



Color patterns of the hermit crab *Calcinus tibicen* (Herbst, 1791) fail to indicate high genetic variation within COI gene

Silvia Sayuri Mandai¹

Raquel Corrêa Buranelli¹  orcid.org/0000-0002-3092-9588

Fernando Luis Mantelatto¹  orcid.org/0000-0002-8497-187X

¹ Laboratório de Bioecologia e Sistemática de Crustáceos (LBSC), Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo (USP). Av. Bandeirantes, 3900. 14040-901 Ribeirão Preto, São Paulo, Brazil.

SSM E-mail: silvia.mandai@usp.br

RCB E-mail: raquel.buranelli@gmail.com

FLM E-mail: fmantel@usp.br

ZOOBANK <http://zoobank.org/urn:lsid:zoobank.org:pub:A7CC24B7-E8BB-4925-A0C7-3929E7534EDF>

ABSTRACT

Apart from traditional characters, other data have been used for taxonomy, like color patterns. Based on the different colors (green and orange) observed for some *Calcinus tibicen* (Herbst, 1761) specimens, we evaluated the genetic distance for cytochrome oxidase subunit I mitochondrial gene of individuals collected in Pernambuco (northern Brazil) and in São Paulo (southeast Brazil). We found low genetic variation (0.2–1.1%), and no evidence of isolation on our molecular tree based on genetic distance. We suggest high levels of gene flow between specimens with different color patterns, which are polymorphisms and might be related to the kind of nutrition as well different ecological and evolutionary predation characteristics.

KEY WORDS

Anomura, COI gene, coloration, Diogenidae, polymorphism.

CORRESPONDING AUTHOR

Fernando Luis Mantelatto
fmantel@usp.br

SUBMITTED 28 August 2017
ACCEPTED 22 November 2017
PUBLISHED 26 March 2018

Guest Editors

Alessandra A. de Pádua Bueno
and Sandro Santos

DOI 10.1590/2358-2936e2018008



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Nauplius, 26: e2018008

Morphological characters have been used as the main tool of identification and description of decapod crustaceans, but other information such as life history and development, habitat, mating behavior, coloration, and molecular data have become useful for species recognition (Knowlton, 1986; Malay and Paulay, 2009). With the improvement of optical photo equipment and more facilities during field collections during the last two decades, taxonomists have used more frequently color patterns of living animals or the residual patterns found in preserved specimens for species-specific recognition or identification in some decapod groups (*e.g.* Salmon *et al.*, 1979; Williams and Felder, 1986; Bruce, 1978; Malay and Paulay, 2009; Negri *et al.*, 2014; Hamasaki *et al.*, 2017).

Among hermit crabs, color pattern differentiation is quite significant between cryptic species (*e.g.* Asakura and Paulay, 2003; Lemaitre and Poupin, 2003; Poupin and Malay, 2009; Negri *et al.*, 2014), and has been considered relevant in the identification of other decapod species (see Poore *et al.*, 2017 for review). In addition, different color patterns may have significance in the reproductive context, such as being decisive in mate choice (*e.g.* Detto *et al.*, 2006), which may therefore be a barrier of reproductive isolation (Malay and Paulay, 2009). Furthermore, camouflage by coloration is considered a usual anti-predator strategy and might be related to behavioral and morphological mechanisms (*e.g.* Hamasaki *et al.*, 2017).

The hermit crab genus *Calcinus* Dana, 1851 includes 43 species (McLaughlin *et al.*, 2010) and is characterized by having morphologically similar species, causing misinterpretations and misidentifications (Haig and McLaughlin, 1983). Even though changes in color are known for some *Calcinus* species, colors might change markedly and fast during preservation (Haig and McLaughlin, 1983) and this is the main reason why color is frequently rejected in some systematic studies of decapod crustaceans (*e.g.* Chassard-Bouchaud, 1965; Malay and Paulay, 2009).

In this context, as color patterns have been considered important taxonomic characters for *Calcinus* species, we sought to evaluate if color differences between some specimens of *Calcinus tibicen* (Herbst, 1761) co-occurring in the same locality were based on high genetic differences.

We evaluated the genetic distance for the cytochrome oxidase subunit I (COI) mitochondrial gene between six differently colored individuals (orange and green) from the states of Pernambuco (PE, 08°16'15.56"S 34°56'40.34"W) and São Paulo (SP, 23°25'20.03"S 45°3'42.61"W), Brazil (Fig. 1A, B) [PE orange: CCDB 5327 – GenBank accesses KT897571, KT897572; PE green: CCDB 5328 – GenBank accesses KT897552, KT897570; SP orange: CCDB 0769 – GenBank access KT897493; SP green: CCDB 0778 – GenBank access KT897522]. The identification of the specimens was confirmed based on morphological characters (Melo, 1999). Here we report that the green pattern has changed its color after preservation in 70% ethanol.

General molecular analysis protocols followed Schubart *et al.* (2000), with modifications according to Mantelatto *et al.* (2006). For DNA extraction, salting-out extraction method (Miller *et al.*, 1988) was used, with changes aiming suitability to the material. The primers COH6 (5'-TADACTTCDGGRTGDCCAAARAAYCA-3') and COL6b (5'-ACAAATCATAAAGATATYGG-3') (Schubart and Huber, 2006) were used in amplification by means of a Polymerase Chain Reaction (PCR) (Sambrook *et al.*, 1989). Thermocycling conditions included: initial denaturation (2 min at 94°C), 35 annealing cycles (94°C for 30 s; 50°C for 30 s; 72°C for 1 min); final extension (2 min at 72°C). PCR products were purified using the kit SureClean and sequenced with the ABI Big Dye Terminator Mix (Applied Biosystem, Carlsba, CA, USA) in an ABI 3730xl DNA Analyzer (Applied Biosystems automated sequencer). Sequences were edited, and a consensus sequence was obtained from both strands (forward and reverse directions), using the program BioEdit 7.2.5.0 (Hall, 1999). Sequences (608 base pairs) were aligned via Clustal W (Thompson *et al.*, 1994) with interface to BioEdit 7.2.5.0 (Hall, 1999). Matrixes of genetic divergence were computed in the program Molecular Evolutionary Genetics Analysis (MEGA) 6, using Kimura 2-parameters (Kimura, 1980). A Neighbor Joining tree was constructed using the software MEGA 6 (Tamura *et al.*, 2013) based on genetic distance. In this analysis we used the following species as outgroups: *Calcinus californiensis* Bouvier, 1898 (GenBank access: KT89759), *Calcinus explorator* Boone, 1930 (GenBank

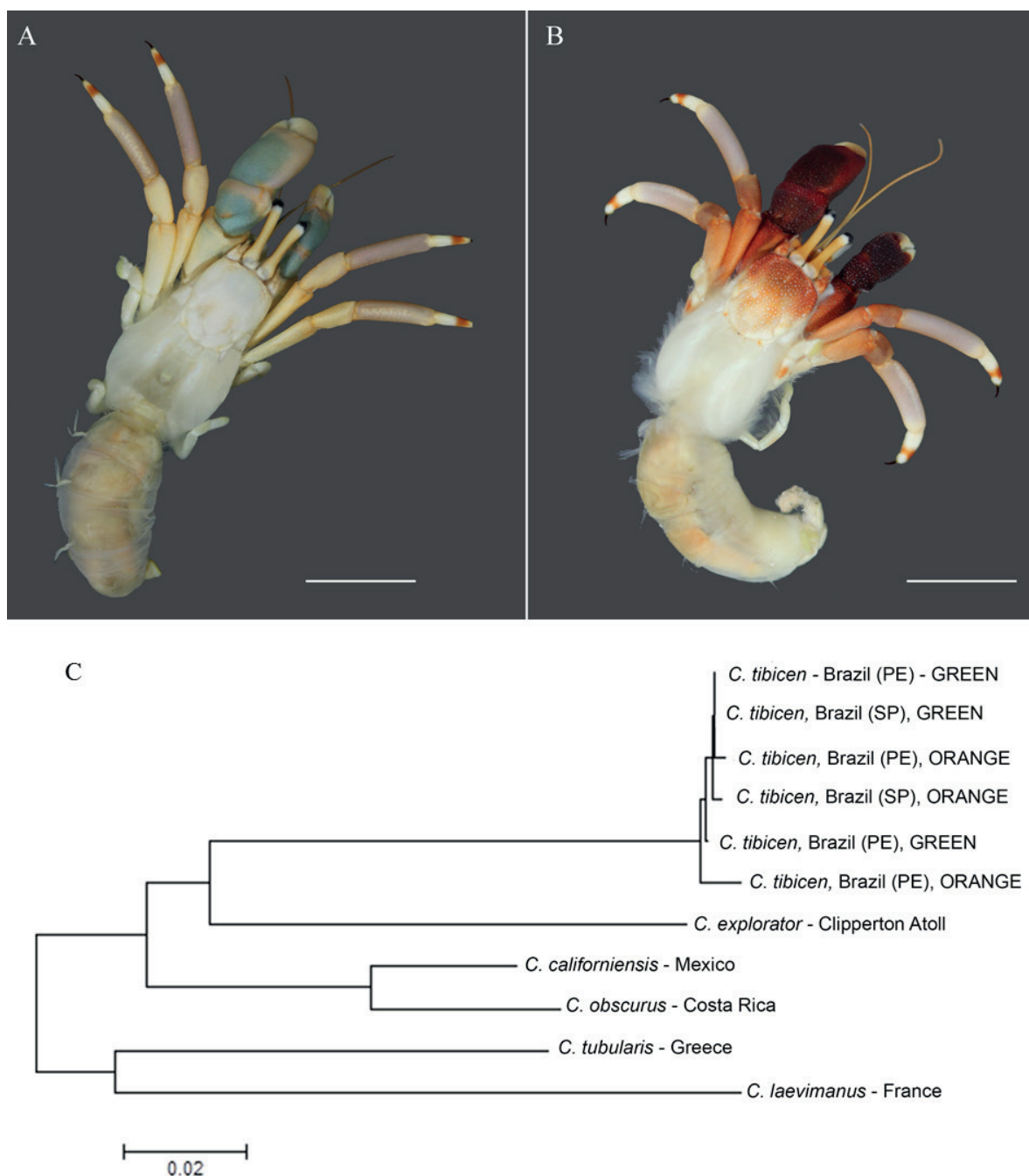


Figure 1. Specimens of *Calcinus tibicen* (Herbst, 1761) from two Brazilian localities (PE: Pernambuco and SP: São Paulo), showing differences in the color patterns of the chelae in life. (A) green claw male, CCDB 5328, scale bar = 10 mm. (B): orange claw male, CCDB 5327, scale bar = 11 mm (both from Pernambuco). (C) Neighbor Joining tree of *C. tibicen* specimens based on genetic distance for COI gene. Photos by R.C. Buranelli.

access: FJ620320), *Calcinus laevimanus* (Randall, 1840) (GenBank access: FJ620270), *Calcinus obscurus* Stimpson, 1859 (GenBank access: KT897589) and *Calcinus tubularis* (Linnaeus, 1767) (GenBank access: KT897590).

Our results showed high levels of genetic homogeneity (0.2–1.1% of variation), and the

Neighbor Joining tree based on the genetic distance also pointed out no isolation amongst the differently colored specimens – geographically separated by more than 2,500 km – of *C. tibicen* from Pernambuco and São Paulo (Fig. 1C); the opposite, *i.e.* high levels of variation (15.5–23.3%), was observed when comparing these colored individuals with the outgroups. These

data indicate that these patterns are polymorphisms of the species as reported for other decapods (e.g. Terossi and Mantelatto, 2010), and are probably related to ecological factors, but hardly to a reproductive barrier.

Here we suggest some ecological factors, non-exclusive, probably related to the color patterns of the chelae: thermoregulation, intraspecific communication, and camouflage (Endler, 1978). The tones of the animals, in general, tend to be similar to their backgrounds (e.g. Poulton, 1890; Duarte *et al.*, 2016), and in this case, some of the animals were collected in association and/or close to the red or green algae banks (FLM, pers. obs.). It means that individuals with orange or green chelae inhabit the same region, nevertheless, they can occupy different habitats with distinct background coloration, what might represent camouflage aspects, as already reported for other decapods (e.g. Bauer, 1981; Duarte *et al.*, 2016). Morphology, coloration, and behavior can be linked to different ecological and evolutionary predation pressure under the same habitat (Hacker and Madin, 1991), related to predator color vision and predator color acuity (Endler, 1978). Natural predators of *C. tibicen* in this situation might be the fish *Diodon hystrix* Linnaeus, 1758 (Randall, 1967), and invertebrates such as *Octopus vulgaris* Cuvier, 1797 and *Octopus briareus* Robson, 1929 (Gilchrist, 2003). Then, the variation in the chelae continued in *C. tibicen*'s populations representing adaptive significance against different predators and the color patterns can be an interesting way of camouflage against visually-hunting predators (Bauer, 1981; Hamasaki *et al.*, 2017). Due to the relationship with the substrate, it can also be conjectured that the tones of colors are also related to the kind of nutrition of the specimens.

Therefore, although in genus *Calcinus* color patterns are taken to species-specific recognition or identification, in the case of *C. tibicen* evaluated here, this characteristic may not be used (in opposition to what was reported in Salmon *et al.*, 1979; Bruce, 1978; Malay and Paulay, 2009; Negri *et al.*, 2014; Hamasaki *et al.*, 2017). Both colors of chelae occur within the same species, which was represented by the low rates of genetic variation among them and when compared with the genetic range of variation (0–9.9%) observed along the entire *C. tibicen* distribution in the Western Atlantic (Mandai *et al.*, unpublished data). Some causes related to these differences in colors of the chelae, orange or

green, were inferred here; however, more studies about *C. tibicen*'s ecology and feeding might be taken to better understand this intriguing pattern in some regions of its distribution.

ACKNOWLEDGEMENTS

This article was part of the Bachelor's thesis of SSM supported by scientific fellowship of Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (2014/10639-1). Major financial support was provided by FAPESP (2002/08178-9, Temático Biota 2010/50188-8; Coleções Científicas 2009/54931-0; APQ 2016/50376-5), Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Procs. 473050/2007-2, 471011/2011-8, 504322/2012-5; Research Scholarship PQ302748/2010-5, 304968/2014-5) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Ciências do Mar II 2005/2014 - 23038.004308/2014-14) to FLM. RCB thanks the Post-Doctoral fellowship by CNPQ (PROTAX 150462/2016-6). We thank anonymous reviewers for their suggestions that help to improve the quality of this paper during revision process. The authors, especially FLM, are honored to recognize the many achievements of our colleague, Ludwig Backup, to development of carcinology in Brazil and for this reason dedicate this contribution for this commemorative edition to him.

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