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Themistoclis Giannoulis
University of Thessaly

Anne-Marie Dutrillaux
Muséum National d'Histoire Naturelle

Julien Touroult
Muséum National d'Histoire Naturelle

Contantina Sarri
University of Thessaly

Zissis Mamuris
University of Thessaly

See next page for additional authors

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Themistoclis Giannoulis
Laboratory of Genetics, Comparative and Evolutionary Biology
Department of Biochemistry and Biotechnology
University of Thessaly
41221 Larissa, Greece

Anne-Marie Dutrillaux
UMR 7205, CNRS/MNHN
Structure et Evolution de la Biodiversité
Département Systématique et Evolution
Muséum National d'Histoire Naturelle
CP 39, 16, rue Buffon
F-75005 Paris, France

Julien Touroult
Muséum National d'Histoire Naturelle
Service du Patrimoine Naturel
CP41, 36 rue Geoffroy Saint-Hilaire
F-75005 Paris, France

Constantina Sarri, Zissis Mamuris
Laboratory of Genetics
Comparative and Evolutionary Biology
Department of Biochemistry and Biotechnology
University of Thessaly
41221 Larissa, Greece

Bernard Dutrillaux
UMR 7205, CNRS/MNHN
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Abstract. Chromosomes of four rare and localized Caribbean Prioninae (Coleoptera: Cerambycidae) species were analysed. All have 26, XY karyotypes. Those of *Solenoptera canaliculata* (Solenopterini) from Guadeloupe nearby islands Les Saintes and Marie-Galante and *S. quadrilineata* from Martinique look similar. They have a single pair of sub-metacentric autosomes. The karyotype of *S. touroulti* from St. Lucia has three sub-metacentric pairs. It appears closer to that of *Hovorodon maxillosum* (Mallodontini) from Marie-Galante which has ten sub-metacentric pairs. The CO1 gene sequence, taking two European species *Aegosoma scabricorne* (Prioninae: Aegosomatini) and *Ergates faber* (Prioninae: Ergatini) as external groups was analysed in *S. canaliculata* and *S. quadrilineata*. In spite of their karyotype similarity, their CO1 genes differ by a strong accumulation of mutations. Thus, either chromosomal or genetic data confirm the species status of the three closely related *Solenoptera* species. Ten different CO1 haplotypes are found among the 21 specimens of *S. canaliculata* studied from les Saintes and Marie-Galante. Both different haplotypes were found in each island and identical haplotypes were found in different islands. Hence, the gene flow was not interrupted. Biogeographical parameters favor the hypothesis that repeated passages between islands were made possible by floating trunks, principally from Les Saintes to Marie-Galante.

Key Words. Chromosomes, CO1 sequence, Lesser Antilles, Prioninae, *Solenoptera*.

Introduction

The genus *Solenoptera* Audinet-Serville, 1832 belongs to the tribe Solenopterini, one of the 21 tribes of Prioninae longhorn beetles (taxonomy following Tavakilian and Chevillotte 2012). This tribe comprises six other genera (*Derancistrodes* Galileo and Martins, 1993, *Derancistrus* Audinet-Serville, 1832, *Elateropsis* Chevrolat, 1862, *Holonotus* Thomson, 1861, *Prosternodes* Thomson, 1861 and *Sphenostethus* Haldeman, 1848) and is composed of 47 species spread in the Antilles and in the American continent from southern USA to Colombia. The genus *Solenoptera* includes 16 species, 8 of which occur in Lesser Antilles, 5 in Greater Antilles, 2 in both Lesser and Greater Antilles and one in Colombia. There is a strong endemism in this genus. Most species are located in one or a small number of islands and each island harbors one or a small number of species. No other genus of Solenopterini occurs in the Lesser Antilles, while *Derancistrodes, Derancistrus, Elateropsis* and *Prosternodes* are found in the Greater Antilles and Bahamas. Only 4 genera are known from the continent: *Holonotus* (3 species) in Central America, Mexico and Colombia, *Sphenostethus* in Texas (1 species), *Elateropsis* in Florida (2 species) and *Solenoptera* (1 species) in Colombia (Monné 2013). Thus, with 85% of species in the Caribbean islands and Bahamas, the speciation process of Solenopterini principally occurred from island to island, with an obligate passage over the seas. In the genus *Solenoptera*, *S. canaliculata* (Fabricius, 1787) is the species with the widest distribution, from Trinidad in the south to Marie-Galante in the north. It is also found in the other satellite islands of Guadeloupe, Les Saintes, 25 km distant from Marie-Galante, but not in the main island of Guadeloupe, less than 10 km distant from Les Saintes. In contrast, *S. touroulti* Dalens and Delahaye, 2007, seems to have a very narrow distribution in a mountain forest of St. Lucia (Dalens and Delahaye 2007). Up to now, it is known by two specimens only. *Solenoptera quadrilineata* (Olivier, 1795) was found in several localities from Martinique. It was also indicated from Guadeloupe, probably erroneously, because no specimen is known from this island (Chalumeau and Touroult 2005). To our knowledge, published genetic data about Prioninae are limited to DNA sequences of 8 species of Prionini (Hunt et al. 2007; Feng et al. 2010) and one species of Callipogonini (Lim et al. 2013), and cytogenetic data are limited to eight species (Dutrillaux 1970; Ferreira and Mesa 1977; Virkki in Smith and Virkki 1978; Ferreira and Mesa 1977; Ferreira et al. 1993). The phylogenetic relationships among the tribes are unclear and even attribution of many species to a given tribe remains a matter of debate.

Here, we propose to (1) report chromosome data about the above cited 3 species of *Solenoptera* and *Hovorodon maxillosum* (Drury, 1773) (Prioninae: Mallodontini) from Marie-Galante with the aim to establish some phylogenic relationships by comparing their karyotypes, (2) compare CO1 mitochondrial gene sequence of *S. canaliculata* and *S. quadrilineata,* (3) understand inter-island dispersion of *S. canaliculata* by comparing the morphotypes (sizes) and CO1 sequences of specimens from 3 islands, and (4) compare their CO1 sequence with that of two other Prioninae, *Aegosoma scabricorne* (Scopoli, 1763) (Aegosomatini) and *Ergates faber* (Linnaeus, 1761) (Ergatini) taken as external groups, and literature data on this subfamily.

Material and Methods

Animals (Fig. 1).

Solenoptera canaliculata: Specimens were collected in decayed trunks or branches from various species of trees as grubs, pupae or imagines. Grubs, captured in December or March during the 2007-2011 years, were further bred in cherry-wood until imago stage, which was generally reached in the following May. Specimens were obtained from Le Chameau, Terre-de-Haut (Les Saintes, 15° 53'N, 61° 52'W), Grande Anse, Terre-de-Bas (Les Saintes 15°85'N, 61°65'W) and the western littoral forest of Marie-Galante (15°58'N, 61°17'W). Two male specimens of each island were used for chromosome analysis, and a total of 21 specimens (7 from each island) was used for DNA sequencing.

Solenoptera touroulti: a single pupa was obtained from Piton Flore in St. Lucia, the type locality, in March 2011. The imago (the second specimen known of this species) was obtained in April.

Solenoptera quadrilineata: four specimens at the grub or pupa stage were captured in dry forests of the southern part of Martinique, near Fort-de-France. One male imago was obtained in August 2011 and

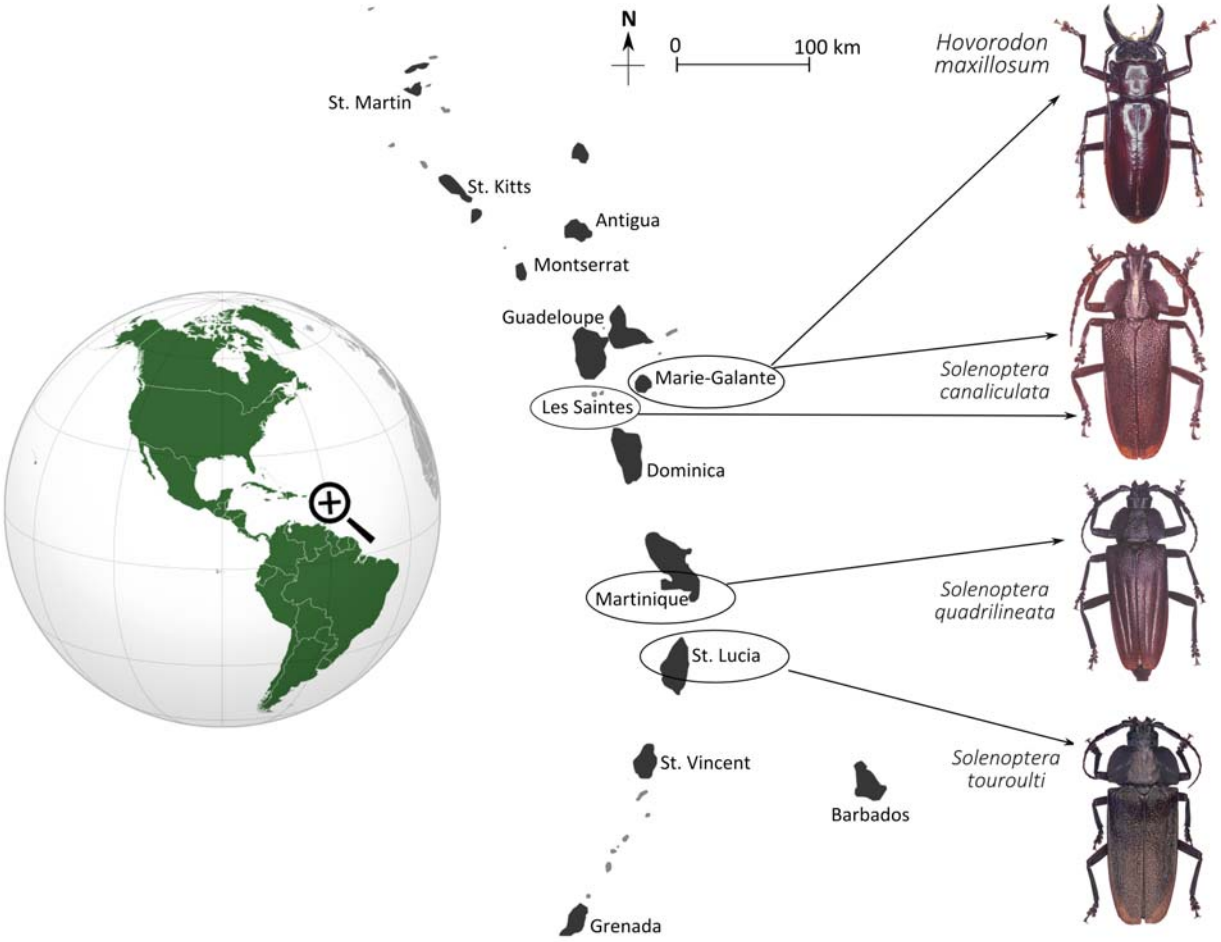


Figure 1. Schematic representation of the Lesser Antilles and photography of the insects under study.

two males and one female imagines in July 2012. All were used for DNA sequencing and the three males were used for karyotyping.

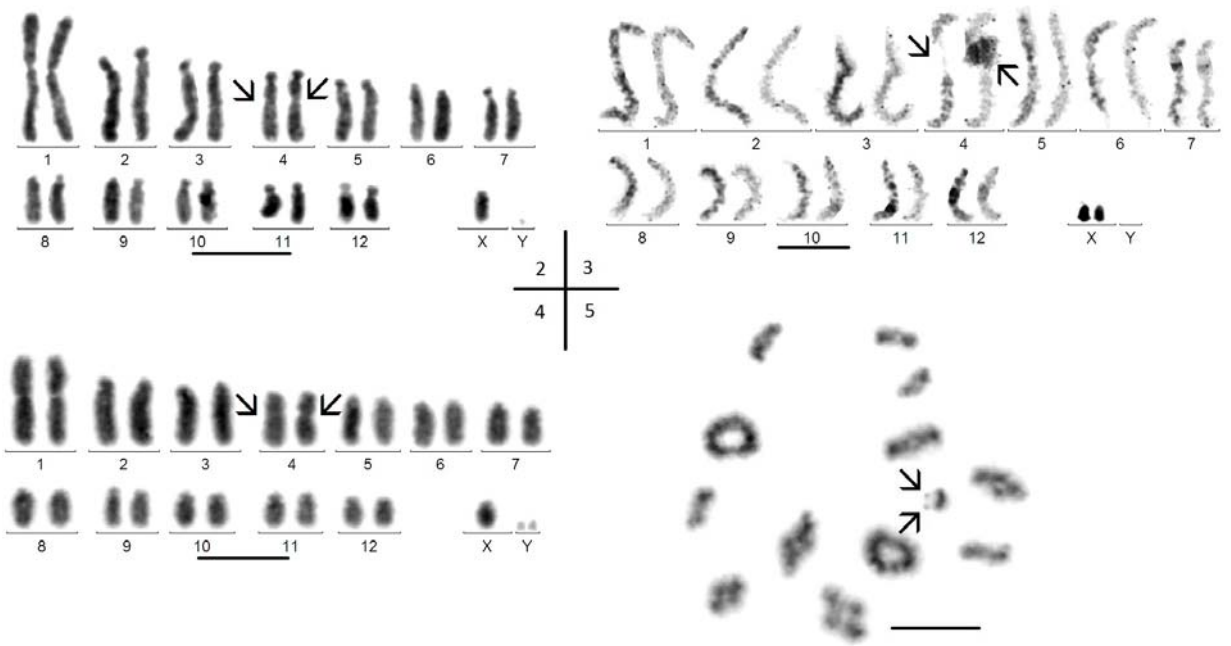
Hovorodon maxillosum: three specimens, one pupa and two grubs were captured in the western littoral forest of Marie-Galante. Grubs were put in cherry-wood until metamorphosis, and young imagines were used for cytogenetic studies in March 2010.

Aegosoma scabricorne: larvae were captured in decayed birch-tree near Irun (Spain, 43°21'N, 1°47'E). They were conserved until young imagines were obtained in May 2011.

Ergates faber: grubs and pupae were captured in decayed trunks in two localities in Greece: near Kalamata (southern Peloponnese, 37°02'20''N, 22°06'51''E) and Parnassos Mountain (Central Greece, 38°32'N 22°37'E) in June 2011.

Cytogenetic Methods. In male Prioninae, gametogenesis is achieved soon after metamorphosis. To obtain mitotic divisions from gonads, it was thus necessary to perform dissections early, before imago maturity. In *Solenoptera* species, we found that imagines with an incompletely pigmented abdomen were yet suitable. This stage allowed us to make a correct species identification. A lateral incision of the abdomen did not alter the morphology, and allowed us to take up to four testicular follicles. Follicles were dropped in 8.8 g/l aqueous KCl solution for 15 min and further submitted to our usual protocols (Dutrillaux et al. 2010).

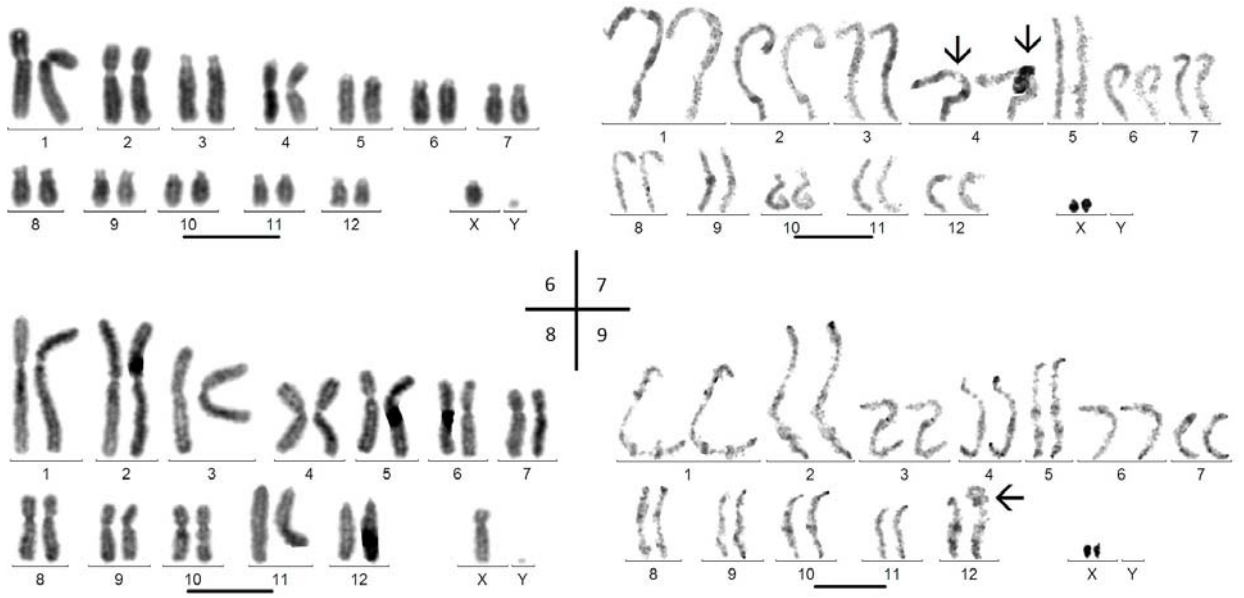
Molecular Methods. Mitochondrial DNA was isolated from 29 specimens (Fig. 10). A 516 bp segment of the *CO1* gene was amplified using the set of primers: C1-J-2195 TTG-ATT-TTT-TGG-TCA-TCC-AGA-AGT and L2-N-3014 TCC-AAT-GCA-CTA-ATC-TGC-CAT-ATT-A. PCR reactions (50 μL) contained 200-500 ng DNA, 1 x Taq buffer, 2 mM MgCl2, 0.2 mM of each dNTP, 50 pmoles of each primer and 1 U



Figures 2-5. Karyotypes of *Solenoptera* species. **2)** Giemsa stained male karyotype of *S. canaliculata*. The arrows on chromosome 4 indicate an elongated region, which is the only region stained by C-banding. Bar = 10 μm, as in other figures. **3)** Karyotype of a spermatocyte I at pachynema of *S. canaliculata*, after Giemsa (left) and silver (right) stainings. The NOR on bivalent 4 looks elongated, and is surrounded by nucleoli after silver staining. **4)** Giemsa stained XYY karyotype of *S. quadrilineata*. Chromosome 4 is elongated at the same position as in *S. canaliculata* (arrow), which indicates the position of the NOR. **5)** Giemsa stained metaphase I of the XYY male of *S. quadrilineata* exhibiting a parachute sex trivalent. Arrows point out the two Ys. The accidental presence of two Ys in male beetles was estimated to about 1 per cent (Dutrillaux and Dutrillaux 2011). Bars = 10μm.

Taq polymerase (Invitrogen, Carlsbad, USA). The cycling conditions consisted of an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 52°C for 40sec and extension at 72°C for 1min, with a final extension at 72°C for 10min. PCR products were purified using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and sequenced bi-directionally. Nucleotide sequences were aligned using ClustalX (Thompson et al. 1997). MEGA4 (Tamura et al. 2007) was used to calculate pairwise and overall nucleotide differences and to construct the UPGMA and the neighbour-joining tree with 10000 bootstrap replicates. According to the findmodel server (http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html) (Guindon et al. 2005) the GTR+G+I model of evolution that fitted the data set. A Bayesian analysis was also performed with MRBAYES version 3.1 (Huelsenbeck and Ronquist 2001), under the GTR+G+I model of sequence evolution, using random starting trees run for 8 x 106 generations and sampled every 100 generations. The burn-in frequency was set to the first 25% of the sampled trees. Direct examination of the sampled log-likelihood values showed that values had reached a stationary equilibrium by this point. All trees preceding this cut-off were discarded when calculating posterior nodal probabilities, mean log-likelihood scores and a summary phylogeny including estimates of branch lengths.

Our data were compared with sequences of species belonging to Prioninae retrieved from Genbank: AF332947.1 *Prionus insularis* Motschulscky, 1857, GU130432.1 *Rhaphipodus fruhstorferi* Lameere, 1903, JN093124.1 *Callipogon relictus* Semenov, 1899, GU130426.1 *Dorysthenes paradoxus* (Faldermann, 1833), GU130427.1 *Dorysthenes granulosus* (Thomson, 1861), GU130429.1 *Dorysthenes zivetta* (Thomson, 1877), GU130431.1 *Prionus murzini* Drumont and Komiya, 2006, GU130430.1 *Prionus gahani* Lameere, 1912, GU130428.1 *Dorysthenes fossatus* (Pascoe, 1857), HM062974.1 *Prionus asiaticus* (Faldermann, 1837).



Figures 6-9. Karyotypes of *Solenoptera* and *Hovorodon* species. **6)** Giemsa stained karyotype of *S. touroulti* exhibiting three pairs (# 1, 2 and 4) of sub-metacentric autosomes. **7)** Karyotype of a spermatocyte I at pachynema of *S. touroulti*, after Giemsa (left) and silver (right) stainings. Notice the lack of synapsis at the NOR locus (arrow) and the silver staining of nucleoli. **8)** Giemsa stained karyotype of *H. maxillosum* exhibiting 10 pairs of sub-metacentric autosomes. **9)** Karyotype of a spermatocyte I at pachynema of *H. maxillosum*, after Giemsa (left) and silver (right) stainings. Arrow: NOR on bivalent 12. Bars = 10μm.

Results

Chromosome analysis. *Solenoptera canaliculata*: all the specimens from the three islands have the same karyotype, composed of 26 chromosomes. Pair #1 and X chromosomes are sub-metacentric, all the others are acrocentric, with a heterochromatic short arm of variable size. The Y chromosome is punctiform: 26, XY (Fig. 2). The C-banding is weak on centromeric regions. The most intense C-bands are observed in intercalary position of two acrocentrics that we classified as pair #4. In Giemsa-stained chromosomes, this C-band corresponds to a pale and elongate region, mimicking a centromere. In meiotic cells, the X and Y bivalent is of the parachute type at metaphase I ($12+Xy_p$). At the pachytene stage, one bivalent exhibits a large intercalary uncoiled region after Giemsa staining. This region is stained by silver, and surrounded by argyrophilic components (nucleoli, Fig. 3). We conclude that this bivalent corresponds to the chromosome pair #4, which carries the nucleolus organizer region (NOR) in intercalary position.

Solenoptera quadrilineata: its karyotype is similar to that of *C. canaliculata*: 26, XY. However, in one of the three males studied, there is an additional punctiform chromosome (Fig. 4). At metaphase I of meiosis, the two punctiform chromosomes are associated with the X, forming a parachute with two "parachutists", demonstrating that there are two Y chromosomes: 27, XYY and $12+XY_pY_p$ (Fig. 5). The NOR is at the same position as in *S. canaliculata* on bivalent 4 at pachynema.

Solenoptera touroulti: the single specimen studied has also a 26,XY formula. However, pairs 2 and 4 are sub-metacentric (Fig. 6), while they are acrocentric in the two former species. Furthermore, pair #1 is less metacentric. Thus, three chromosomal rearrangements at least, presumably inversions, separate this karyotype from that of the other two species. Only pair # 4 exhibits C-bands, at the proximal part of its long arm. This region is also silver stained. At pachynema, this region of bivalent 4 exhibits a marked elongation and is heavily silver stained and surrounded by nucleoli (Fig. 7).

Hovorodon maxillosum: its karyotype is composed of 26 chromosomes, all but pairs #11 and 12 and the Y are meta- or sub-metacentric (26, XY Fig. 8). C-banding is very poor, almost limited to the centromeric region of one of the two acrocentric pairs, presumably #12. The sex chromosomes have a classical

parachute configuration at metaphase I. The NOR is located at the extremity of one bivalent, probably #12 (Fig. 9).

Aegosoma scabricorne and *Ergates faber*: Their karyotypes, composed of 20 and 24 chromosomes, respectively, were described in Dutrillaux (1970).

CO1 DNA sequencing of *S. canaliculata* and *S. quadrilineata*. Out of the 516 sites, 179 were variable while 150 of them were informative for parsimony. Among the 179 variable sites, 77.2% are third codon positions, while first and second codon positions are much more conserved (18.4% and 4.4% respectively), a pattern typically observed in segments under strong functional constraints. Some insect molecular studies using CO1 are in accordance with these findings (Cognato and Sperling 2000; Villalba et al. 2002; Giannoulis et al. 2011). Sequence divergence within groups and species ranged from 0.0% to 41.1%.

The various haplotypes of *S. canaliculata* were clearly separated from that of *S. quadrilineata* with a net sequence divergence of 12.5% (Fig. 10). Thus, although their karyotypes are similar, *S. canaliculata* and *S. quadrilinetata* can be considered as good species.

In *S. canaliculata*, the 21 specimens of this study were distributed into 10 different haplotypes. Seven specimens from each island (Fig. 10), Marie-Galante (MG), Terre-de-Haut (TdeH) and Terre-de-Bas (TdeB) had 3, 4 and 5 different haplotypes, respectively. A same haplotype was shared by MG (4 specimens) and TdeB (2 specimens) and another haplotype was shared by TdeH (2 specimens) and TdeB (2 specimens). The genetic distances remain small (mean net and mean genetic distances between islands d=0.0% and d=1.9%, respectively) between the different haplotypes, and more interestingly, not more important between specimens belonging to different islands than to the same island.

Morphological comparisons between *S. canaliculata* specimens from the three islands. In each island, there was no variation of the general morphology, except that of size. The same was true for inter-island comparison, but in spite of this variation, we found that the size of specimens from Marie-Galante was larger than that from Les Saintes:

- Les Saintes TDH: n=8, range: 31-42 mm, mean: 36.5 mm, standard variation: 4.4 mm;
- Les Saintes TDB: n=10, range: 23-39 mm, mean: 29.8 mm, standard deviation: 4.4 ;
- Marie-Galante: n= 17, range: 32-48 mm, mean: 40.9 mm, standard deviation: 4.6 mm.

Discussion

Overall knowledge of Prioninae phylogeny. Both molecular and chromosomal published data on Prioninae are scarce. In this large subfamily of Cerambycidae composed of more than 1000 species grouped into 21 tribes (Tavakilian and Chevillotte 2012), cytogenetic data of 8 species only were reported (Dutrillaux 1970; Virkki in Smith and Virkki 1978; Ferreira and Mesa 1977; Ferreira et al. 1993). Their chromosome numbers range from 20 in *Aegosoma scabricorne* (Scopoli, 1763) (Aegosomatini) to 34 in *Pyrodes nitidus* (Fabricius, 1787) (Mallaspini). A single species belonging to Solenopterini, *Derancistrus thomae* (Linnaeus, 1767), now considered as *Solenoptera thomae,* was succinctly studied (Virkki in Smith and Virkki 1978). Only its meioformula $13+Xy_p$ was reported, thus its karyotype formula should be 28, XY, instead of 26, XY in the 3 *Solenoptera* species studied here. In the literature, the other Prioninae species with 26 chromosomes are *Mallodon spinibarbe* (Linnaeus, 1758) and *Enoplocerus armillatus* (Linnaeus, 1767) (Virkki in Smith and Virkki 1978; Ferreira and Mesa 1977), which belong to the tribes Mallodontini and Callipogonini, respectively. The morphology of their chromosomes is unknown. *Hovorodon maxillosum,* studied here, is closely related to *M. spinibarbe* and has also 26 chromosomes, including 10 sub-metacentric autosomal pairs. Thus, the number of 26 chromosomes is shared by species belonging to Mallodontini, Callipogonini and Solenopterini. This may indicate a phylogenetic relationship between these three Neotropical tribes. In the other subfamilies of Cerambycidae, most (55% to 62%) of species possess 20 chromosomes (calculated from Smith and Virkki 1978), a number found in most Polyphaga beetles. It is noteworthy that 10/11 species of Prioninae possess more than 20 chromosomes. Thus, the increase of chromosome number may be a characteristic of Prioninae. To the best of our knowledge, data on DNA sequences are limited to 11 species of Prioninae, of which 9 belong to the tribe Prionini, one to Callipogonini and one to Mallodontini (Feng et al. 2010; Hunt et al. 2007; Lim et al.

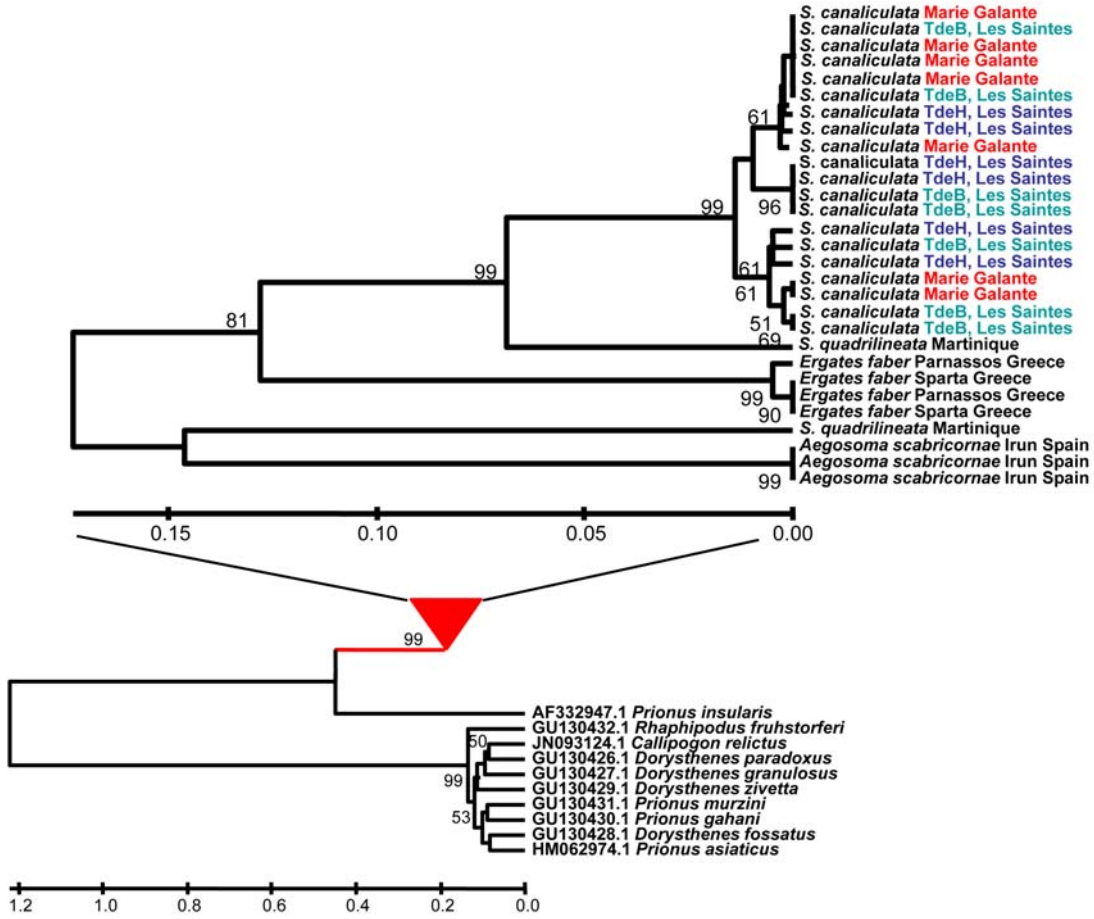


Figure 10. Phylogenetic tree resulting from the Bayesian analysis, clustering the different haplotypes of Prioninae retrieved from the Genbank with those assessed of this study as compressed subtree (red triangle). Expansion of the subtree and phylogenetic relationships between the different haplotypes is also shown. The topology of the clusters was similar for the UPGMA and NJ trees. Numbers above branches of the major clusters correspond to posterior probabilities from the Bayesian analysis. TdeH and TdeB: Terre-de-Haut and Terre-de-Bas islands from Les Saintes.

2013). This supports the possible monophyletic origin of Prioninae, but remains too limited to provide information about the phylogeny of the subfamily.

Chromosome evolution of the three *Solenoptera* species. In our study, the similarity of the karyotypes of the specimens of *S. canaliculata* from three satellite islands of Guadeloupe and that of *S. quadrilineata* from Martinique suggests that the chromosomal evolution is not very active in the genus *Solenoptera*. The chromosomal rearrangements having occurred between these species and *S. touroulti*, from St. Lucia, which differs by three inversions, at least, indicates a probable reproductive separation. Interestingly, one of these inversions involves chromosome 4. Both the acrocentric (in *S. canaliculata* and *S. quadrilineata*) and the sub-metacentric (in *S. touroulti*) forms of this chromosome carry the NOR. Whatever the original morphology of this chromosome, it can be reconstructed that one of the break points of the inversion was located at contact with or in the heterochromatic region harboring the NOR. The location of the NOR on chromosome 12 in *N. maxillosum* and chromosome 3 in the 3 species of *Solenoptera*, shows that translocations and inversions have occurred and that the NOR may represent a site of chromosome instability. With its three pairs of sub-metacentrics, the karyotype of *S. touroulti* looks closer to that of *H. maxillosum* than those of *S. canaliculata* and S. *quadrilineata*, which have a single sub-metacentric pair. It is generally admitted that the ancestral karyotype of polyphagan Coleoptera was composed of 20 chromosomes (Smith and Virkki 1978). This applies to Cerambycidae (Dutrillaux and Dutrillaux 2009). By comparing many karyotypes, we proposed that ancestral chromosomes were meta- or sub-metacentric (Dutrillaux and Dutrillaux 2012). The karyotype of *Aegosoma*

scabricorne (Prioninae, Aegosomatini), with its 20 meta- or sub-metacentric chromosomes (Dutrillaux 1970), may represent a configuration close to that of Prioninae ancestors. The passage from 20 to 26 chromosomes, here observed, indicates that complex chromosome changes (fissions) occurred, possibly in common ancestors of Neotropical tribes. With the caution imposed by the scarcity of the data, we propose that the meta-/sub-metacentric chromosomes of *H. maxillosus* compose a karyotype closer to that of Prioninae ancestors than that of the *Solenoptera* species. In this hypothesis, the karyotype of *S. touroulti*, which would have conserved three sub-metacentrics would be less derived than those of *S. canaliculata* and *S. quadrilineata*. This was not expected because the distribution area of *S. touroulti* seems to be very limited in St. Lucia, while that of the *S. canaliculata* is much larger in the Lesser Antilles. This recalls the observation that, in the genus *Cyclocephala* Latreille, 1829 (Dynastinae), the distribution of *C. insulicola* (ancestral karyotype) is limited to the altitude rain forest of Guadeloupe while that of *C. tridentata* Fabricius, 1801 (highly derived karyotype) is widely spread in Guadeloupe (Giannoulis et al. 2012). In both situations, the species with a limited distribution would not be a vicariant, but a relict species.

Hypothesis on the colonization of Guadeloupe archipelago by *S. canaliculata*. The karyotypes of *S. quadrilineata* and *S. canaliculata* are not different, although these species are morphologically distinct. It indicates that there is no chromosomally induced gametic barrier, while the CO1 sequence data exhibits a clear separation between the two species. Thus, chromosome changes did not play any role in the separation of these species. For *S. canaliculata*, there is neither chromosomal, nor sequence difference in relation with their locations in the different islands under study. The 10 observed haplotypes can be separated into two groups (Fig. 9) in which there are specimens from the three islands, and three to five different haplotypes are found in each island. Thus, the gene flow is not interrupted between islands, although Marie-Galante is 25 km distant from Les Saintes. In this context, the size difference reported above between specimens from Les Saintes and Marie-Galante cannot be explained by a genetic divergence, but rather by external factors such as better nutrition in the large Marie-Galante than in the small and dry Les Saintes islands.

Surprisingly, *S. canaliculata* is unknown from the main island of Guadeloupe, only 10 km north from Les Saintes and 26 km west from Marie-Galante. Two main parameters must be considered to explain oversea colonization: wind orientation and upper layer marine current orientation. Wind orientation in this region is principally East to West, which should have facilitated the passage from Marie-Galante to Guadeloupe. Obviously, this did not work. Marine currents between Marie-Galante and Les Saintes parallel Guadeloupe, alternate with tides, but have a predominant west to east orientation. Thus, the passages of *S. canaliculata* between Les Saintes and Marie-Galante were probably achieved by floating trunks and principally from Les Saintes to Marie Galante. This fits with the presence of *S. canaliculata* in the residual forests along the sandy beaches of the West coast of Marie-Galante facing Les Saintes. It fits also with the higher number (8 vs. 3) of haplotypes in Les Saintes, suggesting a longer presence, in these two small islands than in Marie-Galante. It would be interesting to study *S. canaliculata* specimens from Dominica, 40-50 km distant to the south of Les Saintes and Marie-Galante, but we failed to capture this species in Dominica.

Conclusion

The three *Solenoptera* species studied here are not only separated by their location, St. Lucia, Martinique and satellite islands of Guadeloupe, but also by their chromosomes (*S. touroulti* vs. the two others) and the number of their mutations in CO1 gene (*S. quadrilineata* vs *S. canaliculata*). Their status of good species is thus reinforced. As contrast, there is no genetic separation between *S. canaliculata* specimens from les Saintes and Marie-Galante, which share the same genetic polymorphism. This suggests that repeated passages have occurred from one island to another, presumably from Les Saintes to Marie Galante. The lack of *S. canaliculata* in the neighboring main island of Guadeloupe may be explained by geographical conditions.

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