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Short Communication

Description and characterization of the melanic morphotype of *Rhodnius nasutus* Stål, 1859 (Hemiptera: Reduviidae: Triatominae)

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ABSTRACT

Introduction: For the first time we provide the description of the melanic (dark) morphotype of *Rhodnius nasutus* and determine the pattern of genetic inheritance for this characteristic. **Methods:** *Dark morph R. nasutus* specimens were crossbred with standard (typically patterned) *R. nasutus*. **Results:** We present the first occurrence of the melanic morphotype in the genus *Rhodnius*. The crossbreeding results demonstrate that the inheritance pattern of this characteristic follows Mendel's simple laws of segregation and an independent assortment of alleles. **Conclusions:** Phenotypic variation of *R. nasutus* reinforces the heterogeneity found in the Triatominae. Descriptions of new species in this subfamily require rigorous validation criteria.

Keywords*:* Triatominae. *Rhodnius nasutus.* Dark morph. Cross-experiment. Melanic pattern.

The *Rhodnius* genus contains 19 species, 13 of which occur in Brazil. This genus represents one of the main genera belonging to the subfamily Triatominae, which are of epidemiologic importance for the transmission of Chagas disease. Various authors have reported the importance of palm trees as a natural ecotope for *Rhodnius* species[1-3], particularly palm trees belonging to the genera *Attalea*, *Acrocomia*, *Copernicia*, *Mauritia*, and *Syagrus*[2,3].

Rhodnius nasutus is one of the Triatominae species that is native to Brazil. It is distributed in the semi-arid region of northeastern Brazil, which is predominantly covered by scrub vegetation. This species is considered a vector of secondary importance for the transmission of Chagas disease, and is mainly associated with the carnaúba palm tree (*Copernicia prunifera*)[2-4]; however, this species also infests other species of palm trees[2].

The chromatic pattern of Triatominae is used as an important characteristic for species determination. However, several studies have reported intra-specific variation in this characteristic[2,5]. For instance, dark morphotype (dark morphs or melanic) *Triatoma infestans* individuals have been collected from parrot nests (*Aratinga acuticaudata*) in the Bolivian Chaco[6], with intermediate coloring being recorded in the Andean Valleys and Chaco[7]. More recently, dark morph specimens of *T. infestans* were also found in parrot nests (*Amazona aestiva*) in the Chaco Province of Argentina, which is a region that is free of anthropogenic activity, with the closest domicile being located at a distance of 25km[8]. Chromatic differences were also found in *Triatoma rubrovaria*. Four different morphotypes of this species were identified in the same geographic spot and ecotope in the State of Rio Grande do Sul, Brazil. Genetic studies based on isoenzymes confirmed that the differences in their chromatic patterns signified intra-specific variation only[5]. The current study aimed to present the occurrence of melanic *R. nasutus* and to determine the pattern of genetic inheritance for this characteristic.

Between July 8 and 11 in 2003, parental samples of *R. nasutus* were collected from babaçu palm trees (BA) (*Attalea speciosa*), in the district of Meruoca, and carnaúba palm trees (CA), in Sobral, State of Ceará. Authorization was provided by the Brazilian Institute of Environment and Natural Resources (IBAMA, *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis*, Authorization Nº 007/2002-COMAF; Process Nº 02001.001333/02-71). The palm trees were cut down and dissected to search triatomines, following a previously described method[2]. A total of 11 adults and 11 nymphs were collected at Sobral, while 2 adults and 23 nymphs were collected at Meruoca. The triatomines were transferred to the insectary of the *Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas*, *Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz* (LATEC/CPqRR/FIOCRUZ) to establish colonies. Two groups were formed, each belonging to the palm tree species from which the triatomines had been collected. The two groups were housed under semi-controlled conditions of temperature and humidity (26 ± 2°C; 70 ± 10% UR), and were fed weekly on Swiss mice anesthetized with thiopental.

The occasional appearance of adults with atypical, dark coloring (here named *dark morphs*) was observed in the F3 generation of the *R. nasutus* colony from carnaúba palm trees.

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Dark morph (melanic) nymphs were easily identified. The head and thorax of *R. nasutus dark morphs* are dark colored, whereas these body parts were mostly reddish or chestnut-colored in standard nymphs[2] (**Figure 1**). *Dark morph* nymphs were separated from standard nymphs to form a colony exclusively made up of this phenotype. Fifth stage nymphs were sexed[9] and separated according to head coloring (**Figure 1**). Then the following crosses were made, with five couple being used in each cross group: I) standard male x *dark morph* female; II) *dark morph* male x standard female; III) standard male x standard female; and IV) *dark morph* male x *dark morph* female. The standard bugs were used from the carnaúba colony only. Each couple was placed together for 60 days and fed weekly on Swiss mice anesthetized with thiopental. As soon as the fifth stage nymphs of the F1 generation were produced, all individuals were sexed following the same criteria used to obtain the F2 generation by a crossbreeding experiment.

Couples from all cross directions that exhibited the phenotype according to the standard species description produced 100% offspring with the same characteristic as the F1 generation. The couples that exhibited melanic phenotypes (male and female *dark morphs*) also produced 100% individuals with the same characteristic (*dark morphs*) in the two analyzed generations (F1 and F2). Of the five couples in the crossbreeding experiment between standard females and *dark morph* males, one couple did not copulate, three couples produced standard triatomines in the F1 generation, and one couple produced offspring with the same proportion of both phenotypes (1:1). The couples whose progenitors were melanic females and standard males produced standard bugs only. For one couple in this cross-experiment, fewer offspring were produced in the F1 generation (n = 11) than the other couples **(Table 1)**. This couple also produced a high number of unviable eggs, which appeared to be unfertilized **(Table 1)**.

The results obtained from the triatomines used in the F2 generation of the crossbreeding experiment for *dark morph* male x *dark morph* female and standard male x standard female were similar to the results obtained in the F1 generation. For the F2 generation standard female x melanic male cross, three couples with males and females that had the standard phenotype in F1, produced F2 with both phenotypes. The other two couples in this group did not copulate **(Table 2)**.

The melanic *T. infestans* found in northeastern Argentina was first described as a sub-species, called *T. infestans melanosoma*. Later, it was suggested to be a new species, which was grounded on the argument of its dark coloring[10]. However, today it is well documented that the specimens found in Argentina and the Bolivian Chaco are an intra-specific phenotypic variation. Among the Triatominae species, black forms have only been described for *T. infestans* and *T. brasiliensis*, being the taxonomic question of the *T. brasiliensis* complex recently solved[11]. However, this report is the first to demonstrate this phenotypic variation for triatomine bugs belonging to the genus *Rhodnius.*

Despite the general chromatic pattern of the body representing an extremely useful tool identifying Triatominae species, this criterion should not be considered as absolute. In the case of *Rhodnius,* particularly the species of the *R. prolixus* complex, which are phylogenetically very close, and are still considered undecipherable by some[2,3,12], the chromatic characteristics are very important factors for correctly identifying the species. The triatomines described here as *dark morph* cannot be identified through the current classification keys.

Inter-specific crossbreeding experimental studies are very useful for validating the taxonomic status of Triatominae species, even in allopatric species[13]. In the case of the *R. prolixus* complex, the presence of viable laboratory hybrids has been

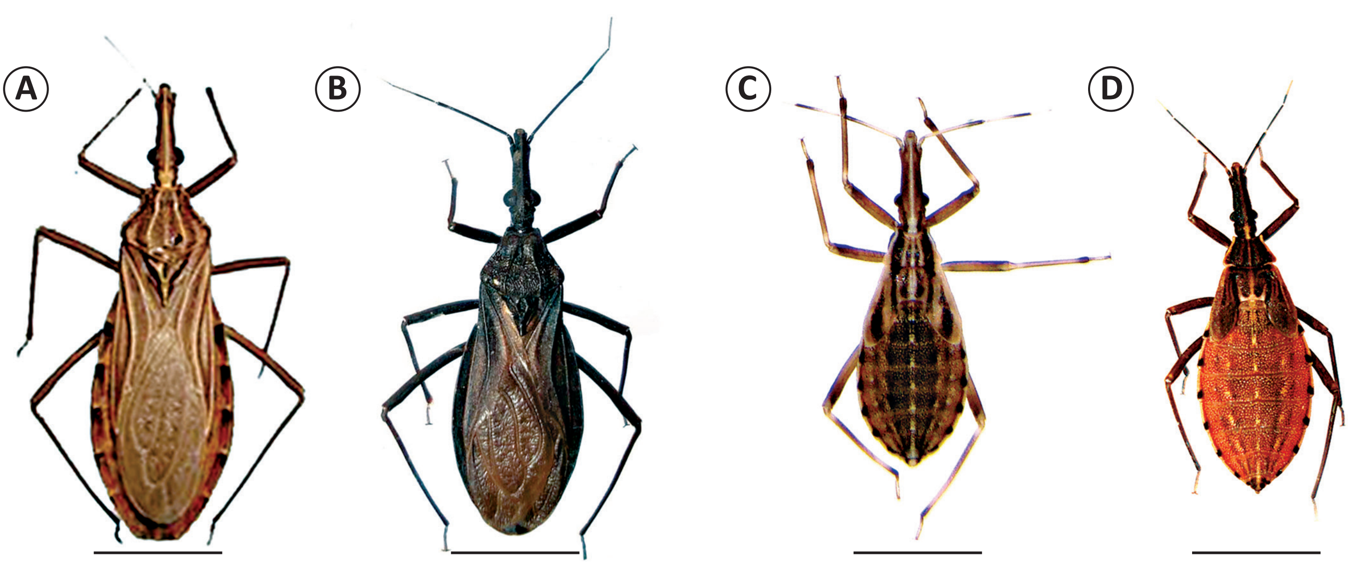

FIGURE 1 - A) Standard *Rhodnius nasutus* adult. B) Dark morph *R. nasutus* adult. C) Standard *R. nasutus* fifth stage nymph used in the intra-specific cross-experiments. D) Dark morph *R. nasutus* fifth stage nymph used in the intra-specific cross-experiments. Fifth stage nymphs were separated according to the head coloring in the intra-specific cross-experiments. Scale bar = 5mm.

TABLE 1 - Crossbreeding of *Rhodnius nasutus* showing the quantity of insects produced by each couple and the possible genotype of the parental and F1 generation.

1. ♀ standard x ♂ dark[a]	Dark (%)	Standard (%)	Genotype (parental and F1)[b]	Probable F1
Couple 1	zero	73 (100.0)	dd x SS = 100% Sd	100% standard
Couple 2	zero	75 (100.0)	dd x SS = 100% Sd	100% standard
Couple 3	NC	NC	NC	NC
Couple 4	zero	65 (100.0)	dd x SS = 100% Sd	100% standard
Couple 5	44 (50.0)	44 (50.0)	dd x Sd = 50% Sd + 50% dd	50% standard and 50% dark
Total	44 (14.6)	257 (85.4)		
2. ♀ dark x ♂ standard	Dark (%)	Standard (%)	Genotype (parental and F1)	Probable F1
Couple 1	zero	39 (100.0)	dd x SS = 100% Dd	100% standard
Couple 2	zero	11 (100.0)*	dd x SS = 100% Dd	100% standard
Couple 3	zero	63 (100.0)	dd x SS = 100% Dd	100% standard
Couple 4	zero	56 (100.0)	dd x SS = 100% Dd	100% standard
Couple 5	zero	39 (100.0)	dd x SS = 100% Dd	100% standard
Total	zero	208 (100.0)		
3. ♀ standard x ♂ standard	Dark (%)	Standard (%)	Genotype (parental and F1)	Probable F1
Couple 1	NC	NC	NC	NC
Couple 2	zero	72 (100.0)	SS x SS = 100% SS	100% standard
Couple 3	zero	65 (100.0)	SS x SS = 100% SS	100% standard
Couple 4	zero	55 (100.0)	SS x SS = 100% SS	100% standard
Couple 5	zero	83 (100.0)	SS x SS = 100% SS	100% standard
Total	zero	275 (100.0)		
4. ♀ dark x ♂ dark	Dark (%)	Standard (%)	Genotype (parental and F1)	Probable F1
Couple 1	83 (100.0)	Zero	dd x dd = 100% dd	100% dark
Couple 2	83 (100.0)	Zero	dd x dd = 100% dd	100% dark
Couple 3	69 (100.0)	Zero	dd x dd = 100% dd	100% dark
Couple 4	87 (100.0)	Zero	dd x dd = 100% dd	100% dark
Couple 5	117 (100.0)	Zero	dd x dd = 100% dd	100% dark
Total	439 (100.0)			

[a]**♀:** female; **♂:** male; standard: *R. nasutus* of typical color; dark: melanic *R. nasutus*. [b]**S:** allele from standard *R. nasutus*; **D or d:** allele from dark morph *R. nasutus.* **F1:** generation one; **NC:** no copulation. *Many unviable eggs

demonstrated[12]. Considering that, in some geographic regions, *Rhodnius* species occur sympatrically, it is possible to find natural hybrids with phenotypic characteristics that are distinct to the parental. *R. nasutus* is described as a species of the Triatominae, with a red-brownish body as its general characteristic. This coloring is very similar to the color of the fibers and stem of the carnaúba palm tree[2,14], which is considered as the main ecotope for *R. nasutus*. Thus, this coloration disguises the triatomines, protecting them from predators. A study of *R. nasutus* collected from five species of palm trees in the south mountain region of the State of Ceará, Brazil, found chromatic variation among the collected individuals, with those from carnaúba showing typical coloring[2]. The other four morphotypes had brownish coloring, which was very similar to the stem and sheath foliage of the palm trees that they inhabited[2]. While chromatic differences were observed, melanic *R. nasutus* were not found at any of the sampling sites. These observations corroborate previous studies, suggesting that ecotopes the inhabited by triatomines may influence the coloration of these insects due to phenotypic plasticity. This association between triatomine bugs and palm trees was observed in the similar chromatic characteristics of *R. pictipes*, which has a darker hue, and *R. robustus*[15], which has a pinker, light-brown hue, with the palm trees that they inhabit.

TABLE 2 - Crossbreeding experiment of *Rhodnius nasutus* showing the quantity of insects produced by each couple and the possible genotype of the F1 and F2 generations.

1. ♀ standard x ♂ dark[a]	Dark (%)	Standard (%)	Genotype (F1 and F2)[b]	Probable F2
Couple 1	28 (36.8)	48 (63.2)	Sd x Sd = SS, 2Sd, dd	75% standard and 25% dark
Couple 2	15 (27.8)	39 (72.2)	Sd x Sd = SS, 2Sd, dd	75% standard and 25% dark
Couple 3	NC	NC	NC	NC
Couple 4	NC	NC	NC	NC
Couple 5	28 (36.8)	48 (63.2)	Sd x dd = 50% Sd + 50% dd	50% standard and 50% dark
Total	71 (34.5)	135 (65.5)		
2. ♀ dark x ♂ standard	Dark (%)	Standard (%)	Genotype (F1 and F2)	Probable F2
Couple 1	25 (24.8)	76 (75.2)	Sd x Sd = SS, 2Sd, dd	75% standard and 25% dark
Couple 2	14 (28.0)	36 (72.0)*	Sd x Sd = NN, 2Sd, dd	75% standard and 25% dark
Couple 3	NC	NC	NC	NC
Couple 4	14 (20.6)	54 (79.4)	Sd x Sd = SS, 2Sd, dd	75% standard and 25% dark
Couple 5	38 (37.3)	94 (62.7)	Sd x Sd = SS, 2Sd, dd	50% standard and 50% dark
Total	91 (25.9)	260 (74.1)		
3. ♀ standard x ♂ standard	Dark (%)	Standard (%)	Genotype (F1 and F2)	Probable F2
Couple 1	-	-	-	-
Couple 2	Zero	57 (100.0)	SS x SS = 100% SS	100% standard
Couple 3	Zero	32 (100.0)**	SS x SS = 100% SS	100% standard
Couple 4	Zero	67 (100.0)	SS x SS = 100% SS	100% standard
Couple 5	Zero	94 (100.0)	SS x SS = 100% SS	100% standard
Total	Zero	250 (100.0)		
4. ♀ dark x ♂ dark	Dark (%)	Standard (%)	Genotype (F1 and F2)	Probable F2
Couple 1	-	-	-	-
Couple 2	63 (100.0)	Zero	dd x dd = 100% dd	100% dark
Couple 3	91 (100.0)	Zero	dd x dd = 100% dd	100% dark
Couple 4	24 (100.0)	Zero	dd x dd = 100% dd	100% dark
Couple 5	37 (100.0)	Zero	dd x dd = 100% dd	100% dark
Total	215 (100.0)	Zero		

[a]**♀:** female; **♂:** male; standard: *R. nasutus* of typical color; dark: melanic *R. nasutus*. [b]**S:** allele from standard *R. nasutus*; **d:** allele from dark morph *R. nasutus.* **F1:** generation one; **F2:** generation two; **NC:** no copulation. *Many unviable eggs. **Unviable eggs.

The emergence of *R. nasutus dark morphs* were very rare in the colonies maintained in the insectary for four years, appearing spontaneously among standard *R. nasutus* collected from carnaúba. This way, we show that the existence of melanic *R. nasutus* is due to the genetic inheritance, and is also very rare in nature. Thus, the crossbreeding experiments between standard *R. nasutus* phenotypes with *dark morph* phenotypes confirmed that this type of inheritance is recessive Mendelian. Only crosses of standard males x *dark morph* females and *dark morph* males x standard females were not fully successful, producing unviable eggs. The phenotypic variation shown here for *R. nasutus* further reinforces the intraspecific heterogeneity in the Triatominae subfamily, demonstrating the importance of using rigorous criteria for describing new species.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Lent H, Wygodzinsky P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bull Amer Mus Nat Hist 1979; 163:520.
2. Dias FBS, Bezerra CM, Machado EMM, Casanova C, Diotaiuti L. Ecological aspects of *Rhodnius nasutus* Stål, 1859 (Hemiptera: Reduviidae: Triatominae) in palms of the Chapada do Araripe in Ceará, Brazil. Mem Inst Oswaldo Cruz 2008; 103:824-830.
3. Abad-Franch F, Monteiro FA, Jaramillo N, Gurgel-Gonçalves R, Dias FBS, Diotaiuti L. Ecology, evolution and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe *Rhodniini* (Triatominae). Acta Trop 2009; 110:159-177.
4. Lima MM, Coutinho CF, Gomes TF, Oliveira TG, Duarte R, Borges-Pereira J, et al. Risk presented by *Copernicia prunifera* palm trees in the *Rhodnius nasutus* distribution in a Chagas disease-endemic area of the Brazilian northeast. Am J Trop Med Hyg 2008; 79:750-754.
5. Almeida CE, Pacheco RS, Noireau F, Costa J. *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera: Reduviidae) I: Isoenzymatic and Chromatic Patterns of Five Populations from the State of Rio Grande do Sul, Brazil. Mem Inst Oswaldo Cruz 2002; 97:829-834.
6. Noireau F, Flores R, Gutierrez T, Dujardin JP. Detection of sylvatic dark morphs of *Triatoma infestans* in the Bolivian Chaco. Mem Inst Oswaldo Cruz 1997; 92:583-584.
7. Cortez MR, Emperaire L, Piccinali RV Gürtler RE, Torrico F, Jansen AM, et al. Sylvatic *Triatoma infestans* (Reduviidae, Triatominae) in the Andean valleys of Bolivia. Acta Trop 2007; 102:47-54.
8. Ceballos LA, Piccinali RV, Berkunsky I, Kitron U, Gürtler RE. First Finding of Melanic Sylvatic *Triatoma infestans* (Hemiptera: Reduviidae) Colonies in the Argentine Chaco. J Med Entom 2009; 46:1195-1202.
9. Rosa JA, Barata JMS, Barelli N, Santos JLF, Belda Neto FM. Sexual distinction between 5th instar nymphs of six species of Triatominae (Hemiptera Reduviidae). Mem Inst Oswaldo Cruz 1992; 87:257-264.
10. Lent H, Jurberg J, Galvão C, Carcavallo RU. *Triatoma melanosoma*, novo status para *Triatoma infestans melanosoma* Martinez, Olmedo & Carcavallo, 1987 (Hemiptera: Reduviidae). Mem Inst Oswaldo Cruz 1994; 89:353-358.
11. Costa J, Correia NC, Neiva VL, Gonçalves TCM, Felix M. Revalidation and redescription of *Triatoma brasiliensis macromelasoma* Galvão, 1956 and an identification key for the *Triatoma brasiliensis* complex (Hemiptera: Reduviidae: Triatominae). Mem Inst Oswaldo Cruz 2013; 108:785-789.
12. Barrett TV. Current research on Amazonian Triatominae. Mem Inst Oswaldo Cruz 1988; 83 (suppl I):441-447.
13. Belisário CJ, Pessoa GCD, Diotaiuti L. Biological aspects of crosses between *Triatoma maculata* Erichson, 1848 and *Triatoma pseudomaculata* Corrêa & Espínola, 1964 (Hemiptera: Reduviidae). Mem Inst Oswaldo Cruz 2007; 102:517-521.
14. Dias FBS, Paula AS, Belisário CJ, Lorenzo MG, Bezerra CM, Harry M, et al. Influence of the palm tree species on the variability of *Rhodnius nasutus* Stål, 1859 (Hemiptera, Reduviidae, Triatominae). Infec Gen Evol 2011; 11:869-877.
15. Gaunt M, Miles M. The ecotopes and evolution of triatomines bugs (Triatominae) and their associated Trypanosomes. Mem Inst Oswaldo Cruz 2000; 95:557-565.