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Plagiometriona emarcida (Boheman, 1855) and *Plagiometriona forcipata* (Boheman, 1855) (Coleoptera: Chrysomelidae: Cassidinae), a single species differing in larval performance and adult phenotype

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Plagiometriona emarcida (Boheman, 1855) and *Plagiometriona forcipata* (Boheman, 1855) (Coleoptera: Chrysomelidae: Cassidinae), a single species differing in larval performance and adult phenotype

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Matings frequently observed between the tortoise beetles *Plagiometriona forcipata* (Boheman, 1855) and *Plagiometriona emarcida* (Boheman, 1855), in the State of Rio de Janeiro, Brazil, led us to compare the ecology, life history and genetics of these two morphologically distinct species. Host plant censuses revealed *P. emarcida* was about five times more abundant, while populations of both species responded in parallel to climate. Laboratory-reared immature *P. forcipata* took 2 days longer to complete development and showed higher mortality rates. No differences were detected in ovipositional behaviour, genitalia morphology or in sex ratio of offspring. The adult offspring of field-caught females of both species contained individuals with both species' elytral patterns. Finally, nearly identical cytochrome oxidase I sequences obtained from individuals of each species suggest that they are a single polymorphic species. Based on these observations and the examination of type material we propose the new synonymy: *Plagiometriona forcipata* = *P. emarcida*, **syn. nov.**

Keywords: Cassidinae; *Plagiometriona*; synonymy; polymorphism; cytochrome oxidase I

Introduction

The presence and maintenance of multiple colour patterns within populations is of substantial interest because colour can directly affect individual fitness by modulating body temperature, the efficacy of intra- and interspecific communication and susceptibility to predation (Endler 1978, 1993a, b; Brakefield 1985). Intraspecific colour differences in insects may result from "proximate" environmental factors (such as crowding, food type, temperature and humidity) acting during ontogeny to affect the expression of colour genes in adults, or their expression may have been genetically fixed by "ultimate" factors acting over evolutionary time (Peschken 1972; Lu and Logan 1994). Studies have documented high levels of colour pattern polymorphism within populations of several chrysomelid beetle species (e.g. Fujiyama and Arimoto 1988; Zvereva et al. 2002; Gonçalves and Macedo 2003; Nahrung and Allen 2005) and evidence suggests that some of these colour pattern morphs are mimicking

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warningly coloured, toxic insects in their surroundings and are thereby gaining protection from predators (Vasconcellos-Neto 1988). Additionally, colour morphs within cryptic species are expected to increase “aspect diversity” making it more difficult for predators to search on a particular image stereotype to find prey (Ricklefs and O’Rourke 1975; Monteiro 1991).

For taxonomists, polymorphisms can generate problems in accurate species diagnosis. Commonly, each morph is given species status until other lines of evidence are carefully examined, as with *Chelymorpha cribraria* Fabricius, 1775, whose numerous morphs were described as distinct species (Vasconcellos-Neto 1988). Problems can also occur when sibling species are erroneously considered “morphs” of a single species (Verma and Kalaichelvan 2004).

We observed numerous matings between two tortoise beetle species with distinctive elytral patterns during fieldwork in southern Brazil, *Plagiometriona emarcida* (Boheman, 1855), whose dorsum is entirely metallic gold, and *Plagiometriona forcipata* (Boheman, 1855) whose dorsum has a conspicuous black annulus (Fig. 1A). We investigated aspects of the ecology, development and genetics of these species for the first time, obtaining results that reveal substantial differences in larval development and survivability yet supporting their synonymy and status as alternative phenotypes within a single species, *Plagiometriona forcipata*.

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Material and Methods

Field site

Field observations were conducted at the Serra dos Órgãos National Park (22°26′56″ S; 42°59′5″W) in the State of Rio de Janeiro, south-eastern Brazil. Data were obtained through 14 monthly surveys of marked *Solanum lhotskyanum* Dunal (Solanaceae) plants along the Pedra do Sino Trail, which ranges from 1100 m to 2263 m above sea level. Thirty plants were chosen sequentially at mid-elevation (1600–1800 m) and another 30 plants were chosen at high elevation (2000–2100 m). This work was part of a larger study in the area in which monthly surveys were carried out on five host plant species at three different elevations from January 2007 to February 2008 (Flinte et al. 2009).

During the study the lowest mean daily temperatures recorded at the Park’s meteorological station (980 m elevation) occurred between May and August 2007 (mean 16.4°C) and the highest occurred between January and March 2007 (mean 21.4°C) and between December 2007 and February 2008 (mean 20.7°C). Monthly precipitation was low between June and August (mean 48.8 mm per month) and greatest in January 2007 and between November 2007 and February 2008 (mean 458.2 mm per month) (Fig. 2C).

Laboratory observations

Ten *P. emarcida* and three *P. forcipata* females were selected from host plants in the field during September and November 2007 and reared with males of the same species in the Laboratory of Insect Ecology at the Federal University of Rio de Janeiro to obtain an F1 generation. Three to four females were placed in each plastic container and temperatures were maintained between 25 and 27°C. Paper towels were

placed on the bottom of the containers to absorb moisture and leaves from *Solanum enantiophyllanthum* Bitter (see host records in Flinte et al. 2008), were changed every 2 days. Fresh leaves were removed as needed from stems of the host plant brought from the field and maintained in plastic bags kept under refrigeration. Eggs laid by these field-collected females (P1, parental generation) were reared to compare number of eggs deposited per female, larval defensive mechanisms and the duration and mortality within each developmental stage. The number of each "species" present in the adult F1 offspring was tallied for each mother. Length of the adult sclerotization period was taken to be the number of days elapsing between adult emergence and full development of golden colouration on the elytra and pronotum.

Finally, the abdomens from 227 F1 individuals were removed and dissected to determine offspring sex ratios. The aedeagi from two males and spermathecae from two females of each species were immersed in very dilute sodium hydroxide for 24 hours to break down the membranes. Preparations were later photographed with a 1.3-megapixel camera and Carl Zeiss lens attached to a SV12 Zeiss stereoscope. Measurements were made with a graduated ocular scale.

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DNA preparation, cytochrome oxidase I sequencing and analysis

As part of a larger study of the genus *Plagiometriona* in South America, the DNA from the gonadal tissues of adult tortoise beetles previously stored in 95% ethanol was extracted. Tissue was ground in extraction buffer (180 µl ATL Qiagen tissue lysis buffer (Qiangen Inc., Valencia, CA 91355, USA) and 20 µl proteinase K) with a sterile pestle, vortexed for 10 s, and heated overnight at 55°C. Following incubation 200 µl AL lysis buffer (Qiagen) was added and the sample was heated at 70°C for 10 min, then 200 µl 96–100% ethanol was added to each sample. The mixture was pipetted into the Dneasy mini spin column and centrifuged at 8000 r.p.m. (~6000 × g) for 1 min, then the flow-through and collection tube were discarded. The Dneasy mini spin column was placed in a new 2-ml collection tube and 500 µl AW1 buffer wash 1 (Qiagen) was added and the sample was centrifuged for 1 min at 8000 r.p.m. (~6000 × g), then the flow-through and collection tube were discarded. Again, a new collection tube was used, 500 µl AW2 buffer wash 2 (Qiagen) was added and the sample was centrifuged for 3 min at 14,000 r.p.m. (20,000 × g); the collection tube was then discarded. The mini column was placed in a 1.5-ml tube and 200 µl AE elution buffer (Qiagen) was added, then the sample was centrifuged for 1 min at 8000 r.p.m. (~6000 × g). Extractions were held at –20°C while in use, or at –80°C for longer storage.

Two fragments of the mitochondrial cytochrome oxidase gene (*CO1*) were extracted, amplified by polymerase chain reaction (PCR) and sequenced from the five described *Plagiometriona* species constituting the ingroup, *P. emarcida*, *P. forcipata*, *P. dodonea* (Boheman, 1855), *P. sahlbergi* (Boheman, 1855) and *P. dorsosignata* (Boheman, 1855) and from the two outgroup species, *Charidotis furunculus* (Boheman, 1855) and *Helocassis flavorugosa* (Boheman, 1855), all tribe Cassidini (GenBank accession nos: GQ268937–GQ268947). The *CO1* was amplified by PCR using two primer pairs (Simon et al. 1994) in separate reactions, C1-J-1718 and C1-N-2191 plus C1-J-2183 and TL2-N-3014 each in a volume of 20 µl: 1 µl DNA sample, 2 µl 10 × buffer (Applied Biosystems Inc., Foster City, CA, USA), 2 µl $MgCl_2$ (25 µM), 1 µl nucleotide mix (8 mM each), 0.8 µl of dimethylsulphoxide 5%, 1 µl each primer (20 mM), 0.2 U *Taq* DNA polymerase (Amplitaq, ABI) plus distilled, deionized

water. The PCR cycling conditions were: 94°C for 2 min, 10 cycles of 94°C for 30 s, 46°C for 30 min, 72°C for 45 min, then 24 cycles of 94°C for 30 s, 48°C for 30 min, 72°C for 45 min, and finally 72°C for 10 min, 10°C for 2 min.

When the two sequences were trimmed and concatenated a single 1179-base-pair fragment was obtained, which when translated to amino acids was free of stop codons for each sample. Evolutionary relationships among the samples were inferred by Neighbour-Joining analysis in MEGA4 of 1179 base pairs of *CO1* sequence. Well-supported nodes were indicated by bootstrapping percentages of 95% or greater (5000 replicates). Branch lengths were computed using the Kimura two-parameter method.

Statistical analysis

The normality of all data were examined using the Kolmogorov–Smirnoff test in GRAPHPAD INSTAT® Version 3.0. Normal data were then submitted to the Student's *t*-test for independent samples, whereas non-normal data were submitted to the Mann–Whitney *U*-test. Analyses were performed using STATISTICA® 6.0 (Statsoft, Inc., Tulsa, OK). Chi-square test contingency tables were used to compare the mortality of each species during developmental stages, and to determine whether the species occurred in different proportions in the field and laboratory. The chi-square tests were also used to detect whether sex ratios differed significantly between species and whether each species differed significantly from an expected 1 : 1.

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Results

***Plagiometriona forcipata* (Boheman, 1855)**

Coptocycla forcipata Boheman, 1855: 198 (type locality: 'Brasilia').
Coptocycla emarcida Boheman, 1855: 342 (type locality: 'Brasilia'), **syn. nov.**

Life cycle and seasonal variation in adult field populations

Observations in the field recorded a total of 26 matings, 16 occurring among *P. emarcida* individuals, two among *P. forcipata* individuals and eight among individuals of both species (Fig. 1A). Because individuals could be sexed with confidence during matings, we were able to observe that both species occurred within each sex on the same host plant species and at the same time of year.

Eggs were deposited either singly or in small groups (maximum of four), mostly on the undersides of leaves and were covered by a thin transparent membrane typical of most *Plagiometriona* species (Fig. 1B). Unidentified species of eulophid wasps were occasionally observed standing on the elytra of females, and on one occasion, on the elytra of an ovipositing female (Fig. 1C). Larvae constructed a fecal–exuvial shield, which they carried above their dorsum as in other genera of Cassidinae (Fig. 1D) and which was retained during pupation (Fig. 1E). Teneral *P. emarcida* adults were greenish (Fig. 1F), whereas *P. forcipata* individuals emerged with a visible annulus, which darkened over time, first on the pronotum and then on the elytra. Adults of both

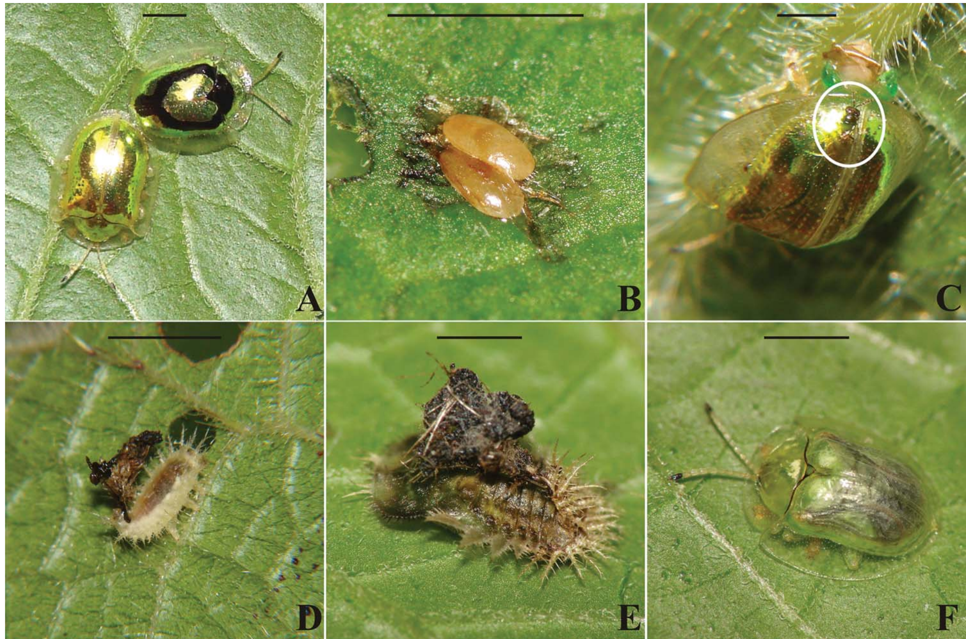

Figure 1. Different stages of development of the studied species. Mating of *Plagiometriona emarcida* and *Plagiometriona forcipata* in the field (A), eggs (B), a *P. emarcida* female ovipositing with a phoretic eulophid wasp circled (B), larva (D), pupa (E), and a *P. emarcida* teneral adult. Scale 2 mm.

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species in the field were found mainly on the underside of host plant leaves (82.2% and 82.9% for *P. emarcida* and *P. forcipata* individuals, respectively; $n = 213$ and $n = 41$).

Finally, surveys in the study areas revealed that adult abundance of the two species varied in a similar manner throughout the year and at both altitudinal sites. Adult individuals of *P. emarcida* were always more common than *P. forcipata* individuals (240 versus 44, respectively). Adults of both species were more numerous from September to December, showed a decrease in abundance at the beginning of the year and were rare from April to August in both altitudinal sites (Fig. 2A,B). This pattern follows that described for other Chrysomelidae species in the area and at the same altitude (Flinte et al. 2009). Larvae became abundant on food plants 1–2 months later than adults, but otherwise were distributed across the year similar to adults, being rare from April to September, numerous from October to February, and peaking in November (Fig. 2A,B). Temperatures were lower from May to August and higher from December to March, while rainfall was scarce from June to August and elevated from November to February (Fig. 2C). Temperature and rainfall therefore seem to correlate closely with the seasonal distribution of these species. The onset of harsher climatic conditions was correlated with decreasing abundance in both species, whereas the onset of milder conditions in spring and summer correlated with increased numbers, first of adults then of immatures.

Although observations of adults of both species were more frequent at the high-elevation site (142 *P. emarcida* and 26 *P. forcipata* versus 98 and 18 at mid-elevation,

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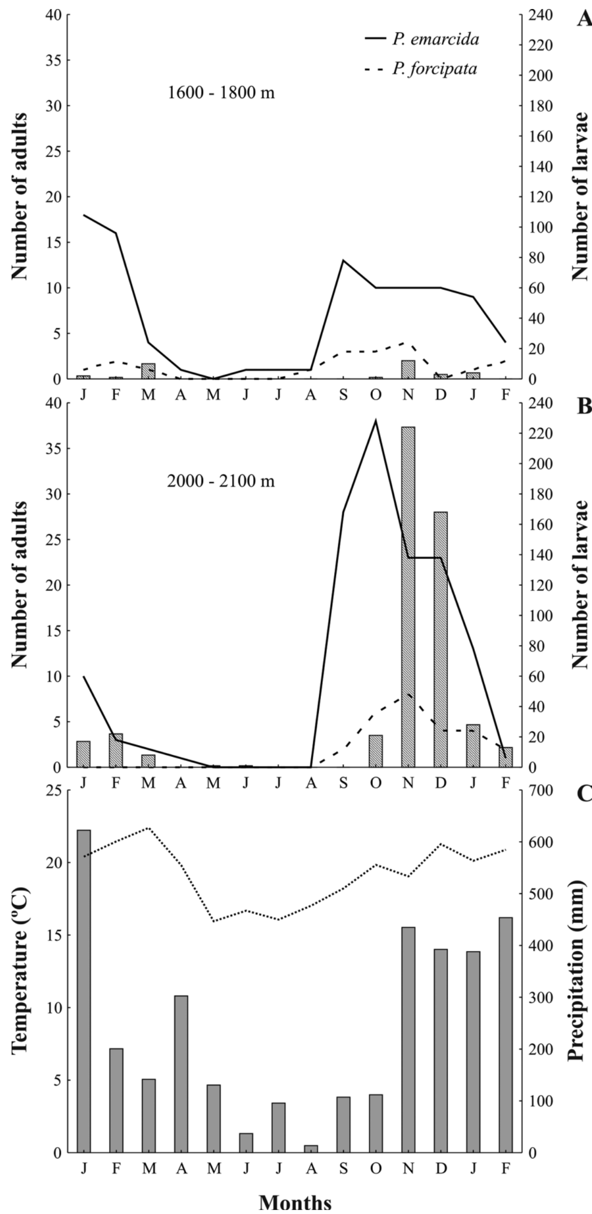

Figure 2. Abundance of *Plagiometriona emarcida* and *Plagiometriona forcipata* adults (lines) and larvae (bars) at the mid-elevation (A) and high-elevation (B) sites; and mean daily temperature (line) and monthly precipitation (bars) (C) (National Institute of Meteorology station located at 980 m) from January 2007 to February 2008 at the study area.

respectively), the overall representation of the species was identical at the two sites, 84.5% *P. emarcida* and 15.5% *P. forcipata* individuals. Individuals of both species were more widely distributed across the year at the mid-altitude site (13 and 9 months for *P. emarcida* and *P. forcipata*, respectively, compared with 10 and 6 months at

high elevation) (Fig. 2A,B). Moreover, the greater abundance of larvae and adults at the high-altitude site occurred during the warmer months of the year. Also, although adults of both species were rare during the less favourable season, they disappeared entirely from censuses only at the high-altitude site (Fig. 2A,B), where temperatures were considerably lower than at the mid-elevation.

Development and mortality of offspring reared in the laboratory from the ovipositions of field-collected females

Plagiometriona emarcida females deposited 4.6 ± 0.9 eggs/day (n = 7) and *P. forcipata* females deposited 5.7 ± 1.7 eggs/day (n = 8), rates which were not significantly different ($t = -1.53$; 13 d.f.; $p = 0.14$). The lengths of the egg and larval stages were significantly greater in offspring of *P. forcipata* females, resulting in a significant 1.8-day difference in the length of immature development between the species (Table 1). Larvae hatched 10–11 days after oviposition and the overall life cycle (oviposition to adult emergence) in captivity lasted *ca.* 41.3 days for offspring of *P. emarcida* females and approximately 43.1 days for offspring of *P. forcipata* females. Sclerotization of teneral adults (Fig. 2F) of both species took 13.4 ± 2.8 days (n = 14).

Mortality in the laboratory during each developmental period was significantly higher for offspring of *P. forcipata* females; 14.5% versus 24.8% during the egg stage (chi-square = 4.9; 1 d.f.; $p < 0.027$), 14.5% versus 41.2% during the larval stage (chi-square = 18.4; 1 d.f.; $p < 0.000$) and 2.5% versus 38.6% during the pupal stage (chi-square = 58.6; 1 d.f.; $p < 0.0001$) for *P. emarcida* and *P. forcipata* individuals, respectively (Table 2). The total mortality rate from egg to adult was also significantly different, 29.2% versus 73.7% for *P. emarcida* and *P. forcipata* individuals, respectively (chi-square = 28.83; 1 d.f.; $p < 0.001$).

In neither species did the adult sex ratio of the F1 generation vary significantly from an expected 1 : 1 ratio (*P. emarcida* offspring: 77 males and 95 females, chi-square = 1.88, 1 d.f., $p < 0.170$; *P. forcipata* offspring: 32 males and 23 females, chi-square = 1.47, 1 d.f., $p < 0.225$). Nor did the sex ratio differ significantly between the two species (chi-square = 3, 1 d.f., $p < 0.0831$) and when combined, the overall numbers of males (109) and females (118) were remarkably similar (1 : 1.08).

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Table 1. Developmental periods (days) for the two different *Plagiometriona* species reared simultaneously in separate containers under identical physical conditions in the laboratory.

Morph	Egg – Larva	Larva – Pupa	Pupa – Adult	Egg – Adult
Plagiometriona emarcida	10.5 ± 1.2 (n = 332)	24.2 ± 1.9 (n = 290)	6.8 ± 1.2 (n = 283)	41.3 ± 2.1 (n = 283)
Plagiometriona forcipata	11.1 ± 1.7 (n = 137) (U = 17963.5; $z = -3.580$; $p < 0.0001$)*	25.6 ± 2.0 (n = 97) (U = 5315.5; $z = -8.325$; $p < 0.0001$)*	6.7 ± 0.8 (n = 70) (U = 11100.0; $z = 1.589$; $p = 0.112$)	43.1 ± 3.3 (n = 70) (U = 8745.5; $z = -5.2775$; $p < 0.0001$)*

Note: *Indicates significant differences.

Table 2. Percentage of mortality and number of individuals alive during each developmental stage for offspring derived from each species and reared in separate containers under identical physical conditions in the laboratory.

Morph	Egg	Larva	Pupa	Total mortality
Plagiometriona emarcida	14.5% (n = 332)	14.5% (n = 290)	2.5% (n = 283)	29.2% (n = 332)
Plagiometriona forcipata	24.8% (n = 137) *(p = 0.027)	41.2% (n = 97) *(p < 0.001)	38.6% (n = 70) *(p < 0.001)	73.7% (n = 137) *(p < 0.001)

Note: *Indicates significant differences.

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Frequencies of the species in adult field populations and in offspring reared in the laboratory

Plagiometriona emarcida individuals constituted 84.5% of the 284 individuals sighted on the field censuses. The 10 *P. emarcida* and three *P. forcipata* field-caught females moved to the laboratory produced a total of 353 offspring, 80.2% of which were *P. emarcida* individuals, proportions not significantly different from those observed in the field (chi-square = 2.01, 1 d.f., $p = 0.16$). The colour pattern of offspring from both "species" overwhelmingly resembled that of their mothers but a minority of individuals were of the alternative colour pattern (Table 3).

Phenotypic variation within P. forcipata

At least 11 variants in the expression of the dark annulus on the elytral disk and pronotum were found among *P. forcipata* offspring (Fig. 3). The two most frequent variants recorded in the F1 offspring were also the two most common variants casually observed during field observations.

Genitalia

There were no obvious between-species differences in the shape or size of male or female genitalia (Fig. 4). The spermatheca was well sclerotized and falcate and the

Table 3. The proportions and numbers of each species among offspring from field-collected females moved to the laboratory and held with males.

Parent	Offspring (F1)	
	Plagiometriona emarcida	*Plagiometriona forcipata*
Plagiometriona emarcida (n = 10)	99.6% (n = 259)	0.4% (n = 1)
Plagiometriona forcipata (n = 3)	25.8% (n = 24)	74.2% (n = 69)
All	80.2% (n = 283)	19.8% (n = 70)

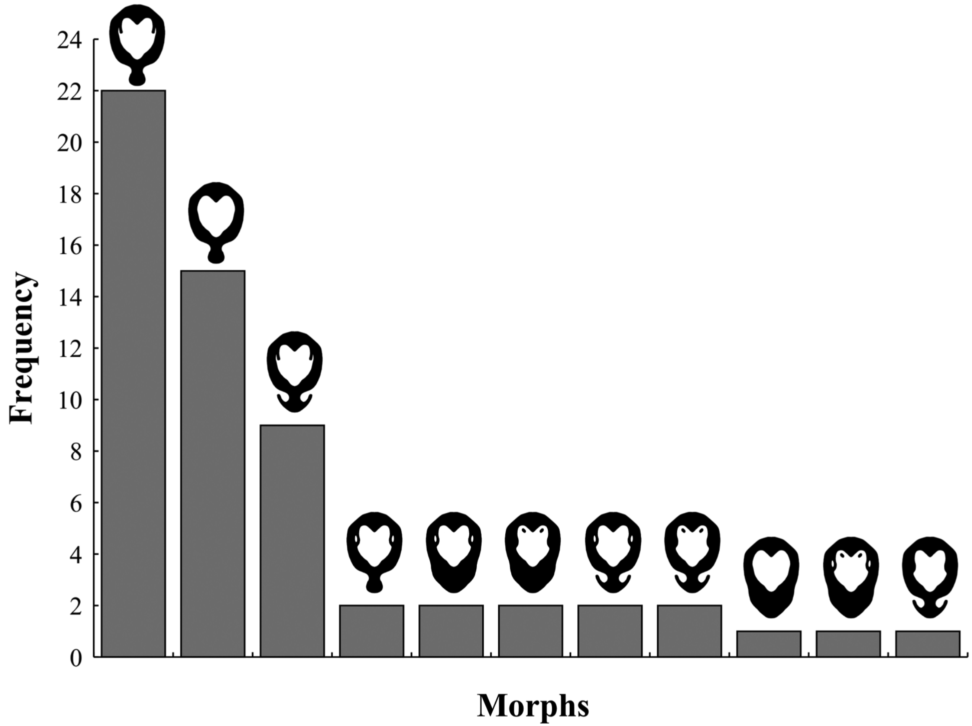

Figure 3. Frequency of the 11 variations recorded in the ring shape of the F1 generation of *Plagiometriona forcipata*.

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spermathecal duct was long (approximately 10.5 mm) and loosely coiled along almost its entire length in each species. The uncoiled male ejaculatory duct was approximately 7 mm in length, the seminal vesicle was 2.6 mm in length and the aedeagus was greenish in colour in fresh specimens and 2.0 mm in length (straight line distance, base to tip) and 0.3 mm at maximum width.

Sequencing

We used 1179 base pairs of sequence data obtained from the mitochondrial cytochrome oxidase gene (*CO1*) to examine the divergence between *P. emarcida* and *P. forcipata* relative to a small number of congeners and two outgroup taxa within the tribe Cassidini. Nucleotide sequences were identical for both *P. forcipata* samples except for an ambiguous read occurring at positions 39–42 in *P. forcipata* no. 2. Accordingly, a single amino acid gap was generated at position 14, the only difference existing in the two *P. forcipata* protein sequences. Nucleotide sequences for both *P. emarcida* individuals were identical, and differed from the *P. forcipata* sequences only at position 513, where an A was present in both *P. emarcida* and a C was present in both *P. forcipata* sequences. This third position mutation had no effect on the amino acid (threonine) produced by this codon in either morph. A great deal more collecting and sequencing would be required to determine whether this mutation is a

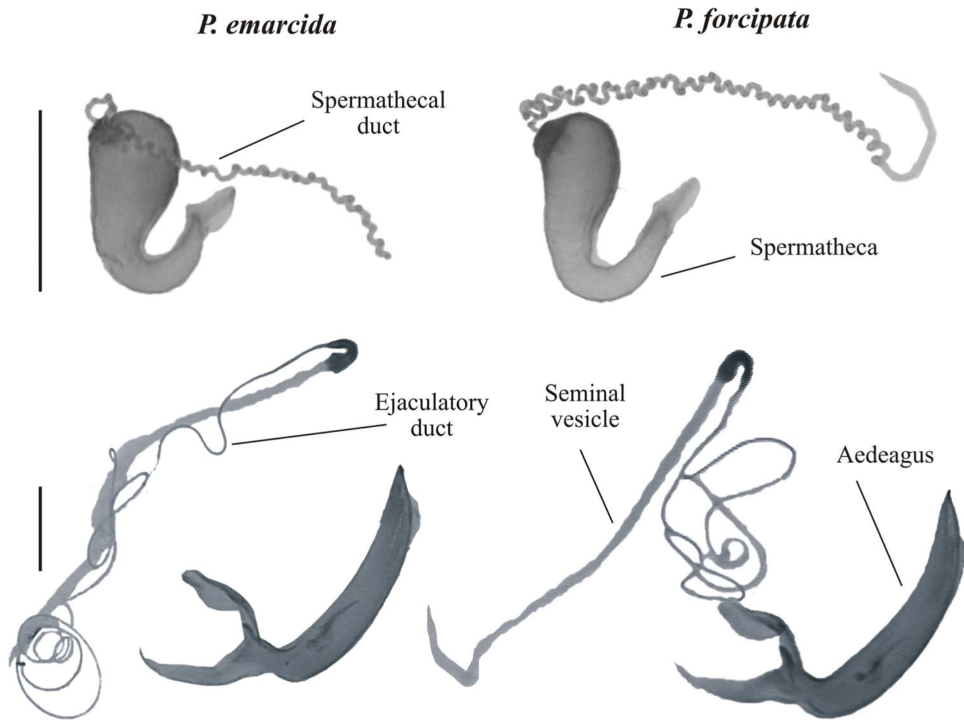

Figure 4. Photograph of spermatheca and spermathecal duct (above) and aedeagal system (below) of both *Plagiometriona emarcida* (left) and *Plagiometriona forcipata* (right). Scale 5 mm.

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fixed genetic difference occurring between the two morphs. The neighbour-joining tree (Fig. 5) shows no significance divergence between *P. emarcida* and *P. forcipata*, clear indication that these taxa belong to the same biological species. In contrast, *Plagiometriona sahlbergi*, the next closest taxon within this small set of species, varies by 5–6%.

Taxonomic comments

Originally, both species were placed in the fourth group of Spaeth's *Plagiometriona* system, characterized by humeral angles normally protruding anterad, epipleural margin not reaching to apical margin of elytra, elytra regularly convex, second and third antennomeres subequal in length and basal antennomeres uniformly yellow, pronotum more or less quadratic with more or less distinct anterior corners, and terminal antennomere black. Spaeth (1937) included in the group five species, three of them from Brazil, *P. forcipata* and *P. emarcida* and *P. gyrata* (Boheman, 1855). *Plagiometriona gyrata* differs in having a subcircular body and regularly rounded lateral sides of the elytra, while *P. forcipata* and *P. emarcida* have oval elytra with lateral sides distinctly emarginate in the anterior third. For separation of *P. forcipata* and *P. emarcida* there is no criterion other than the colour of elytra.

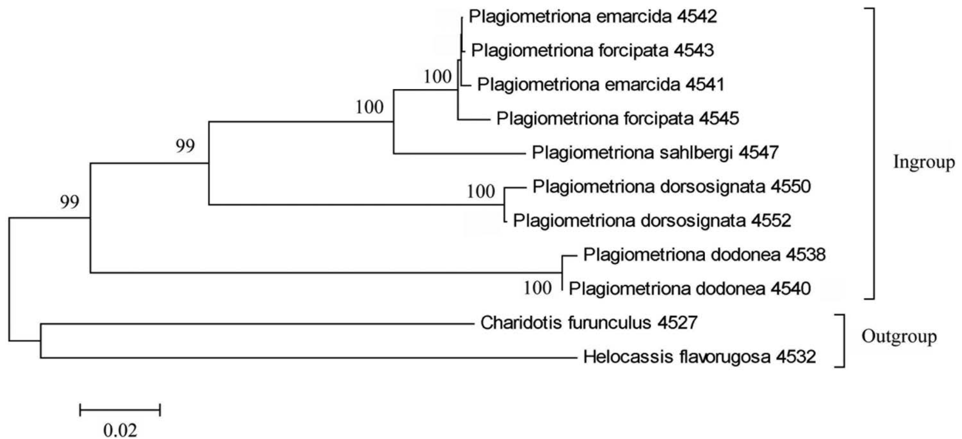

Figure 5. Evolutionary relationships within a small group of Brazilian Cassidinae (Ingroup, five species in the genus *Plagiometriona*; Outgroup, one *Charidotis* species and one species of *Helocassis*) inferred by neighbour-joining analysis in MEGA4 of 1179 base pairs of cytochrome oxidase I sequence.

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Type material examined

Coptocycla emarcida. Lectotype [designated by Borowiec (1999)], pinned: "Brasil [white, printed and soft label] // Bhn. [white, printed and soft label] // Type [white, printed and soft label] // LECTOTYPE / des. L. Borowiec [red, printed and cardboard label]"; paralectotype, pinned: "Brasil [white, printed and soft label] // M. Gall [white, printed and soft label] // PARALECTOTYPE / des. L. Borowiec [red, printed and cardboard label]" (both specimens preserved at Naturhistoriska Rijksmuseet, Stockholm, Sweden).

Coptocycla forcipata. Lectotype [designated by Borowiec (1999)], pinned: "Brasil [white, printed and soft label] // Germ. [white, printed and soft label] // Type [white, printed and soft label] // LECTOTYPE / des. L. Borowiec [red, printed and cardboard label]"; paralectotype, pinned: "Brasil [white, printed and soft label] // Bhn. [white, printed and soft label] // PARALECTOTYPE / des. L. Borowiec [red, printed and cardboard label]"; paralectotype, pinned: "Brasil [white, printed and soft label] // Bhn. [white, printed and soft label]" (all specimens preserved at Naturhistoriska Rijksmuseet, Stockholm, Sweden).

Discussion

Many biological and ecological traits of these formerly two *Plagiometriona* species resemble those found in related groups of Cassidinae, such as the oviposition pattern, egg covering and larval fecal–exuvial shield (Jolivet and Hawkeswood 1995; Jolivet and Verma 2002), although the overall life cycle (oviposition to adult emergence) was 5–7 days longer than for the similarly-sized *P. flavescens* (Boheman, 1855) (Nogueira-de-Sá and Macedo, 1999).

Shorter development times and lower mortality rates for the *P. emarcida* phenotype could at least partially account for its prevalence in the field and in laboratory rearings (Table 3). Cotter et al. (2008) recently addressed how melanism affects immune function and life-history trade-offs in Lepidoptera. Slower development and higher mortality in *P. forcipata* immature stages could represent a developmental "cost" associated with the production of the melanic circle in the adult stage, although it is unclear why these costs would accrue or be so substantial. The proportion of melanic morphs in some insect populations tends to increase with altitude, presumably as a way to maximize heat absorption (see references in Hodkinson 2005; Trullas et al. 2007). The finding that the melanic "ringed" morph was no more frequent at high elevation than at mid-elevation suggests that the ring does not confer a large thermal advantage on that morph in cooler habitats. We recommend continued study of development and survival in both field and laboratory of larvae from *P. forcipata* parents of known maternal and paternal phenotypes.

Both *P. forcipata* phenotypes were active across the warmer part of the year and were rare or absent during the coldest months (Fig. 2). As temperature has an important effect on insect performance (Andrewartha 1952; Rockstein 1964; Howe 1967; Vivan and Panizzi 2005), we suspect that it may be the principal factor affecting activity and presence of this species on our censuses. We were surprised to find that adults and larvae of both morphs were more numerous at the high-altitude site, which is about 2.4°C cooler than the mid-elevation site (see Flinte et al. 2009). This altitudinal pattern may have various causes, including interactions with other species through competition, predation or parasitism, physiological adaptations, water availability and phenology and quality of host plants (see Cordell et al. 1998; Lomolino 2001; Hodkinson 2005). Now that the basic pattern has been described, perhaps the importance of these different factors can be investigated using controlled rearings and caged transplant studies.

While developmental and survival differences observed in the laboratory could indicate more than one species, (1) frequent observations of matings between individuals of different phenotypes in the field, (2) the appearance of both phenotypes within the adult brood of single females, (3) no obvious between-species differences in the shape or size of male or female genitalia, (4) no other morphological difference besides elytral colour pattern and (5) the nearly identical *CO1* mitochondrial sequences lead us to conclude that *P. forcipata* and *P. emarcida* are two phenotypes of a single polymorphic species. We therefore propose the synonymy of *P. emarcida* and *P. forcipata*. Both species were described in the same volume of Boheman's monograph (Boheman 1855), *P. emarcida* on p. 342, *P. forcipata* on p. 198. According to Clause 23.1, Priority law (ICZN 1999), the valid name of a particular taxon is the oldest available name applicable to it. Therefore *P. forcipata* is retained as the valid name and *P. emarcida* becomes its junior synonym.

We would encourage the continued study of the genetic basis of the two principal phenotypes, the number and dominance of alleles controlling elytral pattern, including the genetic modifiers that may determine the variations observed in the shape of the dorsal ring of *P. forcipata*.

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References

Andrewartha, HG. 1952. Diapause in relation to the ecology of insects. Biol Revs. 27:50–107.

Boheman, CH. 1855. Monographia Cassididarum. Tomus Tertius. Stockholm: Holmiae. 543 p.

Borowiec, L. 1999. A world catalogue of the Cassidinae (Coleoptera: Chrysomelidae). Wroclaw: Biologica Silesiae. 476 p.

Brakefield PM. 1985. Polymorphic Müllerian mimicry and interactions with thermal melanism in ladybirds and a soldier beetle: a hypothesis. Biol J Linn Soc. 26:243–267.

Cordell S, Goldstein G, Mueller-Dombois D, Webb D, Vitousek PM. 1998. Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. Oecologia 113:188–196.

Cotter SC, Myatt JP, Benskin CMH, Wilson K. 2008. Selection for cuticular melanism reveals immune function and life-history trade-offs in *Spodoptera littoralis*. J Evol Biol. 21(6):1744–1754.

Endler JA. 1978. A predator view of animals colour patterns. Evol Biol. 11:19–64.

Endler JA. 1993a. Some general comments on the evolution and design of animal communication systems. Phil Trans R Soc Lond. B 340:215–225.

Endler JA. 1993b. The colour of light in forests and its implications. Ecol Monogr. 63:1–27.

Flinte V, Macedo MV, Monteiro RF. 2008. Tortoise beetles (Chrysomelidae: Cassidinae) of a tropical rain forest in Rio de Janeiro, Brazil. In: Jolivet P, Santiago-Blay J, Schmitt M, editors. Research on Chrysomelidae Vol. 1. Leiden: Brill. p. 194–209.

Flinte V, Macedo MV, Monteiro RF. 2009. Chrysomelids and their host plants along an altitudinal gradient in an Atlantic Rain Forest in the State of Rio de Janeiro, Brazil. In: Jolivet P, Santiago-Blay J, Schmitt M, editors. Research on Chrysomelidae Vol. 2. Leiden: Brill.

Fujiyama S, Arimoto K. 1988. Genetics of the two colour morphs of *Chrysolina aurichalcea* (Mannerheim) (Coleoptera: Chrysomelidae) and their gene frequencies in two mountainous areas of central Honshu, Japan. In: Jolivet P, Petitpierre E, Hsiao TH, editors. Biology of Chrysomelidae. Dordrecht: Kluwer Academic Publishers. p. 205–215.

Gonçalves RO, Macedo MV. 2003. Population ecology of the polymorphic species *Chelymorpha cribaria* (Coleoptera: Chrysomelidae) in Rio de Janeiro, Brazil. In: Furth DG, editor. Special topics in leaf beetle biology. Sofia-Moscow: Pensoft Publishers. p. 285–294.

Hodkinson ID. 2005. Terrestrial insects along elevations gradients. Biol Revs. 80:489–513.

Howe RW. 1967. Temperature effects on embryonic development in insects. Annu Rev Entomol. 12:15–42.

Jolivet P, Hawkeswood TJ. 1995. Host-plants of Chrysomelidae beetles of the world: an essay about the relationships between leaf beetles and their food-plants. Leiden: Backhuys Publishers. 281 p.

Jolivet PH, Verma KV. 2002. Biology of leaf beetles. Andover: Intercept Publishers. 337 p.

ICZN. 1999. International code of zoological nomenclature. 4th edition. London: International Trust for Zoological Nomenclature. 306 p.

Lomolino MV. 2001. Elevation gradients of species-density: historical and prospective views. Global Ecol Biog. 10:3–13.

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Lu W, Logan P. 1994. Geographic variation in larval feeding acceptance and permorphance of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Ann Entomol Soc Am. 87(4):460–469.

Monteiro RF. 1991.Cryptic polychromatism in *Rekoa marius* Lucas and *R. palegon* Cramer larvae (Lycaenidae: Theclinae). J Res Lepid. 29(2):77–84.

Nahrung HF, Allen GR. 2005. Maintenance of colour polymorphism in the leaf beetle *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae: Paropsini). J Nat Hist. 39(1):79–90.

Nogueira-de-Sá F, Macedo MV. 1999. Behavior and population fluctuation of *Plagiometriona flavescens* (Boheman) (Chrysomelidae: Cassidinae). In: Cox ML, editor. Advances in Chrysomelidae biology 1. Leiden: Backhuys Publishers. p. 299–305.

Peschken DP. 1972. *Chrysolina quadrigemina* (Coleoptera: Chrysolemidae) introduced from California to British Columbia against the weed *Hypericum perforatum*: comparison of behavior, physiology and colour in association with post-colonization adaptation. Can Entomol. 104:1689–1698.

Ricklefs RE, O'Rourke K. 1975. Aspect diversity in moths: a temperate–tropical comparison. Evolution 29:313–324.

Rockstein M. 1964. The physiology of Insecta, I. New York and London: Academic Press. 640 p.

Simon C, Frati F, Beckenback A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. Ann Entomol Soc Am. 87:651–701.

Spaeth F. 1937. Neue Cassidinen des Rijksmuseums in Leiden und meiner Sammlung. Temminckia 2:135–158.

Trullas SC, van Wyk JH, Spotila JR. 2007. Thermal melanism in ectotherms. J Therm Biol. 32:235–245.

Vasconcellos-Neto J. 1988. Genetics of *Chelymorpha cribraria*, Cassidinae: colour patterns and their ecological meanings. In: Jolivet P, Petitpierre E, Hsiao TH, editors. Biology of Chrysomelidae. Dordrecht: Kluwer Academic Publishers. p. 217–232.

Verma KK and Kalaichelvan T. 2004. Polymorphism and microtaxonomy in Chrysomelidae. In: Jolivet P, Santiago-Blay J, Schmitt M, editors. New Developments in the biology of Chrysomelidae. The Hague: SPB Academic Publishing. p. 213–224.

Vivan LM, Panizzi AR. 2005. Nymphal and adult permorphance of genetically determined types of *Nezara viridula* (L.) (Heteroptera: Pentatomidae), under different temperature and photoperiodic conditions. Neotrop Entomol. 34(6):911–915.

Zvereva EL, Kozlov MV, Kruglova OY. 2002. Colour polymorphism in relation to population dynamics of the leaf beetle, *Chrysomela lapponica*. Evol Ecol. 16:523–539.

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