A new melanistic variant of the caterpillar hunter *Calosoma wilcoxi* LeConte, 1848 from Texas, United States of America and a preliminary phylogeny of the genus *Calosoma* Weber, 1801 (Coleoptera: Carabidae)

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Abstract—Two aberrant ground beetle (Coleoptera: Carabidae) specimens from the genus *Calosoma* Weber, 1801 were collected in Waco, Texas, United States of America, in 2012–2013. The specimens, which are morphologically most similar to *Calosoma wilcoxi* LeConte, 1848, but are dark blue-black instead of the typical metallic green. We employed DNA barcoding and phylogenetic methods to confirm the identities of the aberrant specimens. Preliminary phylogenetic analyses of cytochrome oxidase subunit 1 (COI) sequences of central Texas and southwestern species place the aberrant specimens with 100% confidence as *C. wilcoxi*. The new variant of *C. wilcoxi* presumably occurs at low densities. Frequent collecting from 2011 to 2014 resulted in the discovery of only two of the aberrant coloured individuals among hundreds of typical green specimens. These specimens (to our knowledge) represent the first published record of melanistic *Calosoma* from North America. While the majority of North American species in the genus are naturally black, two of the most widely distributed and abundant species, *C. scrutator* (Fabricius, 1775) and *C. wilcoxi*, are typically green. We sequenced the aberrant form as well as all species co-occurring with the new colour morph at the collection locality and used records from GenBank and the Barcode of Life Data System to generate a preliminary phylogeny of the genus, which suggested that some of the currently established subgenera are likely not monophyletic.

Introduction

Ground beetles of the genus *Calosoma* Weber, 1801 (Coleoptera: Carabidae) are large, conspicuous predatory beetles common throughout North America and most of the Northern Hemisphere. At least three species of the genus were introduced in the United States of America over the last century as biological control agents for caterpillars, although only *C.* (*Calosoma*) *sycophanta* (Linnaeus, 1758) appears to have become established (Burgess 1911; Bousquet 2012). The only major analyses covering the Nearctic fauna (Gidaspow 1959; Lindroth 1961) and the description of a single new species (Dajoz 1997) resulted in the recognition of 41 valid native North American species and subspecies and a single introduced species (Bousquet 2012). Six species of *Calosoma* occur in central Texas, United States of America: *Calosoma* (*Callitropa*) *externum* Say, 1823, *C.* (*Callitropa*) *macrum* LeConte, 1826, *C.* (*Calodrepa*) *scrutator* (Fabricius, 1775), *C.* (*Calodrepa*) *wilcoxi* LeConte, 1848, *C.* (*Camegonia*) *marginale* Casey, 1897, and *C.* (*Castrida*) *sayi* Dejean, 1826.

In April of 2011, an aberrant specimen of this genus was collected in Waco, Texas. The specimen was similar in size and habitus to *C. wilcoxi*, but was

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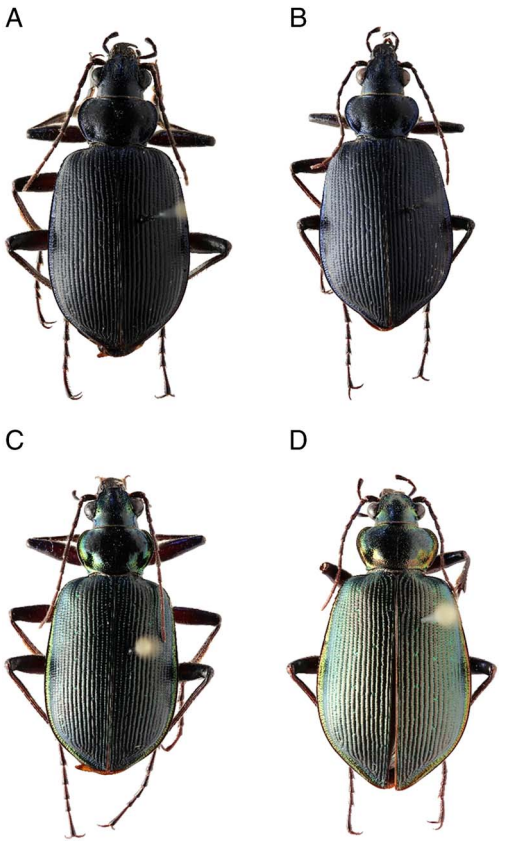
Fig. 1. Habitus photographs of the two colour morphs of *Calosoma wilcoxi*. (A) Male and (B) female of blue morph. (C) Male and (D) female of the green morph.



dark blue-black and not the typical green (Fig. 1). Only one specimen was known until 2013, when a second was found at the same location. In order to properly identify these specimens, a combined morphological and genetic approach, using DNA barcoding methods, was applied.

DNA barcoding has enriched the study of biodiversity by providing quick and cost effective methods for the identification of distinct mitochondrial lineages and robust species-level identification (Hebert *et al*. 2004). For animals, a section of the cytochrome oxidase subunit I (COI) gene is the primary marker for DNA barcoding. By comparing morphological variation to DNA barcoding results, increased confidence in the identification of similar or cryptic taxa can be achieved.

Here we genetically compared the two aberrant *Calosoma* specimens to specimens of all other local species in order to determine whether these specimens represented a separate lineage, or phenotypic plasticity within *C. wilcoxi*. We sequenced the COI gene of the aberrant specimens, as well as the five species sympatric at the sampling location, *C.* (*Carabosoma*) *angulatum* Chevrolat, 1834 from Arizona, United States of America, and two northern African species (*C.* (*Caminara*) *oliveri* Dejean, 1831 and *C.* (*Campalita*) *maderae* (Fabricius, 1775)) as outgroups. In addition, we incorporated previously published sequences as available on GenBank (www.ncbi.nlm.nih.gov/genbank) and the Barcode of Life Data Systems (BOLD) (www.boldsystems.org), providing further coverage of North American, South American, and European species (Table 1). Combined with our newly generated sequences, we generated a preliminary phylogeny covering 20 species of *Calosoma* from four continents. We discuss the results of our phylogenetic reconstruction and compare them to previously established relationships in the genus.

Materials and methods

Laboratory methods

We extracted total DNA of 15 specimens belonging to eight species, of which two specimens belonged to the new phenotype (Cal14, Cal15; specimens preserved in the collection of the Centrum für Naturkunde (CeNak) of the University of Hamburg, Hamburg, Germany). Extracts were prepared from a single mesothoracic leg of dried or ethanol stored material. DNA extractions were carried out according to Paxton *et al*. (1996). Polymerase chain reactions were performed using standard protocols and barcoding primers provided by Folmer *et al*. (1994): LCO1490 (5'-GGTCA ACAAATCATAAAGATATTGG-3') and HCO2 198 (5'-TAAACTTCAGGGTGACCAAAAAATC A-3'). Polymerase chain reactions (total volume 10 µL) contained 0.1 µL GoTaq (5 u/µL) (Promega, Madison, Wisconsin, United States of America), 2 µL GoTaq-Buffer (5 ×) (Promega), 0.2 µL $MgCl_2$ (25 mM), 0.3 µL of both primers (10 µM), 0.2 µL dNTPs (10 mM), 1.0 µL DNA template, and 5.9 µL H_2O. Polymerase chain reaction cycling conditions were as follows: initial denaturation 94 °C for 3.0 minutes, followed by 35 cycles of 94 °C for 30 seconds, 48 °C for 45 seconds, 72 °C for one

Table 1. GenBank/BOLD accession numbers for sequences used in this study.

GenBank/BOLD accession	Species	Subgenus	Location	References
KU521575	*Calosoma angulatum*	*Carabosoma*	United States of America: Arizona: Santa Cruz County	This study
JX279623	*C. breviusculus*	*Callisthenes*	Turkey	Andujar *et al.* (2012)
NEONU1722_13	*C. eremicola*	*Carabosoma*	United States of America: California	BOLD
KM844552	*C. frigidum*	*Calosoma*	Canada	BOLD
JF887528	*C. frigidum*	*Calosoma*	Canada	BOLD
KM849025	*C. frigidum*	*Calosoma*	Canada	BOLD
KM844960	*C. frigidum*	*Calosoma*	Canada	BOLD
KC245651	*C. granatense*	*Castrida*	Ecuador, Galapagos	Hendrickx *et al.* (2015)
KC245652	*C. granatense*	*Castrida*	Ecuador, Galapagos	Hendrickx *et al.* (2015)
KM446586	*C. inquisitor*	*Calosoma*	Germany	Hendrickx *et al.* (2015)
KM447200	*C. inquisitor*	*Calosoma*	Germany	Hendrickx *et al.* (2015)
KM452546	*C. inquisitor*	*Calosoma*	Germany	Hendrickx *et al.* (2015)
KC245578	*C. leleuporum*	*Castrida*	Ecuador, Galapagos	Hendrickx *et al.* (2015)
KC245579	*C. leleuporum*	*Castrida*	Ecuador, Galapagos	Hendrickx *et al.* (2015)
KC245551	*C. linelli*	*Castrida*	Ecuador, Galapagos	Hendrickx *et al.* (2015)
KC245552	*C. linelli*	*Castrida*	Ecuador, Galapagos	Hendrickx *et al.* (2015)
KR132535	*C. macrum*	*Callitropa*	United States of America: Texas: McLennan County	Hendrickx *et al.* (2015)
KU521568	*C. macrum*	*Callitropa*	United States of America: Texas: McLennan County	This study
KU521569	*C. macrum*	*Callitropa*	United States of America: Texas: McLennan County	This study
KU521574	*C. maderae*	*Campalita*	Morocco	This study
KR132537	*C. marginale*	*Camegonia*	United States of America: Texas: McLennan County	Hendrickx *et al.* (2015)
KU521578	*C. marginale*	*Camegonia*	United States of America: Texas: McLennan County	This study
KU521579	*C. marginale*	*Camegonia*	United States of America: Texas: McLennan County	This study
JX259814	*C. marginale*	*Camegonia*	United States of America: Colorado	Gibson *et al.* (2012)
NEONU583_11	*C. marginale*	*Camegonia*	United States of America: Texas	BOLD
KU521576	*C. oliveri*	*Caminara*	Morocco	This study
KU521577	*C. oliveri*	*Caminara*	Morocco	This study
NEONU1105_12	*C. peregrinator*	*Carabosoma*	United States of America: New Mexico	BOLD
NEONU1106_12	*C. peregrinator*	*Carabosoma*	United States of America: New Mexico	BOLD
NEONU1107_12	*C. peregrinator*	*Carabosoma*	United States of America: New Mexico	BOLD
NEONU1108_12	*C. peregrinator*	*Carabosoma*	United States of America: New Mexico	BOLD
NEONU1109_12	*C. peregrinator*	*Carabosoma*	United States of America: New Mexico	BOLD
NEONU588_11	*C. prominens*	*Camegonia*	United States of America: California	BOLD

Table 1. *Continued*

GenBank/BOLD accession	Species	Subgenus	Location	References
KR132536	*C. sayi*	*Castrida*	United States of America: Texas: McLennan County	Hendrickx *et al.* (2015)
KU521581	*C. sayi*	*Castrida*	United States of America: Texas: McLennan County	This study
KU521582	*C. sayi*	*Castrida*	United States of America: Texas: McLennan County	This study
KC245546	*C. scrutator*	*Calodrepa*	United States of America: Texas: McLennan County	Hendrickx *et al.* (2015)
KU521580	*C. scrutator*	*Calodrepa*	United States of America: Texas: McLennan County	This study
KM844287	*C. scrutator*	*Calodrepa*	Canada	BOLD
GU013578	*C. scrutator*	*Calodrepa*	Canada	Park *et al.* (2010)
NEONU576_11	*C. scrutator*	*Calodrepa*	United States of America: Arizona	BOLD
NEONU577_11	*C. scrutator*	*Calodrepa*	Mexico	BOLD
NEONU1726_13	*C. semilaeve*	*Chrysostigma*	United States of America: California	BOLD
NEONU1727_13	*C. semilaeve*	*Chrysostigma*	United States of America: California	BOLD
JQ693413	*C. sycophanta*	*Calosoma*	Spain	Andujar *et al.* (2012)
KM440993	*C. sycophanta*	*Calosoma*	France	Hendrickx *et al.* (2015)
KM451281	*C. sycophanta*	*Calosoma*	France	Hendrickx *et al.* (2015)
KR132538	*C. wilcoxi*	*Calodrepa*	United States of America: Texas: McLennan County	Hendrickx *et al.* (2015)
KU521570	*C. wilcoxi*	*Calodrepa*	United States of America: Texas: McLennan County	This study
KU521571	*C. wilcoxi*	*Calodrepa*	United States of America: Texas: McLennan County	This study
KU521572	*C. wilcoxi*	*Calodrepa*	United States of America: Texas: McLennan County	This study
KU521573	*C. wilcoxi*	*Calodrepa*	United States of America: Texas: McLennan County	This study
KM845926	*C. wilcoxi*	*Calodrepa*	Canada	BOLD
HM909088	*Carabus clathratus*	*Limnocarabus*	Finland	Pentinsaari *et al.* (2014)

BOLD, Barcode of Life Data System.

minute, and final elongation 72 °C for eight minutes. Polymerase chain reactions products were purified using EXOSAP-it (Affymetrix, Santa Clara, California, United States of America) following the manufacturer's instructions. Sanger sequencing was performed by GATC-Biotech (Konstanz, Germany).

Genetic analyses

Sequences were inspected and aligned using the MAFFT (Multiple Alignment using Fast Fourier Transform) algorithm as implemented in Geneious v.6 (Kearse *et al*. 2012). The most suitable substitution model was determined to be GTR + I + G using jModelTest2 (Guindon and Gascuel 2003; Darriba *et al*. 2012). We then used the Bayesian approach implemented in MrBayes v.3.2.5 (Ronquist *et al*. 2012) to reconstruct the phylogeny of *Calosoma*. A sequence of *Carabus clathratus* Linnaeus, 1761 (HM909088) was defined as the outgroup. We ran MrBayes for 20 million generations sampling every 2000 generations resulting in 10 000 sampled trees. Convergence was confirmed by the average split frequencies being consistently below 0.02. A burn-in of 1000 trees was discarded before summarising the results in a consensus tree. The tree was visualised with FigTree v. 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

A total of 15 new COI sequences were generated for this study. After trimming, each sequence was 620 base pairs in length (Table 1). An additional 38 sequences from previous studies were incorporated into our phylogenetic analyses (Table 1). In total, our phylogenetic analyses included 53 sequences belonging to 20 species from the genus *Calosoma*, representing lineages from North America, South America, North Africa, and Europe and one sequence of *Carabus clathratus* as outgroup. The resulting tree (Fig. 2) indicates that *C*. (*Calodrepa*) *wilcoxi* is closely related to the European *C*. (*Calosoma*) *inquisitor* (Linnaeus, 1758), which along with *C*. (*Calodrepa*) *scrutator* are the most basal species in the genus of those investigated in this study.

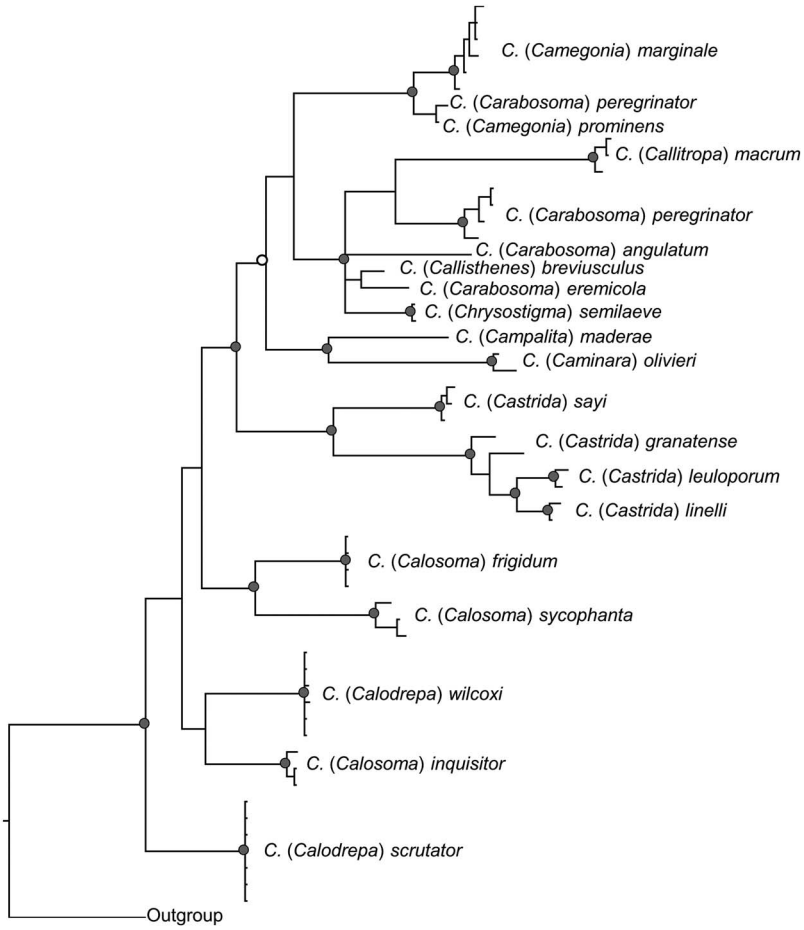
Discussion

Our genetic analyses confirm that the two aberrant *Calosoma* specimens collected in Texas represent black-blue morphological variants of the widespread, typically green *C. wilcoxi*. Melanism is known to occur in a wide variety of Coleoptera, including the families Cantharidae (Brakefield 1985); Carabidae (Davis *et al*. 2007), and the subfamily Cicindelinae (Carabidae) (Pearson *et al*. 2015); Chrysomelidae (Tower 1906); Coccinellidae (Mikkola and Albrecht 1988; Jong *et al*. 1996); Scarabaeidae (Ratcliffe and Mico, 2001); and Tenebrionidae (Harris 1988). Our results confirm the first recorded case of melanism in the genus *Calosoma*. Beetles of the genus, in particular the common North American species *C. scrutator* and *C. wilcoxi*, are well represented in collections; yet, we could find no evidence of previously reported melanism in either of these species, both of which are abundant, widespread, and typically green.

Our phylogenetic analysis, while limited due to our taxonomic and geographic coverage of the genus, does provide some useful information for understanding relationships within the genus. Our study strongly supports the monophyly of the subgenus *Castrida* Motschulsky, 1866 (0.98 posterior probability), based on the four species incorporated into this study (*C. sayi*, *C. granatense* Géhin, 1885, *C. linelli* Mutchler, 1925, and *C. leuloporum* Basilewsky, 1968). However, we did not recover the nominate subgenus *Calosoma* as monophyletic, based on three species incorporated into our analysis (*C. frigidum* Kirby, 1837, *C. sycophanta*, and *C. inquisitor*). Similar results were reported in a previous genetic analysis based on the mitochondrial ND5 marker (Su *et al*. 2005). One specimen of *C.* (*Carabosoma*) *peregrinator* (BOLD ID NEONU1109-12.COI-5P) grouped within a cluster otherwise only consisting of *Camegonia* species (*C. prominens* and *C. marginale*). This is likely based on a misidentification of the specimen.

Our results indicate that *C. wilcoxi* is closely related to the European *C. inquisitor*, and the subgenera of *Calosoma* and *Calodrepa* Motschulsky, 1865 are the most basal of the genus. This does not conform with previous phylogenetic analyses, which indicated that subgenus *Castrida* occupied a basal position (Su *et al*. 2005). However, this may partially be a result of different outgroup choice and taxon sampling. Based on morphological analyses, the subgenus *Calosoma* was originally identified as an ancestral lineage within the subfamily Carabinae (Jeannel 1940); hence, it is not surprising that based on our choice

Fig. 2. Bayesian phylogenetic tree reconstructed with MrBayes; grey circles represent posterior probabilities above 0.95, white circles represent posterior probabilities above 0.90.



of outgroup (*Carabus*) the analyses suggest a basal position of *Calosoma*. While any inference based on the results of our phylogenetic analyses are limited, given the sampling performed to identify the aberrant specimens, our results do indicate that the currently established subgeneric classifications within the genus are likely in need of revision.

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