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Errata

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Errata

On the first page of the Table of Contents in the September 2020 issue, 103 (3), the research paper by Houlihan et al. should show the page range as 369–375. We regret the error.

We have been informed that there was a misplacement of figures in the article beginning on page 619 in the September 2019 issue, 102 (3). We regret the error and have made the correction below.

Natural enemies of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), a recent invasive pest on maize in South India

Sharanabasappa, C. M. Kalleshwaraswamy, J. Poorani, M. S. Maruthi, H. B. Pavithra, and J. Diraviam

The correct captions are:
Fig. 1. Larval parasitoid of *Coccygidium melleum* on *Spodoptera frugiperda*.
Fig. 2. Larval parasitoid of *Odontepyris* sp. on *Spodoptera frugiperda*.

We have been informed that there was an incorrect figure in the article beginning on page 376 in the September 2020 issue, 103 (3). Figure 1 should be the image shown below. We regret the error.

Coloration patterns of the tegmina of *Mahanarva spectabilis* (Hemiptera: Cercopidae): biological, morphological and genetic bases

Ricardo Aquino Borges, Alexander Machado Auad, Marcy das Graças Fonseca, Cristiano Amancio Vieira Borges, Ana Luisa Sousa Azevedo, Tiago Teixeira de Resende, Gervásio Silva Carvalho, and Fernando Rodrigues de Oliveira

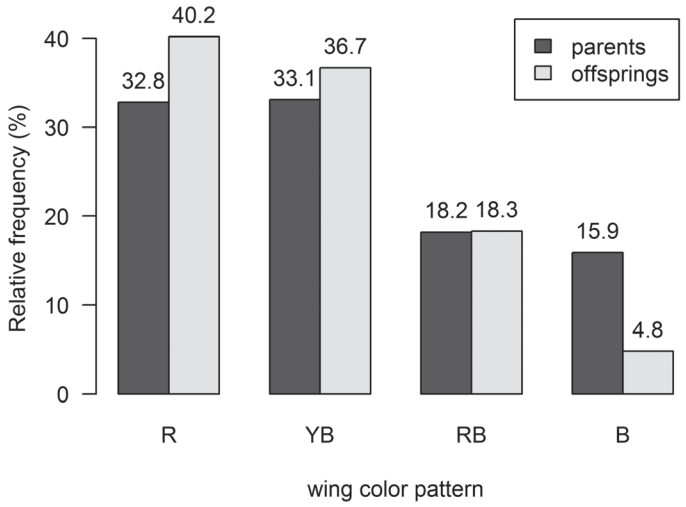


Fig. 1. Relative frequency of 484 adults collected at the Embrapa Dairy Cattle experimental field in the town of Coronel Pacheco, Minas Gerais, Brazil, from which 242 couples were formed, with their 1,484 offspring, with regard to wing color patterns, regardless of sex.

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Natural enemies of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), a recent invasive pest on maize in South India

Sharanabasappa[1,], C. M. Kalleshwaraswamy[1], J. Poorani[2], M. S. Maruthi[1], H. B. Pavithra[1], and J. Diraviam[3]*

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), native to the Americas, is considered one of the important invasive polyphagous pests. It is prevalent in several countries such as Brazil, Argentina, and the USA (Prowell et al. 2004; Clark et al. 2007), instigating economic losses in a variety of crops such as maize, soybean, cotton, beans (Pogue 2002; Nagoshi et al. 2007; Bueno et al. 2010), rice, other grasses, and a number of weeds (Nabity et al. 2011). Because of its wide host range, *S. frugiperda* is one of the most harmful pests threatening annual crops in tropical regions (Andrews 1980; Cruz et al. 1999). Severe incidences of fall armyworm were reported from African countries such as Sao Tome, Nigeria, Benin, and Togo in 2016 (Goergen et al. 2016). The incursion of fall armyworm as an invasive pest into Asia was reported for the first time in India by Sharanabasappa et al. (2018), Ganiger et al. (2018), and Shylesha et al. (2018). Recently this pest has been widely reported in many parts of southern and northern India (Mahadeva Swamy et al. 2018). Other species of *Spodoptera*, such as *S. litura* (F.), *S. exigua* (Hübner), and *S. mauritia* (Boisduval), are major pests of several crops in India with a rich array of indigenous natural enemies. The native bioagents of *Spodoptera* spp. have an opportunity to expand their niche by parasitizing *S. frugiperda*, a closely related pest of foreign origin. It is highly probable that the local bioagents may widen their niche by adapting to *S. frugiperda*, and check its population buildup and further spread. Hence, there is a need to identify the existing natural enemies of fall armyworm in India, which could be used for its management in the future. This study was undertaken to document the natural enemies of fall armyworm, and to record their abundance from different locations in Karnataka and Tamil Nadu in southern India.

Surveys for the occurrence of *S. frugiperda* were conducted in different maize growing areas of Karnataka and Karur District of Tamil Nadu. An attempt was made at each site, e.g., Shivamogga, Davanagere, Chitradurga, Chikmagaluru, Chamarajanagar, Bellary and Belagavi districts of Karnataka, to collect at least 100 larvae of different stages; however, greater numbers of larvae were collected in only a few locations. In the infested field, feeding injury in the leaf whorl and the presence of fresh frass were used to identify the infestation of *S. frugiperda* larvae. Larvae were pulled from the whorl and placed in a circular insect breeding dish (Himedia TCP030, HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India; 90 mm diam, 40 mm height) containing cut maize leaf bits, brought to the laboratory and maintained at 26 ± 2 °C, 75 to 80% relative humidity, and 12:12 h (L:D) photoperiod. These were observed for the emergence of parasitoids; parasitoids emerging from fall armyworm at different stages (larval and pupal) were preserved in 70% ethanol and later identified taxonomically. Larvae from 2 unsprayed maize fields were brought to the laboratory in 25 mL plexiglass vials, and reared individually in the laboratory until the emergence of the parasitoids. Observations were recorded on the number of adult parasitoids that emerged from larvae, and the percentage parasitization and sex ratio were calculated. For the entomofungal pathogens, numbers of field-infected larvae and total larvae were recorded and converted to percentage infection.

In total, we recorded 5 larval parasitoids, 3 predators, and 1 entomopathogenic fungus in our surveys (Table 1), of which 3 parasitoids, namely *Coccygidium melleum* (Roman) (Hymenoptera: Braconidae) (Fig. 1), *Odontepyris* sp. (Hymenoptera: Bethylidae) (Fig. 2), and *Eriborus* sp. (Hymenoptera: Ichneumonidae), are reported for the first time on *S. frugiperda* in the world. The single female specimen reared from *S. frugiperda* matched the description of *C. melleum* by Achterberg (2011). *Coccygidium melleum* is "common in the Afrotropical region (from South Africa up to Senegal and Somalia) and reaching Yemen and the United Arab Emirates" (Achterberg 2011), but it has not been recorded so far in the Indian subcontinent. *Coccygidium* spp. (Hymenoptera: Braconidae) are known to parasitize Noctuidae, including *Spodoptera* spp. (Achterberg 2011). Sisay et al. (2018) reported *Coccygidium luteum* (Brullé) (Hymenoptera: Braconidae) as a parasitoid of *S. frugiperda* in Ethiopia. Three species of *Coccygidium* (*C. luteum*, *C. melleum*, and *Coccygidium sissoo* [Wilkinson]) have been known to parasitize *S. exigua* (Hübner). This is the first report

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Table 1. List of natural enemies of *Spodoptera frugiperda* in maize ecosystem.

Sl. No.	Scientific name	Family	Host stage	Place	Percentage parasitism number of predators per plant
1	*Coccygidium melleum* (Roman)**	Hymenoptera: Braconidae	Endo larval parasitoid	Karnataka: Davanagere: Honnali; 29.VI.2018	0.001
2	*Campoletis chlorideae* Uchida	Hymenoptera: Ichneumonidae	Endo larval parasitoid	Davanagere: Honnali; 29.VI.2018	2–4
3	*Eriborus* sp.**	Hymenoptera: Ichneumonidae	Endo larval parasitoid	Davanagere: Honnali; 29.VI.2018	0.001
4	*Odontepyris* sp.**	Hymenoptera: Bethylidae	Larval parasitoid	Tamil Nadu: Karur: Kulithalai; 20.VIII.2018	0.001
5	*Exorista sorbillans* (Wiedemann)	Diptera: Tachinidae	Endo larval parasitoid	Chitradurga: Basapur; 19.VII.2018	0.001
6	*Forficula* sp.	Dermaptera: Forficulidae	Predator	Karnataka: Shivamogga; 18.VI.2018	1–2
7	*Harmonia octomaculata* (Fabricius)	Coleoptera: Coccinellidae	Predator	Karnataka: Shivamogga: Muttodu; 25.VI.2018	0.5–1.00
8	*Coccinella transversalis* Fabricius	Coleoptera: Coccinellidae	Predator	Karnataka: Shivamogga: Muttodu; 25.VI.2018	0.5–1.00
9	*Nomuraea rileyi* (Farlow) Samson	Ascomycota: Clavicipitaceae	Entomo-pathogen	Shivamogga, Badravati: Kudarekonda, 18.VII.2018. Sogalu, Honnali; Davanagere 25.VIII.2018	10–15

**First report from India

of *C. melleum* as a parasitoid of *S. frugiperda* in the world. We recorded 2 more larval endoparasitoids, *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) and *Eriborus* sp. on *S. frugiperda* in Karnataka. The extent of parasitism by *C. chlorideae* was 2 to 3% in 2 untreated maize fields monitored at Shivamogga and Davanagere districts of Karnataka. Shylesha et al. (2018) also recorded it on *S. frugiperda*. *Campoletis chlorideae* and *Eriborus argenteopilosus* (Cameron) (Hymenoptera: Ichneumonidae) are responsible for the regulation of 2 major noctuid pests in India, *S. litura* and *Helicoverpa armigera* Hübner (Lepidoptera: Noctudae). Both parasitoids attack the host larvae in the first or second instar stage (Bajpai et al. 2006). About 6 species of *Campoletis* have been known to parasitize *S. frugiperda* in the Americas and the Caribbean (Molina-Ochoa et al. 2003). Species of *Odontepyris* are known to be ectoparasitoids of lepidopteran larvae belonging to Noctuidae, Pyralidae, Oecophoridae, and Tortricidae (Lim & Lee 2013), and this is the first report of it as a parasitoid of *S. frugiperda*. We recorded negligible levels of parasitism of fall armyworm by a tachinid, *Exorista sorbillans* (Wiedemann) (Diptera: Tachinidae). Predators such as earwigs and coccinellids (*Harmonia octomaculata* [F.] and *Coccinella transversalis* [F.]) (both Coleoptera: Coccinellidae) also were found to be active in fall armyworm infested maize fields in the surveyed locations. *Harmonia octomaculata* and *C. transversalis*, which were found to be abundant in fall armyworm infested maize fields, may play a significant role in controlling the early stage larvae. Various larger species of Coccinellidae attack caterpillars and other beetle larvae (Hodek et al. 2012), and several genera feed on various insects or their eggs. For instance, in India, *Micraspis vincta* (reported as *Veraniavincta*) (Coleoptera: Coccinellidae) was found feeding on the egg masses of *S. litura* on a groundnut crop (Rajasekhara Rao 1997), and *S. exigua* on an onion crop (Subba Rao 1998; Sailaja Rani 2004). *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is known to feed on eggs and larvae of *S. exigua* in China (Liu et al. 2016), and *Coccinella* sp. is a predator of *S. exigua* in Vietnam (Chau 1995). Shylesha et al. (2018) recorded earwigs (*Forficula* sp.) (Dermaptera: Forficulidae) as predators of fall armyworm on maize. The entomofungal pathogen *Nomuraea rileyi* (Clavicipitaceae), was found to be associated commonly with fall armyworm and caused 10 to 15% larval infection in August.

The present study reports new associations of natural enemies with fall armyworm in India. A wide range of parasitoids has been recorded on fall armyworm worldwide as shown by the inventory of parasitoids and parasites of fall armyworm in the Americas and the Caribbean basin that included approximately 150 species of parasitoids and parasites from 14 families (Molina-Ochoa et al. 2003). Our results clearly indicate that native parasitoids of other *Spodoptera* spp. in India, such as *C. chlorideae* and *E. argenteopilosus*, may also adapt to *S. frugiperda* in due time. It would be worthwhile to evaluate indigenous parasitoids of *Spodoptera* spp. that known to be effective in India against *S. frugiperda*. For instance, *Telenomus remus* Nixon (Hymenoptera: Platygastridae), an effective egg parasitoid of *S. litura*, is known to parasitize *S. frugiperda* as well (Molina-Ochoa et al. 2003). *Nomuraea rileyi* was found to be very effective against *S. litura* (Padanad & Krishnaraj 2009) in Karnataka.

Further information on the occurrence and rates of parasitism of indigenous natural enemies is of paramount importance in designing a biological control program for fall armyworm, either through conservation of native natural enemies or the introduction of new species for augmentative release. The current blanket recommendation and indiscriminate use of pesticides against the fall armyworm may have a negative impact on natural enemies. Application of insecticides that are less toxic to natural enemies should be encouraged, rather than the continued use of conventional broad-spectrum insecticides, so as

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Fig. 1. *Odontepyris* sp.: Larval parasitoid on *Spodoptera frugiperda*.

to protect natural enemies from the adverse effects of insecticides. The design of more comprehensive IPM programs for fall armyworm management in the region would be a useful strategy.

Summary

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a pest recently invading maize in India. Studies were conducted in southern India from Jun to Aug 2018 to identify and assess the abundance of natural enemies attacking *S. frugiperda*. In total, 5 species of larval parasitoids, 3 predators, and 1 entomopathogen were found attacking larvae of *S. frugiperda*. The larval parasitoids were *Coccygidium melleum*, *Campoletis chlorideae*, *Eriborus* sp., *Exorista sorbillans*, and *Odontepyris* sp. Three predators, *Forficula* sp., *Harmonia octomaculata*, and *Coccinella transversalis*, and 1 entomofungal pathogen, *Nomuraea rileyi*, were recorded. The average parasitism caused by *C. chlorideae* was found to be 2 to 3%, whereas the remaining parasitoids showed negligible parasitism. *Nomuraea rileyi* recorded 10 to 15% larval infection in Aug. Three parasitoids, *Coccygidium melleum*, *Eriborus* sp., and *Odontepyris* sp., were reported for the first time attacking *S. frugiperda*. Efforts should be undertaken to identify more natural enemies and to preserve the existing ones, through ecofriendly practices and judicial use of pesticides, allowing them to function effectively.

Key Words: fall armyworm; parasitoids; predators; entomopathogen

Sumario

El cogollero, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) es una plaga invasora reciente sobre el maíz en la India. Se realizaron estudios en el sur de la India desde el junio hasta el agosto de 2018 para identificar y evaluar la abundancia de enemigos naturales que atacan a *S. frugiperda*. En total, se encontraron 5 especies de parasitoides larvales, 3 depredadores y 1 entomopatógeno que atacan las larvas de *S. frugiperda*. Los parasitoides larvales fueron *Coccygidium melleum*, *Campoletis chlorideae*, *Eriborus* sp., *Exorista sorbillans*, y *Odontepyris* sp. Se registraron tres depredadores, *Forficula* sp., *Harmonia octomaculata*, y *Coccinella transversalis* y 1 patógeno entomofúngico, *Nomuraea rileyi.* Se encontró que el promedio de parasitismo causado por *C. chlorideae* fue de 2 a 3%, mientras que los parasitoides restantes mostraron un parasitismo insignificante. *Nomuraea rileyi* registró una infección en las larvas del 10 al 15% en agosto. Se reportan por primera vez tres parasitoides, *Coccygidium melleum*, *Eriborus* sp., y *Odontepyris* sp. atacando a *S. frugiperda*. Se

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Fig. 2. *Coccygidium melleum*: Larval parasitoid on *Spodoptera frugiperda*.

deben realizar esfuerzos para identificar más enemigos naturales y preservar los existentes, a través de prácticas respetuosas con el medio ambiente y el uso prudente de pesticidas, que les permite funcionar de manera efectiva.

Palabras Clave: cogollero; parasitoides; depredadores entomopatógeno

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Coloration patterns of the tegmina of *Mahanarva spectabilis* (Hemiptera: Cercopidae): biological, morphological and genetic bases

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Abstract

This study examines the tegminal coloration pattern and the morphometry of *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) progeny of crosses between parents with differing wing patterns. Genetic studies were used to investigate whether the different coloration patterns of tegmina have resulted from speciation within the *M. spectabilis* clade. We crossed *M. spectabilis* with differing wing patterns to determine percentages of coloration standards of the tegmina and the biometry of the first generation (F1) progeny. DNA of specimens was extracted and analyzed. The results show that parental phenotype was a determining factor in the tegminal coloration pattern of offspring of generation F1. Slight variation exists in the specimens' morphometry; no grouping trend is evident with regard to specimens with different tegminal coloration patterns. Based on the characteristics of the population analyzed herein, it seems that there has been no speciation of *M. spectabilis*.

Key Words: forage; speciation; spittlebug

Resumo

Avaliou-se o padrão de coloração de asas e a morfometria da descendência de cruzamentos entre pais com diferentes padrões de asas de *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae). Estudos genéticos foram utilizados para investigar se os diferentes padrões de coloração das asas resultaram de especiação de *M. spectabilis*. Utilizou-se casais de *M. spectabilis* com diferentes padrões de asas para determinar as porcentagens dos padrões de coloração das asas e a biometria da progênie de primeira geração (F1). O DNA das amostras foi extraído e analisado. Os resultados mostraram que o fenótipo parental foi um fator determinante no padrão de coloração das asas dos descendentes da geração F1. Verificou-se pequena variação na morfometria das amostras; nenhuma tendência de agrupamento é evidente em amostras com diferentes padrões de coloração de asas. Com base nas características da população analisada, não houve especiação de *M. spectabilis*.

Palavras Chaves: forrageira; especiação; cigarrinhas-das-pastagens

Spittlebugs (Hemiptera: Cercopidae) cause extensive damage to pasture in Brazil and many other countries of tropical America (Valério & Nakano 1988). Among the species in Brazil, *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) is a limiting pest in forage production (Auad et al. 2007). The nymphs of *M. spectabilis* suck the plants' sap causing yellow coloration in the entire plant, whereas adults feed on the shoots of the plant causing phytotoxicity (Valério 2009).

Certain cercopid species vary in the color of their tegmina. If a population demonstrates perceptible phenotypic variation among specimens, it is highly probable that evolutionary trends have been at work (Townsend et al. 2006). Hutchinson (1963) and Farish and Scudder (1967) attributed variations of spittlebug tegminal coloration patterns to genetic causes. Phenotypic variation may have resulted from mating behavior differences in attraction signals or from geographic barriers (Townsend et al. 2006).

Variations in tegminal coloration pattern of *Notozulia entreriana* (Berg) (Hemiptera: Cercopidae) have been detected in Brazil (Mendonça Filho 1972; Ramos 1976; Valério 1979; Milanez 1980; Naves 1980; Sá 1981). Variations also were reported for the species *Deois schach* (F.), *Deois flavopicta* (Stål), and *Mahanarva fimbriolata* (Stål) (all Hemiptera: Cercopidae) (Sá 1981). Guagliumi (1972/73) observed that *M. fimbriolata* males have tegminal coloration patterns that may differentiate them into 3 races or co-species. In spite of the importance of the above-mentioned research, investigation of the causes of these variations in the spittlebug started in 2010 with Auad and collaborators. They described 4 tegminal coloration patterns for *M. spectabilis*, namely a straw-yellowish hue with longitudinal black spots, a reddish

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hue with longitudinal black spots, a totally reddish hue, and a totally black hue (Auad et al. 2010). Although the authors proposed the existence of subspecies based on the tegminal coloration pattern, further research is needed to test this hypothesis.

The current paper aims to (i) define tegminal coloration pattern and the morphometry of *M. spectabilis* of generation F1 progeny from parents differing in wing pattern, and (ii) use molecular genetic techniques to determine if variation in tegminal coloration patterns of *M. spectabilis* have resulted from speciation within this clade.

Materials and Methods

BIOASSAY 1. DETERMINATION OF DIFFERENT TEGMINAL COLORATION PATTERNS OF PARENTAL *MAHANARVA SPECTABILIS* RETRIEVED FROM THE FIELD AND THEIR FIRST GENERATION OFFSPRING

Approximately 2,000 nymphs of *M. spectabilis* were collected at an Embrapa Dairy Cattle experimental field in the town of Coronel Pacheco, Minas Gerais State, Brazil. Nymphs were transferred to 500 mL pots with elephant grass as a food substrate and kept in the greenhouse of the Embrapa Dairy Cattle. Emerged virgin adults (n = 242 couples) were classified daily with regard to tegminal coloration pattern, namely a straw-yellowish hue with black spots (YB), a reddish hue with black spots (RB), a totally reddish hue (R), and a totally black hue (B), following Auad et al. (2010). The adults were paired to form the following couples: (i) yellowish with black spots (YB) × yellowish with black spots (YB) (n = 30); (ii) reddish with black spots (RB) × reddish with black spots (RB) (n = 11); (iii) totally reddish (R) × totally reddish (R) (n = 30); (iv) totally black (B) × totally black (B) (n = 7); (v) yellowish with black spots (YB) × reddish with black spots (RB) (n = 22); (vi) yellowish with black spots (YB) × totally reddish (R) (n = 54); (vii) yellowish with black spots (YB) × totally black (B) (n = 24); (viii) reddish with black spots (RB) × totally reddish (R) (n = 25); (ix) reddish with black spots (RB) × totally black (B) (n = 19); (x) totally reddish (R) × totally black (B) (n = 20), with a total of 10 treatments. Replications (n) varied for each treatment due to population oscillation of tegminal coloration patterns of specimens which emerged from the nymphs collected in the field.

Couples were individualized and conditioned on the upper part of elephant grass plants within voile cages so that the F1 generation could be obtained. A piece of gauze was placed at the base of each cage as a substrate for egg laying. After the death of the females, eggs were retrieved from the gauze. The gauze was placed on a set of sieves through which running water was jetted, where eggs remained within the 400 mesh sieve. Eggs from each couple were placed singly in 9 cm Petri plates lined with filter paper and stored in BOD-type acclimatized chambers (28 ± 2 °C, a 14:10 h (L:D) photoperiod, and 70% relative humidity) until the embryo stage was close to hatch (S4). Eggs at stage S4 were placed in 1 × 1 cm filter paper sheets and transferred to 500 mL pots, each with an elephant grass plant whose roots were exposed by jetted water. Exposed roots served as the food substrate for the nymphs. Up to 40 eggs were placed on each plant. Pots were sealed with plastic lids and gauze, and maintained in trays in the greenhouse. Emergence of adults was monitored daily to evaluate the tegminal coloration pattern of the first generation.

Prior to analyzing the different crosses between progenitors, Pearson's χ^2 goodness-of-fit test was employed for testing for the adherence of the frequency distribution for tegminal coloration phenotypes of the F1 (1,484 adults) to the distribution observed for progenitors (484 adults). Next, for each of the 10 crosses established by the progenitors' wing patterns, the occurrence proportions of the 4 tegminal coloration patterns for the F1 were compared. For 65 out of the 242 progenitor couples, at least 8 descendants developed to adulthood. Thus, in order to ensure a minimal reliability of the proportions, progeny data arising from only these couples were employed to evaluate the tegminal coloration patterns of F1 generation. Taking each of the 10 crosses singly as a specific sub-assay regardless of the others, the proportions of each tegminal coloration pattern occurring within the progeny were compared by Analysis of Variance (ANOVA) followed by Tukey's test with significance level of 0.05. Statistical package R vers. 3.1.3 (R Core Team 2015) was used for analysis.

BIOASSAY 2. BIOMETRY OF FIRST GENERATION OF *MAHANARVA SPECTABILIS* FROM PROGENITOR WITH DIFFERING TEGMINAL COLORATION PATTERNS

Biometric analysis was undertaken with 20 males and 20 females of each tegminal coloration pattern of *M. spectabilis*: yellowish-black (YB), reddish-black (RB), reddish (R), and black (B), obtained from the first generation retrieved from Bioassay 1. Insects were monitored with a stereoscopic microscope (Novainstruments, Piracicaba, São Paulo, Brazil), and images were transferred to computer. Anatomic parts were measured with the program Screen Calipers for ProScope (LX - ProScope HR Software, Philadelphia, Pennsylvania, USA). Length and width (mm) of head, pronotum, scutellum, and tegmina were recorded.

Biometric evaluation data were subjected to variance analysis and Tukey's test (α = 0.05). The ANOVA included effects of sex, wing patterns, and the interaction between sex and wing pattern on the width and length of head, pronotum, scutellum, and tegminal. When a significant interaction effect was detected, the levels of one factor were compared across levels of the other by means of ANOVA F-tests, followed by Tukey's post-hoc multiple comparisons with the significance level set at 0.05. Data were analyzed with statistical package R vers. 3.1.3 (R Core Team 2015).

BIOASSAY 3. EXTRACTION AND QUANTIFICATION OF DNA FROM *MAHANARVA SPECTABILIS* OF DIFFERENT TEGMINAL COLORATION PATTERNS

Molecular analysis was performed for 3 *M. spectabilis* specimens of each tegminal coloration pattern, collected in Coronel Pacheco and preserved in 70% alcohol. An external control consisted of 3 specimens of *M. fimbriolata* (Stål) collected in Coronel Pacheco, Minas Gerais, Brazil, and 3 specimens of *Mahanarva* tristis (F.) (Hemiptera: Cercopidae) collected in the state of Pará, Brazil.

Wings, legs, and head of each insect were extracted with a pair of tweezers. The remaining part of each insect was transferred to a separate Eppendorf tube and ground with liquid nitrogen. DNA was extracted from the residual part at the Molecular Genetic Laboratory of Embrapa Dairy Cattle (Juiz de Fora, Minas Gerais, Brazil) following the protocol of Ferreira and Grattapaglia (1995).

Fifteen Random Amplified Polymorphic DNA (RAPD) markers from Operon Technologies Inc. (Alameda, California, USA) (OPA13; OPA16; OPB1; OPB6; OPB7; OPB20; OPD8; OPE4; OPE11; OPE18; OPF1; OPG8; OPG10; OPG11; OPG17) were selected for genotyping the 18 samples. DNA amplification reactions were performed in 35 µL aliquots with 0.4 mM primer, 0.15 mM dNTPs, 1.0 unit of Taq polymerase, 10 mM Tris-HCl (pH 8.0), 2.5 mM $MgCl_2$, and 50 mM KCl. The following cycles were used for amplification: 40 cycles at 94 °C for 30 s, at 37 °C for 60 s, and at 72 °C for 30 s; followed by 1 cycle at 72 °C for 7 min. Products were separated by electrophoresis in

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2% agarose gel, and images were digitized for further analysis using Gel Analyzer 2010 (www.gelanalyzer.com).

A binary matrix was built according to presence (1) and absence (0) of bands between tegminal coloration patterns of *M. spectabilis*. Rates were employed for diversity analysis by the Jaccard Index (Jaccard 1901). A similarity matrix also was employed to perform the unweighted pair group method using arithmetic averages, producing a dendrogram with different tegminal coloration patterns of *M. spectabilis* and control groups (*M. fimbriolata* and *M. tristis*). Molecular marker data underwent molecular variance analysis (Analysis of Molecular Variance, AMOVA) to assess distances between tegminal coloration patterns of *M. spectabilis*.

Results

BIOASSAY 1. DETERMINATION OF DIFFERENT TEGMINAL COLORATION PATTERNS OF PARENTAL *MAHANARVA SPECTABILIS* RETRIEVED FROM THE FIELD AND THEIR FIRST GENERATION OFFSPRING

Estimated relative occurrence frequencies of tegminal coloration patterns of progenitors collected in the field were 33%, 33%, 18%, and 16% for patterns reddish (R), yellowish-black (YB), reddish-black (RB), and black (B), respectively, regardless of sex (Fig. 1). First generation adults showed mean occurrence percentages of 40%, 37%, 18%, and 5%, respectively, revealing an increase in the occurrence of tegminal coloration pattern reddish (R) and a reduction in pattern black (B), when compared to progenitors (Fig. 1). So, the frequency distribution of offspring differed significantly from that of the progenitors (χ^2 = 144.5; gl = 3; $P < 0.0001$).

The evaluation of generation F1 showed that in crosses in which one of the progenitors had tegminal coloration pattern reddish (R), its offspring had a greater chance (more than 50%) of expressing this same phenotypic pattern of coloration than the other patterns (Fig. 2A, B, D), except cross reddish/reddish-black (R/RB) (Fig. 2C). Furthermore, it should be underscored that in the cross between progenitors of the same coloration pattern reddish (R) (Fig. 2A), the least percentage of descendants with the same pattern reddish (R) was 69%, whereas the maximum percentage of all the other coloration patterns was only 15%. Distancing revealed the prevalence of offspring with coloration pattern reddish (R) derived from the cross.

Also, if one progenitor had tegminal coloration pattern yellowish-black (YB) (Fig. 2 E–G), the chance of having progeny with yellowish-black (YB) coloration was greater (> 40%) when compared to the other patterns, except for cross yellowish-black/reddish-black (YB-RB) (Fig. 2F). This observation suggests persistence in this pattern's occurrence when pattern reddish (R) was absent. Furthermore, couples yellowish-black/yellowish-black (YB-YB) significantly generated the highest mean percentage (56%) of offspring with the yellowish-black (YB) pattern, followed by patterns reddish (R) (25%) and reddish-black (RB) (19%). Since pattern black (B) showed the lowest mean occurrence rate (0.5%) (Fig. 2E), yellowish-black/yellowish-black (YB-YB) progenitors were heterozygous; similarly, we are assuming the same inference for cross reddish-reddish (R-R).

Disregarding coloration patterns reddish (R) and yellowish-black (YB), in crosses where one of the *M. spectabilis* progenitors had tegminal coloration pattern reddish-black (RB) (Fig. 2H, I), the proportion of descendants with the reddish-black (RB) pattern also tended to be higher (> 50%) than that of the black (B) pattern. There was no significant predominance of any tegminal coloration pattern in generation F1, with mean percentages ranging between 20 and 30%, in crosses where both parents showed tegminal coloration pattern black-black (B-B) (Fig. 2J).

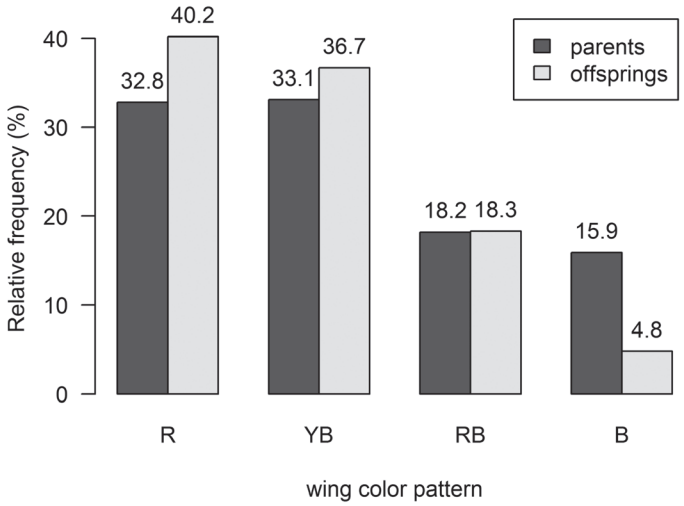


Fig. 1. Relative frequency of 484 adults collected at the Embrapa Dairy Cattle experimental field in the town of Coronel Pacheco, Minas Gerais, Brazil, from which 242 couples were formed, with their 1,484 offspring, with regard to wing color patterns, regardless of sex.

The results of these crosses indicate that different wing patterns vary in their likelihood of occurrence given the same pattern in a parent. They suggest an order of genetic dominance associated with phenotypes reddish > yellowish-black > reddish-black > black (R > YB > RB > B).

BIOASSAY 2. BIOMETRY OF FIRST GENERATION OF *MAHANARVA SPECTABILIS* FROM PROGENITOR WITH DIFFERING TEGMINAL COLORATION PATTERNS

Based on morphological studies, no significant interaction effects of sex and wing pattern on the length or width of the head or pronotum (Fig. 3A–D) or on width of tegmina (Fig. 3H) were found. *Mahanarva spectabilis* females are longer and wider at the head, pronotum, scutellum, and tegmina than males of the same species (Fig. 3A–D, H). Length of head and widths of pronotum and tegmina were not different among specimens of different wing patterns (Fig. 3A, D, H). However, width of head and length of the pronotum were smaller in specimens of the totally black (B) wing pattern (Fig. 3B, C).

Interactions between sex and wing pattern were observed for length (Fig. 3E) and width of scutellum (Fig. 3F) and tegminal length (Fig. 3G). Length and width of the scutellum of pattern reddish (R) and the length of the tegmina of pattern reddish (R) and yellowish-black (YB) females were significantly larger than those of other females. Tegminal length and scutellum width in males did not differ for wing patterns. However, the length of the scutellum was smaller in the black (B) hue specimens (Fig. 3 E–G).

BIOASSAY 3. EXTRACTION AND QUANTIFICATION OF DNA FROM *MAHANARVA SPECTABILIS* OF DIFFERENT TEGMINAL COLORATION PATTERNS

Molecular studies revealed that 101 bands were generated from the 15 markers (Table 1) among the 18 specimens belonging to different tegminal coloration patterns and controls. Twenty-eight exclusive molecular markers were obtained for the 3 species (10 for *M. spectabilis*, 8 for *M. fimbriolata*, and 10 for *M. tristis*) (Table 1).

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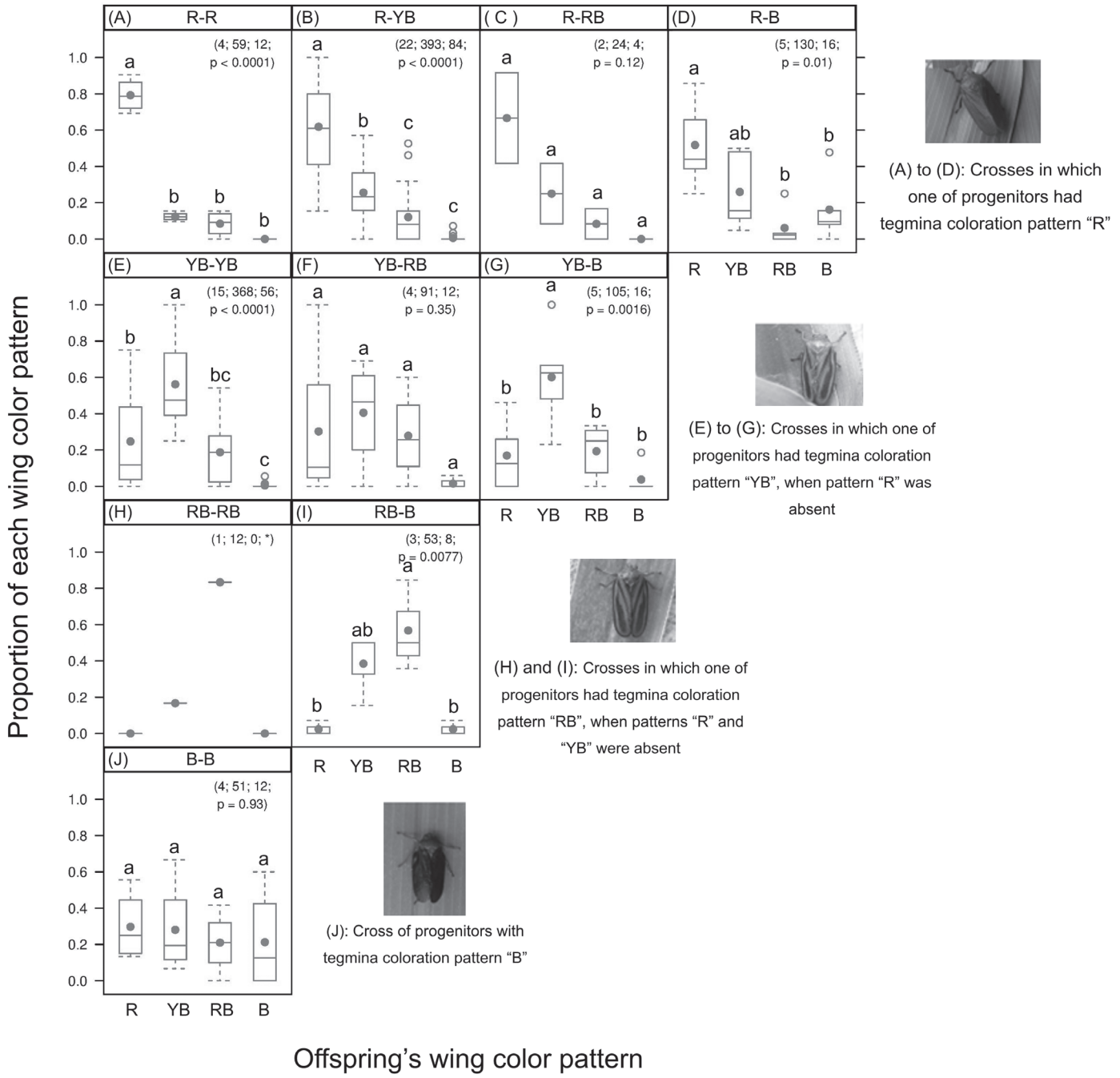


Fig. 2. Box plot representation of the distribution of the offspring's tegminal coloration pattern proportions obtained for each type of mating cross, according to parents' wing color pattern, regardless of sex. Each box spans from the first to the third quartile (interquartile range). The segment inside the box and the filled circle indicate median's and mean's locations, respectively. Whiskers above and below the box extend either to the maximum/minimum data value or to the most extreme value falling within the extent equivalent to 1.5 × interquartile range, starting from the box; points exceeding these limits are considered suspected outliers and are marked with unfilled circles. Different letters within each quadrant indicate significantly different mean proportions (Tukey's test, α = 5%). Value in parentheses are, in the order they appear, the number of replicates (i.e., the number of couples from which at least 8 offspring were obtained, that grew to adulthood), the total number of offspring generated from these replicates that grew to adulthood, the ANOVA residual degrees of freedom, and the ANOVA *F*-test *p*-value. *Insufficient *n* for analysis.

When different morphological patterns of *M. spectabilis* were taken into account, the analysis of molecular variance (AMOVA) detected much greater variation within each tegminal coloration pattern (99%) than among the different patterns (1%) (PhiPt 0.007; $P = 0.365$; df = 3,11). This finding demonstrated that there was no parallelism between phenotypic and molecular differences (Fig. 4).

The dendrogram gives a better view of the approximation degree between the different wing patterns in *M. spectabilis* (Fig. 4). In fact, it reveals that the external group (*M. tristis*) is actually more distant from the other 2 species (*M. fimbriolata* and *M. spectabilis*). However, there is no grouping trend in the latter with regard to similar wing patterns.

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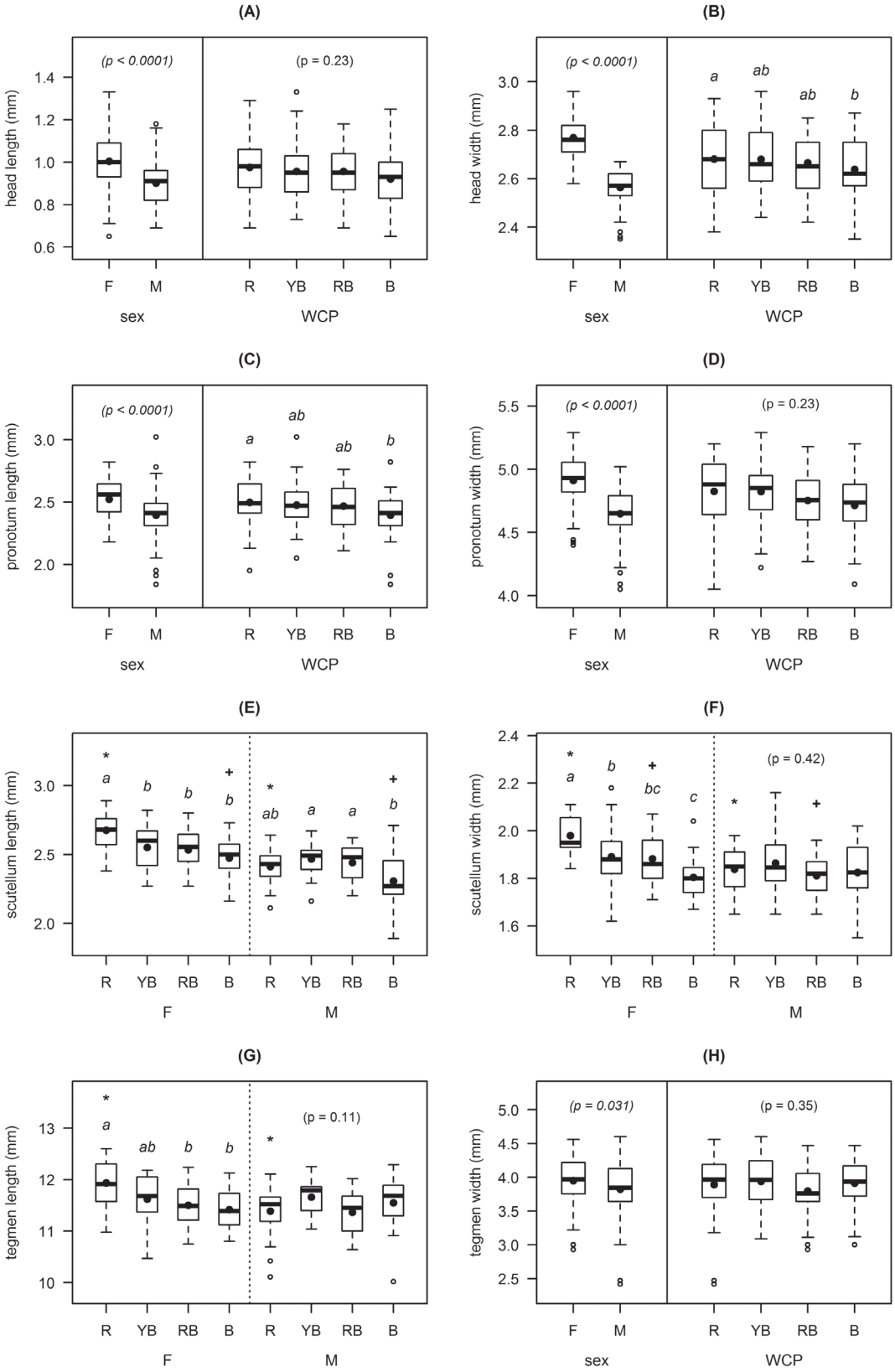


Fig. 3. Comparative biometrics of *M. spectabilis* by sex and wing color patterns. Distributions observed for head length (A) and width (B), pronotum length (C) and width (D), scutellum length (E) and width (F), and tegmen length (G) and width (H). In cases (A) to (D) and (H), sex × wing color pattern interaction was not significant; thus *p*-values and letters refer to mean comparison between sexes or among wing color patterns, where means followed by different letters were found to be significantly different. In the other cases, (E) to (G), sex × wing color pattern interaction was found to be significant, and its analysis was conducted: *p*-values and letters refer to mean comparison among wing color patterns within sex, where different letters indicate significant differences, whereas distributions marked with a same symbol (* or +) indicate a significant difference between the sexes for a given wing color pattern. ANOVA followed by Tukey's test at 5% significance probability level was used in all cases.

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Table 1. Exclusive marks in the 3 species of spittlebugs under analysis (*M. spectabilis*, *M.fimbriolata*, and *M. tristis*).

Species	Primer	Nucleotide sequence	Fragment size (bp)
M. spectabilis	OPA-16	AGCCAGCGAA	680–1,100
	OPB-01	GTTTCGCTCC	810
	OPB-07	GGTGACGCAG	780
	OPB-20	GGACCCTTAC	780
	OPD-08	GTGTGCCCCA	990
	OPE-11	GAGTCTCAGG	990
	OPF-01	ACGGATCCTG	580
	OPG-08	TCACGTCCAC	610
	OPG-17	ACGACCGACA	420
M. fimbriolata	OPB-06	TGCTCTGCCC	770–790
	OPD-08	GTGTGCCCCA	360
	OPE-18	GGACTGCAGA	350
	OPF-01	ACGGATCCTG	800
	OPG-08	TCACGTCCAC	400
	OPG-10	AGGGCCGTCT	430
	OPG-17	ACGACCGACA	600
M. tristis	OPA-13	CAGCACCCAC	630
	OPB-06	TGCTCTGCCC	580
	OPB-07	GGTGACGCAG	800–1,400
	OPD-08	GTGTGCCCCA	400
	OPE-18	GGACTGCAGA	1,000–2,000
	OPF-01	ACGGATCCTG	1,200
	OPG-17	ACGACCGACA	900–1,000

Discussion

The development of complex scenarios in the evolution of insects may be known only when transformations at the phenotypic level of populations are analyzed (Beutel et al. 2011). Issues on the manner by which 2 or more phenotypes exist within the same population have been studied by researchers for more than a century. Wing polymorphism in insects is a good model to investigate the ecological causes and consequences of dispersion dynamics (Dingle 1996; Schwander & Leimar 2011). Within such a context, spittlebug species (Hemiptera: Cercopidae) show a wide variability in tegminal coloration patterns. In northeastern Brazil, Mendonça Filho (1972) reported variation in tegminal coloration patterns in *Aeneolamia selecta* (Walker) and in *Notozulia entreriana* (Berg) (both Hemiptera: Cercopidae). Guagliumi (1972/73) observed chromatic polymorphism in *M. fimbriolata* and showed that, due to their well-defined distributions, 3 races or co-species could be established. Ramos (1976) detected variations in tegminal coloration pattern in *N. entreriana* also in northeastern Brazil. In the center-west region of Brazil, Valério (1979) reported polymorphism in *N. entreriana* and in *D. flavopicta* (Stål 1954), while Naves (1980) observed a high degree of variability in wing designs and hue for *N. entreriana*. Milanez (1980) also noted chromatic variations in the 2 species collected in the southeastern region of Brazil. Within the same region, Auad et al. (2010) reported 4 tegminal coloration patterns for *M. spectabilis*. In the case of this species, the current study supports the results of Auad et al. (2010) determining 4 patterns in wing coloration (Fig. 2).

Variations in tegminal coloration patterns for spittlebugs have yet to be explained. Morphological differences among populations of the same species dispersed through latitude and height gradients have been reported for several insect groups (Smith et al. 2000). They are usually associated with temperature, humidity, photoperiod (Tauber et al. 1986), altitude (Halkka et al. 1980; Sá 1981), and frost (Whittaker 1972).

Although the above-mentioned research has shown that external causes are prevalent in the diversity of tegminal coloration patterns, they were not a determinant factor in the current study. The assay was conducted in greenhouse facilities in which all treatments had the same environmental conditions and the same type of host plant. The 4 wing patterns observed were the same as those reported in the regions of Presidente Prudente, São Paulo, Brazil; Brasília, Distrito Federal, Brazil (Auad et al. 2010), and Coronel Pacheco, Minas Gerais, Brazil (Auad et al. 2012).

Although other investigations have reported differences in tegminal coloration patterns in the spittlebug (Mendonça Filho 1972; Ramos 1976; Valério 1979; Milanez 1980; Naves 1980; Sá 1981; Auad et al. 2010), this study is the first step to understand the predominance of wing patterns from crosses between *M. spectabilis* with differing tegminal coloration patterns. It is clear that parental phenotype is a determinant factor for tegminal coloration in generation F1 of *M. spectabilis*. This information may be useful for taxonomic, ecological and molecular studies, and will be an aid in the discovery of pest control strategies that take advantage of tegminal coloration patterns. It must be stressed that practically all coloration patterns in generation F1 were obtained in crosses of *M. spectabilis* specimens of the same tegminal coloration pattern. This observation showed that specimens were not homozygous. On the other hand, homozygosis seems to occur in *M. spectabilis* specimens collected in the region of Valença, Rio Janeiro, and Campo Grande, Mato Grosso do Sul, Brazil, by Auad et al. (2010), who reported the occurrence of a single tegminal coloration pattern.

Morphometric methods in combination with demographic information may aid greatly in the explanation of issues related to co-variations of characteristics within and between populations. According to Wang et al. (2009), large specimens are prone to greater longevity, fecundity, and reproduction success. However, our research showed only small differences in the size of external structures of *M. spectabilis* specimens of different tegminal coloration patterns, suggesting similarity in performance.

This study identified exclusive markers for the 3 *Mahanarva* species. Variability lies within the expected range for different populations of the species. It should be underscored that the definition of exclusive marks for each species is relevant because insects generally are identified by phenotypic characteristics such as size, coloration, and male genitalia.

When different tegminal coloration patterns of *M. spectabilis* were investigated, differentiation at the genetic level was absent since there were no grouping trends with regard to similar tegminal coloration patterns. This result corroborates that by Paula-Moraes et al. (2006) who reported high variability among *M. spectabilis* samples at genetic and morphological levels, without any parallelism between them. Similarly, Auad et al. (2010) did not register any correlation between genetic variability and tegminal coloration in *M. spectabilis*. In fact, phenotypically similar *M. spectabilis* populations had a greater genetic variability than the more heterogeneous ones. Patterns of genetic and phenotypic variation within populations are affected by a complex interaction of ecological and evolutionary processes. Theory states that gene flow increases diversity within and reduces differences between populations (Tinnert & Forsman 2017). In this case, genetically diverse populations, even though phenotypically more homogeneous, are probably more capable of colonizing new habitats, and resisting and adapting to environmental changes.

The current study revealed that parental phenotype is a factor in the tegminal coloration pattern in generation F1. Results show that there was a slight variation in the specimens' morphometry, and there is no grouping trend with regard to specimens with different tegminal

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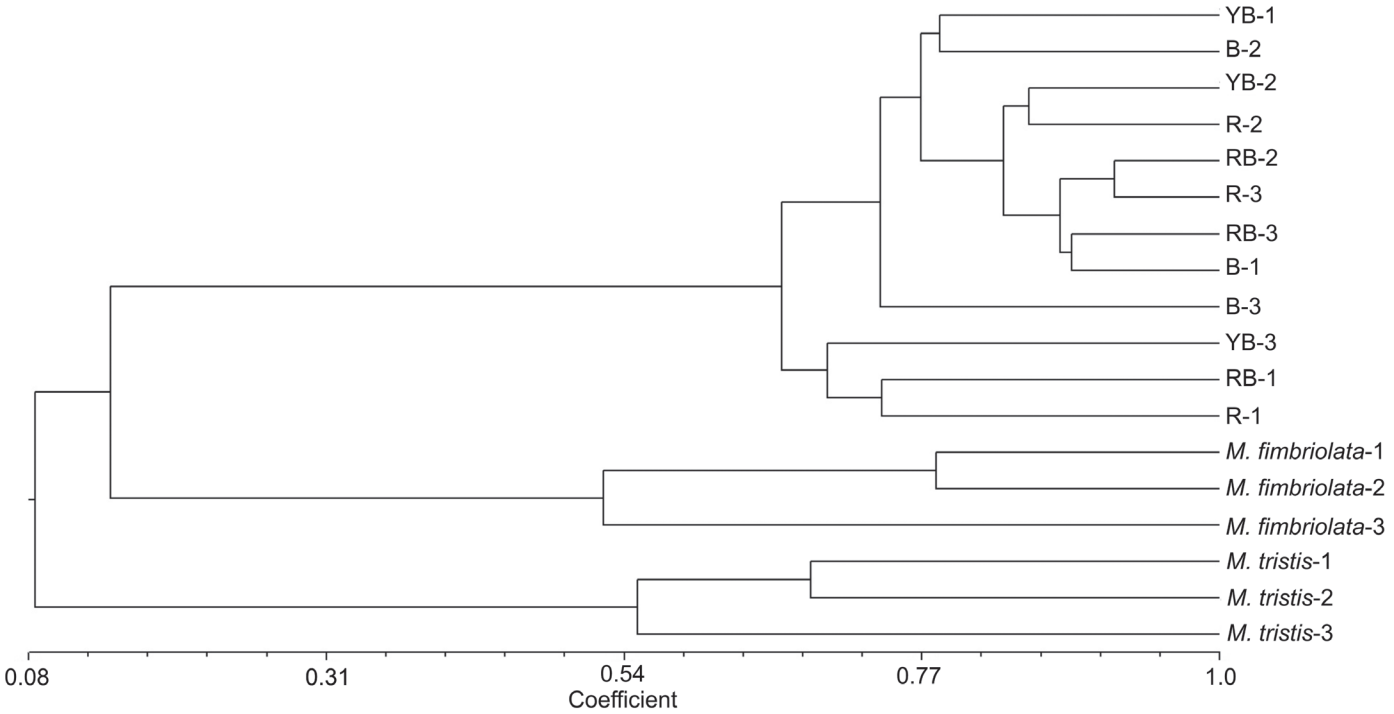


Fig. 4. Genetic distances between different species of spittlebugs and different wing color patterns of *M. spectabilis*, where YB = straw-yellowish hue with black spots; RB = reddish hue with black spots; R = total reddish hue; B = total black hue.

coloration patterns. This observation suggests phenotypic plasticity, or rather, the capacity of the species to alter its phenotype without the need of genotypic modifications. It seems that, based on the characteristics of the evaluated population, no speciation exists within *M. spectabilis*. Further studies should be undertaken to understand the reasons for and consequences of the development of *M. spectabilis* individuals with different tegminal coloration patterns.

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