African Journal of Biotechnology Vol. 9(10), pp. 1470-1480, 8 March, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Phylogenetic information reveals the peculiarity of *Caryedon serratus* (Coleoptera, Chrysomelidae, Bruchinae) feeding on *Cassia sieberiana* DC (Caesalpinioideae)

M. Sembène[1]*, K. Kébé[1], A. Delobel[2] and J. Y. Rasplus[3]

[1]Département de Biologie Animale, Faculté des Sciences et Techniques, BP 5005 Dakar, Sénégal.
[2]247 Avenue Paul-Langevin, F-92260 Fontenay-aux-Roses, France.
[3]INRA - UMR 1062 CBGP (INRA/ IRD/ Cirad/ Montpellier SupAgro), Campus international de Baillarguet, CS 30016, 34988 Montferrier-sur-Lez, France.

Accepted 15 December, 2009

Studies over the past 20 years on natural populations indicated that sympatric speciation may be far more common and widespread among plants and animals than previously thought. By using molecular phylogenetics (on a combined data set of two genes), the relationships between *Caryedon serratus* native forms and forms feeding on groundnut was investigated. The specific objectives were to clarify the taxonomic status of *C. serratus* feeding on *Cassia sieberiana* DC. Morphological analysis was conducted, using parts of the adult specimens which were dry-mounted for the studies. Morphological study comprised extraction of the genitalia, which were cleared and mounted in *Canada balsam* following standard procedures. Results showed a strong differentiation of insects from different hosts' trees, with specimens from *C. sieberiana* possibly representing a sibling species.

Key words: *Caryedon serratus*, phylogeny, taxonomy, Cytochrome B, ITS, groundnut, *Cassia sieberiana.*

INTRODUCTION

The preservation of biodiversity requires knowing the degree of variety of what is to be preserve. Numerous genetic studies have been reported in the literature which aimed at studying the differentiation of the natural populations and at understanding the factors which are due to this differentiation. Generally, species are unevenly distributed in their area of distribution. They are divided into several populations, of which migration is allowed. The rates of migration of individuals from a population to another can vary according to the considered species, their capacities to move on long distances and the nature of the environment that separates the populations (that is, if it is very hostile to the sort or not).

Populations which exchange few individuals in terms of migration tend to be more differentiated genetically than populations where there is a lot of migration. However, migration is not the only parameter to be taken into account when the genetic structuralization of a species is being studied. The demography of the populations constitutes a parameter as important as the migration. For example, at rate of equal migration, the differentiation between populations by genetic drift is made much quicker in small-sized populations than in populations with big strength.

The case of the groundnut seed-beetle, *Caryedon serratus*- Olivier (Coleoptera: Chrysomelidae Bruchinae) is interesting to study. The identity of *C. serratus* (Olivier, 1790) was established by Decelle, who published a comprehensive synonymy in 1966. It has a small number of native host plants in Africa such as *Bauhinia rufescens, Bauhinia* (*Piliostigma*) *reticulata, Bauhinia* (*P.*) *thonningii,* tamarind (*Tamarindus indica*), which all belong to the leguminous subfamily Caesalpinioideae. Less than a century ago, this beetle added groundnut (*Arachis hypogaea*) (Leguminoseae Papilionoideae) to its diet, thus

*Corresponding author. E-mail: sembene@ird.sn or Mbacké.sembene@ucad.edu.sn. Tel: (00221)775541463.

Table 1. Population sampling: origin, host plant, abbreviations and numbers of scored individuals (between parentheses).

Sites and coordinates	Sampling plants, abbreviations of populations and number of scored individuals				
	A. hypogaea	***B. rufescens***	***C. sieberiana***	***P. reticulatum***	***T. indica***
Thies (16°04'W, 15°33'N)	AT(38)	BT (39)	CT (23)	PT (36)	TT (35)
Linguère (15°25'W, 15°88'N)	AL (39)	BL (37)	CL (38)	PL (36)	TL (33)
Fimela (16°41'W, 14°08'N)	AF (35)	BF (33)	CF (42)	PF (35)	TF (38)
Keur Baka (15°57'W, 13°56'N)	AK (42)	BK (35)	CK (39)	PK (40)	TK (33)
Molodo (06°05'W, 14°13')	AM (16)	BM (44)	CM (34)	PM (45)	TM (37)

becoming one of the major African insect pests. The problem is still spreading in some parts of the continent (Delobel, 1995). African countries such as Niger, Mali, Senegal and Congo derive a substantial part of their national income from groundnut cultivation. In these areas, the presence of the seed-beetle has compelled farmers, traders and national storage facilities to make use of large amounts of insecticides, thus dramatically reducing their annual income. *C. serratus* was recently identified in Queensland as a pest of *Cassia brewsteri*, a source of edible gum (Cunningham and Walsh, 2002).

In Asia, *C. serratus* is usually known as the tamarind seed-beetle (Arora, 1977), sometimes as a pest of groundnuts (Vazirani, 1975; Dick, 1987) and it is often also reported as feeding on the seeds of various species of *Acacia* (Mimosoideae) or *Cassia* (Caesalpinioideae) (Mukerji and Chatterjee, 1951; Arora, 1977; Vir and Jindal, 1996). However, Indian specimens of tamarind and/or groundnut seed beetles have often been known as *Caryedon gonagra* (Fabricius, 1798). In 1966, Decelle synonymized *gonagra* and *serratus*, partly on the basis of Southgate and Pope's (1957) redescription and illustration of *C. gonagra*. In 1894, Decaux gave a quite precise description of the morphology and biology of a tamarind seed-beetle, which he named *Caryoborus tamarindi*. The name *tamarindi* has been in use in Egypt (Shomar, 1963) and in India (Vazirani, 1975).

Reviews in the mid-1990s recognized eight confirmed host species associated with *C. serratus*, all of them belonging to the Caesalpiniaceae subfamily with the exception of peanut, *A. hypogaea* L. (Fabaceae) (Pierre and Huignard, 1990; Nilsson and Johnson, 1992; Delobel, 1995). *C. serratus* is widely distributed throughout Africa, from Senegal to South Africa and in southern Asia (Johnson, 1966). In the neotropics and Hawaii, it is not associated with groundnuts (Robert, 1985; Nilsson and Johnson, 1992; Delobel, 1995). *C. serratus* is thought to have shifted to groundnuts from trees of the Caesalpiniaceae family in West Africa in the early 1900s (Delobel, 1995). The ability of *C. serratus* to colonize groundnuts (introduced from South America to Africa towards the end of the 19th century) must be considered in connection with recent advances in our knowledge of the phylogeny of the genus *Caryedon.* Morphological and molecular data indicated that *C. serratus* belongs to a clade of Mimosoideae feeding species and is therefore not directly related with other Caesalpinioid feeders (Silvain and Delobel, 1998). It may be hypothesized that *C. serratus* has acquired a more performant secondary compound detoxification ability than its Mimosoideae feeding relatives and that this ability gave it the potential to expand its host spectrum beyond Mimosoideae (ancestral host range) and Caesalpiniodeae (extended host range).

Studies on food plant selection and larval development (Robert, 1985; Ali-Diallo, 1991), sub-specific variation in morphological (Sembène and Delobel, 1996) and genetic characters (Sembène et al., 1998; Sembène and Delobel, 1998; Sembène, 2004, 2006) in *C. serratus* provided support for the hypothesis that a certain amount of genetic isolation exists between populations with different feeding habits.

In this study, interest is on clarifying the taxonomic status of beetles feeding on *C. sieberiana*. In order to solve the problem, the DNA of *C. serratus* specimens reared from groundnuts, *B. rufescens*, *C. sieberiana*, *Piliostigma reticualtum* and *T. indica* in Senegal and Mali was analyzed. For this, comparisons used involved both allopatric (but feeding on the same host) and sympatric (but feeding on different hosts) seed-beetle populations.

MATERIALS AND METHODS

Study site

Samples were collected in several localities in Senegal: Ouarak (16°04'W, 15°33'N), Thiès (16°56'W, 14°48'N), Fimela (16°41'W, 14°08'N), Keur Baka (15°57'W, 13°56'N). One sample was collected in Molodo (06°05'W, 14°13') in Mali. The sites were within the sahelian zone of Senegal, where the rainy season lasts from July to September (500 to 700 mm rainfall).

C. serratus samples

Beetles used in this study were reared from eggs, larvae or pupae on/inside pods collected on the different host trees between 2004 and 2007 (Table 1). The pods of the five hosts of *C. serratus* (*B. rufescens*, *C. sieberiana*, *P. reticulatum*, *T. indica*) were non-dehiscent. Females laid their eggs on the surface of ripe pods and newly hatched larvae bore through the husk and into the seed, where larval development took place. Five to seven weeks after oviposition, mature larvae exit the pods and fell to the ground where they pupate after spinning a whitish silk cocoon. Emergence of the

adults took place about two weeks after pupation. The period during which ripe pods of *P. reticulatum* and *C. sieberiana* remain on the tree does not exceed, in a given area, 5 or 6 months, which is usually between November and May. After pods have fallen to the ground, they are often eaten by cattle or burnt and only a few seeds remain accessible to *C. serratus* females. Towards the end of the dry season and during the rainy season, seeds become progressively less available. When the first new *P. reticulatum* or *C. sieberiana* pods reach maturity after the rainy season, in November, *C. serratus* levels were at their lowest. Infestation rates observed on *P. reticulatum* at that time were not higher than 2 to 4 eggs per 1000 pods (Sembène, 1997). On hosts which bear pods all year round (*B. rufescens* and to a lesser extent, *T. indica*), generations follow one another in the pods attached to the tree and infestation rates do not exhibit the wide fluctuations observed on *Piliostigma*. Populations, however, experience a definite decline during the rainy season, probably due to increased adult mortality (Pierre and Huignard, 1990; Ndiaye, 1991). Pods of the derived host groundnut were removed from the soil shortly after the end of the rainy season, in October. Oviposition took place on drying pods within a week after harvest. Infestation rates at that time were very low, in the order of 1 egg per 10000 seeds. After the harvest has been taken to the stores, infestation continued and population levels rapidly increased.

Pods were taken from the trees as soon as they reached maturity, at the start of new infestations (between November and January). Groundnut samples were collected from the field during drying. Adult beetles were genetically analysed almost immediately after they emerged from pods. Firstly, however, the genital parts were examined following Prevett (1965) in order to avoid confusion with *Caryedon crampeli* Pic (*C. cassiae* in Prevett), a species which also feeds on *B. rufescens*, *C. sieberiana* and *P. reticulatum*. Voucher specimens were kept in the I.F.A.N. Cheikh Anta Diop (Dakar) collections. In all, a total of 25 different populations (individuals of both sexes) were genetically scored in the study.

DNA protocols

DNA extraction

Total genomic DNA was extracted from prothorax of individual seed-beetle as described by Vogler et al. (1993). The abdomen, elytra and antennae were kept apart to avoid contamination by fungi and nematodes and to permit subsequent morphological observations.

mtDNA amplification and PCR-amplified

A partial CytB end region was PCR-amplified. The CytB end region consisted of primer CB1 (5' - TATGTACTACCATGAGGACAAATATC-3') and CB2 (5'-ATTACACCTCCTAATTTATTAGGAAT-3'). The 25 µl PCR reaction mixtures contained 2.5 µl enzymes buffer supplied by manufacturer, 2.5 mM $MgCl_2$, 0.6 units of Taq polymerase, 17.5 pM of each primer, 25 nM of each DNTP and 1 µl of DNA extract. After an initial denaturation step at 92°C for 3 min, reaction was subjected to 35 cycle of 1 min at 92°C; 1 min 30 s at 48°C and 1 min at 72°C. A pool of two PCR was concentred and loaded on a 1.3 del agarose gel. The PCR gel band was cut and then purified with Quiaquick PCRgel purification kit (Qiagen) and directly sequenced on an Abi 373 automated sequencer using TaqFS and Dye-labelled terminators (Perkin-Elmer).

Nuclear DNA amplification

ITS1 region was PCR-amplified, using the primer CIL (5' GCGT TCGAARTGCGATGATCAA 3') and CIU (5'GTAGGTGAACCTGCA GAAGG3') developed by Vogler and DeSalle (1994). PCR conditions were similar to those used for mitochondrial domain, except that the elongation step was increased to 1 min 30 s.

Data analysis

Sequence alignment was performed using ClustalW (Thompson et al., 1994) as implemented in BioEdit. Aligned sequences were entered in McClade 3.06 (Maddison and Maddison, 1992) for subsequent treatments. The resulting data were subsequently used to calculate Kimura 2-parameter genetic distances between the haplotypes. G-tests (log-likelihood ratio test) were performed to test genetic distances homo-geneity among hosts at the same site and among sites for the same host (Sokal and Rohlf, 1981).

Phylogenetic relationships were reconstructed with PAUP 4^*_B8 (Swofford, 2001) using the maximum parsimony method (MP). The MP analysis was done with the heuristic search option of 50 random stepwise taxon addition replicates, using the branch swapping tree bisection-reconnection (TBR) option. A bootstrap procedure (1000 iterations with the same option of heuristic search) was used to establish the score of each node (Felsenstein, 1985) by retaining group compatible with the 50% majority rule consensus. A strict consensus tree was computed whenever multiple equally parsimonious trees were obtained.

The molecular clock hypothesis was tested following Posada and Crandall (2001), by comparing the log-likehood scores of the best trees (Shimodaira and Hasegawa, 1999), using reel Boostrap with 1000 replications used to test for a significant difference between the score of the different trees obtained. In addition, analyses were conducted using the distance-matrix method with the Neighbour-Joining (NJ) algorithm (Saitou and Nei, 1987) on Kimura 2-parameter distances with PAUP 4^*_B8 (Swofford, 2001). The robustness of inferences was assessed through bootstrap resampling (1000 replicates).

The two analyses were completed separately with Cyt. B and ITS1. The partition homogeneity test (Farris, 1994), as implemented in PAUP was used to determine the appropriateness of combining both partial Cyt. B and ITS1 genes into a single analysis with the same option. The Wilcoxon's signed-Rank test (Templeton, 1983) was applied to compare the statistical significance of the best tree produced by each tree reconstruction method to one another.

Two outgroup were used in this study: *Bruchidius atrolineatus* which belongs to the family Bruchidae and *C. gonagra* (F.) obtained from Bauhinia variegate L. from Egypt (BVC). *C. gonagra* is a species which may be synonymous with *C. serratus* (Delobel et al., 2003).

Morphological analysis

Parts of the adult specimens were dry-mounted for morphological studies. Morphological study comprised extraction of the genitalia, which were cleared and mounted in *Canada balsam* following standard procedures. As often as feasible, slide preparations of male genitalia were made with internal sac everted. This gave a clearer view of the different sclerites and spines that are attached to the sac wall and of their relative position. It thus avoided possible misinterpretations due to variable positions of sclerites in variously retracted internal sacs. Slides were studied through a Leica DMLS light microscope and photographs were taken with a Nikon Coolpix 990 digital camera. Drawings were made with Adobe Illustrator 9.0, using photographs as models.

RESULTS

DNA sequences

468 bp of the partial Cyt. B gene from 25 *C. serratus*

populations was obtained. The alignment was straightforward and involved no insertions/deletions. The sequences could be unambiguously aligned and showed 20 different haplotypes due to 51 polymorphic sites. Of these sites, 96% were parsimony informative. The number of nucleotide differences in pair wise comparisons of *C. serratus* populations ranged from 0 to 17.2%. Between *C. serratus* sampled on *C. sieberiana* (called "Sieberiana") and the other (called "Serratus"), it ranged from 14.4 to 15.8%. In "Serratus" this number ranged from 0 to 0.09%. Within the same host species, the number ranged from 0 to 0.02%.

The PCR products obtained for the ITS1 domain were 874 pb in "Serratus" and 950 pb in "Sieberiana" while in both cases, 6 pb in 18S and 49 pb in 5.8S. The sequences could be unambiguously aligned. Of these sites, 35.4% were variable and were parsimony informative. The number of nucleotide differences in pairwise comparisons of *C. serratus* populations ranged from 0 to 24.6%. Between "Sieberianae" and "Serratus", this number ranged from 24.9 to 35.7%. In "Serratus" this number ranged from 0 to 0.1%. Within the same host species, the number ranged from 0 to 0. 02%. In "Sieberiana" the number of nucleotide differences ranged from 0 to 0.003%.

All sequences have been deposited in GenBank under accession numbers, AY449464. The two sets of sequences could be unambiguously aligned together and were used in the analysis described below.

Distance matrix and phylogenetic trees

Among the *C. serratus*, the genetic distances measured on the total alignment of CytB + ITS1 (1418 pb) ranged between 0 and 0.204, but clearly fall in two classes: "Serratus" from 0 to 0.037 and "Sieberiana" from 0.186 to 0.195, separating *C. serratus* into two major groups. One group ("Serratus") comprises haplotypes sampled in *A. hypogaea*, *B. rufescens*, *P. reticulatum* and *T. indica*. The second group (Sieberiana") gathers the haplotypes raised from *C. sieberiana*. The genetic distance between the two exceeds genetic distances typical of geographical populations or subspecies in invertebrates (Klein and Seitz, 1994; Emelianov et al., 1995; Kerdelhué et al., 2002).

Genetic distance between hosts was highly significant (0.295) and due, for a large part, to sample obtained from *C. sieberiana*, which differed from all other samples. In another study, after considering that *C. serratus* samples raised from *C. sieberiana* might represent a separate species, they were excluded from the original data set and genetic distances within and between host-plants and within and between localities were reanalysed. Genetic distance values fell but remained significant ($P < 0.001$) in both cases (Sembène, 2006 and Sembène et al., 2008 for commentary).

The MP analysis on Cyt B nucleotide data yielded 29 equally parsimonious trees that were 845 steps long. The same methods on the ITS1 data set yielded seven equally parsimonious trees, 245 steps long (CI = 0.963; RI = 0.981). All analyses of combined data set yielded clades, one for all "Serratus" specimens, whatever their host and a second for all "Sieberiana" specimens. Bootstrap values were all over 50%. Separation according to host plant was clear. *C. sieberiana* samples showed a similar trend.

Similar patterns of relationships were obtained with NJ analysis. Samples typically clustered according to host plant. *C. sieberiana* samples were clearly separated from all other samples and showed high bootstrap values. These confirmed results given by Sembène and Delobel (1998), Sembène (2006) and Sembène et al., (2008) for Senegalese specimens and in particular the relative genetic isolation of populations on *B. rufescens* and the close relationship existing between *P. reticulatum* and *A. hypogaea* populations. *C. sieberiana* samples showed a similar trend. These beetles were phylogenetically closer to *C. gonagra* than that of "Serratus".

The genetic distance between *C. serratus* and *C. gonagra* was computed from a portion of the Cyt. B gene that was successfully used to distinguish species in the rodent genus, *Praomys* (Lecompte et al., 2002), or aphids in the genus, *Aphis* (Cœur-d'Acier, unpublished). The present data indicated that Senegalese and Egyptian specimens of *Caryedon* belong to two distinct clades.

For each data set, the topology obtained with MP and NJ methods was compared using the Shimodaira-Hasewaga test and the Wilcoxon's signed-rank test. These tests did not support a significant difference between the trees. The strict consensus trees of MP and NJ trees are presented in Figure 1 for Cyt. B; in Figure 2 for ITS1 and in Figure 3 for the pooled data.

Morphological analysis

A careful study of the external and genital morphology showed that variation occurred between samples of different geographical origins. Colour appeared as rather variable within populations. The density of dark markings ranged from almost absent, with entirely reddish elytral integument, to very dense black mottling on elytra that is almost entirely black underside with the legs being variously dotted with black. However, a constant trend existed in the colour of legs, with Egyptian and Asian specimens showing more contrasted femora and tibiae. Interesting diagnostic characters were the shape of the hind femur, which was more or less oblong and the number of teeth of its ventral pecten. Morphology of male genitalia provided several diagnostic characters, in particular the fine morphology of sclerites in the internal sac and the size and density of spines in its apical part were large and numerous in African specimens but smaller and much less numerous in Asian specimens. Female genitalia, particularly the relative size of dorsal

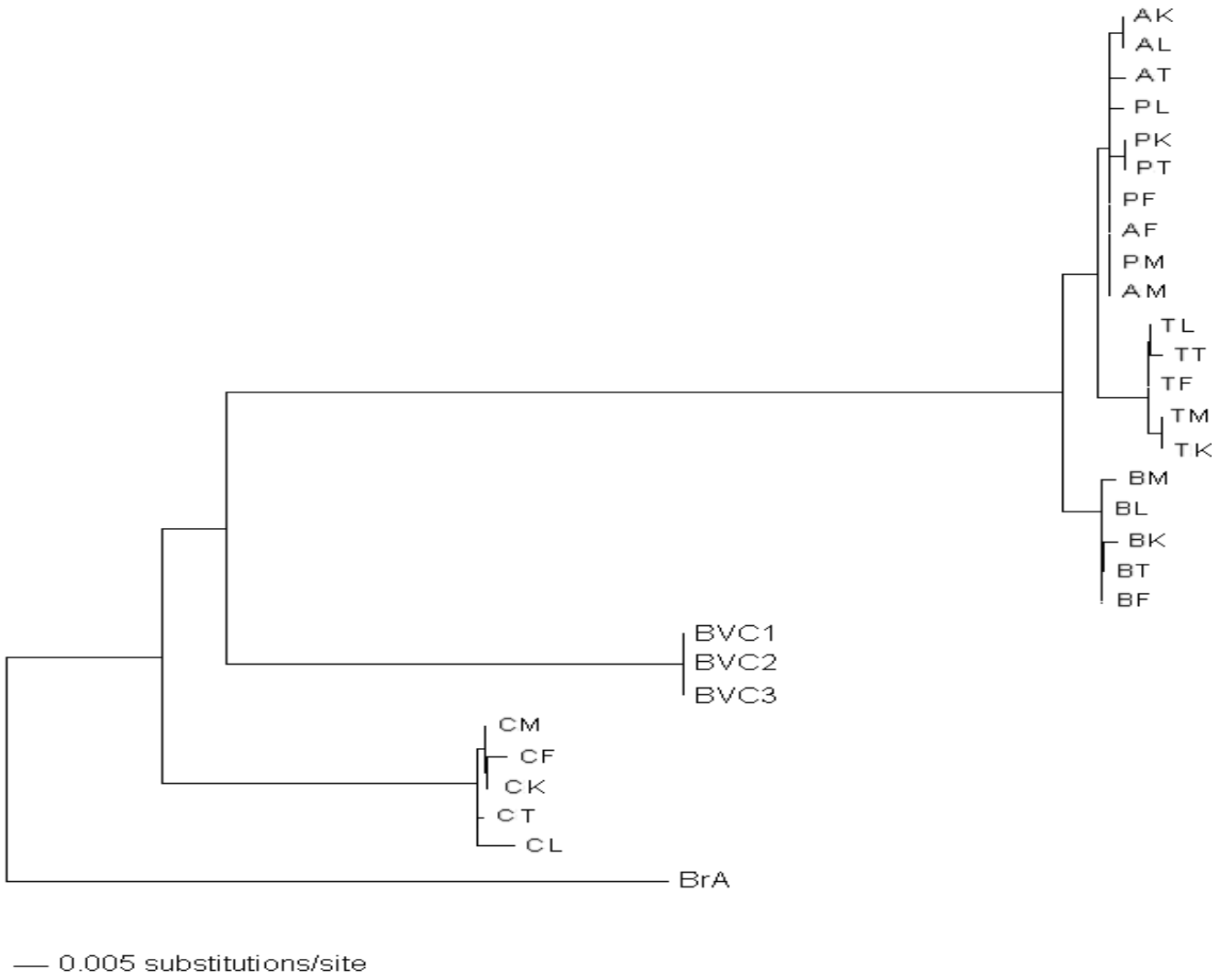

Figure 1. Phylogenetic relationships among nucleotide sequences of partial cytochrome b gene (468 pb) of 25 populations of *Careyedon serratus*. This tree is between a maximum parsimony (MP) consensus tree and a Neighbour-Joining (NJ) consensus tree. The first numbers above or under branches are MP% bootstrap values (1000 replicates) and the second numbers, the NJ % bootstrap values (1000 replicates). *Bruchidius atrolineatus* (BrA) and *Caryedon gonagra* (BVC) are the outgroups.

and ventral vaginal sclerites, also showed constant differences. In West African specimens, these sclerites were almost of equal size, whereas Egyptian specimens had a much smaller dorsal sclerite. Size and density of spines in the bursa copulatrix were also a good diagnostic character.

Diagnosis

"Serratus"

It is a large greyish-brown species, with small black spots, and pale antennae and legs. It has about 10 - 12 teeth at the ventral side of the hind femur. Hind femur is 2.2 times longer than its width.

a) Redescription

"*Serratus*" length was 3.7 - 6.2 mm; and width, 1.9 - 3.2 mm (excluding head). Fresh insects had greyish brown colour. The integument was reddish with small, scattered black or dark brown spots, merging here and there into larger, irregular spots. Antennae and legs (except hind femora and tibiae) were pale. Antennomeres 1 and 5 - 11 were often darker on disk. Their vestiture was dense, but not quite covering the integument, and it was recumbent except for a few erect setae, especially on pygidium. Setae were pale greyish over the red parts of the integument and blackish or dark brown over dark spots. A few areas of the setae had denser dark spots. The third apex of the elytra, especially along the suture, basal half of pygidium, ventro-apical part of hind femora; first

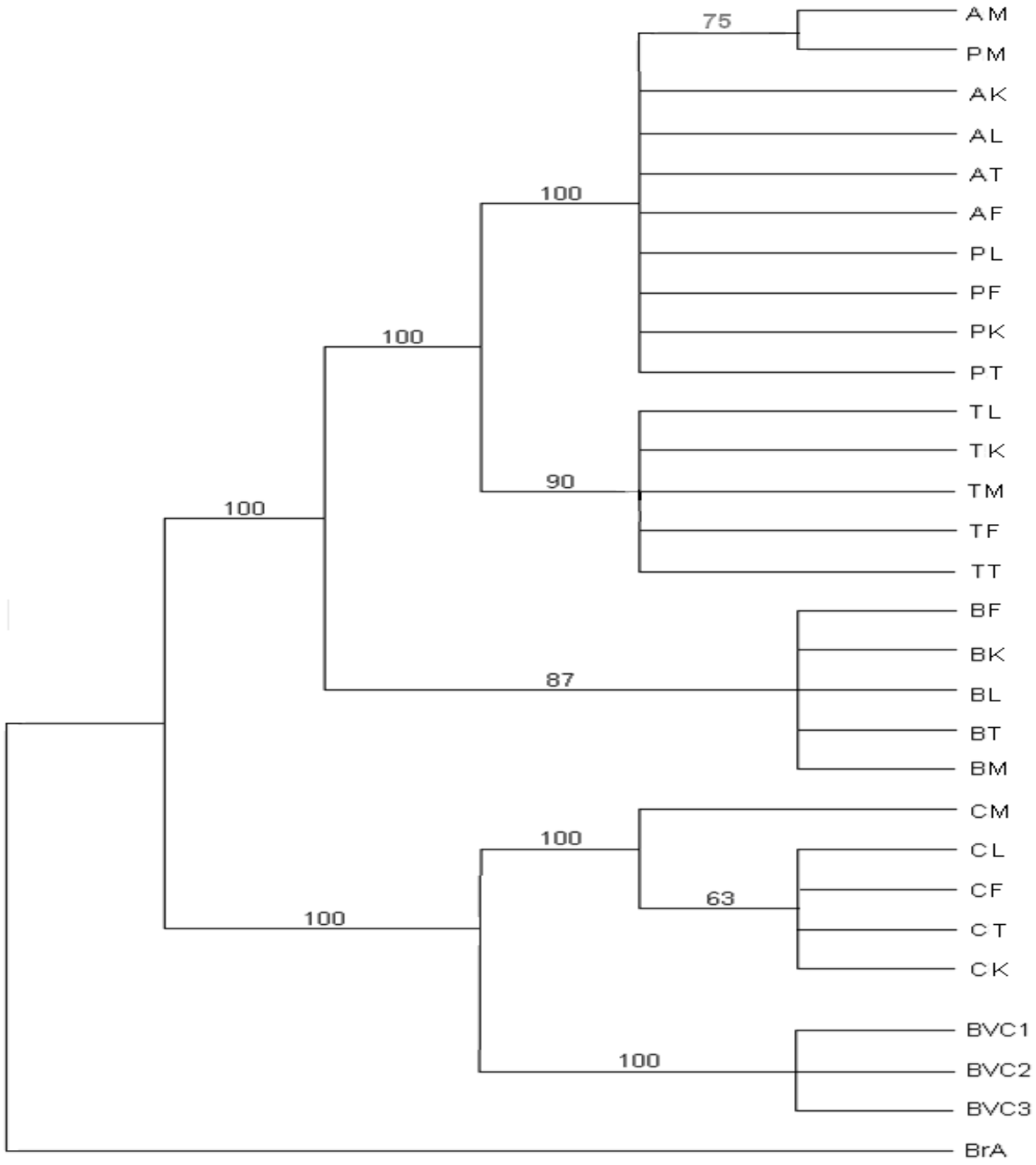

Figure 2. Phylogenetic relationships among nucleotide sequences of the total ITS1 of the total gene (950 pb) of 25 *Caryedon Serratus* populations. This tree is the consensus between a maximum parsimony (MP) consensus tree and a neighbour–Joining (NJ) consensus tree. The first numbers above branches are MP% bootstrap values (1000 replicates) and the second numbers are the NJ% bootstrap values (1000 replicates). *Bruchidius atrolineatus* (BrA) and *Caryedon gonagra* (BVC) are the outgroups.

abdominal sternites were often almost entirely black. Fore and median legs sometimes with the apex of femur and base of tibia were diffusely darker. Pronotum was often devoid of black spots. Apex and median line of pygidium had whitish setae. Colouration varied among individuals: a few entirely black specimens have been found in Congo and Ivory Coast. Other specimens had yellowish instead of reddish ground colour and were almost devoid of dark spots.

The male species had a short head, constricted back eyes, bulging eyes, maximum head width about 1.7 times width for back eyes; ocular sinus well defined; eye width about 2.5 times minimum distance between eyes, sharp median longitudinal carina on frons, vertex without

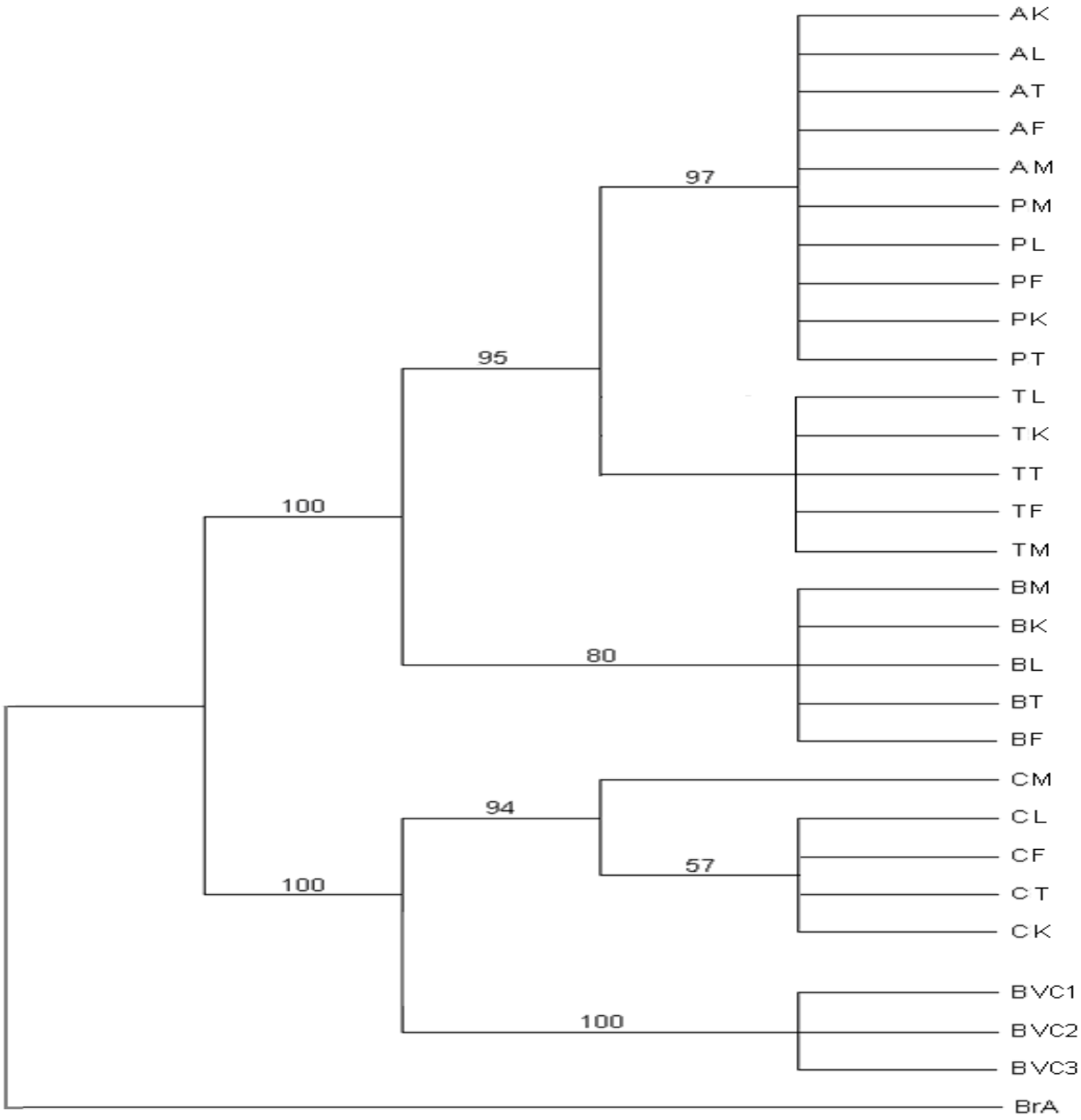

Figure 3. Phylogenetic relationships among nucleotide sequences of the pooled data (partial cytochrome b and ITS1 genes) of 25 *Careyedon serratus* populations. This tree is the consensus between a MP consensus tree and a Neighbour-joining (NJ) consensus tree. The first numbers above branches are MP% bootstrap values (1000 replicates) and the second values are the NJ% bootstrap values replicates. *Bruchidius atrolineatus* (BrA) and *Caryedon gonagra* (BVC) are the outgroups.

interocular tubercle. Antenna reached the fifth base of the elytra; antennal segments 1-4 were cylindrical. Segments 5-10 were serrate, segment 11 was oblong; segment 1 about twice as long as segment 2; segment 3 was 1.6 times longer than 2, segment 4 hardly shorter than 2, segments 5 - 10 as long as antennomere 1 (9 - 10 slightly longer), but widened at the apex; segment 11 is 1.4 times longer than 1 and about 2.8 times as long as the width.

Pronotum was about 1.6 to 1.7 times as wide and as long, with the greatest width at the base. Sides were almost parallel at base, straight or slightly concave, then abruptly converging at about two third of the length, disc feebly convex; punctures on disc irregularly spaced, setous; distances between punctures varied from 1 to 3 diameters; cuticle between coarse puncturation with fine punctures.

Elytra was about 1.5 times as long as their combined width; sides regularly convex; disc convex; striae on disc thin and deep, punctured; punctures elongated, with setae, distances between punctures less or about equal to their diameter; interstriae convex, with strong micropunctation and a very small number (varying from 0

to 2 per interstria) of coarser punctures.

Legs were without sexual dimorphism; hind femora strongly incrassate, at their widest (at base of first spine) 2.2 times longer than width; mesoventral margin with a series of blunt teeth not hidden by hair, followed by a pecten of 10 - 12 sharp teeth, with first tooth 1.5 to 2 times longer than second; hind tibiae was acute, with 5 carinae complete; apex of hind tibia with mucro was as long as tibial width at apex (lateral view).

Abdomen was without modified setae; sternite 5 emerges to about one third of its length; pygidium was 1.2 times as long as width. Females were similar to male; fifth sternite did not emerge.

In Senegal many material was examined: the samples (arachide) were collected at 2 January 1995 and in december 1996 by *A.* Delobel in Keur Baka. *Arachis hypogaea* in Dakar at 27 September 1999 by the same collector ; region of Thiès, Ngazobil, 30 march 1996, seeds of *Tamarindus indica* named Tama 13 (H. & A. Delobel) ; region of Fatick, in Fimela, seeds of *Tamarindus indica*, 6 January 1996 named Tama 09; in Dakar, market, *Tamarindus indica,* 16 October 1994, same collector ; region of Kaolack, Keur Baka, ex seed *Piliostigma reticulatum*, 1er July 1999 (M. T. Gueye); Nianing, ex pods of *Piliostigma reticulatum*, 14 November 1998 (H. & A. Delobel); Birkelane, *P. reticulatum*, 2 January 1995, same collector ; region of Thiès, Bandia, *P. reticulatum*, 17 December 1994, same collector.

b) Host plants

Caesalpinioideae: *B. rufescens* (Niger, Senegal)*, B. (Piliostigma) reticulata* (Niger, Nigeria, Senegal), *B. (Piliostigma) thonningii* (Ivory Coast, Congo), *T. indica* (Senegal). In Nigeria, Prevett (1965, as *C. gonagra*; 1967, as *C. serratus*) reared *C. serratus* from these hosts and also from *Cassia arereh*.

c) Distribution

These species are found in Chad, Congo (People's Republic, capital Brazzaville), Ivory Coast, Niger, Nigeria, Senegal. The groundnut seed-beetle has been reported from many African countries, India (Vazirani, 1975; Dick, 1987), America (Johnson, 1966; Nilsson and Johnson, 1992; Romero and Johnson, 2002) and Australia (Cunningham and Walsh, 2002).

One specimen has been seen from Réunion Island: St Paul, Boucan Canot, 5m, 6.v.1952 (J. Hamon), identified as *C. serratus* Ol. by J. Decelle in 1971 [coll. IRD, Paris]; however the specimen, a female, has no genitalia and cannot therefore be assigned to one or the other species with certainty.

d) Affinities

C. serratus is part of a small group of species with similar genitalia and elytral pattern. Typical of this species are the shape of the larger hooks, the presence of two pairs of thin, and strongly curved sclerites at apex of internal sac. It is most closely related with *C. acaciae* sensu Decelle, from which it may be distinguished by the absence of modified setae on the first two abdominal sternites. It is also closely related with *C. longispinosus* sensu Decelle (nomen nudum) (Silvain and Delobel, 1998; Delobel et al., 2000), which is characterized by a particularly long first tooth of pecten at hind femur, but has a similar genital morphology.

"Sieberianae"

It is a large greyish-brown species, with small black spots, and pale antennae and legs; it has about 13 - 14 teeth at ventral side of hind femur. Hind femur is 1.8 times times longer than its width.

a) Redescription

Its length is 2.8 - 5.2 mm; width: 1.5 - 2.6 mm (excluding head). Fresh insects had a greyish brown colour. The integument was reddish with small, scattered black or dark brown spots, merging here and there into larger, irregular spots. Antennomeres 1 and 5-11 were often darker on disk. Its vestiture was dense, but not completely covering integument, it was recumbent except for a few erect setae, especially on pygidium. Setae were pale greyish over red parts of the integument and blackish or dark brown over dark spots. A few areas had denser dark spots; the third apex of elytra, especially along suture, basal half of pygidium, ventro-apical part of hind femora; first abdominal sternites are often almost entirely black. Fore and median legs with apex of femur and central two-thirds of tibia were darker. Pronotum is often devoid of black spots. Apex and median line of pygidium had whitish setae.

The male species had, a short head, constricted back eyes; bulging eyes, maximum head width about 1.7 times width behind eyes; ocular sinus well defined; eye width about 4 times minimum distance between eyes; sharp median longitudinal carina on frons, vertex without interocular tubercle. Antenna reached the fifth base of the elytra; antennal segments 1 - 4 cylindrical; segments 5 - 10 serrate; segment 11 oblong; segment 1 about twice as long as segment 2; segment 3 1.6 times longer than 2; segment 4 equal to 2; segments 5 - 10 as long as antennomere 1, but widened at apex; segment 11 about 1.2 times longer than 1 and 2.4 times as long as width.

Pronotum was about 1.6 to 1.7 times as wide and as long, with greatest width at base. The sides were almost parallel at base, straight or slightly concave, then abruptly converging at about two third of their length, disc feebly convex; punctures on disc irregularly spaced, setous. Distances between punctures varied from 0 to 2

diameters; cuticle between coarse puncturation with fine punctures.

Elytra was about 1.5 times as long as their combined width; sides regularly convex; disc convex; striae on disc thin and deep, punctured; punctures elongated, with setae, distances between punctures less or about equal to their diameter; interstriae convex, with strong micropunctation.

Legs were without sexual dimorphism; hind femora strongly incrassate, at their widest (at base of first spine) 1.8 times longer than width; mesoventral margin with a series of blunt teeth not hidden by hair, followed by a pecten of 12-14 sharp teeth, with the first tooth 1.5 to 2 times longer than the second, to which it is usually coalescent. Hind tibiae strongly acute, with 5 carinae complete; apex of tibiae with mucro about as long or slightly longer than tibial width.

Abdomen was without modified setae; sternite 5 emerge to about one third its length; pygidium 1.2 times as long as width. Females were similar to male; fifth sternite did not emerge.

DISCUSSION

Different levels of genetic differentiation between co-occurring *Bauhinia*, *Cassia*, *Piliostigma* and *Tamarindus* wild populations and groundnut populations of *C. serratus* have being identified There is evidence of at least four distinct biotypes in Senegal, with restricted gene flow between each other. This genetic differentiation observed in *C. serratus* (a phytophagous insect) is necessarily accompanied by the installation of pre or post-zygotic reproductive isolation in the absence of extrinsic barriers to gene flow. The main factors that could possibly maintain allele frequency differences among ancestral populations and between them and derived groundnut populations include the following: post-mating reproductive isolation between races, differential larval survivorship within the seed, differential host recognition by adult beetles, temporal and spatial differences in adult emergence and host seed availability. Many of these mechanisms are discussed in Sembène (2006) and Sembene et al. (2008).

Serratus versus sieberianae

A strong differentiation clearly exists between *C. serratus* feeding on *C. sieberiana* and on the four other host plants. The average value of Kimura genetic distance between the two groups (0.198) exceeds genetic distances typical of sympatric (Kerdelhué et al., 2002) and geographical populations or subspecies in invertebrates (Klein and Seitz, 1994). The individuality of "*C. sieberiana*" samples may be explained by an incipient sympatric speciation or by the existence on *C. sieberiana* of a sibling species of *C. serratus* as a result of a previous diffe-rentiation of populations in allopatry, with present-day overlap of *C. sieberiana* and other host plants. These hypotheses cannot be tested without further knowledge of biogeographic and temporal variations in host plant distribution. However, host race formation mechanisms as temporal and spatial differences in adult emergence and host availability, certainly play a major part in maintaining homogeneity within the *C. sieberiana* population because *C. sieberiana* trees bear pods over a short period of time, from December to February. *C. serratus* females keep laying eggs on the pods after abscission, but populations must then face interspecific competition with congeneric *C. crampeli* Decelle. In mid-January 1997, a sample of C. sieberiana pods (on the tree) which yielded 1000 adults seed beetle, of which 87 were *C. serratus* and 13 were *C. crampeli* was collected in Sokone (16°22W, 13°53N). A sample of fallen pods collected in March under the same tree yielded a total of 327 beetles, of which only 56 (17%) were *C. serratus* and the rest (83%) were *C. crampeli.* It may be assumed that this drastic population reduction had negative impact on the beetle's capability to utilize host plant species. In the same order, hybridization experiments between individuals from *C. sieberiana* and *P. reticulatum*, which were genetically the most distant indicated that post-mating isolation is not likely, as laboratory F1 progeny were fertile but significant differential survivorship of larvae of the different host populations was observed. The fertility of the beetles reared on the same host plant was significantly greater than that obtained after sexual crossings (Sembène, 2004). However, reproducing in the laboratory does not indicate that the two "species" interbreed in nature as the two gene pools seem isolated. The peculiarity of *C. sieberiana*-associated forms raises the question of an appropriate taxonomic classification of this group. A 76 bp difference existed in the ITS1 nucleotides between "Sieberianae" and "Serratus". The genetic distance observed between the two entities was higher or about existing between species of the same genera. "Sieberianae" is genetically closer to *C. gonagra* than *C. serratus* (Delobel et al., 2003) –Sieberianae" amplifies only three of the 10 microsatellites loci identified in "Serratus" (Sembène et al., 2003) - "Sieberianae" is the only one to present a duplication of locus ADH1 (Sembène et al., 1998).

There are several significant controversies associated with using molecular techniques to study systematics and evolution. These included debates over the relative importance of molecular versus morphological data, and the constancy of the molecular clock

ACKNOWLEDGEMENTS

This study is partially supported by the International Foundation of Science (IFS) and the Institut de Recherche pour le Développement (IRD-DSF). Facilities were provided by the CBGP laboratory (Montferrier-sur-Lez, France).

REFERENCES

Ali-Diallo B (1991). Biologie de *Caryedon serratus* (Ol) en présence de ses plantes hôtes sur le terrain et en conditions expérimentales Thèse Universite de Niamey, Tome 1 : p. 103.

Arora GL (1977). Taxonomy of the Bruchidae of Northwest India Part I Adults Oriental Insects Supplement, 7: 1-132.

Cunningham DC, Walsh KB (2002). Establishment of the peanut bruchid (*Caryedon serratus*) in Australia and two new host species, *C. brewsteri* and *C tomentella.* Aust. J. Exp. Agric. 42: 57-63.

Decaux C (1894). Métamorphoses du *C. tamarindi*, Coléoptère de la famille des Bruchides. Le Naturaliste, 16: 129-131

Decelle J (1966). *Bruchus serratus*Ol, 1790, espèce type du genre *Caryedon* Schönherr, 1823. Revue de Zoologie et de Botanique Africaines, 74: 169-173.

Delobel A (1995). The shift of *Caryedon serratus* Ol from wild Caesalpiniaceae to groundnuts took place in West Africa (Coleoptera: Bruchidae). J. stored Prod. Res., 31: 101-102.

Delobel A, Tran M, Sembène M (2000). Influence du choix alimentaire sur la fécondité et le développement larvaire des *Caryedon* des légumineuses (Coleoptera, Bruchidae) au Sénégal. Annales de la Société entomologique de France (n s), 36: 61-73.

Delobel A, Sembène M, Fédière G, Roguet, D (2003) Identity of groundnut and tamarind seed-beetles (Coleoptera: Bruchidae: Pachymerinae), with the restoration of *Caryedon gonagra* (F), Annales de la Société Entomologiques de France, 39 : pp 197–206

Dick KM (1987). Losses caused by insects to groundnuts stored in a warehouse in India. Trop. Sci. 27: 65-75.

Emelianov I, Mallet J, Baltensweiler W (1995). Genetic differentiation in *Zeiraphera diniana* (Lepidoptera: Tortricidae, the larch budmoth): polymorphism, host races or sibling species? Heredity, 75: 416-424

Fabricius JC (1798). Supplementum entomologiae systematicae Proft & Storch. Haffniae, pp. 158-159.

Farris JS (1994). Methods for computing Wagner trees. Syst. Zool. 34: 21-34.

Felsenstein J (1985). Confidence limits on phylogenies: An approach using the bootstrap Evolution, 39: 783-791

Johnson CD (1966). *Caryedon gonagra* (Fab) established in Mexico. Pan-Pacific Entomologist, 42: p. 162.

Kerdelhué C, Roux-Morabito G, Forichon J (2002). Population genetic structure of *Tomicus piniperda* L (Curculionidae: Scolytinae) on different pine species and validation of *T destruens* (Woll), Mol. Ecol. 14: 483-494.

Klein M, Seitz A (1994). Geographic differentiation between populations of *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae): concordance of allozyme and morphometric analysis, Zoological J. Linn. Soc. 110: 181-191

Lecompte E, Denys C, Granjon L (2002). The phylogeny of the *Praomys* complex (Rodentia: Muridae) and its phylogeographic implications. J. Zool. Syst. Evol. Res. 40: 8-25

Maddison WP, Maddison DR (1992). MacClade: analysis of phylogeny and character evolution version 3.0. Sinauer Associates, Sunderland MA, p. 398.

Mukerji S, Chatterjee SN (1951). Morphology of genital structures of Bruchidae. Indian J. Zool. 13: 1-28.

Ndiaye S (1991). La bruche de l'arachide dans un agrosystème du centre-ouest du Sénégal: Contribution à l'étude de la contamination en plein champ et dans les stocks de l'arachide *(Arachis hypogaea L) par Caryedon serratus* (Ol) (Coleoptera-Bruchidae); rôle des légumineuses hôtes sauvages dans le cycle de cette bruche Thèse Uni de Pau et des Pays de l'Adour, p. 96.

Nilsson JA, Johnson CD (1992). New host, *Bauhinia variegata* L and new locality records for *Caryedon serratus* (Olivier) in the New World. Pan-Pacific Entomologist, 68: 62-63.

Olivier AG (1790). *Encyclopédie méthodique* Histoire naturelle Insectes, Baudonia, Paris 5: 195-202.

Pierre D, Huignard, J (1990). The biological cycle of *Caryedon serratus* Boh (Coleoptera, Bruchidae) on one of its host plants *Bauhinia rufescens* Lam (Caesalpinioideae) in a Sahelian zone, Acta Oecologica, 11: 93-101.

Posada D, Crandall KA (2001). Selecting the best-fit model of nucleotide substitution. Evolution, 50: 580-601.

Prevett PF (1965).The genus *Caryedon* in Northern Nigeria, with descriptions of six new species. Annales de la Société entomologique de France (ns), 1: 523-547.

Prevett PF (1967).The field occurrence of *Caryedon serratus* (Ol), the groundnut seed beetle, in Uganda. J. stored Prod. Res. 3: 267-268.

Robert P (1985). A comparative study of some aspects of the reproduction of three *Caryedon serratus* strains in presence of its potential host plants. Oecologia, 65: 425-430.

Romero J, Johnson CD (2002). *Cassia moschata* HBK, new host for *Caryedon serratus* (Olivier) in the new world. Coleopterists Bulletin, 56: 95-96.

Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

Sembène M (1997). Modalités d'infestation de l'arachide par la bruche *Caryedon serratus* (Olivier) en zone soudano-sahélienne : identification morphométrique et génétique de populations sauvages et adaptées Thèse 3ème cycle, Univ Cheikh Anta Diop, Dakar, p 128.

Sembene M (2004). Inter-strain fecundity and larval mortality in the groundnut beetle Caryedon serratus (Coleoptera: Bruchidae. Int. J. Trop. Insect Sci. 24: 319-322.

Sembene M (2006). The origin of groundnut infestation by the seed beetle caryedon serratus (Coleoptera Bruchidae): results from cytochrome and ITS1 gene sequences. J. Stored Prod. Res. 42: 97-111.

Sembène M, Brizard JP, Delobel A (1998). Allozyme variation among populations of groundnut seed-beetle C*aryedon serratus* (Ol) (Coleoptera: Bruchidae) in Senegal. Insect Sci. Appl. 18: 77-86.

Sembène M, Delobel A (1996). Idenfication morphométrique de populations soudano-sahéliennes de bruche de l'arachide, *Caryedon serratus* (Olivier) (Coleoptera Bruchidae). J. Afr. Zool. 110: 357-366.

Sembène M, Delobel A (1998). Genetic differentiation of groundnut seed-beetle populations in Senegal. Entomologia Experimentalis et Applicata, 87: 171-180.

Sembène M, Vautrin D, Silvain JF, Rasplus JY, Delobel A (2003). Isolation and characterization of polymorphic microsatellites in the groundnut seed beetle, *Caryedon serratus* (Coleoptera, Bruchidae) Mol. Ecol. Notes, 3: 299-301.

Sembène M, Rasplus JY, Silvain JF and Delobel, A (2008) Genetic differentiation in sympatric populations of the groundnut seed beetle *Caryedon serratus* (Coleoptera: Chrysomelidae): new insights from molecular and ecological data. Int. J. Trop. Insect Sci. 28(3): 168-177.

Shimodaira H, Hasegawa M (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16: 1114-1116.

Shomar NFH (1963). A monographic revision of the Bruchidae of Egypt (UAR). Bull. Soc. Ent. Egypt, 47: 141-196.

Silvain JF, Delobel A (1998). Phylogeny of West African *Caryedon* (Coleoptera: Bruchidae): congruence between molecular and morphological data. Mol. Phylogenet. Evol. 9: 533-541.

Sokal RR, Rohlf FJ (1981). Biometry 2nd ed. WH Freeman and Co, New York/San Francisco p. 859.

Southgate BJ, Pope RD (1957). The groundnut seed-beetle, a study if its identity and taxonomic position. Annals and magazine of natural History, (ser 12); 10: 669-674.

Swofford DL (2001). PAUP* Phylogenetic Analysis Using Parsimony (*and other methods), Version 4, Sinauer Associates, Sunderland MA, p 398.

Templeton AR (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution, 37: 221-244.

Thompson JD, Higgins DG, Gibson TJ (1994). Clustal W: improving sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680.

Vazirani TG (1975). A contribution to the knowledge of Oriental Bruchidae. J. Bombay natural History Society, 72: 740-757

Vir S, Jindal SK (1996). Field infestation of *Caryedon serratus* Olivier on the pods and seeds of *Acacia nilotica* in the Thar desert of India. J. Trop. For. Sci. 9: 189-193.

Vogler AP, DeSalle, R (1994). Evolution and phylogenetic information content of the ITS-1 region in the tiger beetle *Cicindela dorsalis*. Mol.

Biol. Evol. 11: 393-405
Vogler AP, Desalle R, Assmann T, Knisley CB, Schultz, TD (1993). Molecular population genetics of the endangered tiger beetle, *Cicindela dorsalis* (Coleoptera: Cicindelidae), Ann. Entomol. Soc. Am. 86 (2): 142-152