



# Using a comprehensive DNA barcode library to detect novel egg and larval host plant associations in a *Cephaloleia* rolled-leaf beetle (Coleoptera: Chrysomelidae)

CARLOS GARCÍA-ROBLEDO<sup>1,2,\*†</sup>, ERIN K. KUPREWICZ<sup>2,3†</sup>, CHARLES L. STAINES<sup>1</sup>, W. JOHN KRESS<sup>2</sup> and TERRY L. ERWIN<sup>1</sup>

<sup>1</sup>*Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, 20560, USA*

<sup>2</sup>*Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC, 20560, USA*

<sup>3</sup>*Organization for Tropical Studies, Duke University, Durham, NC 27708, USA*

Received 30 November 2012; revised 21 March 2013; accepted for publication 22 March 2013

To fully understand the ecology and evolution of plant–herbivore interactions, information regarding the life history of both immature and adult insect stages is essential. However, most knowledge of plant–herbivore associations is derived from observations of adults. One reason for this bias is that species identification of immature stages is usually challenging. DNA barcodes can be used to identify immature stages to the species level. This technique compares short sequences of the appropriate DNA barcode loci [e.g. mitochondrial cytochrome *c* oxidase subunit I (COI) gene for insects] of an unidentified specimen with a known DNA barcode library. The accuracy of DNA-based identifications depends on the comprehensiveness of the DNA barcode library. We generated a comprehensive DNA barcode library for a community of rolled-leaf beetles (Coleoptera: Chrysomelidae) in a premontane tropical forest in Costa Rica. The DNA barcode COI accurately identified all beetle species included in the study. Using this DNA barcode library, we identified eggs and larvae of *Cephaloleia histrionica* Baly with 100% confidence. This new record of *C. histrionica* is unique in that this species completes its life cycle on a bromeliad, whereas most *Cephaloleia* species are associated with plants from the order Zingiberales. The life cycle, diet breadth, immature stages, and sexual dimorphism are described for *C. histrionica*. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **110**, 189–198.

**ADDITIONAL KEYWORDS:** Bromeliaceae – *Cephaloleia histrionica* – COI – herbivory – mitochondrial DNA – *Pitcairnia arcuata* – Zingiberales.

## INTRODUCTION

The life cycles of insect herbivores involve several life stages, which usually differ with respect to diets and environmental requirements. To fully understand plant–herbivore interactions and their effects on ecosystems, it is essential to study the ecology of insects throughout their entire life cycles. One impediment to

understanding the interactions between plants and immature insects is that the morphology of eggs and larvae of most species remain unknown. One alternative to identifying species via egg and larval morphology is to follow the insect life cycle in the field to completion and identify the emerging adult. Direct observations provide unique insights into the natural history of insect herbivores. Unfortunately, direct observations are also time-consuming and can be logistically difficult.

An alternative way to link the different life-history stages of an insect herbivore species is to use DNA

\*Author for correspondence; e-mail: garciac@si.edu

†The authors contributed equally to the present study.

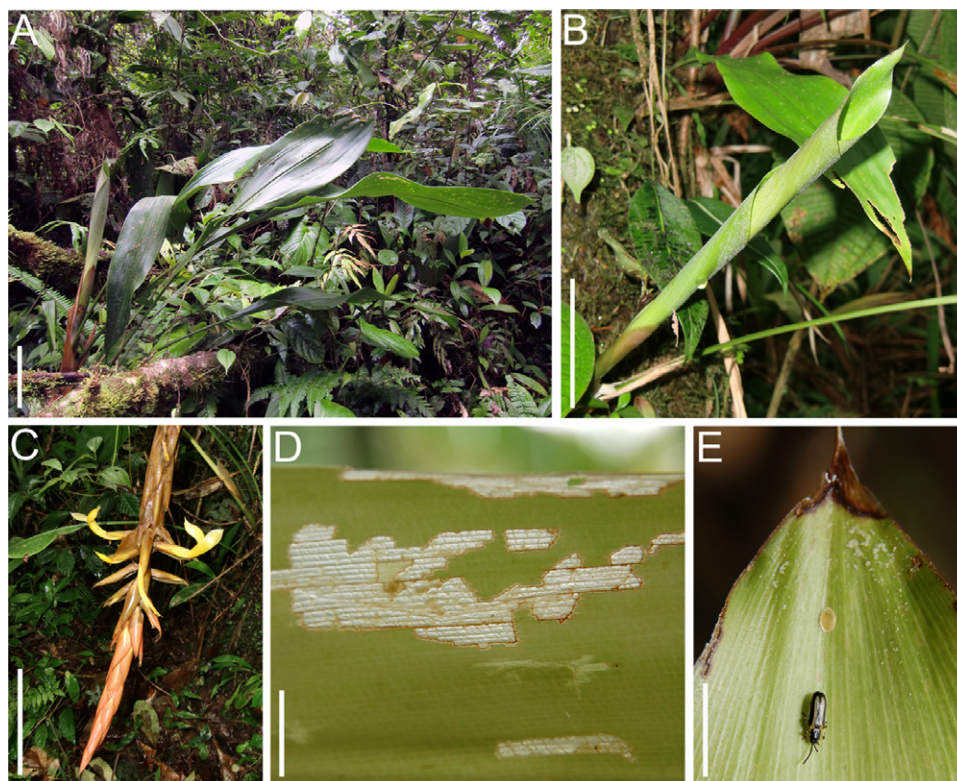
barcoding techniques (Hebert *et al.*, 2003). The primary target in animals is a short (< 650 bp) region of the mitochondrial cytochrome *c* oxidase subunit I (COI) (Hebert *et al.*, 2003). The usefulness of this approach for the present study is that DNA barcode identification techniques can compare DNA extracted from immature insect stages with a DNA reference library (Miller *et al.*, 2005; Caterino & Tishechkin, 2006; Levkanicova & Bocak, 2009; Curiel & Morrone, 2012).

Species identification success using molecular methods depends on the accuracy of taxonomic identifications associated with each sequence in the DNA barcode library (Ekrem, Willassen & Stur, 2007). Another challenge with respect to using DNA barcode identifications is that the reference library must contain sequences for all species potentially found in the community (Ekrem *et al.*, 2007). In cases when the reference library does not include matching sequences, sample DNA fragments will be misidentified or identified at a taxonomic rank above species (Ekrem *et al.*, 2007; Ross, Murugan & Li, 2008; Virgilio *et al.*, 2012).

The association between *Cephaloleia* Chevrolat, 1836 beetles and plants from the order Zingiberales is

well known (García-Robledo & Horvitz, 2011, 2012a, b). *Cephaloleia* beetles are Neotropical and evolved in association with plants in the families Cannaceae, Costaceae, Heliconiaceae, Marantaceae, and Zingiberaceae over the last 35–60 Myr (Wilf *et al.*, 2000; García-Robledo & Staines, 2008). *Cephaloleia* beetles are also known as the ‘rolled-leaf beetles’ because adults feed and mate inside the scrolls formed by young rolled leaves of their host plants (García-Robledo, Horvitz & Staines, 2010). The Neotropical genus *Cephaloleia* comprises 209 recorded species (Staines, 2008). The larvae of only four species from this genus have been described (García-Robledo *et al.*, 2010). At present, there is only one published record of a *Cephaloleia* beetle completing its life cycle on a non-Zingiberales host plant (i.e. *Cephaloleia* aff. *vagelineata* in Arecaceae) (Urueta-Sandino, 1972).

In the Braulio Carrillo tropical premontane forest in Costa Rica, Central America, we found adult *Cephaloleia histrionica* Baly, 1885 feeding on the rolled leaves of the bromeliad *Pitcairnia arcuata* (André) André (Bromeliaceae, Pitcairnioidae). We also found eggs and larvae potentially belonging to the genus *Cephaloleia* in this same bromeliad (Fig. 1).



**Figure 1.** *Pitcairnia arcuata* (Bromeliaceae), host plant of *Cephaloleia histrionica*. A, habit. B, detail of a rolled leaf used as a larval and adult food source and adult oviposition site. C, inflorescence. D, leaf damage produced by a feeding adult *C. histrionica*. E, female ovipositing on a rolled leaf of *P. arcuata*. Scale bars: (A, B, C) = 10 cm; (D, E) = 1 cm.

For the present study, we tested the accuracy of using DNA barcodes to delimit *Cephaloleia* species. First, we assembled a DNA library containing all known species of *Cephaloleia* in our study area. Using this comprehensive local DNA barcode library, we identified the eggs and larvae found on *P. arcuata* bromeliad leaves. DNA barcode-based identifications were complemented with the description of several aspects of the natural history of *C. histrionica*, such as diet breadth, immature stages, and sexual dimorphism.

## MATERIAL AND METHODS

### STUDY SITE

The study was conducted from July to November 2011 on the Atlantic slope of the Cordillera Central of Costa Rica, Central America, in the Rara Avis and Selva Tica reserves, near the border of Braulio Carillo National Park (Coordinates of shelters: 10°18'10"N, 84°02'02"W and 10°16'54"N, 84°02'41"W, respectively). All samples were obtained between 600–800 m.a.s.l. in tropical premontane rain forest (Holdridge, 1947; Hartshorn & Peralta, 1988). At these locations, we recorded 22 species of plants from the order Zingiberales and eight species of *Cephaloleia* rolled-leaf beetles. In this area, we also recorded two species of *Chelobasis* Gray, 1832 (Chrysomelidae), a genus of rolled-leaf beetles that feeds on plants from the genus *Heliconia* (Heliconiaceae: Zingiberales).

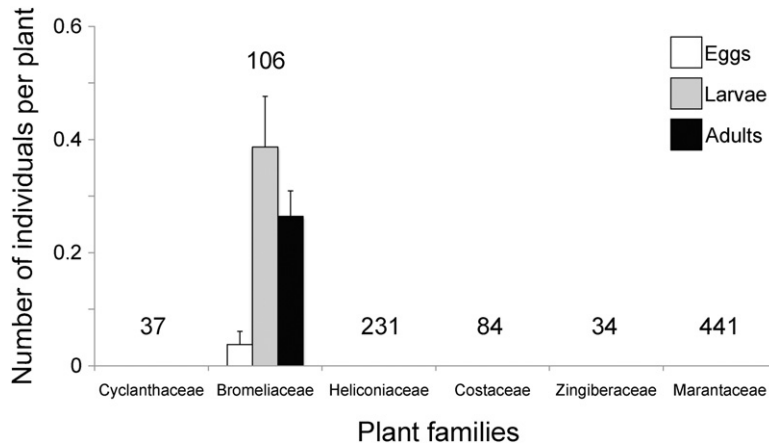
Additionally, we surveyed leaves of *Cyclanthus bipartitus* Poit. ex A. Rich (Cyclanthaceae), the host plant of a chrysomelid species previously included in the genus *Cephaloleia* (*Cephaloleia costaricensis*, later transferred to *Parimatidium costaricensis* Umann) (Staines, 2009a).

### SURVEY OF EGGS, LARVAE, AND ADULT *C. HISTRIONICA* BALY, 1885 IN POTENTIAL HOST PLANTS

To determine the host plants used by *C. histrionica*, we recorded the number of *C. histrionica* adults found on rolled leaves of all the Zingiberales present in the study site (Fig. 2). We also surveyed young leaves of *C. bipartitus* (Cyclanthaceae). Finally, we recorded the numbers of eggs, larvae, and adults on rolled leaves and inflorescences of the bromeliad *P. arcuata*, a new host plant record for *Cephaloleia* beetles. Eggs and larvae found in *P. arcuata* were collected for further taxonomic identification using molecular markers.

### SPECIES IDENTIFICATION OF ADULTS, EGGS, AND LARVAE USING DNA BARCODES

We assembled a reference DNA barcode library containing sequences from all known *Cephaloleia* and *Chelobasis* species in the study area. In our analyses, we also included two chrysomelid species recorded on



**Figure 2.** Number of larvae, pupae, and adults of *Cephaloleia histrionica* recorded inside rolled-leaves of plants used as hosts by *Cephaloleia* beetles in a tropical premontane forest (mean + SD). Numbers on the bars represent the number of host plants surveyed. Sample sizes for each plant species included in the study: Cyclanthaceae: *Cyclanthus bipartitus* ( $N = 37$ ). Bromeliaceae: *Pitcairnia arcuata* ( $N = 106$ ). Heliconiaceae: *Heliconia irrasa* ( $N = 140$ ), *Heliconia mathiasiae* ( $N = 4$ ), *Heliconia pogonantha* ( $N = 39$ ), *Heliconia sarapiquensis* ( $N = 39$ ), *Heliconia* sp<sub>1</sub> ( $N = 9$ ). Costaceae: *Costus bracteatus* ( $N = 60$ ), *Costus laevis* ( $N = 12$ ), *Costus malortieanus* ( $N = 2$ ), *Costus* sp<sub>1</sub> ( $N = 2$ ), *Costus* sp<sub>2</sub> ( $N = 8$ ). Zingiberaceae: *Renealmia cernua* ( $N = 19$ ), *Renealmia* sp<sub>1</sub> ( $N = 15$ ). Marantaceae: *Calathea* aff. *crotalifera* ( $N = 186$ ), *Calathea cleistantha* ( $N = 24$ ), *Calathea foliosa* ( $N = 13$ ), *Calathea guzmanioides* ( $N = 3$ ), *Calathea leucostachys* ( $N = 9$ ), *Calathea micans* ( $N = 45$ ), *Calathea similis* ( $N = 4$ ), *Calathea* sp<sub>1</sub> ( $N = 100$ ), *Ischnosiphon inflatus* ( $N = 16$ ), *Plelostachya leiostachya* ( $N = 11$ ).



*C. bipartitus* (Cyclanthaceae), *Prosopodonta limbata* Baly, 1858 and *P. costaricensis*. Collected beetles were preserved in 95% EtOH. One leg of each voucher specimen was removed and DNA was extracted in accordance with the protocols described by García-Robledo, Erickson, Staines, Erwin & Kress, 2013. Amplification of the mitochondrial gene COI was performed in 96-well plate formats using the COI Folmer primer (see Supporting information, Table S1).

A polymerase chain reaction was followed by ExoSap (Affymetrix) purification of amplified products, followed by standard sequencing using BigDye Di-Deoxy terminator (Applied Biosystems) sequencing. Employing the same protocols as those used for adults, we also obtained COI sequences for eggs and larvae collected from leaves of the bromeliad *P. arcuata*.

Sequences of eggs, larvae, and adults were aligned using multiple sequence alignment with high accuracy and high throughput (Edgar, 2004). To determine the accuracy of using DNA barcodes to identify the beetle species included in the present study, we calculated the similarity of each COI sequence with other sequences included in our DNA barcode library (i.e. measured as the percentage of bases/residuals that are identical). We estimated the frequency distributions for both inter- and intraspecific similarities among sequences. Using these sequence distributions, we estimated a DNA barcode gap (i.e. the thresholds at which a COI sample included in the study can be identified as conspecific or as heterospecific) (Hajibabaei *et al.*, 2006).

We generated a Neighbour-joining tree and estimated bootstrap support after 100 replicates using GENEIOUS PRO, version 5.6.5 (Biomatters-development-team, 2012). We selected *Chelobasis bicolor* Gray, 1832 and *Chelobasis perplexa* (Baly, 1858) (Chrysomelidae) as outgroups. The tree showing species identifications using the COI DNA barcode was edited using MESQUITE (Maddison & Maddison, 2011). DNA sequences were deposited in GenBank (for accession numbers, see the Supporting information, Tables S1, S2).

#### DESCRIPTION OF IMMATURE STAGES OF

##### *C. HISTRIONICA* BALY, 1885

To describe larval microstructures, we obtained scanning electron microscopy (SEM) images for five larvae collected on *P. arcuata* (i.e. *C. histrionica* larvae; see Results). Larvae were dehydrated using 95% and 100% EtOH and a final critical point dehydration using hexamethyldisilazane. Samples were sputter coated using palladium and mounted on carbon adhesive tabs on aluminum stubs. SEM images were

obtained using a TM3000 microscope (Hitachi). Vouchers of larvae and mounted specimens used for SEM imaging were deposited in the Department of Entomology collection, US National Museum of Natural History.

#### SEXUAL DIMORPHISM

To determine whether *C. histrionica* displays sexual size dimorphism, we measured the lengths of males and females from the tip of the head to the end of the last abdominal segment. Measurements were performed using a digital camera (Model 3.2.0; Diagnostic Instruments Inc.) attached to a stereoscope (MZ 12S; Leica). Lengths were estimated on the digital images at an accuracy of  $1 \times 10^{-2}$  mm, using Spot, version 3.5.8 (Diagnostic Instruments Inc.). Differences in lengths between males and females were tested using a Mann–Whitney *U*-test. Additionally, we described morphological differences in the last abdominal segment between males and females.

#### RESULTS

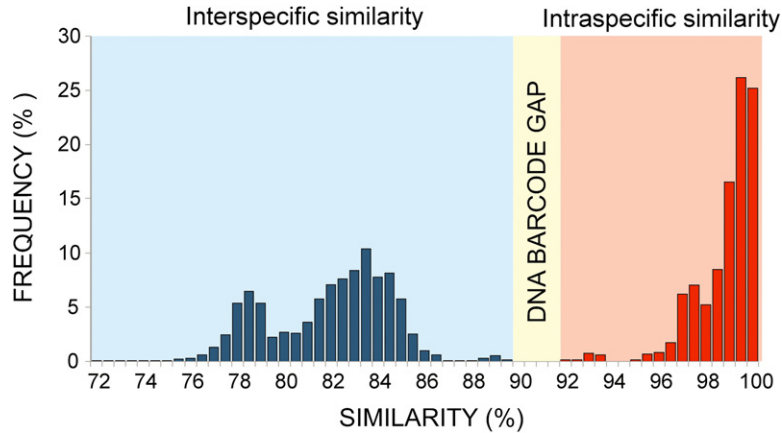
##### SURVEY OF EGGS, LARVAE, AND ADULT *C. HISTRIONICA* BALY, 1885 IN POTENTIAL HOST PLANTS

We surveyed a total of 949 rolled leaves from 24 plant species. The survey included plants from the families Cyclanthaceae (*C. bipartitus*), Bromeliaceae (*P. arcuata*, a new host plant record for *C. histrionica*), Costaceae, Heliconiaceae, Marantaceae, and Zingiberaceae (for a list of Zingiberales species included in the survey, see Fig. 2). We only found adult *C. histrionica* inside the rolled leaves of *P. arcuata* (Bromeliaceae) (Fig. 2). After dissecting each bract in ten inflorescences of *P. arcuata*, we observed no eggs, larvae or adult *Cephaloleia* (Fig. 1C).

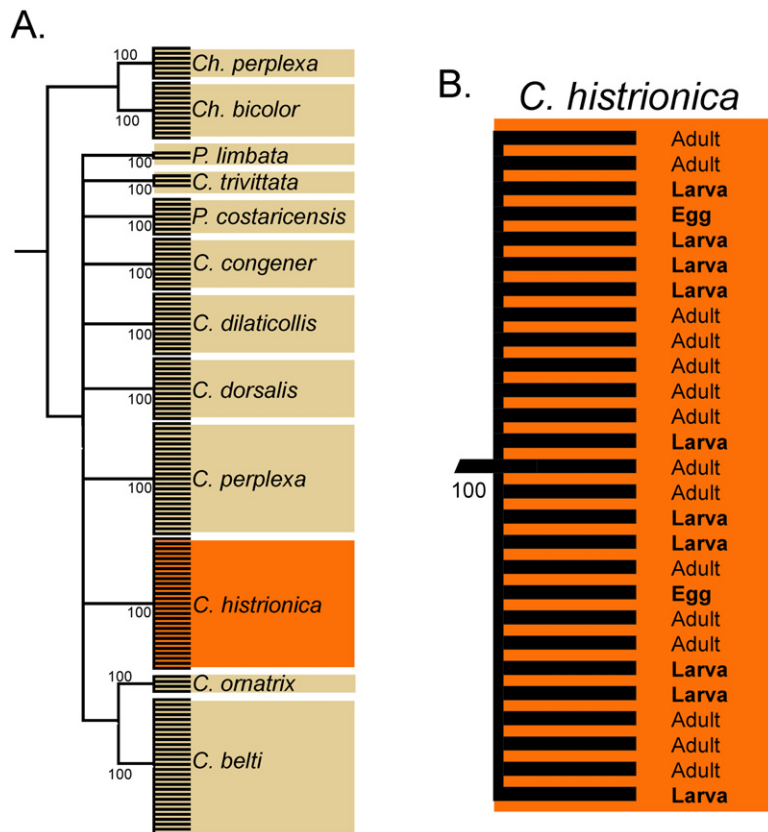
##### SPECIES IDENTIFICATION OF ADULTS, EGGS, AND LARVAE USING DNA BARCODES

COI sequences of individuals from the same species displayed a similarity between 92–100%. Heterospecific sequences were only 72–89% similar (Fig. 3). There was no overlap between intra and interspecific similarity frequency distributions (Fig. 3). Most COI sequences of individuals within the same species (i.e. 95% of all intraspecific comparisons) were 97–100% similar (Fig. 3).

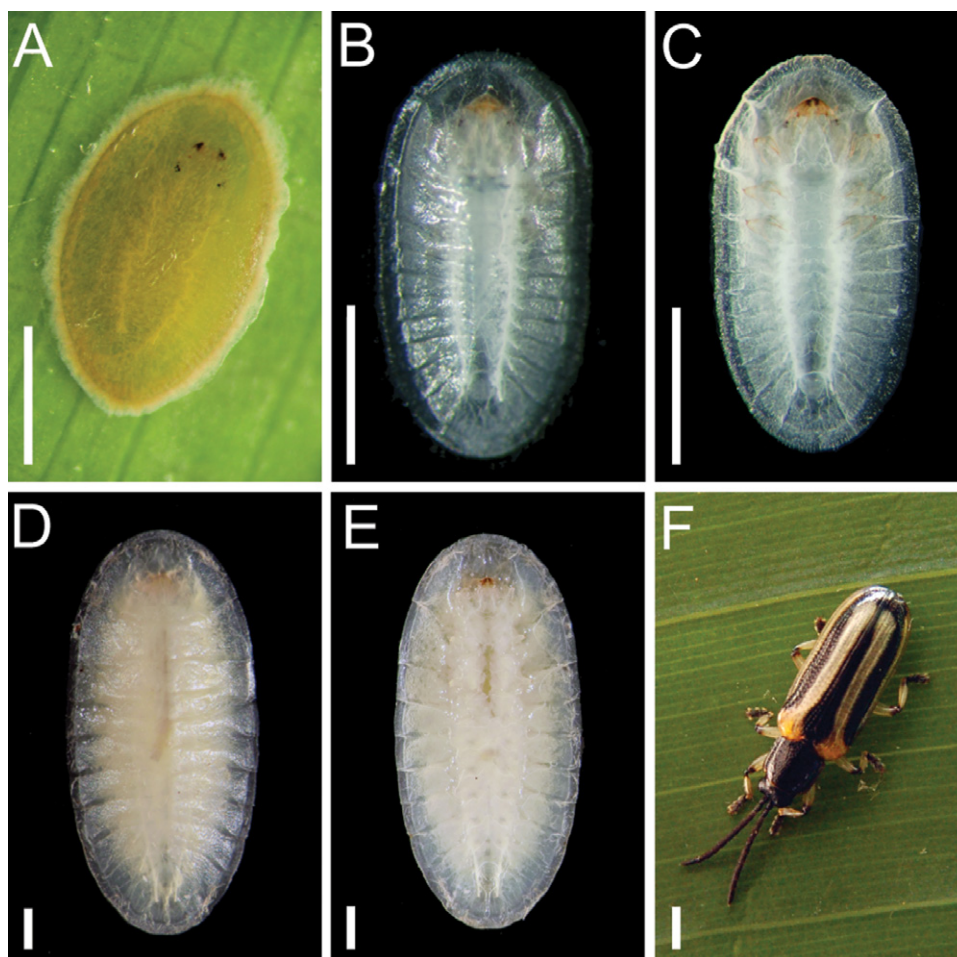
The Neighbour-joining tree of COI sequences grouped all individuals within each insect herbivore species into monophyletic groups. All nodes grouping individuals within species had a bootstrap support of 100% (Fig. 4A). All DNA sequences extracted from



**Figure 3.** Frequency distributions of inter- and intraspecific similarities for beetle cytochrome *c* oxidase subunit I (COI) sequences (percentage of bases/residuals that are identical for each comparison). Interspecific frequency distribution is based on 10 007 paired comparisons. Intraspecific frequency distribution is based on 1316 paired comparisons. The sample size for each beetle species is included in the Supporting information (Table S1).



**Figure 4.** Identification of *Cephaloleia* species and their immature stages using cytochrome *c* oxidase subunit I (COI) sequences. A, Neighbour-joining tree includes bootstrap values (%) supporting species identifications. Boxes group individuals within each species. B, results of the identification of eggs and larvae collected in *Pitcairnia arcuata* (Bromeliaceae) using the DNA barcode COI. All eggs and larvae were identified as *Cephaloleia histrionica*. For more information on each specimen, see the Supporting Information (Table S1).



**Figure 5.** A, egg. B, C, first-instar larva (dorsal and ventral views). D, E, second-instar larva (dorsal and ventral views). F, adult *Cephaloleia histrionica*. Scale bars (all panels) = 1 mm.

eggs and larvae collected from leaves of *P. arcuata* (Bromeliaceae) corresponded to the species *C. histrionica* (Fig. 4B).

DESCRIPTION OF IMMATURE STAGES OF  
*C. HISTRIONICA* BALY, 1885

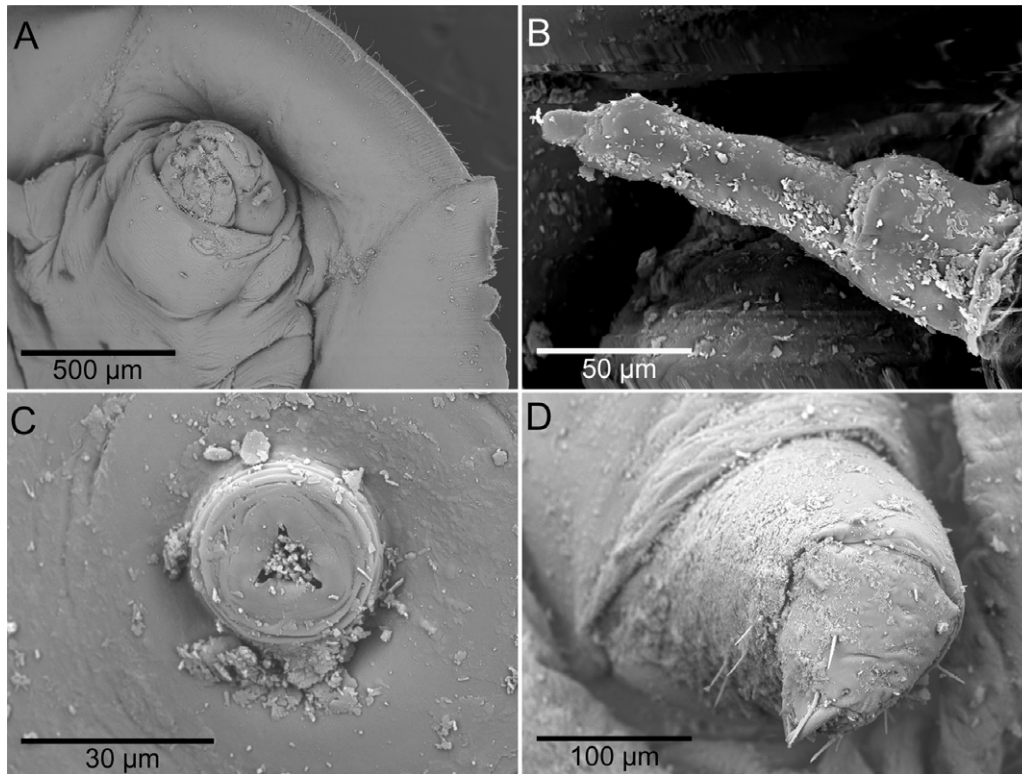
*Cephaloleia histrionica* eggs are pale yellow (Fig. 5A). The attachment tissue of the egg to the substrate is pale cream coloured. Eggs were found attached to the inner surfaces of rolled leaves of *P. arcuata* (Figs 1E, 5A). Egg length (mean  $\pm$  SD) was  $2.55 \pm 0.09$  mm and egg width was  $1.59 \pm 0.10$  mm ( $N = 6$ ). Larva colour when alive was creamy-white becoming translucent laterally and apically, with some yellowish areas medially (Fig. 5B, C, D, E). When fixed in EtOH, this colour becomes yellowish-brown. Dorsum without medial setose ridge. Total length: 8.6–9.3 mm; width 4.6–4.9 mm ( $N = 4$ ).

**Dorsum.** Prothorax without raised central area; micropustulate; with pale setae along lateral and

apical margins; lateral and apical margins with numerous shallow sulci. Mesothorax without raised central area or carina or sulcus; micropustulate; laterally with numerous shallow sulci on expansion. Metathorax with central portion micropustulate; without carina or sulcus. Abdominal tergites 1–6 slightly narrowed in middle; without carina laterally; spiracle near basal margin; each spiracle appears as spot with darker margin, orifice (Fig. 6C). Abdominal tergites 7–10 without surface plicae or carinae.

**Venter.** Surface of expansions smooth, sulcate laterally. Head with surface sparsely punctate; labrum smooth, without setae; clypeus with fringe of setae at apex, with four large punctures each with a single seta; mandibles bidentate; maxillary palps with two palpomeres, without setae at apex; maxilla robust, clavate, with fringe of long setae at apex; labium densely setose (Fig. 6A). Antenna with antennomere (1) short, robust,  $\frac{1}{2}$  length of (2); (2) cylindrical, longer than (1) and (3) combined; (3) shortest, with ring of 19





**Figure 6.** Details of larval structures in *Cephaloleia histrionica*. A, head. B, antenna. C, spiracle. D, leg.

setae at apex (Fig. 6B). Prothorax longer than others, wider than long, slightly depressed in middle; surface rugose-striate. Meso- and metathorax wider than long, slightly depressed in middle; surface rugose-striate. Abdominal sternites 1–8 wider than long, decreasing in width; with transverse sulcus just beyond middle and second transverse sulcus near apex; sternite 9–10 fused, rounded at apex. Leg femur wider and longer than tibiotarsus; tibiotarsus sub-conical, with a robust claw and six setae at apex (Fig. 6D).

#### SEXUAL DIMORPHISM IN *C. HISTRIONICA* BALY, 1885

Female *C. histrionica* were found to be longer than males (Mann–Whitney *U*-test,  $W = 117$ ,  $P < 0.0001$ , mean  $\pm$  SD: females =  $5.84 \pm 0.14$ , males =  $5.31 \pm 0.20$ ,  $n_{\text{females}} = 20$ ,  $n_{\text{males}} = 6$ ). This species displays a marked sexual dimorphism in the shape of the last abdominal sternite and the pygidium. In females, the last sternite is slightly acuminate (Fig. 7). The end of the last sternite in males is U-shaped (Fig. 7).

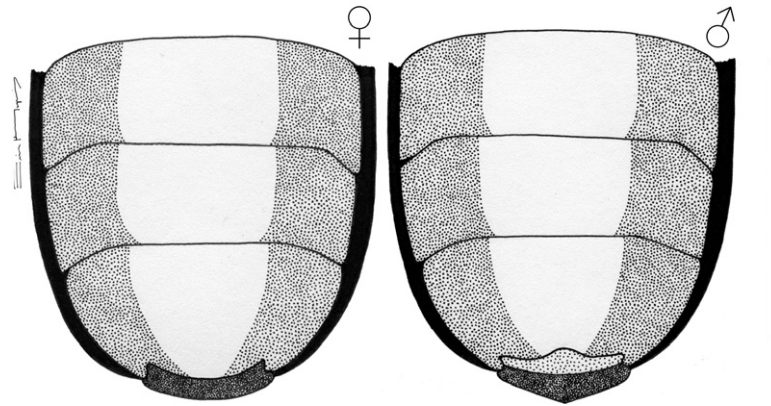
#### DISCUSSION

In this community of rolled-leaf beetles, we found no discrepancies between morphological and molecular identifications or any evidence of cryptic species. This

indicates that the DNA barcode COI has sufficient low intraspecific variation and sufficiently high interspecific variation to delimit all the *Cephaloleia* species included in the study.

Part of our success can be attributed to the careful identification of each sample by an expert taxonomist before it was included in our DNA barcode library. The taxonomy of *Cephaloleia* and *Chelobasis* beetles is very stable as a result of decades of research on this group of insect (Staines, 1996; Staines, 2009b), which is not the case for most insect groups (Cardoso *et al.*, 2011). This taxonomic impediment is a serious issue when using public DNA databases. For example, it is acknowledged that public DNA databases such as GenBank contain many misidentified specimens, leading to incorrect identifications (Meier *et al.*, 2006).

Another potential reason for our success in delimiting rolled-leaf beetle species is that the eggs, larvae, and adults examined represent individuals within the same locality. Theory suggests that intraspecific genetic variation of DNA barcode libraries will increase when using individuals from multiple populations (Dasmahapatra & Mallet, 2006). Consequently, the accuracy of identifications using DNA barcodes will be higher in local studies than that in studies conducted at broad geographical scales (Lukhtanov *et al.*, 2009). There is some evidence that COI intraspecific variation increases when including



**Figure 7.** Sexual dimorphism of the last sternite of *Cephaloleia histrionica*. Scale bar = 1 mm. Illustration by E. K. Kuprewicz.

individuals from multiple populations (Lukhtanov *et al.*, 2009). However, this increase in genetic variation is sufficiently small to avoid interspecific sequence overlaps and ensure successful species identification (Lukhtanov *et al.*, 2009).

The identification of eggs and larvae of *C. histrionica* is only one of several examples of the potential for DNA barcodes to identify the immature stages of insects. Previous studies include the identification of immature terrestrial and aquatic arthropods (Miller *et al.*, 2005; Caterino & Tishechkin, 2006; Ekrem *et al.*, 2007; Traugott *et al.*, 2008; Levkanicova & Bocak, 2009; Zhang & Weirauch, 2011; Curiel & Morrone, 2012). The general conclusions of these studies are that identification success improves when a comprehensive DNA barcode library is available.

Surveys in the field combined with DNA barcode analyses reveal that the life cycle of the population of *C. histrionica* studied is closely associated with the bromeliad *P. arcuata*. On the Pacific coast of southern Costa Rica, *C. histrionica* is a specialist on plants from the family Costaceae (Zingiberales) (C. García-Robledo, unpubl. data). Future studies using DNA barcodes need to be performed to determine whether or not these differences in host plant use are the result of diet variation among populations or whether the southern Pacific coast populations of *C. histrionica* correspond to a morphologically-similar/identical cryptic species.

Previous accounts of *Cephaloleia* rolled-leaf beetles feeding on bromeliads are based on unpublished observations (McKenna & Farrell, 2005). Documented interactions between chrysomelids and bromeliads are rare. Some recorded interactions include *Acentroptera pulchella* Guérin-Ménéville and *Calliaspis rubra* Olivier, herbivores of bromeliads in the Peruvian Amazon (Lowman, Wittman & Murray, 1996; Mantovani *et al.*, 2005; Frank & Lounibos, 2009).

*Cephaloleia* beetles have rarely been recorded feeding on plants other than Zingiberales. There are only a few records of adults collected from or feeding on plants from the families Arecaceae, Cyclanthaceae, Cyperaceae, Orchidaceae, and Poaceae (Staines, 2008). The present study shows that at least one species of *Cephaloleia* can complete its life cycle in a non-Zingiberales host plant.

In conclusion, the DNA barcode COI can delimit species of rolled-leaf beetles with great accuracy. DNA barcoding is a practical tool that can be used to discover novel plant–herbivore associations such as the interaction between *C. histrionica* and its primary host plant, the bromeliad *P. arcuata*.

#### ACKNOWLEDGEMENTS

The authors thank the staff of Selva Tica – Rara Avis Reserves, I. Lopez (Smithsonian Institution) and J. Hurtado (TEAM project Barva Transect, Conservation International) for logistical support. We thank S. Nagi and D. Erickson (Smithsonian Institution) for assistance during the laboratory work. S. Whittaker (Smithsonian Institution) provided advice with respect to obtaining SEM images. Comments by Hugh Loxdale and an anonymous reviewer improved the manuscript substantially. This research was funded by a Smithsonian Postdoctoral Fellowship, a National Geographic-Waitt Institute grant (W149-11), a Heliconia Society International grant, and a Rubenstein Fellowship – Encyclopedia of Life to C.G.

#### REFERENCES

- Biomatters Development Team.** 2012. *Geneious® Pro 5.6.5: created by Biomatters*. Available at: <http://www.biomatters.com/>



- Cardoso P, Erwin TL, Borges PAV, New TR. 2011.** The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation* **144**: 2647–2655.
- Caterino MS, Tishechkin AK. 2006.** DNA identification and morphological description of the first confirmed larvae of *Hetaeriniinae* (Coleoptera : Histeridae). *Systematic Entomology* **31**: 405–418.
- Curiel J, Morrone JJ. 2012.** Association of larvae and adults of Mexican species of *Macrelmis* (Coleoptera: Elmidae): a preliminary analysis using DNA sequences. *Zootaxa* **3361**: 56–62.
- Dasmahapatra KK, Mallet J. 2006.** DNA barcodes: recent successes and future prospects. *Heredity* **97**: 254–255.
- Edgar RC. 2004.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.
- Ekrem T, Willassen E, Stur E. 2007.** A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution* **43**: 530–542.
- Frank JH, Lounibos LP. 2009.** Insects and allies associated with bromeliads: a review. *Terrestrial Arthropod Reviews* **1**: 125–153.
- García-Robledo C, Erickson DL, Staines CL, Erwin TL, Kress WJ. 2013.** Tropical plant–herbivore networks: reconstructing species interactions using DNA barcodes. *PLoS ONE* **8**: e52967.
- García-Robledo C, Horvitz CC. 2011.** Experimental demography and the vital rates of generalist and specialist insect herbivores on novel and native host plants. *Journal of Animal Ecology* **80**: 976–989.
- García-Robledo C, Horvitz CC. 2012a.** Jack of all trades masters novel host plants: positive genetic correlations in specialist and generalist insect herbivores expanding their diets to novel hosts. *Journal of Evolutionary Biology* **25**: 38–53.
- García-Robledo C, Horvitz CC. 2012b.** Parent–offspring conflicts, ‘optimal bad motherhood’ and the ‘mother knows best’ principles in insect herbivores colonizing novel host plants. *Ecology and Evolution* **2**: 1446–1457.
- García-Robledo C, Horvitz CC, Staines CL. 2010.** Adult and larval morphology, host plants, adult longevity and notes on natural history in *Cephaloleia* ‘rolled-leaf beetles’ (Coleoptera: Chrysomelidae: Cassidinae). *Zootaxa* **2610**: 50–68.
- García-Robledo C, Staines CL. 2008.** Herbivory in gingers from latest Cretaceous to present: is the ichnogenus *Cephaloleichnites* (Hispiinae, Coleoptera) a rolled-leaf beetle? *Journal of Paleontology* **82**: 1035–1037.
- Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN. 2006.** DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 968–971.
- Hartshorn GS, Peralta R. 1988.** Preliminary description of primary forests along the La Selva-Volcan Barva altitudinal transect, Costa Rica. In: Almeda F, Pringle CM, eds. *Tropical rain forest: diversity and conservation*. San Francisco, CA: California Academy of Sciences, 281–295.
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003.** Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B, Biological Sciences* **270**: 313–321.
- Holdridge LR. 1947.** Determination of world plant formations from simple climatic data. *Science* **105**: 367–368.
- Levkanicova Z, Bocak L. 2009.** Identification of net-winged beetle larvae (Coleoptera: Lycidae) using three mtDNA fragments: a comparison of their utility. *Systematic Entomology* **34**: 210–221.
- Lowman MD, Wittman PK, Murray D. 1996.** Herbivory in a bromeliad of the Peruvian rain forest canopy. *Journal of the Bromeliad Society* **46**: 52–55.
- Lukhtanov VA, Sourakov A, Zakharov EV, Hebert PDN. 2009.** DNA barcoding Central Asian butterflies: increasing geographical dimension does not significantly reduce the success of species identification. *Molecular Ecology Resources* **9**: 1302–1310.
- Maddison WP, Maddison DR. 2011.** *Mesquite: a modular system for evolutionary analysis*, Version 2.75. Available at: <http://mesquiteproject.org>
- Mantovani A, Magalhaães N, Teixeira M, Leitaão G, Staines C, Resende B. 2005.** First report on host plants and feeding habits of the leaf beetle *Acentroptera pulchella* Guérin-Ménéville (Chrysomelidae, Hispiinae). *Contributions to systematics and biology of beetles. Papers celebrating the 80th birthday of Igor Konstantinovich Lopatin. Pensoft Series Faunistica No 43. Pensoft. Sofia, Bulgaria*: 153–157.
- McKenna DD, Farrell BD. 2005.** Molecular phylogenetics and evolution of host plant use in the Neotropical rolled leaf ‘hispiine’ beetle genus *Cephaloleia* (Chevrolat) (Chrysomelidae: Cassidinae). *Molecular Phylogenetics and Evolution* **37**: 117–131.
- Meier R, Shiyang K, Vaidya G, Ng PKL. 2006.** DNA barcoding and taxonomy in diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* **55**: 715–728.
- Miller KB, Alarie Y, Wolfe GW, Whiting MF. 2005.** Association of insect life stages using DNA sequences: the larvae of *Philodytes umbrinus* (Motschulsky) (Coleoptera : Dytiscidae). *Systematic Entomology* **30**: 499–509.
- Ross HA, Murugan S, Li WLS. 2008.** Testing the reliability of genetic methods of species identification via simulation. *Systematic Biology* **57**: 216–230.
- Staines CL. 1996.** The genus *Cephaloleia* (Coleoptera: Chrysomelidae) in Central America and the West Indies. *Revista de Biología Tropical Special Publication No. 3*: 3–87.
- Staines CL. 2008.** A new species of *Cephaloleia* Chevrolat, 1837 (Coleoptera: Chrysomelidae: Cassidinae) from Dominica. *Insecta Mundi* **30**: 1–4.
- Staines CL. 2009a.** Generic reassignment of species in the tribe Cephaloleiini Chapuis, 1875 (Coleoptera: Cassidinae). *Insecta Mundi* **0107**: 1–4.
- Staines CL. 2009b.** A review of the genus *Chelobasis* Gray, 1832 (Coleoptera: Chrysomelidae: Cassidinae). *Zootaxa* **2033**: 58–68.
- Traugott M, Bell JR, Broad GR, Powell W, Van Veen JF, Vohlhardt IMG, Symondson WOC. 2008.** Endoparasitism

- in cereal aphids: molecular analysis of a whole parasitoid community. *Molecular Ecology* **17**: 3928–3938.
- Urueta-Sandino E. 1972.** *Cephaloleia* sp. cerca a *variegata* Pic, una plaga de la Palma Africana. *Revista Facultad Nacional de Agronomía* **26**: 75–77.
- Virgilio M, Jordaens K, Breman FC, Backeljau T, De Meyer M. 2012.** Identifying insects with incomplete DNA barcode libraries, African fruit flies (Diptera: Tephritidae) as a test case. *PLoS ONE* **7**: e31581.
- Wilf P, Labandeira CC, Kress WJ, Staines CL, Windsor DM, Allen AL, Johnson KR. 2000.** Timing the radiations of leaf beetles: hispines on gingers from latest Cretaceous to recent. *Science* **289**: 291–294.
- Zhang G, Weirauch C. 2011.** Matching dimorphic sexes and immature stages with adults: resolving the systematics of the *Bekilya* group of Malagasy assassin bugs (Hemiptera: Reduviidae: Peiratinae). *Systematic Entomology* **36**: 115–138.

### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Samples included in the DNA barcode analyses. Tables summarize collection information, the life stage of each sample (egg, larva or adult), host plant family/species, and sequence quality (percentage of untrimmed bases of high-quality; HQ%). DNA amplifications were performed with the Folmer primers: GGTC AACAAATCATAAAGATATTGG (F) and TAAACTTCAGGGTGACCAAAAAATCA (R).

**Table S2.** DNA sequences (COI) of limited quality and not deposited in GenBank (FASTA format). Sequences in this supplement were included in the analyses because they successfully identified individuals to the species level with 100% confidence. Numbers at the beginning of each sequence represent collection numbers. Information for each collection was included in Table S1.