S c , Ra . a . ∩ Ca ab . B . , (B , c . ∩ . : *M c a*) . ∩ N . Z a a ∩

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Abstract

Table 1. List of Broscini species used in this study with information regarding authority, distribution (S.I. = South Island New Zealand, N.I. = North Island New Zealand; Ch.Is. = Chatham Islands) and number of individuals employed.

Species	Distribution	# used in study	Authority
M. alternans	S.I. & Ch.Is.	12	Laporte de Castelnau, 1867
M. alternans hudsoni	The Snares	1	Broun, 1909
M. crenicolle	N.I. & S.I.	9	Laporte de Castelnau, 1867
M. crenaticolle	N.I.	5	Redtenbacher, 1868
M. curvidens	N.I.	1	(Broun, 1915)
M. fulgidum (cf fulgidum)	S.I.	5	Broun, 1881
M. howittii	S.I.	2	Laporte de Castelnau, 1867
M. longicolle	N.I.	1	Broun, 1923
M. lucidum	S.I.	2	Laporte de Castelnau, 1867
M. occiputale	N.I.	3	Broun, 1923
M. cf oconnori	N.I.	4	Broun, 1912
M. oregoides	S.I.	2	(Broun, 1894)
M. rugiceps	S.I.	1	Sharp, 1886
M. sculpturatum	S.I.	4	Blanchard, 1843
M. huttense (cf huttense)	S.I.	3	Broun, 1915
M. simplex	N.I.	4	Laporte de Castelnau, 1867
M. spinifer	N.I.	4	Broun, 1880
M. strictum	S.I.	1	Britton, 1949
M. sulcatum	N.I. & S.I.	1	(Sharp, 1886)
M. validum	N.I.	1	Broun, 1923
M. rectolineatum	S.I.	1	Laporte de Castelnau, 1867
M. politanum	S.I.	1	Broun, 1917
M. impressum	S.I.	1	Laporte de Castelnau, 1867
M. constrictum	S.I.	3	Broun, 1881
M. costellum lewisi	S.I.	1	Broun, 1908
M. costellum obesum	S.I.	1	Townsend, 1965
M. allani	S.I.	1	Fairburn, 1945
M. laterale	S.I.	1	Broun, 1917
M. minax	S.I.	2	Britton, 1949
M. elongatum	S.I.	1	Laporte de Castelnau, 1867
M. metallicum	S.I.	1	Sharp, 1886
M. ducale	S.I.	1	Sharp, 1886
M. morio	S.I.	1	(Laporte de Castelnau, 1867)
M. infimate	S.I.	1	Lewis, 1902
M. punctatum	S.I.	1	(Laporte de Castelnau, 1867)
Meta. moniliferum	S.I.	2	Bates, 1867
Meta. aberrans	S.I.	5	Putzeys, 1868
Meta. tibiale	S.I.	1	(Laporte de Castelnau, 1867)
Brullea antarctica	N.I. & S.I.	1	Laporte de Castelnau, 1867
Bountya insularis	Bounty Is.	1	Townsend, 1971
O. aereus	S.I.	6	(White, 1846)
O. inaequalis	S.I.	2	(Laporte de Castelnau, 1867)
O. crypticus	S.I.	1	Pawson, 2003
O. septentrionalis	S.I.	2	Pawson, 2003
D. clivinoides	S.I.	4	(Laporte de Castelnau, 1867)
D. obtusum	S.I.	2	(Broun, 1886)
D. seclusum	S.I.	1	(Johns, 2007)

doi:10.1371/journal.pone.0086185.t001

Table 2. Cont.

Sample ID	Species	Genes	Location
MB 128*	M. impressum	d	S.I., Queenstown, Kinloch
MB 134*	M. constrictum	d	S.I., Craigieburn Forest P., Education Ct.
MB 35*	M. constrictum	c	S.I., Fog Peak, Porter's Pass
MB 27*	M. constrictum	d	S.I., Canterbury, Craigieburn Rec. area
MB 121*	M. ducale	c	S.I., Lewis Pass, Lake Daniels Walk
MB 20*	M. cf oconnori	d	N.I., Levin, 30B The Avenue
MB 73*	M. cf oconnori	c	N.I., Te Urewera, Ngamoko Trig Tr.
MB75*	M. cf oconnori	d	N.I., Dannevirke, Norsewood Res.
MB 21*	M. cf oconnori	a	N.I., Wellington, Levin, Ohou
MB 76*	M. simplex	d	N.I., Tararua Forest Park, Putara
MB 77*	M. simplex	c	N.I., Tararua Ra., Mt Holdsworth
MB 25*	M. simplex	b	N.I., Manawatu, Pahiatua Track
MB 64*	M. simplex	a	N.I., Manawatu, Palmerston North
MB 63*	M. longicolle	a	N.I., Ruahine Ra., Pohangina Valley
MB 69*	M. validum	a	N.I., Tongariro NP, Whakapapanui Track
MB 90*	M. oregoides	a	S.I., Christchurch, Ahuriri Scenic Res.
MB 147.1*	M. oregoides	c	S.I., Christchurch, Ahuriri Scenic Res.
MB 19*	M. lucidum	a	S.I., Otago, Carrick Range
MB 111*	M. lucidum	d	S.I., Pisa Range
MB 03*	M. rugiceps	a	S.I., Fiordland, Lake Harris
MB 45*	M. sculpturatum	a	S.I., Dunedin, Ross Reserve
MB 125*	M. sculpturatum	d	S.I., Catlins Forest Park, River Walk
MB 04*	M. sculpturatum	d	S.I., Dunedin, Leith Saddle
MB 06*	M. sculpturatum	d	S.I., Dunedin, Mosgeil, Silver St.
MB 09*	M. huttense	c	S.I., Canterbury, Peel Forest
MB 46*	M. cf huttemse	d	S.I., Canterbury, Peel Forest
MB 108*	M. cf huttense	b	S.I., Canterbury, Peel Forest
MB 96*	M. strictum	a	S.I., Nelson, Takaka Hill, Canaan
MB 95*	M. sulcatum	a	S.I., Kaikoura, North of Ohau Point
MB 66*	M. curvidens	a	N.I., BOP, Rotorua
MB 68*			

Table 2. Cont.

Sample ID	Species	Genes	Location
MB 199	Bountya insularis	d	Bounty Is., Proclamation Is.
MB 192	Chylnus ater	d	Australia
MB 13	O. aereus	b	S.I., Otago, Danseys Pass
MB 41	O. aereus	a	S.I., Dunedin, Morrison St.
MB 28	O. aereus	d	S.I., Dunedin, 46 Morrison St.
MB 29	O. aereus	d	S.I., Dunedin, Sandfly Bay
MB 47	O. aereus	d	S.I., Dunedin, Silver Peaks
MB53	O. aereus	d	S.I., L. Onslow, Lammarlaws
MB 5	O. inaequalis	d	S.I., Dunedin, Miller Rd.
MB 48	D. clivinoides	b	S.I., Seaward Kaikoura Ra., Tinline Va.
MB 31	D. clivinoides	a	S.I., NW Nelson, Heaphy Track
MB 161	D. obtusum	d	S.I., Fiordland, Kepler Track
MB 162	D. obtusum	c	S.I., Otago, Catlins Coast, Tautuku
MB 159	D. clivinoides	c	S.I., Otago, Kinloch
MB 8	D. clivinoides	d	S.I., Nelson, Cobb Valley
MB 175	D. seclusum	d	S.I., Fiordland, Spey River Valley

(M. = Mecodema; Meta = Metaglymma; O. = Oregus; D. = Diglymma; S.I. = South Island New Zealand; N.I. = North Island New Zealand; BOP = Bay of Plenty; genes analysed: a = COI, COII, 16S, 18S; b = COI, COII, 16S; c = COI, 18S; d = COI; LUNZ = Lincoln University Entomological Research Museum; P = S.M. Pawson collection); COI sequences from samples of the Mecodema ingroup marked with * have previously been deposited in Genbank (#).

naturally an increasing interest in how diversification is distributed through time and space.

In this study we examine the phylogenetic relationships and timing of radiation of Mecodema (Blanchard, 1843) carabid beetles (tribe Broscini). This endemic genus of large, flightless beetles constitutes a prominent species radiation in New Zealand and presents a good opportunity to explore species level diversification. We utilise the fact that the genus is represented on the Chatham Islands, which are located approximately 850 km east of the South Island, New Zealand in the Pacific Ocean. Geological evidence for the formation of this archipelago within the last 4 Myr is compelling [3,32,33,34] and corroborated by genetic data for many taxa (e.g. insects – [18,35], plants – [36,37,38], parakeets – [39], pigeons – [40,41], cicadas – [42], invertebrates and plants – [43]). In this study the earliest possible establishment of an island biota (4 Myr) on the Chathams [32] is used as a maximum possible calibration for estimating the timing of diversification in Mecodema. Furthermore a substitution rate for Coleoptera [44] is employed to further explore timing of lineage diversification of this beetle genus.

Materials and Methods

Sampling

The genus Mecodema (Blanchard, 1853) belongs to the tribe Broscini (Carabidae). Broscini has a worldwide distribution but has its main diversity in the southern hemisphere (subfamily Nothobroscinae) [45] and consists of at least 27 genera, comprising about 80 species (see http://www.landcareresearch.co.nz/research/biosystematics/invertebrates/carabid/carabidlist) [46,47]. Six endemic genera of Broscini are recognized in New Zealand, but Mecodema is especially species-rich. Adult Mecodema beetles are relatively slow-moving, nocturnal, flightless (with fused elytra), generally active throughout the year, and usually scarce [48]. As with other Carabidae, adults and larvae of the New Zealand taxa are predatory. Mecodema is a diverse genus with species distributed throughout the New Zealand mainland from alpine to coastal habitats. In contrast, there is a single species (Mecodema alternans) on the Chatham Islands. The same species occurs in southeast New Zealand near Dunedin. Although M. alternans may be better treated as a species complex [47], no morphological characters have yet been described that distinguish Chatham Island populations from those in mainland New Zealand ([49] & I. Townsend pers. obs.).

In total our sampling comprised 113 specimens, with 88 representing 35 described species, and 4 undescribed Mecodema species (after [45,46] and http://www.landcareresearch.co.nz/research/biosystematics/invertebrates/carabid/carabidlist), see Table 1 for details and authorities. Putative outgroup New Zealand Broscini in our sample included Oregus (Putzeys, 1868), Diglymma (Sharp, 1886), Brullea antarctica (Laporte de Castelnau, 1867), Metaglymma (Bates,

1867), and *Bountya insularis* (Townsend, 1971); plus one representative of Broscini from Australia, *Chylnus atex* (Putzeys, 1868) (Table 2). As many of the species in *Mecodema* are scarce and therefore difficult to collect we also made use of material from museum collections, and supplemented outgroup sampling with available GenBank sequences (*Oregus septentrionalis* AF466847 & AF466848; *Oregus cryptus* AF554423; *Oregus inaequalis* AF466850 [50]; *Calathus azteca* GU254333 [51]; *Broscosoma relictum* AF012502; *Promecodesus* sp. AF012499; *Creobius eydouxi* AF012498 [52]). The resulting sample represents the geographic and ecological range of *Mecodema* in New Zealand (Table 2). *Mecodema* species show examples of allopatric, parapatric and sympatric distribution. Species but not always species groups are limited to particular areas and only few species (e.g. *M. crenicolle*

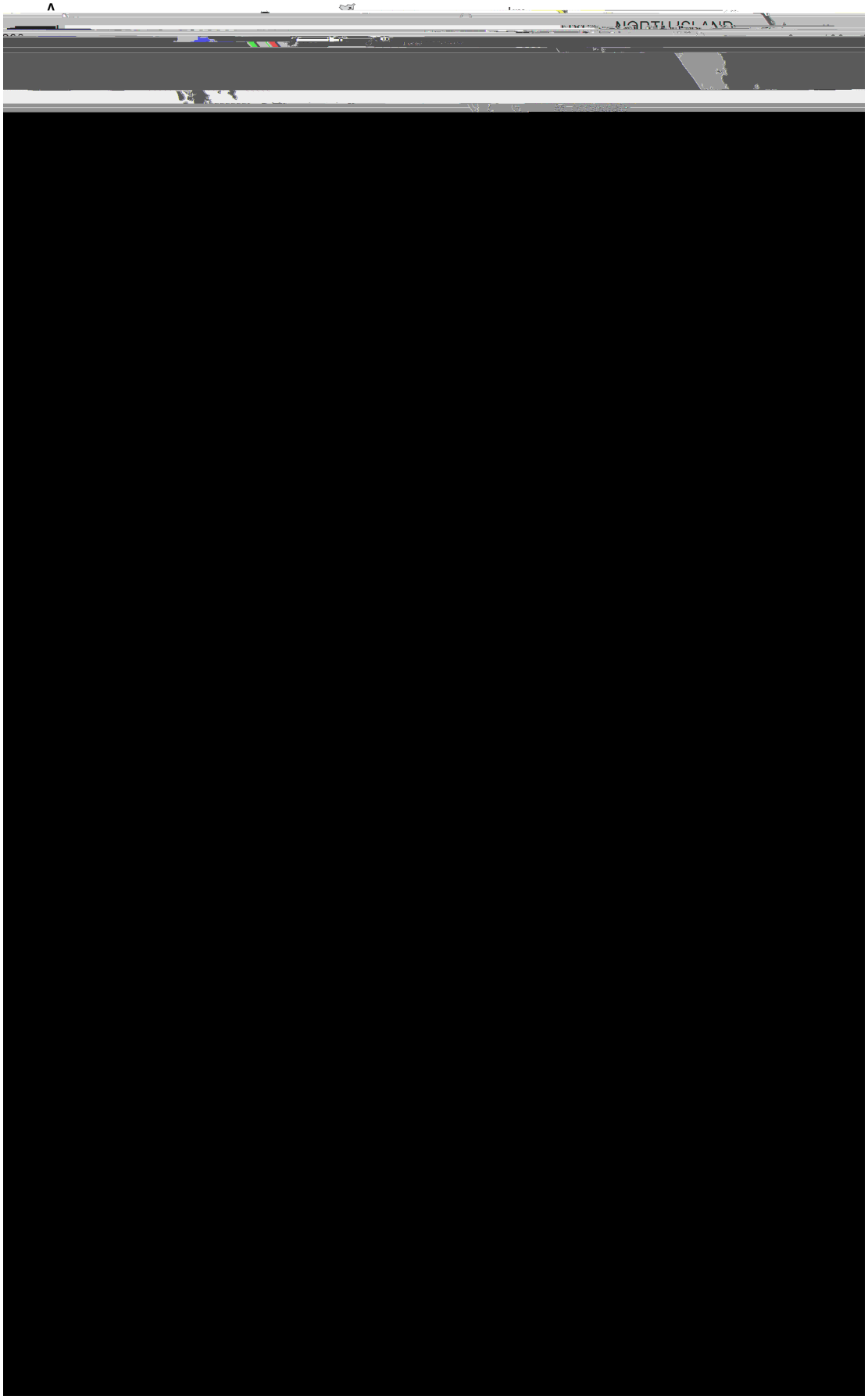

Figure 2. Maximum likelihood phylogeny for *M c a*. A) Spatial distribution of samples used in this analysis. Symbols correspond to those in Fig. 2B and code for different clades. B) Analysis of concatenated dataset including mitochondrial COI, COII and 16S plus nuclear 18S. Values at nodes indicate ML bootstrap support returned by analysis using RaxML. Specimen numbers at tips are given as in Table 2.
doi:10.1371/journal.pone.0086185.g002

Polymerase chain reactions were performed in 10 volumes and the amplified products then checked on a 1% agarose gel and purified using SAP/EXO1 digest (USB Corporation). Purified PCR products were sequenced using standard protocols for the ABI Prism BigDye Terminator Ready Reaction Kit (Applied Biosystems, Mulgrave, Australia) and run on an ABI Prism 377 automated sequencer (Applied Biosystems). Sequence identity was confirmed by comparison with published data, checked for nucleotide ambiguities in Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI, www.genecodes.com) and aligned using SeAl v2.0a11 [58]. The sequences have been deposited with accession numbers KF913050–913193 at GenBank (16S: KF913050–913088; 18S: KF913089–913130; COII: KF913131–913169; COI: KF913170–913193).

Phylogenetic analysis

To test whether *Diglymma* and *Oregus* species are the natural sister group to *Mecodema* we first analysed data from two genes separately (COI and 18S) as it was not possible to gain sequences for outgroup taxa outside of New Zealand for all the employed species and sequence availability in GenBank within Broscini was also very poor. Although *Oregus* and *Diglymma* represent two of the New Zealand Nothobroscina genera considered closest to *Mecodema* [45], it was crucial to verify this relationship within Broscini as two other potential outgroup taxa, *Metaglymma* and *Brullea*, exist. MrBayes 3.1.2 [59] was used to implement Bayesian analysis with the datasets applying a GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. Analyses with MrBayes used four independent

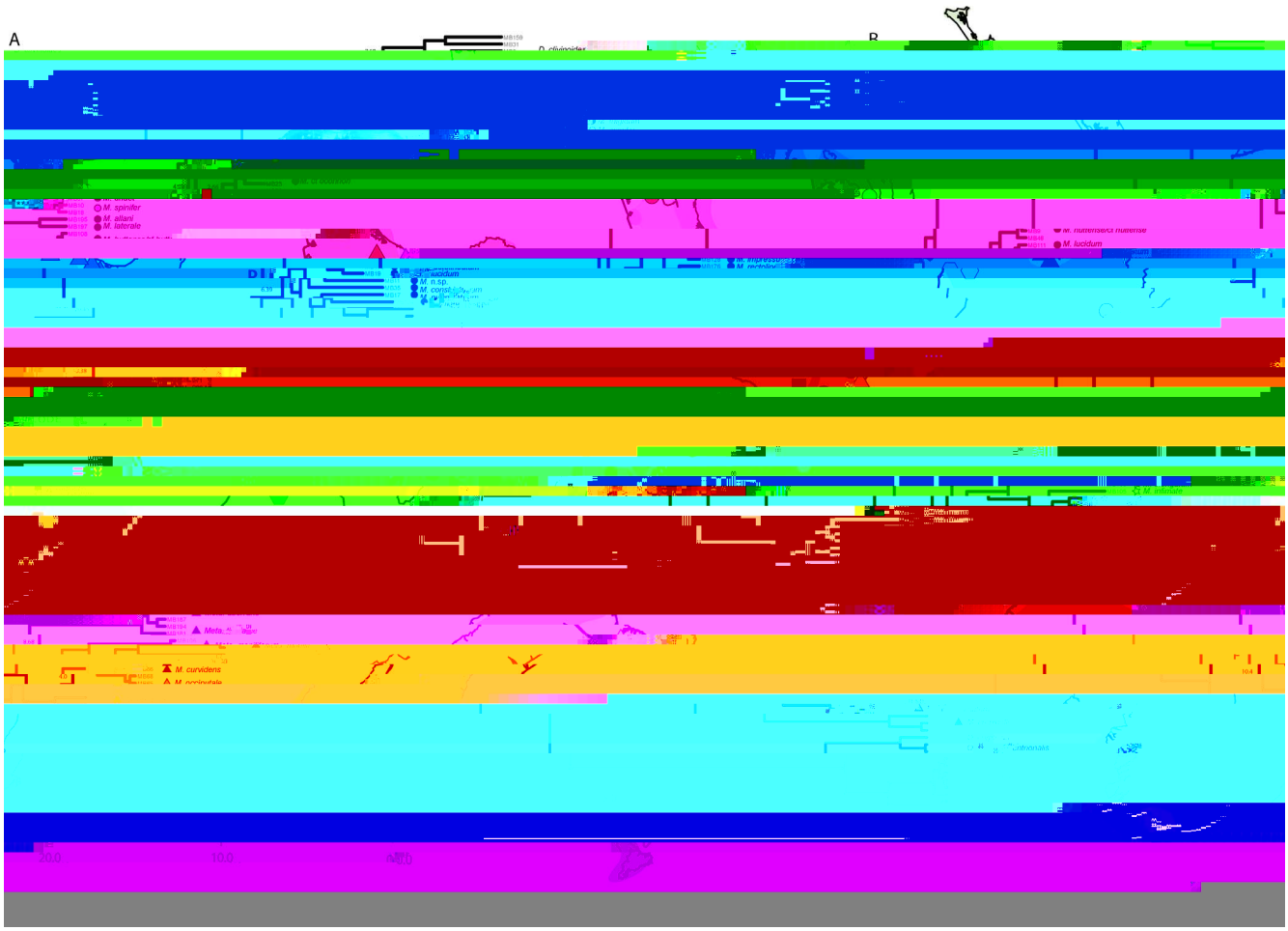

Figure 3. The timing of *M c a* diversification. A) COI Bayesian tree generated in BEAST. Numbers on nodes show age estimates based on stratigraphic calibration of 4 Myr. Outgroup taxa were the same as used in the outgroup test (Fig. 1). Grey branches indicate lineages present in North Island New Zealand, and black branches indicate South Island lineages. Tip symbols correspond to clade and location identifiers in Fig. 2. Coloured symbols match symbols in Fig. 3B, highlighting disjunct lineages in southern North Island and northern South Island. Asterisks indicate age estimates between N.I. and S.I. lineages (* = 1.60 Myr, ** = 1.69 Myr, *** = 2.03 Myr). B) Reconstruction of the paleogeographic environment in lower North Island, New Zealand ca. 3 million years ago, green areas indicating likely land above sea level during this time (modified from [28]). Black outlines indicate present day New Zealand land area with coloured symbols corresponding to those in Fig. 3A, showing the present sampling

Table 4. BEAST time estimates based on stratigraphic and COI substitution rate of Coleoptera [44] calibration.

Node	Estimates Myr (95% HPD) with stratigraphic calibration	Estimates Myr (95% HPD) with rate calibration
A	**4**	**0.27 (0.08–0.46)**
B	1.24 (0.5–2.09)	0.07 (0.02–0.15)
C	8.38 (5.29–12.06)	0.52 (0.22–0.9)
D	6.39 (3.97–9.41)	0.4 (0.18–0.72)

Markov Chain Monte Carlo (MCMC) runs for ten million generations with a burn-in of 10% and a tree sampling frequency of 1000. Results were checked for convergence. Resulting posterior probabilities on the nodes were recorded.

To examine the species phylogeny of the *Mecodema* group in New Zealand we employed all four genes (three mitochondrial and one nuclear) with a subset of 50 taxa (44 ingroup and 6 outgroup samples). The outgroup sampling was chosen after consideration of the results from the prior outgroup analyses. All taxa with data missing for no more than one of four genes were included in the phylogenetic analysis (Table 2). Partition-homogeneity tests (PHT [60]) were implemented in PAUP*4.0b10 [61] with 500 replicates for the combination of the gene regions to detect significant heterogeneity among the data sets.

MrBayes 3.1.2 [59] was then used to implement Bayesian analysis with the concatenated dataset, applying a GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. The same model was applied to the partitions with rates and nucleotide frequencies for each gene unlinked. Analyses with MrBayes used four independent Markov Chain Monte Carlo (MCMC) runs for two million generations with a burn-in of 25% and a tree sampling frequency of 1000. Resulting posterior probabilities on the nodes were recorded. The same data were subjected to Maximum Likelihood analysis with bootstrap resampling incorporating a GTR model with gamma-distributed rate variation. ML analysis used RaxML [62] implemented via the CIPRES portal [63]. The data were partitioned by gene (COI, COII, 16S, 18S) and bootstrap resampling was halted by RaxML

Molecular dating

As fossil remains of *Mecodema* that could provide information for calibrating a molecular clock have not been found, yet, we had to rely on geological information and a substitution rate calculated for Coleoptera COI [44] for calibration. In order to gauge the timing and extent of species radiation in *Mecodema* within New Zealand COI sequences were obtained for additional specimens in addition to previous analyses (Table 2). In some cases, this drew upon museum specimens to further assess the stability of our inferences about the distribution of diversity and timing of radiation in this beetle group.

In total 113 unique COI sequences were used for molecular dating in *Mecodema* (Table 2). To obtain estimates for the maximum age of lineage formation within the genus *Mecodema* we used this dataset of COI with two different calibration strategies. First we employed a stratigraphic calibration to estimate divergence times using the split between Chatham Island and its closest relative on mainland New Zealand. We assumed a normal distribution for the age around a calibration value of 4 Myr, derived from the maximum age for the Chatham Islands land surface [3,33], assuming that colonization was most likely sooner after emergence of the islands than later. Alternatively, to capture the minimum likely diversification dates we also calibrated the COI dataset with the substitution rate estimated by Pons et al. [44] for Coleoptera COI. This included a normally distributed prior on the substitution rate of 0.08606 subst/site/myrs/l, and a 95% HPD interval from 0.0253–0.147 subst/site/myrs/l as an approximation to the posterior distribution provided by Pons et al. [44]. This rate obtained from analysis of numerous beetle taxa is amongst the highest estimated for any animal gene, and other rates obtained for particular beetle lineages are much slower (e.g. 0.0211 subst/site/myrs/l [64], 0.02 subst/site/myrs/l [65]). Even these rates are nearly twice the widely employed 0.0115 subst/site/myrs/l estimate of Brower [66]. Age estimation for both datasets and both calibration strategies were conducted under the assumption of a strict molecular clock as well as assuming a relaxed molecular clock with a lognormal distribution of rates along the phylogenies [67]. The fit of both priors was compared using Bayes Factors. For all datasets and calibration strategies the relaxed lognormal clock fitted the data decisively better than the strict clock [68].

We used the software BEAUTI 1.4.8 [69] and BEAST 1.7.5 [70] for molecular dating with the given calibrations. All analyses were conducted with a Birth-Death tree prior and a random starting tree under the GTR+I+C model of nucleotide substitution. The MCMC was run for 50–100 million generations, sampling every 5000th–10,000th step after a discarded burn-in of 5–10 million steps. Each analysis was run at least two times. The program Tracer 1.4 [71] was used to summarize posterior distributions of all parameters in question, to verify convergence of the MCMC and to estimate Effective Sample Sizes (ESS). If the effective sample size was less than 200, a third MCMC run was conducted for the respective analysis. After convergence of the MCMC was confirmed, the posterior distributions of all parameters were estimated from the combined posterior distributions of all runs conducted for each analysis. The program FigTree 1.4.0 [69] was used to visualize the reconstructed phylogenies.

Results

Three widely used mitochondrial gene regions were employed to gauge the scale of genetic diversity among the *Mecodema* specimens. Overall we observed relatively low genetic distances among species of *Mecodema* with a maximum ML-distance of 0.0179 in COII (COI: 0.0161, 16S: 0.053). Lower values for 16S

compared to COI and COII reflect the comparatively low proportion of variable sites in this gene (16.9%).

Separate analyses of COI and 18S DNA sequences from 41 specimens of *Mecodema* and 6 outgroup taxa (Table 2) resulted in similar topologies even though sequence variation in 18S was low. These analyses confirmed *Oregus* and *Diglymma* as the sister group to *Mecodema*, and revealed the placement of *Metaglymma* and *Brullea* within the *Mecodema* radiation (Figs. 1A & B).

The alignment of data from four gene regions comprising 50 specimens sampled across New Zealand (including 6 outgroup specimens –Table 2, Fig. 2) was 3114 bp long in total. All three mitochondrial genes displayed the average insect A-T content of about 75%. The partition homogeneity test (PHT) revealed no significant heterogeneity of lineage partitioning among the data sets ($p = 0.866$), suggesting their concatenation was appropriate. The GTR+I+C model of nucleotide substitution was identified as the best fitting model by the hLRT and the AIC as implemented in Modeltest 3.5 [72].

Bayesian and ML analysis of the concatenated dataset supported a single topology with the same groupings and branching order and well-supported nodes (Fig. 2). *Mecodema* was confirmed as paraphyletic with respect to *Metaglymma* and *Brullea* as these fall inside the *Mecodema* complex throughout all datasets and analyses in this study. We note that in all cases, *Metaglymma* and *Brullea* are placed within the *M. curvidens/origoides* group. This phylogenetic position contradicts the current taxonomic classification and needs to be addressed further in the future. Clades revealed in this analysis comprise species that are, in many cases,

by the setting of priors. In this study the inferred mitochondrial divergence times based on the fast COI rate obtained for Coleoptera [44] are consistently substantially younger than would be expected for a highly diversified genus even in the relatively youthful landscape of New Zealand.

Despite a perception that New Zealand is an ancient land mass

demonstrating the population genetic and ecological mechanisms of diversification (e.g. [89]).

Increasingly, the fields of species phylogenetics and population phylogeography are merging as it becomes easier to generate appropriate DNA data, and the focus in taxonomy is shifted towards an evolutionary paradigm (e.g. [90]. Teasing apart the interaction of abiotic and genetic processes on population subdivision remains challenging but *Mecodema* is one taxon group that will provide helpful insight, and it is already evident that *Mecodema* is an impressive example of recent species radiation in the New Zealand fauna. In recent years, synthesis of phylogenetic, ecological and taxonomic evidence has indicated that the biology of New Zealand is primarily the story of recent adaptation and speciation [8,9,19]. Understanding properly how our view of ''recent'' geological time relates to the intergenerational population genetics of large invertebrate populations will provide the basis of exciting research.

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Acknowledgments

We would like to thank Steve Pawson and Peter Johns for contributing specimens to this study. Mary Morgan-Richards and Frank Wieland gave valuable comments on earlier drafts of the manuscript. The Department of Conservation assisted with permitting.

Author Contributions

Conceived and designed the experiments: JG SAT. Performed the experiments: JG. Analyzed the data: JG MK SAT. Contributed reagents/materials/analysis tools: JG MK RME JIT SAT. Wrote the paper: JG SAT.

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