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Revalidation and redescription of three distinct species synonymized as *Plagiometriona sahlbergi* (Coleoptera: Chrysomelidae: Cassidinae)

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Abstract. *Plagiometriona sahlbergi* (Boheman, 1855), *P. multisignata* (Boheman, 1855) and *P. scenica* (Boheman, 1855) were described over a century and half ago as separate species based on dry museum specimens. The latter two were later synonymized with *P. sahlbergi*. While observations in nature find both *P. sahlbergi* and *P. multisignata* co-occurring on the same host plant, subtle morphological differences suggested they may be distinct species. We tested the validity of the original species separation using additional morphological characters and available molecular sequence data. The new evidence supports Boheman's original separation of species. Accordingly we remove *P. multisignata* stat. restit. and *P. scenica* stat. restit. from synonymy with *P. sahlbergi* and restore their species status. Mitochondrial gene sequence data suggest that *P. sahlbergi* and *P. multisignata* bear a closer relationship to other sympatric *Plagiometriona* species than they do to one another, raising the possibility that these two species have convergent morphologies due to their shared food plant. Biological and ecological notes are also given for these two sympatric species.

Key words. Coleoptera, Chrysomelidae, Cassidinae, *Plagiometriona*, synonymy, phylogeny, cytochrome oxidase I, morphology, Atlantic Forest, Brazil, Neotropical Region

Introduction

Plagiometriona Spaeth, 1899 is a large Neotropical genus comprising 84 described species exclusively associated with various species of Solanaceae (Riley 1986, Borowiec 1999, Borowiec & Świętojańska 2016, Sekerka 2016). Species occur largely at mid to high elevations (500–4000 m) and in wet cloud forests in two faunal zones. The largest of these zones includes the Andes from southern Mexico to central Bolivia and contains at least 45 species. The second faunal zone is limited to the Brazilian Atlantic coast where 33 species occur in the Serra do Mar. The vast Amazon region sitting between these faunal zones, by contrast, is presently known to contain only six species (Sekerka, unpubl. data).

Spaeth (1937) described 20 new species, divided the genus *Plagiometriona* into 17 species groups and devised a key to them. He also pointed out the importance of male aedeagi as diagnostic characters to separate individual taxa. The genus *Plagiometriona* contains numerous species with only slight variation in aedeagi but exhibiting high variability in dorsal coloration, and remains without complete revision. Numerous taxa remain unstudied by taxonomic specialists, and as a result identification of species is often problematic, especially so for field biologists (Sekerka & Windsor 2012, Borowiec & Świętojańska 2013).

Located in the Brazilian Atlantic coast, the State of Rio de Janeiro holds records of 16 species of *Plagiometriona* (Flinte et al. 2008, 2010; Simões & Monné 2011), including seven that are a challenge to separate in the field based on external morphological characters alone (Flinte et al. 2011). Among the most difficult to identify are two distinct phenotypes, identified and published (Flinte et al. 2008, 2011) as *Plagiometriona sahlbergi* (Boheman, 1855) and *Plagiometriona* sp. feeding on the same plant species, *Cestrum bracteatum* Link & Otto (Solanaceae). Both have spots similar in number and position on the dorsum, but differing in color hue and shape. Mating was never observed between individuals differing in phenotype in the field nor when placed in mixed groups in the same container in the laboratory. Further, adults reared from eggs of known phenotype mothers closely matched the maternal phenotype.

In an effort to confirm species identifications, we (LS) examined type specimens and found evidence that *P. sahlbergi* represents three distinct species, including the two studied above. Spaeth (1937) synonymized *P. sahlbergi* (Boheman, 1855), *P. multisignata* (Boheman, 1855) and *P. scenica* (Boheman, 1855) because the dry specimens of all three had similar dorsal pattern and color, however, it is not known whether Spaeth examined type specimens of the three nominal taxa. Now, based on morphological characters, and supported by available molecular data, we propose the validity of these three different taxa. Additionally, biological and ecological aspects are given for two of these species studied in field and laboratory.

Material and methods

Recently killed voucher specimens and older dead specimens of two focal species (*P. sahlbergi* and *P. multisignata*) were observed and photographed under a Leica M205C ste-

reomicroscope with image acquisition system for external morphology description and measurements. Specimens of these two species were boiled in water for 5 minutes and dissected; the abdomen was then separated from each and boiled in 10% potassium hydroxide for a few seconds, the contents, including genitalia, removed and washed in distilled water and placed on a slide for observation and illustration. Observations on *P. scenica* were restricted to the lectotype deposited at NHRS (Naturhistoriska Riksmuseet) in Stockholm. Identifications and descriptions of all three species were based on comparison with type specimens, additional non-type specimens and original descriptions of similar species. Terminology follows Borowiec & Świętojańska (2013), with the description of the internal sac of male genitalia as in Sekerka & Windsor (2012).

The following abbreviations are used: BL – body length; BR – body ratio (length/width); BW – body width (the largest width of elytra); PL – pronotum length; PR – pronotum ratio (width/length); PW – pronotum width (the largest width of elytra). All data from collection labels are verbatim and organized in sequence from the top where each record is given in single quotations (‘’), each line on the label is separated by a single backslash (|) and different labels are separated by double backslashes (||).

Acronyms of the collections are as follows:

CLEI Entomological Collection of the Laboratory of Insect Ecology, Universidade Federal do Rio de Janeiro, Brazil (Ricardo Monteiro);
NHRS Naturhistoriska Riksmuseet, Stockholm, Sweden (Johannes Bergsten);
ZMHB Museum für Naturkunde, Berlin, Germany (Johannes Frisch).

DNA was extracted from the gonadal tissues of adult beetles of *P. sahlbergi* and *P. multisignata* previously stored at -20°C in 95% ethanol. Protocols of DNA preparation, cytochrome oxidase I sequencing and phylogeny construction followed that described in Flinte et al. (2010).

In a previous study, we censused with replacement seven (total) *Plagiometriona* species occurring on seven Solanaceae species over one year (2006–2007) at six sites along an elevational transect (1300 to 2050 m a.s.l.) in the Serra dos Órgãos National Park (22°32′S and 43°07′W), a mountainous terrain within the Atlantic rain forest in the State of Rio de Janeiro, Southeast Brazil (Flinte et al. 2011). Adult beetles were separated into morphospecies in the field based on elytral morphology. Data are presented as relative abundance calculated as the number of individuals at each altitude for all months (or each month for all altitudes, when describing the occurrence along the year) divided by the total abundance of the species encountered within the study. Plant density (obtained in Flinte et al. 2011) is also given along with species altitudinal distribution. Five females and some males of each species were brought from field to the laboratory and reared at room temperature (ca. 25°C) to establish developmental time. *Plagiometriona sahlbergi* was reared from August to October 2006, and *P. multisignata* from November 2006 to January 2007. We tested whether time of development (egg to adult) differed significantly between species using the Kolmogorov-Smirnov two-sample test as data for *P. multisignata* were non-normally distributed (Shapiro-Wilk test, 0.85, $p < 0.001$).

Taxonomy

Plagiometriona multisignata (Boheman, 1855) stat. restit.

(Fig. 1)

Coptocycla multisignata Boheman, 1855: 379 (original description).

Plagiometriona sahlbergi [misidentification]: Flinte et al. (2008): 205 (biology and host plant record); Flinte et al. (2009): 596 (faunistics); Flinte et al. (2010): 901 (phylogeny); Flinte et al. (2011): 18 (altitudinal and temporal distribution).

Type locality. 'Brasilia'.

Type material examined. Lectotype (designated by Borowiec 1999): ♂, pinned, '29582 [white and printed label] || Brasilia | Hecht. [green and handwritten label] || multisignata Dej [white label handwritten by Boheman] || LECTOTYPE | des. L. Borowiec [red and printed label]' (ZMHB).

Additional material examined. BRAZIL: Rio de Janeiro: Teresópolis, Parque Nacional da Serra dos Órgãos, trilha da Pedra do Sino, 1.ix.2005, 1 spec., 13.–14.x.2005, 1 spec., viii.2006, 3 spec., 25.viii.2006, 1 spec. (Adhesive panel, 1880 m alt.), V. Flinte leg. Additional 38 specimens reared in laboratory conditions, all deposited at CLEI.

Description. Measurements: BL: 5.95–6.28 mm, BW: 5.25–5.40 mm, PL: 1.73–1.90 mm, PW: 3.51–3.76 mm, BR: 1.16, PR: 2.00. Body slightly longer than wide.

Living and fresh specimens (Fig. 1A): Pronotum with translucent yellow explanate margin, greenish yellow stripe and large black semicircular discal spot with two yellow shiny subtriangular to circular spots near scutellum. Scutellum black. Elytra with translucent yellow explanate margins, greenish yellow stripe and wide black central spot with three yellow and four to five red shiny spots. Greenish yellow strip between explanate margins and black central spot of elytra, from base to apex, at base from 9th to last row of punctures and broader apically.

Table 1. Characters distinguishing the three revalidated *Plagiometriona* species.

	P. multisignata (Boheman, 1855)	***P. sahlbergi*** (Boheman, 1855)	***P. scenica*** (Boheman, 1855)
scutellar spots on elytra	present	absent	present
coloration of elytral spots	red and yellow	yellow	yellow
humeral spots	elongate, running along humeral callus	short, situated in front of humeral callus	elongate, running along humeral callus
size of spots	large, black color mostly line form	small, black color extensive	small, black color extensive
punctation of elytra	fine and sparse, spots are smooth with regular surface that appears impunctate	moderate, spots distinctly punctate	moderate, spots distinctly punctate
anterior margin of black pronotal pattern	convex	convex	truncate
aedeagus	truncated on apex, lateral margins slightly concave; in lateral profile, median lobe straight at basal ¾ and apical ¼ curved dorsally (Figs 1E–F)	rounded on apex, lateral margins straight; in lateral profile, median lobe regularly curved dorsally (Figs 2E–F)	unknown

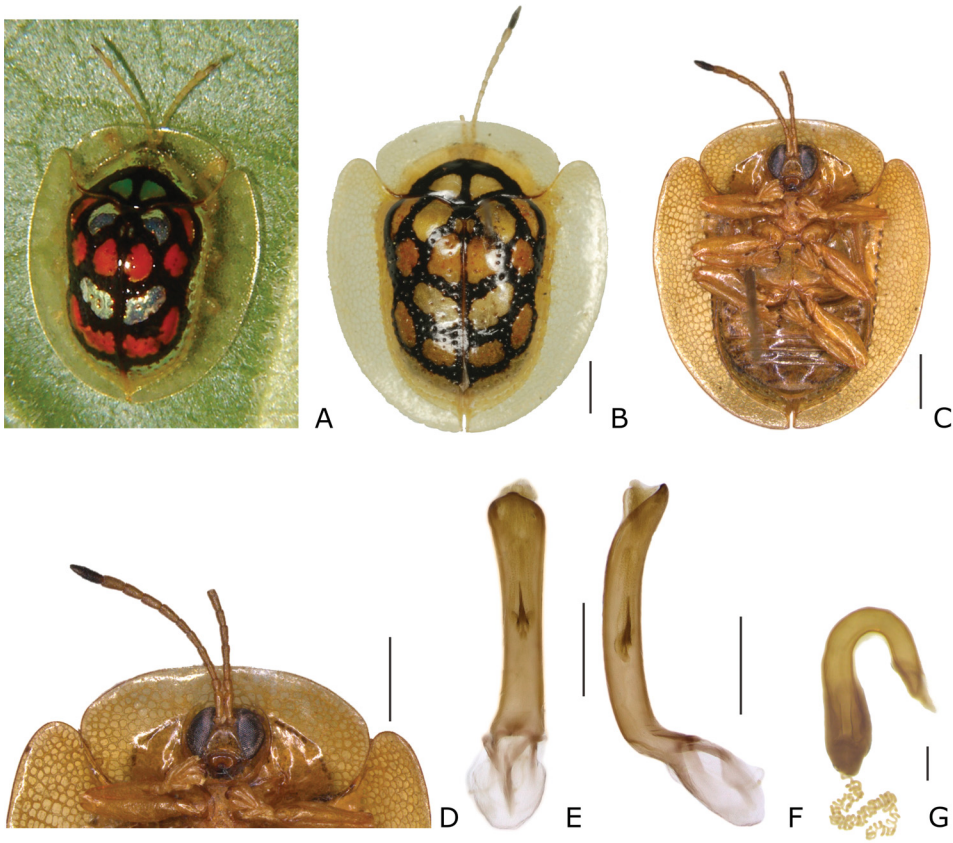

Fig. 1. *Plagiometriona multisignata* (Boheman, 1855). A – live specimen dorsal; B – dried specimen dorsal; C – dried specimen ventral; D – head, antennae, prosternum and fore legs; E – aedeagus dorsal; F – aedeagus lateral; G – spermatheca. Scales: B–D – 1 mm; E–F – 0.5 mm; G – 0.1 mm.

Wide black central spot, covering most of elytra from base and internal margins to 9th row of punctures, with three yellow and five red shiny spots: one wide yellow spot at basal margin from 2nd to 4th row of punctures close to anterolateral margins of scutellum, one small yellow spot with comma form between internal margin of elytra and 1st row of punctures, one red spot at basal margin from 4th row of punctures to humerus, two red spots at subbasal region with almost the same size from the middle of 1st interstitial to 2nd row of punctures and 3rd to 5th row of punctures, one yellow spot from 1st to 4th row of punctures at mid region and submedian region with two red spots from 5th to 6th row of punctures and from 1st to 5th row of punctures, sometimes last two spots connected. Underside with translucent yellow color and remaining body with greenish yellow color, except eyes, last antennomere and claws

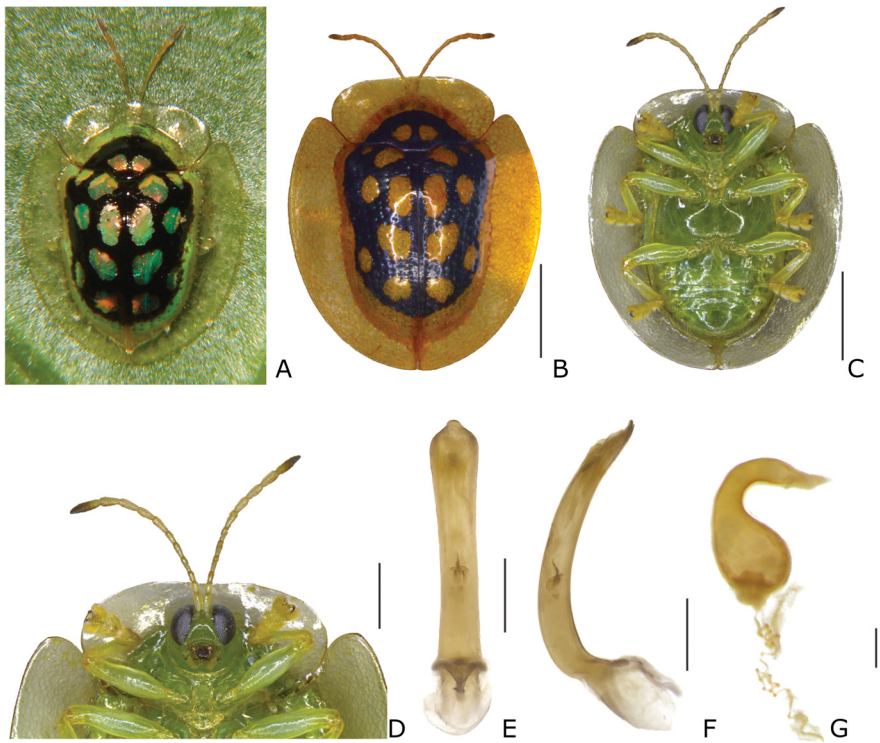

Fig. 2. *Plagiometriona sahlbergi* (Boheman, 1855): A – live specimen dorsal; B – dried specimen dorsal; C – live specimen ventral; D – head, antennae, prosternum and forelegs; E – aedeagus dorsal; F – aedeagus lateral; G – spermatheca. Scales: B–C – 2 mm; D – 1 mm; E – 0.5 mm; G – 0.1 mm.

Fig. 3. *Plagiometriona scenica* (Boheman, 1855), lectotype, dorsal aspect.

black. Dead and dried specimens change translucent, greenish, shiny yellow color to opaque yellow and shiny red becomes opaque light orange (Figs 1B–C).

Male genitalia. Aedeagus slim, truncated on apex and lateral margins slightly concave. Apex in lateral profile bent ventrally, median lobe straight at basal ¾ and apical ¼ curved dorsally (Figs 1E–F).

Female genitalia. Spermathecal capsule C-shaped, slightly expanded basally (Fig. 1G).

Distribution. Brazil (?Espírito Santo, Rio de Janeiro)

Plagiometriona sahlbergi (Boheman, 1855)

(Fig. 2)

Coptocycla sahlbergi Boheman, 1855: 375 (original description).

Plagiometriona sp.: Flinte et al. (2008): 205 (biology and host plant record); Flinte et al. (2011): 18 (altitudinal and temporal distribution).

Type locality. 'Brasilia'.

Type material examined. Holotype: ♂, pinned, 'Rio Jan [white and printed label] || F. Sahlb. [white and printed label] || Type. [white and printed label] || NHRS-JLKB | 000022594 [white and printed label]' (NHRS).

Additional material studied. BRAZIL: Rio de Janeiro: Teresópolis, Parque Nacional da Serra dos Órgãos, trilha da Pedra do Sino, ~1800 m a.s.l., 17.xii.2005, 4 spec., V. Flinte leg. Additional 13 specimens reared in laboratory conditions, all deposited at CLEI.

Description. Measurements: BL: 5.29–6.54 mm, BW: 4.92–5.85 mm, PL: 1.46–1.72 mm, PW: 2.94–3.66 mm, BR: 1.13, PR: 2.08. Body slightly longer than wide.

Living and fresh specimens (Figs 2A,C): Pronotum with translucent yellow explanate margins, greenish yellow strip and large black semicircular basal spot with two yellow shiny circular spots near scutellum. Scutellum black. Elytra with translucent yellow explanate margins, greenish yellow strip and wide black central spot with seven yellow shiny spots. Greenish yellow strip between explanate margins and black central spot of elytra, from base to apex, at base from 8th to last row of punctures and broader apically. Wide black central spot, covering most part of elytra from basal and internal margins to 8th row of punctures, with seven yellow shiny spots: one wide spot at basal margin from 1st to 3rd row of punctures close to anterolateral margins of scutellum, one small subtriangular spot near basal margin from 3rd to 4th row of punctures, two at basal region with almost the same size from 1st to 2nd row of punctures and 3rd to 5th row of punctures, one at subbasal region from 1st to 4th row of punctures, one small spot at mid region from 6th to 7th row of punctures and one wide spot at submedian region from 1st to 4th row of punctures. Underside with visible translucent yellow elytra and remaining body with greenish yellow color, except black eyes and apical region of last antennomere, mouthparts, claws and joints between femurs, tibiae and first tarsomere dark yellow. Color changes from translucent, greenish and shiny yellow in living specimens to opaque yellow in dead and dry specimens (Fig. 2B).

Male genitalia. Aedeagus slim, rounded on apex and lateral margins straight. Apex in lateral profile slightly bent ventrally, median lobe regularly curved dorsally (Figs 2E–F).

Female genitalia. Spermathecal capsule C-shaped with base greatly expanded (Fig. 2G).

Distribution. So far this species is with certainty known only from Serra dos Órgãos in Rio de Janeiro, Brazil.

Plagiometriona scenica (Boheman, 1855) stat. restit.

(Fig. 3)

Coptocycla scenica Boheman, 1855: 377 (original description).

Type locality. 'Brasilia'.

Type material examined. Lectotype (designated by Borowiec 1999): ♀, pinned, 'Brasil [white and printed label] || M. Berl [white and printed label] || Type. [white and printed label] || LECTOTYPE | des. L. Borowiec [red and printed label] || NHRS-JLKB | 000022596 [white and printed label]' (ZMHB).

Remarks. Validity of this species requires further study as we examined only the lectotype which is female and hence we could not compare aedeagi, which are often diagnostic in *Plagiometriona*. The specimen has a postsctutellar spot as in *P. multisignata*, however, all elytral spots are pale yellow (humeral, middle four and posterior four red in the latter). The red color in elytra of some museum specimens has vanished, however, we have examined numerous historical specimens and the red color was always distinct in fully sclerotized individuals. The lectotype of *P. scenica* is a fully sclerotized specimen entirely lacking red color. Also, *P. multisignata* always has large elytra spots while these are small in *P. scenica* and therefore we assume it is a distinct species from both *P. multisignata* and *P. sahlbergi*. For further characters see Table 1.

Molecular analysis of *Plagiometriona sahlbergi* and *P. multisignata*

The mitochondrial gene tree (Fig. 4) contains eight *Plagiometriona* species, all of which are associated with Solanaceae in the altitudinal transect. This analysis supports the view that the two species found exclusively on *Cestrum bracteatum*, *P. multisignata* and *P. sahlbergi*, are distinct, with each having closer relationships to *Plagiometriona* species associated with other species of Solanaceae than to each other. A sister relationship between *Plagiometriona multisignata* and *P. forcipata* is strongly indicated (boot strap = 100), while the relationship between *P. sahlbergi* and the clade composed of *P. dorsosignata* (Boheman, 1855), *P. multisignata* and *P. forcipata* (Boheman, 1855) received only weak support (bootstrap = 45).

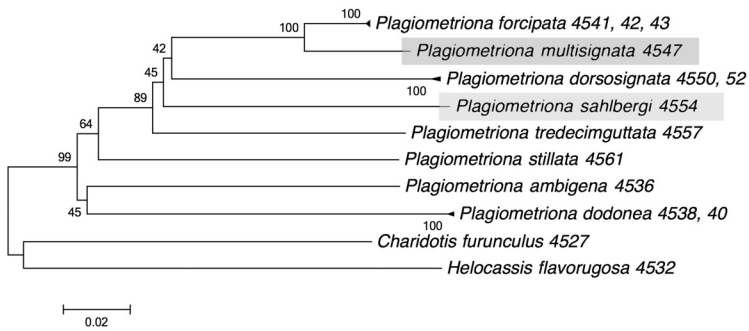

Fig. 4. Relationships inferred from a neighbor-joining analysis using 1179 base pairs of the mitochondrial Cytochrome Oxidase I gene for ten co-occurring Brazilian Cassidinae. Multiple numbers after the species name indicate clades consisting of more than one specimen.

Plagiometriona forcipata feeds on *Solanum lhotskyanum* Dunal. and *S. enantiophyllanthum* Bitter (Flinte et al. 2010), while *P. dorsosignata* feeds on *Aureliana fasciculata* (Vell.) Seudtn. (Flinte et al. 2011). Although *Solanum* and *Aureliana* seem to be closely related genera in Solanaceae, *Cestrum* is considerably distant from these two genera (Barkman, unpubl. data), indicating that host plant phylogeny does not explain genetic proximity between *P. forcipata* and *P. multisignata*.

Aspects of ecology and biology of *Plagiometriona sahlbergi* and *P. multisignata*

Although both *Plagiometriona* species feed exclusively on *Cestrum bracteatum* (Solanaceae) at Serra dos Órgãos National Park (Flinte et al. 2008), the total abundance of *P. multisignata* was more than five times that of *P. sahlbergi* (n = 137 and n = 27, respectively). Beetles were censused on host plants at each of six elevational sites, with both beetle species displaying greatest abundance at 1800 m. *Plagiometriona sahlbergi* was encountered only at 1800 m elevation, while *P. multisignata* occurred at four sites from 1600 to 2050 m, suggesting that it may have a less restricted distribution (Fig. 5A). Further, beetle abundance seemed to be related to plant density, which was higher on the upper two sites (Fig. 5A). Seasonally, approximately 70% of all individuals of *P. sahlbergi* were encountered in February, while *P. multisignata* individuals were more evenly spread over the year, although they too peaked in abundance in February (Fig. 5B). High abundances were also recorded in February for other *Plagiometriona* species studied in the same area (Flinte et al. 2011), likely reflecting the favorable high temperatures and rainfall typical of this time of the year.

Both *P. sahlbergi* and *P. multisignata* lay single, flattened, membranous eggs, which can be covered with feces (Flinte et al. 2008). Larvae carry an exuvial-fecal shield, retained as pupae, and are very similar in shape to other *Plagiometriona* (see Flinte et al. 2010). Egg to adult development under identical conditions in the laboratory was significantly ($p < 0.001$) more rapid in *P. multisignata* than in *P. sahlbergi*, due largely to faster larval development (Table 2). Another species of the same genus, *P. forcipata*, which occurs in the same area has an overall developmental time (oviposition to adult emergence; reared from September to December) in captivity of ca. 42 days (Flinte et al. 2010), similar to *P. sahlbergi*.

Table 2. Developmental time (days) for two *Plagiometriona* species reared under similar physical conditions in the laboratory.

Species	Egg – Larva	Larva – Pupa	Pupa – Adult	Egg – Adult
Plagiometriona sahlbergi	8.3 ± 1.5 (n = 48)	26.5 ± 2.4 (n = 27)	7.6 ± 0.8 (n = 23)	40.9 ± 2.1 (n = 18)
Plagiometriona multisignata	7.4 ± 1.8 (n = 167)	19.0 ± 2.9 (n = 48)	6.7 ± 1.8 (n = 40)	32.7 ± 2.9 (n = 39)

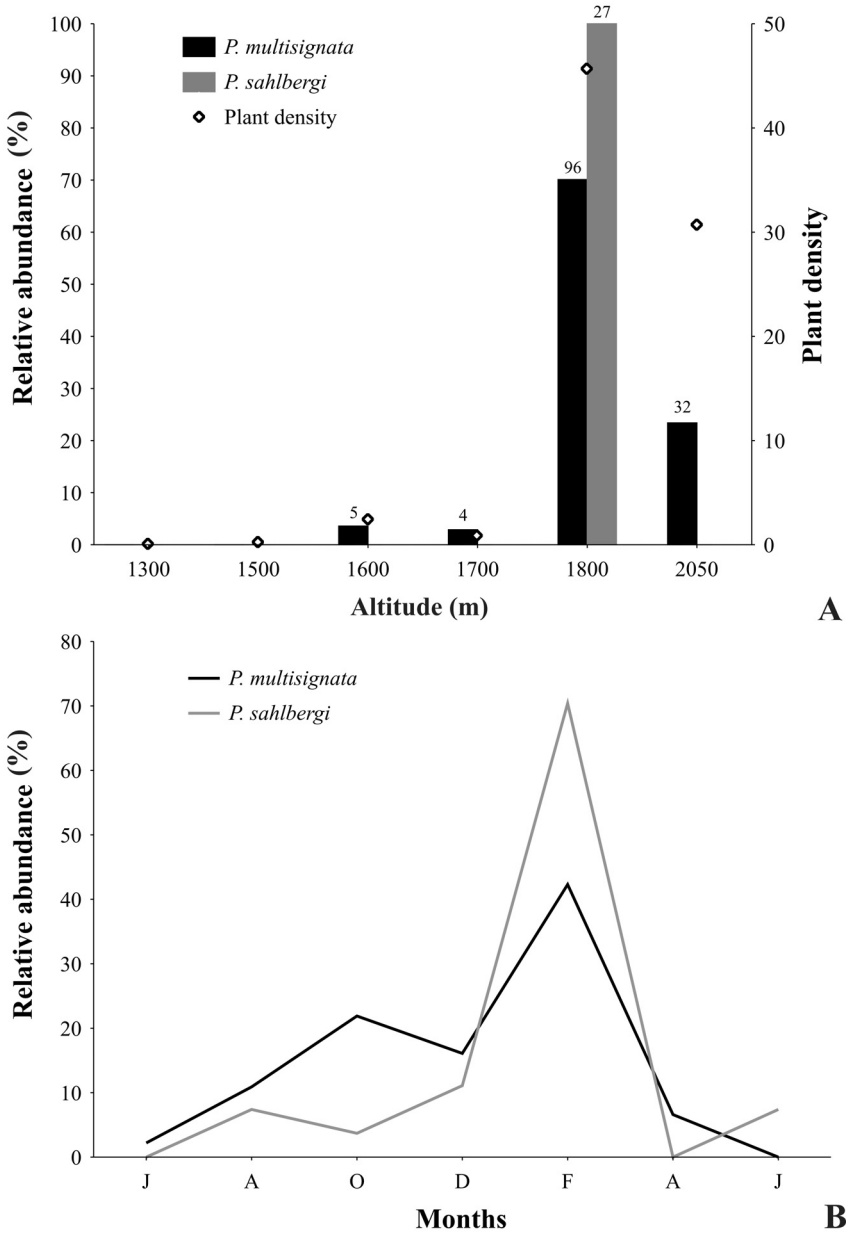

Fig. 5. A – Relative abundance (%) of *Plagiometriona multisignata* (Boheman, 1855) and *P. sahlbergi* (Boheman, 1855) and host plant density along the altitudinal gradient within Serra dos Órgãos National Park, Brazil. Numbers above bars indicate absolute abundances. B – Seasonal differences in abundance occurring in the same species populations from June 2006 to June 2007. (Redrawn from data in Flinte et al. 2011).

Geographical distribution of species

All three species were initially described only as occurring in Brasilia (Boheman 1855). The only detailed records were published by Borowiec (1996) for *P. sahlbergi* (in sense of Spaeth thus including *P. multisignata* and *P. scenica*). One specimen was collected near Rio de Janeiro (Petrópolis) and one from Espírito Santo (Castelo). The specimen from Petrópolis belongs to *P. multisignata*, however, we were unable to verify the specimen from Castelo, which thus might belong to any of the three species. Or, it might belong to *P. multisignata*, the most frequent species in collections and the species identical to Spaeth's concept of *P. sahlbergi*.

We recorded *P. multisignata* and *P. sahlbergi* in Teresópolis (Parque Nacional da Serra dos Órgãos), and *P. multisignata* also in the city of Rio de Janeiro (Floresta da Tijuca) (Flinte et al. 2009, where *P. multisignata* is cited as *P. sahlbergi*) and in Itatiaia (Parque Nacional do Itatiaia; unpubl. data), all in the State of Rio de Janeiro, Brazil.

Conclusions

Based on morphological characters, and supported by further molecular analysis and observations in field and laboratory, we propose the revalidation of *Plagiometriona multisignata* stat. restit. and *Plagiometriona scenica* stat. restit., previously considered synonyms of *P. sahlbergi*. The possibility that *P. multisignata* and *P. sahlbergi*, i.e. sympatric species utilizing the same host plant, have converged in morphology deserves future investigation, as do relationships between other *Plagiometriona* beetles and their Solanaceae host plants.

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