cycas* or an antecedent and then adapted to the more toxic *Bowenia*.

**Table 3. Feeding over five days by *Lilioceris nigripes* collected from (a) nominate *Bowenia spectabilis* on glabrous juvenile foliage of *Cycas media* subsp. *banksii* and (b) from *C. media*, on the foliage of *B. spectabilis*.**

The amount of foliage eaten by *L. nigripes* in the previous 24 hours was scored on a scale of 1 – 4 where 1 = none eaten, 2 = sample bites only, 3 = 5-50% eaten and 4 = >50% eaten.

Day of trial	(a) <i>C. media</i>	
	subsp. <i>banksii</i>	(b) <i>B. spectabilis</i>
1	3	3
2	4	4
3	4	4
4	4	4
5	4	4

The point of particular interest in respect to plant systematics is that *L. nigripes* distinguishes between *B. spectabilis* and *B. serrulata* but not between populations of nominate and putative *B. spectabilis*. This and other data, e.g. of pollination vectors (Wilson 2002) and karyology (Kokubugata *et al.* 2001), support the recognition of the two named species but not of a proposed third species. As the MAM glycoside contents of the two species of *Bowenia* are similar, 5.04 and 4.33% fresh weight respectively (Moretti *et al.* 1983), this suggests some other chemical differentiation has occurred during their long period of separation and now prevents *L. nigripes* feeding on *B. serrulata*. That *L. nigripes* can change from browsing on *Cycas* to *B. spectabilis* and the reverse but will not browse on *B. serrulata*, even in the absence of other food, supports this hypothesis.

The parallels of the results of this study and those of Windsor *et al.* (1999) of *Aulacoscelis* (Chrysomelidae) and *Nomotus* (Languriidae) on Central American cycads are striking. They show feeding by leaf beetles on cycads is more common than previously recognized. The finding by Windsor *et al.* (1999) that an exclusively New World insect species feeds on *Cycas revoluta*, a species in a family to which they have had no evolutionary exposure, suggests no substantive barrier exists to feeding by chrysomelids across the two families. The observation that *Aulacoscelis* distinguishes between species in *Zamia* (Windsor *et al.* 1999),

and in this study that *L. nigripes* distinguishes between *B. spectabilis* and *B. serrulata*, suggest subtle but important chemical differences exist between some species. The data in both studies are consistent with the third tenant of coevolution of Ehrlich & Raven (1964), i.e. older plant lineages are associated with older insect lineages.

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