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Contemporary genetic structure of an endemic freshwater turtle reflects Miocene orogenesis of New Guinea

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The island of New Guinea lies in one of the most tectonically active regions in the world and has long provided outstanding opportunity for studies of biogeography. Several chelid turtles, of clear Gondwanal origin, occur in New Guinea; all species except one, the endemic *Elseya novaeguineae*, are restricted to the lowlands south of the Central Ranges. *Elseya novaeguineae* is found throughout New Guinea. We use mitochondrial and nuclear gene variation among populations of *E. novaeguineae* throughout its range to test hypotheses of recent extensive dispersal versus more ancient persistence in New Guinea. Its genetic structure bears the signature of Miocene vicariance events. The date of the divergence between a Birds Head (Kepala Burung) clade and clades north and south of the Central Ranges is estimated to be 19.8 Mya [95% highest posterior density (HPD) interval of 13.3–26.8 Mya] and the date between the northern and southern clades is estimated to be slightly more recent at 17.4 Mya (95% HPD interval of 11.0–24.5 Mya). The distribution of this endemic species is best explained by persistent occupation (or early invasion and dispersal) and subsequent isolation initiated by the dramatic landform changes that were part of the Miocene history of the island of New Guinea, rather than as a response to the contemporary landscape of an exceptionally effective disperser. The driving influence on genetic structure appears to have been isolation arising from a combination of: (1) the early uplift of the Central Ranges and establishment of a north-south drainage divide; (2) development of the Langguru Fold Belt; (3) the opening of Cenderawasih Bay; and (4) the deep waters of the Aru Trough and Cenderawasih Bay that come close to the current coastline to maintain isolation of the Birds Head through periods of sea level minima (–135 m). The dates of divergence of turtle populations north and south of the ranges predate the telescopic uplift of the central ranges associated with oblique subduction of the Australian Plate beneath the Pacific Plate. Their isolation was probably associated with earlier uplift and drainage isolation driven by the accretion of island terranes to the northern boundary of the Australian craton that occurred earlier than the oblique subduction. The opening of Cenderawasih Bay is too recent (6 Mya) to have initiated the isolation of the Birds Head populations from those of the remainder of New Guinea, although its deep waters will have served to sustain the isolation through successive sea level changes. The molecular evidence suggests that the Birds Head docked with New Guinea some time before the Central Ranges emerged as a barrier to turtle dispersal. Overall, deep genetic structure of the species complex reflects events and processes that occurred during Miocene, whereas structure within each clade across the New Guinea landscape relates to Pliocene and Pleistocene times.

ADDITIONAL KEYWORDS: Birds Head – Chelidae – *Elseya novaeguineae* – Indonesia – Langguru Fold Belt – molecular clock – Papua – tectonics – Vogelkop.

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INTRODUCTION

Phylogeography strives to understand contemporary distribution patterns of species by integrating information on biological relationships among populations with information on historical connectivity (Avise *et al*., 1987). Depending on the timescale, past connectivity is influenced by such processes as plate tectonics (Sanmartíin & Ronquist, 2004), sea level change (Schultz *et al*., 2008), landscape surface processes (e.g. river capture: Hurwood & Hughes, 1998; Burridge, Craw & Waters, 2006), habitat change (e.g. aridification: Douady *et al*., 2003; Maguire & Stigall, 2008), and ecological interactions (Kennedy *et al*., 2002). The island of New Guinea lies in one of the most tectonically active regions in the world and has long provided outstanding opportunity to study the impact of these processes on biogeography (Wallace, 1860; Mayr, 1944; Polhemus & Polhemus, 1998; Heads, 2002; Rawlings & Donnellan, 2003; Wüster *et al*., 2005; Deiner *et al*., 2011; Nyári & Joseph, 2013). Originating from the collision of the northward-moving Indo-Australian plate and the westward-moving Pacific plate, the current topographic configuration of New Guinea is a relatively young (approximately 10 Mya). It consists of a complex composite of accreted oceanic and continental terranes in the north, a relatively stable Australian continental block underlying the lowlands in the south, and a central range that has undergone dramatic uplift and deformation arising from collision rates of up to 100 mm $year^{-1}$ (Pigram & Davies, 1987; van Ufford & Cloos, 2005; Stanaway, 2008). New Guinea lies at the critical junction between the Asian and Australasian bioregions, and so has played an important role both in the invasion of Australia by faunal elements of Asian origin (e.g. the murine rodents: Rowe *et al*., 2008) and as a refuge for Australasian diversity (Hope & Aplin, 2007), decimated elsewhere by progressive aridification of the Australian continent during the Tertiary (Magee *et al*., 2004; Cohen *et al*., 2011). The exchange of fauna between New Guinea and Australia has been complicated by their recurrent interconnection and separation as sea levels have varied in response to Pleistocene glacial cycles (Lambeck & Chappell, 2001; Reeves *et al*., 2008; Cook *et al*., 2012). Freshwater turtles provide exemplary examples of the interplay between dispersal, vicariance, time, and morphological or genetic divergence.

New Guinea, and particularly the tropical southern lowlands, supports the highest species richness of freshwater turtle species in Australasia. Species of Asian origin include two softshell turtles in the genus *Pelochelys* (Trionychidae), commonly found in estuarine areas, and considered to be capable of extensive marine dispersal (Rhodin, Mittermeier & Hall, 1993). *Pelochelys bibroni* occurs south of the Central Ranges, and *Pelochelys signifera* occurs to the north (Georges & Thomson, 2010). Both are closely related to *Pelochelys cantori* of south-east Asia. Neither has reached Australia. *Carettochelys insculpta*, now restricted to southern New Guinea and northern Australia (Georges & Thomson, 2010), belongs to a family (Carettochelyidae) that was widespread in the Tertiary, its distribution covering much of Laurasia by the Eocene (Meylan, 1988). A fossil *C. insculpta* from marine beds at the mouth of Mariana Creek, Vailala River, Papua New Guinea (PNG), has been dated as upper Miocene (Glaessner, 1942). *Carettochelys insculpta* is considered to be of south-east Asian origin (Cogger & Heatwole, 1981).

All remaining species of turtle in Australia and New Guinea belong to the family Chelidae. These are of clear Gondwanan origin because they are not found outside their current range of South America and Australasia even in the fossil record. Their fossil record in Australia dates back to the mid Cretaceous, approximately 100–110 Mya (Smith, 2010). In Australasia, chelid turtles achieve their highest species richness in the Fly drainage of PNG (Georges, Guarino & Bito, 2006). The species *Chelodina parkeri*, *Chelodina rugosa*, *Chelodina pritchardi*, *Chelodina novaeguineae*, *Elseya branderhorsti*, and *Emydura subglobosa* each have clear relationships to sister taxa in Australia (Georges & Adams, 1992, 1996). The endemic short-necked chelid turtle *Elseya novaeguineae* (Meyer, 1874) is unusual in that its phylogenetic relationship with other Australasian taxa is unclear (Boulenger, 1889; Goode, 1967; McDowell, 1983; Georges & Thomson, 2010), confounded by the combination of absence of an alveolar ridge on the triturating surfaces of the mouth (prominent in *Elseya*), an expanded parietal bridge leading to extension of the head shield as lateral processes extending almost to the tympanum (characteristic of *Myuchelys*), and the usual presence of a cervical scute (usually so in *Emydura* but not *Elseya* or *Myuchelys*) (Georges & Thomson, 2010). Molecular data have *E. novaeguineae* as sister to *E. branderhorsti* (Le *et al*., 2013), sister to a clade consisting of *Elseya dentata*, *Elseya* sp. aff. *dentata* [Magela] (Georges & Adams, 1996) and *E. branderhorsti* (Todd *et al*., 2013), or as a lineage falling between the Queensland *Elseya* (*Elseya albagula* and relatives) and the northern *Elseya* (*E. dentata* and relatives) (Georges & Adams, 1992).

Dispersal of most chelid turtles between Australia and New Guinea probably occurred relatively recently, in the late Pliocene, Pleistocene and Holocene, because these species are restricted to the lowlands south of the Central Ranges. *Elseya*

novaeguineae departs from this otherwise ubiquitous distributional pattern in being abundant and widespread throughout New Guinea, in the tributaries and flooded forests of the lowlands of southern and northern New Guinea, and the Birds Head (Kepala Burung) of West Papua (Georges & Thomson, 2010). In the present study, we explore three potential hypotheses to explain this unusual distribution. The first and only published hypothesis (Rhodin *et al*., 1993) is that *E. novaeguineae* dispersed to New Guinea from Australia after the Central Ranges were established but, by chance or exceptional dispersal capability, made its way to the north of the island and across to the Birds Head. This hypothesis suggests a relatively recent dispersal throughout New Guinea, and would have the southern form sister to a clade comprising the populations of Birds Head and north of the Central Ranges. A second explanation, herein referred to as the 'docking hypothesis', is that *E. novaeguineae* came to occupy and speciated on an island terrane of continental origin that now forms part of the Birds Head, presumably after it broke away from the Australian craton in the Cretaceous but before its current connection to the island of New Guinea proper (Polhemus, 2007). After Birds Head docked with greater New Guinea, *E. novaeguineae* could have dispersed to the north and south of the emerging Central Ranges. This hypothesis would have the Birds Head populations as sister to a clade comprising the southern and northern forms. A third '*in situ* hypothesis' is that *E. novaeguineae* is a long-standing and persistent resident of the area that now forms New Guinea before becoming fragmented by vicariance associated with the development the Langguru Fold Belt, the opening of Cenderawasih Bay, and uplift of the Central Ranges. We address these hypotheses using a fossil calibrated analysis of mitochondrial and nuclear DNA from Australian short-necked chelid turtles combined with a broad geographical sampling of *E. novaeguineae* with multiple mitochondrial genes, and relate this structure to current interpretations of the geological history of New Guinea. We also explore phylogeographical patterns within each of the major clades emerging from our analysis, relative to topography and opportunity to disperse along exposed continental shelf during low sea levels.

MATERIAL AND METHODS

Specimens of *E. novaeguineae* were collected from throughout their range in Indonesian New Guinea (Fig. 1) by Indonesian nationals under contract to Bill McCord in support of other studies. The region referred to in the present study as the Birds Head (literally translated in Indonesian as Kepala Burung) refers to the entire crustal block west of Cenderawasih Bay, including Vogelkop Peninsula (also known as Doberai Peninsula), Bomberai Peninsula, Binuturi Basin, as well as associated islands of Salawati, Waigeo, and Misool (Fig. 1). Specimens of *E. novaeguineae* and *E. branderhorsti* (outgroup taxon) from the Bensbach, Morehead, Fly, and Kikori rivers of PNG were collected as part of general surveys (Georges *et al*., 2006, 2008). A sample of skin was taken from the trailing edge of the vestigial toe on the hind foot of each specimen and immediately preserved in 90% ethanol. Samples were transported to the University of Canberra or the American Museum of Natural History where they were stored at −20 °C until analyzed. Total genomic DNA was extracted by salt extraction (*sensu* Miller, Dykes & Polesky, 1988; FitzSimmons, Moritz & Moore, 1995), or using Chelex (Bio-Rad) beads, or by using a commercially available DNeasy Tissue Kit (Qiagen Inc.) in accordance with the manufacturer's instructions for animal tissues. The success of genomic extraction was confirmed by gel electrophoresis and quantification using a Nanodrop ND-1000 spectrophotometer (Fisher Thermo).

For each specimen, we amplified 1038 bp of mitochondrial (mt)DNA sequence, comprising 257 bp of control region (primers EmyThr 5′-CACCACCC TCCTGAAATACTC-3′; H. B. Shaffer, pers. commun.; TCR500, Engstrom, Shaffer & McCord, 2002), 533 bp of the NADH dehydrogenase subunit 4 (ND4), a further 70 bp of ND4 coding region, together with 71 bp of tRNA His, 59 bp of tRNA Ser, and the first 47 bp of tRNA Leu [primers ND4: Arévalo, Davis & Sites (1994); ND4Int: Fielder *et al*. (2012); Leu+G 5′-GCATTACTTTTACTTGGATTTGCA CCA-3′ *sensu* Arévalo *et al*. (1994)]. For each DNA fragment, two products from two independent reactions were sequenced in both directions to ensure sequence fidelity. This is referred to as the reduced gene set. Following preliminary analysis to identify major clades, a further 269 bp of 12S [primers L1091 (pos 491) and H1478 (pos 947): Kocher *et al*. (1989)], 370 bp of 16S [primers M89(L) and M90(H): Georges *et al*. (1998)], 393 bp of CO1 [primers M72(L) and M73(H): Georges *et al*. (1998)], 846 bp of cytochrome *b* (cyt *b*) [primers GLUDGE: Palumbi *et al*. (1991); mt-E-Rev2: Barth *et al*. (2004)] and a larger fragment of the ND4 gene [866 bp, primers ND4/ND4_672(f): Engstrom *et al*. (2002); Leu: Arévalo *et al*. (1994)] were sequenced for three representative specimens from each major clade and two specimens of *E. branderhorsti*. This is referred to as the full gene set (total 2744 bp).

Polymerase chain reaction (PCR) products (50 μL of each sample) were either precipitated with 50 μL of 20% polyethylene glycol, washed with 80% ethanol and re-suspended in 13 μL of water or cleaned on a

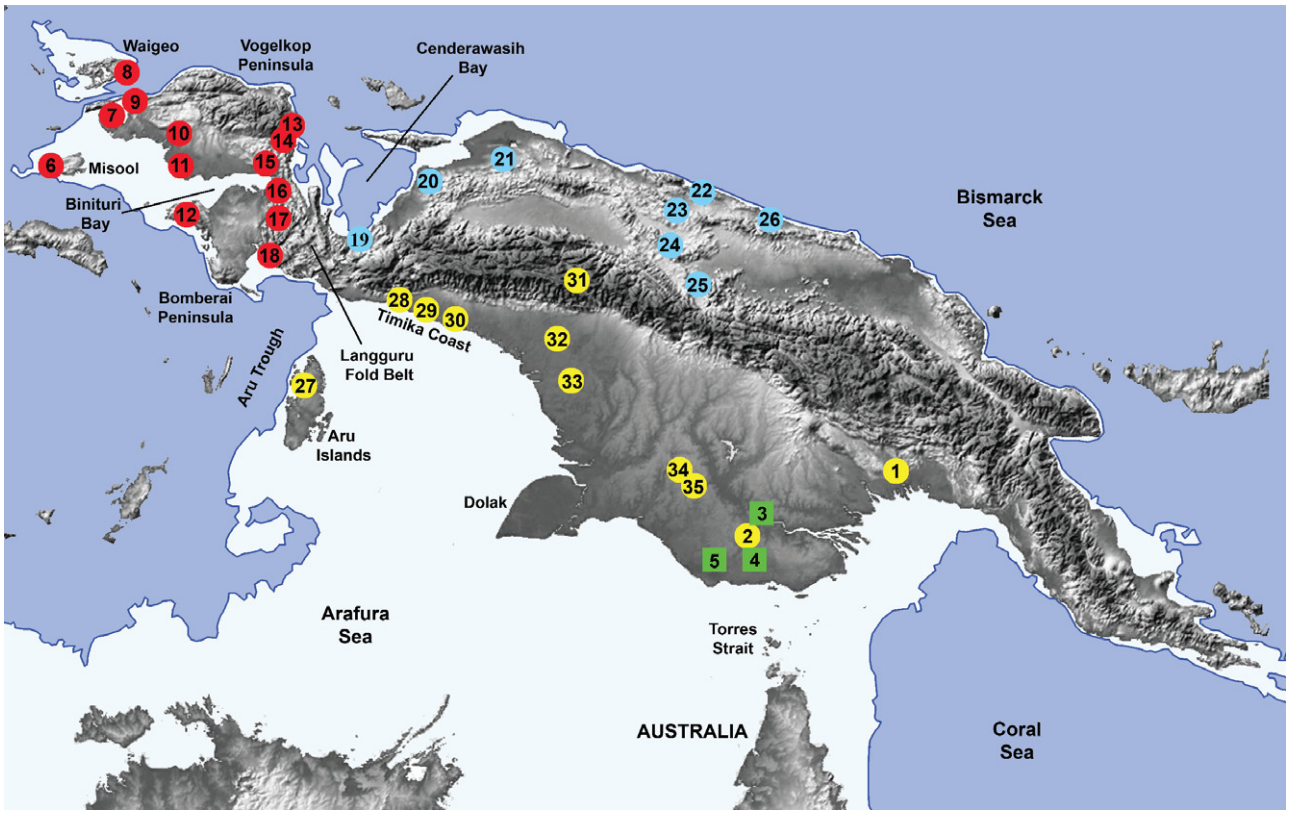

Figure 1. Sampling locations and distribution of major clades for *Elseya novaeguineae* (●) and *Elseya branderhorsti* (■, green) on New Guinea and associated islands. The region comprising Vogelkop and Bomerai peninsulas is collectively referred to as the Birds Head, the narrow area containing the Langguru Fold Belt as the Birds Neck, and the remainder of the island as greater New Guinea. Distribution of haplotypes from the Birds Head clade is shown in red, the northern clade in blue and the southern clade in yellow. Additional details on locations referred to in the text are provided in the text (Specimens Examined) using the site numbers as a cross-reference. The light shaded oceanic region shows the extent of exposure of the Arafura Shelf and coastal New Guinea at the sea level minima (approximately –135 m).

Biomek automated apparatus using the Ampure system (Beckman-Coulter Inc.). The purified PCR products were either packaged and sent to Macrogen Inc. (World Meridian Venture Centre 10F) for sequencing or cycle sequenced in-house at the American Museum of Natural History's Sackler Institute for Comparative Genomics using BigDye reagents (Perkin Elmer), after which cycle sequencing products were ethanol-precipitated and run on an ABI3770 automated sequencer (Applied Biosystems) [GenBank Accession numbers: JN188812–188926, sequence alignment deposited in Dryad (Georges *et al*., 2013)]. Sequences were edited and aligned using GENEIOUS PRO, version 5.3.3 (http://www.geneious.com) and with final alignment by eye.

Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed using PAUP* 4.0b10 (Swofford, 2002). Gaps were excluded from all analyses. MP analyses were undertaken using default parameter values. Support for clades was calculated using 10 000 bootstrap replicates obtained by heuristic search, each of which was based on 100 random addition sequence replicates. We consider bootstrap values in excess of 70% to be indicative of support for the associated node, and bootstrap values in excess of 90% to be strong support. ML analyses were performed as heuristic searches (as-is stepwise addition followed by tree bisection–reconnection branch swapping) under the best fit model of molecular evolution (TrN+G+I; *sensu* Tamura & Nei, 1993) and the substitution estimates and gamma parameter estimated by MODELTEST, version 3.06 (Posada & Crandall, 1998). Support for clades was calculated using 1000 bootstrap replicates. Mean rates of nucleotide substitution calculated from the reduced gene dataset (uncorrected and corrected TrN+I+G distances) were compared between major clades within *E. novaeguineae* (*E. branderhorsti* as outgroup) using relative rate tests (Takezaki, Rzhetsky & Nei, 2004) as implemented in PHYLTEST (Kumar, 1996). *Elseya*

branderhorsti was used as the outgroup based on the analysis of Le *et al*. (2013).

To provide broader context for dating the divergences between *E. novaeguineae* lineages, we calibrated a molecular clock with known fossils and incorporating broader taxonomic sampling than just *E. novaeguineae* and *E. branderhorsti*. We used mitochondrial sequences for ND4, cyt *b*, several tRNAs and the nuclear R35 intron from Le *et al*. (2013) for the Australian short-necked chelid radiation (hereafter taken to consist of the genera *Elseya*, *Elusor*, *Emydura*, *Myuchelys*, and *Rheodytes*, but not *Pseudemydura*). The Le *et al*. (2013) dataset was reduced to single representatives per species (to comply with the assumption of the Yule model of complete taxon sampling, with each operational taxonomic unit representing a different taxon; Ho *et al*., 2008). *Myuchelys purvisi* (*Flaviemys purvisi* of Le *et al*., 2013) was excluded from the analysis because there is substantial conflict between mitochondrial DNA and nuclear DNA topologies for that species. The sequences for *E. dentata* referred to by Le *et al*. (2013) were identified as *Elseya irwini* and the misidentification was corrected. Sequence data for *Elusor macrurus*, *Myuchelys latisternum*, and *Elseya dentata* used by Le *et al*. (2013) were missing certain genes, in which case we replaced their entire sequences with data from unpublished whole mitochondrial genome sequences and added additional data for the nuclear R35 locus [sequence alignment was deposited in Dryad (Georges *et al*., 2013)]. Sequences were aligned with the online version of MAFFT, version 7.046 (Katoh & Standley, 2013) using the very slow G-INS-i algorithm with the scoring matrix for nucleotide sequences set to 1PAM/K = 2, a gap opening penalty of 1.53, and an offset value of 0.5.

BEAST 2.0.2 (Bouckaert *et al*., 2013) was used to estimate molecular divergence times of lineages based on fossil age estimates. Input files were generated using BEAUti 2.0.2 (Bouckaert *et al*., 2013). The analysis used an uncorrelated lognormal relaxed molecular clock with rate variation following a tree prior using the calibrated Yule model. We separated the data into two partitions: one for the mitochondrial data and one for the nuclear data. For the model of nucleotide substitution, we used the RB BEAST addon, which automatically adjusts the analysis to choose the best model of nucleotide substitution for each partition. The topology was fixed based on a previous BEAST run, which included a sequence of *Chelodina rugosa* (GenBank: NC_015986.1 and AY339641.1) as an outgroup along with our fossil calibrations. This provided a suitable tree with branch lengths consistent with our priors. We fixed this topology in our analysis and allowed BEAST to estimate branch lengths only.

Turtles are commonly found in the Australian fossil record, although the osteology of extant forms has been poorly studied (Thomson & Georges, 2009; Smith, 2010). Hence, diagnostic characters are often unavailable and placing fossils into a phylogeny of living species is difficult. Fossils from only two time horizons (Thomson & Mackness, 1999; Mackness, Whitehead & McNamara, 2000; de Broin & Molnar, 2001) have sufficient information that they can be used as calibrated constraints in a molecular clock analysis. Fossil remains from the Redbank Plains Formation were identified as representing two species from the Australian short-necked chelid radiation (the *Emydura* group of de Broin & Molnar, 2001) but could not be assigned to genus because of a lack of diagnostic features (de Broin & Molnar, 2001). These were placed at the basal node of the Australian short-necked chelid radiation. The age of the Redbank Plains Formation is Eocene, with an estimated age of 55.0–58.5 Mya (Langford *et al*., 1995), and the Redbank Plains fossils are consistent with other similarly aged fossils likely to be part of the Australian short-necked radiation from the Pilbara (Boongerooda Greensand, Paleocene) and Proserpine (possibly Eocene) (de Broin & Molnar, 2001). We used this calibration in our analysis with a lognormal distribution and an offset of 52 Mya to set the minimum age (allowing for some error in the geological age estimation of the formation), a mean of 4.75 and an SD of 0.5. Turtle fossils from Bluff Downs in the Allingham Formation are closely related to *Elseya irwini* (Thomson & Mackness, 1999). The age of the Allingham Formation is between 3.6–5.2 Mya based on dating of lava flows (Mackness *et al*., 2000). The turtle fossils were found in the lower sections of the formation, suggesting that they were deposited earlier in the history of the formation. For this calibration in our analysis we used a lognormal distribution with an offset of 3.6 Mya to set the minimum age, with a mean of 1 and an SD of 0.5 on the node between the sister species *E. irwini* and *E. lavarackorum*. Three separate analyses were conducted using both calibration points in the same analysis, plus one analysis with each calibration used individually to evaluate their influence on estimated dates. Analyses were also conducted excluding sequence data to check that posterior distributions were not heavily driven solely by our priors rather than the sequence data.

BEAST analyses were run for 50 million generations, with parameters logged every 10 000 generations. Multiple runs were conducted to check for stationarity and to ensure that independent runs were converging on a similar result. The log and tree files from four runs were combined in LOGCOMBINER, version 2.0.2 (Bouckaert *et al*., 2013), with a 10% burn-in. Individual and combined log files were

examined in TRACER, version 1.5 (Rambaut & Drummond, 2007), whereas the combined tree file was summarized using TREEANNOTATOR, version 1.7.5 (Bouckaert *et al*., 2013) (version 2.0.2 was providing false values) with the mean values placed on the maximum clade credibility tree.

Specimens examined (Fig. 1)

Data are the species, drainage (drainage number of Fig. 1), latitude and longitude, and specimen number(s) (Wildlife Tissue Collection, University of Canberra, UC<Aus> in GenBank).

Papua New Guinea: *Elseya novaeguineae*, Kikori River [7] (7.3056S 144.1684E) AA036613/15/17, (7.2326S 144.0110E) AA036607, (7.0975S 143.9929E) AA036609, (7.1367S 144.3653E) AA036130/33; Morehead River [2] (8.4450S 141.7940E) AA042861/62; *Elseya branderhorsti*, Fly River [3] (8.294S, 141.91E) AA042986; Merauke River [35] (7.5104S 140.8609E) AA042067; Morehead River [4] (8.93S, 141.561E) AA042628; Bensbach River [5] (8.618S, 141.135E) AA42682. West Papua, Indonesia: Aer Besar River [12] (2.9316S 132.3340E) AA042047/97; Aika River [29] (4.7801S 136.8457E) AA042026/63; Bian River [34] (7.3289S 140.6641E) AA042256/80; Bira River [11] (2.1246S 132.1657E) AA042044/049/148; Kaimana Peninsula [18] (3.6606S 133.7613E) AA042122; Klamaloe River(?) [9] (0.8711S 131.2535E) AA042037/69; Kuri River [16, 17] (2.9806S 134.0313E) AA042132/50 (2.5323S 133.9655E) AA042077/88; Lorenz River [31] (4.0949S 138.9471E) AA042247; Mamberamo River [21] (2.1448S 137.8375E) AA042039/125; Memika River [30] (4.6184S 136.4716E) AA042133/58; Merauke River [35] (7.5104S 140.8609E) AA042035/111; Misool Island [6] (1.8304S 129.8235E) AA042081/94; Mumi River [14] (1.6144S 134.0654E) AA042131/147/186/194/255; Muturi River [15] (2.0682S 133.7212E) AA042027/038/050/143/213/217; Pauwasi River [24] (3.5522S 140.5706E) AA042195/234; Ransiki River [13] (1.5065S 134.1669E) AA042058; Salawati Island [7] (1.0132S 131.0774E) AA042141; Sanoringga River [20] (2.5019S 136.5568E) AA042029/34; Sepik River [25] (4.2967S 140.9572E) AA042123/91; Tami River [22, 23] (2.9105S 140.7678E) AA042024/210, (2.6939S 140.9798E) AA042053/151/172/236/283, (2.6777S 140.9835E) AA042041, (2.6330S 141.1410E) AA042028/32; Tunguwatu River, Aru Island [27] (5.7689S 134.4163E) AA042030/046/114/204/257; Urumbuwe River [32] (5.1683S 138.6343E) AA042100/142/154/229; Uta River [28] (4.5351S 135.9938E) AA042033/40; Waigeo Island [8] (0.3335S 131.1698E) AA042083; Wanggar River [19] (3.4636S 135.3174E) AA042055/59; Waromge River [10] (1.5031S 132.1681E) AA042157/84; Yalingi River [26] (3.2056S 142.1935E) AA042057. Voucher numbers are for the Wildlife Tissue Collection at the University of Canberra (http://iae.canberra.edu.au/cgi-bin/locations.cgi); photo vouchers are available on request.

RESULTS

For the reduced gene set, we identified 34 haplotypes from the 82 specimens of *E. novaeguineae* for which we had sequence data for control region, ND4, and associated tRNAs. Of the 1038 bp of combined sequence, 848 positions were invariant, and 22 were parsimony uninformative, leaving 168 parsimony informative characters (increasing to 190 when outgroup *E. branderhorsti* is included). Indels accounted for 11 positions that were excluded from the phylogenetic analysis of sequence data. Some were, however, parsimony informative. A single nucleotide indel in control region united the haplotypes from the Kikori drainage of the Gulf Province of PNG. A single nucleotide indel in the control region, a second indel of 3 bp in control region, and a single nucleotide indel in $tRNA^{Ser}$ were concordant as a synapomorphy uniting the northern populations of Mamberamo [21], Sepik [25], Tami [22,23], Sanoringga [20], and Wanggar [19] (Fig. 1).

The MP analysis of the reduced gene set yielded 57 equally shortest trees (378 character state changes) and the strict consensus tree is shown in Figure 2. There are three distinct and well supported clades: one comprising haplotypes from the Birds Head and associated islands (hereafter the Birds Head Clade), one comprising haplotypes from north of the Central Ranges (hereafter the Northern Clade), and one comprising haplotypes from south of the Central Ranges, including the island of Aru (hereafter the Southern Clade) (Fig. 1). All three clades received 100% bootstrap support. Within these clades, there was strong support for all structure within the Birds Head Clade, and for a distinct Kikori clade within the Southern Clade (Fig. 1). Differences between the 57 trees arose from rearrangements of closely-related haplotypes within the Northern and Southern Clades. The topology of the ML tree (single tree, –log likelihood 3405.62) did not differ in any important respects from that of the MP tree (Fig. 2).

Addition of further sequence data from 12 s, 16 s, CO1, and cyt *b* for the full gene set (total of 2572 characters, 2210 of which were constant and 57 parsimony uninformative and 305 informative characters) did not alter the topology and marginally increased bootstrap support for the node uniting the Northern and Southern Clade to the exclusion of the Birds Head Clade (Fig. 2). It rose to 86% for the MP analysis and 83% in the ML analysis compared to the respective values of 79% and 78% for the full and

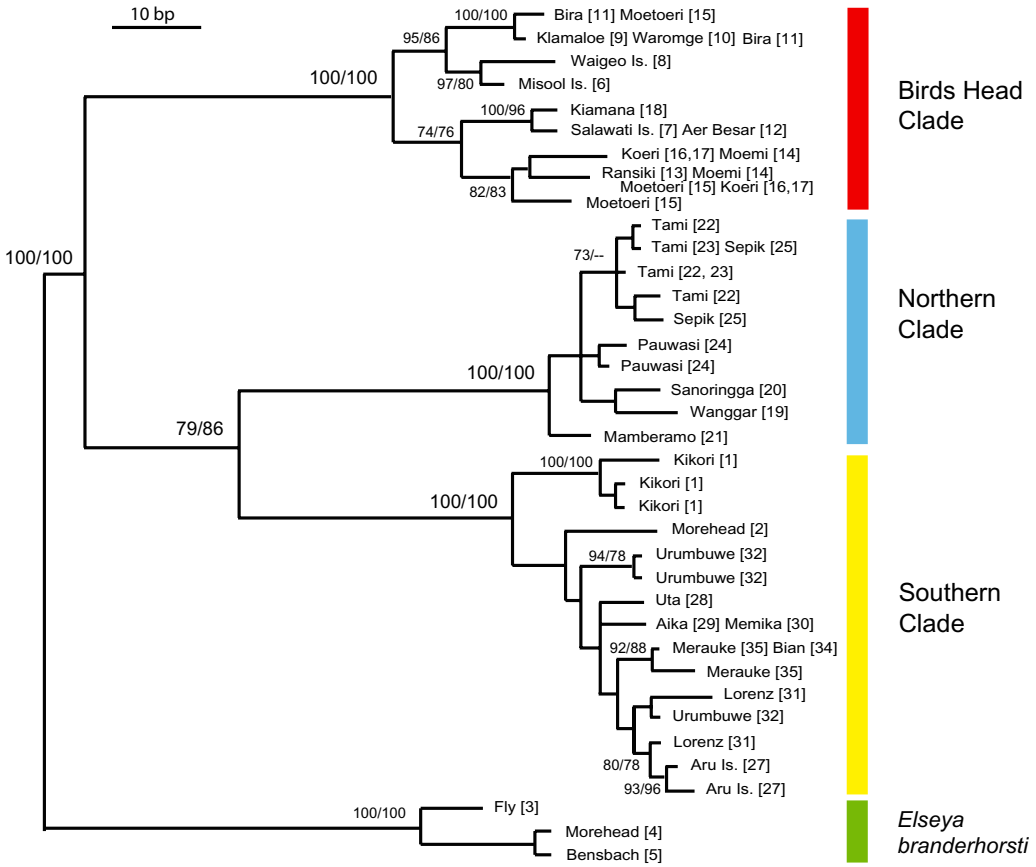

Figure 2. Maximum parsimony (MP) phylogeny for the mitochondrial haplotypes of the New Guinea turtle *Elseya novaeguineae* from the full gene set. Terminal names are those of drainage basins; the reference numbers refer to locations shown in Fig. 1 in square brackets. Colours for the three major clades are Birds Head in red, northern clade in blue and southern clade in yellow with the outgroup (*Elseya branderhorsti*) in green. Bootstrap values (> 70%) for the major clades are drawn from analysis of the full gene set, with the MP values followed by the maximum likellihood (ML) values. Bootstrap values for minor clades are drawn from analysis of the reduced gene set. The topology of the ML tree did not differ in any substantial way from the MP tree.

reduced gene sets respectively. Thus, the best supported topology has the Birds Head Clade as basal to the Northern and Southern Clades with significant, although there is less than 100% bootstrap support. There were no informative indels in the additional sequences of the full gene set.

Rates of sequence divergence for cyt *b* and ND4 (Table 1) did not differ between the three *E. novaeguineae* clades measured against the outgroup taxon *E. branderhorsti* (Birds Head versus Southern: $Z = 0.10$, $P = 0.92$; Birds Head versus Northern: $Z = 0.97$, $P = 0.32$; Northern versus Southern: $Z = 1.18$, $P = 0.24$, PHYLOTEST, version 2; Kumar, 1996), suggesting that the rate of sequence evolution is constant across these clades.

Dates of divergence using the two calibration constraints singly and in combination in BEAST for *E. novaeguineae* are presented in Table 2 and Figure 3 (see also Supporting information, Fig. S1).

Table 1. Mean among and within clade *p*-distances for *Elseya novaeguineae* from the Birds Head (BH), north and south of the New Guinea Central Ranges for coding ND4 and Cyt*b* mitochondrial DNA genes (from the full gene set)

	Elbran	BH	North	South
Elbran	1.2%			
Vogel	7.7%	1.2%		
North	7.0%	6.7%	0.5%	
South	7.8%	7.3%	6.2%	0.3%

Elseya branderhorsti (Elbran) is from the Transfly of Papua New Guinea. Lower matrix, percentage divergence based on uncorrected *p*-distances; diagonal, mean within-clade *p*-distances.

Table 2. Results of BEAST dating analyses using different combinations of calibrations: calibration based on fossils from both the Redbank Plains and the Bluff Downs formations, on the Redbank Plains formation only, and on the Bluff Downs formation only

Comparison	BH versus Rest (Mya)	Nth versus Sth (Mya)	mtDNA (%/Mya)	nDNA (%/Mya)
Both	18.8 (12.5–25.6)	16.5 (10.2–23.1)	0.40	0.34
Redbank Plains	19.8 (13.3–26.8)	17.4 (11.0–24.5)	0.38	0.32
Bluff Downs	9.3 (3.6–15.8)	8.2 (3.0–14.1)	0.86	0.74

The mean and 95% highest posterior densities are given for the specific nodes of interest: Birds Head (BH) Clade versus Northern and Southern Clades (BH versus Rest) and Northern versus Southern Clades (Nth versus Sth). The mean percentage (pairwise) per million year rate of evolution estimated for the mitochondrial (mt)DNA and nuclear (n)DNA are also given from each BEAST analysis

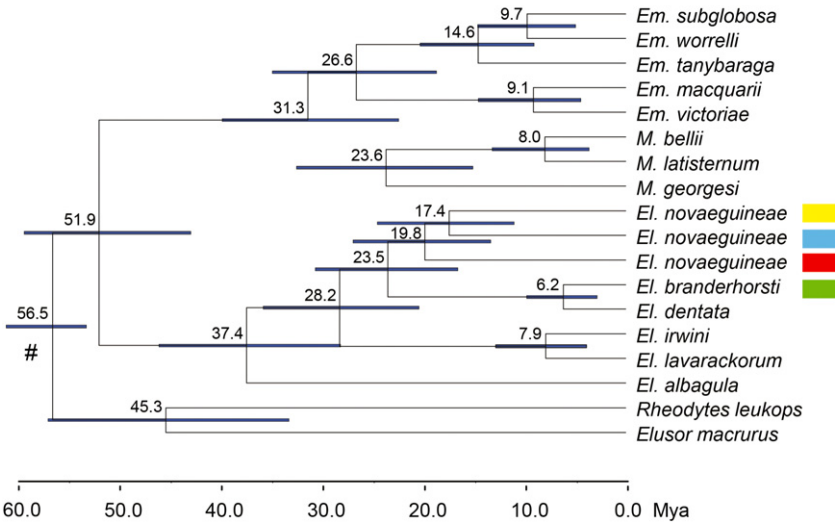

Figure 3. Bayesian molecular clock estimates for the Australian short-necked chelid radiation based on analysis of mitochondrial and nuclear DNA. The numbers by the nodes represent the mean ages in millions of years; horizontal bars represent the 95% highest posterior density ranges. The hash symbol (#) indicates the node where the fossil calibration was placed. The colour by the operational taxonomic unit (OTU) name matches the distribution of the clades in Fig. 1 and the identification of clades in Fig. 2.

Running the identical analysis (but without data) confirmed that our input settings reproduced the prior probability distributions on our calibrated nodes and that our data were responsible for our results rather than our priors. Most statistics from all three analyses had equivalent sample size scores > 3000, demonstrating the chains were well sampled. When the single calibration for the Bluff Downs fossils was used, all dates were much younger than for the Redbank Plains analysis and for the combined analysis (Table 2; see also Supporting information, Fig. S1). The results from the combined calibrations were similar to the results from Redbank Plains analysis alone (typically within 10%), except that the node defined by the Bluff Downs calibration had a mean age estimate of 4.9 Mya [95% highest posterior density (HPD) interval of 3.9–6.2 Mya] versus 7.9 Mya (95% HPD interval of 3.9–12.8 Mya) (Fig. 3). Using the single Bluff Downs calibration doubled the rates of evolution for both genes in comparison with the rate estimates involving the Redbank Plains fossil (Table 2). Mean rates of evolution were moderately low (which is consistent with turtles having an overall slower rate of evolution than many vertebrates (Shaffer *et al.*, 2013), with the Bluff Downs calibrated analysis rates being slightly more than twice the rate for those from Redbank Plains or the combination analyses (Table 2).

DISCUSSION

Mitochondrial and nuclear sequences of the populations of *E. novaeguineae* from north of the Central Ranges, south of the Central Ranges, and on the Birds Head are highly divergent, which suggests a history of isolation that extends deep in time. Dating these divergences using molecular data is challenging, particularly calibration of the molecular clock that is needed to convert the relative rates of DNA change to a temporal scale (Muller & Reisz, 2005; Joyce *et al*., 2013). Furthermore, the chelid fossil record is difficult to interpret because knowledge of osteology of extant forms is poor, and assigning fossils even to genus is problematic (Gaffney, 1979; de Broin & Molnar, 2001). We could identify only two fossil-bearing formations with sufficient certainty of identity to provide calibration constraints useful in dating. Using the Redbank Plains fossils alone yielded mean estimates for dates of divergence of the Birds Head Clade from the Northern and Southern Clades of 19.8 Mya (95% HPD interval of 13.3–26.8 Mya) and the divergence of the clades north and south of the Central Ranges at 17.4 Mya (95% HPD interval of 11.0–24.5 Mya; Fig. 3). Using the Bluff Downs fossils alone yielded somewhat younger mean estimates of 9.3 Mya (95% HPD interval of 3.6–15.8 Mya) and 8.2 Mya (95% HPD interval of 3.0–14.1 Mya), respectively, with limited overlap between their 95% HPD intervals (see Supporting information, Fig. S1).

It is clear that our Redbank Plains and Bluff Downs calibrations are in conflict because all age estimates differ by almost half when the latter calibration is used (see Supporting information, Fig. S1). When the two calibrations are used in the same analysis, the only node with different estimates to the analysis with Redbank Plains alone is the one calibrated by Bluff Downs (mean 7.9 Mya, 95% HPD interval of 3.9–12.8 Mya versus mean 4.5 Mya, 95% HPD interval of 3.8–5.3 Mya; see Supporting information, Fig. S1). We argue the Redbank Plains fossil calibration is more reliable than the Bluff Downs calibration. Placing fossils within a molecular phylogeny is greatly influenced by the nearest sister lineage to the lineage to which the fossil belongs. The Bluff Downs fossils were described as *Elseya nadibajagu*, which is the sister species to *E. irwini* (Thomson & Mackness, 1999). Because we are limited to extant species in our molecular phylogeny, we had to place the fossil calibration for *E. nadibajagu* fossils at the node for *E. irwini* and *E. lavarackorum*. We argue that this calibration is underestimating divergence times and that the Bluff Downs fossils most likely represent separation from *E. irwini* that is more recent than the separation of *E. irwini* and *E. lavarackorum*. For these reasons, we have greater confidence in the placement of our Redbank Plains fossils (Fig. 3) than those from Bluff Downs (see Supporting information, Fig. S1).

The Redbank Plains formation calibration is not without its difficulties. All fossil calibrations have uncertainty associated with them (Donoghue & Benton, 2007) arising from inaccuracy in the molecular phylogeny, in the geological dates of the formation in which the fossils are found, in identification of the fossils, which are commonly fragmentary, in their placement within the phylogeny, and arising from operational decisions to accommodate the time lag between lineage divergence and evolution of diagnostic synapomorphies in fossils for both sister lineages. Fossils do not provide a calibration event at a particular time of lineage divergence because they are more likely to reside on branches of the phylogeny than on nodes. Fossils yield instead a minimum age constraint, by placing the fossil on the appropriate node within the topology (Donoghue & Benton, 2007). Placing the fossils within our phylogeny was a principal limitation because the Redbank Plains fossils could only be assigned broadly to the Australian short-necked chelid radiation. Thus, the calibration constraint was placed deeper in the phylogeny than might have been the case had more definitive information been available on morphology to allow the fossils to be resolved to specific genera. However, if the two fossil taxa from Redbank Plains had been assigned to extant genera, the result would be to increase the age estimates for *E. novaeguineae* divergences. From this perspective, our estimates and the credible ranges associated with them are minimum age estimates. It is also possible that the Redbank Plains fossils (and other likely related fossils of similar age from the Pilbara and Proserpine; de Broin & Molnar, 2001) represent multiple genera that diverged earlier than the Australian short-necked chelid radiation we have defined, although those genera subsequently went extinct. If this were the case, our estimates would be too old. However, no Tertiary Australian chelid fossil turtles have been assigned to extinct genera (Thomson & Mackness, 1999; de Broin & Molnar, 2001).

Irrespective of which calibration is considered accurate, it is clear that *E. novaeguineae* has an old history in New Guinea. The Central Ranges of New Guinea formed as a result of the collision of the Australian craton with oceanic terranes, a process that began in the Late Oligocene with the docking of the Sepik Terrane, approximately 25 Mya (Pigram & Davies, 1987). The ranges continued to form with increasing vigour through the late Miocene, Pliocene, and Pleistocene with the docking of the East Papua composite terranes (14 Mya, latest middle Miocene),

the docking of the West Papua composite terrane, and the northern island-arc terranes of central New Guinea (10 Mya, early late Miocene) (Pigram & Davies, 1987; Pigram & Symonds, 1991). However, it is generally accepted that the telescopic uplift of the central fold belt to form the Central Ranges began in the late Miocene, 8–11 Mya, with the commencement of oblique subduction of the Australian Plate beneath the Pacific Plate. At some point in the above process, estimated by our dating to be early Miocene (mean age 19.8 Mya, 95% HPD interval of 13.3–26.8 Mya; Fig. 3), the Central Ranges became a barrier to dispersal of *E. novaeguineae* that has not been subsequently breached. Our dates suggest that this isolation occurred during the early phases of uplift, driven by the accretion of island terranes to the north, rather than the subsequent telescopic uplift associated with the oblique subduction that came later (Pigram & Davies, 1987; Pigram & Symonds, 1991). Isolation from the perspective of the turtles would have occurred early in the uplift process, when lowland river tributaries no longer interdigitated and their drainages became isolated by uplands that were modest relative to the relief of the current Central Ranges.

The unusual distribution of *E. novaeguineae* in relation to the Central Ranges is thus best explained as the species having a former distribution in the Miocene that extended into the continental region now supporting the island of New Guinea. There, its populations were isolated by the early stages of the formation of the Central Ranges to yield two distinct and highly divergent clades. Other species of chelid turtle appear to have invaded New Guinea after its orogenesis was well established, and are consequently restricted to the lowlands south of the Central Ranges. The proposition that *E. novaeguineae* was among them but, by chance, dispersed across the Central Ranges to the north of the island, is not supported by our data, neither by our dates, nor the topology of our phylogeny.

Interpretation of the divergence of the Birds Head Clade from the Northern and Southern Clades is more complex. One interpretation called the 'docking hypothesis' is that, in the early Miocene, *E. novaeguineae* came to occupy and speciated on an island terrane of continental origin that now forms part of the Birds Head, presumably after it broke away from the Australian craton in the early Cretaceous but before its current docking to mainland New Guinea (Polhemus, 2007). Presumably, *E. novaeguineae* dispersed to the Birds Head during an earlier connection, which may have been possible as the result of a persistent close relationship of the island terrane and the Australian continent (including New Guinea) (Polhemus & Polhemus, 1998). Two of the major terranes that make up the Birds Head, Kemum and Misool, are clearly continental in origin: both Australia and the Birds Head share fossil *Glossopteris* flora from the late Paleozoic–early Mesozoic (Chaloner & Creber, 1990), and the two have similar paleomagnetic polar wander paths from the late Carboniferous and Triassic (Giddings, Sunata & Pigram, 1993). Paleomagnetic data indicate that the Kemum Terrane detached from the main continental landmass in the early Cretaceous and had a history of movement independent of the Australian craton until at least the Miocene (Pigram & Davies, 1987; Giddings *et al*., 1993). During this period, the Kemum Terrane was expanded by the fusion of both continental terranes (e.g. the Misool Terrane to its western margin in the Late Oligocene) and oceanic terranes (e.g. the Tamrou Terrane to its northern edge in the late Miocene-early Pliocene) (Pigram & Davies, 1987). The composite is then assumed to have moved eastward to integrate with greater New Guinea in the late Miocene, via the Langguru Terrane, which, at that time, may have already been attached to the Australian craton (Pigram & Davies, 1987; Decker *et al*., 2009). Once docked, *E. novaeguineae* would have been able to disperse between the Birds Head and mainland New Guinea, before the collisional process described above drove the development of the Langguru Fold Belt as an effective barrier to turtle dispersal.

An alternative interpretation, called the '*in situ* hypothesis', arises because some geologists regard the evidence for an allochthonous origin for the continental terranes of Kemum and Misool as unconvincing (Dow & Sukamto, 1984; Charlton, 2000). They argue that, on the contrary, the geological evidence strongly supports a relatively local origin. Charlton (2000) argues that the present structural isolation of the Birds Head terranes from autochthonous Australia has resulted from processes acting after initial collision of a coherent Australian continent with an island arc system, rather than the pre-collisional disaggregation of the Australian margin of the allochthonous terrane models. Here, the formation of the Langguru Fold Belt arose through deformation from the counterclockwise rotation of the Birds Head, rather than a more direct collisional process.

Under the *in situ* hypothesis, *E. novaeguineae* was widespread before becoming fragmented by vicariance events associated with the development of the Central Ranges, the Langguru Fold Belt, and Cenderawasih Bay. Formation of the Langguru Fold Belt in the Birds Neck region (Bailly *et al*., 2009), coupled with the opening of Cenderawasih Bay by counterclockwise rotation of the Birds Head that began in the Early Pliocene (Charlton, 2000), would have effectively isolated the populations to the west, on Birds Head and

associated islands. It is important to note that the very narrow continental shelf surrounding the Birds Neck region would have maintained this isolation through the periods of Pleistocene sea level lows (+1 to −135 m; Clark & Mix, 2002); the deep waters of the Aru Trough and Cenderawasih Bay occur close to the current coastline on either side of the Birds Neck (Fig. 2) (Jongsma *et al*., 1989; Voris, 2000).

The *in situ* hypothesis fits less comfortably with the molecular dates than the docking hypothesis because the key geological events (creation of Cenderawasih Bay – early Pliocene, 6 Mya; formation of Langguru Fold Belt – Late Miocene, 11 Mya) (Charlton, 2000; Bailly *et al*., 2009) are much younger than our mean molecular dates of DNA divergence (19.8 Mya). Also, the topology of the three major clades of *E. novaeguineae* in the phylogeny is more directly consistent with the docking hypothesis than the *in situ* hypothesis because the Birds Head haplotypes are collectively sister to a clade comprising the Northern and Southern Clades (Fig. 3). Regardless of which hypothesis comes to prevail, the deeper divergence of the Birds Head Clade than between the Northern and Southern Clades in the present study is evidence that the mechanisms of isolation of the Birds Neck region predate the emergence of the Central Ranges as a barrier to turtle dispersal. The opening of Cenderawasih Bay is too recent (6 Mya) to have initiated the isolation of the Birds Head populations from those of the remainder of New Guinea, although its deep waters will have served to sustain the isolation through successive sea level changes.

Our data challenge aspects of the geological history of the relationship between the drifting Birds Head terrane relative to mainland New Guinea because our fossil calibrated molecular clock results find much earlier divergences than predicted. This incongruence with the geological history is best evaluated by comparison with other biogeographical and phylogeographical studies across the region. Unfortunately, the taxonomy and biogeography of many groups is poorly known within New Guinea. Mitochondrial sequence variation among the passerine Little Shrike-Thrush *Collurincincia megarhyncha*, common and widespread in New Guinea, also showed remarkable divergence among lineages, comparable to that observed among different species or even genera of birds (5–11%). These divergences were considered to be concordant with the estimated time of formation of topographical barriers (Deiner *et al*., 2011). A pattern of high genetic divergence north and south of the Central Ranges has been demonstrated for a range of taxa (LeCroy & Diamond, 1995; Polhemus & Polhemus, 1998; McGuigan *et al*., 2000; Dumbacher & Fleischer, 2001; Rawlings & Donnellan, 2003; Zwiers, Borgia & Fleischer, 2008; Unmack, Allen & Johnson, 2013), which suggests isolation via central montane orogenesis. Other species do not respect the Central Ranges as a barrier and, instead, show an east–west pattern of genetic structure (Joseph *et al*., 2001; Murphy, Double & Legge, 2007); for some, this may reflect ancient vicariance origins on emergent terranes (Heads, 2002). Several studies highlight the significance of the biota of the Birds Head. Within birds, differences between Birds Head and the remainder of New Guinea are referred to as the 'zoogeographers gap' (Hartert *et al*., 1936), with many species or subspecies of birds having concordant disjunctions between Birds Head and northern and southern regions (LeCroy & Diamond, 1995). In aquatic organisms, Birds Head is noted as a distinct biogeographical region for fishes (Allen, 1991: 268). Aquatic Heteroptera of the Birds Head show strong local endemism at the species level, and the Birds Head shares almost all genera with greater New Guinea, indicating close proximity of these two regions since at least the beginning of the Tertiary (Polhemus & Polhemus, 1998). Unfortunately, few phylogenetic results exist for widespread taxa in New Guinea that are comparable to the distribution of *E. novaeguineae*. One study examined phylogenetic and morphological patterns in New Guinea logrunners (Joseph *et al*., 2001). They found a deep divergence (7.2% sequence divergence) between Birds Head and species from remaining New Guinea.

The strongest evidence for congruence in phylogenetic and molecular clock estimates with *E. novaeguineae* comes from rainbowfishes (McGuigan *et al*., 2000; Unmack *et al*., 2013). Rainbowfishes yielded remarkably similar dates of divergence for three clades concordant with those of *E. novaeguineae*. The majority of species in the family (approximately 77) are in the genera (*Melanotaenia*, *Chilatherina*, and *Glossolepis*), distributed over most lowland regions of New Guinea. Unmack *et al*. (2013) found three major clades across New Guinea, with Birds Head comprising the first branching lineage, followed by a northern and a southern clade. Molecular clock estimates were based on a standard rate of molecular evolution. Separation of Birds Head and mainland New Guinea was estimated to have a mean age of 32.7 Mya (95% HPD interval of 28.4–37.3 Mya), whereas separation north and south of the Central Ranges was estimated with a mean age of 27.0 Mya (95% HPD interval of 23.8–30.8 Mya) (Unmack *et al*., 2013). Although these age estimates are older than for *E. novaeguineae*, the use of a standard rate is only an approximate estimation. Similar to the results for *E. novaeguineae*, the rainbowfish ages also challenge aspects of the geological interpretations for New Guinea. Only examination of additional groups can shed further light on the generality of these results.

Mitochondrial variation within each of the three major clades of *E. novaeguineae* shows some structure, which can be interpreted in the context of different geomorphic conditions that influence connectivity between rivers occupied by the three clades. Low sea levels during glacial maxima will have increased connectivity between drainages in the southern lowland owing to river coalescence on the exposed continental shelf (Fig. 1). By contrast, the northern region and most of the Birds Head changes little because the continental shelf is very narrow (Fig. 1). The topography of the three regions differs considerably too because much of the lowlands in the southern region has relatively little topographic relief between drainages that would facilitate turtle movements between rivers (Fig. 1). Northern New Guinea is more topographically complex, although there are three major rivers (Ramu, Sepik, Mamberamo rivers) with vast east–west extents that extend, and thus facilitate, turtle movement across most of northern New Guinea. By contrast, the Birds Head lacks larger river basins and many drainages are isolated by rugged topography, although some extensive flood plain regions exist in the south (Fig. 1). These geomorphic settings predict lower genetic divergences across southern New Guinea, moderate divergences in northern New Guinea, and highest divergences in the Birds Head.

Based on haplotype divergences, our results are broadly consistent with predicted patterns. Haplotype divergences average only 0.3% in the south, 0.5% in the north and 1.2% in the Birds Head (Table 1). Quite a number of drainages in all three regions had evidence of contemporary interconnections provided by the shared haplotypes, which is partially indicative of the mobility of *E. novaeguineae* across lowland terrestrial environments. In the southern region, shared haplotypes were found between the Bian [34] and Merauke [35] rivers, and along the Timika Coast (Uta [28], Aika [29], and Memika [30] rivers). Similar haplotype exchange has occurred between the Tami [23] and Sepik [25] rivers in the northern lowlands and in the eastern Vogelkop Peninsula, between the Ransiki [13], Mumi [14], Muturi [15], and Kuri [16, 17] rivers (facilitated by the wetlands of the Binituri basin); between the Klamaloe [9], Waromge [10], and Bira [11] rivers; and between the Bira [11] and Muturi [15] rivers of the southern Vogelkop Peninsula.

The main divergence within the Southern Clade is the group of haplotypes in the Kikori Delta [1] (Fig. 2), presumably isolated from the drainages to the west by the southern projection of the Darai Plateau and associated uplands, as well as being separated from other populations by a greater geographical distance (Fig. 1). In both the southern and northern regions, most drainages show some structure between them, although, in the Southern Clade, divergences are slightly lower and Lorentz [31] and Urumbuwe [32] are paraphyletic, whereas most drainages in the Northern Clade are reciprocally monophyletic (except Tami [22, 23] and Sepik [24]) (Fig. 2). Results for the Southern Clade suggest that the past few glacial cycles of low sea levels (which exposed a large area of continental shelf; Fig. 1) have not led to extensive sharing of turtle haplotypes; thus, either the rivers that we sampled remained somewhat isolated or the turtles avoided the exposed continental shelf (they are yet to be recorded from Australia which was connected via the exposed continental shelf). In the Birds Head region, there was a greater number of shared haplotypes between rivers but much deeper divergences within the clade (Fig. 2). The deepest split within Birds Head Clade (with 100% bootstrap support) separates most drainages of the southern lowlands of Vogelkop Peninsula (drainages [9], [10], [11], and [15] of Fig. 1) from those to the east across the Binaturi Gulf in the Kiamana Peninsula [12], from the north-eastern and eastern portions of Birds Head [13, 14, 16–18], and a geographically eclectic population from Salawati Island [7], which is otherwise geographically nested within the clade on eastern Vogelkop Peninsula. Another unexpected result was the lack of deeper divergence for the population from Waigeo Island [8] because the channel between Waigeo Island and Vogelkop Peninsula is too deep to have been exposed by sea level change. Overall, the stronger mtDNA structuring within the Birds Head Clade has most likely resulted from the more complex landscape of the region compared with the north and south of the remainder of New Guinea.

The taxonomy of *E. novaeguineae* is clearly in need of revision given the deep divergences found between the three clades (Fig. 2). The three major clades of *E. novaeguineae* have long independent evolutionary trajectories. Rhodin & Genorupa (2000) regard *E. novaeguineae* as restricted to the north of the Central Ranges and to the Birds Head, from the Popondetta region of north-eastern PNG to the Vogelkopf Peninsula in the west. They admit the possibility that *Elseya schultzei* from the Tami River near Jayapura might represent a distinct taxon, as might the north-western Vogelkop populations from around Sarong, the Sepik and eastern PNG, and the isolated population on Waigeo Island. They regard the populations to the south of the Central Range as a distinct but undescribed species (they refer to it as *Elseya* sp. 1), distinguished from those of the north and Birds Head by the combination of a striking red plastron in juveniles and subadults, and a generally rounder carapace: the northern forms have a yellow plastron at all ages and a more oval shell. This

southern species is distributed from the Purari drainage of PNG to the Timika region of West Papua. Rhodin & Genorupa (2000) identify another possible species (*Elseya* sp. 2) in the Berau Gulf region, distinguished by a prominently serrated and keeled shell. The Aru Islands population is also identified as warranting further taxonomic investigation.

Our data provide support for the contention of Rhodin & Genorupa (2000) that the populations to the north of the central ranges comprise a distinct species, *schultzei* (Vogt, 1911), and that the populations represented by our southern clade require further investigation as a possible undescribed third species. However, there is no strong evidence in our data to warrant further subdivision at the species level. The mtDNA of the Aru Island form falls clearly within the south clade, and the Berau Gulf form falls clearly within the Birds Head clade. The northern and southern clades show lower genetic structure, and the Birds Head clade shows somewhat more structure. However, in the absence of a rigorous morphological analysis to identify characters that can be used consistently to diagnose the taxa, and also infer reproductive isolation, we do not regard there to be more than one species represented within any of our three clades.

Conclusions

The present study is one of a number of recent molecular studies that have demonstrated a surprisingly strong signature of geological history on genetic substructuring of populations within species or among closely-related species in New Guinea. Collectively, these previous studies, together with the present study, demonstrate the value of biogeographical data in corroborating geological evidence for the historical processes that have formed the modern island of New Guinea, notwithstanding the limitations on geological dating arising from the complexity of the tectonic history of New Guinea, and the uncertainties of molecular clock dating arising from a paucity of fossils with sufficient definition to assign reliable and precise dates for calibration. Our data do not support the hypothesis of *E. novaeguineae* as a relatively recent disperser to New Guinea in the Pleistocene, as well as its subsequent chance dispersal across contemporary physiography that has served as a barrier to the other six species of chelid turtles in southern New Guinea. By contrast, the exceptionally deep divergences of the three clades of *E. novaeguineae* establish the distribution of this endemic species as one best explained by early occupation (or invasion and dispersal) and subsequent isolation by the dramatic landform changes that are part of the middle and late Miocene of the island of New Guinea. Our data favour the drifting Birds Head terrane hypothesis over the *in situ* hypothesis of an early and persistent residence by *E. novaeguineae* in the region of the Australian craton that is now the Birds Head because both the topology of the phylogeny and the estimated dates of divergence more strongly support the former than the latter hypothesis. This controversy is likely to be resolved definitively, if at all, only after we have concordance in phylogeographical patterns from comparative studies including other freshwater fauna, which support one hypothesis more strongly than the other. What we can say is that the historical driving influences on contemporary genetic structure of *E. novaeguineae* appear to have been early isolation, as subsequently enforced by a combination of: (1) the early uplift of the Central Ranges; (2) the development of the Langguru Fold Belt; (3) the opening of Cenderawasih Bay; and (4) the deep waters of the Aru Trough and Cenderawasih Bay that come close to the current coastline to maintain isolation of the Birds Head through periods of sea level minima. Deep genetic structure of the species complex reflects events and processes that occurred during Miocene, whereas structure within each clade across the New Guinea landscape relates to Pliocene and Pleistocene times.

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ARCHIVED DATA

Data deposited in the Dryad digital repository (Georges *et al.*, 2013).

SUPPORTING INFORMATION

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

Figure S1. Bayesian molecular clock estimates for the Australian short-necked chelid radiation based on analysis of mitochondrial and nuclear DNA. The numbers by the nodes represent the mean ages in Mya; horizontal bars represent the 95% highest posterior density intervals. A hash symbol (#) indicates the node where the fossil calibrations were placed.