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Discovery of new species of New Caledonian *Arsipoda* Erichson, 1842 (Coleoptera: Chrysomelidae) and insights on their ecology and evolution using DNA markers

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New Caledonia is among Earth's biodiversity hotspots, and we are far from knowing how many species it sustains. We applied DNA-based methods for quick biodiversity assessment of New Caledonian *Arsipoda* flea beetles, enhancing the discovery and description of new species. Mitochondrial DNA phylogenetic analysis (*cox1*, *rrnS*) for four out of five known neocaledonian taxa hints at the existence of additional species, and two are confirmed and described based on morphology: *Arsipoda geographica* Gómez-Zurita sp. nov. and *Arsipoda rostrata* Gómez-Zurita sp. nov. Timing this small radiation using standard insect mitochondrial substitution rates places its origin in the Miocene. A DNA-based approach to investigate potential food plants for these herbivorous insects reveals associations with Myrsinaceae and Ericaceae, which have not yet been found in New Caledonia, suggesting that this indirect methodology may help in discovering undetected flora. Traditional taxonomy and molecular approaches cooperate here, boosting our knowledge on species inventory and ecological interactions where it is most needed.

Keywords: *Arsipoda*; Alticinae; molecular ecology; mitochondrial DNA phylogeny; new species; Pacific Islands; taxonomy

Introduction

New Caledonia is deservedly recognized as one of the 34 hotspots of our planet's biodiversity (Myers et al. 2000; Lowry et al. 2004; Mittermeier et al. 2004) because of its high biodiversity and the endemicity of its singular fauna and flora (Holloway 1979; Daugherty et al. 1993). Although this is well known, a renewed interest in the diversity of life in this area, in part fuelled by the controversy of its recent or ancient origins (Grandcolas et al. 2009), has resulted in the discovery of many new species, revealing indeed how poor our knowledge remains, particularly for some groups, e.g. insects (Balke et al. 2007; Smith et al. 2007).

The present work is another example of the much needed research on producing an inventory of biodiversity that is still required to obtain a comprehensive view of the insect fauna of New Caledonia. In the course of a single recent fieldtrip, sponsored by

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the National Geographic Society, to the Grande Terre, the largest and oldest island of New Caledonia, and among samples from Malaise trap surveys by Trichoptera specialists (K.A. Johanson, pers. comm.), we found some specimens that were clearly ascribable to a particular genus of flea beetles, *Arsipoda* Erichson, 1842 (= *Eratosthenes* Clark, 1864), but presenting noticeable morphological differences to the known types.

Arsipoda is a genus of flea beetles mostly endemic to the Australasian and Indomalayan ecozones with over 60 species present in Australia, New Guinea, the Solomon Islands and in New Caledonia, and only one widespread from the Philippines to Vietnam and northeast India (Baly 1864; Blackburn 1896; Bryant 1941; Samuelson 1973). The highest species diversity of *Arsipoda* occurs in Australia, with over 50 species described. Remarkably, in spite of the large difference in surface between these two islands (1 : 400), New Caledonia bears the second largest species diversity in the range of the genus, with five known endemic species. These species were recognized by G. Allan Samuelson (Bishop Museum, Honolulu, HI, USA), who worked intensively on Pacific chrysomelids, mainly in the group of the flea beetles (Samuelson 1973), but this author was also aware of a higher diversity that remained undescribed (Samuelson 1989).

In the original description, Erichson (1842) compared his new genus *Arsipoda* (type species: *Arsipoda bifrons* Erichson from Tasmania) with the Palaearctic genera *Psylliodes* and *Dibolia*, with which, unsurprisingly, he could find remarkable diagnostic differences. In the accompanying comments he stressed several characters, including the broad separation of antennal bases, a basal pronotal impression, ordered elytral punctation, and a particular shape of the maxillary palpi and legs. Baly (1864) expanded the generic concept to cover a range of variation precisely in the basal furrow of the pronotum and striated elytral punctation, both sometimes independently absent or reduced. Chapuis (1875) erected the group Arsipodites – including the genera *Arsipoda*, *Nisotra*, *Balanomorpha* and *Podagrica* – based on the closed procoxal cavities, the conformation of the abdominal segments and precisely (and interestingly) the lack of the basal transversal furrow of the pronotum. Later, Blackburn (1896) thought that he had found an important diagnostic character for the genus, a median longitudinal furrow on the fifth abdominal ventrite of males, which had little systematic impact in later works. More recently, the genus *Arsipoda* was ranked allied to genera with closed anterior coxal cavities, bearing a basal transverse impression on pronotum, as in the Old World *Crepidodera* (Heikertinger 1924), or devoid of this impression, such as *Panilurus* Jacoby or *Erystus* Jacoby (Jacoby 1885, 1904; Maulik 1926). Nevertheless, the lack of a solid suprageneric system for the flea beetles precludes any formal attempt to date to place *Arsipoda*, and recent generic catalogues (Seeno and Wilcox 1982) only place it near *Crepicnema* Scherer, proposed by this author to include Jacoby's *Crepicnema tenimberensis*, a species from Assam, Philippines (Palawan) and Vietnam (Scherer 1969; Gruev 1982), otherwise treated successively as *Chaetocnema*, *Crepidodera* or *Arsipoda*.

From a systematic point of view, New Caledonian species show all the typical characters of the genus, like the obliteration of postantennal swellings, 11-segmented antennae, pronotum with prebasal transverse impression flanked by short longitudinal striae or small fossettes, regular striation of elytra, closed procoxal cavities and appendiculate claws, among others (Samuelson 1973).

Although the affiliation of New Caledonian species to *Arsipoda* seems unambiguous, their closest relative is still unknown, but it is probable that they evolved *in situ* as a result of isolation or are the product of a few colonization events from a common stock. This group constitutes a characteristic assemblage of rather small (1.5–3.0 mm; only *Arsipoda agalma* Samuelson reaches 3.0 mm) yellowish species, whereas Australian species are typically larger in size (4.1 ± 1.28 mm), often with metallic tints or very dark coloured. Our first aim is to provide additional data and formal descriptions on the diversity and structure of this assemblage, highlighting the relevance of using molecular markers to speed up the discovery of biodiversity, but also, as described below, the often neglected study of ecological interactions. In the past few years there has been a boost of initiatives exploiting the use of DNA variation to recognize undetected diversity, culminating in large-scale DNA barcoding of life projects taking up on the idea of using a single, universal short variable genetic marker with low overlap between intraspecific and interspecific divergence to diagnose species (Hebert et al. 2003). Other widely used approaches exploit the phylogenetic content of appropriate markers to unravel the structure and limits of diversity (e.g. Pons et al. 2006). What is less common is that the insight gained from the use of DNA, simultaneously provides the basis and/or reinforcement for a formal taxonomic exercise resulting in the description of new species. This practical divorce creates the illusion of a gap, actually nonexistent, between new and traditional taxonomy to discover and characterize the diversity of life.

The investigation of biodiversity focusing on herbivorous insects, such as flea beetles, benefits enormously from the understanding of host-plant associations and their degree of specialization. Even though some species of *Arsipoda* have been recognized as crop pests, there is not much biological information available for the species in this genus. *Arsipoda tenimberensis* (Jacoby) has been associated with the culture of sweet potato in New Guinea (Kimoto et al. 1984), *Arsipoda concolor* (Blackburn) has been found in Australia on *Juncus* (Hawkeswood and Furth 1994) and there exist reports of associations with up to 13 plant families (Jolivet and Hawkeswood 1995). The New Caledonian species were in turn associated with several plant families and recorded as feeding specifically on pollen in some cases (Samuelson 1973, 1989), and these beetles are known in Australia to cause chew-damage to flowers in several botanic groups, causing great prejudice to the development of the plants they attack (e.g. Duncan et al. 2004). Studies on biodiversity usually avoid investigating ecological relationships for the inventoried species, and this is understandable when the magnitude of the task is considered. In particular, the information relative to host-plant associations in *Arsipoda* from New Caledonia is scarce and it reveals a very broad range of potential food, a remarkable finding taking into account that flea beetles tend to be rather restricted in their ecology (Samuelson 1973, 1989). We have recently developed a molecular strategy to investigate insect host plants by polymerase chain reaction (PCR) amplification of taxonomically diagnostic food DNA remains recovered from total DNA extractions of beetles, which can help to investigate these relationships (Jurado-Rivera et al. 2009). Therefore, our second aim is to apply our new molecular tool to explore the food preferences of these species and advance our understanding of the ecology and diversification of New Caledonian *Arsipoda*.

In summary, in this paper we present a preliminary molecular species phylogeny based on two mitochondrial markers. We discover the existence of new species,

which are subsequently corroborated from the analysis of their morphology and are formally described. We also apply a recently developed molecular-based approach to make inferences on host range and specificity of these beetles in New Caledonia.

Materials and methods

Specimens and genetic data

Individuals for molecular analyses were collected in the field by sweeping and beating vegetation or from Malaise traps (Table 1, Figure 1). Samples including specimens of all New Caledonian species of *Arsipoda* except the morphologically distinct *Arsipoda evax* Samuelson plus specimens not ascribable to any of the known species, were stored in ethanol and at –20°C until they were processed for DNA extraction. Extractions were performed non-destructively with a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) from whole specimens, splitting them at the junction of prothorax and meso- or metathorax. *Cox1* was amplified using the primers C1-J-2183

Table 1. Samples of New Caledonian *Arsipoda* used for phylogenetic analysis, geographic source (as in Figure 1), and voucher numbers.

Species	*n*	Source	Voucher no.
Arsipoda agalma	1	Prov. Nord, Mont Panie (1)	IBE-JGZ-1063
Arsipoda isola	1	Prov. Sud, Plaine des Lacs (2)	IBE-JGZ-1058
	1	Prov. Sud, Monts Kwa Ne Mwa, 2 km E Pic Mouirange (3)	IBE-JGZ-1065
Arsipoda shirleyae	3	Prov. Sud, Monts Kwa Ne Mwa, 2 km E Pic Mouirange (3)	IBE-JGZ-1064, 1066–1067
Arsipoda yiambiae	2	Prov. Sud, Mont Do (4)	IBE-JGZ-1061
Arsipoda sp. 1	3	Prov. Sud, Mont Kouakoué (5)	IBE-JGZ-1022, 1056–1057
Arsipoda sp. 2	1	Prov. Sud, Mont Koghi (6)	IBE-JGZ-1062
Arsipoda sp. 3	1	Prov. Sud, Mont Do (4)	IBE-JGZ-1060

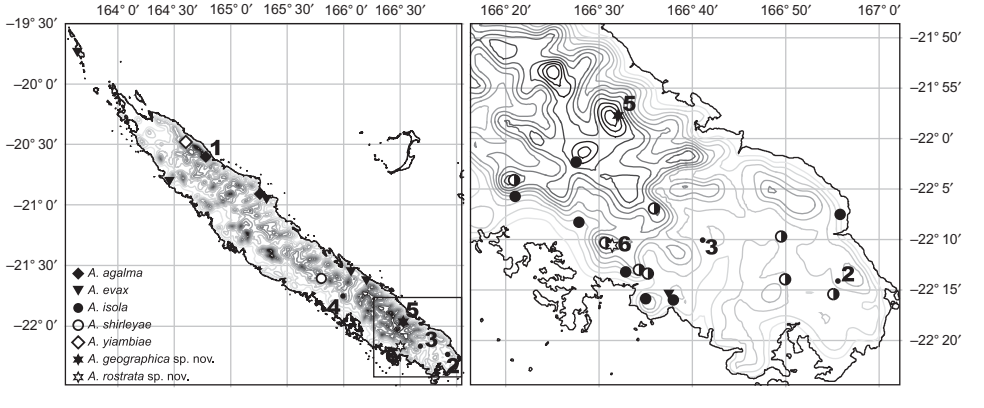

Figure 1. Distribution of *Arsipoda* Erichson in New Caledonia (from Samuelson, 1973) and geographic source of samples available for phylogenetic analysis with locality data as in Table 1.

and TL-N-3014 (Simon et al. 1994) for a fragment of 829 base pairs; when the amplification failed, two complementary internal primers (5′-ACR TAA TGA AAR TGG GCT ACW A-3′ with C1-J-2183 and its reverse-complement with TL-N-3014; Ribera et al. 2010) were used to amplify the same fragment in two smaller segments of 401 and 406 base pairs, respectively. A fragment of 512–513 base pairs of *rrnS* was amplified using primers SR-N-14759 and SR-J-14233 (Simon et al. 1994). The PCR conditions used 35 cycles of 30 seconds denaturation, 30 seconds annealing and 1 minute elongation, with annealing temperature of 50°C for both markers, lowered to 48°C in the case of *cox1* for specimens 1022 and 1065. Trees were rooted using the homologous sequences obtained from *Arsipoda concolor* Blackburn from Victoria in Australia (Grampians, Wannon River, 5 km north of Dunkeld, 236 m 37°37.494′ S, 142°20.226′ E; 27 November 2006; Lars Hendrich leg.). All sequences obtained for this study have been deposited at the EMBL Nucleotide Sequence Database (Accession nos. FN773500–FN773528).

External body and genital characters were analysed for all specimens, and measures and proportions were estimated with a calibrated micrometer mounted on a Leica M80 stereomicroscope, also used to photograph the specimens using a Leica DFC420 digital camera and the freely available software COMBINEZM for frame stacking (http://www.hadleyweb.pwp.blueyonder.co.uk/index.htm).

Sequence analysis

Sequences for phylogenetic analysis were unambiguously aligned manually in the case of *cox1* and for *rrnS* we used the MAFFT 6 multiple sequencing alignment algorithm implementing the L-INS-i strategy and default parameters (Katoh et al. 2005). The alignment included seven positions with gaps, three exclusively due to an extra nucleotide in the outgroup sequence. The matrix thus aligned was analysed with PAUP* 4.0b10 (Swofford, 2003) using parsimony and maximum likelihood methods. Parsimony searches used the branch-and-bound algorithm and both unweighted and *a posteriori* successive reweighting of characters based on the rescaled consistency index (Farris 1969); gaps were considered either as missing data or a fifth character state. Maximum likelihood searches implemented a GTR+G+I substitution model best fitting the data as inferred with MODELTEST 3.7 (Posada and Crandall 1998), and a heuristic strategy using a 100 random starting topologies and the tree bisection and reconnection branch-swapping algorithm, saving multiple trees. Node support was added by bootstrapping the original dataset 10,000 times in each tree search strategy.

Sequence diversity measures within and between groups of sequences were computed with MEGA 4 implementing *p*-distances and a bootstrap-based estimation of standard deviation (Tamura et al. 2007).

Molecular clock analysis

Our sampling being centred exclusively on New Caledonian *Arsipoda*, which is in principle a large genus with wider distribution, makes the investigation of events on the phylogeny, including dating of nodes, a preliminary exercise. However, we opted to investigate our data using a molecular clock approach to provide a hypothetical framework for discussion. Two fashions of molecular clock were implemented using

BEAST v1.5.2 (Drummond and Rambaut 2007), strict and uncorrelated log-normal relaxed clocks, applying a single GTR+G+I substitution model for the data and a normal distribution for the substitution rate prior, with mean substitution rate per lineage and million years of 0.0115 (standard substitution rate for arthropod mitochondrial DNA; Brower 1994) and a standard deviation of 0.002. Nodes of interest, including the ingroup depth, were also specified and the Markov chain Monte Carlo search involved 10^7 generations, sampling every thousandth tree. The input file for BEAST was prepared using BEAUTI v.1.5.2 and the obtained results were analysed with TRACER v1.4.1 (Rambaut and Drummond 2007).

Food plant data

The specimens investigated in this study come either from Malaise trapping or indiscriminate sampling of vegetation, so information on host-plant associations has not been available, requiring an alternative approach to recover these important data. Potential host-plant DNA sequences of *Arsipoda* were investigated by PCR amplification from total beetle DNA extracts using specific primers, *c* A49325 and *d* B49863, for an intron in the *trn-L*(UAA) gene, one leucine transfer RNA of the chloroplastic genome (Taberlet et al. 1991). Taxonomic identification of the plant source for these sequences was inferred applying a phylogenetic approach by comparison of these hypothetical diet sequences with homologous data available in public databases following the strategy outlined in Jurado-Rivera et al. (2009).

Results and discussion

Phylogenetic diversity and time framework for New Caledonian Arsipoda

Parsimony analysis of the combined *cox1* and *rrnS* data produced two most parsimonious trees each of 691 steps [gaps treated as missing data; consistency index (CI) = 0.757, retention index (RI) = 0.783] or 700 steps (gaps as new state; CI = 0.760, RI = 0.784), identical in each analysis except for the relative positions of two *Arsipoda shirleyae* Samuelson terminals (Figure 2B). Treating gaps both as missing data or a new character state took two rounds of rescaled consistency index reweighting to obtain a single tree stabilized at tree length 444.03334 steps (CI = 0.886, RI =0.892) or 453.03334 steps (CI = 0.889, RI = 0.893), respectively. *A. posteriori* reweighting produced the same topology as the unweighted analysis, selecting for the alternative topology placing *A. shirleyae* (1067) as sister to their conspecifics. Major support differences were obtained depending on the tree search strategy, with few terminal or all nodes receiving high support in unweighted and weighted tests, respectively (Figure 2B). The maximum likelihood strategy rendered a single tree of likelihood score –4710.73157 and very similar topology to the parsimony tree, except for the placement of the undetermined *Arsipoda* sp. 3 (IBE-JGZ-1060) as sister to *Arsipoda isola* Samuelson. The high bootstrap support for this tree concentrated exclusively on the same terminal nodes as in the unweighted parsimony analyses (Figure 2C).

The mean divergence between the outgroup and ingroup sequences was 0.179 ± 0.009. Despite general low support for basal nodes (in unweighted tree search strategies), the trees showed very low intraspecific sequence divergence (mean

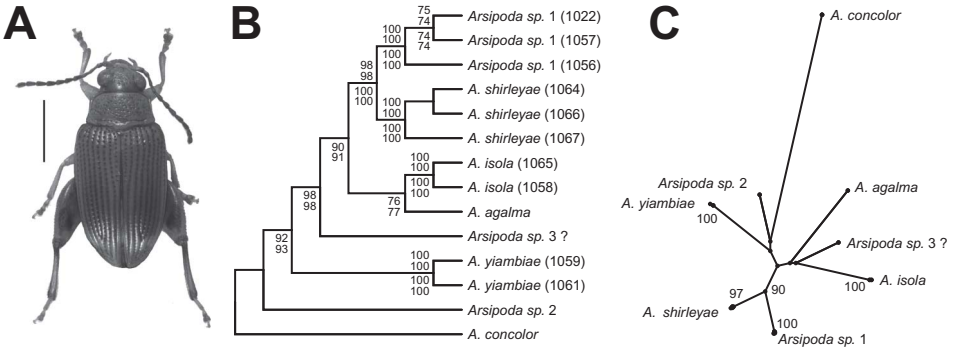

Figure 2. Mitochondrial phylogeny of New Caledonian *Arsipoda* Erichson. (A) *Arsipoda agalma* Samuelson, a representative New Caledonian species (scale bar = 1 mm). (B) Most parsimonious tree for mitochondrial DNA data of New Caledonian *Arsipoda* with bootstrap support measures in unweighted (above branches) and weighted (below branches) analyses, treating gaps as missing data (top value) or a fifth character state (bottom value). (C) Best topology of maximum likelihood analysis for the same data showing bootstrap support values.

p-distance: from 0.003 ± 0.002 in *A. isola* to 0.006 ± 0.002 in *A. shirleyae*) with high statistical support (100%) in all cases, and high divergence (from 0.081 ± 0.012 between *A. shirleyae* and *Arsipoda* sp. 2, to 0.144 ± 0.009 between *A. isola* and *Arsipoda yiambiae* Samuelson) with unsupported relationships for interspecific data, except between *A. shirleyae* and an unidentified morphologically distinct species, *Arsipoda* sp. 1, with mean divergence 0.082 ± 0.007 (Figure 2). Besides these two species, five other mitochondrial lineages could be recognized, three corresponding to the species *A. isola*, *A. agalma* Samuelson and *A. yiambiae*, and two singletons for *Arsipoda* spp. 2 and 3, respectively (Figure 2, Table 1).

Our ingroup node was established at 11.5 million years ago [Mya; 95% confidence interval (95%CI): 7.4–16.7 Mya] using a strict molecular clock or slightly older at 14.1 Mya (95%CI 6.5–24.1 Mya) using a relaxed alternative. This result, in the absence of samples of *A. evax*, provides a maximum age for the New Caledonian assemblage in the Miocene. The only strongly supported relationship in our phylogeny, that of *A. shirleyae* with *Arsipoda* sp. 1 is dated at 5.5 Mya (strict clock; 95%CI 3.4–7.9 Mya) or at 6.2 Mya (relaxed clock; 95%CI 2.2–11.2 Mya) in the Miocene–Pliocene boundary.

The study of external and genital morphological characters for the lineages identified as *Arsipoda* spp. 1 to 3 revealed that they correspond to previously unrecognized species. In the case of *Arsipoda* sp. 3, it is represented by a single female for which we lack the spermatheca. Even though it is an individual genetically different from other specimens available in the study and its appearance departs in some respects from the other known and newly discovered species, we prefer to delay its formal description until new material is available for study because we lack the important taxonomic information supplied by the male genitalia, or to a minor extent by the spermatheca.

Taxonomic discoveries

Arsipoda agalma Samuelson, 1973

This species, characterized by its relatively large size (3.0 mm) and a distinct anterior sulcus on vertex, was described based on two male specimens collected in two nearby localities (~ 65 km apart) on the eastern slope of the Massif du Panié, northeast of Grande Terre (Samuelson 1973). Both types are deposited at the J.L. Gressitt Center for Research in Entomology (Bishop Museum, Honolulu, HI, USA). The female was unknown so far. However, among the specimens investigated in our study, there was one female *Arsipoda* collected on the trail to the top of Mt Panié, below the limit of the National Park (Province Nord, New Caledonia) on the 23–28 November 2001 (K.A. Johanson, T. Pape and B. Viklund leg.), which conforms very well to the male holotypes of *A. agalma* in most respects, including the diagnostic supraocular groove reaching the region behind the antenna.

Differential description of female

Specimen slightly more slender than male holotype (3.08 mm long, 1.42 mm wide) and paler. Frons punctate-rugose, more impressed than in male, and antennae proportionally shorter, not reaching middle of elytra. Coarser punctation on disc of pronotum, with deeper, better-defined antebasal transversal furrow. Apex of last abdominal ventrite broadly rounded as in all female *Arsipoda*. The spermatheca (Figure 3A) of this species remained unknown. Its shape is perhaps more reminiscent of that in *A. shirleyae*, lacking as the latter a beaked apex (or appendix; present in all other species) and with a relatively thick and long ductus, inserted anteriorly to base of basal part, and strongly bent against the body of this basal part. It can be distinguished from the homologous structure in *A. shirleyae* by a less elongated form, with a relatively large and stout apical part and a shorter, more convex basal part.

Arsipoda geographica Gómez-Zurita sp. nov.

Type material

Holotype: one male, Nouvelle Caledonie: Prov. Sud, Mt Kouakoué 21°95.758′ S, 166°53.830′ E, 1315 m, 17 March 2008, J.A. Jurado-Rivera leg. Voucher no. (specimen

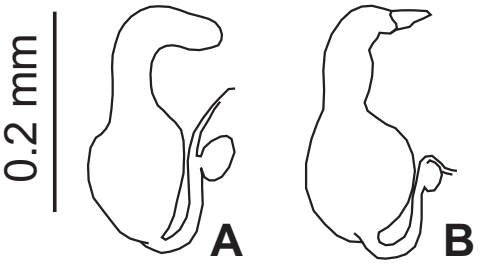

Figure 3. Spermathecae of (A) *Arsipoda agalma* Samuelson and of (B) *Arsipoda geographica* Gómez-Zurita sp. nov.

and DNA): IBE-JGZ-1022. Paratypes: two females, same data as holotype. Voucher nos.: IBE-JGZ-1056, IBE-JGZ-1057. The holotype will be deposited at the Musée National d'Histoire Naturelle (Paris, France), one paratype at the Naturhistoriske riksmuseet (Stockholm, Sweden) and the other paratype at the Institut de Biologia Evolutiva (CSIC, Barcelona, Spain).

Description

Habitus (Figure 4A). Body elongate oval, 2.9–3.1 mm long (holotype: 2.9 mm) and 1.3–1.4 mm wide (holotype: 1.3 mm); dorsally brown testaceous, darker at four apical antennal segments, behind eyes, disc and antebasal transverse furrow of pronotum, sutural area, basal margin, rows of punctures and lateral declivity of elytra, and epipleura; anterior head parts (except labrum), mouth pieces and legs dirty pale yellow. Winged species.

Head. Frons microreticulated, densely and finely punctured, slightly depressed as weakly impressed furrow near dorsal margin of eye, bearing single erect seta medially on furrow; separated from clypeus by obsolete broadly U-shaped impression. Postantennal tubercles obsolete. Interantennal space weakly convex, slightly rugose,

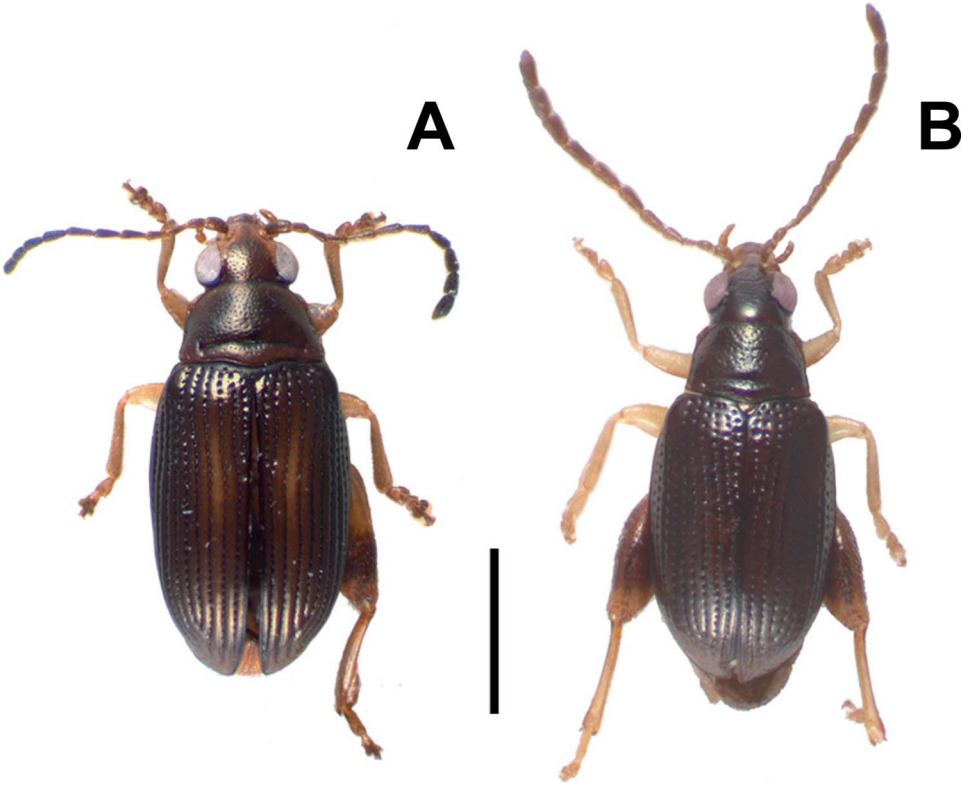

Figure 4. (A) Holotype of *Arsipoda geographica* Gómez-Zurita sp. nov. (male). (B) Holotype of *Arsipoda rostrata* Gómez-Zurita sp. nov. (male). Scale bar = 1 mm.

1.57 × wider than longitudinal diameter of antennal socket. Eyes large, convex, almost round (1.22 × longer than wide). Clypeus almost smooth, depressed laterally; anteriorly slightly concave, with transversal row of eight very fine, translucent setae. Labrum semicircular, almost smooth. Maxillary palpi elongated, elbowed at second segment; last segment elongated with rounded acuminated apex. Antennae relatively long, almost reaching midlength of elytra; two basal segments elongated ovoid; third segment and following slender, gradually expanding apically; third and fourth segments subequal, longer than second; five to seven segments longer than preceding, slender; eight to ten segments subtrapezoidal, rugose, densely pubescent; last segment with acuminated lancet-like apex.

Pronotum (Figure 5). Subtrapezoidal, slightly transverse (midlength: 0.60–0.57 mm; 0.60 mm in holotype), convex, laterally sloping downwards more acutely at anterior half; anterior margin (0.77–0.83 mm wide; 0.77 mm in holotype) very finely bordered, slightly concave medially; sides weakly concave, strongly bordered; basal margin (0.90–1.03 mm wide; 0.97 mm in holotype) bi-sinuose, very finely bordered except medially on slightly protruding median convex lobe; anterior angles as obliquely elongated explanate rounded callus, dorsally and posteriorly with large setigerous pore bearing long seta obliquely bent backwards; posterior angles broadly rounded at right angle with median setigerous pore at margin bearing seta sublaterally bent forwards; surface of pronotum delicately microgranulose, with uniformly dense fine punctures, comparable to punctures on frons, finer at sides, bearing minute translucent setae on disc; relatively deep basal transverse and weakly sinuose furrow at about one-quarter of pronotal length, with both ends marked by fovea or small longitudinal folding. Proepisterna smooth, unpunctured, slightly concave. Prosternum long, anteriorly concave, unpunctured, with sparse fine yellowish setae; proepisternal suture oblique, deep; intercoxal process narrow between coxae, less than half diameter of coxae, broadly expanded laterally at broadly rounded apex, almost enclosing

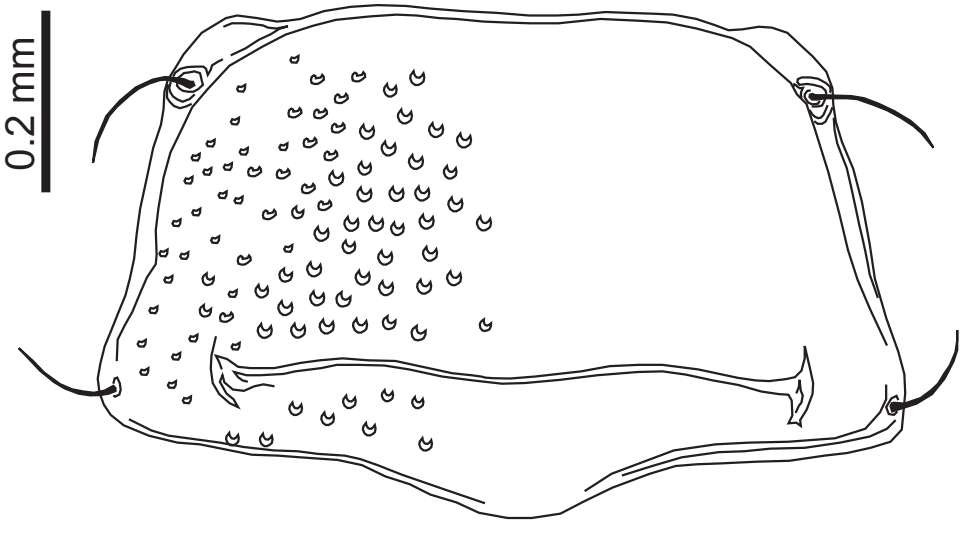

Figure 5. Pronotum of *Arsipoda geographica* Gómez-Zurita sp. nov.

coxal cavities behind. Scutellum wider at base than longer, broadly rounded at apex, unpunctured, extremely finely rugose.

Elytra. Long (1.93–2.13 mm long; 2.03 mm in holotype; and 0.67–0.7 mm wide at middle; 0.67 mm in holotype), flattened at disc, evenly convex at lateral declivity, very slightly broader at base than pronotum basally; anterior angles broadly rounded, sides weakly convex towards broadly rounded apex; humeral calli convex, protruding; sides margined from humeral angle almost reaching sutural angle, with margin almost concealed from above, especially at apical third owing to lateral convexity of elytra; surface very finely microsculpted with delicate transverse microreticulation and sparse, minute, very weakly impressed punctures, sparsely covered with minute, very short translucent setae; surface regularly striate-punctate, with 10 longitudinal rows of regular dense, deeply impressed round punctures, except at apical angle, unordered and weaker; scutellar row short (10–12 punctures); rows 2–4 running from elytral base reaching almost elytral apex (second row almost converging with suture apically); rows 5 and 6 slightly sinuose basally running from base of elytra converging apically at middle of apical declivity of elytra; rows 7–10 starting behind smooth humeral calli, running parallel to elytral margin, gradually disappearing before apex; marginal bead of punctures present; interstriae convex, more so apically and laterally on elytra as weakly raised costae. Epipleura wide, biconcave, sloping downwards, so visible laterally; gradually but quickly narrowing apically before apical curvature of elytra; surface smooth, unpunctured; punctured all along external margin.

Ventral parts. Meso- and metasternal processes almost twice as wide as prosternal process; metaventrite smooth, glossy, with scattered fine punctures bearing short fine setae; longitudinally impressed medially; apical margin of each half sinuous, producing broadly triangular median emargination. Front and mid femora spindle-shaped, uniformly sparsely covered by fine whitish setae; hind femora strongly swollen (ratio length : width = 1.69), wider near base because of strong dorsal expansion, gradually narrowing towards apex; front and mid tibiae straight, gradually widening to apex; hind tibiae curved, externally concave to fold over femora, considerably gradually expanded apically, dorsally excavated; first segment of protarsi and mesotarsi subtriangular, densely uniformly pubescent underneath, slightly expanded in male (holotype); second segment subtriangular, only apically pubescent underneath; third segment bilobed, broader than first segment, with long, dense yellowish setae ventrally; onychium long and slender, club-shaped; first segment of hind tarsi elongated, as long as three remaining segments together; claws appendiculate. In male (holotype), first abdominal segment long, weakly convex apically, with scattered punctures and fine whitish setae; three intermediate segments slightly concave apically, very finely transversely striate, with scattered whitish setae except at median longitudinal quarter; last visible ventrite largely concavely emarginated at apex, with whitish setae laterally and broadly denuded medially, longitudinally impressed medially by dark line. In females (paratypes), median longitudinal glabrous area of ventrites narrower; last visible ventrite not emarginated apically, regularly rounded, devoid of dark impression.

Reproductive organs. Aedeagus 3.8 × longer than broad medially (1.03 mm long, 0.27 mm wide); almost parallel-sided with weakly widened apical quarter; slightly

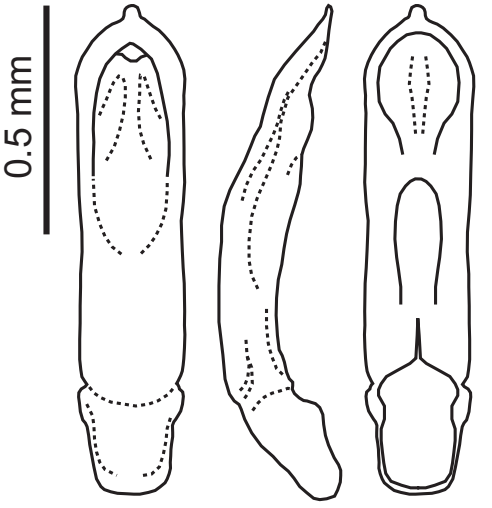

Figure 6. Aedeagus (dorsal, lateral and ventral views) of *Arsipoda geographica* Gómez-Zurita sp. nov.

curved, mostly basally, on lateral view, tapering at slightly dorsally bent apex; apex expanded as small (0.03 mm) semicircular projection; dorsal apical opening large, elongated oval; ventrally with median elongated depression (Figure 6). Spermatheca small (0.23 mm long), with basal part almost spherical, distally projecting an elongated neck ending at relatively short apical part, provided with darkened pointed appendix; ductus short, narrow, inserted slightly sideways to basal part and strongly recurved, with sclerotized part ending next and at midlevel of basal part (Figure 3B).

Diagnosis

Because of its size, darker tint and costate elytra, this species could be confounded with *A. agalma*, but they can be distinguished by the lack of supraocular furrow in *A. geographica* Gómez-Zurita sp. nov., its finer punctation on frons and pronotum, and by the shape of aedeagus, with regularly rounded apex and elongated oval operculum, and that of spermatheca (Figure 3). In the key presented by Samuelson (1973), this species would be paired with *A. shirleyae*, with which it may also be confounded, in spite of this species being smaller (2.1–2.5 mm). They can be distinguished nevertheless by the shape of the basal antennal segments, with third segment not longer than fourth in *A. geographica* Gómez-Zurita sp. nov., the lack of elytral costae in *A. shirleyae*, and very clearly by the shape of the aedeagus (not apically expanded in *A. geographica* sp. nov.) and the spermatheca (spherical and not elongated basally, with U-shaped duct in *A. geographica* Gómez-Zurita sp. nov.).

Distribution

Endemic to New Caledonia.

Etymology

The species name is in recognition of the National Geographic Society, which sponsored the work in New Caledonia resulting in the discovery of this species.

Arsipoda rostrata Gómez-Zurita sp. nov.

Type material

Holotype: one male, Nouvelle Caledonie: Prov. Sud, Mt Koghi, ca. 600 m, 12–16 November 2001, K.A. Johanson, T. Pape and B. Viklund leg. Voucher no. (specimen and DNA): IBE-JGZ-1062. The holotype is deposited in the Entomology collection of the Naturhistoriska Riksmuseet (Stockholm, Sweden). Left antenna of the holotype is broken, but all segments are mounted together with the specimen.

Description

Habitus (Figure 4B). Body elongate oval, 2.43 mm long and 1.07 mm wide, moderately convex; body uniformly brown with hind femora, antennae and frontally on head paler, and last five antennal segments darker; hind femora preapically broadly infuscate; anterior and median legs, metatibiae and metatarsi yellowish. Winged species.

Head. Frons smooth, glossy, unpunctured, with delicate oblique striae behind eyes; surface of vertex slightly irregular, with sparse shallow punctures between eyes; short longitudinal furrow dorsolaterally above and next to eye, proximally ending at small fovea bearing erect seta. Postantennal calli oblique, obsolete. Interantennal space almost flat, narrow (1.33 × transverse diameter of antennal socket). Eyes large, convex, elongated anteroposteriorly (ratio 12 : 21.5). Genae long (1.33 × transverse diameter of antennal socket); clypeus depressed at sides, also long, unpunctured, with transverse anterior row of six very fine, whitish setae, two median setae shorter. Labrum transverse, relatively long (0.38 × as long as wide), unpunctured, curved at apical angles, straight anteriorly, with very weak dorsal emargination at apex and two short very fine setae transversally at each side of disc. Maxillary palpi elongated; last segment slightly narrower than preceding, cylindrical, with rounded apex. Antennae long, slender; first segment elongated, gradually expanding to midlength and subparallel-sided apically; second segment elongated ovoid, shorter (0.73 ×) than first; third segment slightly shorter than second, narrower, weakly gradually expanded apically; fourth segment as long as first, club-shaped, clearly wider than third; segments 5 to 10 longer than fourth, subcylindrical, with segments 7 and 8 longest (1.5 × longer than first), slightly granulose and rather pubescent; last segment longest (1.7 × longer than first), tapering at two-thirds to rounded apex.

Pronotum. Trapezoidal, slightly transverse (longitudinal midlength: 0.47 mm), disc weakly convex with sides laterally sloping, strongly on apical half; anterior margin (0.5 mm wide between anterior angles) almost straight, basal margin convex (0.70 mm wide), with slightly protruding median lobe, both very finely bordered; sides straight, with margin feebly explanate laterally bearing four or five rather deep spaced punctures alongside margin internally; anterior angles protruding as obliquely swollen calli with rather large round fovea bearing a single long erect seta;

posterior angles straight, rounded, with setigerous pore at angle. Surface of pronotum bright, delicately shagreened, with dense shallow punctures, slightly deeper at sides of disc, each puncture bearing a very fine and small, appressed backwards seta; deep basal transverse, weakly sinuous furrow at about one-quarter of pronotal length, with ends at level with humeral internal depression of elytra, marked by very short longitudinal fold. Proepisterna almost flat, but perceptibly decliving at apex; glossy, very sparsely and finely punctured. Prosternum transversally convex, almost as long as longitudinal section of procoxae; anterior margin concave, very finely bordered, fringed anteriorly by dense long pale yellowish setae; surface smooth, glossy, impunctate, except at small area near anterior angles and six setigerous punctures on median area bearing appressed backwards fine long setae; prosternal process very narrow, as broad as maxillary palp segments, but quickly expanding apically surrounding and enclosing procoxae posteriorly, with apex slightly convex and very finely bordered laterally and apically. Scutellum almost twice as broad basally as long, nearly semicircular, smooth, unpunctured.

Elytra. Long (1.67 mm long, 0.53 mm wide at middle), flattened at disc, regularly convex at lateral declivities and posteriorly; broader than pronotum at round humeral angles; humeral calli protruding, glossy, unpunctured; sides regularly but weakly curved, broadest at middle, gradually converging to broadly rounded apex; finely margined from humeral to sutural angles, with margin slightly explanate, entirely visible from above except shortly below humerus and apical declivity of elytra; surface of elytra glossy, smooth, with very few secondary very minute punctures on intervals and sparse, very fine and minute scattered setae, slightly denser on disc; surface regularly striate/punctate, with punctures becoming unordered, obsolete at apical declivity; scutellar row reaching one-third of elytra, with nine relatively deep punctures; rows 2 to 6 running from base to blurred apical area of elytra, weakly sinuous, more markedly for second row, approaching suture beyond apical end of sutural row; rows 7 to 10 starting behind humeral prominence, running parallel to elytral margin; striae interspace slightly convex, particularly at lateral declivity; margin of elytron regularly punctured. Epipleura broad basally, glossy, unpunctured, thinning gradually, almost disappearing towards apex, laterally oblique, entirely visible from side; inner margin punctured in all its length.

Ventral parts. Mesoventrite concave, apically narrowly subquadrate between mesocoxae with apical angles produced as small teeth. Metaventrite long, rather convex in transverse section, finely bordered, with longitudinal median impression, glossy, unpunctured except for uniformly sparse setigerous punctures bearing very fine translucent appressed backwards setae. Procoxae slightly transverse; mesocoxae swollen but depressed posteriorly to receive retracted femora; metacoxae narrow, transverse, depressed below level of metaventrite. Profemora and mesofemora spindle-shaped, sparsely pubescent; metafemora very strongly developed, greatly enlarged dorso-anteriorly (1.86 × longer than wide), minutely, almost imperceptibly punctured, finely sparsely pubescent, with denser hairs towards apex, longitudinally depressed posterodorsally at apex to receive flexed metatibiae. Pro- and mesotibiae almost straight, gradually expanding towards apex, finely punctured and relatively densely setose; metatibiae straight in lateral view, slightly bent outwards and flattened in dorsal view; small tooth dorsally, near apex of metatibia on external margin

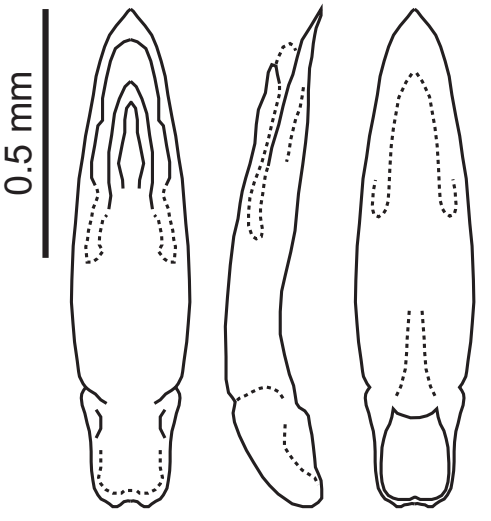

Figure 7. Aedeagus dorsal, lateral and ventral views of *Arsipoda rostrata* Gómez-Zurita sp. nov.

followed by row of minute spines; apex of metatibiae armed externally with relatively large, darkened spur. First protarsal and mesotarsal segments enlarged, twice as long as broad, and as long as next two segments together; metatarsi narrow, elongated, with first segment 1.33 × longer than next two segments together. Claws appendiculate. Abdominal sternites very convex laterally, very feebly shagreened, pubescent with fine translucent, relatively long setae, except medially on four last segments; last segment longer than two preceding together, apically trilobate, with median lobe depressed and internal marginal ends of lateral lobes slightly superimposed on depressed median area as short ridges, possibly assisting locking mechanism of the genital orifice by the pygidium.

Aedeagus. 3.75 × longer than widest point at slightly less than two-thirds from apex (1.00 mm long, 0.27 mm wide); spearhead-shaped, with sides regularly curved to rather acuminated apex; dorsally presenting a large elongated membranous operculum covered by a chitinous lancet-like ligule; straight in lateral view, slightly bent basally at level with widest point on ventral view (Figure 7).

Diagnosis

This small and stylized species of *Arsipoda* could be confounded at first sight with the two other slender New Caledonian species in the genus with straight or slightly concave sides of the pronotum, namely *A. shirleyae* and *A. isola*. However, *A. rostrata* Gómez-Zurita sp. nov. has several remarkable diagnostic traits, including the darker, more reddish-brown upper body coloration, a more elongated head, the punctation on elytra slightly sparser (for instance it bears nine punctures on sutural row compared with 13 or 14 in the other species), but chiefly on the shape of the aedeagus, which unequivocally differentiates these species. The aedeagus in *A. shirleyae* has a broadened apex with a

small median apical tooth as opposed to a gradually tapering pointed end in the new species. That of *A. isola*, although it is also lancet-like, is narrowed in the middle and slightly broadened apically, not widest postmedially and gradually narrowing towards apex as in *A. rostrata* Gómez-Zurita sp. nov., besides showing an apically expanded ligule.

Distribution

Endemic to New Caledonia.

Etymology

The holotype of this species has a particularly long head, owing to an elongated rostrum, hence the Latin adjective (fem.) for beaked, curved at the end, or rostrated, *rostratus*, *-a*, *-um*.

Ecology of New Caledonian Arsipoda

The information regarding the ecology of the genus *Arsipoda* is scarce. Different species have been reported as feeding on several plant tissues, including leaves, flowers and, most remarkably, pollen of several plant families. Host records exist for the Australian *A. concolor* on Juncaceae (Hawkeswood and Furth 1994), *Arsipoda holomelaena* (Germar) on Hemerocallidaceae (Duncan et al. 2004), and *Arsipoda chrysis* (Olivier) on Asteraceae (Bruzzese 1996), and the Solomon's *Arsipoda salomonensis* Bryant, the southeast Asian *A. tenimberensis* (Jacoby) and the Australian *Arsipoda parvula* Jacoby on Convolvulaceae (Samuelson 1967; Kimoto et al. 1984; Hutton et al. 2008); the Solomon's endemic was also recorded on Araceae (Bryant 1941). Some New Caledonian species were found on Ericaceae and Proteaceae in the case of *A. evax*, Cunoniaceae, Myrtaceae, Phellinaceae and also Proteaceae in the case of *A. isola*, and Winteraceae and Anacardiaceae, as well as Phellinaceae, for several unidentified species of *Arsipoda* (Samuelson 1973, 1989). Most interestingly, some of these records, particularly those for Anacardiaceae, Phellinaceae and Winteraceae, but also Proteaceae, were confirmed for pollen consumption, which is a reportedly rare feeding strategy in leaf beetles (Samuelson 1989, 1994). In any case, the trophic spectrum for New Caledonian *Arsipoda* reveals a relatively low degree of specialization, or an opportunistic behaviour, certainly for flower consumption, with the same species able to change diet even along temporal or altitudinal gradients (Samuelson 1994).

Two-thirds of beetle specimens checked for the presence of chloroplast DNA sequences in their total DNA extracts either failed to amplify anything or produced complex band patterns on the gel. This may be in great part related to the relatively low DNA quality obtained from specimens that remained dead on Malaise traps, maybe for days, in tropical weather before finally being recovered and transferred into absolute ethanol. Another possible explanation, particularly for examples producing unspecific amplifications, may be related to the expected mixed diet of these animals. However, one-third of the specimens tested produced single band patterns that yielded high quality *trn-L*(UAA) intron sequences. One of the females of *A. geographica* Gómez-Zurita sp. nov. produced one *trn-L*(UAA) intron sequence (EBI acc. no. FN773527) sister to that of the species *Ardisia speciosa* (posterior probability

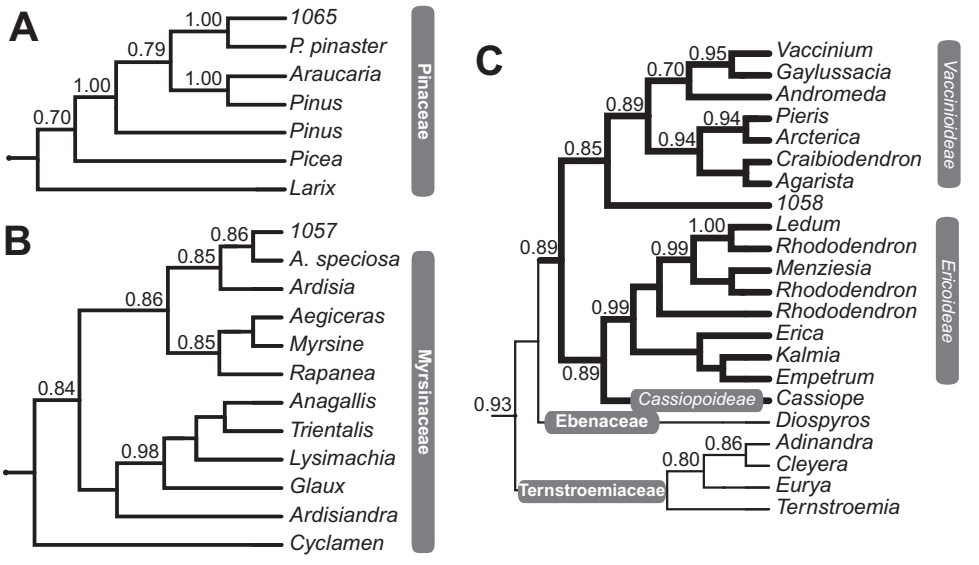

Figure 8. Diet molecular phylogenetic inference for *Arsipoda* spp. Synthetic trees showing the phylogenetic placement of trnL(UAA) sequences obtained from (A, C) *Arsipoda isola* Samuelson and (B) *Arsipoda geographica* Gómez-Zurita sp. nov. (see main text for details).

(*pp*) = 0.86), within a clade of *Ardisia* (*pp* = 0.85), a genus of Myrsinaceae related to the generotypic taxon of the family, the genus *Myrsine* (*pp* = 0.86) (Figure 8B). The food plant inference can be clearly restricted to the Myrsinaceae of the Ericales. Myrsinaceae is a diversified family in New Caledonia, with species in the genera *Maesa* (1), *Rapanea* (14) and *Tapeinosperma* (39) (Jaffré et al. 2004; Schmid 2006).

Ardisia has been recognized as an important pest in many Indian and Pacific islands, but so far has been prevented from invading New Caledonia (e.g. Meyer et al. 2006; Soubeyran 2008). The absence of *Ardisia* in Grande Terre and the recovery of this *Ardisia*-like sequence from a phytophagous beetle collected in a remote and isolated area of the island raise the question of a potentially undiscovered myrsinaceous plant and *Arsipoda* host in the Special Reservation for Fauna and Flora of the Kouakoué.

A specimen of *A. isola* (1065) from Monts Kwa Ne Mwa, even if captured from a trap, yielded a clear sequence (EBI acc. no. FN773528) very closely related to the only available homologous sequence of *Pinus pinaster* in GenBank (*pp* = 1.00) (Figure 8A). This is a problematic result in several respects. First of all, unlike most angiosperms, gymnosperm pollen carries chloroplast DNA, which makes it a potential contamination source for this molecular technique, considering that this type of pollen is almost ubiquitous. Besides, contamination is more likely to affect samples of low DNA quality, as expected for the specimens at hand.

Second, there are no native species of *Pinus* in New Caledonia. Nevertheless, the introduction of *Pinus caribaea* in the island for commercial use in 1966 is well documented (Crémière and Ehrhart 1990), and most remarkably that of *P. pinaster* in the Nouméa region in 1900, although not for economic purposes (MacKee 1985).

The sequence of *P. caribaea* was not available for analysis, but it belongs to the section Trifoliae of the subgenus *Pinus*, together with other representatives included in the *trn-L*(UAA) phylogeny, which none the less did not attract the hypothetical diet sequence to their vicinity, whereas *P. pinaster* and *Pinus canariensis* (both in the section *Pinus*) did with high support. In any case, considering the pollinivorous and arguably polyphagous nature of these animals, although sample contamination is the most likely explanation for this result, finding gymnosperm remains in their diet falls within the possible range of purely biological explanations as well (see below).

Finally, another *A. isola* specimen, this time from Plaine des Lacs and freshly collected, produced a sequence (EBI acc. no. FN773526) clearly belonging in the family Ericaceae (*pp* = 0.89), and sister to the available representatives of the subfamily Vaccionioideae (*pp* = 0.85). In this case, fixing the number of GenBank hits to 100 as in our default procedure did not allow finding the relevant plant family node for the diet sequence; increasing it to 250 clearly showed its placement within Ericaceae and related to Vaccionioideae (Figure 8C). The flora of New Caledonia only includes two known native Ericaceae in this same subfamily, the species *Paphia neocaledonica* and *Paphia paniensis* (Jaffré et al. 2004; Venter and Munzinger 2007). Interestingly, both species have very restricted ranges in the Plateau de Dogny and Mont Panié, respectively, so relatively far away from the collecting site of the beetle specimen. This situation also raises the question whether there is yet another undiscovered ericaceous plant in New Caledonia, an idea supported by the phylogenetic position of the purported diet sequence from the beetle, basal to Vaccinioideae and removed from the available representatives of the tribe Vaccinieae, which includes *Paphia* and the related genus *Agapetes* (Kron et al. 2002).

The results obtained for *A. isola* associated with *P. pinaster* need to be interpreted with some caution with the preliminary information at hand, but those from the other specimens can be put into a reliable ecological context. Our empirical data obtained from plant remains in the beetle DNA extractions reveal two different food choices, specifically on Myrsinaceae and Ericaceae, respectively, both belonging to the Ericales (Figure 8B,C). The association of New Caledonian *Arsipoda* with Ericaceae had been proposed already, not so in the case of the other family. But, as described above, and supported by our observations, the food choice for these species is extraordinarily broad, even within a single species, and so far covers representatives of seven plant orders.

This ecological versatility has been noted already in the ability of an unidentified species to feed on mango flowers, when this is a plant that was recently introduced in New Caledonia for commercial purposes (Samuelson 1994). If confirmed, the identification of an introduced gymnosperm as food resource for *Arsipoda* would be a remarkable finding, expanding the trophic potential even across Spermatopsida or the seed plants, for a group already known to use a huge spectrum of the angiosperms, the flowering plants.

Notes on the distribution of New Caledonian Arsipoda

The genus *Arsipoda* includes species distributed throughout the geography of Grande Terre, from low to high altitudes, and from sclerophyll and rainforests to the

maquis minier (Samuelson 1973). This ample distribution, which may be a reflection or another indication of the ecological versatility discussed above, seems to be true as well for some of the species (Figure 1). *Arsipoda evax* has been found from as far south as the environments of Mont-Dore, to one of the Belep Islands, an archipelago of small islets about 50 km offshore from the northwestern tip of Grande Terre. In turn, *A. isola* and *A. shirleyae* have been found, often in sympatry as in our samples from Pic Mourrange, in the drier southern third of the island, suggesting more restricted ranges. As for *A. agalma* and *A. yiambiae*, they were exclusively known from their type localities on the eastern mountainous tropical part of the island, in the environment of Massif du Panié.

Our sample of *A. agalma* indeed comes from the type locality, but this is not the case for our specimens of *A. yiambiae*, collected together with an unidentified species more than 150 km southeast of the type locality, in the also mountainous range of Mont Do. The two new species described here are only known so far from their respective type localities: one already prospected in the past, Mt Koghi not far from Nouméa (*A. rostrata* Gómez-Zurita sp. nov.), but the other from a very isolated point in the Massif du Humboldt, very remarkable from a biological point of view and clearly understudied faunistically, the Massif du Kouakoué (*A. geographica* Gómez-Zurita sp. nov.).

Concluding remarks

In one of the most recent attempts to quantify the terrestrial animal diversity in New Caledonia, some 4500 known species were recorded, estimating that a five- to fifteen-fold increase in actual species numbers could be expected (Chazeau 1993). Indeed, over the past 15 years many new taxa have been added to prove how far we are from reaching the ultimate goal of inventorying the whole of the New Caledonian diversity. A keyword-based search in *Zoological Records* (14 October 2009) for publications since 1993 including the words "New Caledonia" and "Coleoptera" yielded 47 taxonomic publications with 231 new species descriptions, an increase of 15% over the estimated figure by Chazeau (*c.* 1500; 1993). In relatively better-known zoological groups, for example arachnids and reptiles, the same search strategy produced 27 taxonomic papers and 140 new species in the case of 'Arachnida' and 23 papers describing 41 species in the case of 'Reptilia', in both cases doubling the number of species known in 1993.

In this work we focus on a single genus of a leaf beetle subfamily, the Alticinae, including 11 genera and some 27 species in New Caledonia (Jolivet et al. in preparation). A relatively small sampling obtained from a variety of collecting techniques not necessarily aiming at flea beetles showed that it included new species increasing at least 30% the known species diversity of *Arsipoda*. Our approach using molecular markers to characterize the animals allowed us, as non-specialists in this group, to quickly recognize the new species, directing our efforts to these new entities for their formal description, while the use of xenobiotic markers also helped in identifying some aspects of the interaction of these animals with their environment, in particular pointing at potential hosts. Interestingly, the inference of plant hosts compatible with the New Caledonian flora yet not covered by the current catalogues, hinted in turn to some undiscovered botanical diversity, this time using an indirect approach. Even though we are aware of New Caledonia bearing a higher Alticinae diversity than currently characterized, with seven species, *Arsipoda* stands out already as a

particularly species-rich genus of flea beetles in the island. It was beyond the scope of this study to prove the monophyly of New Caledonian *Arsipoda*, but it is likely that it constitutes a natural group, radiating in isolation, as has occurred for several animals and plants (e.g. Eibl et al. 2001; Smith et al. 2007). According to our molecular clock estimates it would also be a relatively recent addition to the island fauna, in the mid-Miocene. The data available on the distribution and biology of these species suggest that in general there are no geographical or ecological (or a mixture of both, e.g. altitude) barriers to dispersal, therefore the high species richness remains intriguing, at least under allopatric or host-shift models of speciation. Alternatively, this species assemblage richness might be the result of several independent colonization events in the Miocene or later, as observed for other insect groups (Balke et al. 2007; Grandcolas et al. 2009). The presence on the island of several species, independently of them being the result of single or multiple colonization events, may be favoured by their eclectic nature at least regarding food choice. The singularity of the flora of New Caledonia may condition the success rate of herbivorous newcomers to finding an appropriate niche to thrive, unless they are generalists or versatile in behaviour and/or metabolism, as has been shown in the case of *Arsipoda*, able to use new resources, such as introduced plant species (Samuelson 1994). This same versatility may prove an advantage against extinction, given the alarming rates of loss of original flora in New Caledonia, currently supporting for instance only 2% of its original tropical dry forest (Myers et al. 2000; Gillespie and Jaffré 2003). Understanding both the origins and speciation patterns in this genus will require a thorough phylogenetic examination of *Arsipoda* from neighbouring areas.

This study highlights the importance of investing in fieldwork on biodiversity hotspots as allowed by funding sources. Most importantly, it prompts us to design fieldwork with the appropriate strategies to allow the implementation of molecular-based approaches to taxonomy and the analysis of biodiversity, both from a cataloguing point of view and for the study of biotic interactions. The investigation of these isolated communities, lacking the framework of the broader evolutionary lineage into which they belong, generates perhaps more questions than answers about their evolution, but clearly constitutes the first step towards more extensive approaches, with a wealth of evolutionary hypotheses to test.

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