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ORIGINAL ARTICLE

The effect of environmental diversification on species diversification in New Caledonian caddisflies (Insecta: Trichoptera: Hydropsychidae)

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ABSTRACT

Aim To test whether environmental diversification played a role in the diversification of the New Caledonian Hydropsychinae caddisflies.

Location New Caledonia, south-west Pacific.

Methods The phylogeny of the New Caledonian Hydropsychinae caddisflies was hypothesized using parsimony and Bayesian methods on molecular characters. The Bayesian analysis was the basis for a comparative analysis of the correlation between phylogeny and three environmental factors: geological substrate (ultrabasic, non-ultrabasic), elevation and precipitation. Phylogenetic divergence times were estimated using a relaxed clock method, and environmental factors were mapped onto a lineage-through-time plot to investigate the timing of environmental diversification in relation to species radiation. The correlation between rainfall and elevation was tested using independent contrasts, and the gamma statistic was calculated to infer the diversification pattern of the group.

Results The diversification of extant *Orthopsyche–Caledopsyche* species began in the Middle–Late Oligocene, when much of the island of New Caledonia was covered by ultrabasic substrate and mountain forming was prevalent. Most lineages originated in the Middle–Late Miocene, a period associated with long-term climate oscillation. Optimization of environmental factors on the phylogeny demonstrated that the New Caledonian Hydropsychinae group adapted to ultrabasic substrate early in its evolutionary history. The clade living mostly on ultrabasic substrate was far more species-rich than the clade living mostly on non-ultrabasic substrate. Elevation and rainfall were significantly correlated with each other. The lineage-through-time plot revealed that the main environmental diversification preceded species diversification. A constant speciation through time was rejected, and the negative gamma indicates that most of the diversification occurred early in the history of the clade. According to the inferred phylogeny, the genus *Orthopsyche* McFarlane is a synonym under *Caledopsyche* Kimmins, and *Abacaria caledona* Oláh & Barnard should also be included in *Caledopsyche*.

Main conclusions The age of the radiation does not support a vicariance origin of New Caledonian Hydropsychinae caddisflies. Environmental diversification pre-dates lineage diversification, and thus environmental heterogeneity potentially played a role in the diversification of the group, by providing a variety of fragmented habitats to disperse into, promoting speciation. The negative gamma indicates that the speciation rate slowed as niches started to fill.

Keywords

Character optimization, environmental heterogeneity, evolutionary radiation, freshwater insects, Hydropsychidae, lineage-through-time plot, New Caledonia, phylogeny, species diversification.

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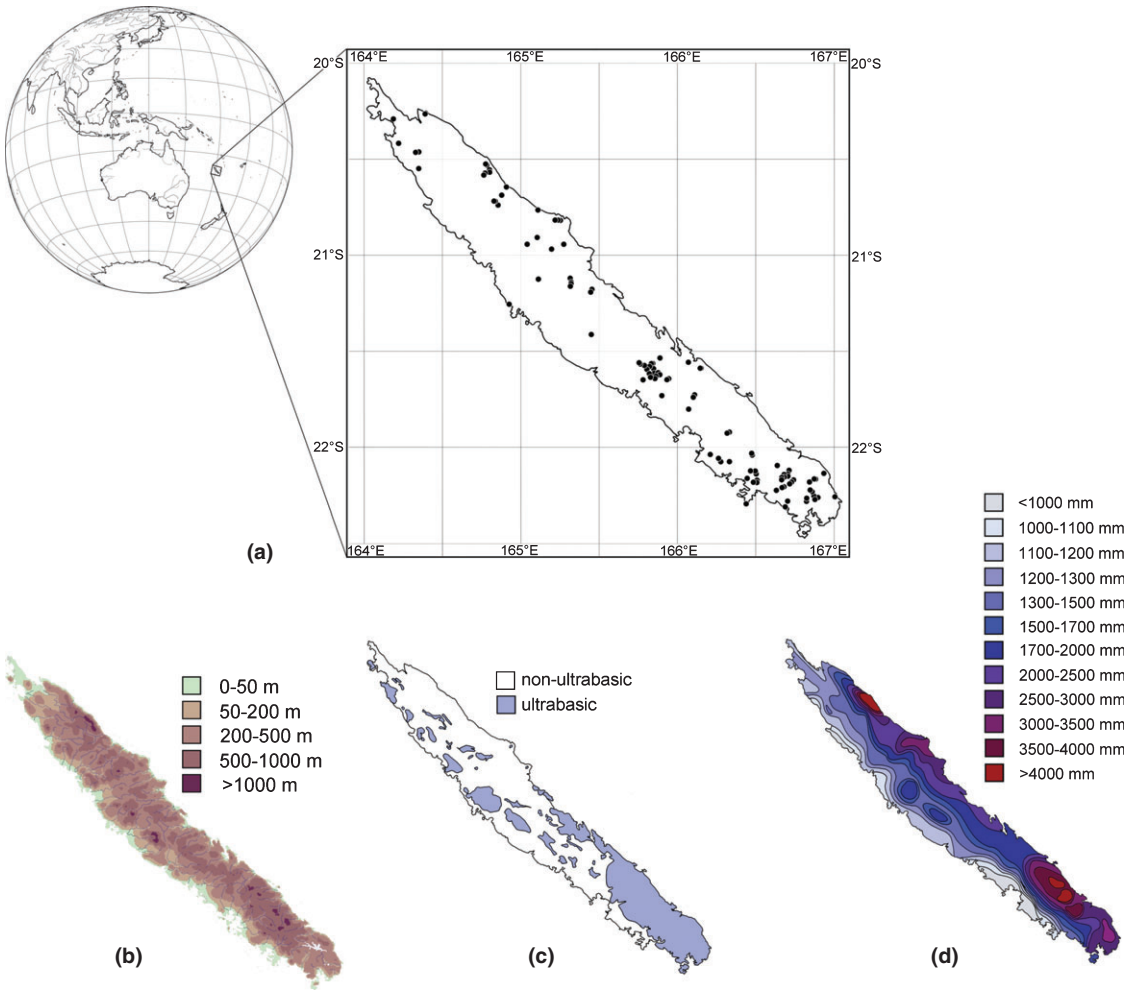

Figure 1 (a) Map of New Caledonia with sampling localities. (b) General topography of New Caledonia. (c) The distribution of ultrabasic substrate (blue) and non-ultrabasic substrate (white) on New Caledonia. (d) Annual rainfall on New Caledonia. Data are taken from ORSTOM (1981).

INTRODUCTION

New Caledonia (Fig. 1a) is a fragment of the former Gondwana landmass, and the presence of certain relict taxa on the island led earlier researchers to believe that the New Caledonian biota represented fragments of a wider Gondwanan fauna (Raven & Axelrod, 1972; Holloway, 1979; Morat *et al.*, 1986; Cooper & Millener, 1993). It was also implied that the high diversity on the island was a result of its antiquity. This idea has recently been disputed, as geological data support the hypothesis that New Caledonia (Fig. 1a) underwent a series of submersions during the Palaeocene and Eocene (Paris *et al.*, 1979; Aitchison *et al.*, 1998). Contemporary studies applying molecular dating methods to diverse organism groups have revealed that numerous groups of New Caledonian organisms diverged more recently, in many cases during the last few million years (e.g. Murienne *et al.*, 2005; Page *et al.*, 2005; Harbaugh & Baldwin, 2007).

Local endemism is high on New Caledonia, and many organism groups display large species radiations (Grandcolas *et al.*, 2008). In Trichoptera, one-third of the genera and all but two of the nearly 400 known species are endemic (Espeland *et al.*, 2008). Rapid radiations may be adaptive, non-adaptive or a combination of the two. Non-adaptive radiations are a result of diverse, fragmented habitats and occur without apparent niche differentiation (Gittenberger, 1991). Adaptive radiations occur when there is differential adaptation to the variety of niches that the resulting species occupy (Gittenberger, 1991; Schluter, 2000). Proposed explanations for the radiations on New Caledonia are combinations of variations in rock chemistry, topographic features and climatic factors, which lead to a high diversity of habitats (Chazeau, 1993; Morat, 1993). Adaptive radiation has contributed to New Caledonian species radiations in, for example, springtails (Collembola) (D'Haese, 2003), crickets (Orthoptera) (Robillard & Desutter-Grandcolas, 2004) and skinks (Squamata) (Bauer *et al.*, 2006). This is a well-known phenomenon on islands, where colonization is often a matter of chance and opportunity (Losos *et al.*, 1998; Gillespie, 2004).

Trichoptera larvae are fully aquatic and greatly affected by water quality. They occupy a large number of different microhabitats and trophic niches and generally have a low tolerance to pollution (Mackay & Wiggins, 1979). These features make them ideal bio-indicators for freshwater quality, and they are frequently used in such studies (Rosenberg & Resh, 1993).

Ultrabasic substrate covers around one-third of the main island of Grande Terre (Jaffré *et al.*, 1987), and rivers and streams running over this substrate are much affected by high concentrations of heavy metals and generally have a very high pH (Trescases, 1974). This environment is potentially harsh for caddisflies, and it is intriguing that the number of species is especially high in the southern parts of the island, where ultrabasic rock-substrate is widely distributed (Fig. 1c). A previous study also demonstrated that the ancestor of the New Caledonian endemic genus *Xanthochorema* (Hydrobiosidae) diversified on ultrabasic substrate (Espeland *et al.*, 2008).

In this study we use phylogenetic comparative methods, divergence time estimation and a lineage-through-time (LTT) plot to investigate whether ultrabasic rock substrate, elevation and rainfall (Fig. 1b–d) have affected the diversification of the genera *Orthopsyche* McFarlane, 1976 and *Caledopsyche* Kimmins, 1953 (Hydropsychidae). Whereas *Caledopsyche* is endemic to New Caledonia, *Orthopsyche* is represented on New Zealand as well (Oláh *et al.*, 2006). These genera are morphologically diverse on New Caledonia, ranging from 3.1 to 17.5 mm in male forewing length (Schefter & Ward, 2002; Oláh *et al.*, 2006; M. Espeland & K.A. Johanson, unpublished data), and include species with different distributions, from widespread to very restricted.

Our hypothesis is that environmental factors are involved in driving species diversification on New Caledonia, and should therefore be detected as environmental diversification preceding lineage diversification on a LTT plot. If, on the other hand, environmental diversification lags behind the species radiation, it may be assumed that it is not involved in the speciation process. We tested this hypothesis using a species-level phylogeny hypothesis based on molecular data from all but one of the New Caledonian Hydropsychidae species, together with the methods developed by Hardy & Linder (2005), which include mapping environmental characters on a chronogram and plotting the derived data on a LTT plot.

MATERIALS AND METHODS

Taxon sampling and specimens

In order to cover the maximum possible geographical and ecological distribution, all specimens of the genera *Abacaria*, *Orthopsyche* and *Caledopsyche* from each New Caledonian sampling site were included in the analysis. Holotypes and paratypes were included for species delimitations when these were questionable. *Orthopsyche amiena* (Sykora, 1967) was excluded as it was unavailable for sequencing. The three genera were found at 67 of the 158 localities sampled on New Caledonia between 2001 and 2006. Distribution maps of the New Caledonian species are given in Appendix S1 in Supporting Information. Seven of the nine *Orthopsyche* species from New Zealand were also incorporated in the study, and several Hydropsychinae species from other areas were included in the analysis as near outgroups, based on the Hydropsychinae revision of Oláh & Johanson (2008). Representatives of the Hydropsychidae subfamilies Diplectroninae, Smicrideinae and Macronematinae, as well as specimens of the closely related families Philopotamidae, Ecnomidae, Polycentropodidae, Psychomyiidae, Dipseudopsidae and Stenopsychidae, were included as far outgroups to stabilize the phylogeny. The phylogenetic trees were rooted on the hydroptilid species *Hydroptila simulans* Mosley, 1920. Appendix S2 lists the specimens, localities and GenBank accession numbers. All material was preserved in ethanol (80%) and stored at −20 °C. Vouchers and DNA aliquots were deposited at the Entomology Department, Swedish Museum of Natural History (NHRS).

DNA extraction, polymerase chain reaction and sequencing

Genomic DNA was extracted from abdomen or left hind leg tissue using the GeneMole automated extraction system (Mole Genetics, Oslo, Norway) or the Blood & Tissue Genomic Mini prep System (Viogene, Taipei, Taiwan). Hotstart Ready-To-Go pcr beads (GE Healthcare Lifesciences, Piscataway, NJ, USA) were used for polymerase chain reactions (PCRs) to amplify fragments of the nuclear genes elongation factor-1α (EF-1α, 1099 bp), RNA polymerase II (RP2, 772 bp) and cadherin (CAD, 850 bp), and of the mitochondrial gene cytochrome *c* oxidase subunit I (COI, 658 bp). Each 25-μL reaction contained 2 μL of DNA, 1 μL of each primer (10 μm) and 22 μL of dH_2O. PCRs were performed under the following conditions: 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, annealing temperature (Appendix S3) for 30 s and 72 °C for 40–50 s, followed by a final extension of 8 min at 72 °C. See Appendix S3 for primers used and annealing temperatures. PCR products were purified using EXOSAP (GE Healthcare Lifesciences). The same primers were used for both sequencing and PCR. Sequencing reactions were prepared using the BigDye™ Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) and cycle-sequencing was run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems Inc.). Each 20-μL sequencing reaction mixture included 1 μL of BigDye™, 1 μL of 1.6-μm primer, 2–4 μL of DNA template and the appropriate amount of dH_2O, and was run at 96 °C (1 min) followed by 25 cycles of 96 °C (30 s), 50 °C (15 s) and 60 °C (4 min). Finally, the sequencing reactions were purified using the DyeEx 96 Kit (QIAGEN Nordic, Solna, Sweden).

Raw sequence data and contigs were viewed and assembled using the Pregap4 and Gap4 modules of the Staden software package (Staden *et al.*, 1998). Each sequence region was sequenced using both forward and reverse primers, and EF-1α was amplified in two overlapping regions. Primer sequences

were removed from the beginning of each sequence, and matching forward and reverse sequences for each gene region ensured the accuracy of sequence data. Heterozygous sites were replaced with N and treated as missing data. Alignments were straightforward, and done by hand, as all genes are protein coding. No gaps were produced except for vouchers EI5 and EI8 (Dipseudopsidae), which lack one codon (bases 472–474) of the COI.

Phylogenetic methods

Maximum parsimony (MP) analyses of individual gene datasets and the combined dataset were executed in TNT (Goloboff *et al.*, 2004) using 2000 replications of tree fusing, 1000 iterations of parsimony ratchet, and branch swapping on trees in memory. Gaps were treated as missing data in all analyses. A strict consensus tree was constructed when more than one shortest tree was found. Estimates of branch support were calculated using jackknife analyses (Farris *et al.*, 1996) with 2000 replicates and a deletion probability of 36% for each character. The results were shown as GC values (groups present/contradicting) for a more conservative approach than non-GC jackknife values (Goloboff *et al.*, 2004).

Bayesian phylogenetic inference was applied using the mpi version of MrBayes 3.2. (Ronquist & Huelsenbeck, 2003; Altekar *et al.*, 2004). The most appropriate substitution model was chosen with MrModeltest 2.3 (Nylander, 2004) under the Akaike information criterion. Bayesian analyses were run for 1,000,000 generations on all genes separately, and for 5,000,000 generations on the partitioned combined dataset, with sampling every 500 generations. Burn-ins of 500 and 2500 samples, respectively, were discarded after assessment of the convergence diagnostics in Tracer 1.4 (Rambaut & Drummond, 2007). All Bayesian analyses where run twice to ensure they converged towards the same topology. Both Bayesian and parsimony analyses where also run with a reduced dataset, with EX3 *Hydropsyche* sp. n. 1 as outgroup to infer whether any topological instability was caused by outgroup choice.

Divergence-time estimation

For divergence time estimation a partitioned dataset with 32 taxa was constructed, including one representative of all New Caledonian species and potentially cryptic species. In addition, one specimen of the Fijian *Abacaria* sp. (DJ7), the New Zealand *Orthopsyche* sp. (BM2) and the African (CW2) and European (EX3) *Hydropsyche* species were included.

The molecular clock hypothesis was tested using Bayes factors [$2\ln(B_{10})$], which were calculated based on the harmonic means from analyses of the dataset in MrBayes 3.2 with and without the clock assumption. The result was evaluated according to the criteria in Kass & Raftery (1995).

Divergence times were estimated using an uncorrelated lognormal relaxed clock model (Drummond *et al.*, 2006) under a GTR model with gamma + invariants sites in BEAST 1.4.8 (Drummond & Rambaut, 2007). The dataset was partitioned into genes and codon positions [1 + 2] and [3]. The input files were prepared in BEAUTI 1.5b.

Hydropsyche viduata Ulmer, 1912, a fossil species from Baltic amber dated to the Lutetian, 43.6–52 Ma (Ritzkowski, 1997; Weitschat & Wichard, 2002), was used as calibration point. It was implemented as a lognormal prior on the root height of the tree with mean 0.0, a standard deviation of 1.0 and an offset of 43.6 to accommodate for fossil uncertainty. This means that the age of the root node can be older than 43.6 Ma, but not younger. The tree prior was set to Yule process; all other priors were kept as default. The analysis was run twice for 12,000,000 generations with sampling every 1000 generations and a burn-in of 3000. Results were assessed in Tracer 1.4 (Rambaut & Drummond, 2007).

Environmental and lineage diversification

The tree topology used in environmental and lineage diversification analyses was a modified version of the relaxed clock tree, which included only the New Caledonian species. *Caledopsyche atalanta* and *Orthopsyche nadauna* have high genetic heterogeneity and were divided into three and two lineages, respectively, resulting in a dendrogram with 28 terminals.

The effects of ultrabasic substrate, precipitation and elevation on diversification were studied using MP character optimization. Elevation and precipitation data were taken from ORSTOM (1981), in which the precipitation data is based on measurements from the time period 1956–1975. Presence/absence on ultrabasic rock substrate was coded as a discrete character and optimized using two optimization schemes: standard Fitch optimization in Mesquite 1.2 (Maddison & Maddison, 2009), and presence coding to account for ancestral polymorphisms in DIVA 1.1 (Ronquist, 1996). MaxMin coding (Graham *et al.*, 2004; Hardy & Linder, 2005) was used for rainfall and elevation data to avoid losing information that would result from coding them in increments, and to allow ancestral polymorphisms. The minimum and maximum values were coded as two separate characters for each taxon, optimized independently on internal nodes using squared-change parsimony (SqCP, Maddison, 1991), and weighted using branch lengths from the Bayesian relaxed clock tree. The correlation between precipitation and elevation was calculated using independent contrasts (Felsenstein, 1985). We tested whether the contrasts were phylogenetically independent by plotting the absolute value of the standardized contrast against the standard deviation of the contrast, following Garland *et al.*'s (1992) recommendations. Untransformed data gave the best results and were used in all analyses. These methods were executed in Mesquite (Maddison & Maddison, 2009).

A LTT plot calculated using the R package Ape (Paradis *et al.*, 2004) was applied to test whether environmental diversification preceded lineage diversification. Continuous rainfall and elevation characters were divided into increments of 500 mm and 200 m, respectively. The discrete geological substrate character was implemented using the presence/

absence coding from DIVA 1.1 with three characters, namely non-ultrabasic, ultrabasic and both. Environmental diversity was estimated by summing the states gained across time for each character separately and together, and expressing them as proportions of total diversity for the characters, following Hardy & Linder (2005). Gamma statistics were calculated, and the hypothesis of constant speciation rate through time was tested using the constant rate (CR) test (Pybus & Harvey, 2000). To account for incomplete taxon sampling we simulated 1000 trees under a birth–death model using functions in APE and GEIGER (Harmon *et al.*, 2008). A number of tips corresponding to the number of missing taxa were then randomly pruned from each tree, and the gamma statistic for the pruned trees was calculated [Monte Carlo constant rates (MCCR) test, Pybus & Harvey, 2000]. Because at least one taxon is missing in the analysis, but probably more, we pruned five taxa from each tree in the simulations.

RESULTS

Phylogenetic analyses

The genera *Caledopsyche*, *Orthopsyche* and *Abacaria* were not separate monophyletic groups in any of the resulting trees, and a few of the species were non-monophyletic (Fig. 2). The terminal clades in the phylogeny did not correspond to the described species in all instances. The species *Orthopsyche cerberus*, *O. vrupama* and *O. sematho* are clearly synonymous with other species (*O. cerberus* = *O. phallaina*, *O. vrupama* = *O. pakaha* and *O. sematho* = *O. naduana* and *O. pakaha*) and are included under these names in further analyses. *Orthopsyche nadauna* and *Caledopsyche atalanta* (as currently described) seem to consist of two or three cryptic sibling species and are divided into two and three groups, respectively, in the following analyses. The genus *Hydropsyche* is also non-monophyletic with African (CW1, CW2) and European species in separate clades.

The MP analysis of the combined dataset gave four shortest trees (L = 14,665). The Bayesian analyses were run under a GTR+G+I model for all partitions as given by MRMODELTEST. All analyses of separate partitions gave different topologies (not shown), but no conflicting nodes had high support in any analysis. The combined dataset (Fig. 2) was highly resolved and well supported by high posterior probabilities and jackknife values for most nodes. The main differences between the trees generated by the different methods and included genes were the placements of the New Zealand *Orthopsyche*, the non-New Caledonian *Abacaria*, and the African *Hydropsyche alluaudina* and *Hydropsyche* sp. n. These clades were either placed as sister groups to the New Caledonian species, or within the New Caledonian species.

The analyses with full and reduced sets of outgroups (not shown) gave identical topologies, but the latter received less support.

Closely related species are mostly allopatric or have overlapping ranges. The three sympatric species *Caledopsyche apide*, *Orthopsyche rashala* and *O. ayapona* were found in only one stream at Mount Panié and are not closely related to each other. The two sympatric species *Orthopsyche edhamasa* and *O. kina* are sister taxa.

Timing caddisfly diversification

Support for the clock hypothesis was very low [$2\ln(B_{10})$ = 0.03], and thus the use of a relaxed clock model is justified. Divergence time estimation with a relaxed clock returned a mean root age of 44.5 Ma [95% credibility interval (CI): 43.6–45.8 Ma], and the ancestor of the New Caledonian diversification was estimated to a mean age of 28.2 Ma (95% CI: 22.4–32.5 Ma) (Fig. 3). Our results show that most of the lineage splitting on New Caledonia took place between 16 and 4 Ma (95% CI: 19–3 Ma).

Geology, elevation and rainfall

Both Fitch and DIVA optimization of substrate on the phylogeny support the idea that there was a shift in substrate association from non-ultrabasic to ultrabasic at the ancestral node leading to *Orthopsyche/Caledopsyche* (Fig. 4a). The ancestor speciated into one clade associated with non-ultrabasic substrate (three extant species) and one clade associated with ultrabasic substrate (25 extant species). Secondary dispersal to the alternative substrate type occurred independently more than once in both clades.

Elevation and precipitation are, as expected from the maps (Fig. 1), significantly positively correlated (minimum elevation–minimum rainfall, $P = 4.4 \times 10^{-7}$; maximum elevation–maximum rainfall, P = 0.01). Many extant species are confined to low elevations or to low and intermediate elevations (Fig. 4b). Few species are found only at high elevations. Some species, such as *Orthopsyche schefterae*, are found over a wide range, from almost sea level to above 800 m. The ancestral species were mostly limited to intermediate elevations, from around 240 to 600 m, and to intermediate rainfall, from 1800 to 2800 mm. As shown in Fig. 4b, the variation in body size measured by mean forewing length is very pronounced within the group.

Lineage and environmental diversification through time

The gamma statistic was negative, −2.36, and the hypothesis that diversification was constant over time was rejected by the CR test (P = 0.018). This indicates that most branching events occurred early in the history of the group. The result was not biased as a result of incomplete taxon sampling (MCCR test, 5 taxa pruned, P = 0.006). It is evident from the LTT plot (Fig. 5) that the main environmental diversification occurred before the main species diversification of *Orthopsyche–Caledopsyche*. Three major changes in speciation rate were identified, occurring *c.* 23, 15 and 7 Ma, of which the first two are associated with environmental diversification.

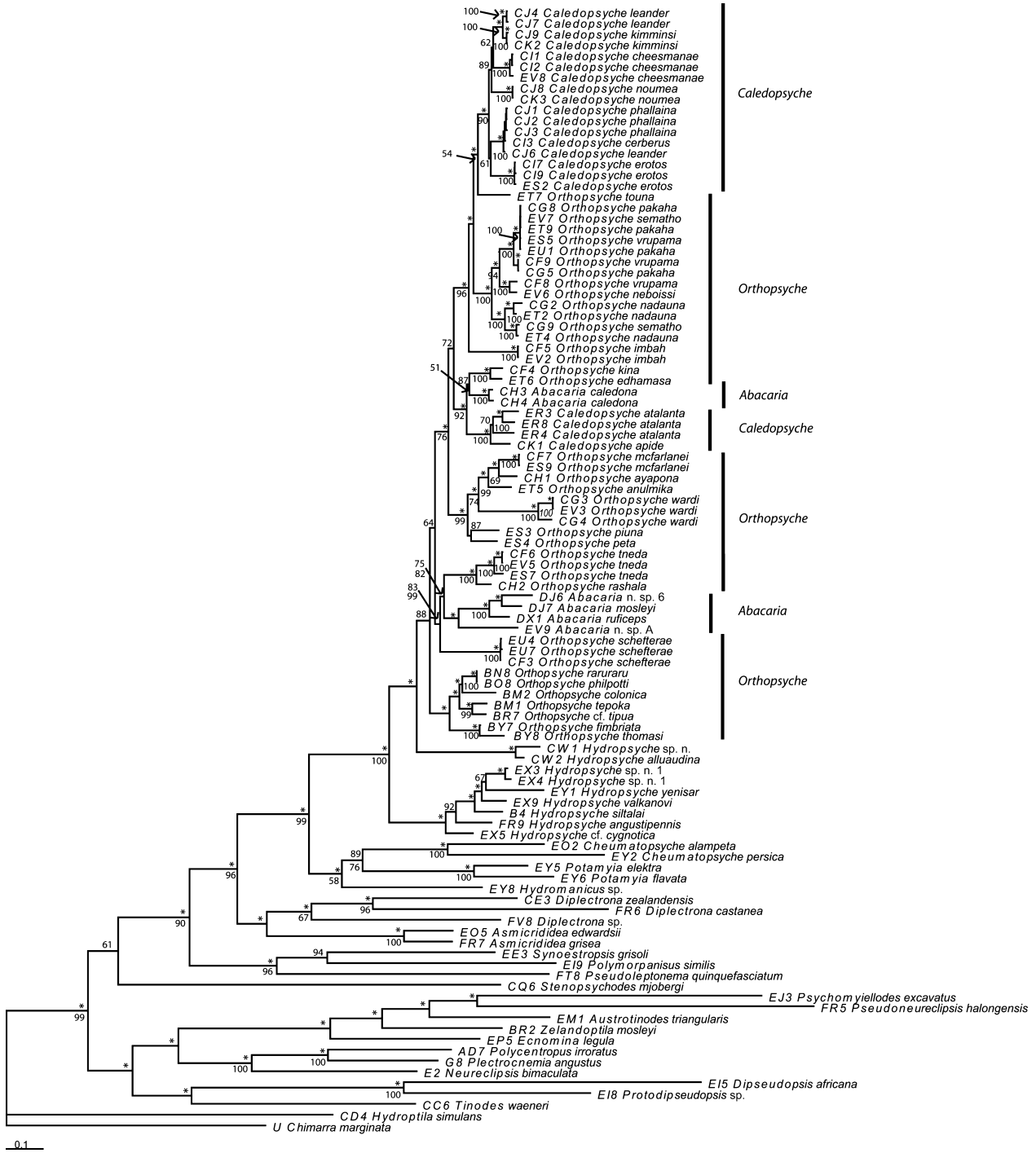

Figure 2 The 50% majority rule consensus tree from the Bayesian analysis of the combined dataset of New Caledonian Hydropsychinae. Posterior probabilities ≥ 95% are indicated with an asterisk and posterior probabilities from ≥ 50% to < 95% are denoted with the appropriate probability above branches. Below branches are jackknife values (GC) from the parsimony analysis.

DISCUSSION

Phylogenetic analysis and taxonomy

According to the phylogeny inferred in this study, *Orthopsyche*, *Caledopsyche* and *Abacaria* are not monophyletic. Furthermore, only three of the nine species groups of *Caledopsyche* and *Orthopsyche* proposed by Oláh & Johanson (2008) are monophyletic. The generic division of these two genera was based mainly on the number of hindwing forks (Schefter, 2005), which is demonstrated above to be a plastic character. Our phylogeny also shows that the New Caledonian *Abacaria caledona* belongs in the *Orthopsyche–Caledopsyche* group, and that the New Caledonian *Orthopsyche* and

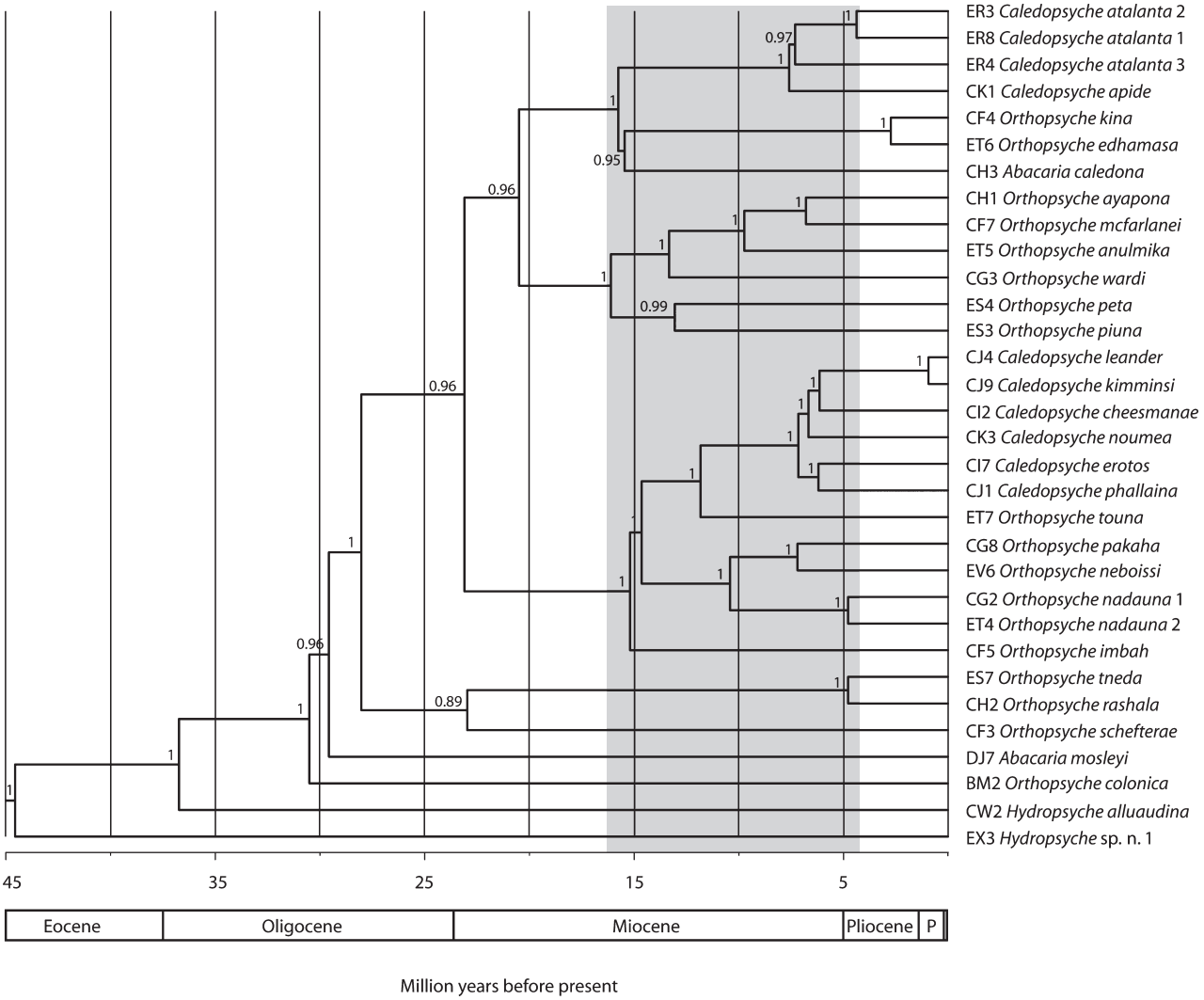

Figure 3 Chronogram showing lineage diversification of the Hydropsychinae caddisflies from New Caledonia under a relaxed clock model. The grey area indicates the time period when most of the lineage splitting occurred. Numbers on branches are posterior probabilities. On the epoch bar, P indicates Pleistocene.

the New Zealand *Orthopsyche* should be placed in different genera. Our findings support the hypothesis that Hydropsychinae colonized New Caledonia only once, rather than several times as indicated in previous classifications. It is, however, not possible to infer the sister group to the New Caledonian species group from our analysis, as the placements of the potential candidates, *Abacaria* (Papua New Guinea, Solomon Islands, Fiji) and New Zealand *Orthopsyche*, are not stable in the phylogeny. We found a frequent incongruence between morphological- and molecular-based species delimitations, a phenomenon observed in many studies (Funk & Omland, 2003, and citations therein). The varying position of some groups in trees inferred using different methods and genes is not caused by outgroup choice, but might be a result of long-branch attraction (Felsenstein, 1978), incomplete lineage sorting or the combination of genes used in the analysis. More sequence data are necessary to obtain a better resolved phylogeny.

Diversification on ultrabasic substrate

The ancestor to the New Caledonian *Caledopsyche–Orthopsyche* species adapted to a new substrate, which might have facilitated speciation within the group. The radiation primarily on ultrabasic substrate produced far more species than did the radiation on non-ultrabasic substrate. Based on these data alone we cannot interpret whether the radiation was adaptive, linked to substrate or caused by allopatric speciation on the fragmented and varying environments offered by the ultrabasic substrate. Espeland *et al.* (2008) found that the ancestor of the caddisfly genus *Xanthochorema* (Hydrobiosidae) quickly colonized ultrabasic substrate, and that early colonization of ultrabasic substrate was followed by speciation associated with that substrate. When the radiation began *c.* 28 Ma, Grande Terre was more extensively covered by ultrabasic substrate than it is today (Guillon, 1969), which partly explains the low diversification on non-ultrabasic substrate. As more

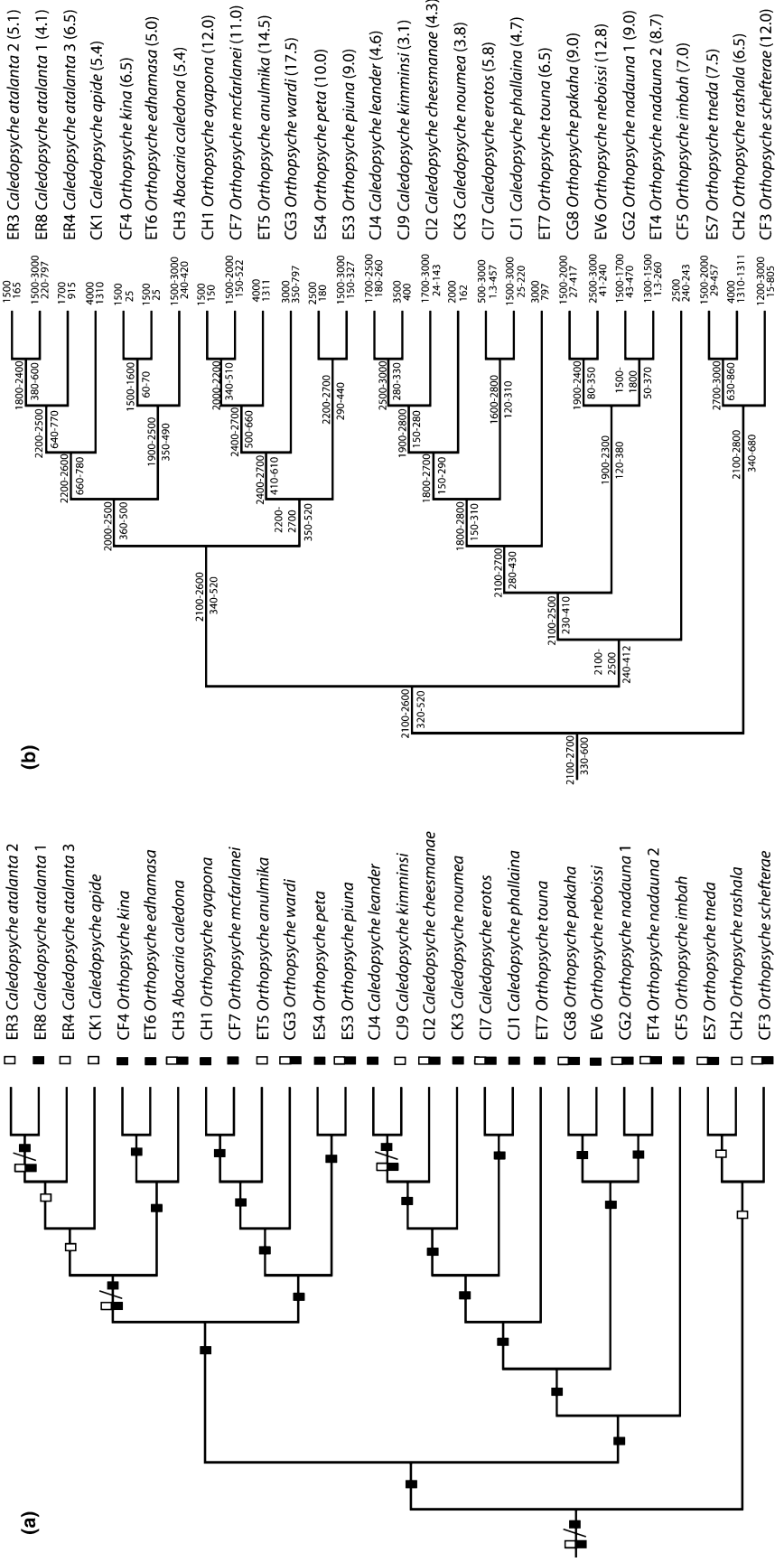

Figure 4 (a) DIVA and Fitch optimizations (before and after slash, respectively, when different) of geological substrate on the New Caledonian Hydropsychinae phylogeny. The white box denotes presence on non-ultrabasic substrate, the black box denotes that on ultrabasic substrate and the combined black and white box denotes that on both substrates. (b) Squared-change parsimony optimization of annual rainfall in millimetres (above branches) and elevation in metres (below branches). Ranges at terminals are for today's elevation and rainfall for each taxon. Numbers in parentheses after taxon names are mean body size as measured by male forewing length (in mm).

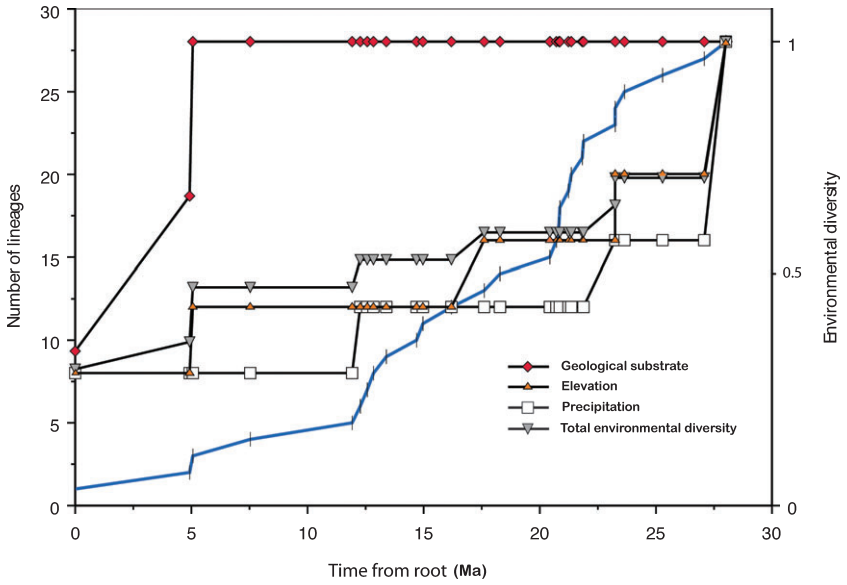

Figure 5 Geological substrate, elevation and precipitation of New Caledonia, and all factors combined (total environmental diversity) mapped on a lineage-through-time (LTT) plot (blue).

non-ultrabasic substrate became available, that substrate was colonized by species initially living on ultrabasic substrate. In the *atalanta–caledona* clade the ancestor dispersed from ultrabasic to non-ultrabasic substrate, which was followed by speciation on non-ultrabasic substrate. It is, therefore, possible that a shift in substrate was part of the driving force behind the speciation of this clade.

Rainfall, elevation and diversification

It is expected that rainfall and elevation are correlated in New Caledonia, as high-elevation areas have higher rainfall, as indicated in Fig. 1b,d. Rainfall patterns are important for caddisfly phenology, and can be a factor limiting niche diversity even in permanent water bodies (Resh & Rosenberg, 1984). Flint (1991) found correlations between rainfall pattern and emergence of adults, and de Moor & Ivanov (2008) noted that a high degree of endemism is often found in areas with high rainfall. The New Caledonian topography is diverse, and dispersal between streams might be prevented by dense vegetation and steep mountainsides. This allopatric isolation is probably more important than the elevation itself, as the maximum elevation on New Caledonia (1628 m) is not pronounced and would probably not require physiological adaptations.

Divergence times and ecological heterogeneity

It is evident that the ancestral New Caledonian Hydropsychidae evolved in the Middle–Late Oligocene (25–30 Ma), a period associated with intensive mountain formation and increased environmental heterogenity (Chevillotte *et al.*, 2006). As inferred in this study, the initial radiation is older than those of most other New Caledonian species radiations (Fig. 2 in Grandcolas *et al.*, 2008), but not as old as needed to support a vicariant origin of the biota as hypothesized previously (Raven & Axelrod, 1972; Holloway, 1979; Morat *et al.*, 1986; Cooper & Millener, 1993). Most of the diversifications occurred in the Middle–Late Miocene (around 4–16 Ma), a time associated with long-term climate change (Chevillotte *et al.*, 2006).

High environmental heterogeneity was clearly already present when the main species radiation occurred. This corresponds to Vinson & Hawkins (1998) findings that physical heterogeneity promotes biodiversity in freshwater insects. The first two apparent increases in speciation rate (Fig. 5) were associated with changes in environmental diversification, whereas the last increase was not. This indicates that both non-adaptive (fragmented habitats free to colonize) and adaptive (avoiding, for example, competition, predation) radiation have played a role in the diversification of the clade. According to the gamma statistic, the main radiation occurred early in the evolution of the group, a phenomenon found in most clades (McPeek, 2008). Rabosky & Lovette (2008) explain this phenomenon as probably resulting from a decreased speciation rate over time as niches fill rather than from an increased extinction rate. McPeek (2008) hypothesized that clades with a negative gamma statistic should be ecologically well differentiated and also display differences in characters not directly related to reproduction, and especially in characters that promote coexistence. The sixfold variation in average forewing size, and thus body size, between the species observed here supports this view. Jannot & Kerans (2003) reported that, within Hydropsychidae genera in general, body size differences are not very pronounced.

Large variation in body size is also found in other New Caledonian animals, such as skinks and geckos. Radiation in these groups has been explained by a combination of

fragmentation of a continuous habitat over time, and subsequent ecological adaptation to different foraging niches (Bauer & Sadlier, 2000; Bauer & Jackman, 2006). New Caledonian diving beetles (Dytiscidae) living sympatrically on Mount Panié are apparently ecologically segregated (Balke *et al.*, 2007). These insects occupy much of the same habitats as caddisflies, and thus adaptive radiation might also be a plausible explanation for the evolution of a high diversity of caddisflies in New Caledonia.

As in all members of the Trichoptera suborder Annulipalpia, the aquatic larvae of *Orthopsyche–Caledopsyche* species reside inside a fixed retreat and filter particles from the water using a capture net (Wiggins, 2004). Nothing is known about the specific ecological niches of *Orthosyche–Caledopsyche* species, but the difference in body size might be an adaptation towards feeding on different-sized particles or a different placement of the net. A study by Pauls *et al.* (2008) indicates that shifts in feeding ecology facilitate species diversification in caddisflies.

CONCLUSIONS

According to this study the *Orthopsyche–Caledopsyche* caddisflies on New Caledonia form a monophyletic group, which started diverging *c.* 28 Ma. Environmental diversification preceded the species diversification of this group, and high environmental heterogeneity thus opened up a variety of fragmented habitats and ecological niches, which facilitated caddisfly diversification, leading to a diverse and ecologically well-segregated fauna. Caddisflies, with their varied ecology and extreme species richness, provide an excellent model system for the study of species diversification on New Caledonia and elsewhere.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Distribution maps of the New Caledonian *Orthopsyche*, *Caledopsyche* and *Abacaria* species included in the analyses.

Appendix S2 Specimen information, collecting locality area with coordinates, and GenBank accession numbers.

Appendix S3 Primers and annealing temperatures used in this study.

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