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Diversity and diversification of Eumolpinae (Coleoptera: Chrysomelidae) in New Caledonia

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Contemporary taxonomic work on New Caledonian Eumolpinae (Chrysomelidae) has revealed their high species richness in this Western Pacific biodiversity hotspot. To estimate total species richness in this community, we used rapid DNA-based biodiversity assessment tools, exploring mtDNA diversity and phylogenetic structure in a sample of 840 specimens across the main island. Concordance of morphospecies delimitation with units delimited by phenetic and phylogenetic algorithms revealed some 98–110 species in our sample, twice as many as currently described. Sample-based rarefaction curves and species estimators using these species counts doubled this figure (up to 210 species), a realistic estimate considering taxonomic coverage, local endemism, and characteristics of sampling design, amongst others. New Caledonia, compared with larger tropical islands, stands out as a hotspot for Eumolpinae biodiversity. Molecular dating using either chrysomelid specific rates or tree calibration using palaeogeographical data dated the root of the ingroup tree (not necessarily a monophyletic radiation) at 38.5 Mya, implying colonizations after the Cretaceous breakage of Gondwana. Our data are compatible with the slowdown in diversification rates through time and are also consistent with recent faunal origins, possibly reflecting niche occupancy after an initial rapid diversification. Environmental factors (e.g. soil characteristics) seemingly played a role in this diversification process.

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ADDITIONAL KEYWORDS: biodiversity – biodiversity and conservation hotspot – diversification patterns – DNA barcoding – Eocene – generalized mixed Yule–coalescent method – mtDNA – phylogeny – species delimitation.

INTRODUCTION

Our current views on the distribution of Earth's biodiversity, particularly in relation to conservation, have been significantly influenced by the concept of hotspots, which amalgamates perceptions of the degree of endemicity with those of vulnerability (Myers *et al*., 2000). Several refinements to and also criticism of this concept have been proposed based on different taxonomic choices and/or metrics (e.g. Orme *et al*., 2005), or excluding taxonomic considerations altogether (e.g. Kareiva & Marvier, 2003; Hoekstra *et al*., 2005). However, regardless of the pertinence of the concept or how its implementation is attempted, the corroboration of the existence of areas with exceptionally rich, singular, and threatened biotas prevails (Myers, 2003; Possingham & Wilson, 2005); this pattern demands an academic explanation and can help conservation efforts. One such place is New Caledonia. At 18 500 km^2 (88% corresponding to the largest island, Grande Terre), it is the smallest of the biodiversity hotspots, yet it shows similar biodiversity indexes as other recognized hotspots one or two orders of magnitude larger (Myers *et al*., 2000).

This Western Pacific archipelago has been host to an interesting riddle relating to the origins of its biodiversity. The archipelago is constituted by a combination of plate tectonic and volcanic (oceanic) land aggregations (Pelletier, 2006) on the Norfolk Ridge, midway between New Guinea and New Zealand. The opening of the Coral and Tasman Seas, initiated some

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85 Mya, separated this oceanic feature from the fragmenting supercontinent Gondwana, and with this process the oldest terrains of New Caledonia became isolated (Kroenke, 1996). Thus, the historical biogeography of New Caledonia, its high endemism rates, and the presence of relicts have been traditionally interpreted in the context of late Cretaceous, Gondwanan vicariance, much as for New Zealand (Jolivet & Verma, 2010). Recent debate stemming from geological and biogeographical research in New Zealand (Sanmartín & Ronquist, 2004; Gibbs, 2006; Campbell & Hutching, 2007) and extrapolated to New Caledonia, as well as cumulative geological and analytical evidence on several groups of organisms (Pelletier, 2006; Grandcolas *et al*., 2008), have advocated alternative dispersive models for the origin of the New Caledonian biota. Data support in most cases a recent, late Eocenic or Oligocenic origin for species assemblages in New Caledonia (Murienne *et al*., 2005; Smith *et al*., 2007; Espeland & Johanson, 2010; Nattier *et al*., 2011), a theory that has been linked to geological evidence for the island's complete submergence below the ocean surface during the Eocene (Espeland & Murienne, 2011). Therefore, if this theory is correct, all the terrestrial biodiversity that we see today on New Caledonia would be descended from *de novo* transmarine colonizers after the pre-Oligocenic biota had been washed away as a result of this catastrophic event. However, some data seem to corroborate the Gondwanan hypothesis (Sharma & Giribet, 2009), leaving open as possibilities either partial submergence or nearby refugia that could have served as sources of immediate colonization with a depauperate, but more or less original, biota (Heads, 2010). In any case, the origin of the New Caledonian biota appears as a valid question that needs to be solved case by case.

For years, we have been interested in characterizing the diversity of a species-rich group of New Caledonian leaf beetles, the Eumolpinae (Jolivet, Verma & Mille, 2005, 2007a, b, c; 2009a, b; Samuelson, 2010; Gómez-Zurita, 2011a, b). This Chrysomelidae subfamily is one of the largest, with some 7000 species worldwide, and is particularly diverse in the tropics (Jolivet & Verma, 2008). In New Caledonia, the species count so far is 65 species (with only three in the tribe Nodinini, not treated in this study) in some eight genera, but the perception from study of available collections is that many more await discovery. Considering the size of the island and its remoteness, this figure stands out as relatively high compared with other faunas, adding to the idea of New Caledonia being a hotspot of diversity for Chrysomelidae beetles. This distinction may be shared by the neighbouring Fiji archipelago, of nearly the same size and only slightly closer to the Equator, which hosts up to 68 species of Eumolpinae (Bryant & Gressitt, 1957). These observations contrast with those from the whole of North America north of Mexico, with some 145 species of Eumolpinae (Arnett *et al*., 2002), with the fauna of Madagascar, at similar latitude but more than thirty times larger than New Caledonia, and only having between five and six times as many Eumolpinae species (http://www.chrysomelidae.it/afr_Eum/index.html; Stefano Zoia), and with that of Borneo, on the Equator and forty times larger, but with only 88 species of Eumolpinae recorded (Mohamedsaid, 2004). Besides a high species count, five of the known genera are endemic to New Caledonia, and all the species also qualify as endemic. Thus, this group fits most others that have been investigated to date in the archipelago with regard to high diversity, uniqueness, and singularity (Grandcolas *et al*., 2008).

The approach used so far for the characterization of this fauna has followed a traditional perspective based on the study of external and genitalic morphological features. This approach alone, practiced intensively in a very short time period of five years, has nearly quadrupled the number of known species compared to historical 19th and early 20th century research (Montrouzier, 1861; Fauvel, 1862; Perroud & Montrouzier, 1864; Heller, 1916). However, the approach has also resulted in some examples of taxonomic inaccuracy (Gómez-Zurita, 2011a, b). In any case, the challenge ahead is to characterize a very diverse and complex zoological group, relying on operational criteria to minimize taxonomic subjectivity, and with a certain component of urgency to address the conservation needs inherent to biodiversity hotspots. These circumstances demand the use of rapid biodiversity assessment (RBA) approaches, i.e. quickly collecting information on the species present in a given area (Alonso *et al*., 2011). It has been recognized that RBA helps in reducing costs and simplifying otherwise very complex ecological assemblages (New, 1998), but in the context of our work, it also supplements taxonomic expertise, which may take years or entire careers to develop (e.g. Ward & Larivière, 2004). In particular, we advocate here an approach that exploits DNA sequences as barcodes or within a phylogenetic framework to speed up the recognition of species limits, already used in the context of arthropod island radiations (e.g. Monaghan *et al*., 2006), and also increases accuracy, reliability, and chiefly objectivity in taxonomic assessment.

Despite the initial controversy generated when the idea of DNA taxonomy was originally put forward (Tautz *et al*., 2003), DNA-based species delimitation has been established as a common practice in ecological and evolutionary studies and a wide range of relevant methodologies have been developed over the past decade. Recently, with the rapid accumulation of genome-level data, the statistical rigour of species

delimitation has increased considerably, as coalescent theory provides powerful models for testing alternative hypotheses of evolutionary independence (Fujita *et al*., 2012). However, these multilocus coalescent-based approaches are not yet applicable to routine large-scale biodiversity assessments and species discovery when there is no previous taxonomic information available, although they may be employed at a later stage to evaluate specific hypotheses of species status. Phenetic analyses based on DNA sequence pairwise distances (Hebert *et al*., 2004; Blaxter *et al*., 2005; Jones, Ghoorah & Blaxter, 2011) are the most widely applied clustering methods as they are quick and easy to implement, but they have been criticized for failing to consider evolutionary processes (Cognato, 2006), and for relying on largely arbitrary fixed thresholds (DeSalle, Egan & Siddall, 2005; Meier *et al*., 2006) whose performance critically depends on the existence of a 'barcode gap' between intra- and interspecific variation (Meyer & Paulay, 2005).

Although DNA-based phenetic methods doubtlessly have operational advantages, the main asset of DNA-based surveys for RBA is that the availability of DNA sequence data in taxonomic surveys provides more robust species delimitation criteria and also offers a unique glimpse into the evolution of the communities (Monaghan *et al*., 2006). Several methods have been proposed for tree-based species delimitation with different sets of underlying assumptions (Fujita *et al*., 2012). Amongst these, the generalized mixed Yule–coalescent model (GMYC; Pons *et al*., 2006) provides an evolutionary alternative to distance-based clustering methods based on population genetics and phylogenetic theory, and it is suited for single-locus data. It has been employed successfully for the characterization of hyperdiverse faunas in biodiversity hotspots (Monaghan *et al*., 2009; Pagès *et al*., 2010; Isambert *et al*., 2011), including New Caledonia (Espeland & Johanson, 2010), and for the discovery of cryptic diversity in a variety of understudied taxa (Barraclough *et al*., 2009; Fontaneto *et al*., 2009). Several studies have evaluated the performance of the method using simulations and have shown that it is sensitive to certain characteristics of species history, such as high speciation rates or large effective population sizes (Esselstyn *et al*., 2012; Reid & Carstens, 2012), to the effect of sampling (Lohse, 2009), and to artefacts related with phylogenetic inference (Reid & Carstens, 2012). Nonetheless, independently evolving groups delimited by the model in real data sets correspond well to morphological species (Pons *et al*., 2006; Monaghan *et al*., 2009; Hendrich *et al*., 2010) and represent meaningful entities from ecological (Powell *et al*., 2011) and biogeographical (Papadopoulou *et al*., 2011) points of view.

In this study, we aimed to discover how many species of New Caledonian Eumolpinae there are in our sample using molecular and phylogenetic tools in an RBA framework and further how representative this species diversity is of the expected species richness in the archipelago. This information is important not only for the assessment of biodiversity in the island but also in the context of New Caledonia as a biodiversity hotspot. We were also interested in timing the origin of this assemblage and testing this against the classical Gondwanan biogeographical paradigm and the most recent revisionist transoceanic colonization views. This is in turn a very valuable piece of information to understand the origins of biodiversity in New Caledonia. Finally, knowing how many Eumolpinae species there are and their temporal framework, we examined the mode of diversification of this group in this remarkable biodiversity hotspot.

MATERIAL AND METHODS

Beetle sampling

Extensive leaf beetle sampling was carried out in a total of 38 localities covering a variety of habitats across the main island in New Caledonia (Grande Terre), including sclerophyll forests in the western part of the island, the so-called *maquis minier* in the south, and localities from mid to high altitude in the central mountain chains, as well as a few incursions into rainforests of the eastern and northern ranges (Fig. 1, Table S1). Three additional samples were available from the same number of surrounding islands (Ouvéa, Maré, and Île-des-Pins). Most samples were collected by beating and sweeping vegetation and were immediately stored in 100% ethanol in the field. Relatively fewer samples were collected using Malaise traps from other entomological surveys and donated for our research, and still fewer samples included in the study were dry specimens from the Institut Agronomique néo-Calédonien (IAC) beetle collection (La Foa, New Caledonia). The specimens are currently vouchered in J. Gómez-Zurita's research collection (IBE-CSIC, Barcelona), but type material for the species discovered as part of this research will eventually be deposited in the Entomological Collection of the Muséum national d'Histoire naturelle (Paris, France).

DNA extraction and sequencing

A total of 840 Eumolpinae specimens covering as much morphological and geographical diversity as possible was selected for genetic characterization (Table S1). DNA extractions were performed nondestructively from whole specimens using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) was ampli-

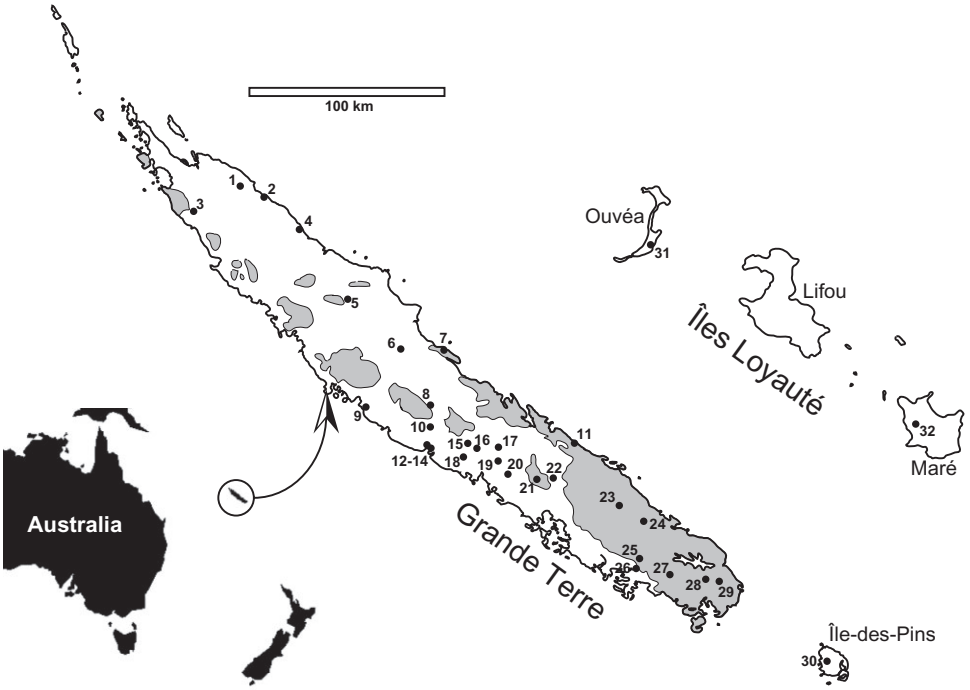

Figure 1. Map showing the position of New Caledonia relative to Australia and New Zealand, with details on the distribution of ophiolitic nappe (shaded areas; redrawn from Cluzel *et al*., 2001) discussed in the main text, and the geographical sources of samples used in this study: 1, Mandjélia; 2, Pouebo; 3, Koumac; 4, Cascade de Tao; 5, Bopope; 6, L'Aoupinié; 7, Baie Ugué; 8, Col des Roussettes; 9, Poya; 10, Bourail; 11, environs Thio; 12, Baie des Tortues; 13, Roche Percée; 14, Delta de la Nera; 15, Table Unió; 16, Farino; 17, Col d'Amieu; 18, Moméa; 19, Sarramea; 20, La Foa; 21, Mont Do; 22, route Boulouparis-Thio; 23, Mount Humboldt; 24, Kouakoué; 25, Barrage de la Dumbea; 26, Monts Koghis; 27, Kwa Ne Mwa; 28, Chutes de la Madeleine; 29, Plaine des Lacs; 30, Île-des-Pins; 31, Ouvéa; 32, Maré.

fied using TL-N-3014 (Simon *et al*., 1994) and a modified C1-J-2183 (Gómez-Zurita *et al*., 2012) for a fragment of 829 bp, or alternatively by using 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*., 2011) to produce two smaller and non-overlapping fragments of 401 and 406 bp for degraded template DNA. Primers SR-N-14759 and SR-J-14233 (Simon *et al*., 1994) were used to amplify a fragment of 512–513 bp of the small subunit of the mitochondrial ribosomal RNA (*rrnS*). PCR conditions used in every case were 35 cycles of 30 s denaturation, 30 s annealing (50 °C for *cox1* and 55 °C for *rrnS*), and 1 min elongation. PCR products were purified using ammonium acetate and isopropanol and sequenced in both directions using a BigDye Terminator v. 3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Matrix assembly

Sequences were assembled and edited using GENEIOUS PRO 5.3.6 (Biomatters Ltd, http://www.geneious.com/). The *cox1* sequences were unambiguously aligned manually as they were not length-variable, whereas for the *rrnS* sequences we used the MAFFT 6 multiple sequencing alignment algorithm implementing the E-INS-i strategy and default parameters (Katoh *et al*., 2005). Prior to the phylogenetic analyses, specimens with identical haplotypes for both *cox1* and *rrnS* were identified and the redundant sequences were removed from the data set. Best-fit nucleotide substitution models for each gene and codon position were selected by the Akaike information criterion in jModelTest (Posada, 2008). Different data partitioning schemes were evaluated using the AIC after conducting preliminary maximum likelihood analyses in RAxML 7.2.6 (Stamatakis, 2006), and the selected partitioning scheme was used for all subsequent phylogenetic analyses. Four alternative data partition treatments were compared: no data partitioning (1P); partitioning by locus (2P); *cox1* first and second codon positions, *cox1* third codon positions and *rrnS* (3P); and partitioning by locus and further partitioning *cox1* by each codon position (4P).

Species delimitation

We used three different approaches for species delimitation of sampled Eumolpinae: sorting to

morphospecies, and both phenetic and phylogenetic DNA-based approaches.

Morphospecies designation

Specimens were sorted to morphospecies and assigned to known species when possible. Sorting to morphospecies used information not only on external appearance and resemblance, but also on male and female genitalic characters as these typically have high species diagnostic value in Coleoptera (e.g. Sharp & Muir, 1912). These species hypotheses, consistent with traditional taxonomic practice, constituted the basis for comparison with the results from the DNA-based approaches to species delimitation.

Phenetic approach

We used the recently developed 'Automated Barcode Gap Discovery' (ABGD) software (Puillandre *et al*., 2011) to explore the existence of a 'barcode' gap in the New Caledonian Eumolpinae data set. The method aims to detect a gap in the distribution of pairwise genetic distances, distinguishing between intraspecific and interspecific distances, and sorting the sequences into hypothetical species accordingly. Given a prior intraspecific divergence value chosen by the user, the ABGD software identifies the first significant gap beyond this threshold and this is used to partition the data. Inference of the gap and data partitioning are then recursively applied to the obtained groups until no further partitioning is possible. The procedure is repeated for a range of *a priori* intraspecific divergence values. Extremely low *a priori* values would lead to the separation of each haplotype as a different group, whereas extremely high values would place all haplotypes into a single group. If there is a barcode gap in the data set, intermediate *a priori* thresholds are expected to lead to similar partitions (Puillandre *et al*., 2011, 2012). We applied the method on each gene separately as well as on the two gene fragments together, using a reduced data set without any missing data. Distances were calculated under the Jukes–Cantor model and we explored a range of prior intraspecific divergences between 0.001 and 0.1.

Phylogenetic species delimitation

We applied the GMYC model (Pons *et al*., 2006) on the Eumolpinae mtDNA gene tree. This method is increasingly used for species delimitation in taxonomically understudied faunas, and was specifically developed for mtDNA data. The method delimits putative species (or 'independently evolving lineages') by identifying a shift in the branching rates of the gene tree, which corresponds to the transition from interspecific diversification (Yule model) to intraspecific genealogical branching (coalescent). A threshold value is optimized for this transition at the point of highest likelihood and confidence intervals are calculated as solutions within two log-likelihood units from the maximum. A recent modification of the original model allows the age of the transition point to vary amongst lineages (Monaghan *et al*., 2009). The GMYC model was applied using functions of the R package 'splits' (SPecies LImits by Threshold Statistics; available at http://r-forge.r-project.org/projects/splits/) in R 2.13.2 (R Development Core Team, 2011). We assessed both single- and multiple-threshold models and evaluated which fitted the data significantly better.

GMYC analyses rely on a pre-estimated ultrametric phylogenetic tree, which we obtained following three alternative approaches, trying to account for biases in topology and branch-length estimation. The first tree was obtained based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) without enforcing a clock, with six parallel runs of 20 000 000 generations each, using one cold and three incrementally heated Markov chains ($\lambda = 0.1$) and sampling every 1000 steps. A separate substitution model was applied to each partition, as selected by AIC. After checking standard convergence diagnostics, all post-burn-in trees were summarized in an all-compatible consensus tree. This consensus tree was subsequently converted to an ultrametric tree using either: (1) penalized likelihood as implemented in r8s 1.7 (Sanderson, 2003) with the optimal smoothing parameter selected by cross-validation of values between 0.01 and 1000 or (2) the PATHd8 algorithm (Britton *et al*., 2007). In both cases, the root of the tree was arbitrarily fixed to 100 time units. The second tree was obtained under maximum likelihood (ML) in RAxML 7.2.6 (Stamatakis, 2006) with 100 replicates, applying a separate general time-reversible + gamma ($GTR + \Gamma$) model to each partition [as RAxML only implements GTR-based models and the program's author does not recommend the use of $GTR + \Gamma + I$ (where I = proportion of invariant sites)]. The tree with the highest likelihood was transformed into ultrametric using r8s and PATHd8 approaches as above. Finally, a third tree was inferred under an uncorrelated lognormal relaxed clock in BEAST 1.6.2 (Drummond *et al*., 2006; Drummond & Rambaut, 2007) using a coalescent tree prior with constant size. The mean substitution rate of the two gene fragments was fixed arbitrarily to 1, and a separate clock model was applied to each partition, with an ucld.mean drawn from a uniform distribution [0,100]. Moreover, a separate substitution model was applied to each partition, with a uniform prior [0,100] for each of the relative substitution rate parameters, and default values for all other priors and operator settings. Three independent runs were conducted of 50 million generations each (sampling every 5000th generation). The convergence and mixing of the Markov chain Monte Carlo chains were assessed by inspection of the trace

plots and the effective sample sizes using TRACER 1.5 (Rambaut & Drummond, 2007). Samples from the three independent runs were then pooled after removing a 10% burn-in using LogCombiner 1.6.2 (Drummond & Rambaut, 2007) and the means of node heights were summarized on a 'maximum clade credibility' tree using TreeAnnotator 1.6.2 (Drummond & Rambaut, 2007). Both MrBayes and RAxML analyses included the Australian species *Edusella* sp. (voucher no. IBE-JGZ-0225) as an outgroup, which was pruned (either in r8s or in R) before applying the GMYC model. BEAST analyses were performed without using an outgroup. MrBayes and BEAST analyses were run on the Cipres Science Gateway server (Miller, Pfeiffer & Schwartz, 2010).

Species richness estimation

Species accumulation curves were constructed in order to assess the comprehensiveness of the sampling, and species estimators were computed in order to get an estimate of the expected total species richness of Eumolpinae in New Caledonia. Specimens were assigned to putative species based on our different proxies for species delimitation, and incidence data were recorded for each independent sample (collected from a different locality and/or different date). EstimateS (Colwell, 2005) was used to compute sampled-based rarefaction curves with the Mao Tau function and four incidence-based nonparametric species richness estimators (incidence-based coverage estimator, Chao 2, and first and second order jackknife) with 100 randomizations of sample order.

Estimation of time-framework

Molecular dating analyses were conducted in order to infer the age and the diversification rates of the group. These analyses were performed at the species level using a reduced data set (112-taxon set), which included one individual per GMYC group and additionally the three individuals collected from the surrounding islands (Ouvéa, Maré, and Île-des-Pins). In the absence of fossil evidence, estimation of divergence times was based either on previously calibrated rates from other studies or on a tree calibrated with geological information. In the first case, two strategies were considered: (1) a mean rate of *cox1* and *rrnS* (0.018 substitutions per site per Myr per lineage) calculated for the same two gene fragments for a different group of chrysomelids based on biogeographical evidence (Gómez-Zurita *et al*., 2012); and (2) the so-called standard insect mtDNA clock rate (0.0115 substitutions per site per Myr per lineage; Brower, 1994), which is widely used in the entomological literature, even though its general applicability across taxa and gene regions has been questioned (Papadopoulou, Anastasiou & Vogler, 2010; Pons *et al*., 2010). There is a *cox1* rate proposed for Coleoptera (0.0861 substitutions per site per Myr per lineage; Pons *et al*., 2010), but we opted to overlook it here as it proposes an unreliably high value within a prohibitive confidence interval, suggesting some kind of analytical artefact (Andújar, Serrano & Gómez-Zurita, 2012). In both cases we applied an uncorrelated lognormal relaxed clock in BEAST 1.6.1 (Drummond *et al*., 2006; Drummond & Rambaut, 2007) under a Yule tree prior and partitioning data (both substitution and clock models) using the 3P scheme. Three independent runs of 50 000 000 generations (sampling every 5000th generation) were performed for each of the alternative substitution rates. After checking convergence diagnostics and removing a 10% burn-in, the samples from the three independent runs were pooled, and the means of the node heights, with 95% highest posterior density intervals were summarized on a maximum clade credibility tree using TreeAnnotator 1.6.1. The results of the three analyses were evaluated in the light of the palaeogeographical history of the region.

A biogeographical tree calibration was attempted by dating the phylogenetic split between two samples from two of the Îles Loyauté (Maré and Ouvéa) and their sister from Grande Terre conservatively at a maximum of 2 Mya, the purported time of emergence of the former (Dubois, Launay & Recy, 1974; Kroenke & Rodda, 1984; Grandcolas *et al*., 2008). In this case BEAST was used as above, under a strict clock and the 1P partitioning scheme, and calibration data were implemented effectively as single-point information by using an age prior for the split of interest defined by a narrow normal distribution (mean = 2, SD = 0.001). In the absence of other calibration points to modulate this information, this strategy aimed at estimating the oldest possible age for the ingroup, as allowed by oceanic island colonization scenarios (maximum age), and as an alternative to uniform priors with hard left bounds generally used when other age priors are available. (Nonetheless, other calibration strategies, including relaxed clocks, uniform, wide or truncated normal priors for the same node or for this and the one defined by the sample from Île-des-Pins, were used and resulted in convergence problems.)

Estimation of diversification rate

The dated phylogenies were subjected to diversification rate analyses using two alternative approaches:

1. Lineage through time plots were constructed and the gamma statistic (Pybus & Harvey, 2000) was calculated, which assesses whether splitting events are evenly distributed across the phylogeny

or accumulated towards either the root or the tips of the tree. Negative gamma values indicate that nodes are accumulated towards the root of the phylogeny and are interpreted as a signature of a slowdown in diversification rates. These analyses were performed using functions of the R packages laser (Rabosky, 2006a) and ape (Paradis, Claude & Strimmer, 2004). In order to account for incomplete taxon sampling, we conducted a Monte Carlo constant rates (MCCR) test as implemented in laser (Rabosky, 2006a,b). For this purpose, 5000 phylogenies were simulated under the null hypothesis of a constant rate birth–death diversification process and a number of tips were randomly pruned from the tree to mimic incompleteness of sampling as assessed by the species richness estimators (we used the most extreme of the calculated values i.e., total clade size = 200, missing taxa = 90). Moreover, nine models of diversification were fitted using ML and compared under the AIC criterion. These included two rate-constant models (Yule 1-rate; birth–death model), two rate-variable (Yule 2-rate; Yule 3-rate), two density-dependent (diversity dependent linear; diversity dependent exponential), and three models with continuous-time varying speciation/extinction rates (SPVAR; EXVAR; BOTHVA) (Rabosky & Lovette, 2008). The significance of the dAICrc statistic (difference between the best-fit rate-constant and the best-fit rate-variable model) was evaluated against a null distribution of AIC scores created on the basis of 5000 trees simulated as explained above.

2. A recently developed coalescent ('backwards-in-time') approach was applied (Morlon, Potts & Plotkin, 2010), which, amongst other advantages, is particularly suited for incomplete phylogenies. This approach aims to investigate: (1) whether diversity is constant (saturated) or expanding, (2) whether diversification rates are time-constant or time-variable, and (3) whether extinction can be detected. We tested the nine diversification models proposed by Morlon et al. (2010) and compared their second-order AIC (AICc) scores under a ML framework, using R scripts provided by the authors. The total number of species at present was set to 200 (i.e. the maximum value calculated by the species richness estimators). For each model we tried several initial values, as recommended by the authors, in order to avoid getting trapped in local optima.

RESULTS

Eumolpinae sequence diversity

From a starting set of 840 Eumolpinae individuals from New Caledonia, 797 were amplified successfully for at least one of the two mitochondrial fragments. The remaining 5.1% of samples consistently failed to produce PCR products or the expected sequence, and these were mostly dry collection specimens or specimens collected from Malaise traps. However, for some others amongst these difficult samples at least *rrnS* or one of the smaller *cox1* fragments were amplified and sequenced. In summary, 725 specimens were sequenced for both markers (1.9% with 5–11%, and 17.7% with < 5% of missing data), 46 lacked *cox1* entirely, seven lacked one of the two shorter *cox1* fragments, 17 lacked *rrnS*, and two were only available for one of the smaller *cox1* segments. Sequences are available in the EMBL nucleotide sequence database (http://www.ebi.ac.uk/ena/) under accession numbers HF920681–HF921458 (*rrnS*) and HF921478–HF922228 (*cox1*).

Out of the 797 concatenated sequences, there were 349 unique haplotypes. The final alignment comprised a total of 1386 characters [817 nucleotides (nt) for *cox1* and 569 nt for *rrnS*], including 727 variable positions (687 of them informative for parsimony) and 77 positions with gaps within the *rrnS* fragment. Uncorrected pairwise distances averaged for both markers ranged from 0.0007 to 0.2024 (mean 0.1427) and considering each marker individually were 0–0.238 (mean 0.150) for *cox1* and 0–0.202 (mean 0.130) for *rrnS*.

Delimitation of morphospecies

The initial 840 Eumolpinae specimens were prepared dry after DNA extraction and their sclerotized genitalic structures mounted with the specimen, available for future reference in the collection IBE-JGZ (Barcelona, Spain). Their comparison allowed the identification of 102 morphospecies, completely independently from information derived from sequence data, and there were sequences available for 98 of them.

Phenetic species delimitation

For the distance-based analyses of Eumolpinae mtDNA sequence data using the ABGD method (Puillandre *et al*., 2011) a reduced data set was compiled including identical haplotypes but free from missing data, which could distort divergence estimates. These data included 715 individuals belonging to 94 morphospecies and for slightly shorter DNA sequence fragments (650 nt of *cox1* and 518 nt of aligned *rrnS*).

The distribution of pairwise distances indicated the potential existence of a 'barcode gap' in the data set, i.e. a gap that divides the distribution between a left and a right mode (Fig. S1). After inspecting a broad range of *a priori* intraspecific divergence values

(0.001–0.1), we focused on an intermediate range of values (0.01–0.08 for *cox1*, 0.005–0.035 for *rrnS*), within which two candidate thresholds were identified for each marker (Table 1). Following Puillandre *et al*. (2011, 2012), these putative thresholds were identified based on the observation that the same number of groups was produced by the initial partitioning procedure across a broad range of *a priori* intraspecific divergence values (Table 1). The groups defined by the first threshold had a better one-to-one match with morphospecies than those defined by the second threshold (Table 1), and the exact matches were higher for *cox1* (87%) or the combined *cox1* + *rrnS* data set (87%) than for *rrnS* (82%). For the combined *cox1* + *rrnS* data set we explored further the results of the recursive partitioning procedure, which gave a slightly higher number of groups. In one of the examined cases the recursive partitioning appeared to improve slightly the match with the morphospecies, up to 88%, whereas in the others it gave the same or a poorer match (Table 1; Fig. S1).

Table 1. Automated barcode gap discovery analysis of mtDNA data of New Caledonian Eumolpinae

Gene fragment	Threshold*	Intraspecific divergence range	No. of groups	Exact matches†
cox1	1i	0.011–0.040	105	82
	2i	0.041–0.080	87	72
rrnS	1i	0.005–0.009	115	77
	2i	0.009–0.032	92	76
cox1 + *rrnS*	1i	0.007–0.020	102	82
	1r	0.013–0.016	113	77
	1r	0.020–0.022	105	83
	2i	0.030–0.066	88	74
	2r	0.037–0.050	91	74

*First (1) and second (2) thresholds are distinguished, as well as their recovery in an initial (i) or recursive (r) procedure.

†Referred to 94 morphospecies present in the reduced data set without missing data.

Phylogenetic species delimitation

The selected partitioning scheme for the phylogenetic analyses based on the complete 349-haplotype data set was 3P, i.e. two partitions for *cox1*, and the corresponding substitution models were a GTR + Γ + I for the first and second codon positions of *cox1*, and a GTR + Γ for *rrnS* and the third codon position of *cox1*. The results for the reduced 112-terminals data set were identical except for a best fit of the Hasegawa-Kishino-Yano (HKY + Γ + I) model for *rrnS* data. All phylogenetic analyses resulted in similar tree topologies (Fig. 2). Clade support was generally higher towards the tips of the tree and lower near the root, i.e. tree topology appeared rather unstable at the deeper level. There was however a highly inclusive and relatively deep clade that was consistently recovered as monophyletic across all ML and Bayesian analyses performed [bootstrap (BS) > 90%, posterior probability (PP) = 1.00]. This clade, referred to as Clade A hereafter (Fig. 2), included four highly supported lineages: two with most samples currently ascribed to the endemic genus *Montrouzierella*, and closely related to *Montrouzierella nana* (Clades A1 and A3; BS = 99%, PP = 1.00), one with the species *Dematocroma terastiomerus* (lineage A2) and one with *Samuelsonia* species and closely related to *Samuelsonia bicolor* (Clade A4; BS = 74%, PP = 0.99). Other supported clades with four or more species included Clade B, composed of *Dematocroma difficilis* and four to seven relatives (BS = 100%, PP = 0.99); Clades C and D, both with species closely related to *Dematocroma pilosa* (BS = 78–94%, PP = 0.99); Clade E composed of *Dematocroma laboulbenei* and five to seven relatives (BS = 79%, PP = 0.98); Clade F grouping all *Samuelsonia* species with metallic teguments (nine to ten species; BS = 94%, PP = 0.99); Clade G with *Dematocroma maculifrons* and three relatives (BS = 97; PP = 0.99); Clade H with *Dematocroma culminicola* and three or four close relatives (BS = 100%, PP = 0.99); and Clade I with *Taophila subsericea* and its close relatives (BS = 100%, PP = 0.99).

The results of the GMYC analyses differed slightly depending on the method used to obtain the ultrametric tree (Table 2). In most cases, except when

Figure 2. Ultrametric and time-calibrated haplotype tree of New Caledonian Eumolpinae using BEAST under an uncorrelated log-normal (ULN) relaxed clock and a coalescent tree prior. Node support is indicated as maximum likelihood bootstrap values > 70% and posterior probabilities > 0.95 (black dot). Supported clades with four or more species are indicated (Clades A–I), as well as the number of individuals sharing a haplotype (in parentheses; haplotype name matches voucher number of the individual used to represent it). Summary of species delimitation results following three alternative approaches shown next to the tree: (1) morphospecies; (2) automated barcode gap discovery (ABGD) method, first threshold; (3) ABGD, second threshold; (4) generalized mixed Yule-coalescent (GMYC) method, single threshold (confidence interval represented by grey vertical line on tree); and (5) GMYC, multiple threshold model (based on a maximum likelihood + r8s tree). Ple., Pleistocene; Pli., Pliocene.

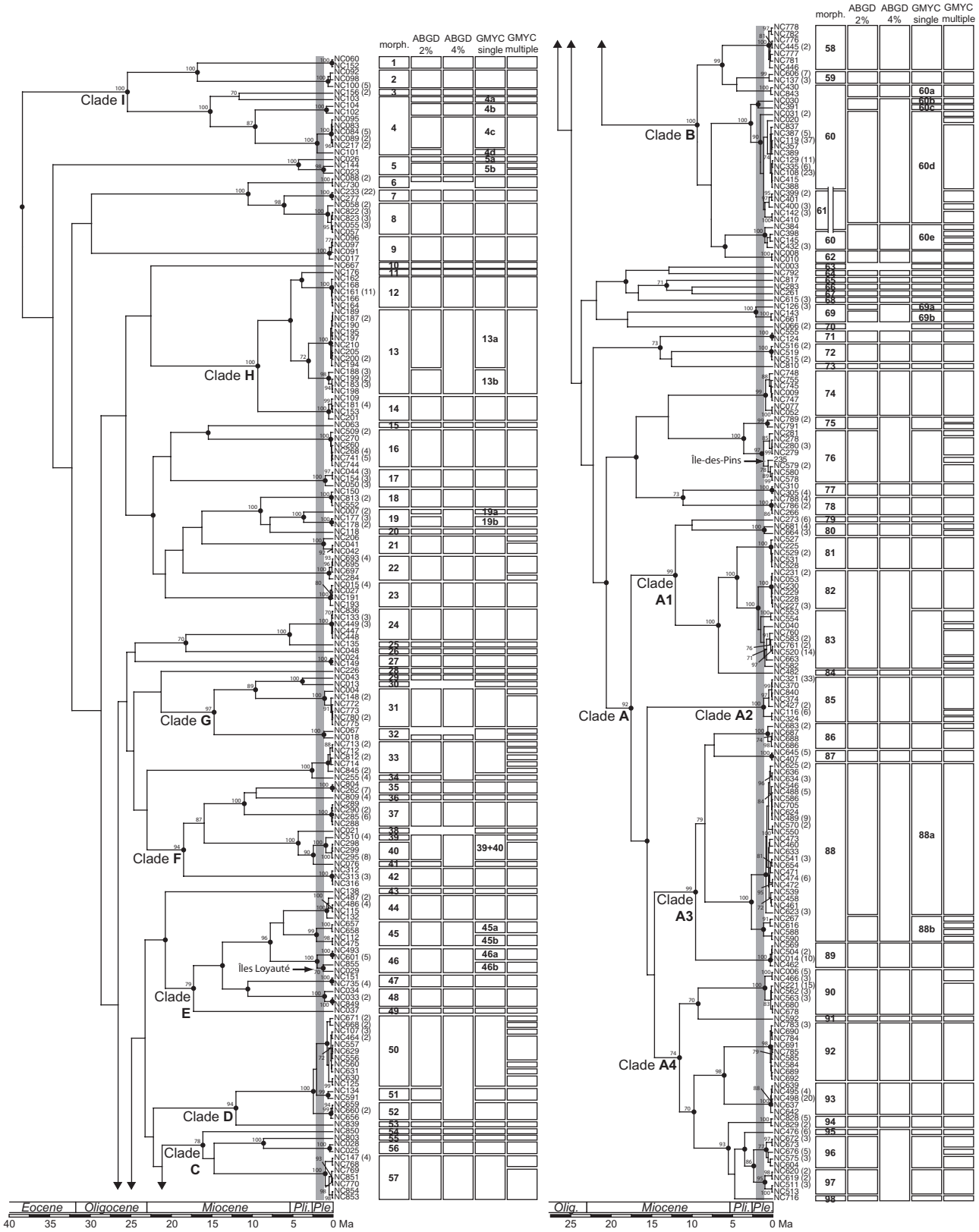
morph.
ABGD 2%
ABGD 4%
GMYC single
GMYC multiple
Clade I
Clade H
Clade G
Clade F
Clade E
Clade D
Clade C
Clade B
Clade A
Clade A1
Clade A2
Clade A3
Clade A4
Îles Loyauté
Île-des-Pins
Eocene
Oligocene
Miocene
Plio.|Ple
Olig.
40 35 30 25 20 15 10 5 0 Ma
25 20 15 10 5 0 Ma

Table 2. Comparison of results of the generalized mixed Yule-coalescent (GMYC) model with either single or multiple thresholds between species diversification and population coalescence based on New Caledonian Eumolpinae mtDNA trees obtained using different methods

Tree building	Threshold	$N_{entities}$ [CI]	$N_{clusters}$ [CI]	L_{null}	L_{GMYC}	LR	d.f.	Exact matches
MrBayes + r8s	Single	110 [102–115]	72 [67–72]	637.092	763.208	252.232***	3	83
	Multiple	148 [146–150]	76 [74–77]		775.3601	24.305***	9	69
MrBayes + d8	Single	103 [94–111]	69 [62–72]	664.950	792.547	255.193***	3	85
	Multiple	148 [148–154]	77 [77–78]		800.102	15.110	9	68
RAxML + r8s	Single	111 [107–121]	68 [68–73]	606.952	769.940	325.975***	3	81
	Multiple	152 [90–156]	70 [57–71]		780.108	20.338***	6	74
RAxML + d8	Single	103 [97–108]	67 [63–69]	917.640	1083.406	331.532***	3	82
	Multiple	122 [114–148]	79 [70–79]		1090.558	14.304	18	77
BEAST	Single	110 [103–114]	71 [68–72]	2564.708	2694.702	259.987***	3	86
	Multiple	150 [148–151]	76 [75–77]		2707.112	24.81951*	12	64

$N_{entities}$, total number of delimited entities; CI, confidence intervals as solutions within two log-likelihood units of the maximum likelihood solution; $N_{clusters}$, number of entities with more than one individual; L_{null}, likelihood of the null model, i.e. a single coalescence for the entire tree; L_{GMYC}, likelihood of the GMYC model with either single or multiple thresholds; LR, chi-square value of the likelihood ratio test comparing either the multiple-threshold against the single-threshold GMYC model, or the latter against the null model; d.f., degrees of freedom for the likelihood ratio test; *, $P < 0.05$; ***, $P < 0.005$.

the starting tree was obtained using the PATHd8 algorithm, the null model, a single coalescence for the entire tree, was rejected (likelihood ratio test, $P = 0$). The GMYC model with a single threshold delimited 103–111 independently evolving entities, depending on the phylogenetic method used, with 83–88% of them matching perfectly the morphospecies designation (Table 2). The multiple-threshold model identified a much greater number of entities (122–152, depending on the method used), mainly because of an increased number of singletons (43–82 vs. 34–43 for the multiple and single thresholds, respectively), whereas the number of identified clusters did not increase greatly (70–79 vs. 67–72 for the multiple and single thresholds, respectively). The entities identified by the multiple-threshold model provided a poorer match with the morphospecies designation (65–78%) than the single-threshold entities (Table 2).

Figure 2 presents the results of the single-threshold GMYC analysis based on the BEAST tree, which showed the highest number of exact matches with morphospecies, i.e. 86 out of 98 morphospecies. (There were three morphospecies – 4, 60, and 61 – that were not retrieved as monophyletic mtDNA groups, and they could not be identified, by definition, as independently evolving entities by the GMYC model.) The disagreements between these two approaches to species recognition were mostly a result of over-splitting (Fig. 2; Table 3). Thus, in the best-case scenario, the 12 examples of morphospecies that did not coincide with the GMYC entities included nine cases of splitting groups of otherwise anatomically homogeneous animals. In seven of these cases a single morphospecies was split into two reciprocally monophyletic groups, and in five of them the two GMYC groups were actually allopatric. In one case, the disagreement was because of lumping and two involved both lumping and splitting. When the multiple-threshold GMYC model was applied, in principle providing a better fit to the data, the over-splitting was more extreme. For example, in the case of the RAxML + r8s tree, which showed the highest number of exact matches between GMYC entities and morphospecies, all 24 cases of disagreement were because of over-splitting morphologically and geographically coherent morphospecies.

Species richness estimators

The results from the analysis based on a single-threshold GMYC on a BEAST tree (showing the best fit to morphospecies delimitation) were used subsequently to select one representative from each GMYC entity for species richness estimation and molecular dating (see below). Species accumulation curves showed an increasing trend with sampling size without showing evidence of approaching an asymptote (Fig. S2). The estimated species richness based on single-threshold GMYC entities (mean of 100 randomizations of sample order ± 1 SD) oscillated between 168 ± 12 (first order jack-knife) and 199 (second order jack-knife), with intermediate values for Chao 2 (174 ± 22) and Incidence-based Coverage Estimator (ICE) (197). When the estimators were

Table 3. Disagreements between the assessments of New Caledonian Eumolpinae based on morphospecies designation and generalized mixed Yule-coalescent (GMYC) groups, exploring the cases of disagreements between morphospecies and GMYC entities

Morphospecies	Monophyly	GMYC entities*	Allopatry	No. ind.	No. loc.	Mean *d* (range)
4	No	4a–d	No	15	5	4.57% (2.73–13.47)
5	Yes	5a–b	Yes	3	3	4.67% (3.97–5.89)
13	Yes	13a–b	No	20	2	2.49% (1.93–3.25)
19	Yes	19a–b	Yes	7	2	2.74% (2.11–5.80)
39/40	Yes (recipr.)	39+40	Yes	14	2	0.01% (0–0.02)
45	Yes	45a–b	Yes	4	2	1.89% (1.85–1.95)
46	Yes	46a–b	Yes	8	4	1.28% (0.81–2.90)
60	No	60a–e	No	100	16	1.38% (0.53–10.85)
61	No	60d–e	Yes	12	2	3.41% (1.79–7.74)
69	Yes	69a–b	Yes	5	3	1.35% (0.71–1.92)
88	Yes	88a–b	No	52	14	0.80% (0.51–1.04)

*Entities based on the single-threshold GMYC results for the tree obtained with BEAST, i.e. the one maximizing agreement with morphospecies (see Fig. 2).
No. ind., number of individuals included in the GMYC group; No. loc., number of localities providing these individuals; Mean *d*, mean uncorrected distance amongst haplotypes included in the sample expressed as percentages; recipr., reciprocal.

calculated using the designated morphospecies, the corresponding values were slightly lower: 148 ± 10 (first order jack-knife), 160 ± 23 (Chao 2), 169 (ICE), and 177 (second order jack-knife), whereas they were slightly over 10% higher when calculated based on the results of the APGD method (combined *cox1 + rrnS* analysis, first threshold, initial procedure): 159 ± 11 (first order jack-knife) 186 ± 30 (Chao 2), 196 (second order jack-knife), and 210 (ICE). However, these values need to be treated with caution, as the species richness estimators did not provide stable estimates across different sample sizes. ICE and Chao 2 showed some signs of levelling off, whereas the first and second order jack-knife estimates appeared to increase steadily with sampling size (Fig. S2).

Molecular dating

Table 4 shows the results of dating the tree using a single individual per GMYC entity and applying different rates and calibration strategies. Using the rate calculated by Gómez-Zurita *et al*. (2012) for *Cryptocephalus* leaf beetles and with the same mtDNA markers studied here, and a relaxed clock, the root age was estimated at approximately 38.5 Mya (95% high posterior density intervals, HPD = 32–45 Mya; Fig. 3A, Table 4). The age of divergence between the Îles Loyauté (Ouvéa and Maré) lineage and their sister taxon from Grande Terre was estimated at 1.85 Mya (HPD = 1.2–2.6 Mya), whereas the age of divergence between the individual from Île-des-Pins and its conspecific from Grande Terre was 0.35 Mya (HPD = 0.15–0.56 Mya). Furthermore, the origin of the monophyletic endemic Clade A was dated at approximately 20.5 Mya (HPD = 17.7–23.3 Mya). This clade would be contemporaneous with others, such as Clade F, that include fewer sampled species. The oldest and statistically supported intra-island radiation would correspond to the species group of *Taophila subsericea* in the endemic genus *Taophila* (Clade I), at some 26.3 Mya (HPD = 10.9–31.9 Mya). Using the standard insect mtDNA rate, slower than the previous, marker-specific rate, the estimated ages were in every case 55% older compared to the previous values (Table 4).

The former analyses retrieved a low posterior value for the variance in the rates across branches ($0.207 \leq$ ucld.stdev ≤ 0.356, depending upon the gene fragment), consistent with a small deviation from the clock-like behaviour of data (C. Andújar, V. Soria, J. Serrano & J. Gómez-Zurita, unpubl. data). In this scenario, under a strict clock model, we investigated the possibility of single-point calibration analyses using a maximum age for the Îles Loyauté colonization of 2 Mya. These analytical conditions retrieved for example a maximum age for the root of 35.1 Mya (HPD = 24.1–45.0 Mya) and for the colonization of Île-des-Pins of 0.48 Mya (HPD = 0.22–0.78 Mya), and generally age estimates only slightly lower than those based on the marker-specific rate (Table 4).

Diversification analyses

Initially, the diversification analyses were conducted using the tree calibrated with the available leaf beetle

cox1 + rrnS rate (Gómez-Zurita *et al.*, 2012). The gamma statistic was negative ($\gamma = -4.401$) and remained significantly negative after correcting for incomplete taxon sampling (MCCR test, critical value: –3.143104, $P < 0.05$), which suggested a slowdown in diversification rates through time. Rate-variable models fitted the data significantly better than rate-constant models (δ[AICrc] = 23.12841, $P << 0.001$). ML estimates of the parameters of all rate-variable models indicated a decrease in diversification rates through time. Out of the nine models that were tested, the 'yule3rate' model, which assumes two diversification rate shifts, was found to fit the data best (AIC = –40.396; Table 5). According to the best-fit model, the initial diversification rate of the group was 0.2 lineages/Myr, which decreased to 0.1 at around 23 Mya, and later (approximately at 16 Mya) to 0.05 lineages/Myr (Fig. 3A; Table 4).

The results of the coalescent approach (Morlon *et al.*, 2010) also supported a decrease in diversification rates through time. According to the AICc values (Table 6), the best-fit model was one with expanding diversity, exponentially declining speciation rate (speciation rate at present $\lambda_0 = 0.055$ lineages/Myr, exponential variation in speciation rate $\alpha = 0.044$), and no extinction (model 6), whereas similar AICc scores were also recorded for models 4a and 4b (expanding diversity, time-variable speciation rates, and a very low extinction rate, either constant or variable). Therefore, these results rejected a saturated-diversity model as well as a constant-speciation model, instead pointing towards relatively low extinction rates.

The same analyses using the other calibrations available also retrieved significantly negative gamma statistics and the same best-fit models, although the actual parameter values changed. When the standard insect mtDNA rate was used, the estimated diversification rates were lower. Thus, under Rabosky's (2006a, b) models the estimated diversification rates were 20–40% lower: initial diversification rate at 0.16 lineages/Myr, decreasing to 0.07 at around 40 Mya and to 0.03 at 15 Mya. In turn, the coalescent approach produced a speciation rate at present = 0.034 lineages/Myr. When the biogeographical calibration was applied, the inferred rates under the preferred yule3 model were 0.18 lineages/Myr, decreasing to 0.06 at around 18 Mya and to 0.02 at 1.6 Mya (i.e. slower rates than with the marker-

Table 4. Inferred ages (in Myr) of 15 supported focal nodes taken from Figure 2 and based on two previously proposed substitution rates and an upper-bound biogeographical calibration. Mean ages and 95% high posterior density intervals given as estimated using BEAST

Clade	Morphospecies	*cox1 + rrnS* specific rate (age range)	Standard rate (age range)	Biogeography (age range)
Root age	1–98	38.46 (32.12–45.02)	59.96 (50.09–70.97)	35.14 (24.10–45.94)
Clade A	79–98	20.54 (17.73–23.3)	31.92 (27.58–36.56)	18.82 (13.76–24.90)
Clade A1 (gr. of *Montrouzierella nana*)	79–84	14.74 (11.88–17.59)	22.85 (18.25–27.65)	12.96 (9.10–17.33)
Clade A3 (gr. of *M. nana*)	86–89	10.96 (8.70–13.30)	17.01 (13.46–20.76)	10.11 (7.10–13.56)
Clade A4 (gr. of *Samuelsonia bicolor*)	90–98	13.54 (11.20–15.95)	21.12 (17.36–25.16)	12.68 (9.01–16.73)
Clade B (gr. of *Dematocroma difficilis*)	58–62	9.78 (7.95–11.92)	15.27 (12.27–18.49)	9.03 (6.39–12.05)
Clade C (gr. of *Dematocroma pilosa*)	54–57	19.02 (15.54–22.89)	29.68 (14.00–35.70)	17.85 (12.72–23.75)
Clade D (gr. of *D. pilosa*)	50–53	14.13 (10.28–18.31)	22.03 (16.00–28.69)	12.23 (8.22–16.72)
Clade E (gr. of *Dematocroma laboulbenei*)	44–49	20.39 (16.70–23.88)	31.79 (26.15–37.75)	18.27 (12.98–24.29)
Clade F (gr. of metallic *Samuelsonia*)	35–42	20.50 (17.01–24.00)	31.91 (26.25–37.61)	18.32 (13.04–24.29)
Clade G (gr. of *Dematocroma maculifrons*)	29–32	16.35 (12.62–19.99)	25.50 (20.06–31.74)	15.63 (10.89–20.88)
Clade H (gr. of *Dematocroma culminicola*)	11–14	10.77 (7.87–13.84)	17.04 (12.17–22.14)	9.49 (6.50–12.87)
Clade I (gr. of *Taophila subsericea*)	1–4	26.29 (10.94–31.87)	40.91 (32.57–50.12)	24.06 (16.91–32.16)
Îles Loyauté (Ouvéa, Maré)	46(b)	1.85 (1.18–2.58)	2.88 (1.80–4.05)	Fixed to 2 Myr
Île-des-Pins	76	0.35 (0.15–0.56)	0.6 (0.24–0.90)	0.48 (0.22–0.78)

gr., group.

Figure 3. Timing and rate of diversification of New Caledonian Eumolpinae. A, species-level phylogeny dated using an uncorrelated lognormal clock in BEAST under a Yule tree prior, and a marker-specific mean substitution rate from Gómez-Zurita *et al.* (2012). Grey bars indicate the 95% highest posterior density intervals of node heights. Terminals are labelled according to the morphospecies/generalized mixed Yule-coalescent numbers shown in Figure 2, and the strongly supported Clades A–I recovered by all phylogenetic analyses are also indicated. B, lineage-through-time plot of the above phylogeny and diversification rates as estimated for the best fit model (yule3rate; Rabosky, 2006a, b).

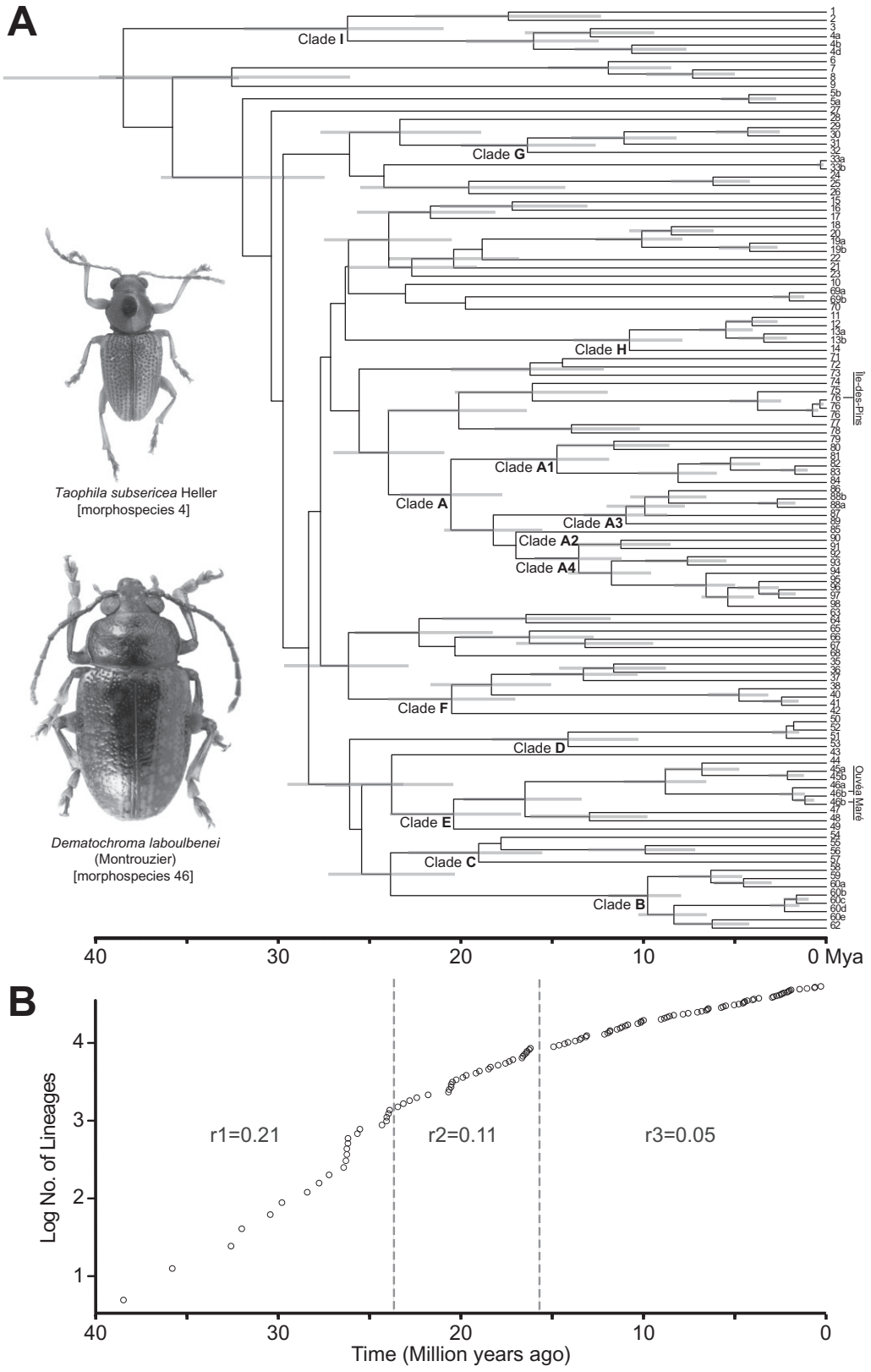
A
Clade I
Clade G
Clade H
Clade A1
Clade A
Clade A3
Clade A2
Clade A4
Clade F
Clade D
Clade E
Clade C
Clade B
Ile-des-Pins
Ouvéa Maré
Taophila subsericea Heller
[morphospecies 4]
Dematochroma laboulbenei
(Montrouzier)
[morphospecies 46]
40
30
20
10
0 Mya
B
Log No. of Lineages
r1=0.21
r2=0.11
r3=0.05
Time (Million years ago)

Table 5. Diversification rate model comparisons in LASER (Rabosky, 2006b), based on the tree of New Caledonian Eumolpinae calibrated using a marker-specific rate

Model	LH	r_0 or λ_0	μ_0	Extra parameters	AIC	dAIC
pureBirth	9.634	0.068	NA	NA	–17.268	23.128
bd	9.634	0.068	0	NA	–15.268	25.128
DDL	20.267	0.137	NA	K = 140.422	–36.533	3.863
DDX	20.663	0.540	NA	xp = 0.522	–37.325	3.071
yule2rate	22.674	0.135	NA	r1 = 0.049, t1 = 16.019,	–39.348	1.048
yule3rate	25.198	0.208	NA	r1 = 0.107, t1 = 23.777 r2 = 0.049, t2 = 16.019	–40.396	0.000
SPVAR	20.849	0.278	0.001	k = 0.053	–35.698	4.698
EXVAR	9.469	0.069	0.001	z = 1.006	–12.938	27.459
BOTHVAR	20.849	0.279	0.001	k = 0.053, z = 0.500	–33.698	6.698

LH, log-likelihood of corresponding model; r_0 (= λ_0–μ_0), initial net diversification rate for diversity dependent linear (DDL), diversity dependent exponential (DDX), yule2rate, and yule3rate (or constant net diversification rate for pureBirth and bd model) models; λ_0, initial speciation rate for the SPVAR, EXVAR, and BOTHVAR models; μ_0, the initial extinction rate when applicable; NA, not applicable; K, carrying capacity parameter of DDL model; xp, exponent of DDX model; r1, net diversification rate after the first shift at time t1; r2, net diversification rate after the second shift at time t2; k, parameter of the exponential change in speciation rate for the models SPVAR and BOTHVAR; z, parameter of exponential change in extinction rate for models SPVAR and BOTHVAR; dAIC, the difference in Akaike information criterion score from the best-fit model (SPVAR, model with time-varying speciation only; EXVAR, model with time-varying extinction only; BOTHVAR, model with both speciation and extinction varying through time).

Table 6. Diversification rate model comparisons using a coalescent approach (Morlon *et al*., 2010) based on the tree of New Caledonian Eumolpinae calibrated using a marker-specific rate

	Diversity	Speciation	Extinction	Parameters	LH	AICc
Model 1	Saturated	Constant	Constant	τ_0 = 0.204	–42.346	86.730
Model 2	Saturated	Varying	Varying	τ_0 = 0.042, γ = 0.151	20.347	–36.582
Model 3	Expanding	Constant	Constant	λ_0 = 0.096, μ_0 = 1.17e–09	10.272	–16.431
Model 4a	Expanding	Varying	Constant	λ_0 = 0.073, α = 0.045, μ_0 = 0.052	26.449	–46.672
Model 4b	Expanding	Constant	Varying	λ_0 = 0.096, μ_0 = 3.46e–10, β = –0.027	10.272	–14.317
Model 4d	Expanding	Varying	Varying	λ_0 = 0.079, α = 0.038, μ_0 = 0.079, β = –0.062	26.767	–45.153
Model 5	Expanding	Constant	No	λ_0 = 0.096	10.272	–18.506
Model 6	Expanding	Varying	No	λ_0 = 0.055, α = 0.044	25.641	–47.169

τ_0, turnover rate; γ, exponential variation in turnover; λ_0, speciation rate at present; μ_0, extinction rate at present; α, exponential variation in speciation rate; β, exponential variation in extinction rate; LH, log-likelihood of corresponding model; AICc, second order Akaike information criterion.

specific rate and different corresponding times for the inferred points of rate shifts), whereas under model 6 of the coalescent approach, the inferred speciation rate at present was 0.061 lineages/Myr (slightly higher than with the marker-specific rate).

DISCUSSION

Insights on rapid mtDNA species delimitation of New Caledonian Eumolpinae

We adopted here a rapid mtDNA-based biodiversity assessment approach to characterize the Eumolpinae fauna of New Caledonia, and evaluated its performance by contrasting the results with an independent traditional taxonomic evaluation, based on the study of external and genitalic morphological characters. A study like ours shows that for rapid biodiversity inventories, the single-locus approach remains very valuable. It clearly provides a necessary first step for an exploratory assessment of poorly known biota by clustering the sequences into putative species in the absence of any *a priori* taxonomic hypothesis, and aids in the actual process of building this initial taxonomic backbone.

In our phenetic clustering overview of these data and to avoid the use of a predefined cut-off value (DeSalle *et al*., 2005; Meier *et al*., 2006), we employed the recently developed ABGD method (Puillandre

et al., 2011), which aims to infer such a gap from the distribution of pairwise distances in a sequence data set. In the case of New Caledonian Eumolpinae, the ABGD method inferred two candidate thresholds, the first of which produced sequence clusters more congruent with morphospecies designations (88% vs. 79% of exact matches when both markers were used). Both the 2% and 3% cut-off values of *cox1* sequence divergence that are commonly applied in animal barcoding studies (Hebert *et al*., 2003; Barret & Hebert, 2005; Hendrich *et al*., 2010; Prado *et al*., 2011) fall within the prior intraspecific divergence range of the first threshold. The presence of two candidate 'barcode gaps' for our data indicates a partial overlap between intra- and interspecific variation in the data set, as found in many other taxonomic groups and might be attributed to species history and/or demography (Meyer & Paulay, 2005) or to geographical scale effects (Bergsten *et al*., 2012). It is thus likely that neither of the two inferred thresholds represents a real limit between intra- and interspecific divergence across the whole data set. In the absence of any other independent criterion, such as morphological evaluation, the choice between the two alternative solutions would be arbitrary, yet it is encouraging that there is 87% agreement between them, and a high correspondence of the resulting clusters with the morphospecies designations in both cases.

In our tree-based approach to species delimitation, we compared two alternative versions of the GMYC model: the single-threshold model (Pons *et al*., 2006), which assumes a fixed depth of transition from speciation to coalescent across all lineages (i.e. all speciation events are assumed to be older than all coalescent events), and the multiple-threshold (Monaghan *et al*., 2009), which relaxes this assumption. The latter can account for greater variation in divergence times and effective population sizes amongst species and thus it is theoretically expected to perform better than the single-threshold approach (Reid & Carstens, 2012). Indeed, the multiple-threshold model showed a better fit to our data set according to the likelihood ratio test. However, the results of these analyses varied greatly across the different phylogenetic inference methods (49–78% similarity of results amongst methods, calculated using Dice's coefficient), and had a relatively low match (65–79%) with morphospecies designation, whereas the single-threshold version appeared more robust to phylogenetic inference biases (86–96% similarity amongst methods), and delimited groups that were more congruent with morphospecies (83–88% match). Notably, the multiple-threshold approach seemed to over-split a proportion of the inferred species and to produce a great number of singletons, with the affected species being different amongst analyses. This observation is consistent with recent simulations that have shown that this model overestimates the number of inferred species under a wide range of conditions (Esselstyn *et al*., 2012). One possible cause for over-splitting in the New Caledonian Eumolpinae data set might be the effect of sampling. Simulations (Lohse, 2009) suggest that highly incomplete sampling at the population level (high proportion of demes not sampled) may produce artificial clusters recognized as separate GMYC groups. Given the patchy sampling scheme of the current study, it would not be surprising that this issue had an effect on the New Caledonian Eumolpinae data set, but as the true distribution of these species is completely unknown, it is impossible to evaluate its magnitude. If this were the case, it appears that the single-threshold approach is more robust to these sampling artefacts, presumably because the optimization of the single transition point averages over well-sampled and undersampled demes. Multiple-threshold optimization relies on a heuristic algorithm that can be trapped in local optima for large data sets (Fujisawa, 2011); the disparity of results in our different implementations of this optimization for the New Caledonian Eumolpinae data may illustrate this problem. These reasons combined persuade us to consider the results of the single-threshold method to be more reliable for the New Caledonian Eumolpinae data set.

Most disagreements between morphospecies and GMYC single-threshold entities (apart from two cases) can be linked either with nonmonophyly or with allopatry. Paraphyly (or polyphyly) at the species level owing to incomplete lineage sorting or introgression is well-documented (Funk & Omland, 2003; Bergsten *et al*., 2012), and will unavoidably impede the performance of the GMYC model, but it only appears to affect a very small proportion of the studied taxa here. The split between allopatric GMYC entities may either reflect true isolation owing to lack of gene flow between populations (Papadopoulou *et al*., 2008) or under-sampling of linking populations (Lohse, 2009) as explained above. In any case, allopatry of closely related taxa represents a major challenge for species delimitation when assessing morphological or other characters that serve as proxies for reproductive isolation (Fujita *et al*., 2012); therefore, the status of these allopatric lineages needs to be evaluated further using multiple genetic markers.

The independent approaches to species delimitation attempted here produce largely congruent results for the number of evolutionary entities in the available sample of New Caledonian Eumolpinae. These methodologies moreover agree to a surprising degree in the limits of these entities (88% for both ABGD-first threshold and GMYC-single threshold when

compared with morphospecies delimitation; and up to 95% agreement between both DNA-based approaches). Additionally, where there is disagreement, except in the case of paraphyletic morphospecies 60 and 61, one monophyletic morphospecies is further split into additional reciprocally monophyletic entities or more rarely two sister morphospecies are lumped into a single evolutionary entity. Overall, our results indicate that a single locus DNA-based approach has been very effective to assess the diversity of New Caledonian Eumolpinae, providing fast, objective, and reliable measures to our question on species diversity. mtDNA appears to track relatively well the morphospecies delimitation, and thus could be applied in the future for further studies to improve our knowledge of this group. This might imply that in this group of leaf beetles, effective population sizes and ages do not differ enormously amongst species, and that there are not many other confounding factors, such as recent rapid radiations, widespread incomplete lineage sorting, or introgression. Such a high match between mtDNA groups and morphological species has not been found in recent and rapidly diversifying island radiations (Monaghan *et al*., 2006), neither it is expected in other groups of leaf beetles where there is known extensive introgression (e.g. Gómez-Zurita, Funk & Vogler, 2006; Gómez-Zurita *et al*., 2012).

New Caledonia: also a hotspot for Eumolpinae diversity

Congruence amongst species delimitation approaches allows us to propose with relatively high confidence that the evolutionary entities uncovered with these procedures are a defensible proxy for species diversity in our sample of New Caledonian Eumolpinae, which would be in the order of 98–110 species. This estimate nearly doubles the number of known (formally defined) species, but at the same time it only represents approximately half of the predicted eumolpine species diversity in the archipelago (148–210 species, depending on the estimator used). However, is the latter a defensible expectation for the total species diversity of Eumolpinae in New Caledonia? Some indirect evidence suggests that these high species number predictions may be indeed realistic and, actually, quite conservative. Most of our samples (71.9%) were collected in a single campaign right at the end of the main rainy season (March–April) and mostly focused on the central chains and western plains in the island, with very few incursions into rainforests and high elevations of the northern and eastern parts of the island. We have no information on leaf beetle species seasonality in New Caledonia, but it is likely that at least some species will show population peaks in other periods typically more suitable for insect activity, e.g. following the first rains after a dry period (e.g. Grimbacher & Stork, 2009). Considering that these are herbivorous beetles, each species with varying degrees of specialization, it is however unquestionable that expanding or intensifying the sampling effort in more environments (with different plant communities) would result in sampling more Eumolpinae diversity. The areas in particular where we sampled less intensively, such as high elevations and/or the rainforests, are well known for their botanical diversity and endemicity in New Caledonia (Jaffré *et al*., 2004), which may be mirrored by Eumolpinae specialized in exploiting these plant resources. In summary, our species estimates more precisely reflect the expectations for the same environments and season that we sampled, which are a partial representation of the island niches, instead of global expectations.

Other indirect evidence for our diverse Eumolpinae sample representing at most a mere 50–75% of the actual diversity of this group, as suggested by species accumulation curves, can be inferred from the proportion of described species actually present in our sample. Our ingroup intentionally excluded eumolpines of the tribe Nodinini, present but poorly represented in New Caledonia (Gómez-Zurita, 2011b). By focusing on the tribe Colaspoidini, to which > 95% of described New Caledonian Eumolpinae belong, the root of the trees stayed as close as possible to the island colonization event(s) by this group of beetles, rather than the basal diversification of Eumolpinae (likely to have been in the upper Cretaceous; Gómez-Zurita *et al*., 2007). However, most importantly, for the groups included, although we revealed a remarkable undescribed diversity, there were nonetheless notable absences from the described diversity: out of 12 currently described species of *Samuelsonia*, only six were present in our sample; only two out of seven *Montrouzierella*; only eight out of 12 *Taophila*; and only 12 out of 19 *Dematochroma*, were available for DNA analysis. These cases alone represent 22 additional species to add to our available hundred.

All in all, the evidence, treated from a very conservative perspective, points to a rich Eumolpinae fauna in New Caledonia, with well over 150 species, more than in the entire Nearctic region (Arnett *et al*., 2002). Although equivalent studies on Eumolpinae from neighbouring areas are lacking, the data available so far suggest that all New Caledonian eumolpines are endemic, and at least two genera (*Taophila* and *Montrouzierella*) also qualify as endemic. As for the vulnerability of these species, our present knowledge is clearly insufficient to make a sound assessment, and it is also beyond the scope of this study, but in general it is possible to propose

some arguments for their assembled treatment as threatened, even if their populations could be considered currently healthy. New Caledonia at large is already a priority for conservation (Myers *et al*., 2000; Myers, 2003; Mittermeier *et al*., 2004), and New Caledonian eumolpine species (as assessed in this study) reveal in general very narrow ranges – even compatible with their treatment as critically endangered (IUCN Species Survival Commission, 2001) – as well as several examples of geographical and ecological segregation, in some cases associated with highly threatened ecosystems, such as the dry forest, minimally represented in Grande Terre (Bouchet, Jaffré & Veillon, 1995). Again, considering the surface of the island, in terms of empirically demonstrated and analytically estimated species richness, their local endemicity and also an idea of potential threats for these species, it is possible to assert New Caledonian Eumolpinae as a significant component of the New Caledonian biodiversity hotspot.

New Caledonian Eumolpinae: island radiation(s) of transoceanic travellers

Where did this high diversity of eumolpine beetles come from? How did it originate? In the context of New Caledonian biogeography, we consider three main competing hypotheses about these origins: a mostly ancient Gondwanan stock; one or several post-Eocene colonizations; or a combination of both, giving rise to co-existing ancient and recent evolutionary lineages on the islands (Murienne, 2009). In every case, given the long-standing geographical isolation of the archipelago, subsequent *in situ* diversification can be assumed. The most suitable test for these hypotheses would be a phylogenetic analysis based on comprehensive sampling of Eumolpinae in the western Pacific (or an updated and sound taxonomic revision of the group for this same area), but this kind of taxonomic coverage is currently beyond our possibilities. However, an analysis exclusively based on New Caledonian data already provides a convincing argument for the origins of this community of Eumolpinae.

The implementation of the averaged insect mtDNA substitution rate (Brower, 1994) to New Caledonian Eumolpinae (Colaspoidini) resulted in an inferred root age for this clade at around the Cretaceous–Tertiary boundary (approximately 50–71 Mya), precisely the hypothesized time of geographical isolation of New Caledonia from Gondwana (also the approximated age for the entire subfamily; Gómez-Zurita *et al*., 2007). This age is supportive of both the first and the third hypotheses. However, it is not old enough to suggest ancient persistence of Eumolpinae lineages since Gondwana (Murienne, 2009). Moreover, extrapolating substitution rates amongst genetic markers has been shown to be a poor decision when marker-specific rates are available, as taxon-specific rate peculiarities possess in general less variance than that observed between markers, and even lower levels if considering closely related taxa (Andújar *et al*., 2012). Indeed, using the rate calculated by Gómez-Zurita *et al*. (2012) for other leaf beetles but for the same mtDNA markers studied here, and alternatively an internal tree calibration using the Pleistocene age of the Îles Loyauté as the maximum age for their colonization, the root age of New Caledonian Eumolpinae was estimated as younger than 45 Mya in every case, with a mean of around 35–38 Mya. These results fit instead with the second hypothesis, with the re-emergence time of New Caledonia in the Late Eocene following a period of island submersion (Pelletier, 2006). Moreover, in support of this Eocenic origin, in the case of the *cox1 + rrnS* rate-based calculation, the age of divergence between the Îles Loyauté (Ouvéa and Maré) lineage and their sister taxon from Grande Terre was estimated at 1.85 Mya, a date compatible with the time of emergence of these islands, around 2 Mya (Pelletier, 2006). Conversely, the standard insect mtDNA rate renders a puzzling age of divergence between these lineages as marginally older than the time of emergence of the Îles Loyauté (2.9 Mya; 95% HPD: 1.8–4.1 Mya). A similar pattern has been described for other organisms, with an age pre-dating that of the recent islands and has been explained by assuming that they travelled from older and currently submerged oceanic islands on the Loyalty Ridge (Heads, 2008, 2011). However, this argument applies to the endemic organisms in the Îles Loyauté, susceptible to a history of previous isolation elsewhere. In our particular case, the samples from Maré and Ouvéa belong (or are very closely related) to *Dematochroma laboulbenei*, a member of an Early Miocene species complex in Grande Terre (Clade C, Fig. 2) and distributed on its eastern slopes, facing the Îles Loyauté, thus fitting a scenario of recent colonization when the current terrains of these uplifted reef islands became suitable for this species. Facing these data and being critical of the procedure and results based on the standard insect mtDNA rate, we are inclined to favour the recent, Late Eocenic origin of New Caledonian Eumolpinae from as yet unknown biogeographical sources.

For any of the scenarios discussed here, the age estimates given above represent highly conservative solutions if we were to consider that the whole of the New Caledonian Eumolpinae diversification – if we sampled every lineage, including the oldest in the island – stems from a unique ancestor (i.e. dated root). If we relax this assumption and assume two or more colonizations of Grande Terre by Eumolpinae, the nodes representing these events would be neces-

sarily younger than this root, providing a yet stronger challenge against the Gondwanan scenario. The analysis of diversification rates seemingly also supports the scenario of a recent origin for the Eumolpinae in New Caledonia. Different approaches to the study of trends in these diversification rates suggest that our data are compatible with a slowdown in lineage accumulation towards the Recent. This may reflect the effects of progressive niche occupancy and saturation after a rapid initial diversification, i.e. after a radiation following a successful post-Eocene recolonization of the archipelago, as has been suggested for several other New Caledonian taxa (Espeland & Murienne, 2011).

Admittedly, the analysis of diversification rates could be confounded by independent colonization events, which we are unable to detect here, as well as by seasonal or ecological sampling bias. These limitations prevent us from making use of these data to provide solid conclusions about the diversification of these beetles. Yet, some interesting trends can be detected from these data that provide clues about this diversification and a basis for new, educated working hypotheses for the evolutionary analysis of this diverse group of organisms. One possibility for the rich fauna of the New Caledonian Eumolpinae would be that these evolutionary lineages exhibit intrinsically rapid diversification dynamics. These dynamics, as evaluated in our analyses, go from a faster initial phase (0.16–0.20 lineages/Myr) to a slower later stage (0.02–0.05 lineages/Myr). When single birth–death or birth-only models are fitted and an average value is estimated across the phylogeny, permitting comparison with similar studies, the obtained diversification rate (depending upon the evolutionary rate implemented) ranges between 0.04 and 0.07 lineages/Myr, well within the range of analogous values that are commonly found in arthropods (mode of distribution 0.05–0.1; McPeek & Brown, 2007), and close to the average rates estimated for beetles (0.05–0.07; Hunt *et al*., 2007). Therefore, the highly diverse New Caledonian Eumolpinae represent a relatively recent group without evidence for an intrinsically high rate of diversification, justifying the question as to how this remarkable species diversity originated. One possibility is that the community of New Caledonian Eumolpinae actually represents an assemblage resulting from many independent and successful colonizations established on the island, and followed by moderate local diversification. We argue that this is an unlikely scenario based on the geographical isolation of New Caledonia, but also on the taxonomic and phylogenetic closeness of the Eumolpinae species in the island. It would also be an unheard of, unique example of an important component of New Caledonian diversity originating *outside* the archipelago. Instead, some of the reasons for the high number of Eumolpinae species must be sought after *inside* New Caledonia.

Despite the relatively small geographical scale considered here, spatial isolation of beetle populations is predicted to promote differentiation leading to speciation. The evidence for this assertion stems from the observation that there is a remarkable degree of local differentiation and allopatry of closely related species (reminiscent of 'Jordan's Law'; Allen, 1907), with most species as defined above showing very narrow ranges. Sixty-five of the single-threshold GMYC clusters were found at a single locality, and for the whole sample, 74.2 (morphospecies) to 75.7% (GMYC) of species were found in localities less than 25 km apart. Only 4.5 (GMYC) to 5.2% (morphospecies) were found in localities more than 200 km apart (approximately half the length of Grande Terre), one of them *D. laboulbenei*, with populations in the Îles Loyauté. Based on 72 nodes with bootstrap support > 50% in the ML tree, 78.8% of terminal nodes (i.e. as proxy for sister-species relationships) represented the split of allopatric lineages, whereas 18.2% related to sympatric lineages (the remaining were treated as parapatric as a result of partial geographical overlap). Conversely, 51.3% of internal nodes were recorded as allopatric, and 41.0% as sympatric. Despite the challenge of correlating geographical ranges with the geography of speciation (e.g. Losos & Glor, 2003; Fitzpatrick & Turelli, 2006; Ribera *et al*., 2011), these values are suggestive of a vicariant model of speciation, with sister taxa being predominantly allopatric and this marked geographical pattern washed out by range expansion of phylogenetically divergent lineages (Coyne & Orr, 2004: 94). Also in support for this model, most cases in which the DNA-based procedures further split morphospecies based on high genetic divergence or not fitting coalescent expectations, these splits affected allopatric groups of samples. Our knowledge of the actual species ranges and species relationships is rather schematic, limited to our current sampling and our choice of phylogenetic markers, but the observed trends are unmistakably of allopatric speciation at work, and possibly acting at a very local scale (judging from the small species ranges), surely reinforced by the island orography and its local environmental diversity (the microendemicity of Grandcolas *et al*., 2008).

As noted above, a relatively high proportion of internal nodes also represented allopatric ranges for their subtending lineages, sometimes reflecting relatively deep splits amongst species groups (e.g. clade D, species 35–42). This pattern is suggestive of some degree of regional diversification as well, as a result of episodic or environmental factors explaining the diversity of Eumolpinae in New Caledonia. The

indication of regional diversification is also supported by the corroboration of what appear to be, with our fragmentary data, nonrandom species distributions. For those species with more than one locality sampled (33.7%), the localities nearly always grouped in two main regions: localities north-west and south-east, respectively, of an imaginary boundary roughly running from Baie de St-Vincent and perpendicular to the main island axis. The interesting point about this biogeographical pattern is that it roughly fits two geological domains in the island, the dominance of ultramafic ophiolitic nappe in the south versus basement, sedimentary, and basaltic terranes elsewhere (Pelletier, 2006). This same pattern has been described and invoked to explain the diversification of New Caledonian flora (Jaffré *et al*., 1987), reptiles and amphibians (Bauer & Sadlier, 2000), and that of Trichoptera (Espeland & Johanson, 2010), amongst others, although its importance has been questioned for other organisms such as cockroaches in the genus *Lauraesilpha* (Murienne *et al*., 2008). Indeed, soil type may be an important driving force for diversification in this group of beetles through both indirect and direct causes. The indirect effects would be through specialization imposed on the host-plants upon which these phytophagous beetles depend, whereby the plant communities diversify themselves and become structured in response to soil types (Jaffré *et al*., 1987; Pillon *et al*., 2010). However, there may be direct effects of soil type resulting in beetle adaptation and eventual speciation because these animals spend their immature stages as soil-dwelling, blind, root-feeding larvae, eventually pupating into the soil as well (Jolivet & Verma, 2008).

As for most biodiversity inventorying efforts of invertebrates in hotspot areas, ours is not the last word for New Caledonian Eumolpinae. Despite this, we hope that our RBA approach to this catalogue will enable something that could be understood as an 'accelerated RBA' enterprise in the future. In other words, enhancing species discovery and cataloguing by reference to a previously existing database with information amenable to quick, objective comparisons and assessments (i.e. DNA). Moreover, we also believe that our approach highlights the importance of investing in the slower, perhaps more subjective, but extremely rewarding, cultivation of the holistic Linnean approach to taxonomy. Indeed, our results could be interpreted and validated relatively quickly by reference to solid knowledge of the morphological diversity of the group under study, which is only acquired after countless hours preparing, dissecting, and definitively observing this biological diversity in the field and under a microscope. However, RBA approaches remain unfinished business if not pursued to their ultimate conclusion, which is to formalize the new entries in the inventory of biodiversity. Amongst just the eumolpine samples from which data were collected for this study, there are a few dozen new species for science that must be formally described. This work is only suitable for trained specialists, the professionals with the greatest responsibility for a meaningful catalogue of life.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Output of the automated barcode gap discovery (ABGD) method, assessing the existence of a barcode gap in the New Caledonian Eumolpinae mtDNA data set. A, distribution of pairwise distances in the data set, for each marker separately and in combination. B, results of the ABGD method indicating the number of groups obtained (in either the primary or recursive partition) for each prior intraspecific divergence value.
Figure S2. Sample-based species accumulation curves and four species richness estimators, as calculated based on: A, morphospecies; B, phenetic clusters (first automated barcode gap discovery threshold, initial partition procedure, two markers combined); and C, generalized mixed Yule–coalescent entities (single threshold, based on the tree from a BEAST analysis). Standard deviations for the final estimate of observed species richness, first order jack-knife, and Chao 2 estimators are indicated with their corresponding interval.
Table S1. Sampling localities (numbered as in Fig. 1) and morphospecies of New Caledonian Eumolpinae (numbered as in Fig. 2), with the corresponding number of specimens (*N*) studied and with DNA sequences.