The radiation of truncatelloidean gastropods across the South Pacific

I n a u g u r a l d i s s e r t a t i o n

zur

Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Ernst-Moritz-Arndt-Universität Greifswald

vorgelegt von Susan Zielske

geboren am 24.05.1985

in Berlin

Greifswald, 16.06.2016

Dekan: Prof. Dr. Werner Weitschies

1. Gutachter : PD Dr. Haase

2. Gutachter: Prof. Dr. Wilke

Tag der Promotion: 25.11.2016

Contents

Abbreviations

AH	aperture height
AI	Austral Islands
AW	aperture width
BWW	width of the first body whorl
COI	Cytochrom Oxidase subunit I
ITS	Internal transcribed spacer
LHI	Lord Howe Island
ml	maximum likelihood
mt	mitochondrial
MY/myr	Million years (two different abbreviations due to differing Journal stiles)
MYA/ma	Million years ago (two different abbreviations due to differing Journal stiles)
nc	nuclear
NC	New Caledonia
NZ	New Zealand
SH	shell height
SP	South Pacific
SW	shell width

Attachment

The attached CD containes a digital version of this thesis and the primary data/shell measurements underliing the species descriptions presented herin.

All genetic sequences are available from Genbank and therefore not attached.

1. Synopsis

1.1. Introduction and leading questions

The original idea behind this study was to reconstruct the radiation of truncatelloidean gastropods across the south pacific following the anatomy based idea that they dispersed in a stepping stone like pattern from New Zealand in the south-west via New Caledonia, Vanuatu and Fiji to the Austral Islands, belonging to French Polynesia in the east (Haase 2010a; **Figure 1**).

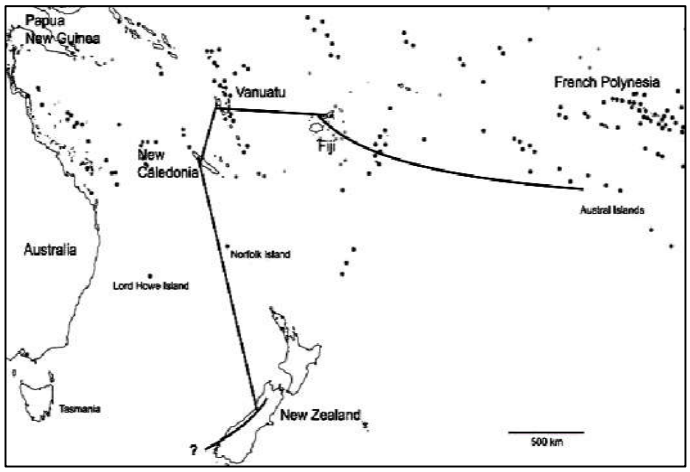

Figure 1: Anatomy based dispersal hypothesis. Figure source: Haase 2010a.

As a basis for this project, funded by the German Science Foundation (grant HA4752/2-1) two expeditions were conducted. In 2011 Vanuatu and the Austral Islands and in 2012 New Caledonia and Fiji were surveied. These expeditions were meant to complete earlier collecting efforts, especially on Vanuatu and Fiji which had not been surveyed conclusively to that point. Based on the material collected during those expeditions and a wide range of material provided by the Muséum national d`Histoire naturelle in Paris, the Australian Museum Sydney as well as the Museum of New Zealand (Te Papa Tongarewa) the first step was to focus on each island/archipelago individually.

The first study (see 3.1) focused on the archipelago Vanuatu aiming to describe new species and giving a time frame for the complex colonization history across the islands belonging to this archipelago.

The second study (3.2) focused on the islands attributed to Fiji. The aim was to describe the new species and reconstruct the colonization history.

The third study (3.3) addressed the main island of New Caledonia. It focused on a dated phylogeny trying to solve the question if New Caledonia is a Darwinian island or Gondwanan relict. Furthermore this study was meant to solve taxonomic ambiguities considering molecular data.

The fourth study (3.4) joined the results of the three previous studies examining them in the context of the whole family Tateidae including species from the Austral Islands, New

Zealand, Australia and even Indonesia. In this study the dispersal pathways of tateid gastropods across the South Pacific were to be reconstructed.

In the following paragraphs some basic knowledge about the South Pacific itself, the studied organisms and molecular genetics methods will be illustrated before the most important findings are summarized in more detail.

1.2. The South-Pacific

The South Pacific defines the whole area between South America in the east and Australia/Southeast Asia in the west, south of the equator (**Figure 2**). It covers more than 40 M km^2 of which less than 1 % is above sea level. The major islands and archipelagos are from west to east: Solomon Islands, Bismarck Archipelago, New Caledonia, Vanuatu, New Zealand, Fiji, Tonga, Samoa, Cook Islands as well as the islands and archipelagos attributed to French Polynesia. All these islands and archipelagos are of oceanic origin except for New Caledonia and New Zealand, which are continental fragments.
However as both have probably experienced long phases of submergence until the late Eocene and late Oligocene respectively they may also be considered as oceanic (Waters & Craw 2006, Murienne 2009, Carr *et al*. 2015).

New Caledonia[1] representing the third largest island in the South Pacific is one of the worlds biodiversity hotspots (Myers *et al*. 2000). It has a high degree of endemism, which holds especially for the flora with more than 74 % endemics but also for reptiles (> 80 %) and invertebrates (Chazeau 1993; Jaffré & Veillon 1994). The unique geological history of the island with a long history of isolation, a very heterogeneous topography and geochemistry (Grandcolas *et al*. 2008; Murienne 2009) results in most of the endemics being so called narrow range endemics occurring only in very restricted areas on the island (Grandcolas *et al.* 2008; Wulf *et al*. 2013).

[1] This paragraph is in large parts taken from the manuscript focussing on New Caledonia: Molecular phylogeny and a modified approach of character-based barcoding refining the taxonomy of New Caledonian freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae). Zielske S, Haase M (2015) Molecular Phylogenetics and Evolution 89: 171-181.

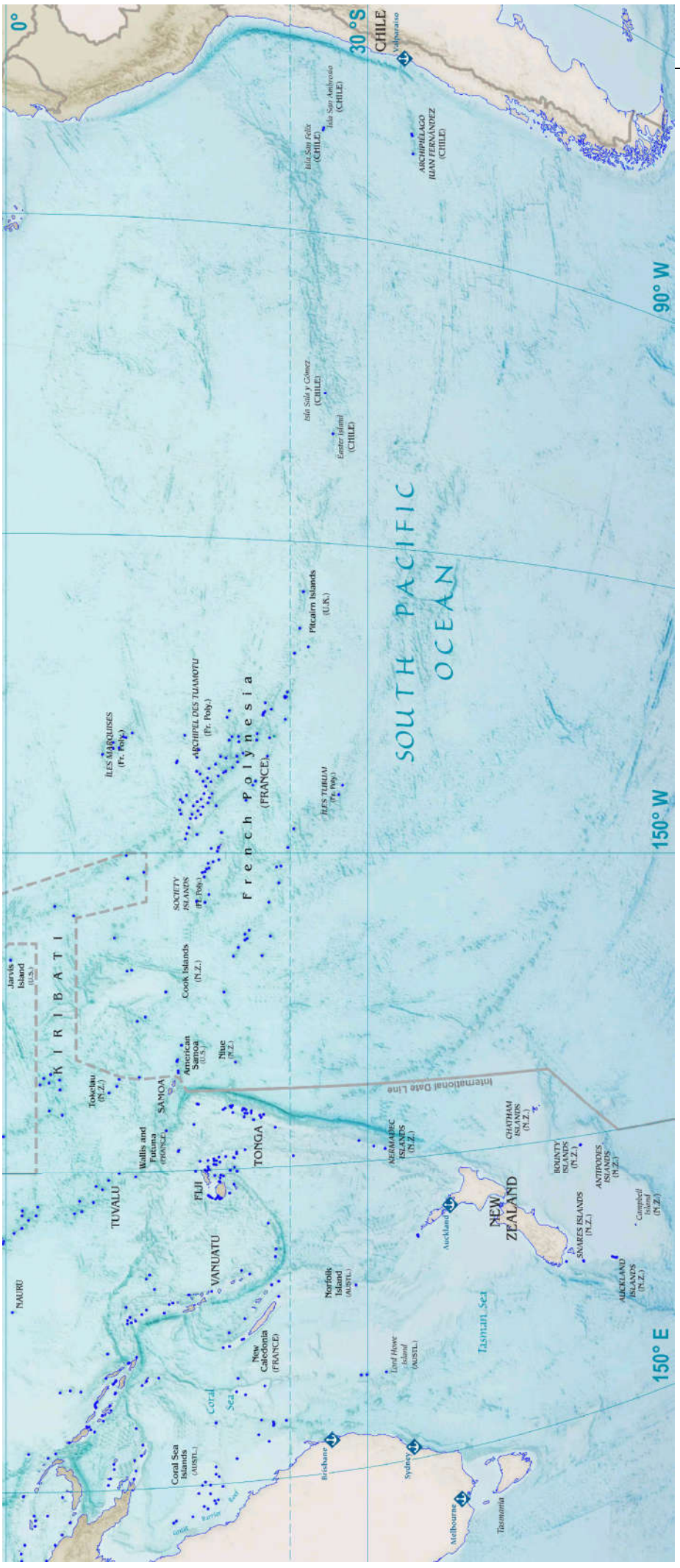

Figure 2: the South Pacific Region, Mercator projeition scale 1:36.000.000 (edited from the Map of the World Oceans, CIA world fact book).

The main island of New Caledonia (Grande Terre) covers more than 16.000 km^2 comprising more than 85 % of the whole landmass of the archipelago. Furthermore the Loyalty Islands in the east, the Isle of Pines in the south and the Belep archipelago in the north are attributed to New Caledonia. Grande Terre is a is a fragment of the continental crust of Zealandia and began to separate about 65 MYA (Neall & Trewick 2008; Schellart *et al*. 2006). The current position of the main island was reached about 50 MYA in Eocene but further severe geological changes occurred in the context of the collision with the Loyality arc including the main phase of uplift of the main island in Oligocene (Grandcolas *et al*. 2008; Neall & Trewick 2008). Subsequent quaternary uplift in the context of the subduction of the Vanuatu trench resulted in the emergence of the Loyality islands (Neall & Trewick 2008) which are of volcanic origin. Grande Terre is divided in a humid east coast and a drier west cost by a central chain of mountains.

Most recently published biological studies support the theory that the main island of New Caledonia was entirely submerged between 65 to 45 MYA and re-colonized in a stepping stone like fashion out of Southeast Asia or directly from Australia (Cruaud *et al*. 2012; Espeland & Murienne 2011; Swenson *et al*. 2014). Consequently the main island of New Caledonia seems to be rather a Darwinian island than a Gondwanan refuge as originally thought (Holloway 1979; Lowry 1998; Morat *et al.* 1986). However, the potential existence of ephemeral islands acting as refuge-habitats in the region during times of submergence, supported by the existence of relictual groups (Heads 2008; Ladiges & Cantrill 2007), has not been ruled out with certainty (Grandcolas *et al*. 2008; Murienne 2009).

The Archipelago of Vanuatu[2] is situated about 2000 km east of Australia and 400 km northeast of the main island of New Caledonia in the Pacific Ocean. It consists of more than 80 islands spread over more than 900 km. According to Simeoni (2009; see also references cited therein) the Archipelago can be divided into three parts being the result of different volcanic episodes: The western belt comprising the largest Islands Malekula and Santo as well as the Torres Islands is the presumably oldest part with active volcanism from the Oligocene to the middle Miocene. The formation of the eastern belt comprising Pentecost and Maewo dates to volcanism from the end of the Miocene to the early Pliocene. The Central Chain comprising

[2] This paragraph is in large parts taken from the manuscript focussing on Vanuatu: Zielske S., Haase M. (2014) When snails inform about geology: Pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda). *Journal of Zoological Systematics and Evolutionary Research* **215**: 217-236.

all islands between Aneityum and the Banks Islands is the presumably youngest part with continuous volcanism from the late Pliocene to today.

However, our knowledge about the final emergence of the different islands is in parts ambiguous due to the complex history of formation and many stages of submergence and re-emergence. The Torres Islands are entirely covered by Quaternary coral formations indicating a very young subaerial history (Taylor *et al*. 1985). Large parts of Malekula are covered by Pliocene (southern part) to Pleistocene (northern part) coral limestone. A window of Miocene rocks in the north, has been interpreted as paleoisland, which, however, probably also drowned and re-emerged only in the Pleistocene (Taylor 1992). Total submergence since the early Pliocene is assumed for Santo. But at least parts have probably remained above sea level since uplift in the latest Pliocene. However, Santo has also wide areas covered by Quaternary coral reef limestone. This indicates that, similar to Malekula, there are younger and older parts regarding the time since emergence (Robin *et al.* 1993). The eastern belt islands are practically entirely covered by Pliocene to Pleistocene coral reef limestone suggesting a late Quaternary uplift (Taylor 1992). Most islands of the central chain including Aneityum, Efate and Gaua owe their emergence to Pleistocene volcanic activity (Robin *et al.* 1993; Greene *et al.* 1994). In contrast, the western part of Erromango seems to be continuously emergent since 2.6 to 2.3 MY except for the Mount Rantop pensinula (Neef & Hendy 1988). The earliest volcanism of Erromango dates back to the late Miocene, though. The island seems to have surfaced and drowned repeatedly since then. However, interpretations if volcanism was submarine or subaerial differ among authors (cf. Robin *et al.* 1993; Greene *et al.* 1994).

The flora and fauna of Vanuatu have predominantly western affinities with a comparably low degree of endemism. So far most studies focused on plant species and despite the spatial proximity of Vanuatu and New Caledonia, these studies showed relationships to species from Fiji, the Solomon Islands, Malaysia or Australia. However, the archipelago is only poorly studied with some islands never been explored so far. (Munzinger 2009)

The archipelago Fiji[3] is located amidst the islands of Tonga, Samoa, Vanuatu, Solomon Islands, New Caledonia and New Zealand. The whole archipelago consists of over 300 islands covering nearly 18300 km^2 of land (Neall & Trewick 2008) of which nearly 90 % are covered

[3] This paragraph is in large parts taken from the manuscript focussing on Fiji: Zielske S., Haase M. (2014) New insights into tateid gastropods and their radiation on Fiji based on anatomical and molecular methods (Caenogastropoda: Truncatelloidea). *Zoological Journal of the Linnean Society* **172**: 71-102.

by the two main islands Viti Levu and Vanua Levu. The formation of Fiji dates back to Late Eocene island arc volcanism (Neall & Trewick 2008) caused by the westward subduction of the Pacific beneath the Australian plate. The first significant landmass, the south-west and south of Viti Levu, was uplifted in the Middle to Late Miocene and established by approximately 7 MYA (Neall & Trewick 2008; Rodda 1994). The center of Viti Levu emerged in the early and/or late Pliocene (Rodda 1994). At about the same time, Vanua Levu was formed (Neall & Trewick 2008). However the island emerged only between 4 and 3 MYA with the east and south-eastern peninsula having an even younger subaerial history of at most 2 MY or considerably less (Rodda 1994).
The vast majority of Fijis flora is of Indo-Malesian origin. A smaller percentage has Australien, New Zealand, New Caledonian or French Polynesian affinities. Comprehensive data does not exist for anaimals as terrestrial ecosystems are widely understudied but several analysis point in the same direction as described for the flora (Ryan 2009).

The Austral Islands are the southernmost archipelago of French Polynesia. They extend over 1200 km from the island of Rimatara in the west to the McDonald Seamount volcano in the east and are formed by three volcanic hotspot chains with the oldest island being emergent since max 12.1 MY. The geological history is associated with the Cook-Islands in the northwest and rather complex with overlapping volcanism, multiplicity of hot spot tracks and movement of the pacific plate (Dickinson 1998; Bonneville 2009).
The species diversity of the Austral Islands and French Polynesia in general is characterized by the geographic isolation and habitat complexity of the islands, causing a comparably low species number with a high degree of endemism at the same time. However, French Polynesia is one of the worlds key biodiversity areas. Species affinities are mostly Indo- and Austro-Malesian with few New Zealand and American taxa. Also taxa otherwise only found in Hawaii or Fiji are described (Meyer 2009).

As shown for the different archipelagos and islands the Flora and Fauna in the South Pacific is mainly of Malesian / Southeast Asian and Australian origin. Also the continental islands of New Zealand and New Caledonia are described as sources for dispersal (see Figure 3). The American continent is rather known as a source for the colonization of the Hawaian Islands in the North Pacific (Keppel *et al.* 2009). In general as a result of isolation and area effects species density decreases from west to east (Keppel *et al.* 2009). But due to the complex geology of most islands, the current flora and fauna evolved conditioned by several unique or

multiple colonization events followed up by in situ speciation and/or adaptive radiation events (e.g. Muellner *et al*. 2008; Keppel *et al.* 2009; Murienne *et al*. 2011; Birch & Keeley 2013).

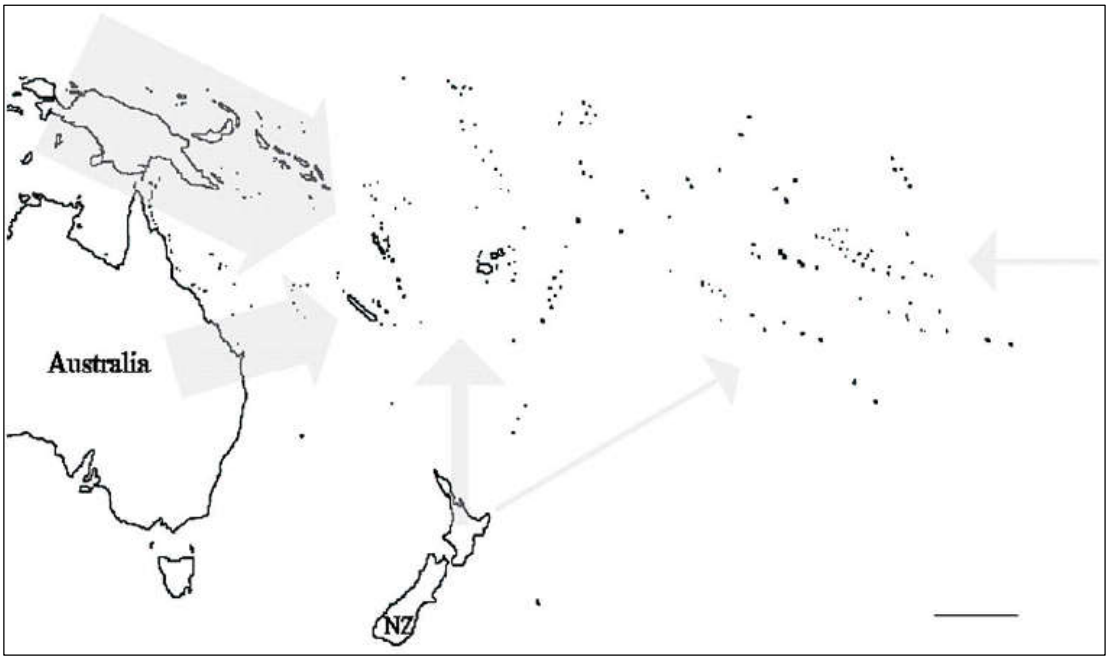

Figure 3: Main dispersal routes in the South Pacific; thickness of lines is an indicator for frequency of use. Scale bar: 500 km

1.3. Truncatelloidean gastropods

The superfamily Truncatelloidea in the order of Littorinomorpha belongs to the largest clade of all living gastropods, the Caenogastropoda. Within this superfamily this study focuses on species belonging to the Family Tateidae, small and minute aquatic snails with an operculum.

Gastropods of the family Truncatelloidea may represent one of the oldest radiations across South Pacific Islands. Including this thesis they are known from 1) Lord Howe Island harbouring 15 species-group taxa in three genera (Ponder 1982); 2) New Caledonia harbouring seven genera with more than 50 species (Haase & Bouchet 1998; Zielske & Haase 2015); 3) Vanuatu harbouring 20 species all attributed to *Fluviopupa* Pilsbry 1911 (Haase *et al*. 2010a; Zielske & Haase 2014a); 4) Fiji harbouring 28 known *Fluviopupa* species (Haase *et al.* 2006; Zielske & Haase 2014b) and finally 5) the Austral Islands attributed to French Polynesia harbouring six species of the genus *Fluviopupa* (Haase *et al.* 2005).

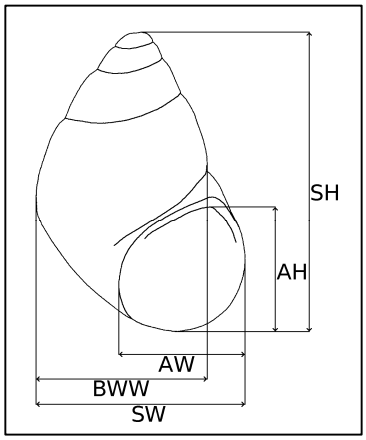

Figure 4: Shell parameters for description of new gastropod species; AH aperture height, AW aperture width, BWW width of first body whorl, SH shell height, SW shell width.

Despite the minute size of the studied taxa the following

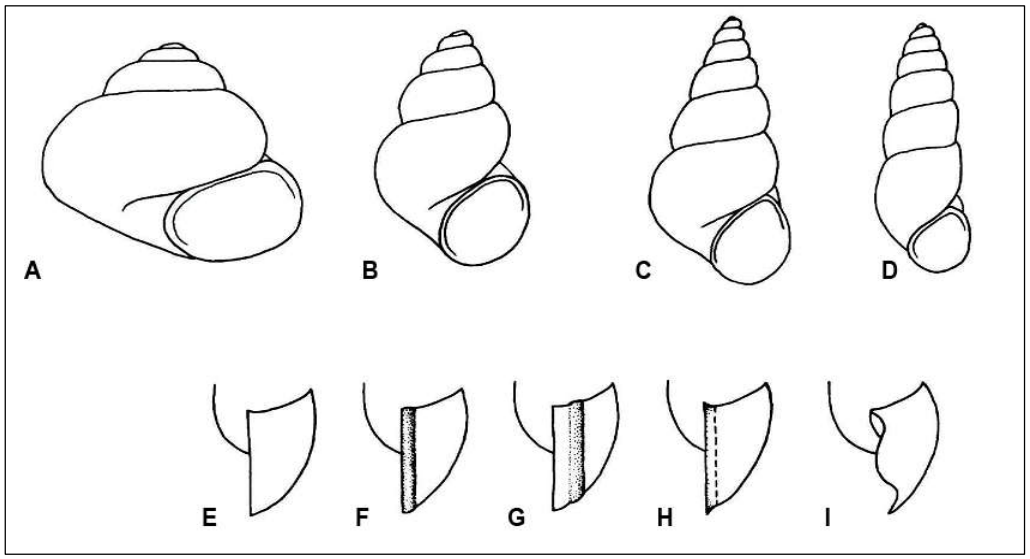

Figure 5: shell form (A-D) and condition of outer lip of shell (E-I); A trochiform, B ovate conic, C conic, D ovate turriform, E simple outer lip, F outer lip with varix, G varix behind outer lip, H fluted outer lip, I sinuate outer lip (edited from Hershler & Ponder 1998)

attributes were used to identify and characterize different species by eye. Size and overall shell-shape, including the number of whorls and number of whorls of the protoconch (the larval shell) (see Figure 4, Figure 5). Also the shape of the operculum and colour of the shell serve as good identifiers but shells are often covered with organic coating or calcifications from different Protozoas.

Regarding the snail itself the pigmentation of epidermis and eyes as well as possible ciliations at the tentacles are important features. However those may only be seen under a dissecting- or even scanning electron microscope.

Considering the mantle cavity the number of filaments of the ctenidium and possible occurrence and position of the osphradium are important features. Further details of the digestive system are studied, especially the coiling of the rectum around the prostate in males, which can be seen without dissecting the animal through the epidermis and the overall shape of the stomach (Figure 6). This includes occurrence and shape of the caecum and coiling of the intestine around the style sack. Moreover the detailed number of cusps

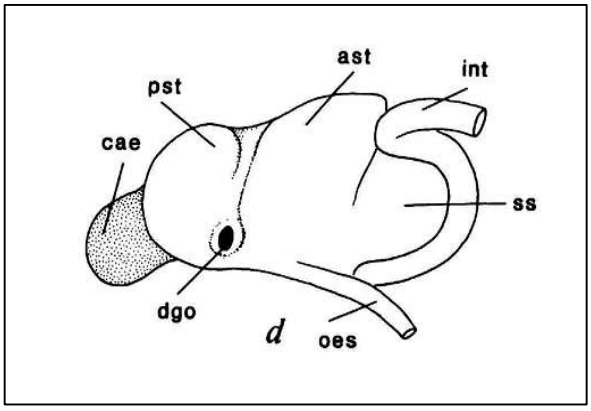

Figure 6: Stomach; ast anterior stomach chamber, cae caecum, dgo opening from stomach to digestive gland, int intestine, oes oesophagus, pst posterior stomach chamber, ss style sac. (Figure from Hershler & Ponder 1998)

and shape of each kind of teeth of the taenioglossate (seven teeth per row) radula is a descriptive feature.

Finally, also the genitalia of these dioecic animals are studied to describe new species with the focus lying on the size of oviduct glands, overall shape of the distal female genitalis (Figure 7) and penis shape in males.

A very detailed introduction to the anatomy of Truncatelloidean gastropods and their relatives traditionally declared as Hydrobioid gastropods is given in: A review of Morphological Characters of Hydrobioid Snails. Hershler & Ponder 1998.

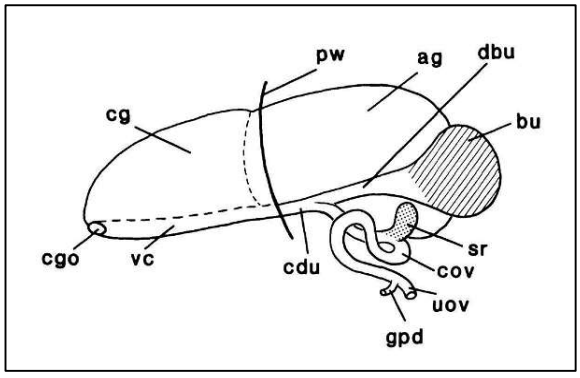

Figure 7: Distale female genitalia; ag albumen gland, bu bursa copulatrix, cdu common oviduct, cg capsule gland, cgo capsule gland opening, cov coiled oviduct, dbu bursal duct, gpd gonopericardial duct, pw pallial wall, sr seminal receptacle, uov upper oviduct, vc ventral channel of capsule gland. (Figure from Hershler & Ponder 1998)

For this thesis species descriptions were based on the measurements of 20 (or all available) schells of each species. Furthermore at least each three male and female specimens of each species were dissected to study the anatomy and to prepare opercula, radulae and cephalopods for scanning electrone microscopy.

However, despite a correct and conclusive description of the anatomy and morphology simple conclusions from similarity to relationship can not necessarily be drawn as convergent evolution is described to occur occasionally (Wilke *et al*. 2003).

1.4. Molecular Phylogenetics

Until less than 100 years ago species/genera and other taxonomic units could only be described using anatomical and morphological data or the description of metabolic processes. Similarities between different species were used to draw conclusions about taxonomic relationships. The general rule was and still is “the more similar, the closer related”. However already in non-molecular times many cases of analogous development (the same character or characterstate developed twice or even more times independently, also called convergence) were known.

In general one can divide three types of characters (Figure 8): monophyletic means that all descendants have the same character state as their common ancestor; paraphyletic means not all descendants have the same character state as their common ancestor; polyphyletic means

the character state developed analogous / there is no common ancestor showing the same character state.

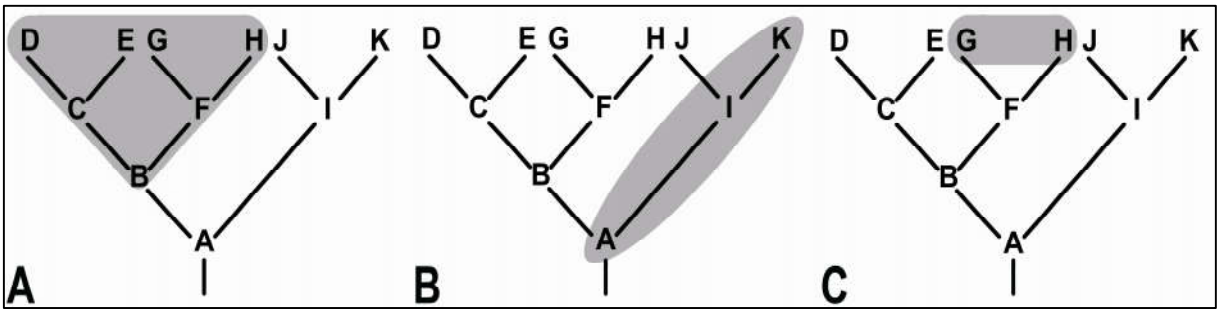

Figure 8: comparison of A) monophyletic; B) paraphyletic; C) polyphyletic groups.

Additionally, the following terms are used to describe phylogenetic trees (information taken from Knoop & Müller 2006 pp. 52-57):

- Terminal taxon: Taka representing the leafs of the tree (D;E;G;H;J;K in Figure 8).
- Node: represents an ancestral taxon or character state (B;C;F;I in Figure 8)
- Branch: connection between two nodes or a node and a terminal taxon, represents the process of anagenesis between the same. Depending on the type of tree the length of the branch may be non informative (cladogram); an indicator for the number of character state changes (phylogram) or the number of character state changes in relation to time (dendrogram).
- Outgroup/Ingroup: the outgroup is a closely related sister group of the ingroup (I, J, K may be the outgroup for B-H in Figure 8)
- Root: "starting" branch indicating the most recent common ancestor (MRCA; A in Figure 8) of the ingroup and outgroup or of all surveyed taxa. Only shown in rooted trees.

With the final understanding of the genetic code in the middle of the 20th century a new kind of data, namely molecular data became available to describe and study the processes of inheritance, speciation and evolution. In this chapter a short introduction to the methods of molecular phylogenetics will be given.

Molecular phylogenetics uses molecular characters or in other words sequence data of nucleotides (with the four character states A adenin, C cytosine, G guanin, T thymin) or amino acids. Besides the study of the simple order of nucleotides in a DNA sequence further methods like fragment-length analyses or MLPA -methods are used. Furthermore, methods are being developed regularly in the fast-paced field of molecular methods.

In this thesis the nucleotide sequences of different genes were used to study phylogenetic relationships. To obtain the sequences of a specific gene, from a specific animal, the DNA has to be isolated from enzymatically dissolved pieces of tissue. Afterwards the DNA of the target gene is multiplied performing a PCR (polymerase chain reaction), before the sequencing itself is conducted. Today many different methods for the sequencing of single genes, whole exomes or even genomes are known but the standard method to sequence single genes, which was also used in this study is still the so called Sanger-sequencing method. For this method another PCR using a small amount of chain-determinating and dye- or radioactively-marked nucleotides is performed. The resulting DNA fragments are subsequently separated electrophoretically to read the sequences (Knoop & Müller, 2006 pp. 33, 34). For this study a dye based method using capillary electrophoresis was used which enabled a fast and automated sequencing.

After sequencing a specific gene or gene-fragment the sequences from all surveyed specimens are assembled in a so called alignment, in which all homologous sites of the different sequences form columns (Figure 9). Protein coding regions may usually be aligned by eye as they only show transitions (nucleodide substitution that exchanges a purine by a purine [A↔G] or a pyrimidine by a pyrimidine [C↔T]) and transversions (nucleodide substitution that exchanges a purine by a pyrimidine or vice versa [A↔T; G↔T; A↔C; G↔C]). Aligning sequences of ribosomale RNA´s or non protein-coding genes is usually more difficult as they may also show deletions (loss of nucleotides) and insertions (additional nucleotides) resulting in gaps in the alignment (for further informations about protein coding genes and RNAs reed indroductive chapters of e.g. Knoop & Müller 2006; Lemey, Salemi & Vandamme 2009). In protein coding genes those deletions or insertions of single nucleotides would change the complete amino-acid order leading to wrongly synthesized proteins (Knoop & Müller 2006 p. 67).

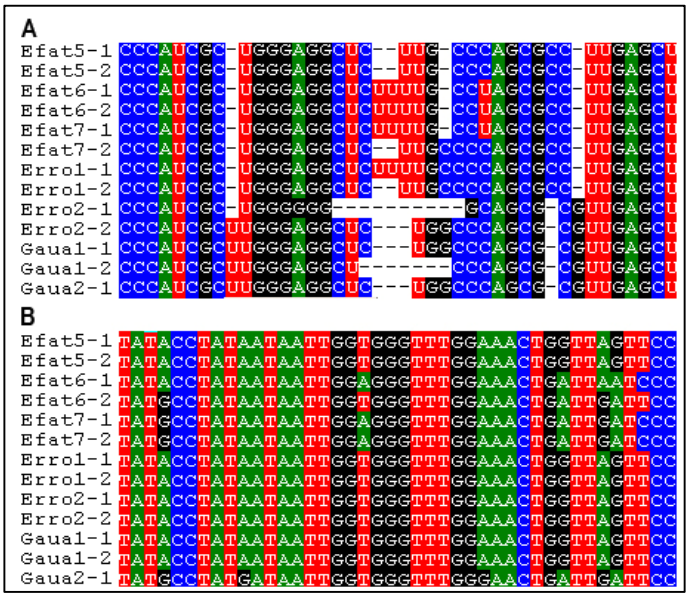

Figure 9: Alignments of A) ribosomale RNA B) protein coding DNA

There are many different software programs that can be used for evaluation in this step. Some of them

may even consider the secondary structure of the resulting protein or RNA to align sequences of the respective genes. Further, the alignments may be evaluated searching for randomly similar or ambiguously aligned sequence sections, which might than be excluded for following analyses.

For this thesis the CLUSTAL W 2.1 (Thompson *et al.* 1994) alignment method implementet in the program BIOEDIT 7.0.5.3 (Hall 1999) was used. The program RNASALSA 0.8.1 (Stocsits *et al.* 2009) served to refine the alignements using secondary structure informations and ALISCORE 2.0 (Misof & Misof 2009) to search for randomly similar positions.

After preparing the alignments, phylogenetic trees may be calculated. For this there exist numerous different programs using several different algorithms. Thus only the most basic methods and algorithms used in this study will be presented in the next paragraphs.

The maximum parsimony analysis is the simplest way to reconstruct a phylogenetic tree and it may also be used to reconstruct trees from morphological data. Using this criterion the optimal tree is the tree that requires the fewest number of character-state changes (i.e. substitutions). Albeit an exhaustive search counting all changes for each possible tree is technically hardly possible when examining an alignment of more than 20 taxa. A second approach is the use of distance methods. Those calculate a measure of distance for each pair of sequences, which reduces the amount of data to be analysed in the tree calculation, but is necessarily associated with a loss of information. The advantage of distance methods is that they are comparably fast and allow the use of substitution models (see below) (Knoop & Müller 2006 pp. 70-72). However this is to wide a field do be explained in detail. For more details see e.g. Knoop & Müller 2006; Lemey, Salemi & Vandamme 2009).

In this study the focus was set to the widely used maximum likelihood and Bayesian methods. Using maximum likelihood methods the best tree is the tree that maximises the likelihood of the given data (Schmidt & von Haeseler 2009). In contrast, Bayesian methods calculate the most probable tree for the given data using the Bayes factor (Ronquist, van der Mark & Huelsenbeck 2009). Both methods are mathematically complex, for more details please refer to the given references. In this thesis the program used to run likelihood methods was GARLI 2.0 (Zwickl, 2006) and MRBAYES 3.2.2 (Ronquist & Huelsenbeck, 2003) was used to run Bayesian reconstructions.

However both methods require the usage of so called substitution models. Those models of evolution are statistical descriptions of the stochastic process of substitution in nucleotide (or amino acid) sequences. There exist many different more or less complex models to correct the

effects of an increasing number of multiple substitutions at the same site with time, relatively high rate of transversions compared to transititons and varying substitution-rates in different alignment-positions (Strimmer & von Haeseler 2009). There are different programs and methods to identify those models but all of them aim to find the least complex model having the highest likelihood for the given sequence data (using the Bayesian criteria it would be the most probable and least complex model for the given data). Here the program PARTITION FINDER 1.1.1 (Lanfear *et al.,* 2012) was used which additionally helps to decide if stem and loop structures in rRNAs or codon positions in protein coding genes should have separate substitution models.

Bayesian methods have the advantage that they directly give a posterior probability for each node of the calculated tree. Using likelihood methods such confidence values can only be calculated using bootstrapping methods. This means that, trees are calculated for replicate datasets of the same size which are created by randomly resampling alignment columns of the original dataset. Finally the percentage of bootstrap-trees resolving a clade is given as a measure of robustness of its monophyly (de Peer 2009).

Besides the pure calculation of phylogenetic trees, further applications of molecular phylogenetic data and methods were developed in the last years. One of them is the use of the molecular clock. The basic idea behind the molecular clock is that genetic distances are correlated with time. The clock may be calibrated using geological events or the age of fossils that may be associated with specific nodes in the tree (Lemey & Posada 2009). Further the use of molecular clock rates calculated in other analyses (of the same gene of preferably closely related species) is possible to date the nodes of a tree. However as geological events and fossil calibrations are usually associated with wide confidence intervals, as many different calibrators as possible are used at the same time. Current programs working with the molecular clock can consider many different calibrators, they allow for varying rates among different parts of the tree, they can work with given phylogenies or calculate trees from alignments on their own, like the program BEAST 1.7.5 (Drummond *et al.,* 2012) used in this thesis. It is important to note that the programs are getting more and more complex and demand great skills of the user to set the different program settings, often without being aware of the detailed algorithms behind those programs.

A further use of phylogenetic trees is the reconstruction of ancestral states. Using the known character states of the terminal taxa of a phylogram or dendrogram the character states of the common ancestors (nodes in the tree) are calculated. The surveyed characters may be

molecular, morphological or also geological ranges. While using programs for ancestral state reconstructions the user often has to define the likelihood for the conversion of one character state to another, which also shows one of the major issues. Thus two different programs based on different algorithms to reconstruct ancestral ranges: RASP 2.1 (Yu *et al.,* 2015) using the Bayesian Binary method and MESQUITE 2.75 (Maddison & Maddison, 2011) with implementation of the maximum likelihood approach were applied.

1.5. Synopsis of results

In the following the findings of each of the four original articles attributed to the radiation of truncatelloidean gastropods in the South Pacific will be shown in detail. The individual characteristics of each article, may it be a new methodological approach or a specific result will be summarized. Furthermore a conclusive picture will be drawn.

When snails inform about geology: Pliocene emergence of Islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda: Tateidae)

Major findings:

- 10 new species
- New Zealand origin of Vanuatu tateids
- Espiritu Santo was colonized 3 MYA and is propably the place of origin for the subsequent colonization of Erromango and later the whole archipelago
- Ambigiouty of the young radiation du to incomplete lineage sorting
- Cryptic possible stem species *Fluviopupa espiritusantoana* Haase, Fontaine & Gargominy 2010

In this study I surveyed material collected on the islands of Vanuatu complemented with material from the Museum National d`Histoire Naturelle Paris. The species record from Vanuatu was doubled with the description of 11 new species attributed to the genus *Fluviopupa*, accompanied with the species descriptions being a major part the article. The descriptions are widely based on anatomical and morphological data.
Further I reconstructed a molecular phylogeny based on sequences of the nuclear 16S rRNA, the mitochondrial cytochrome oxidase subunit I (COI) and the nuclear internal transcribed spacer 2 region (ITS2). The monophyly of the Vanuatu taxa was well supported in this analysis. However it showed that outgroup taxa from New Zealand were closer related to the

Vanuatu species than the ones from Australia which was a first hint that truncatelloidean gastropods did not follow the most common dispersal ways. Most specimens surveyed genetically formed a large hardly resolved clade (I) with only little geographical structure. Only the specimens from Erromango formed a well supported clade (II) of two reciprocally monophyletic species. The third clade (III) comprised three specimens of species attributed also to the unresolved clade I and thus being polyphyletic. The oldest lineage of a Vanuatu species was formed by one specimen of *Fluviopupa narii* Haase, Fontaine & Gargominy 2010 from Santo. The most plausible reason for the found taxonomic incongruence and poorly resolved phylogeny is incomplete lineage sorting which is typical for young and rapid radiations (Funk & Omland 2003). The paraphyly of *F. espiritusantoana* might be explained by ancient incomplete lineage sorting (Takahashi *et al*. 2001; McGuire *et al*. 2007) or cryptic species, respectively. However solving this question would require a more comprehensive study.

Using this phylogenetic tree we tested three different hypothesis of island colonization compared to the unconstrained tree: (1) every island was colonized only once, hence samples from each island formed reciprocally monophyletic groups; (2) samples from Santo and Malekula, the presumably oldest islands, are sister group to the samples of the remaining islands, of which each was colonized only once; and (3) samples from Santo were paraphyletic with respect to the samples from the other islands, of which each was colonized only once. As none of these hypothesis was at least as likely as the unconstrained tree multiple introductions to the different islands have to be assumed.

Further we calculated a dated phylogeny calibrated with the COI substitution rate estimated for New Zealand tateid gastropods. Running the molecular clock approach I focused on the age estimation for the three well defined nodes of the phylogeny using (1) the average genetic distances between the clades and (2) the Bayesian approach implemented in BEAST. The results indicated that tateids have reached Vanuatu at least as early as in the late Pliocene with Santo being the first islandreached. There they could establish at least 3 MYA which increased the time frame for the colonization of Vanuatu assumed in the literature so far (Hamilton *et al.* 2010). According to the calculated phylogeny and time frame Santo was the first island of the archipelago where Truncatelloideans could establish at least in the late Pliocene, followed by Erromango in the early Pleistocene and a final subsequent radiation on the remaining islands of the archipelago in Pleiocene and Holocene. This young age is supported by short branch length and a lack of morphological differentiation. Besides Erromango and Santo, Efate species seem to be the only ones descending from only one colonizer.

New insights into tateid gastropods and their radiation on Fiji based on anatomical and molecular methods (Caenogastropoda: Truncatelloidea)

Major findings:

- 18 new species
- New Zealand origin of Fijian tateids
- Radiation over Fiji began in southern Viti Levu, with subsequent dispersal over the western and central parts of the island and Vanua Levu

The focus of this study was on the species from the archipelago Fiji. Analogous to the first study on Vanuatu the major part of the manuscript enclosed the description of new species as the species record of the genus *Fluviopupa* from Fiji was nearly increased threefold.
Further I calculated a molecular phylogeny using sequences of the COI gene and the 16S rRNA. The Fijian taxa build a well supported monophylum which was closest related to the New Zealand outgroup taxa, which again was contrary to the most common dispersal way from Southeast Asia via the Solomon Islands or Australia and thus is rather exceptional. The Fijian taxa were genetically divided into five well supported lineages (three larger clades and two single species-lineages): 1) *Fluviopupa seasea* Haase, Ponder & Bouchet 2006*;* 2) the oldest clade (I) comprising all samples from southern Viti Levu except for *F. seasea* and 3) *Fluviopupa dromodromo* Zielske & Haase 2014, which was a sister taxon to clade II. 4) Clade II comprised all samples from central and western Viti Levu and 5) clade III all samples from Vanua Levu and eastern Viti Levu. The relationships between and within the described clades were only poorly resolved, representing three young radiations which similar to the species from Vanuatu were blurred due to incomplete lineage sorting.
Based on the calculated phylogeny an ancestral range reconstruction was performed to survey the dispersal pathways across the different islands of Fiji. It showed that the radiation on Fiji most likely started in southern Viti Levu from where the remaining parts of the island and Vanua Levu were reached. Depending on the definition of ranges (each islands representing one range or islands being subdivided in different ranges) one or two subsequent colonization events on Viti Levu were found. However the unevenly distribution of sampling sites due to inaccessibility of boondocks and anthropogenic environmental changes may have influenced the ancestral range reconstruction. Finally the picture drawn by this analyses is in congruence with the geological history of Fiji as southern Viti Levu was part of the first significant landmass emerging in the late Miocene. The emergence of the younger parts of the remaining

Viti Levu and Vanua Levu began only in Pliocene (Rodda 1994) with various tectonic events and volcanism during Pliocene and Pleistocene.

Molecular phylogeny and a modified approach of character-based barcoding refining the taxonomy of New Caledonian freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae)

Major findings:

- Two of the known genera are cryptic
- Character based diagnosis of genera using a modified DNA barcoding approach
- Hints for hybridization between *Hemistomia cockerelli* Haase & Bouchet, 1998 and *Hemistomia fabrorum* Haase & Bouchet, 1998
- The most recent common ancestor is 24.6 (±9.5) MY old suggesting New Caledonia is rather a Darwinian island, than being a fragment of Gondwana land

As truncatelloidean gastropod species from New Caledonia were already studied exhaustively using morphological and anatomical data this study focused on a molecular examination of in large parts described species. The genus *Fluviopupa* that was in the focus of the previous studies is not known from the islands of New Caledonia. A molecular phylogeny was calculated based on sequences of COI, the 16S rRNA and the ITS2.
The phylogenetic analyses analyses could not prove the monophyly of the New Caledonian taxa unambiguously. Further it remained unclear if the New Caledonian taxa are closer related to New Zealand or Australia tateid gastropods. However three clades of which at least the two larger ones were well supported monophyleticwere found: clade I representing the genus *Hemistomia* Crosse, 1872 and clade II the genus *Leiorhagium* Haase & Bouchet, 1998. The unstable clade III comprised species of the genera *Kanakyella* Haase & Bouchet, 1998 and *Hemistomia*, both being paraphyletic or even polyphyletic. In contrast to the previous studies focusing on the genus *Fluviopupa* except for three all species surveyed in this study were represented by well supported monophyletic specimen groups in the molecular phylogeny. The phylogenetic relationships of specimens morphologically unambiguous allocated to *Hemistomia fabrorum* Haase & Bouchet, 1998 or *Hemistomia cockerelli* Haase & Bouchet, 1998 were incongruent which is also shown by using Neighbour Networks instead of rooted trees. This might be due to incomplete lineage sorting or introgression by hybridization (Maddison 1997), phenomena known also for truncatelloidean gastropods (e.g. Haase 2005;

Prier and Bichain 2009; Zielske & Haase 2014a).

Furthermore, the relationships between the *Leiorhagium* species remained widely unclear. The three *Hemistomia* species attributed to clade III were monophyletic but paraphyletic in respect of the remaining *Hemistomia* species. Thus the new genus *Crosseana* Zielske & Haase 2015 was defined. *Kanakyella gentilsiana* Crosse, 1874 and *Kanakyella numee* Haase & Bouchet, 1998 were weakly supported affiliated to clade III but in no analysis were these species sister taxa. Thus another new genus *Novacaledonia* Zielske & Haase 2015 was introduced for *K. numee*.

Moreover we used the CAOS (Characteristic Attribute Organization System) network to identify molecular diagnostic character states for the different genera to extend the existing descriptions beyond morphology and anatomy as defining species or higher taxa based on this data is often difficult due to convergence and morphostasis (Falniowski & Szarowska 1995; Hershler & Ponder 1998; Liu *et al.* 2003; Wilke 2003; Colgan *et al.* 2007; Haase 2008; Wilke *et al*. 2013). However the existing CHAOS method (Sarkar *et al*. 2008) that searches for simple pure characters which are unique characters that occur in all investigated specimens of a particular genus but not in any specimen of the other genera had to be adapted. Instead characters with alternatively fixed states among pairs of genera were defined. The strings of all pair-wise diagnostic characters were then unique for each genus. Using this approach 180 characters were defined that can be used to separate one genus from at least one other.

Further a dated phylogeny for the New Caledonian taxa using again the COI substitution rate for New Zealand taxa, testing three different clock constraints (different combinations assuming a strict or relaxed molecular clock for each of the three genes, respectively) was calculated. The clock relaxed for all three genes was identified as the best fitting model resulting in an origin of all New Caledonian taxa estimated at 24.6 MYA (15.84-34.14 MYA). However this might be an underestimate due to incomplete sampling as two known genera of New Caledonian Truncatelloideans are missing in this analysis. Even so the results are in line with the finding that New Caledonia was colonized after a long phase of submergence, despite being a fragment of Gondwanaland and thus rather is a Darwinian island.

The enigmatic pattern of long distance dispersal of minute freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae) across the South Pacific.

Major findings:

- The South Pacific Tateidae originated in Australia from were they colonized Sulawesi,

Lord Howe Island, New Caledonia and New Zealand

- Findings about the evolutionary history of Tateidae in the South Pacific are not in accordance with the published dominant pattern
- *Fluviopupa* originated in New Zealand and dispersed westwards from the Austral Islands as far as Lord Howe Island
- The genus *Hemistomia* from Lord Howe Island and New Caledonia is polyphyletic
- Geographic distance is no predictor for relationship

This study draws a comprehensive picture about the radiation and diversification of truncatelloidean gastropods across the south pacific. It combines the results of the prior more specific studies and enhances them using species from the Austral Islands, Lord Howe Island, the Indonesian island Sulawesi as well as several species from New Zealand and Australia.
A molecular phylogeny was calculated using three additional nuclear gene fragments (18S rRNA; 28S rRNA and Histone 3) besides the mitochondrial COI and 16S rRNA known from the previous studies. In this phylogeny the sistergroup to the monophyletic Tateidae was formed by the Australian *Beddomeia*-group and *Oncomelania minima* (Pomatiopsidae). The Tateidae were divided in three clades:
1) The Australian genera *Austropyrgus* Cotton, 1942*, Posticobia* Iredale, 1943 and *Tatea* Tenison Woods, 1879 as well as *Hemistomia gemma gemma* Ponder, 1982 from Lord Howe Island. The affiliation of the species from Sulawesi to this clade remained unclear.
2) Two well supported lineages: one comprising the species from New Caledonia and the other comprising the Australian genera *Fonscochlea* Jenkins, 1989, *Caldicochlea* Ponder, 1997 and *Trochidrobia* Ponder, Hershler & Jenkins 1989.
3) The well supported third clade comprised three smaller clades of New Zealand species and a clade of all *Fluviopupa* species, the interrelationships within clade 3 remained unresolved.

The genus *Hemistomia* with representatives on Lord Howe Island and New Caledonia was polyphyletic throughout all analyses. The monophyly of the New Caledonia species was well supported which is in contrast to the study presented prior to this one. This might be due to the higher number of gene fragments surveyed. Also the monophyly of the genus *Fluviopupa* was well supported. The taxa from the Austral Islands build a well supported clade with two subclades, one comprising the species from Raivavae and the other comprising those from Rurutu and Tubuai as well as two specimens from Santo/Vanuatu. The monophyly of the Austral Islands sister clade was only weakly supported. It included the Lord Howe species

and a clade of Vanuatu and Fiji species, with the latter being subdivided into three well supported clades of Fijian species, the species from Erromango/Vanuatu and one clade comprising the remaining species from Vanuatu.

Based on this phylogeny ancestral range reconstructions and possible dispersal hypothesis were tested for the genus *Fluviopupa*. The hypotheses were: 1) radiations on each island archipelago are monophyletic and 2) Fluviopupa dispersed in a stepping stone like fashion according to a minimum spanning tree based on geographic distances. However none of the hypothesis was at least as likely as the unconstrained tree. Thus the suggestion that geographic distance is a good predictor of relationship could not be confirmed. The ancestral range reconstruction showed that the common ancestor of the south pacific Tateidae occurred in Australia. Its descendants colonized Sulawesi and Lord Howe Island and gave rise to the radiations on New Caledonia and New Zealand. The latter harboured the ancestor of the genus *Fluviopupa* which evolved on the Austral Islands. This is a presumably rare pattern because dispersal from New Zealand involves long distances and survival under different climatic conditions (Keppel *et al.* 2009). The Austral Islands were settled against the progression rule for stepping stone like dispersal (Cowie & Holland, 2008) from the youngest to the oldest island i.e. from south-east to north-west (Maury *et al.* 2014). Vanuatu was colonized twice from the Austral Islands and served as a hub for dispersal events to Fiji and Lord Howe Island. How often the islands of Fiji were reached and if there was a subsequent return to Vanuatu could not be determined unambiguously. Thus the pattern concerning Vanuatu is more complex than assumed in first study presented herein. However the polyphyly of *F. espiritusantoana* and *Fluviopupa pascali* Haase, Fontaine & Gargominy, 2010 remains confusing as these taxa appear in the Austral Island clade. The effect of ancient lineage fusion (Garrick *et al.* 2014) might be an explanation for this finding. Also the radiation on Fiji which could not be reconstructed as being monophyletic here is more complex than assumed in the study focussing on this archipelago. The complex and largely westward orientated dispersal history of *Fluviopupa* is not in concordance with the dominant pattern published for other taxa. It's described only for few plant genera (Berry *et al*. 2004; Birch & Keeley, 2013) and supports the increasing evidence (e.g. Bellemain & Ricklefs, 2008; Lapoint *et al.* 2013) against the hypothesis that the colonization of remote islands is a dead end journey (Mayr & Diamond, 2001).

Further a dated phylogeny testing two different dating strategieswas calculated, one using all available emergence times of islands as calibration points and the other using only the age of

Rapa Iti based on the results of the phylogenetic tree reconstruction, besides the COI substitution rate used in both approaches. The age estimates resulting from the latter approach were in general 20 % - 30 %, and for the genus *Fluviopupa* up to 43 % younger. Although using less calibration points the second approach is preferable as it is supported by the ancestral range reconstruction suggesting that *Fluviopupa* originated on the Austral Islands and that the Austral Islands were settled from south-east to north-west. Further it resulted in more plausible age estimates in accordance with the geological evidence. According to this approach the most recent common ancestor of the New Caledonia radiation was dated to 28.5 MYA, which is in accordance with the prior study. Thus New Caledonian tateids remain inconclusive regarding the question whether New Caledonia is a Gondwanan refuge or a Darwinian island (Espeland & Murienne 2011).

The genus *Fluviopupa* originated 5.0 MYA, with the Austral Islands clade having an age of 2.7 MY. The Fiji/Vanuatu clade has an age of 3.6 MY. However the main radiation across Vanuatu started only 1.4 MYA and the Fiji clades have an age of 1.4-2.8 MY which is somewhat older and presumably due to the complex geological patterns with phases of repeated emergence and submergence (Taylor 1992; Robbin 1993; Rodda 1994). The radiations on Lord Howe were estimated as being younger than one MY, which is in concordance with the geological evidence but may be questionable due to the small number of studied species. The most recent common ancestor of the species from Sulawesi was estimated as being 10.8 MY old. This is significantly older than the lakes where those species occur (Hall 2009; Zielske *et al*. 2011) suggesting a more complex evolutionary pattern than assumed for other ancient lake species in this region (von Rintelen *et al*. 2014; Stellbrink *et al*. 2014).

Conclusion:

As described in all the above studies the most likely vectors for long distance dispersal of minute gastropods are birds, as suggested for gastropods in general (Rees 1965; van Leeuwen *et al*. 2012b) and truncatelloideans in particular (e.g. Liu *et al*. 2003; Haase *et al.* 2010b). It has been demonstrated that small snails can even survive the passage thru the gut of water fowl (van Leeuwen *et al*. 2012a; Wada *et al*. 2012). Several species of extant and recently extinct waterbirds have wide distributions across the south pacific (Steadman 2006) and as all

major bird lineages were established already in the Palaeogene (Mayr 2009) this assumption also holds against the deep time frame set in the presented analyses.

Whilst the first three studies attributed to this thesis focus on taxonomic questions and local geological questions of single islands or archipelagos the latest study serves as a synopsis drawing a big picture from the previous results. Based on a much bigger and diverse datasets it solves questions that could not be answered based on the prior datasets like the monophyly of the New Caledonian species. Further it shows that patterns may seem simpler when focussing only on parts of the whole picture than they actually are: for example the complex dispersal pattern described for Vanuatu and Fiji, was only found in the comprehensive analysis.

On the other hand the number of surveyed specimens from the single archipelagos is reduced by nearly 90 % and thus the more specific studies are important as they serve the room for the description of new species and surveillance of taxonomic questions like the genus uncertainties from the analysis based solely on morphological data from New Caledonia. We refrained from a study focussing on the Austral Islands as the species found on the islands were the same that had been described previously. Moreover we were not even able to recollect all known species from the archipelago and thus a molecular study would have probably been very inconclusive. However the material colected was sufficient to be representative for the archipelago in the comprehensive study covering the whole South Pacific.

1.6. Literature

Adam, C. & Bonneville, A. (2005) Extent of the South Pacific superswell. *Journal of Geophysical Research*, **110**, B09408.

Bellemain, E. & Ricklefs, R. E. (2008) Are islands the end of the colonization road. *Trends in Ecology and Evolution*, **23**, 461-468.

Berry, P. E., Hahn, W. J., Systma, K. J., Hall, J. C. & Mast, A. (2004) Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. *American Journal of Botany,* **91**, 601-614.

Birch, J. & Keeley, S.C. (2013) Dispersal pathways across the Pacific: the historical biogeography of *Astelia* s.l. (Asteliaceae, Asparagales). *Journal of Biogeography,* **40**, 1914–1927.

Bonneville, A. (2009) French Polynesia, Geology. In: Gillespie RG, Clague DA (eds), *Encyclopedia of islands*. University of California press, Berkley. pp 338-343.

Carr, L. M., McLenachan, P. A., Waddell, P. J, Gemmell, N. J. & Penny, D. (2015) Analyses of the mitochondrial genome of *Leiopelma hochstetteri* argues against the full drowning of New Zealand. *Journal of Biogeography*, **42**, 1066-1076.

Chazeau, J. (1993) Research on New Caledonian terrestrial fauna: achievements and prospects. *Biodiversity Letters,* **1**, 123–129.

Colgan, D. J., Ponder, W. F., Beacham, E. & Macaranas, J. (2007) Molecular phylogenetics of Caenogastropoda (Gastropoda: Mollusca). *Molecular Phylogenetics and Evolution*, **42**, 717–737.

Cowie, R. H. & Holland, B. S. (2008) Molecular Biogeography and diversification of the endemic terrestrial fauna of the Hawaiian Islands. *Philosophical Transactions of the Royal Society B*, **363**, 3363-3376.

Cruaud, A., Jabbour-Zahab, R., Genson, G., Ungricht, S. & Rasplus, J.-Y. (2012) Testing the emergence of New Caledonia: Fig wasp mutualism as a case study and a review of evidence. *Plos One,* **7**, e30941.

Dickinson, W. R. (1998) Geomorphology and geodynamics of the Cook-Austral Island seamount chain in the South Pacific Ocean: implications for hotspots and plumes. *International Geology Review,* **40,** 1039-1075.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution,* **29,** 1969–1973.

Espeland, M. & Murienne, J. (2011) Diversity dynamics in New Caledonia: towards the end of the museum model? *Bmc Evolutionary Biology*, **11**, 254.

Falniowski, A. & Szarowska, M. (1995) Can poorly understood new characters support a poorly understood phylogeny? Shell-structure data in Hydrobiid systematics (Mollusca: Gastropoda: Prosobranchia: Hydrobiidae). *Journal of Zoological Systematics and Evolutionary Research,* **33,** 133-144.

Funk, D. J. & Omland, K. E. (2003) Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics,* **34,** 397–423.

Garric, R.C., Benavides, E., Russello, M. A., Hyseni, C., Edwards, D. L., Gibbs, J. P., Tapia, W., Ciofi, C. & Caccone A. (2014) Lineage fusion in Galapagos giant tortoises. *Molecular ecology*, **23**, 5276-5290.

Grandcolas, P., Murienne, J., Robillard, T., Desutter-Grandcolas, L., Jourdan, H., Guilbert, E. & Deharveng, L. (2008) New Caledonia: a very old Darwinian island? *Philosophical Transactions of the Royal Society B*, **363**, 3309–3317.

Greene, H. G., Collot, J.-Y., Fisher, M. A. & Crawford, A. J. (1994) Neogene tectonic evolution of the New Hebrides Island arc: a review incorporating ODP drilling results. In: Greene, H.G., Collot, J.-Y., Stokking, L.B. *et al*. *Proeedings of the Ocean Drilling Program*, *Scientific Results* 134: College Station, TX (Ocean Drilling Program), 19–46.

Haase, M. (2005) Rapid and convergent evolution of parental care in hydrobiid gastropods from New Zealand. *Journal of Evolutionary Biology*, **18**, 1076–1086.

Haase, M. (2008) The radiation of hydrobiid gastropods in New Zealand: A revision including the description of new species based on morphology and mtDNA sequence information. *Systematics and Biodiversity*, **6**, 99–159.

Haase, M. & Bouchet, P. (1998) Radiation of crenobiontic gastropods on an ancient continental island: the *Hemistomia*-clade in New Caledonia (Gastropoda: Hydrobiidae). *Hydrobiologia,* **367**, 43-129.

Haase, M., Fontaine, B. & Gargominy, O. (2010a) Rissooidean freshwater gastropods from the Vanuatu archipelago. *Hydrobiologia*, **637**, 53–71.

Haase, M., Gargominy, O. & Fontaine, B. (2005) Rissooidean freshwater gastropods from the middle of the Pacific: the genus *Fluviopupa* on the Austral Islands (Caenogastropoda). *Molluscan Research*, **25**, 145–163.

Haase, M., Naser, M. D. & Wilke, T. (2010b) *Ecrobia grimmi* in brackish Lake Sawa, Iraq: indirect evidence for long-distance dispersal of hydrobiid gastropods (Caenogastropoda: Rissooidea) by birds. *Journal of Molluscan Studies*, **76**, 101 –105.

Haase. M., Ponder, W. F. & Bouchet, P. (2006) The genus *Fluviopupa* Pilsbry, 1911 from Fiji (Caenogastropoda, Rissooidea). *Journal of Molluscan Studies*, **72**, 119-136.

Hall, R. (2009) Southeast Asia's changing palaeogeography. *Blumea*, **54**, 148-161.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hamilton, A., Klein, E. & Austin, C. (2010) Biogeographic breaks in Vanuatu, a nascent oceanic archipelago 1. *Pacific Science*, **64**, 149–159.

Heads, M. (2008) Panbiogeography of New Caledonia, south-west Pacific: basal angiosperms on basement terranes, ultramafic endemics inherited from volcanic island arcs and old taxa endemic to young islands. *Journal of Biogeography*, **35**, 2153–2175.

Hershler, R. & Ponder, W. F. (1998) *A review of morphological characters of hydrobioid snails*. Washington DC: Smithonian institution press.

Holloway, J. D. (1979) *A survey of the Lepidoptera, biogeography, and ecology of New Caledonia*. Series entomologica vol. 15. The Hague: W. Junk.

Jaffré, T., Morat, P. & Veillon, J.-M. (1994) La flore: caractéristiques et composition floristique des principales formations végétales. *Bois et forét des tropique*, **242**, 7–30.

Keppel, G., Lowe, A. J. & Possingham, H. P. (2009) Changing perspectives on the biogeography of the tropical South Pacific: influences of dispersal, vicariance and extinction.*Journal of Biogeography*, **36**, 1035-1054.

Knoop, V. & Müller, K. eds (2006) Gene und Stammbäume - Ein Handbuch zur molekularen Phylogenetik. Spektrum akademischer Verlag, München.

Ladiges, P. Y. & Cantrill, D. (2007) New Caledonia-Australian connections: biogeographic patterns and geology. *Australian Systematic Botany*, **20**, 383–389.

Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution,* **29**, 1695–1701.

Lapoint, R. T., O`Grady, P. M. & Whiteman, N. K. (2013) Diversification and dispersal of the Hawaiian Drosophilidae: the evolution of *Scaptomyza*. *Molecular Phylogenetics and Evolution*, **69**, 95-108.

Van Leeuwen, C. H. A., van der Velde, G., van Groenendael, J. M. & Klaassen, M. (2012a) Gut travellers: internal dispersal of aquatic organisms by waterfowl. *Journal of Biogeography*, **39**, 2031–2040.

Van Leeuwen, C. H. A., van der Velde, G., van Lith, B. & Klaassen, M. (2012b) Experimental Quantification of long distance dispersal potential of aquatic snails in the gut of migratory birds. *PLoS ONE*, **7**, e32292.

Lemey, P., Salemi, M. S. & Vandamme A.-M. eds (2009) The Phylogenetic Handbook – A Practical Approach to Phylogenetic Analysis and Hypothesis Testing. Cambridge University Press, Cambridge.

Lemey, P. & Posada, D. (2009) Phylogenetic Molecular clock analysis / Theory. In Lemey P., Salemi M., Vandamme A.-M. (eds) *The Phylogenetic Handbook*. Cambridge University press.Cambridge. pp. 362-371.

Liu, H.-P., Hershler, R. & Clift, K. (2003) Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Molecular Ecology*, 12, 2771-2782.

Lowry, P. (1998) Diversity, endemism and extinction in the flora of New Caledonia: a review. in: Peng, C.-I., Lowry, P. (Eds.): *Rare, threatened and endangered floras of the Pacific rim*. Monograph Series No 16. Institute of Botany, Academica Sinica, Taipei, pp. 181–206.

Maddison, W. P. (1997) Gene trees in species trees. *Systematic Biology*, **46**, 523-536.

Maddison, W.P. & Maddison D.R. (2011) Mesquite: a modular system for evolutionary analysis. Version 2.75 http://mesquiteproject.org

Maury, R., Legendre, C., Chauvel, C., Guille, G., Blais, S., Giullou, H. & Rossi, P. (2014) Geology An atypical hotspot chain. In: Meyer, J.-Y. & Claridge, E. M. (eds.) *Biodiversity of the Austral Islands, French Polynesia*. Publications scientifiques du *MNHN*, Paris.

Mayr, G. (2009) Paleogene fossil birds. Springer-Verlag, Berlin.

Mayr, E. & Diamond, J. (2001) The Birds of Northern Melanesia Speciation, ecology and biogeography. Oxford University Press, New York.

McGuire, J. A., Linkem, C. W., Koo, M. S., Hutchison, D. W., Lappin, A. K., Orange, D. I., Lemos-Espinal, J., Riddle, B. R. & Jaeger, J. R. (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: Phylogenetics of crotaphytid lizards. *Evolution*, **61**, 2879–2897.

Meyer, J.-Y. (2009) French Polynesia, Biology, In: Gillespie RG, Clague DA (eds), *Encyclopedia of islands*. University of California press, Berkeley. pp 332-338.

Misof, B. & Misof, K. (2009) A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: A more objective means of data exclusion. *Systematic Biology*, **58**, 21-34.

Morat, P., Veillon, J.-M. & MacKee, H. S. (1986) Floristic relationships of New Caledonian rainforest phanerogams. *Telopea*, **2**, 631–679.

Muellner, A. N., Pannell, C. M., Coleman, A. & Chase, M. W. (2008) The origin and evolution of Indomalesian, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography*, **35**, 1769–1789.

Munzinger, J. (2009) Vanuatu, in: *Encyclopedia of Islands*. University of California Press, Berkley, pp. 939-941.

Murienne, J. (2009) New Caledonia, Biology, in: Gillespie RG, Clague DA (eds), *Encyclopedia of Islands*. University of California Press, Berkley, pp. 643–645.

Murienne, J., Edgecombe, G. D. & Giribet, G. (2011) Comparative phylogeography of the centipedes *Cryptops pictus* and *C. niuensis* (Chilopoda) in New Caledonia, Fiji and Vanuatu. *Organsims, Diversity & Evolution*, **11**, 61-74.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Neall, V. E. & Trewick, S. A. (2008) The age and origin of the Pacific islands: a geological overview. *Philosophical Transactions of the Royal Society* B, **363**, 3293–3308.

Neef, G. & Hendy, C. (1988) Late Pleistocene-Holocene acceleration of uplift rate in southwest Erromango Island, southern Vanuatu, South Pacific: Relation to the growth of the Vanuatuan Mid Sedimentary Basin. *Journal of Geology*, **96**, 481–494.

Van de Peer, Y. (2009) Phylogenetic inference based on distance methods / Theory. In Lemey P., Salemi M., Vandamme A.-M. (eds) *The Phylogenetic Handbook*. Cambridge University press.Cambridge. pp. 142-159.

Ponder, W. F. (1982) Hydrobiidae of Lord Howe Island (Mollusca: Gastropoda: Prosobranchia). *Australian Journal of Marine and Freshwater Research*, **33**, 89–159.

Prie, V. & Bichain, J.-M. (2009) Phylogenetic relationships and description of a new stygobite species of *Bythinella* (Mollusca, Gastropoda, Caenogastropoda, Amnicolidae) from southern France. *Zoosystema*, **31**, 987–1000.

Rees, W. J. (1965) The aerial dispersal of Mollusca. *Proceedings of the Malacological Society of London*, **36**, 269–282.

Von Rintelen, T., Stelbrink, B., Marwoto, R. M. & Glaubrecht M. (2014) A snail perspective on the biogeography of Sulawesi, Indonesia: Origin and intra-island dispersal of the viviparous freshwater gastropod *Tylomelania. PloS One*, **9**, e09017.

Robin, C., Mozier, M., Crawford, A. J: & Eggins, S. M. (1993) The geology, volcanology, petrology-geochemistry, and tectonic evolution of the New Hebrides Island Arc, Vanuatu: IAVCEI, Canberra 1993: Excursion Guide. Record 1993/059. Australian Geological Survey Organisation, Canberra.

Rodda, P. (1994) Geology of Fiji, in: *Geology and submarine resources of the Tonga-Lau-Fiji region*, SOPAC Technical Bulletin, pp 131–151.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Ronquist, F., van der Mark P. & Huelsenbeck, J.P. (2009) Bayesian phylogenetic analysis using MrBayes / Theory. In Lemey P., Salemi M., Vandamme A.-M. (eds) The Phylogenetic Handbook. Cambridge University press.Cambridge. pp. 210-236.

Ryan, P. (2009) Fiji, Biology. In: Gillespie RG, Clague DA (eds), *Encyclopedia of islands*. University of California press, Berkley. pp 298-305.

Sarkar, I. N., Planet, P. J. & DeSalle, R. (2008) CAOS software for use in character-based DNA barcoding. *Molecular Ecology Resources*, **8**, 1256–1259.

Schellart, W. P., Lister, G. S. & Toy, V. G. (2006) A Late Cretaceous and Cenozoic reconstruction of the Southwest Pacific region: Tectonics controlled by subduction and slab rollback processes. *Earth-Science Reviews*, **76**, 191–233.

Schmidt, H. A. & von Haeseler, A. (2009) Phylogenetic inference using maximum likelihood methods / Theory. In Lemey P., Salemi M., Vandamme A.-M. (eds) *The Phylogenetic Handbook*. Cambridge University press.Cambridge. pp. 181-198.

Steadman, D. E. (2006) Extinction and biogeography of tropical pacific birds. University of Chicago Press, Chicago.

Stocsits, R.R., Letsch, H., Hertel, J., Misof, B. & Stadler, P.F. (2009) Accurate and efficient reconstruction of deep phylogenies from structured RNAs. *Nucleic Acids Research*, **37**, 6184 –6193.

Strimmer, K. & von Haeseler, A. (2009) Genetic distances and nucleotide substitution models / Theory. In Lemey P., Salemi M., Vandamme A.-M. (eds) *The Phylogenetic Handbook*. Cambridge University press.Cambridge. pp. 111-125.

Swenson, U., Nylinder, S. & Munzinger, J. (2014) Sapotaceae biogeography supports New Caledonia being an old Darwinian island. *Journal of Biogeography*, **41**, 797-809.

Takahashi, K., Terai, Y., Nishida, M. & Okada, N. (2001) Phylogenetic relationships and ancient incomplete lineage sorting among cichlid fishes in Lake Tanganyika as revealed by analysis of the insertion of retroposons. *Molecular Biology and Evolution*, **18**, 2057–2066.

Taylor, F. W. (1992) Quaternary vertical tectonics of the central New Hebrides Island arc. In: Collot, J.-Y., Greene, H.G., Stokking, L.B. *et al*. *Proceedings of the Ocean Drilling Program, Initial Reports*, **134**: College Station, TX (Ocean Drilling Program). 134, 33–42.

Taylor, F. W., Jouannic, C. & Bloom, A. L. (1985) Quaternary uplift of the Torres Islands, Northern New Hebrides Front Arc – Comparison with Santo and Malekula Islands, Central New Hebrides Frontal Arc. *Journal of Geology*, **93**, 419-438.

Wada, S., Kawakami, K. & Chiba, S. (2012) Snails can survive passage through a bird's digestive system. *Journal of Biogeography*, **39**, 69–73.

Waters, J. M. & Craw, D. (2006) Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. *Systematic Biology*, **55**, 351-356.

Wilke, T. (2003) *Salenthydrobia* n.gen. (Rissooidea: Hydrobiidae): a potential relict of the Messinian salinity crisis. *Zoological Journal of the Linnean Society,* **137**, 319-336.

Wilke, T., Haase, M., Hershler, R., Liu, H.-P., Misof, B. & Ponder, W. F. (2013) Pushing short DNA fragments to the limit: Phylogenetic relationships of "hydrobioid" gastropods (Caenogastropoda: Rissooidea). *Molecular Phylogenetics and Evolution*, **66**, 715–736.

Yu,Y., Harris, A.J., Blair, C. & He, X.-J. (2015) RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. *Molecular Phylogenetics and Evolution*, **87**, 46-49.

Zielske, S., Glaubrecht, M. & Haase, M. (2011) Origin and radiation of rissooidean gastropods (Caenogastropoda) in ancient lakes of Sulawesi. *Zoologica Scripta*, **40**, 221-237.

Zielske, S. & Haase, M. (2014a) When snails inform about geology: Pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda: Tateidae). *Journal of Zoological Systematics and Evolutionary Research*, **52**, 217-236.

Zielske, S. & Haase, M. (2014b) New insights into tateid gastropods and their radiation on Fiji based on anatomical and molecular methods (Caenogastropoda: Truncatelloidea). *Zoological Journal of the Linnean Society*, **172**, 71-102.

Zielske, S. & Haase, M. (2015) Molecular phylogeny and a modified approach of character-based barcoding refining the taxonomy of New Caledonian freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae). *Molecular Phylogenetics and Evolution*, **89**, 171-181.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. dissertation, The University of Texas at Austin.

2. Manuscripts

2.1. When snails inform about geology

JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

Accepted on 28 October 2013

J Zoolog Syst Evol Res doi: 10.1111/jzs.12053

Vogelwarte/Zoological Institute and Museum, Greifswald University, Greifswald, Germany

When snails inform about geology: Pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda: Tateidae)

Susan Zielske and Martin Haase

Abstract

The South Pacific archipelago Vanuatu has a very complex geological history including three major phases of volcanism creating island belts and phases of repeated submergence and re-emergence. An important issue for the evolution of the biota of Vanuatu ambiguously discussed in the geological literature is the question whether the entire archipelago has been submerged until the early Pleistocene or if at least parts of the island of Espiritu Santo have remained subaerial throughout the Pliocene. We used a time-calibrated phylogenetic analysis of freshwater gastropods of the family Tateidae based on *COI*, *16S rRNA* and *ITS2* to infer the colonization history of Vanuatu. Our analyses suggested that Espiritu Santo was colonized c. 3 Mya. Espiritu Santo was probably the place of origin for the subsequent colonization of the island of Erromango (2 Mya) and the Pleistocene radiation across the remaining archipelago. We describe 10 new species largely based on morphological and anatomical data. The genetic data in particular of the species from the young islands are taxonomically incongruent probably due to incomplete lineage sorting typical for young radiations. In contrast, the paraphyly of *Fluviopupa espiritusantoana* appearing in three distant clades indicates either the existence of cryptic species or the long survival of the stem species of almost the entire radiation.

Key words: Biogeography – *Fluviopupa* – phylogeny – South Pacific – time-trees

Introduction

Small freshwater gastropods of the family Tateidae may represent one of the oldest radiations across South Pacific islands (Haase et al. 2010a; compare Gillespie et al. 2008; Neall and Trewick 2008; Keppel et al. 2009). They occur in New Zealand (Haase 2008), New Caledonia (Haase and Bouchet 1998); Vanuatu (Haase et al. 2010a), Fiji (Haase and Bouchet 2006) and the Austral Islands, the southernmost archipelago of French Polynesia (Haase et al. 2005). These islands and archipelagos have at least partly been above the Ocean surface since at least the late Miocene (Kroenke 1996; Bonneville et al. 2002). Only for Vanuatu, the geological record is ambiguous (see below). Tateids have not been found on any of the many younger, interspersed islands. The only younger islands that do have a tateid record are Norfolk Island whose single species probably got extinct in historical times (Ponder 1981) and Lord Howe Island (Ponder 1982). Marine or brackish water representatives occur only in coastal waters of Australia (Ponder and Clark 1988) and New Zealand (Haase 2008). The freshwaters of the latter were invaded probably three times independently (Haase 2005). These large land masses in the west and south-west were thus probably the source areas for the colonization of the tropical Pacific islands.

In the course of a project aiming at the reconstruction of the tateid 'conquest' of the South Pacific, six islands of Vanuatu were visited in 2011, viz. Aneityum, Erromango, Efate, Malekula, Pentecost and Gaua. This expedition complemented earlier collecting efforts on Espiritu Santo (short: Santo) and the Torres Islands in 2006 and 2007 (Bouchet et al. 2011), which led to the description of 10 new species (Haase et al. 2010a). The only other islands Tateidae – recently separated from Hydrobiidae and raised to family status within Truncatelloidea and no longer Risooidea (Criscione and Ponder 2013; Wilke et al. 2013) – were reported from until then are Efate and Gaua. The samples from Efate and Santo were attributed to *Fluviopupa brevior* (Ancey 1905) (Ancey 1905; Solem 1959; Starmühlner 1976) and the one from the crater lake on Gaua was classified as *Potamopyrgus* sp. (Baker 1929).

The Archipelago of Vanuatu (Fig. 1) consisting of more than 80 islands spread over more than 900 km is situated about 2000 km east of Australia and 400 km north-east of the main island of New Caledonia in the Pacific Ocean. Following Simeoni (2009; see also references cited therein), the Archipelago can be divided into three parts being the result of different volcanic episodes: The western belt comprising the largest Islands Malekula and Santo as well as the Torres Islands is the presumably oldest part with active volcanism from the Oligocene to the middle Miocene. Volcanism leading to the formation of the eastern belt consisting of Pentecost and Maewo dates from the end of the Miocene to the early Pliocene. The Central Chain comprising all islands between Aneityum and the Banks Islands is the presumably youngest part with continuous volcanism from the early Pleistocene to today. In addition to the complex history of formation, the archipelago has experienced stages of submergence and re-emergence. However, the knowledge about the final emergence of the different islands is partly ambiguous. The Torres Islands are entirely covered by Quaternary coral formations indicating a very young subaerial history (Taylor et al. 1985). Large parts of Malekula are covered by Pliocene (southern part) to Pleistocene (northern part) coral limestone. A window of Miocene rocks in the north has been interpreted as paleoisland, which, however, probably also submerged and re-emerged only in the Pleistocene (Taylor 1992). Total submergence since the early Pliocene is assumed for Santo. But at least parts have probably remained above sea level since uplift in the earliest Pleistocene (Taylor 1992; Robin 1993). Yet, Santo has also wide areas covered by Quaternary coral reef limestone. This indicates that, like on Malekula, there are younger and older parts regarding the time since emergence (Robin 1993). The Eastern belt islands are practically entirely covered by Pliocene to Pleistocene coral reef limestone suggesting a late Quaternary uplift (Taylor 1992). Most islands of the central chain including Aneityum, Efate and Gaua owe their emergence to Pleistocene volcanic activity (Robin 1993; Greene et al. 1994). In contrast, the western part of Erromango seems to be continuously emergent since 2.6–

Corresponding author: Susan Zielske (susan.zielske@googlemail.com)
Contributing author: Martin Haase (martin.haase@uni-greifswald.de)

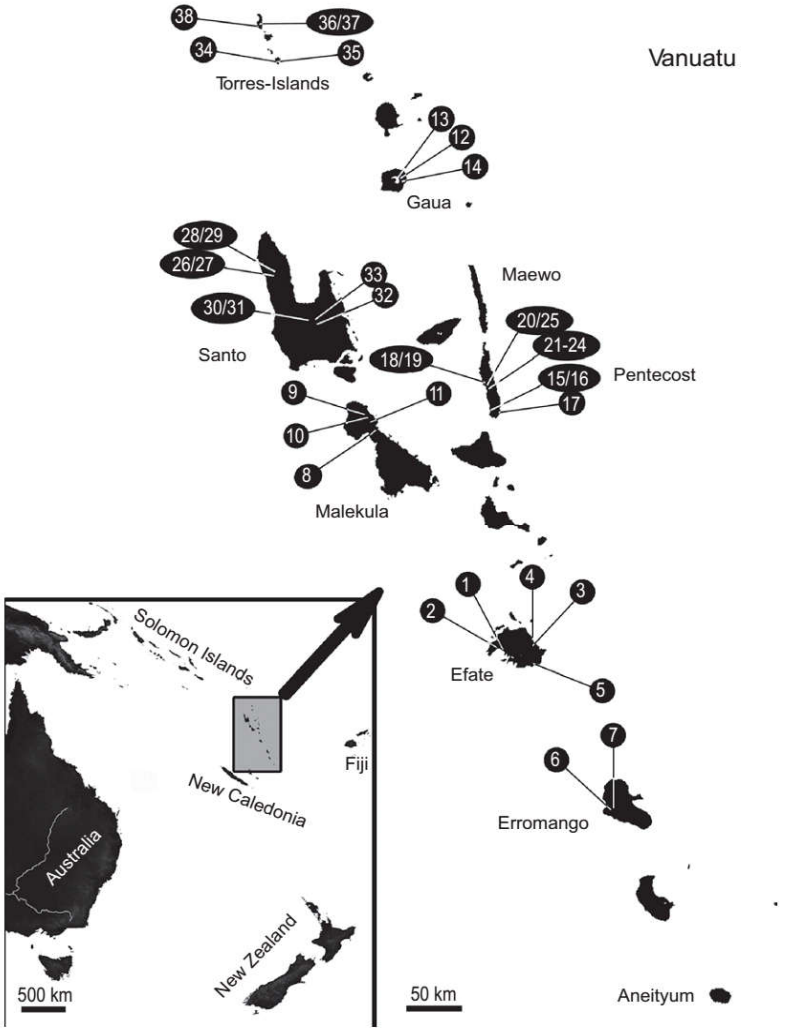

Fig. 1. Map. Vanuatu with sampling localities and location in the Southwest Pacific

2.3 Myr except for the Mount Rantop peninsula (Neef and Hendy 1988). The earliest volcanism of Erromango dates already to the late Miocene, though. The island seems to have surfaced and submerged repeatedly since then. However, interpretations if volcanism was submarine or subaerial differ between authors (cf. Robin 1993; Greene et al. 1994). The uncertainty concerning the emergence of the islands of Vanuatu is illustrated by Figure 12 of Akimoto (1994) where the sea surface is tangent to the peaks of the islands throughout Plio- and Pleistocene. Clear islands exist only in the panels representing Holocene and present age. Neglecting the ambiguities, Hamilton et al. (2010) assumed 'the last period of emergence only in the last 2 myr'. (p. 150) in their recent biogeographical analysis of 'a nascent oceanic archipelago'.

The aims of our paper are threefold: (1) we describe the new species found during our expedition in 2011 based on morphology, anatomy and molecular phylogenetic analyses, (2) based on the phylogenetic analyses, we inferred the colonization history of the archipelago, and (3) the age of the radiation, the latter with possible implications for the reconstruction of the geological history of Vanuatu. Due to their low potential of active dispersal, snails often preserve old distribution patterns (e.g. Cook et al. 1990; Haase et al. 2007; Holland and Cowie 2009). These patterns may be informative also for the reconstruction of geological histories (e.g. Upton and Murphy 1997; Pfenninger et al. 2010). To understand the origin and evolution of flora and fauna of oceanic islands, it is important to know when islands emerged. Regarding in particular the older islands of Vanuatu with their complex history of sea-level fluctuations, the above estimates had to be presented in subjunctive. Therefore, we implemented a molecular clock approach to test the geological scenario outlined above and eventually refine hypotheses regarding the emergence of islands.

Material and Methods

Material

Most specimens examined in this study were collected in May and June 2011. They were preserved in 70% ethanol in the field and transferred to propylene glycol for shipment. Upon arrival in our laboratory, snails were returned to 96% ethanol. Sampling stations are indicated in Fig. 1 and described in detail in the supplementary material. Material from Santo and the Torres Islands was provided by the Museum National d'Histoire Naturelle Paris (no exact collection numbers available), and species from

New Zealand included in the outgroup of phylogenetic analyses came from the Museum of New Zealand Te Papa Tongarewa in Wellington. As outgroup taxa, we selected two tateid species from New Zealand [*Opacuincola delira* Haase 2008 (MNZ M.174126), *Potamopyrgus estuarinus* Winterbourn 1971 (MNZ M.174052/1)], as well as one each from New Caledonia (*Hemistomia winstonefi* Haase and Bouchet 1998), Australia (*Tatea huonensis* Tension Woods 1876) and Brazil (*Potamolithus ribeirensis* Pilsbry 1911) based on the latest phylogeny of rissooidean/truncatelloidean gastropods (Wilke et al. 2013). Sequence data of the three latter outgroup taxa were taken from GenBank (Appendix 2).

Museum acronyms: MNHN, Museum National d'Histoire Naturelle Paris; MNZ, Museum of New Zealand Te Papa Tongarewa; ZMB, Museum für Naturkunde Berlin.

DNA isolation and sequencing

DNA was isolated from two snails of each sample (we crushed entire snails after taking photographs without leaving remnants qualifying as voucher) using QIAGEN's DNeasy Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany). The primers used to amplify a 715-bp-long fragment of the nuclear (nc) *16S rRNA* gene (*16S*) were 16Sar (Palumbi et al. 1991) and 16Sr (5′-tcttctgccacctttatt-3′; this study). The primers L1460 and H1298 (modified at position 12; G → A) by Folmer et al. (1994) amplified a 658-bp-long fragment of the mt cytochrome oxidase subunit I gene (*COI*) gene and the primer ITSfn (5′ gacacattgaacatcgaca 3′; this study) and ITSr4 (Oliverio and Mariottini 2001) a ca. 435-bp-long fragment of the nuclear (nc) internal transcribed spacer 2 region (*ITS2*). Polymerase chain reactions were performed in 12.5 µl containing 1.1 µl 10X BH4 buffer (BIOLINE GmbH; Luckenwalde, Germany), 4.4 mM MgCl, 0.28 pM of each primer, 0.2 mM dNTP, 0.5 µl BSA (1%), 0.25 U DNA-Polymerase (BIOLINE), 5–50 ng DNA and water.

The PCR conditions were 5 min at 95°C for initial denaturation, followed by 40 cycles with a denaturation step at 95°C for 60 s (45 s for *ITS2*), an annealing step as outlined in the following, and an extension step at 72°C for 60 s (75 s for *ITS2*), as well as a final extension step at 72°C for 10 min. Annealing was performed at 46°C for 90 s for *COI*, 53°C for 45 s for *ITS2* and as a touchdown with a temperature drop of one degree in each of the first 10 cycles starting at 60°C followed by 30 cycles at 51°C for 60 s for *16S*. Products were purified enzymatically using exonuclease and shrimp alkaline phosphatase (ExoSAP-IT Affymetrix, Santa Clara, CA, USA) and sequenced using ABI's Big Dye Terminator Ready Reaction Mix v3.1 (Carlsbad, CA, USA) and the PCR primers on an ABI 3130xl Genetic Analyzer. All Sequences are available from GenBank with accession numbers listed in Appendix 2.

For a better resolution of deeper nodes, we attempted to sequence also the nc genes *ATP synthetase* α and the *Elongation factor 1*α, however, failed to establish primers working in at least the majority of specimens. The lack of markers amplifying across a wider range of gastropod groups is commonly deplored (Dayrat et al. 2011).

Phylogenetic analyses

Sequences were edited using BioEdit (Hall 1999). Due to the lack of indels, the protein coding mt *COI* gene could be aligned by eye. The mt *16S* and the nc *ITS2* sequences were initially aligned using Clustal W (Thompson et al. 1994). This alignment was then refined in RNAsalsa (Stocsits et al. 2009) using secondary structure information of *Cacozeliana lacertina* Gould 1861 [*16S*, http://www.rna.icmb.utexas.edu/SIM/4D/Mollusk/ (Cannone et al. 2002), AF101007] and *Haliotis discus* Reeve 1846 [*ITS2*, http://its2.bioapps.biozentrum.uni-wuerzburg.de (Koetschan et al. 2010), GI 28627842] and finally manually edited in BioEdit (the final Alignment is available from TreeBASE http://purl.org/phylo/treebase/phylows/study/TB2:S14586).

Partition Finder (Lanfear et al. 2012) was used to decide whether stem and loop structures in *16S* and *ITS2* and codon positions in *COI* should be treated as partitions with individual substitution models and to find the best fitting models according to the corrected Akaike information criterion. A test for substitution saturation was performed in DAMBE (Xia and Xie 2001) treating gaps as unknown states. Phylogenetic analyses were performed in a maximum likelihood framework. Initial attempts of implementing Bayesian analyses using MrBayes (Ronquist and Huelsenbeck 2003) had to be aborted, because the Markov chains did not reach stationarity (see below). For tree reconstruction, we used Garli (Zwickl 2006) with 500 search-replicates for the concatenated data set and 100 replicates for separated nc and mt data, respectively. To assess the robustness of the topology based on the concatenated data, a bootstrap analysis with 500 replicates was performed. The bootstrap consensus tree was composed using Phyutiliy (Smith and Dunn 2008). Bootstrapping was repeated after exclusion of three taxa with leaf stability (calculated also with Phyutility) index <0.6. Trees were rooted with *Potamolithus ribeirensis* which is the least closely related to the genus *Fluviopupa* among the selected outgroup taxa (Wilke et al. 2013).

Regarding the low resolution in parts of the resulting tree, the analysis of the concatenated data set was repeated using constraint files to test different hypotheses of island colonization. The hypotheses compared were (1) unconstrained tree; (2) every island was colonized only once, hence samples from each island formed reciprocally monophyletic groups; (3) samples from Santo and Malekula, the presumably oldest Islands, are sister group to the samples of the remaining islands of which each was colonized only once; and (4) samples from Santo were paraphyletic with respect to the samples from the other islands, of which each was colonized only once. The resulting trees were tested against each other using the approximately unbiased test implemented in Consel (Shimodaira and Hasegawa 2001).

The phylogeny was dated based on the substitution rate estimated for COI in tateid gastropods from New Zealand ($3.26 \pm 0.14\%$ MY^{-1}), the closest relatives for which rates have been estimated (Haase et al. 2007). A likelihood ratio test for the molecular clock was performed in DAMBE (Xia and Xie 2001). Divergence times were estimated in two approaches, first, based on average *COI*-distances between clades calculated in Paup*4.10b (Swofford 2003) and corrected after the HKY + G substitution model, the best fitting model according to the corrected Akaike information criterion as shown by jModeltest (Posada 2008). The second approach considering all three gene fragments was a Bayesian analysis implemented in BEAST 1.7.5 (Drummond et al. 2012) assuming a birth–death model and a strict clock based on the *COI* substitution rate to calculate divergence times. As these analyses had no outgroup taxa, *COI* was no longer partitioned due to the very restricted variability of first and second codon positions. Topologically instable taxa were omitted. Using the whole taxon set resulted again in problems of parameter optimization most likely caused by the many short branches in clade I (Fig. 2). Therefore, clade I was reduced to nine randomly chosen taxa. Finally, eight independent analyses each run for 40 Mio generations logging every 10,000th were combined to obtain effective sample sizes of >200 for all parameters setting a burn in of 10%.

Morphology

Five shell dimensions, shell height and width, aperture height and width, and width of the body whorl, were measured (parallel and perpendicular to the coiling axis, respectively) from digital photographs taken under a dissecting microscope using Zeiss Axio Vision 4.8 of up to 20 adult individuals of each sample. Whorls were counted to the nearest eighth of a whorl. Statistical comparisons including the multivariate techniques CVA, MANOVA and Hotelling's T^2-tests and univariate Student's *t*-tests were performed using PAST 2.0 (Hammer et al. 2001).

Snails were dissected and anatomies drawn after dissolving shells in 1N HCL. A 5% sodium hypochlorite solution was used to clean shells, radulae and opercula for scanning electron microscopy. Cephalopodia were dried using hexamethydisilazane (Nation 1983). The objects were coated in gold and investigated in a Zeiss EVO LS10 Scanning Microscope.

Species descriptions

The phylogenetic analysis based on molecular data revealed both morphologically and genetically cryptic species (see below), that is, species, which are morphologically very similar and can reliably only be distinguished genetically, and other species, which are genetically indistinguishable but differ in morphology or anatomy. In the following, we describe new species only if the evidence was unambiguous. Populations, for whose variability it was impossible to decide whether it was intra- or

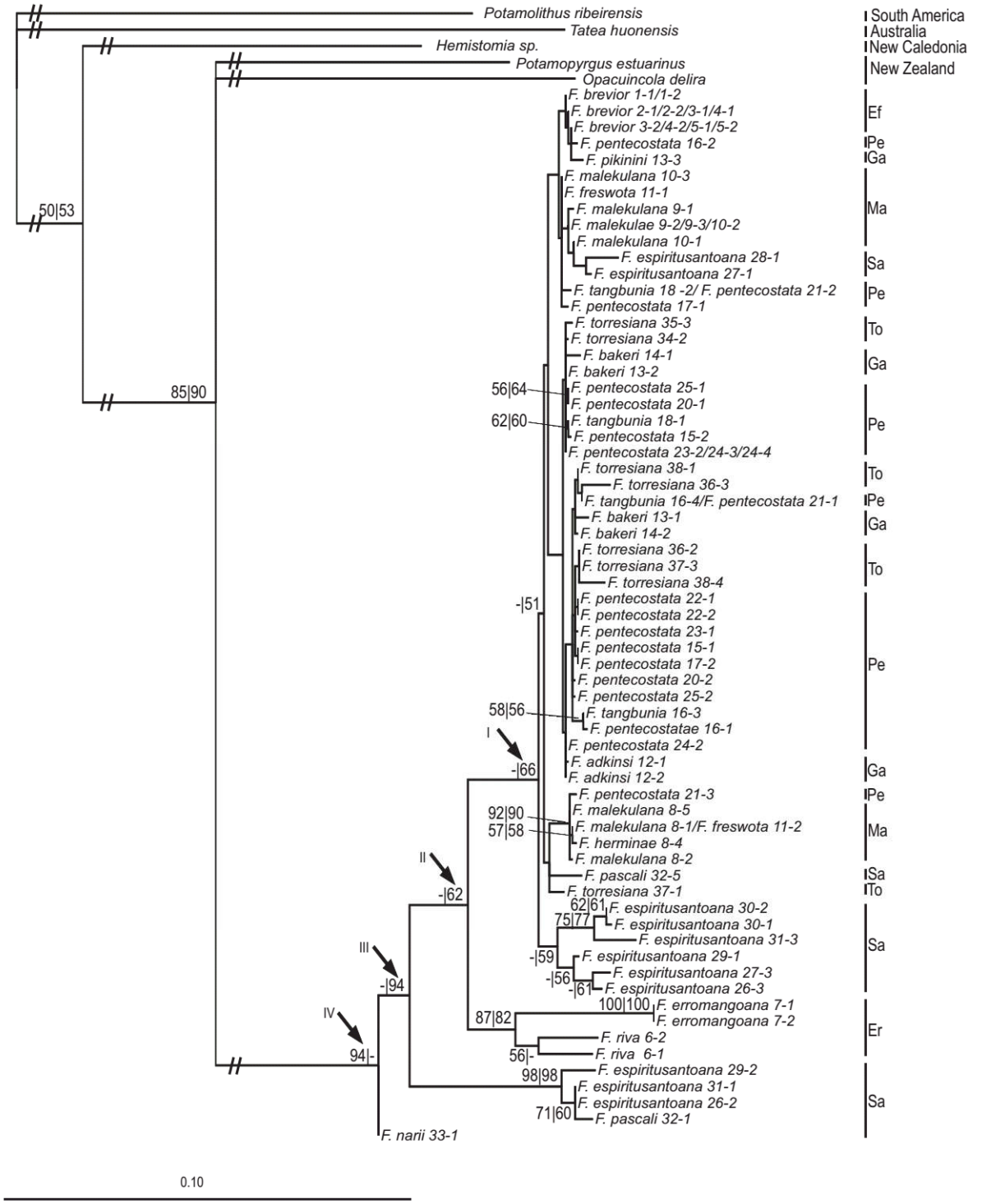

Fig. 2. Maximum likelihood phylogenetic tree obtained from the combined *16S*, *COI*, *ITS2* dataset; bootstrap support values: left with/right without instable taxa, nodes without any support values had <50% support; diagonal lines indicate that branches have been shortened by 50%; arrows indicate dated nodes; numbers behind species names indicate sampling localities and individuals (see Appendices 1 and 2); scale bar = substitutions per site

interspecific, were regarded as single species. For the taxonomic treatment of morphological and anatomical characters, we refer to the 'pragmatic species concept' of Haase and Bouchet (2006).

As a service to the reader, we use the new names already in the phylogenetic analyses although they are formally introduced only in the second part of the article.

Abbreviations

In the figures, the islands of Vanuatu are indicated by two letters: Am, Ambae; An, Aneityum; Ef, Efate; Er, Erromango; Ga, Gaua; Ma, Malekula; Me, Maewo; Pe, Pentecost; Sa, Espiritu Santo; Ta, Tanna; To, Torres Islands.

Results

Phylogenetic analysis

Partition Finder suggested the following partitioning scheme with respective substitution models: HKY + I + Γ for combined stems and loops of *16S*; K81 + Γ for *ITS2* loop structures and F81 + Γ for the stems; and F81 + I, HKY and GTR+Γ for first to third codon positions of *COI*. The test for substitution saturation reported only negligible saturation for all three genes and all three codon positions of *COI*. Sequence divergence based on pairwise distances in the ingroup reached up to 5.1% in *16S*, 10.2% in *COI* and 5.4% in *ITS2*.

In the phylogenetic analysis (Fig. 2), the snails from Vanuatu formed a well-supported monophylum. Interestingly, the closest relatives to the Vanuatu clade were the species from New Zealand. This relationship was also well supported. The sister group relationship of *Hemistomia winstonefi* from New Caledonia with the other island taxa received a bootstrap support of only 67. The Australian *Tatea huonensis* was drawn to *Potamolithus ribeirensis*, the root. Most of the specimens collected in Vanuatu were genetically very similar. Animals from Efate, Malekula, Pentecost, Gaua and the Torres Islands and some from Santo formed the large, hardly resolved clade I with the moderate support of 66. There was also only little geographical structure in this clade characterized by short branch lengths. *Fluviopupa brevior* and *F. adkinsi* nov. sp. were the only species with more than one representative that were recovered each in a single subclade and only few subclades were composed of a single species. Only one subclade comprising more than two specimens received bootstrap support > 50. This subclade contained three specimens of *F. malekulana* nov. sp., the single representative of *F. herminae* nov. sp. and one of *F. freswota* nov. sp., all from Malekula, and one snail ascribed to *F. pentecostata* nov. sp. Clade II, the sister clade of clade I, was well defined and formed by the two reciprocally monophyletic species from Erromango. The next deeper clade, clade III, contained three specimens again of *F. espiritusantoana* and a single one of *F. pascali*, both occurring on Santo and both also represented in the youngest clade I. The oldest lineage in the Vanuatu clade was formed by *F. narii* Haase, Fontaine & Gargominy 2010 from Santo. However, together with *F. pikinini* nov. sp. (13-3) and *F. espiritusantoana* (28-1), this specimen of *F. narii* was topologically unstable with a leaf stability index of 0.53 and removed in a second bootstrap analysis. Thus, there is not much confidence in the branch connecting nodes III and IV. Bootstrap support of nodes I-III was only achieved after removal of these three unstable individuals. The topologies of the analyses separately based on either *ITS2* or the mt fragments were practically identical to the concatenated analysis with only slight differences in the positions within clade I and *F. riva* nov. sp. being paraphyletic in the mtDNA tree.

The comparison of different hypotheses of island colonization revealed that none of the alternative hypotheses was at least as likely as the unconstrained tree (AU-test, $p < 0.01$).

The molecular clock was not rejected (likelihood ratio test, $p > 0.79$) for all three genes. Assuming a sequence divergence of $3.26 \pm 0.14\%$ MYR^{-1}, the following ages for the three nodes relevant for the global picture of the geological evolution of Vanuatu (Figs 2 and 3) were estimated based on the average *COI*-distances between clades: node I: 0.68 ± 0.03 Mya, node II: 2.62 ± 0.11 Mya and node III: 4.81 ± 0.20 Mya. The deepest but uncertain (see above) split concerning *F. narii* could not be dated because sequencing *COI* failed in this individual. The phylogenetic tree resulting from the BEAST analysis (Fig. 3) was topologically basically identical to the ML tree; only the relationships within clade I were differently reconstructed. All basal nodes including nodes I-III were supported by posterior probabilities of 1. The following ages were estimated: node I: 0.84 ± 0.26 Mya, node II: 2.20 ± 0.57 Mya and node III, 3.01 ± 0.77 Mya.

Systematic descriptions

Fluviopupa Pilsbry, 1911

Type species: *Fluviopupa pupoidea* Pilsbry, 1911 (original designation, by monotypy).

Synonymy: *Fluviopupa* Pilsbry, 1911.

The following description is based on the latest comprehensive diagnosis of the genus by Haase et al. (2010a).

Shell measurements and whorls are given in Appendix 1 and therefore not repeated in species descriptions. The following features are invariant across all species and therefore not mentioned again in species descriptions:

Shell (Figs 4 and 5): conical; protoconch well differentiated from teleoconch, surface with wrinkles gradually becoming finer towards the teleoconch; teleoconch smooth apart from growth lines; umbilicus narrow; aperture simple.

Operculum: Corneous, yellow, elongate-ellipsoidal, paucispiral; nucleus submarginal, with variable extent of white smear and small, rudimentary peg (Fig. 6).

External features: tentacles without conspicuous pattern of ciliation.

Mantle cavity: Ctenidium well developed, abutting directly on pericardium; osphradium ovate-elongate; hypobranchial gland not recognized in dissections; kidney not protruding into pallial roof.

Digestive system: Radula (Fig. 7) taenioglossate; stomach with fan-shaped caecum (Fig. 8).

Female genitalia (Fig. 9): Oviparous; ovary sac-shaped, only occasionally extending to stomach; renal oviduct coiling first 180° clockwise and than 270° counter-clockwise, the proximal loop often bent towards albumen gland; one distal receptaculum seminis globular with moderately wide, short duct, lying against left side of middle part of bursa copulatrix; bursa copulatrix behind albumen gland, pyriform to elongate; pallial oviduct with ovate cross-section; genital opening terminal or subterminal.

Male genitalia: Testis lobate, usually covering proximal chamber of stomach; vas deferens initially coiling as vesicular seminalis; vas deferens entering prostate in posterior third; penis simple, usually tapering more or less continuously from broad

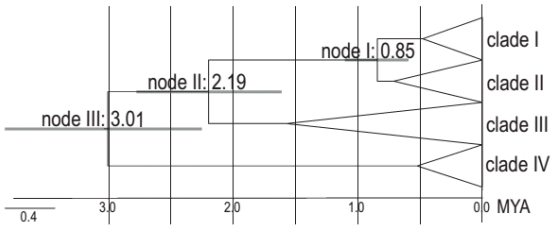

Fig. 3. Maximum clade credibility tree from BEAST analysis; node ages in MY; node bars indicate 95% highest posterior density intervals; posterior probabilities of analysed nodes are 1; scale bar = substitutions per site

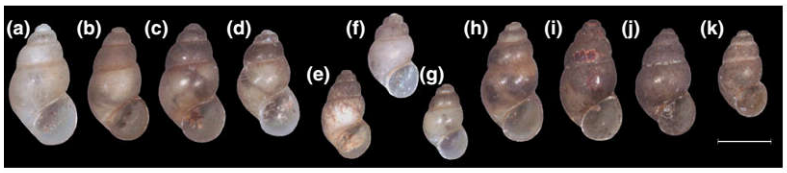

Fig. 4. Shells of Holotypes. (a) *Fluviopupa brevior*; (b) *F. riva*; (c) *F. erromangoana*; (d) *F. malekulana*; (e) *F. herminae*; (f) *F. freswota*; (g) *F. tangbunia*; (h) *F. pentecostata*; (i) *F. adkinsi*; (j) *F. bakeri*; (k) *F. pikinini*; Scale bar = 1 mm

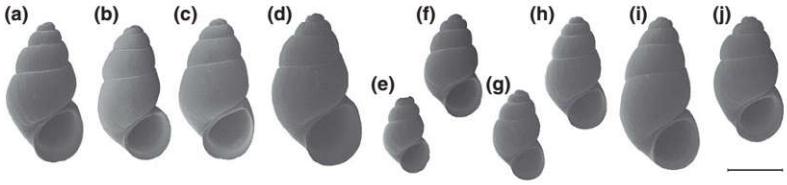

Fig. 5. Shells, SEM pictures. (a) *Fluviopupa brevior* station 3; (b) *F. riva*; (c) *F. erromangoana*; (d) *F. malekulana* station 10; (e) *F. herminae* (apex broken); (f) *F. freswota*; (g) *F. tangbunia* station 27; (h) *F. pentecostata* station 20; (i) *F. adkinsi*; (j) *F. bakeri* station 13; Scale bar = 1 mm

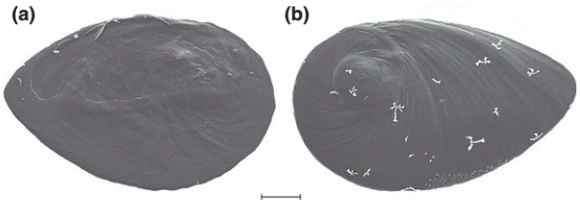

Fig. 6. Operculum. *Fluviopupa pentecostata*; (a) inside, (b) outside; Scale bar = 100 μm

base, occasionally bulging out to form a flange on right side (Fig. 10).

Fluviopupa brevior (Ancey 1905)
Synonymy: *Potamopyrgus brevior* Ancey 1905, Nautilus 19: p. 46.

Type material: Holotype lost; neotype here designated; neotype: ZMB Moll 117880, Paratypes: ZMB moll 117891 (n = 50).

Type locality: Efate Island, Mangaliliu village, station 1 (17°38′19.5″S, 168°12′23.7″E).

Additional material: Efate Island; road to Benjor Beachclub, station2 (17°41′00.6″S, 168°14′53.6″E; MNZ C477745 n > 50, MNHN n = 15); Lamin, station 3 (17°39′10.0″S, 168°31′16.5″E; MNZ C477746 n > 50, MNHN n > 50); East coast, station 4 (17°38′43.7″S, 168°30′48.0″E; MNZ C477748 n > 50, MNHN n = 15) and White Sands, station 5 (17°48′31.7″S, 168°24′01.4″ E; MNZ C477747 n = 50, MNHN n = 15).

Diagnosis: Due to its variable shell size and shape, *F. brevior* is not easy to distinguish from several species occurring on the other islands of Vanuatu. Among species with a terminal penial lappet and similar shell shape and size, *F. pentecostata* nov. sp. is significantly more slender (Hotelling's T^2-test: $p < 0.01$; t-tests: $p < 0.01$ for sw and sw/sh at type localities), *F. espiritusantoana* Haase, Fontaine & Gargominy 2010 has less denticles on the outer teeth and lacks the U-shaped loop in the male's rectum, and *F. pascali* Haase, Fontaine & Gargominy 2010, anatomically similar to *F. espiritusantoana*, is much broader (Haase et al. 2010a).

Description: Shell (Figs 4a and 5a): Grayish-white to light brown; conical; about 1.75–1.8 times higher than wide; protoconch 0.875–1.125 whorls; aperture little higher than wide.

External features: Variable black epidermal pigmentation, with some animals being nearly unpigmented, mantle rim and head with very little or no pigment.

Mantle cavity: Ctenidium with 18–22 filaments; osphradium short, behind middle of ctenidium, reaching third to half of its length.

Digestive system: Rectum close to pallial oviduct in females but making U-shaped loop at anterior end of prostate in males; radula R: 3-5 1 3-5/3-4 3-4, L: 3-4 1 3-4, M1: 20-23, M2: 29-31.

Female genitalia (Fig. 9a, b): Ovary starting 0.75–1.5 whorls below apex, comprising up to 1.5 whorls; receptaculum seminis pyriform, lying against anterior third to middle of bursa copulatrix, bursal duct short, entering slightly below anterior edge of bursa; length of albumen gland two-third of capsule gland, about 1/3 of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky-white, posterior one opaque white, albumen gland translucent; renal oviduct without any special features, proximal loop bent towards albumen gland.

Male genitalia: Testis lobate starting 0.75–1.5 whorls below apex, comprising 0.75–1.5 whorls, partly overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.25–0.5 (in samples 2 and 5 up to 0.75) whorls proximal to anterior end; penis (Fig. 10a) with terminal lappet.

Remarks: The samples from stations 3 and 5 showed a sexual dimorphism with the females being significantly larger in sh, sw, ah and bww at station 3 and in sw, ah, aw and bww at station 5 (t-tests, $p < 0.05$).

Fluviopupa brevior was the only species we found on Efate. Shell size and shape varied between samples (Fig. 11) and partly between sexes. Genetically, the samples were practically identical and formed a monophylum (Fig. 2).

The species has first been described by Ancey (1905) as *Potamopyrgus brevior* based on a single specimen. This holotype has apparently been lost which is why we designated a neotype for the species now described more comprehensively.

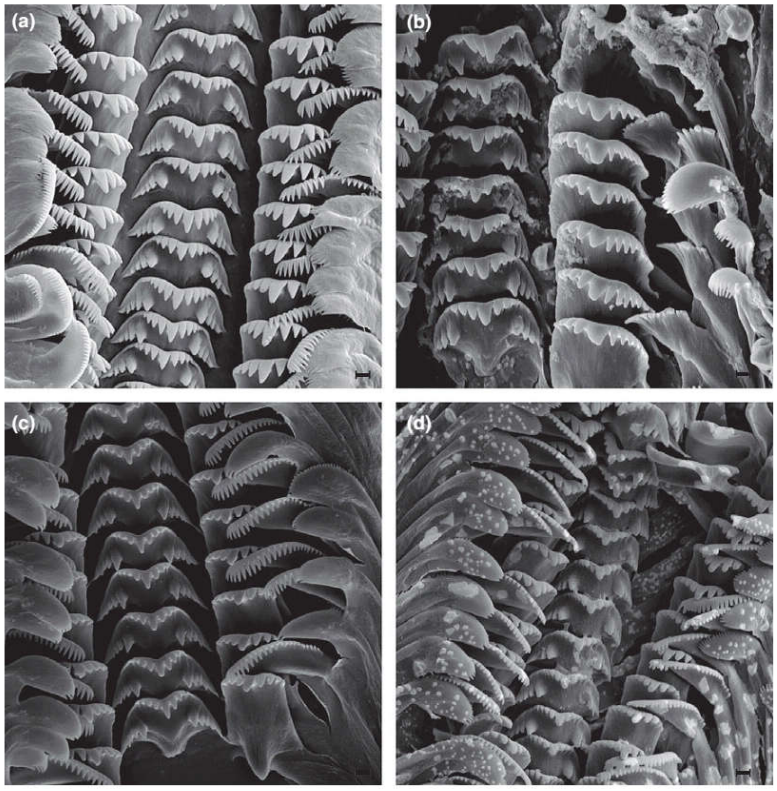

Fig. 7. Radula. (a) *Fluviopupa riva*; (b) *F. malekulana*; (c) *F. pentecostata*; (d) *F. bakeri*; Scale bar = 2 μm

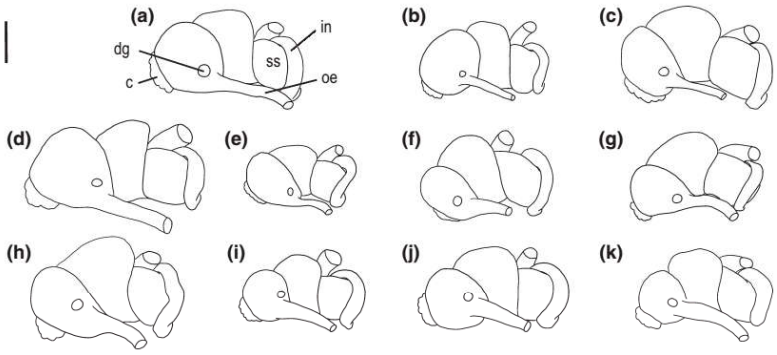

Fig. 8. Stomach. (a) *Fluviopupa brevior*; (b) *F. riva*; (c) *F. erromangoana*; (d) *F. malekulana*; (e) *F. herminae*; (f) *F. freswota*; (g) *F. tangbunia*; (h) *F. pentecostata*; (i) *F. adkinsi*; (j) *F. bakeri*; (k) *F. pikinini*; abbreviations: c caecum, dg opening to digestive gland, in intestine, oe oesophagus, ss style sac; Scale bar = 200 μm

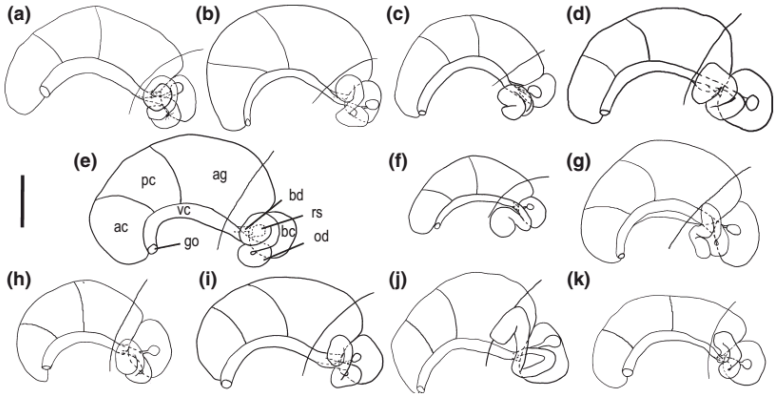

Fig. 9. Distal female genitalia. (a) *Fluviopupa brevior* station 1; (b) *F. brevior* station 2; (c) *F. riva*; (d) *F. erromangoana*; (e) *F. malekulana* station 10; (f) *F. herminae*; (g) *F. freswota*; (h) *F. tangbunia* station 27; (i) *F. pentecostata* station 21; (j) *F. adkinsi*; (k) *F. bakeri* station 13; abbreviations: ac, anterior capsule gland; ag, albumen gland; bc, bursa copulatrix; bd, bursal duct; go, genital opening; od, oviduct; pc, posterior capsule gland; rs, receptaculum seminalis; vc, ventral channel; Scale bar = 200 μm

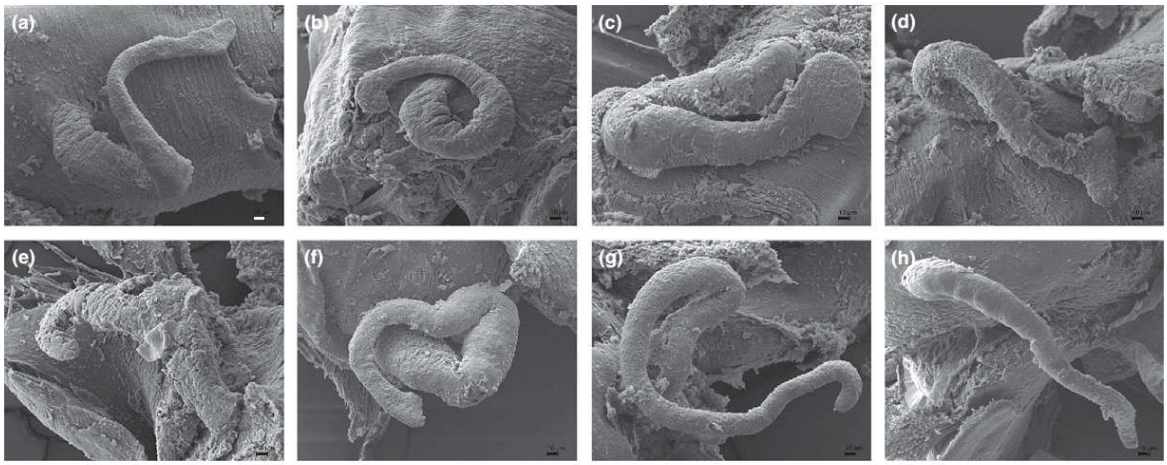

Fig. 10. Penis. (a) *Fluviopupa brevior* station 3; (b) *F. riva*; (c) *F. erromangoana*; (d) *F. malekulana* station 10; (e) *F. freswota*; (f) *F. pentecostata* station 24; (g) *F. adkinsi*; (h) *F. bakeri* station 13; Scale bar = 10 μm

Fluviopupa riva nov. sp

Type material: holotype ZMB Moll 117881; paratypes ZMB Moll 117892 (n = 50), MNZ C477766 (n = 20), MNHN (n = 10).

Type locality: Erromango Island, south-east of the village Dillons Bay, station 6 (18°49′24.3″S, 169°01′36.2″E).

Etymology: The noun riva means river in Bislama, the lingua franca of Vanuatu, and is reminiscent of our 10 fordings of the Williams River before we discovered the type locality.

Diagnosis: *F. riva* nov. sp. is significantly different from its sister species *F. erromangoana* nov. sp. described below (Hotelling's T^2-test: $p < 0.01$). It is smaller in all five dimensions and wider considering the relation of shell height and width (t-tests: $p < 0.01$). The species also differ in the denticle number of the marginal teeth. *F. melissae* Haase, Fontaine & Gargominy 2010 has about the same size and shape but a more ovate aperture and is almost entirely unpigmented (Haase et al. 2010a,b).

Description: Shell (Figs 4b and 5b): Light brown-grey; conical, about 2 mm high and 1.85 times higher than wide; protoconch comprising one whorl; aperture nearly as wide as high.

External features: black epidermal pigmentation, head and mantle rim with less or no pigmentation.

Mantle cavity: Ctenidium with 16–18 filaments; osphradium short, behind middle of ctenidium, reaching half of its length.

Digestive system: Rectum close to pallial oviduct in females but making U-shaped loop at anterior end of prostate in males; radula R: 4 1 4/3 3, L: 4-6 1 4-5, M1: 24-25, M2: 30-32 (Fig. 7a).

Female genitalia (Fig. 9c): Ovary starting 1.25–1.5 whorls below apex, comprising up to one whorl; receptaculum seminis pyriform, lying against anterior third of bursa copulatrix, bursal duct short, entering slightly below anterior edge of bursa; length of albumen gland two-third of capsule gland, about two-third of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky-white, posterior one opaque white, albumen gland translucent milky-white; renal oviduct without any special features.

Male genitalia: Testis lobate starting one whorl below apex, comprising 1.25–1.5 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.25–0.5 whorls proximal to anterior end; penis (Fig. 10b) with terminal lappet.

Remarks: In the phylogenetic analysis (Fig. 2), specimens of *F. riva* nov. sp. formed a well-defined monophyletic group.

Fluviopupa erromangoana nov. sp

Type material: holotype ZMB Moll 117882; paratypes ZMB Moll 117883 (n = 50), MNZ C477767 (n = 25), MNHN (n = 10).

Type locality: Erromango Island, property of Harry and Emili along road between Dillons Bay and Airfield, station 7 (18°46′56.0″S, 169°01′29.3″E).

Etymology: This new species is named after the Island of Erromango where it occurs.

Diagnosis: for distinction from *F. riva* nov. sp. see above.

Description: Shell (Figs 4c and 5c): Light brown-grey; conical, more than 2 mm in height and about 1.8 times higher than wide; protoconch comprising 7/8 whorls; aperture nearly as wide as high.

External features: Black epidermal pigmentation, mantle rim, pallial roof over genital glands, snout and tentacles with less or nor pigmentation.

Mantle cavity: Ctenidium with 17–20 filaments; osphradium short, behind middle of ctenidium, reaching third of its length.

Digestive system: Rectum close to pallial oviduct in females but making U-shaped loop at anterior end of prostate in males; radula R: 4 1 4/4 4, L: 3 1 4, M1: 21-22, M2: 26-27.

Female genitalia (Fig. 9d): Ovary starting 1–1.5 whorls below apex, comprising up to 1.25 whorls; receptaculum seminis pyriform, lying against central third of bursa copulatrix, bursal duct short, entering slightly below anterior edge of bursa; length of albumen gland two-third of capsule gland, about three-fourth of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky-white, posterior one opaque white, albumen gland translucent opaque white; renal oviduct without any special features, proximal loop bent towards albumen gland.

Male genitalia: Testis lobate starting one to 1.25 whorls below apex, comprising one to 1.5 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.5 whorls proximal to anterior end; penis (Fig. 10c) with terminal lappet.

Remarks: The species showed a sexual dimorphism (Hotelling's T^2-test: $p < 0.01$) with females being significantly larger than males in all dimensions (t-tests: $p < 0.01$). In the phylogenetic analysis, specimens of *F. erromangoana* nov. sp. formed the well-supported sister clade to the other species occurring on Erromango, *F. riva* nov. sp. (Fig. 2).

Fluviopupa malekulana nov. sp

Type material: holotype ZMB Moll117883; paratypes ZMB Moll 117894 ($n > 50$), MNZ C477769 ($n = 25$), MNHN ($n = 15$).

Type locality: Malekula Island, Teboune, station 10 (15°59′ 18.2″S, 167°19′37.6″E).

Additional material: Malekula Island; Toros, station 9 (16°01′ 27.6″S, 167°21′22.8″E; MNZ C477749 $n = 14$); Lakatoro, station 8 (16°06′40.8″S, 167°25′13.7″E; MNZ C477750 $n > 50$, MNHN $n = 20$).

Etymology: Malekulana refers to the island of Malekula, where this species occurs.

Diagnosis: *F. malekulana* nov. sp. is significantly larger (Hotelling's T^2-tests: $p < 0.01$) in all shell dimensions (*t*-tests: $p < 0.01$) than *F. freswota* nov. sp. and *F. herminae* nov. sp., both also occurring on Malekula and described below (see also CVA Fig. 11). Moreover, it has a unique hammer-shaped terminal penial lappet. Genetically, *F. malekulana* nov. sp. could not be differentiated from the other two species (Fig. 2). Compared with the equally sized *F. brevior*, *F. malekulana* is in general less pigmented and has a more pronounced terminal penis lappet.

Description: Shell (Figs 4d and 5d): Light brown; conical, height and width quite variable, 1.7–1.75 times higher than wide; protoconch comprising 0.875 to one whorl; aperture always higher than wide.

External features: Intensity of epidermal pigmentation varies, many individuals nearly unpigmented, tentacles, snout and mantle roof over genital glands always less pigmented, mantle rim always unpigmented.

Mantle cavity: Ctenidium with 18–23 filaments; osphradium behind middle of ctenidium reaching one-third of its length.

Digestive system: Rectum close to pallial oviduct in females, making U-shaped loop at anterior end of prostate in males; radula R: 4-1-4/4 4, L: 3-6 1 3-4, M1: 19-20, M2: 26-27 (Fig. 7b).

Female genitalia (Fig. 9e): Ovary starting 0.75–1.25 whorls below apex, comprising up to one whorl; receptaculum seminis globular to pyriform, lying against anterior third of bursa copulatrix, bursal duct short, entering slightly apical to ventral edge of bursa; length of albumen gland two-third of capsule gland, about two-third of albumen gland extending into pallial roof, anterior section of capsule gland translucent white, posterior one opaque yellowish white, albumen gland translucent milky-white; renal oviduct without any special features, proximal loop bent towards albumen gland.

Male genitalia: Testis lobate starting 1–1.25 whorls below apex, comprising 0.75–1.5 whorls, partially covering posterior chamber of stomach; seminal vesicle leaving testis 0.5–0.75 whorls proximal to anterior end; penis (Fig. 10d) with terminal lappet.

Remarks: At station 10, the species showed a sexual dimorphism (Hotelling's T^2-test: $p < 0.05$) with females being significantly larger in sh, aw and bww than males (*t*-test: $p < 0.05$). For some shells collected at station 9, not all parameters could be measured because of calcareous incrustations. Therefore, the smaller appearance of these shells could not be confirmed statistically. Anatomically, there was no difference to the other two samples which is why they were deemed conspecific. In the phylogenetic analysis (Fig. 2), station 8 seemed to be well separated from stations 9 and 10. However, *F. freswota* nov. sp. known from a single site occurred in both clades, indicating recent common ancestry. The short branch lengths of clade I (Fig. 2) obviously have no taxonomic relevance (see Phylogenetic Analysis and Discussion).

Fluviopupa herminae nov. sp

Type material: holotype ZMB Moll 117884; paratype ZMB Moll 117895 ($n = 1$).

Type locality: Malekula Island, Lakatoro, station 8 (16°06′ 40.8″S, 167°25′13.7″E).

Etymology: This species is named after the first author's niece Hermine-Maria who was born in August 2011 at about the time we discovered this species.

Diagnosis: *F. herminae* nov. sp. is significantly smaller (Hotelling's T^2-test: $p < 0.01$) in all shell dimensions than *F. malekulana* nov. sp. and *F. freswota* nov. sp. (*t*-tests: $p < 0.01$) which also occur on Malekula. In comparison with the other species this one is entirely unpigmented except for the eyes and the proximal loop of the renal oviduct is not bent towards the albumen gland.

Description: Shell (Figs 4e and 5e): White to light brown; conical; less than 1.7 mm high and about 1.7 times higher than wide; protoconch comprising 0.875 whorls; aperture higher than wide.

External features: No epidermal pigmentation, eyes black.

Mantle cavity: Ctenidium with 17–18 filaments; osphradium short, behind middle of ctenidium, reaching one-third to one half of its length.

Digestive System: Rectum close to pallial oviduct in females, making U-shaped loop at anterior end of prostate in males; caecum small (Fig. 8e).

Female genitalia (Fig. 9f): Ovary starting 1–1.5 whorls below apex, comprising up to 0.75 whorls; receptaculum seminis pyriform, lying against anterior third of bursa copulatrix, bursal duct short, entering slightly apical to ventral edge of bursa; length of albumen gland two-third of capsule gland, about two-third of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky-white, posterior one opaque white, albumen gland translucent white; renal oviduct without any special features.

Fluviopupa freswota nov. sp

Type material: holotype ZMB Moll 117885; paratypes ZMB Moll 117896 ($n = 20$).

Type locality: Malekula Island, Betel, station 11 (16°02′21.5″ S, 167°22′03.3″E).

Etymology: Freswota, used as noun in apposition, means fresh water or river in Bislama, the lingua franca of Vanuatu.

Diagnosis: This species is significantly smaller than *F. malekulana* nov. sp. and significantly larger than *F. herminae* nov. sp. (see above). The U-shaped loop of the rectum is lying under the anterior half and not under the end of the prostate in contrast to the other species.

Description: Shell (Figs 4f and 5f): Whitish gray to light brown; conical, quite variable in size but always less than 2 mm height, about 1.7 times higher than wide; protoconch comprising 0.875–1 whorl; aperture higher than wide.

External features: Black epidermal pigmentation varying in its intensity with some animals being unpigmented except for the head; pallial roof over genital glands, mantle rim and snout with very little or no pigmentation.

Mantle cavity: Ctenidium with 17–20 filaments; osphradium short, behind middle of ctenidium, reaching one-third of its length.

Digestive system: Rectum close to pallial oviduct in females, making U-shaped loop at anterior half of prostate in males; radula R: 4-5 1 4-5/4 4, L: 4 1 4, M1: 21-22, M2: 27-29; caecum very small (Fig. 8f).

Female genitalia (Fig. 9g): Ovary starting 1–1.5 whorls below apex, comprising up to 1 whorl; receptaculum seminis globular to pyriform, lying against anterior third of bursa copulatrix, bursal duct short, entering slightly apical to ventral edge of bursa; length of albumen gland three-fourth of capsule gland, about

three-fourth of albumen gland extending into pallial roof, anterior section of capsule gland yellowish milky-white, posterior one opaque white, albumen gland translucent milky-white; renal oviduct without any special features, proximal loop bent towards albumen gland.

Male genitalia: Testis lobate starting 1–1.25 whorls below apex, comprising 0.75–1.25 whorls, covering posterior chamber of stomach; seminal vesicle leaving testis 0.375 whorls proximal to anterior end; distal end of penis (Fig. 10e) blunt.

Remarks: In this species, females seemed to be larger than males, but statistical tests were not significant, which may have been caused by the unbalanced sample structure.

Fluviopupa tangbunia nov. sp

Type material: holotype ZMB Moll 117886; paratypes ZMB Moll 117897 (n = 50).

Type locality: Pentecost Island, Litli, station 16 (15°56′18.7″S, 168°12′23.3″E).

Additional material: Pentecost; Waterfall Village, station 18 (15°47′08.5″S, 168°09′56.4″E; MNZ C477751 n = 4); Vetlul, station 20 (15°49′05.2″S, 168°10′22.0″E; MNZ C477752 n = 1); Vanlvibang, station 24 (15°50′58.3″S, 168°11′20.8″E; MNZ C477753 n = 6).

Etymology: This species is named after the Tangbunia bank based on Pentecost, which is dealing in items of customary wealth such as mats, shells or pig tusks rather than monetary currencies.

Diagnosis: This species is smaller and proportionally wider than *F. pentecostata* nov. sp. described below ($p < 0.01$ for Hotelling's T^2-test and t-tests of shell parameters and sh/sw ratio at type localities; see also CVA Fig. 11). Moreover, it is in general less pigmented and has unpigmented tentacles, and the posterior section of the capsule gland is always without a tinge of yellow. Genetically, both species are indistinguishable (Fig. 2).

Description: Shell (Figs 4g and 5g): Very light brown transparent to brown; conical, less than 1.8 mm high, about 1.75 times higher than wide; protoconch comprising 1 whorl; aperture higher than wide.

External features: Variable black epidermal pigmentation, most animals only little pigmented; mantle rim, snout and tentacles unpigmented, pallial roof over genital glands less pigmented.

Mantle cavity: Ctenidium with 17–19 filaments; osphradium in middle of ctenidium, reaching one-third of its length.

Digestive System: Rectum close to pallial oviduct in females, making U-shaped loop at anterior end of prostate in males; radula R: 4-5 1 4-5/3-4 3-4, L: 4-5 1 4-5, M1: 21-23, M2: 25-26.

Female genitalia (Fig. 9h): Ovary starting 1–1.25 whorls below apex, comprising up to 1 whorl; receptaculum seminis globular to pyriform, lying against anterior third of bursa copulatrix, bursal duct short, entering slightly apical to ventral edge of bursa; length of albumen gland three-fourth of capsule gland, about two-third of albumen gland extending into pallial roof, anterior section of capsule gland milky-white, posterior one opaque white, albumen gland translucent milky-white; renal oviduct without any special features, proximal loop bent towards albumen gland.

Male genitalia: Testis lobate starting 0.75–1 whorl below apex, comprising 1–1.25 whorls, covering posterior chamber of stomach; seminal vesicle leaving testis 0.25 whorls proximal to anterior end; terminal end of penis with terminal lappet.

Fluviopupa pentecostata sp. nov

Type material: Holotype ZMB Moll117887; paratypes ZMB Moll 117898 (n > 50), MNZ C477765 (n > 50), MNHN (n = 15).

Type locality: Pentecost Island,Ranwas, station 17 (15°58′03.6″S, 168°15′53.3″E).

Additional material: Pentecost Island; Salap, station 15 (15°56′55.6″S, 168°11′48.0″E; MNZ C477763 n = 8); Litli, station 16 (15°56′18.7″S, 168°12′23.3″E; MNZ C477754 n = 18); Vanibunger, station 25 (15°49′09.3″S, 168°10′40.3″E; MNZ C477755 n > 50, MNHN n = 15); Ravesedle, station 21 (15°51′01.3″S, 168°11′05.4″E; MNZ C477756 n > 50, MNHN n = 15) and 22 (15°51′02.2″S, 168°11′06.4″E; MNZ C477757 n = 39, MNHN n = 15); Vasovee, station 23 (15°51′01.7″S, 168°11′09.9″E; C47758 n = 10); Vanlvibang, station 24 (15°50′58.3″S, 168°11′20.8″E; MNZ C477759 n = 19); Waterfall Village, station 19 (15°47′07.6″S, 168°09′53.8″E; MNZ C477760 n = 25).; Vetlul, station 20 (15°49′05.2″S, 168°10′22.0″E; MNZ C477761 n = 12).

Etymology: This species is named after the island of its occurrence Pentecost.

Diagnosis: For distinction from *F. tangbunia* nov. sp., see above.

Description: Shell (Figs 4h and 5h): Light to dark brown; conical, quite variable in size about 1.85–1.9 times higher than wide; protoconch comprising 0.875–1.125 whorls; aperture higher than wide.

External features: Variable black epidermal pigmentation, which covers the whole visible part of the visceral mass; mantle rim, snout and pallial roof over genital glands with less or no pigmentation.

Mantle cavity: Ctenidium with 17–21 filaments; osphradium short behind middle of ctenidium reaching one-third to half of its length.

Digestive System: Rectum close to pallial oviduct in females, making U-shaped loop at anterior end of prostate in males; radula R: 4-5 1 4-5/3- 4 3-4, L: 4-5 1 4-5, M1: 20-24, M2: 29-35 (Fig. 7c).

Female genitalia (Fig. 9i): Ovary starting 0.75–1.5 whorls below apex, comprising up to 1.5 whorls; receptaculum seminis globular to pyriform, lying against anterior third of bursa copulatrix, bursal duct short, entering slightly apical to ventral edge of bursa; length of albumen gland two-third of capsule gland, about two-third of albumen gland extending into pallial roof, anterior section of capsule gland milky-white, posterior one opaque white to yellowish, albumen gland translucent milky-white; renal oviduct without any special features, proximal loop bent towards albumen gland.

Male genitalia: Testis lobate starting 0.75–1.25 whorls below apex, comprising 0.75–1.75 whorls, covering posterior chamber of stomach; seminal vesicle leaving testis 0.375–0.5 whorls proximal to anterior end; penis (Fig. 10f) with terminal lappet.

Remarks: In the type locality and samples taken from station 19 and 25, this species showed a sexual dimorphism (Hotelling's T^2-test $p < 0.05$). Males were significantly smaller than females in all shell dimensions (t-tests: $p < 0.05$). At stations 16 and 20, the five shell dimensions sh, sw, ah, aw and bww indicated a sexual dimorphism (t-tests: $p < 0.05$), but the Hotelling's T^2-tests were not significant, which may have been caused by the unbalanced sample structure with 12 females and 3 males at station 16 and the very small sample size (n = 8) at station 20. For the sample taken at station 22, only sh, aw and bww showed a sexual dimorphism (t-tests: $p < 0.05$). At stations 21 and 24, no sexual dimorphism was found. For samples taken from stations 15 and 23, statistical tests could not be performed due to lack of material.

Fluviopupa adkinsi nov. sp

Type material: Holotype ZMB Moll 117888; paratypes ZMB Moll 117899 (n > 50), MNZ C477768 (n = 25), MNHN (n = 15).

Type locality: Gaua Island, track to Lake Letas starting at airfield, station12 (14°14′45.5″S, 167°33′55.3″E).

Etymology: This species is dedicated to John Adkins, who, in drenching rain, guided us to Lake Letas, the crater lake of Gaua.

Diagnosis: This species is distinguished from *F. bakeri* nov. sp. co-occurring on the island of Gaua and described below by its larger and more slender shell ($p < 0.01$ for Hotelling's T^2-test and *t*-tests of all five shell dimensions and the sh/sw ratio). Genetically, the species from Gaua are not differentiated (Fig. 2). Regarding species of similar size, *F. pentecostata* nov. sp. and *F. brevior* nov. sp. have a terminal penis lappet and *F. espiritusantoana* has a higher number of denticles on the outer marginal tooth.

Description: Shell (Figs 4i and 5i): Brown; conical, more than 2.1 mm high and about 1.9 times higher than wide; protoconch comprising 0.875 to one whorl; aperture higher than wide.

External features: Epidermis black except for snout, tentacles and mantle rim.

Mantle cavity: Ctenidium with 19–22 filaments; osphradium short, behind middle of ctenidium, reaching one-third of its length.

Digestive system: rectum close to pallial oviduct in females but making U-shaped loop at anterior end of prostate in males; radula R: 4 1 4/4 4, L: 4 1 4, M1: 21-23, M2:28-30.

Female genitalia (Fig. 9j): Ovary starting 1.25 whorls below apex, comprising up to 1.25 whorls; receptaculum seminis pyriform to elongate, lying against central third of bursa copulatrix, bursal duct short, entering slightly apical to ventral edge of bursa; length of albumen gland three-fourth of capsule gland, about three-fourth of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky-white, posterior one opaque white, albumen gland translucent white; renal oviduct without any special features, proximal loop bent towards albumen gland.

Male genitalia: Testis lobate starting 0.75–1.25 whorls below apex, comprising 1.25–1.5 whorls, reaching posterior chamber of stomach; seminal vesicle leaving testis 0.625–0.5 whorls proximal to anterior end; distal end of penis (Fig. 10g) blunt.

Remarks: This species showed a sexual dimorphism with females being significantly larger than males (Hotelling's T^2-test: $p < 0.01$) in all five shell dimensions (*t*-tests: $p < 0.01$, for aw $p < 0.05$).

Fluviopupa bakeri nov. sp

Synonymy: *Potamopyrgus* sp. – Baker 1929, Man and Animals in the New Hebrides: p. 148.

Fluviopupa brevior – Solem 1959; Fieldiana Zoology 43: p. 195, pl. 6, Fig. 11; pl. 27, Figs 5 and 6.

Type material: Holotype ZMB Moll 117889; paratypes ZMB Moll 117900 ($n > 50$), MNZ C477764 ($n = 25$), MNHN ($n = 15$).

Type locality: Gaua Island, Lake Letas, station 13 (14°15′42.4′′S, 167°32′21.9″E).

Additional material: Gaua Island, Medsamavud, station 14 (14°15′48.3″S, 167°36′15.6″E; MNZ C477762 $n = 15$).

Etymology: This species is named after the British biologist John R. Baker who was the first to describe the occurrence of a *Potamopyrgus*-like snail occurring in the crater lake on Gaua without naming or describing it in detail (Baker 1929).

Diagnosis: For differentiation from *F. adkinsi* nov. sp., see above.

Description: Shell (Figs 4j and 5j): Brown; conical, about 2 mm in height and about 1.75–1.8 times higher than wide; protoconch comprising 7/8 to one whorl; aperture slightly higher than wide.

External features: Variable black epidermal pigmentation, snout and mantle rim nearly unpigmented.

Mantle cavity: Ctenidium with 18–21 filaments; osphradium short, behind middle of ctenidium, reaching one-third of its length.

Digestive system: Rectum close to pallial oviduct in females but making U-shaped loop at anterior end of prostate in males; radula R: 4-5 1 4-5/4 4, L: 4 1 5, M1: 23-24, M2:28-30 (Fig. 7d).

Female genitalia (Fig. 9k): Ovary starting 1.25–1.5 whorls below apex, comprising up to 0.75 whorls; receptaculum seminis pyriform to elongate, lying against central third of bursa copulatrix, bursal duct short, entering slightly apical to ventral edge of bursa; length of albumen gland three-fourth of capsule gland, about three-fourth of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky-white, posterior one opaque white, albumen gland translucent white; renal oviduct without any special features, proximal loop bent towards kidney.

Male genitalia: Testis lobate starting 0.75–1 whorl below apex, comprising 0.75–1.25 whorls, reaching posterior chamber of stomach; seminal vesicle leaving testis 0.25–0.375 whorls proximal to anterior end; distal end of penis (Fig. 10h) blunt.

Remarks: The sample from station 13 showed a sexual dimorphism in sh and bww (*t*-test: for sh $p < 0.01$, for bww $p < 0.05$), for station 14 tests could not be performed due to the lack of material.

Fluviopupa pikinini nov. sp

Type material: Holotype ZMB Moll 117890; paratypes ZMB Moll 117901 ($n = 1$).

Type locality: Gaua Island, Medsamavud, station 14 (14°15′ 48.3″S, 167°36′15.6″E).

Etymology: Pikinini, used as noun in apposition, is the Bislama word for child and refers to the relatively small size of this species.

Diagnosis: With a height of less than 1.7 mm, this species is significantly smaller than the other two species occurring on Gaua, *F. bakeri* nov. sp. and *F. adkinsi* nov. sp. (see Remarks).

Description: Shell (Fig. 4g): Colour varies from whitish gray to light brown; conical, about 1.6 mm high and 1.7 times higher than wide; protoconch comprising one whorl; aperture about as wide as high.

External features: Partially black epidermal pigmentation.

Mantle cavity: Ctenidium with 15 filaments ($n = 1$).

Remarks: Statistical comparisons with other species were not possible, because only four individuals were found. This is also the reason why the anatomical description remained incomplete. Genetically, this species is not distinct from the sympatric *F. bakeri* nov. sp.

Morphometrics

Despite the apparent similarity of the shells (Figs 4 and 5), the plot of the CVA based on five shell measurements of population samples illustrates yet considerable differentiation (Fig. 11). The MANOVA was highly significant ($p < 0.01$). Hotelling's pairwise *post hoc* T^2 comparisons are summarized in Table 1 and reported in the Diagnoses of the species descriptions above if taxonomically relevant. However, it is also obvious that shell shape alone cannot be used to identify all species. Anatomical and genetic data have to be taken into consideration as well.

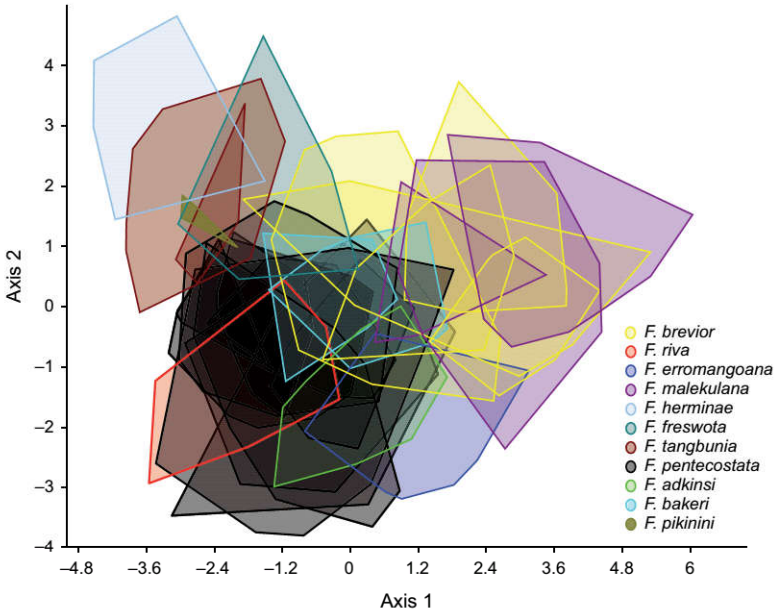

Fig. 11. Canonical variates analysis based on five shell measurements; populations representing the same species are shown in equal colours

Discussion

Our analyses revealed that tateids have reached Vanuatu at least as early as in the late Pliocene, indicating unambiguously that Santo as the first island where they could establish has at least partly remained above the sea level for more than 3 My. This even predates the assumptions of Taylor (1992) and Robin (1993) who did not rule out that Santo has re-emerged during the earliest Pleistocene. Our analyses thus significantly increases the time frame assumed by Hamilton et al. (2010) for the colonization of Vanuatu and the evolution of its flora and fauna *in situ*. The general relevance of these conclusions cannot be assessed, yet, because we are not aware of many other phylogenetic analysis concerning taxa from Vanuatu. In an analysis of the trichopteran genus *Apsilochorema*, the mean time of arrival on Vanuatu was estimated to 5.4 Mya within the highest posterior density interval of 12–2 Mya (Strandberg and Lohanson 2010). This time frame covers more than the ambiguities reported in the geological literature, which remained undiscussed in this paper, though, and would thus still support also Hamilton et al.'s (2010) assumption of a very young subaerial history. Our Bayesian estimate of the origin of the tateids from Vanuatu of 3.01 ± 0.77 Mya was much more accurate but still encompassed the early Pleistocene at the younger end of the density interval at 2.25 Mya. However, considering that islands, once emergent, still have to 'evolve' before they can support biota, we can safely assume that the subaerial history of Santo began considerably earlier already in the late Pliocene. The *COI*-distance-based estimate for node 3 of 4.81 ± 0.20 Mya even suggested a much earlier time of emergence and did not even overlap with the Bayesian estimate in contrast to the younger nodes. This may indeed be an overestimate balanced in the Bayesian analysis by the substitution rates of the other two gene fragments.

According to our reconstruction, Erromango was the next island to be settled after Santo in the early Pleistocene about 2 Mya, which fits into the geological scenario of Neef and Hendy (1988) and means that Erromango is much older than the other central chain islands. Erromango may well have served as stepping stone towards the southern-more and definitely much younger islands Tanna and Aneityum. Yet, there is no record of tateids from either island. We could not visit Tanna in 2011 and on Aneityum the northern part with presumably more suitable habitats (personal communication of locals) could not be surveyed due to bad weather conditions, while in the southern part, no truncatelloidean gastropods where found.

Eastern belt and central chain radiation with the inclusion of species from Malekula have to be of Pleistocene or even Holocene origin as indicated by the starburst-like clade I with short branch lengths lacking a clear geographical structure. Morphological and anatomical differentiations are limited as well. The origin of this radiation is certainly Santo. Scenarios suggesting single colonization events for Malekula, Efate, Pentecost, Gaua and the Torres Islands and somewhat relaxed assumptions were rejected. Only the samples from Efate seem to descend from a single colonizer. For the other islands, multiple introductions and possibly also multiple sources have to be inferred, although the representatives from Pentecost and Gaua (like those from Efate and Erromango) can be characterized by penial morphology suggesting reciprocal monophyly.

The fact that all species found on Malekula are part of the starburst of clade I suggests that Malekula has surfaced only in the rapid uplift phase in the late Quaternary, which has affected the entire region (Taylor 1992). However, we have surveyed only a small part of this large island and may have missed older lineages.

The most plausible vectors of the snails are probably birds (see Haase et al. 2010b for a recent discussion concerning truncatelloidean gastropods). Van Leeuwen et al. (2012a) and Wada et al. (2012) have demonstrated that small snails can even survive the passage through the gut of water fowl. Considering the relatively small distances between the islands, one can safely assume that birds have frequently crossed the open water and thereby occasionally transported snails and other invertebrates (Frisch et al. 2007; Green et al. 2008; Van Leeuwen et al.

Table 1. Hotelling's T^2-tests comparing morphometric data. p-values of pairwise comparisons, Bonferroni corrected. For species represented by more than one sample, the number of the sampling locality is indicated

	F. adkinsi	*F. bakeri* 12	*F. bakeri* 13	*F. brevior* 1	*F. brevior* 2	*F. brevior* 3	*F. brevior* 4	*F. brevior* 5	*F. erromangoana*	*F. freswota*	*F. herminae*	*F. malekulana* 10	*F. malekulana* 8	*F. malekulana* 9	*F. pentecostata* 19	*F. pentecostata* 20	*F. pentecostata* 15	*F. pentecostata* 24	*F. pentecostata* 25	*F. pentecostata* 16	*F. pentecostata* 17	*F. pentecostata* 21	*F. pentecostata* 22	*F. pentecostata* 23	*F. pikinini*	*F. riva*	*F. tangbunia* 24
F. bakeri 12	***																										
F. bakeri 13	**	ns																									
F. brevior 1	***	***	*																								
F. brevior 2	***	***	***	ns																							
F. brevior 3	***	***	***	ns	ns																						
F. brevior 4	***	***	***	ns	ns	ns																					
F. brevior 5	***	**	ns	ns	ns	***	**																				
F. erromangoana	***	***	***	***	***	***	***	***																			
F. freswota	***	***	**	***	***	***	***	***	***																		
F. herminae	***	***	***	***	***	***	***	***	***	ns																	
F. malekulana 10	***	***	***	***	ns	ns	***	***	***	***	***																
F. malekulana 8	***	***	***	***	*	**	***	***	***	***	***	ns															
F. malekulana 9	**	*	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns														
F. pentecostata 19	***	***	*	***	***	***	***	**	***	***	***	***	***	ns													
F. pentecostata 20	*	***	ns	***	***	***	***	ns	***	***	***	***	***	ns	ns												
F. pentecostata 15	ns	ns	ns	ns	*	**	**	ns	*	**	ns	***	**	ns	ns	ns											
F. pentecostata 24	***	***	ns	***	***	***	***	**	***	***	***	***	***	*	ns	ns	ns										
F. pentecostata 25	***	***	***	***	***	***	***	***	***	***	***	***	***	ns	ns	ns	ns	ns									
F. pentecostata 16	***	***	ns	***	***	***	***	*	***	***	***	***	***	ns	ns	ns	ns	ns	ns								
F. pentecostata 17	***	***	**	***	***	***	***	***	***	***	***	***	***	ns	ns	ns	ns	ns	ns	ns							
F. pentecostata 21	***	***	ns	***	***	***	***	ns	***	***	***	***	***	ns	ns	ns	ns	ns	ns	ns	ns						
F. pentecostata 22	***	***	ns	**	***	***	***	ns	***	***	***	***	***	ns	ns	ns	ns	ns	ns	ns	ns	ns					
F. pentecostata 23	***	**	ns	***	***	***	***	ns	***	ns	ns	***	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns				
F. pikinini	**	ns	ns	*	**	***	**	ns	*	ns	ns	***	***	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns			
F. riva	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***	ns	ns	ns	**	**	**	*	***	ns	ns		
F. tangbunia 24	***	**	ns	***	***	***	***	*	***	ns	ns	***	***	ns	ns	ns	ns	ns	**	ns	*	ns	ns	ns	ns	ns	
F. tangbunia 16	***	***	***	***	***	***	***	***	***	ns	ns	***	***	***	***	***	**	***	***	***	***	***	***	ns	ns	***	ns

Significance values: ***p < 0.001; **p < 0.01; *p < 0.05; ns, not significant; *F.*, *Fluviopupa.*

2012b). In recent times, also humans have to be considered as transporters, for example, through the transplantation of plants cultivated in or at springs and streams. Yet, the young subaerial age of these islands and the short branches within the clade concerned also suggest that the picture of colonization events is probably blurred through incomplete lineage sorting.

Incomplete lineage sorting is probably also the main reason for the obvious taxonomic incongruence, which, however, is typical for young and rapid radiations (Funk and Omland 2003). Confusion and mislabelling of samples during laboratory work can be excluded. The taxonomic incongruence had already been realized after a preliminary analysis during data collection. Yet, adding specimens did not change the picture. In addition, clade I is not entirely chaotic. There are several subclades which are at least partly taxonomically and biogeographically coherent, and the topologies of the separate analyses only differ in their resolution. A systematic handling error concerning more than a few samples should have been more destructive.

A more sever taxonomical problem is caused by *F. espiritusantoana* and *F. pascali* from Santo, which are represented both in clades I and III. In clade I, *F. espiritusantoana* formed a larger sister subclade to the remaining clade members including another two specimens belonging to this species. Not only was *F. espiritusantoana* distributed across three clades, but also all but one individual samples were split. The same holds for *F. pascali*, which, however, has been known only from a single locality (Haase et al. 2010a). According to our dating, clades I and III were separated by c. 2.2 My. The problem could be reduced to a single species if we argued that *F. pascali* were an aberrant, morphologically well-defined form of *F. espiritusantoana* (see Haase et al. 2010a), which is, however, not convincing. Similarly, unconvincing is the assumption that the lineages of *F. es-*

piritusantoana represent cryptic species occurring sympatrically, although Haase et al. (2010a) did not rule out that the populations in the north and the centre of Santo represent two different species. Alternatively, *F. espiritusantoana* has to be considered the paraphyletic stem species of almost the entire radiation including the species from Erromango and clade I. The preservation of the unusually divergent lineages would probably be a consequence of repeated bottlenecks the species (and possibly also *F. pascali*) went through on a few islands remaining during repeated periods of partial submergence of Santo during the Pliocene. This would represent a case of ancient incomplete lineage sorting as described for radiations of cichlid fishes or crotaphydid lizards (Takahashi et al. 2001; McGuire et al. 2007). Provided *F. espiritusantoana* is indeed a single species, we would expect recombination between nc and mt lineages. We observed only a single case, where an individual switched position among the subclades of clade I between mt and *ITS2*-trees. Yet, our analysis included only two specimens per site. Therefore, disentangling the alternative hypotheses of cryptic species versus old stem species would require a far more comprehensive analysis and more extensive collecting of fresh material.

The phylogenetic position of *F. narii* was instable. However, according to the most common positions in the bootstrap replicates either as part of clade III or as sister species to all other samples from Vanuatu, it most likely descends from a very deep split.

Considering that fauna and flora of Vanuatu have predominantly western affinities (reviewed by Munzinger 2009; Siméoni 2009; Hamilton et al. 2010), it was surprising that the New Zealand taxa were closer related to the species from Vanuatu than *H. winstonefi* from New Caledonia and *T. huonensis* from Australia. However, considering that there are at least two more lineages of Tateidae occurring on New Caledonia that are unrelated to *Hemistomia* (Haase and Bouchet 1998) and that representatives from the majority of regions harbouring tateids were not included in our analyses, we refrain from a more comprehensive discussion of the potential origin of the species of the Vanuatu archipelago. This will be subject of a forthcoming analysis.

Acknowledgements

The authors like to thank a countless number of field guides and hosts, in particular Lissian Tabi from Pentecost, Donald Lovo from Erromango and Rodolphe Malero from Malekula. Touasi Tiwok from the Department of Environmental Protection and Conservation in Port Vila issued the research permit in accordance with the Convention on Biological Diversity. Philippe Bouchet and Benoît Fontaine from the Museum National d′ Histoire Naturelle Paris are acknowledged for providing material from Santo and the Torres Islands and Bruce Marshall from the Museum of New Zealand Te Papa Tongarewa for local samples. At the University of Greifswald, we thank Rabea Schlüter, head of the Laboratory of Electron Microscopy, and our technician Christel Meibauer, who helped with DNA work. Comments of two reviewers helped to improve an earlier version of the manuscript. Financial support was received from the Deutsche Forschungsgemeinschaft (Grant HA4752/2-1).

References

Akimoto K (1994) Cenozoic benthic foraminiferal biostratigraphy, paleobathymetry, paleoenvironments and paleoceanography of the New Hebrides Island arc and north d'Entrecasteaux Ridge area. In: Greene HG, Collot J-Y, Stokking LB et al. (eds), Proeedings of the Ocean Drilling Program, Scientific Results **134**. College Station, TX (Ocean Drilling Program), pp. 265–291.

Ancey CF (1905) Remarks on some land and fresh-water shells from the New Hebrides, with description on new species. Nautilus **19**:42–46.

Bonneville A, Le Suavé R, Audin L, Clouard V, Dosso L, Gillot PY, Janney P, Jordahl K, Maamaatuaiahutapu K (2002) Arago seamount: the missing hotspot found in the Austral Islands. Geology **30**:1023–1026.

Bouchet P, Le Guyader H, Pascal O (2011) The Natural History of Santo. Publications scientifiques du Muséum national d'histoire naturelle, Paris.

Cannone J, Subramanian S, Schnare M, Collett J, D'Souza L, Du Y, Feng B, Lin N, Madabusi L, Muller K, Pande N, Shang Z, Yu N, Gutell R (2002) The Comparative RNA Web (CRW) Site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics **3**:2. [Correction: *BMC Bioinformatics*. **3**:15.]

Cook LM, Cameron RAD, Lace LA (1990) Land snails of eastern Madeira: speciation, persistence and colonization. Proc R Soc Lond B Biol Sci **239**:35–79.

Criscione F, Ponder WF (2013) A phylogenetic analysis of rissooidean and cingulopsoidean families (Gastropoda: Caenogastropoda). Mol Phylogenet Evol **66**:1075–1082.

Dayrat B, Conrad M, Balayan S, White TR, Albrecht C, Golding R, Gomes SR, Harasewych MG, de Frias Martins AM (2011) Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): new insights from increased taxon sampling. Mol Phylogenet Evol **59**:425–437.

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol **29**:1969–1973.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol **3**:294–299.

Frisch D, Green AJ, Figuerola J (2007) High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. Aquat Sci **69**:568–574.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu Rev Ecol Evol Syst **34**:397–423.

Gillespie RG, Claridge EM, Goodacre SL (2008) Biogeography of the fauna of French Polynesia: diversification within and between a series of hot spot archipelagos. Philos Trans R Soc Lond B Biol Sci **363**:3335–3346.

Green AJ, Jenkins KM, Bell D, Morris PJ, Kingsford RT (2008) The potential role of waterbirds in dispersing invertebrates and plants in arid Australia. Freshw Biol **53**:380–392.

Greene HG, Collot J-Y, Fisher MA, Crawford AJ (1994) Neogene Tectonic Evolution of the New Hebrides Island Arc: A Review Incorporating ODP Drilling Results. In: Greene HG, Collot J-Y, Stokking LB et al. (eds), Proeedings of the Ocean Drilling Program, Scientific Results **134**: College Station, TX (Ocean Drilling Program): 19–46.

Haase M (2005) Rapid and convergent evolution of parental care in hydrobiid gastropods from New Zealand. J Evol Biol **18**:1076–1086.

Haase M (2008) The radiation of hydrobiid hastropods in New Zealand: a revision including the description of new species based on morphology and mtDNA sequence information. Syst Biodivers **6**:99–159.

Haase M, Bouchet P (1998) Radiation of crenobiontic gastropods on an ancient continental island: the *Hemistomia*-clade in New-Caledonia (Gastropoda: Hydrobiidae). Hydrobiologia **367**:43–129.

Haase M, Bouchet P (2006) The radiation of hydrobioid gastropods (Caenogastropoda, Rissooidea) in Ancient Lake Poso, Sulawesi. Hydrobiologia **556**:17–46.

Haase M, Gargominy O, Fontaine B (2005) Rissooidean freshwater gastropods from the middle of the Pacific: the genus *Fluviopupa* on the Austral Islands (Caenogastropoda). Molluscan Res **25**:145–163.

Haase M, Marshall B, Hogg I (2007) Disentangling causes of disjunction on the South Island of New Zealand: the Alpine fault hypothesis of vicariance revisited. Biol J Linn Soc Lond **91**:361–374.

Haase M, Fontaine B, Gargominy O (2010a) Rissooidean freshwater gastropods from the Vanuatu archipelago. Hydrobiologia **637**:53–71.

Haase M, Naser MD, Wilke T (2010b) *Ecrobia grimmi* in brackish Lake Sawa, Iraq: indirect evidence for long-distance dispersal of hydrobiid gastropods (Caenogastropoda: Rissooidea) by birds. J Mollusc Stud **76**:101–105.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **41**:95–98.

Hamilton A, Klein E, Austin C (2010) Biogeographic breaks in Vanuatu, a nascent oceanic archipelago 1. Pac Sci **64**:149–159.

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron **4**:9.

Holland BS, Cowie RH (2009) Land snail models in island biogeography: a tale of two snails. Am Malacol Bull **27**:59–68.

Keppel G, Lowe AJ, Possingham HP (2009) Changing perspectives on the biogeography of the tropical South Pacific: influences of dispersal, vicariance and extinction. J Biogeogr **36**:1035–1054.

Koetschan C, Förster F, Keller A, Schleicher T, Ruderisch B, Schwarz R, Müller T, Wolf M, Schultz J (2010) The ITS2 database III—sequences and structures for phylogeny. Nucleic Acids Res **38**:D275–D279.

Kroenke LW (1996) Plate tectonic development of the western and southwestern Pacific Mesozoic to the present. In: Keast A, Miller SE (eds), The Origin and Evolution of Pacific Islands Biotas, New Guinea to Eastern Polynesia: Patterns and Processes. SPB Academic Publishing, Amsterdam, pp 19–34.

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol **29**:1695–1701.

McGuire JA, Linkem CW, Koo MS, Hutchison DW, Lappin AK, Orange DI, Lemos-Espinal J, Riddle BR, Jaeger JR (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. Evolution **61**:2879–2897.

Nation JL (1983) A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. Biotech Histochem **6**:347–351.

Neall VE, Trewick SA (2008) The age and origin of the Pacific islands: a geological overview. Philos Trans R Soc B **363**:3293–3308.

Neef G, Hendy C (1988) Late Pleistocene-Holocene acceleration of uplift rate in southwest Erromango Island, southern Vanuatu, South Pacific: relation to the growth of the Vanuatuan Mid Sedimentary Basin. J Geol **96**:481–494.

Oliverio M, Mariottini P (2001) Contrasting morphological and molecular variation in *Coralliophila meyendorffii* (Muricidae, Coralliophilinae). J Mollusc Stud **67**:243–246.

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Gabowski G (1991) The Simple Fool's Guide to PCR. Dept. of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu.

Pfenninger M, Vela E, Jesse R, Arantzazu Elejalde M, Liberto F, Magnin F, Martinez-Orti A (2010) Temporal speciation pattern in the western Mediterranean genus *Tudorella* P. Fischer, 1885 (Gastropoda, Pomatiidae) supports the Tyrrhenian vicariance hypothesis. Mol Phylogenet Evol **54**:427–436.

Ponder WF (1981) *Posticobia norfolkensis* (Sykes), an apparently-extinct, fresh-water snail from Norfolk Island (Gastropoda: Hydrobiidae). Proc Linn Soc NSW **105**:17–21.

Ponder WF (1982) Hydrobiidae of Lord Howe Island (Mollusca: Gastropoda: Prosobranchia). Mar Freshw Res **33**:89–159.

Ponder W, Clark G (1988) A morphological and electrophoretic examination of *Hydrobia Buccinoides*, a variable brackish-water gastropod from temperate Australia (Mollusca, Hydrobiidae). Aust J Zool **36**:661–689.

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol **25**:1253–1256.

Robin C, Australian Geological Survey Organisation (1993) The Geology, Volcanology, Petrology-geochemistry, and Tectonic Evolution of the New Hebrides Island Arc, Vanuatu: IAVCEI, Canberra 1993: Excursion Guide. Record 1993/059. Australian Geological Survey Organisation, Canberra, ACT.

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**:1572–1574.

Shimodaira H, Hasegawa M (2001) CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics **17**:1246–1247.

Siméoni P (2009) Atlas du Vanouatou (Vanuatu). Éditions Géo-consulte, Port Vila, Vanuatu.

Smith SA, Dunn CW (2008) Phyutility: a phyloinformatics tool for trees, alignments and molecular data. Bioinformatics **24**:715–716.

Solem A (1959) Systematics of the land and fresh-water Mollusca of the New Hebrides. Fieldiana Zool **43**:1–238.

Starmühlner F (1976) Beiträge zur Kenntnis der Süßwasser-Gastropoden pazifischer Inseln. Ann Nat Hist Mus Wien Ser B Bot Zool **80**:473–656.

Stocsits RR, Letsch H, Hertel J, Misof B, Stadler PF (2009) Accurate and efficient reconstruction of deep phylogenies from structured RNAs. Nucleic Acids Res **37**:6184–6193.

Strandberg J, Lohanson KA (2010) The historical biogeography of *Apsilochorema* (Trichoptera, Hydrobiosidae) revised, following molecular studies. J Zool Syst Evol Res **49**:110–118.

Swofford DL (2003) Paup*. Phylogenetic Analysis Using Parsimony (*and other methods).Version 4. Sinauer Associates, Sunderland, MA.

Takahashi K, Terai Y, Nishida M, Okada N (2001) Phylogenetic relationships and ancient incomplete lineage sorting among cichlid fishes in Lake Tanganyika as revealed by analysis of the insertion of retroposons. Mol Biol Evol **18**:2057–2066.

Taylor FW (1992) Quaternary Vertical Tectonics of the Central New Hebrides Island Arc. In: Collot J-Y, Greene HG, Stokking LB et al. (eds), Proceedings of the Ocean Drilling Program, Initial Reports **134**: College Station, TX (Ocean Drilling Program): 33–42.

Taylor FW, Jouannic C, Bloom AL (1985) Quaternary uplift of the Torres Islands, Northern new hebrides front arc – comparison with Santo and Malekula Islands, Central new hebrides frontal arc. J Geol **93**:419–438.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **22**:4673–4680.

Upton DE, Murphy RW (1997) Phylogeny of the side-blotched lizards (Phrynosomatidae: Uta) based on mtDNA sequences: support for a midpeninsular seaway in Baja California. Mol Phylogenet Evol **8**:104–113.

Van Leeuwen CHA, van der Velde G, van Groenendael JM, Klaassen M (2012a) Gut travellers: internal dispersal of aquatic organisms by waterfowl. J Biogeogr **39**:2031–2040.

Van Leeuwen CHA, van der Velde G, van Lith B, Klaassen M (2012b) Experimental quantification of long distance dispersal potential of aquatic snails in the gut of migratory birds. PLoS ONE **7**:e32292.

Wada S, Kawakami K, Chiba S (2012) Snails can survive passage through a bird's digestive system. J Biogeogr **39**:69–73.

Wilke T, Haase M, Hershler R, Liu H-P, Misof B, Ponder W (2013) Pushing short DNA fragments to the limit: phylogenetic relationships of "hydrobioid" gastropods (Caenogastropoda: Rissooidea). Mol Phylogenet Evol **66**:715–736.

Xia X, Xie Z (2001) DAMBE: software package for data analysis in molecular biology and evolution. J Hered **92**:371–373.

Zwickl DJ (2006) Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion. Ph.D. dissertation, The University of Texas at Austin, Austin, TX.

Appendix 1. Shell morphometry and sex ratio

Species/locality/n/sex ratio		sh	sw	ah	aw	bww	Sh/sw	Ah/aw	Sw/bww	Sh/ah	Sw/aw	w
F. brevior	Holotype	2.31	1.29	0.90	0.81	1.16	1.79	1.11	1.11	2.56	1.59	4.5
Station 1	Mean	2.13	1.20	0.84	0.75	1.09	1.78	1.13	1.10	2.54	1.61	4.4
N = 20	Median	2.14	1.20	0.84	0.75	1.09	1.79	1.13	1.10	2.54	1.61	4.375
8f/12m	Min	1.97	1.10	0.74	0.69	1.01	1.66	1.04	1.00	2.41	1.51	4.125
	Max	2.33	1.31	0.92	0.81	1.17	1.87	1.19	1.18	2.77	1.68	4.75
	SD	0.11	0.06	0.04	0.03	0.05	0.06	0.03	0.03	0.08	0.05	0.16
	cv	5.38	4.66	5.12	4.53	4.91	3.38	3.12	3.13	3.17	2.92	3.69
F. brevior	Mean	2.18	1.22	0.86	0.76	1.10	1.79	1.12	1.11	2.55	1.60	4.43
Station 2	Median	2.21	1.23	0.87	0.76	1.10	1.78	1.11	1.11	2.56	1.60	4.5
N = 40	Min	1.69	0.98	0.66	0.60	0.86	1.65	1.06	1.06	2.44	1.51	4.125
10f/10m	Max	2.56	1.43	0.99	0.88	1.27	1.89	1.20	1.20	2.68	1.73	4.75
	SD	0.22	0.11	0.09	0.07	0.11	0.05	0.04	0.03	0.06	0.06	0.18
	cv	10.17	9.01	10.04	9.34	9.66	2.90	3.60	3.16	2.33	3.51	4.11
F. brevior	Mean	2.33	1.31	0.93	0.81	1.17	1.78	1.15	1.13	2.51	1.62	4.5
Station 3	Median	2.33	1.31	0.92	0.81	1.16	1.78	1.14	1.12	2.51	1.62	4.5
N = 20	Min	2.17	1.23	0.87	0.76	1.10	1.68	1.05	1.06	2.41	1.53	4.25
9f/11m	Max	2.56	1.43	1.00	0.86	1.23	1.84	1.22	1.18	2.67	1.73	4.875
	SD	0.10	0.05	0.04	0.03	0.05	0.04	0.04	0.03	0.06	0.06	0.2
	cv	4.47	3.80	4.65	3.71	3.97	2.30	3.79	2.58	2.41	3.44	4.54
F. brevior	Mean	2.12	1.22	0.85	0.75	1.11	1.74	1.13	1.09	2.50	1.63	4.44
Station 4	Median	2.10	1.21	0.85	0.74	1.11	1.74	1.13	1.09	2.50	1.63	4.375
N = 20	Min	1.82	1.11	0.75	0.66	1.00	1.64	1.04	1.00	2.33	1.54	4.125
11f/9m	Max	2.40	1.33	0.96	0.83	1.30	1.85	1.21	1.15	2.66	1.70	5
	SD	0.15	0.06	0.05	0.05	0.07	0.06	0.04	0.03	0.08	0.04	0.2
	cv	7.02	5.11	6.31	6.15	6.57	3.45	3.39	2.98	3.19	2.46	4.46
F. brevior	Mean	2.06	1.15	0.80	0.71	1.02	1.79	1.13	1.12	2.57	1.63	4.44
Stattion 5	Median	2.06	1.14	0.79	0.71	1.02	1.80	1.13	1.13	2.58	1.62	4.5
N = 25	Min	1.59	0.97	0.65	0.63	0.87	1.64	1.03	1.07	2.41	1.52	3.875
7f/13m	Max	2.49	1.38	0.95	0.82	1.22	1.89	1.28	1.23	2.74	1.79	4.75
	SD	0.23	0.10	0.08	0.06	0.09	0.07	0.06	0.04	0.09	0.08	0.22
	cv	11.15	8.97	9.72	7.90	8.66	4.14	5.39	3.55	3.49	5.19	5.06
F. riva	Holotype	2.14	1.16	0.78	0.75	1.03	1.85	1.05	1.12	2.73	1.55	4.25
Station 6	Mean	2.03	1.10	0.74	0.70	0.94	1.85	1.07	1.17	2.73	1.57	4.33
N = 20	Median	2.06	1.10	0.75	0.70	0.94	1.85	1.05	1.16	2.72	1.56	4.25
11f/9m	Min	1.81	0.98	0.66	0.64	0.79	1.76	1.01	1.11	2.59	1.49	4
	Max	2.15	1.17	0.80	0.76	1.04	1.96	1.14	1.41	2.87	1.65	4.75
	SD	0.10	0.05	0.04	0.03	0.05	0.05	0.04	0.06	0.08	0.05	0.19
	cv	4.94	4.36	5.30	4.00	5.63	2.66	4.01	5.37	2.83	3.27	4.5
F. erromangoana	Holotype	2.24	1.26	0.81	0.81	1.14	1.78	1.00	1.10	2.79	1.56	4.375
Station 7	Mean	2.26	1.26	0.83	0.81	1.12	1.80	1.03	1.12	2.72	1.56	4.25
N = 20	Median	2.28	1.27	0.84	0.82	1.12	1.80	1.03	1.12	2.68	1.55	4.25
11f/9m	Min	2.02	1.15	0.72	0.74	1.00	1.71	0.97	1.08	2.62	1.46	3.875
	Max	2.44	1.33	0.89	0.89	1.19	1.86	1.12	1.18	2.89	1.73	4.625
	SD	0.12	0.06	0.05	0.04	0.06	0.05	0.04	0.03	0.08	0.05	0.21
	cv	5.35	4.50	5.52	5.62	5.04	2.62	3.47	2.37	2.94	3.52	5.07
F. malekulana	Holotype	2.02	1.18	0.84	0.76	1.01	1.70	1.10	1.18	2.40	1.55	4.125
Station 10	Mean	2.21	1.30	0.91	0.81	1.13	1.71	1.12	1.15	2.43	1.60	4.33
N = 20	Median	2.27	1.32	0.92	0.81	1.13	1.70	1.12	1.14	2.45	1.59	4.375
10f/10m	Min	1.82	1.11	0.77	0.71	0.98	1.62	1.02	1.10	2.32	1.51	3.875
	Max	2.53	1.41	1.08	0.91	1.23	1.82	1.20	1.26	2.60	1.72	4.625
	SD	0.19	0.09	0.08	0.06	0.07	0.06	0.05	0.04	0.08	0.05	0.21
	cv	8.47	6.68	8.35	6.94	6.09	3.34	4.25	3.52	3.40	3.40	4.88
F. malekulana	Mean	2.19	1.25	0.90	0.80	1.08	1.75	1.12	1.16	2.44	1.56	4.25
Station 8	Median	2.18	1.24	0.90	0.80	1.08	1.74	1.12	1.14	2.44	1.56	4.25
N = 20	Min	1.87	1.11	0.78	0.70	0.97	1.63	1.04	1.10	2.24	1.47	3.875
10f/10m	Max	2.63	1.38	1.02	0.89	1.23	1.92	1.19	1.32	2.58	1.65	4.625
	SD	0.19	0.08	0.07	0.05	0.08	0.07	0.03	0.05	0.08	0.04	0.19
	cv	8.86	6.89	7.85	6.41	7.08	4.19	3.04	4.45	3.15	2.91	4.51
F. malekulana	Mean	2.06	1.10	0.77	0.73	1.01	1.78	1.06	1.09	2.53	1.50	4.125
Station 9	Median	2.09	1.06	0.79	0.74	0.99	1.74	1.04	1.09	2.55	1.50	4.125
N = 11 (for sh and w *n* = 5)	Min	1.79	1.03	0.67	0.66	0.92	1.73	0.99	1.04	2.42	1.43	4
4f/2m	Max	2.18	1.26	0.89	0.84	1.14	1.86	1.16	1.14	2.63	1.58	4.25
	SD	0.16	0.08	0.07	0.06	0.07	0.07	0.06	0.03	0.09	0.05	0.13
	cv	8.08	7.55	8.94	8.36	7.48	4.02	5.50	2.85	3.75	3.58	3.07

Appendix 1. (continued)

Species/locality/n/sex ratio		sh	sw	ah	aw	bww	Sh/sw	Ah/aw	Sw/bww	Sh/ah	Sw/aw	w
F. herminae	Holotype	1.67	0.96	0.69	0.62	0.82	1.75	1.11	1.17	2.42	1.53	4
Station 8	Mean	1.45	0.83	0.60	0.51	0.72	1.73	1.17	1.15	2.42	1.63	3.75
N = 8	Median	1.46	0.82	0.59	0.49	0.70	1.71	1.13	1.14	2.41	1.59	3.8
3f/2m	Min	1.22	0.73	0.50	0.45	0.66	1.65	1.06	1.11	2.30	1.49	3.375
	Max	1.68	0.97	0.69	0.63	0.83	1.88	1.47	1.25	2.61	2.00	4
	SD	0.19	0.09	0.08	0.06	0.07	0.08	0.13	0.04	0.09	0.16	0.21
	cv	13.47	11.37	13.07	12.67	9.34	4.48	11.20	3.98	4.01	9.85	5.81
F. freswota	Holotype	1.59	0.96	0.65	0.57	0.86	1.66	1.14	1.11	2.46	1.69	3.875
Station 11	Mean	1.66	0.98	0.67	0.60	0.87	1.70	1.11	1.13	2.49	1.62	3.97
N = 20	Median	1.65	0.97	0.66	0.59	0.87	1.69	1.09	1.13	2.50	1.60	3.94
5f/15m	Min	1.37	0.87	0.60	0.55	0.78	1.57	1.03	1.07	2.28	1.53	3.625
	Max	2.00	1.16	0.77	0.69	1.00	1.85	1.27	1.28	2.68	1.81	4.5
	SD	0.18	0.08	0.06	0.04	0.05	0.07	0.06	0.05	0.10	0.08	0.23
	cv	10.88	8.67	8.60	6.94	6.41	4.16	5.20	4.46	4.07	4.87	5.93
F. tangbunia	Holotype	1.43	0.84	0.57	0.56	0.78	1.69	1.02	1.08	2.51	1.51	3.75
Station 16	Mean	1.58	0.91	0.61	0.57	0.81	1.73	1.07	1.12	2.61	1.61	4.10
N = 27	Median	1.60	0.91	0.61	0.57	0.81	1.73	1.07	1.12	2.61	1.61	4.125
10f/10m	Min	1.38	0.82	0.55	0.50	0.72	1.58	1.00	1.07	2.30	1.44	3.75
	Max	1.75	1.04	0.71	0.66	0.90	1.88	1.15	1.21	2.81	1.72	4.375
	SD	0.11	0.05	0.04	0.04	0.05	0.07	0.04	0.03	0.11	0.06	0.18
	cv	6.73	5.63	6.41	6.49	5.92	3.92	4.02	3.13	4.42	3.78	4.46
F. tangbunia		1.38	0.83	0.57	0.54	0.76	1.66	1.06	1.09	2.42	1.54	3.875
Station 18; *N* = 1												
F. tangbunia		1.52	0.85	0.55	0.54	0.75	1.79	1.02	1.13	2.76	1.57	4.125
Station 20; *N* = 1												
F. tangbunia	Mean	1.62	0.94	0.62	0.60	0.83	1.72	1.04	1.13	2.59	1.56	3.98
Station 24	Median	1.64	0.93	0.63	0.60	0.85	1.75	1.03	1.12	2.61	1.57	4.00
N = 6	Min	1.46	0.91	0.59	0.58	0.76	1.59	1.00	1.10	2.35	1.49	3.75
	Max	1.71	0.98	0.64	0.63	0.86	1.76	1.09	1.21	2.75	1.61	4.125
	SD	0.09	0.03	0.02	0.02	0.04	0.07	0.04	0.04	0.14	0.04	0.15
	cv	5.61	3.21	2.93	3.70	4.63	4.15	3.70	3.86	5.69	2.58	3.83
	cv	5.67	4.91	5.75	6.63	5.27	3.34	4.78	2.79	3.75	4.85	3.13
F. pentecostata	Holotype	2.26	1.17	0.81	0.77	1.08	1.94	1.05	1.08	2.81	1.52	4.5
Station 17	Mean	2.12	1.12	0.78	0.72	1.02	1.89	1.08	1.10	2.72	1.55	4.5
N = 50	Median	2.13	1.12	0.77	0.72	1.02	1.89	1.08	1.11	2.74	1.54	4.5
30f/20m	Min	1.72	0.94	0.65	0.61	0.85	1.79	0.99	1.03	2.59	1.39	4
	Max	2.52	1.28	0.89	0.85	1.18	2.01	1.15	1.19	2.88	1.69	5
	SD	0.18	0.09	0.06	0.05	0.08	0.06	0.04	0.04	0.07	0.07	0.20
	cv	8.71	7.76	7.82	6.63	7.90	2.95	3.73	3.52	2.53	4.21	4.57
F. pentecostata	Mean	2.20	1.17	0.81	0.72	1.04	1.89	1.12	1.12	2.74	1.62	4.5
Station 15	Median	2.14	1.11	0.79	0.69	1.01	1.91	1.14	1.12	2.72	1.62	4.5
N = 6	Min	1.99	1.04	0.68	0.68	0.97	1.81	1.00	1.07	2.46	1.53	4.125
-f/-m	Max	2.54	1.33	0.93	0.79	1.16	1.94	1.19	1.18	3.13	1.68	4.875
	SD	0.21	0.12	0.09	0.05	0.08	0.06	0.07	0.04	0.25	0.06	0.31
	cv	10.12	10.89	12.19	7.53	7.81	3.42	6.46	3.61	9.42	3.69	7.22
F. pentecostata	Mean	1.98	1.07	0.73	0.67	0.97	1.85	1.09	1.10	2.71	1.59	3.33
Station 16	Median	2.00	1.07	0.73	0.66	0.96	1.85	1.09	1.09	2.70	1.59	4.375
N = 24	Min	1.73	0.97	0.66	0.58	0.86	1.70	1.03	1.06	2.54	1.50	4
12f/3m	Max	2.24	1.19	0.83	0.78	1.10	1.94	1.22	1.16	2.89	1.74	4.725
	SD	0.13	0.06	0.04	0.05	0.06	0.06	0.04	0.03	0.09	0.05	0.17
	cv	6.77	5.71	6.20	7.00	6.15	3.26	3.64	2.67	3.55	3.40	4.01
F. pentecostata	Mean	1.98	1.05	0.74	0.68	0.96	1.89	1.09	1.09	2.68	1.55	4.40
Station 19	Median	1.98	1.05	0.75	0.68	0.98	1.90	1.08	1.08	2.68	1.55	4.375
N = 20	Min	1.71	0.96	0.65	0.61	0.88	1.76	1.01	1.05	2.61	1.41	4.125
11f/9m	Max	2.25	1.15	0.84	0.72	1.05	1.97	1.18	1.14	2.84	1.64	4.875
	SD	0.14	0.06	0.04	0.03	0.05	0.05	0.04	0.03	0.05	0.06	0.19
	cv	6.94	5.44	5.99	4.57	5.04	2.76	4.00	2.43	1.99	3.72	4.47
F. pentecostata	Mean	2.06	1.12	0.77	0.70	0.99	1.85	1.11	1.13	2.67	1.60	4.33
Station 20	Median	2.02	1.10	0.78	0.69	0.99	1.86	1.10	1.13	2.68	1.60	4.375
N = 15	Min	1.82	1.00	0.69	0.64	0.90	1.78	1.03	1.05	2.55	1.46	4.125
5f/3m	Max	2.33	1.29	0.86	0.77	1.08	1.92	1.17	1.20	2.73	1.72	4.625
	SD	0.14	0.08	0.05	0.04	0.05	0.05	0.04	0.04	0.05	0.06	0.13
	cv	6.76	7.51	6.68	5.37	5.21	2.68	3.60	4.02	1.86	3.88	3.02
F. pentecostata	Mean	2.11	1.14	0.79	0.73	1.03	1.85	1.08	1.11	2.68	1.57	4.5
Station 21	Median	2.10	1.14	0.79	0.73	1.01	1.84	1.09	1.11	2.68	1.58	4.5
N = 20	Min	1.91	1.03	0.67	0.61	0.92	1.75	0.93	1.04	2.49	1.35	4.125
7f/13m	Max	2.28	1.26	0.85	0.83	1.14	1.96	1.16	1.17	2.87	1.69	4.75
	SD	0.12	0.06	0.04	0.05	0.05	0.06	0.05	0.03	0.10	0.08	0.14
	cv	5.67	4.91	5.75	6.63	5.27	3.34	4.78	2.79	3.75	4.85	3.13

Appendix 1. (continued)

Species/locality/n/sex ratio		sh	sw	ah	aw	bww	Sh/sw	Ah/aw	Sw/bww	Sh/ah	Sw/aw	w
F. pentecostata	Mean	2.08	1.13	0.78	0.73	1.03	1.85	1.07	1.09	2.68	1.55	4.40
Station 22	Median	2.09	1.14	0.77	0.72	1.02	1.84	1.07	1.10	2.69	1.56	4.375
N = 20	Min	1.93	1.05	0.72	0.69	0.98	1.72	1.00	1.00	2.56	1.41	4.125
9f/11m	Max	2.23	1.19	0.83	0.80	1.11	1.95	1.13	1.15	2.82	1.68	4.625
	SD	0.08	0.04	0.03	0.03	0.04	0.07	0.04	0.04	0.08	0.07	0.11
	cv	3.89	3.19	3.63	3.94	4.06	3.69	3.52	3.39	3.09	4.30	2.62
F. pentecostata	Mean	1.81	0.99	0.67	0.64	0.89	1.84	1.04	1.12	2.70	1.54	4.25
Station 23	Median	1.81	0.98	0.66	0.63	0.86	1.85	1.05	1.12	2.71	1.53	4.25
N = 7	Min	1.65	0.94	0.63	0.62	0.84	1.74	1.02	1.08	2.62	1.49	4.125
1f/1m	Max	2.00	1.06	0.73	0.71	0.98	1.89	1.08	1.14	2.74	1.60	4.375
	SD	0.11	0.05	0.03	0.03	0.05	0.05	0.02	0.02	0.04	0.04	0.07
	cv	6.11	4.91	4.99	4.98	5.72	3.00	2.13	2.23	1.68	2.80	1.76
F. pentecostata	Mean	1.96	1.07	0.73	0.68	0.94	1.84	1.08	1.13	2.69	1.58	4.40
Station 24	Median	1.97	1.07	0.73	0.68	0.95	1.84	1.08	1.13	2.68	1.59	4.375
N = 34	Min	1.75	0.96	0.64	0.59	0.80	1.71	0.99	1.07	2.49	1.48	4.125
13f/14m	Max	2.21	1.21	0.81	0.77	1.05	1.99	1.16	1.20	2.86	1.69	4.625
	SD	0.14	0.07	0.05	0.05	0.06	0.06	0.04	0.04	0.08	0.05	0.15
	cv	7.04	6.76	6.76	6.73	6.88	3.09	3.80	3.17	3.12	3.21	3.49
F. pentecostata	Mean	2.18	1.14	0.80	0.74	1.03	1.92	1.09	1.10	2.72	1.54	4.5
Station 25	Median	2.19	1.14	0.81	0.75	1.04	1.91	1.08	1.11	2.68	1.54	4.5
N = 20	Min	1.74	0.95	0.65	0.61	0.86	1.80	1.01	1.00	2.49	1.48	4
12f/8m	Max	2.55	1.26	0.92	0.82	1.17	2.07	1.15	1.15	2.99	1.62	4.875
	SD	0.20	0.09	0.07	0.05	0.08	0.08	0.04	0.04	0.12	0.04	0.22
	cv	9.15	7.78	8.63	7.51	7.65	4.03	3.76	3.52	4.51	2.42	5.08
F. adkinsi	Holotype	2.28	1.17	0.80	0.75	1.08	1.95	1.07	1.08	2.84	1.55	4.75
Station 12	Mean	2.35	1.24	0.86	0.74	1.11	1.89	1.15	1.12	2.74	1.67	4.75
N = 20	Median	2.39	1.23	0.87	0.74	1.11	1.89	1.16	1.13	2.77	1.67	4.75
8f/12m	Min	2.13	1.14	0.75	0.69	1.04	1.79	1.04	1.00	2.55	1.55	4.5
	Max	2.52	1.33	0.95	0.78	1.21	2.01	1.22	1.16	2.84	1.74	5.125
	SD	0.12	0.06	0.05	0.03	0.04	0.05	0.05	0.04	0.08	0.05	0.17
	cv	5.34	4.92	6.15	3.76	3.75	2.94	4.62	3.74	2.86	3.27	3.67
F. bakeri	Holotype	2.03	1.14	0.75	0.69	1.05	1.78	1.09	1.09	2.70	1.65	4.375
Station 13	Mean	2.02	1.17	0.76	0.70	1.05	1.74	1.08	1.11	2.66	1.66	4.2
N = 20	Median	2.00	1.16	0.75	0.70	1.05	1.74	1.08	1.10	2.67	1.66	4.2
10f/10m	Min	1.90	1.08	0.70	0.65	0.93	1.65	1.00	1.06	2.54	1.57	3.875
	Max	2.21	1.24	0.82	0.77	1.16	1.84	1.17	1.16	2.83	1.80	4.5
	SD	0.10	0.05	0.04	0.03	0.06	0.04	0.03	0.03	0.07	0.06	0.14
	cv	5.12	4.37	5.16	4.72	5.36	2.54	3.24	2.69	2.70	3.40	3.44
F. bakeri	Mean	1.98	1.11	0.74	0.68	1.01	1.79	1.09	1.10	2.69	1.64	4.2
Station 14	Median	1.99	1.11	0.75	0.69	1.01	1.79	1.09	1.09	2.66	1.63	4.25
N = 14	Min	1.78	1.02	0.67	0.61	0.90	1.70	1.04	1.05	2.58	1.54	3.875
6f/1m	Max	2.18	1.23	0.82	0.72	1.10	1.89	1.17	1.17	2.85	1.76	4.5
	SD	0.12	0.05	0.05	0.03	0.05	0.06	0.04	0.03	0.09	0.06	0.17
	cv	5.95	4.91	6.28	4.64	5.31	3.53	3.56	3.16	3.23	3.55	4.17
F. pikinini	Holotype	1.64	0.94	0.61	0.59	0.84	1.75	1.03	1.11	2.66	1.57	4
Station 14	Mean	1.61	0.93	0.60	0.58	0.85	1.73	1.04	1.09	2.69	1.61	4
N = 4	Median	1.61	0.93	0.60	0.56	0.85	1.73	1.04	1.09	2.68	1.61	4
-f/1m	Min	1.54	0.88	0.58	0.56	0.83	1.72	1.00	1.06	2.66	1.56	3.875
	Max	1.67	0.97	0.62	0.62	0.89	1.75	1.09	1.11	2.72	1.68	4.125
	SD	0.06	0.04	0.02	0.03	0.03	0.01	0.04	0.02	0.03	0.06	0.10
	cv	3.72	4.27	3.62	5.48	3.24	0.89	3.72	1.89	1.16	3.61	2.68

Ah, aperture height; aw, aperture width; bww, body whorl width; cv, coefficient of variation adjusted for sample size; f, females; m, males; max, maximum; min, minimum; SD, standard deviation; sh, shell height; sw, shell width; w, whorls; *F.*, *Fluviopupa,* all measurements are given in mm.

Appendix 2. List of individuals and sequences analysed as well as GenBank accession numbers

Specimen	*16S*	*COI*	*ITS2*
F. adkinsi 12-1	KC875032	KC875104	KC875177
F. adkinsi 12-2	KC875033	KC875105	KC875178
F. bakeri 13-1	KC875034	KC875106	KC875179
F. bakeri 13-2	KC875035	KC875107	KC875180
F. bakeri 14-1	KC875036	KC875108	KC875181
F. bakeri 14-2	KC875037	KC875109	KC875182
F. brevior 1-1	KC874996	KC875076	KC875147

Appendix 2. (continued)

Specimen	*16S*	*COI*	*ITS2*
F. brevior 1-2	KC874997	KC875077	KC875148
F. brevior 2-1	KC874998	KC875078	KC875149
F. brevior 2-2	KC874999	KC875079	KC875150
F. brevior 3-1	KC875000	KC875080	KC875151
F. brevior 3-2	KC875001	KC875082	KC875153
F. brevior 4-1	KC875002	KC875081	KC875152
F. brevior 4-2	KC875003	KC875083	KC875154
F. brevior 5-1	KC875005	KC875085	KC875156
F. brevior 5-2	KC875004	KC875084	KC875155
F. erromangoana 7-1	KC875008	KC875088	KC875159
F. erromangoana 7-2	KC875009	KC875089	–
F. espiritusantoana 26-2	KC875010	KC875090	–
F. espiritusantoana 26-3	KC875011	KC875091	KC875160
F. espiritusantoana 27-1	KC875013	–	KC875161
F. espiritusantoana 27-3	KC875012	KC875092	–
F. espiritusantoana 28-1	KC875014	–	KC875162
F. espiritusantoana 29-1	KC875016	KC875093	–
F. espiritusantoana 29-2	KC875015	–	KC875163
F. espiritusantoana 30-1	KC875018	KC875095	KC875164
F. espiritusantoana 30-2	KC875019	KC875096	KC875165
F. espiritusantoana 31-1	KC875017	KC875094	–
F. espiritusantoana 31-3	KC875020	–	KC875166
F. freswota 11-1	KC875050	KC875121	KC875195
F. freswota 11-2	KC875040	KC875111	KC875185
F. herminae 8-4	KC875042	KC875113	KC875187
F. malekulana 8-1	KC875039	KC875110	KC875184
F. malekulana 8-2	KC875041	KC875112	KC875186
F. malekulana 8-5	KC875043	KC875114	KC875188
F. malekulana 9-1	KC875044	KC875115	KC875189
F. malekulana 9-2	KC875045	KC875116	KC875190
F. malekulana 9-3	KC875046	KC875117	KC875191
F. malekulana 10-1	KC875048	KC875119	KC875193
F. malekulana 10-2	KC875047	KC875118	KC875192
F. malekulana 10-3	KC875049	KC875120	KC875194
F. narii 33-1	KC875021	–	KC875167
F. pascali 32-1	KC875022	KC875097	KC875168
F. pascali 32-5	KC875023	KC879098	KC875169
F. pentecostata 15-1	KC875057	KC875128	KC875202
F. pentecostata 15-2	KC875058	KC875129	KC875203
F. pentecostata 16-1	KC875059	KC875130	KC875204
F. pentecostata 16-2	KC875060	KC875131	KC875205
F. pentecostata 17-1	KC875063	KC875135	KC875209
F. pentecostata 17-2	KC875064	KC875136	KC875210
F. pentecostata 20-1	KC875068	KC875140	KC875214
F. pentecostata 20-2	KC875069	KC875141	KC875215
F. pentecostata 21-1	KC875062	KC875134	KC875208
F. pentecostata 21-2	KC875067	KC875139	KC875213
F. pentecostata 21-3	KC875070	KC875142	KC875216
F. pentecostata 22-1	KC875071	KC875143	KC875217
F. pentecostata 22-2	KC875072	KC875144	KC875218
F. pentecostata 23-1	KC875073	KC875145	KC875219
F. pentecostata 23-2	KC875054	KC875125	KC875199
F. pentecostata 24-2	KC875051	KC875122	KC875196
F. pentecostata 24-3	KC875052	KC875123	KC875197
F. pentecostata 24-4	KC875053	KC875124	KC875198
F. pentecostata 25-1	KC875055	KC875126	KC875200
F. pentecostata 25-2	KC875056	KC875127	KC875201
F. pikinini 14-3	KC875038	–	KC875183
F. riva 6-1	KC875006	KC875086	KC875157
F. riva 6-2	KC875007	KC875087	KC875158
F. tangbunia 16-3	–	KC875132	KC875206
F. tangbunia 16-4	KC875061	KC875133	KC875207
F. tangbunia 18-1	KC875065	KC875137	KC875211
F. tangbunia 18-2	KC875066	KC875138	KC875212
F. torresiana 36-2	KC875028	–	KC875174
F. torresiana 36-3	KC875029	KC875101	KC875175
F. torresiana 37-1	KC875030	KC875102	–
F. torresiana 37-3	KC875031	KC875103	KC875176
F. torresiana 34-2	KC875027	KC875100	KC875173
F. torresiana 35-3	KC875026	–	KC875172

Appendix 2. (continued)

Specimen	*16S*	*COI*	*ITS2*
F. torresiana 38-1	KC875025	KC875099	KC875171
F. torresiana 38-4	KC875024	–	KC875170
Hemistomia sp.	JX970548	JX970617	–
Opacuincola delira	KC875075	KC875146	–
Potamolithus ribeirensis.	JX970549	JX970618	–
Potamopyrgus estuarinus	KC875074	–	KC875220
Tatea huonensis	JX970550	JX970619	–

Supporting Information

Additional Supporting Information may be found in the online version of this article:
Data S1. Detailed description of sampling localities.

When snails inform about geology: Pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda: Tateidae)

Zielske & Haase

Supporting Information:

Detailed description of sampling localities

Stations 1 to 25 have been visited by the authors in 2011; for more information about stations 26 to 38 see Haase *et al.* (2010), original station names in brackets.

Station 1: Efate Island; 17°38'19.5"S, 168°12'23.7"E; 11/05/2011; Mangaliliu village, spring used for drinking water of the village, snails found on wood and limestone; guide: Harry Kalkoa.

Station 2: Efate Island; 17°41'0.6"S, 168°14'53.6"E, 12/05/2011; seepage near the road to Benjor Beachclub on the property of Loulou Mamelin, snails found on limestone, heavily damaged.

Station 3: Efate Island; 17°39'10.0"S, 168°31'16.5"E; 13/05/2011; East coast of the Island, near Lamin, spring with associated basin, snails found on leaves; guide: Joseph John.

Station 4: Efate Island; 17°38'43.7 "S, 168°30'48.0"E; 14/05/2011; East coast of the island, swamp just on the western bank of the road, snails found on leaves.

Station 5: Efate Island; 17°48'31.7"S, 168°24'01.4"E; 15/05/2011; South coast of the island in “White Sands”, spring-pond, snails found on leaves; guide: Wilson Tula.

Station 6: Erromango Island; 18°49'24.3"S, 169°01'36.2"E; 22/05/2011; south east of Dillons Bay, spring used for drinking water next to a cascade of a small river used for washing etc., snails found on limestone, leaves and in the gravel; guide: Donald Lovo.

Station 7: Erromango Island; 18°46'56.0"S, 169°01'29.3"E; 23/05/2011; Untou village next to the road from Dillons Bay to the airfield, on the property of Harry Nare and Emily Uvoi, spring used for drinking water, snails found on leaves; guide: Donald Lovo.

Station 8: Malekula Island; 16°06'40.8"S, 167°25'13.7"E; 27/05/2011; Metened in western Lakatoro, spring, small stream used for drinking water, snails found on leaves; guide James Simo.

Station 9: Malekula Island; 16°01'27.6"S, 167°21'22.8"E; 28/05/2011; Toros, spring used for drinking water, snails found on wood, stones and leaves; guides: Rodolphe Malere, Sam Krem.

Station 10: Malekula Island; 15°59'18.2"S, 167°19'37.6"E; 30/05/2011; Teboune, spring with water basin just above a tarot field, snails found on leaves; guide Rodolphe Malere.

Station 11: Malekula Island, 16°02'21.5"S, 167°22'03.3"E; 31/05/2011; Betel, small spring on river bank almost on level of river surface, certainly submerged after heavy precipitation, used for drinking water, snails found on leaves; guide: Rodolphe Malere.

Station 12: Gaua Island; 14°14'45.5"S, 167°33'55.3"E; 04/06/2011; spring next to the track to lake Letas (starting from airfield), used for drinking water, snails found on leaves; guide: John Atkins.

Station 13: Gaua Island; 14°15'42.4"S, 167°32'21.9"E; 04/06/2011; Lake Letas, snails found in algae and on wood; guide: John Atkins.

Station 14: Gaua Island; 14°15'48.3"S, 167°36'15.6"E; 06/06/2011; Medsamavud, branch of river redirected for irrigation, heavily damaged, snails found on leaves.

Station 15: Pentecost Island; 15°56'55.6"S, 168°11'48.0"E; 12/06/2011; Salap, spring with small water basin, heavily damaged, snails found in the sediment; guide: Lilian Tamata.

Station 16: Pentecost Island; 15°56'18.7"S, 168°12'23.3"E; 13/06/2011; Litli, spring in tarot garden, snails found on leaves; guides: Solomon, Simeon and Samuel.

Station 17: Pentecost Island; 15°58'03.6"S, 168°15'53.3"E; 14/06/2011; Ranwas, stream with spring at the beginning of the track to Bunlap, snails found on leaves and stones; guide: Simeon.

Station 18: Pentecost Island; 15°47'08.5"S, 168°09'56.4"E; 15/06/2011; Waterfall Village, spring, small stream coming out of rock, snails found on leaves; guide Lissian Tabi.

Station 19: Pentecost Island; 15°47'07.6"S, 168°09'53.8"E; 15/06/2011; Waterfall Village, spring, small stream emerging from cave and irrigating garden, snails found on limestone and leaves; guide Lissian Tabi.

Station 20: Pentecost Island; 15°49'05.2"S, 168°10'22.0"E; 15/06/2011; Vetlul, spring in secondary forest, snails found on leaves; guide Lissian Tabi.

Station 21: Pentecost Island; 15°51'01.3"S, 168°11'05.4"E; 16/06/2011; Ravesedle, small stream just slightly beneath the spring, in secondary forest, snails found on leaves; guide Lissian Tabi.

Station 22: Pentecost Island; 15°51'02.2"S, 168°11'06.4"E; 16/06/2011; Ravesedle, spring in secondary forest, snails found on leaves; guide Lissian Tabi.

Station 23: Pentecost Island; 15°51'01.7"S, 168°11'09.9"E; 16/06/2011; Vasove, spring in secondary forest, snails found on heavily rotten leaves; guide Lissian Tabi.

Station 24: Pentecost Island; 15°50'58.3"S, 168°11'20.8"E; 16/06/2011; Vanlvibang, small stream, slightly beneath the spring, snails found on leaves and stones; guide: Lissian Tabi.

Station 25: Pentecost Island; 15°49'09.3"S, 168°10'40.3"E; 17/06/2011; Vanibunger, small stream, used for drinking water, snails found on leaves, guide Lissian Tabi.

Station 26 (SA32): Santo; 14°57'52.1"S, 166°38'11.8"E; Penaorou.

Station 27 (SA39): Santo; 14°58'01.5"S, 166°39'30.5"E; Penaorou.

Station 28 (SA43): Santo; 14°56'36.2"S, 166°40'00.1"E; Béésel valley.

Station 29 (SA48): Santo; 14°56'43.0"S, 166°39'56.5"E; Béésel valley

Station 30 (SAPb2): Santo; 15°18'19.1"S, 166°52'05.9"E; river Ora.

Station 31 (SAPb1): Santo; 15°18'27.4"S, 166°51'35.3"E; river Ora.

Station 32 (SA110): Santo; 15°22'26.8"S, 166°58'28.2"E; Butmas.

Station 33 (SAPb3): Santo; 15°20'21.5"S, 166°58'19.6"E; Fapon.

Station 34 (To28): Torres; 13°25'35.9"S, 166°40'53.8"E; North of Riara Bay.

Station 35 (To24): Torres; 13°26'47.5"S, 166°41'27.2"E; Sothern Tip of Toga.

Station 36 (To2): Torres; 13°08'35.2"S, 166°34'07.8"E; northeast of Mt Wonvara.

Station 37 (To5): Torres; 13°09'18.7"S, 166°34'33.6"E; Hiu.

Station 38 (To15): Torres; 13°09'17.9"S, 166°32'49.6"E; Yuwutu Bay.

Literature cited herein:

Haase M, Fontaine B, Gargominy O (2010) Rissooidean freshwater gastropods from the Vanuatu archipelago. Hydrobiologia **637**: 53–71.

2.2. Tateid gastropods on Fiji

Zoological Journal of the Linnean Society, 2014, **172**, 71–102. With 10 figures

New insights into tateid gastropods and their radiation on Fiji based on anatomical and molecular methods (Caenogastropoda: Truncatelloidea)

SUSAN ZIELSKE* and MARTIN HAASE

Vogelwarte, Zoological Institute and Museum, Greifswald University, Soldmannstr. 23, 17489 Greifswald, Germany

Received 10 December 2013; revised 12 March 2014; accepted for publication 12 March 2014

The South Pacific archipelago of Fiji is characterized by a predominantly Indo-Malesian flora and fauna. We provide a first systematic study on Fiji's tateid gastropods – previously classified as Hydrobiidae – describing 18 new species, combining morphological, anatomical, and molecular data. The molecular phylogeny of tateid gastropods based on *16S* rRNA and cytochrome *c* oxidase subunit I (*COI*) showed that the species from Fiji were closer related to New Zealand than to Australian or New Caledonian taxa, which is rather exceptional. Performing an ancestral range reconstruction we inferred the colonization history across the two main islands. The radiation had its origin in southern Viti Levu, with a subsequent dispersal over the western and central parts of the island. The chronology of the radiation over eastern Viti Levu and Vanua Levu remained unresolved because of incomplete lineage sorting, a phenomenon typical for young radiations.

doi: 10.1111/zoj.12153

ADDITIONAL KEYWORDS: ancestral range reconstruction – biogeography – *Fluviopupa* – new species – phylogeny – Rissooidea – South Pacific.

INTRODUCTION

Truncatelloidean gastropods belonging to the family Tateidae are largely confined to freshwaters. Marine and brackish water species occur only in the coastal waters of Australia (Ponder & Clark, 1988) and New Zealand (Haase, 2008). New Zealand freshwaters were invaded three times, independently (Haase, 2005). Respective investigations for Australia have not yet been performed. These land masses were probably the source areas for the spread North to New Guinea (Bernasconi, 1995) and Sulawesi (Zielske, Glaubrecht & Haase, 2011), on the one hand, and across the South Pacific, on the other hand, where tateids have colonized Norfolk and Lord Howe Island (Ponder, 1981, 1982), New Caledonia (Haase & Bouchet, 1998), Vanuatu (Haase, Fontaine & Gargominy, 2010a; Zielske & Haase, in press), Fiji (Haase, Ponder & Bouchet, 2006), and the Austral Islands, the southernmost archipelago of French Polynesia (Haase, Gargominy & Fontaine, 2005).

In 2012, in the course of a project aiming to reconstruct the tateid 'conquest' of the South Pacific, we visited the two main islands of Fiji, Viti Levu and Vanua Levu (see map Fig. 1), that cover nearly 90% of the land mass of Fiji. The whole archipelago of Fiji consists of over 300 islands covering nearly 18 300 km^2 of land (Neall & Trewick, 2008). The formation of Fiji dates back to the Late Eocene island arc volcanism (Neall & Trewick, 2008) caused by the westward subduction of the Pacific beneath the Australien plate. The first significant land mass, the south-west and south of Viti Levu, was uplifted in the Middle to Late Miocene, and was established by *c.* 7 Mya (Rodda, 1994; Neall & Trewick, 2008). The centre of Viti Levu emerged in the Early and/or Late Pliocene (Rodda, 1994). At about

*Corresponding author.
E-mail: susan.zielske@googlemail.com

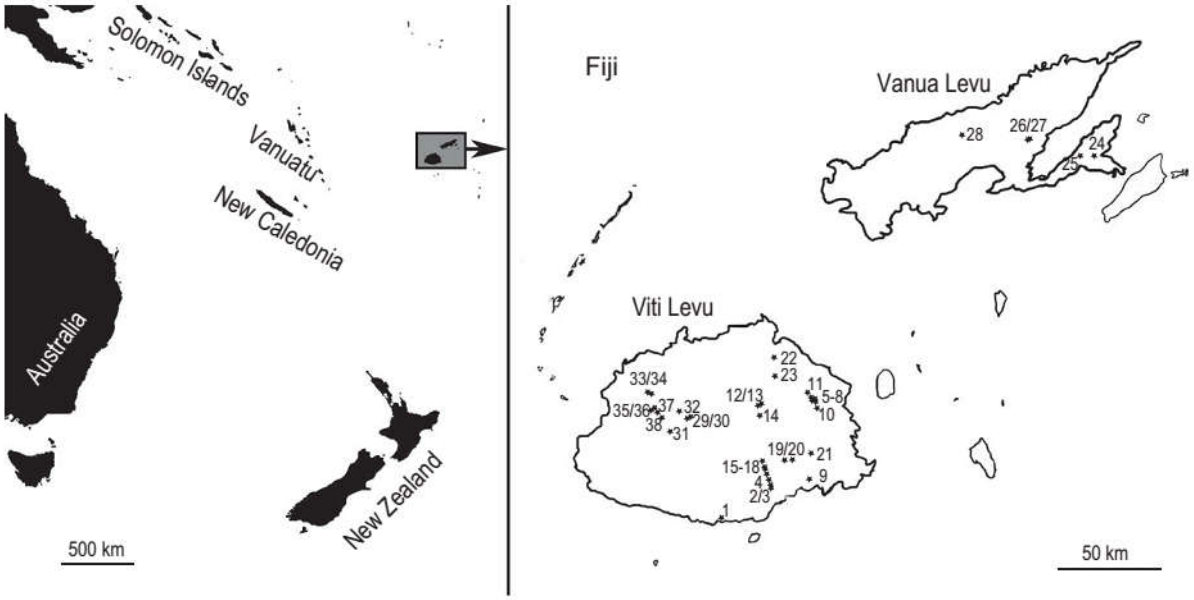

Figure 1. Map of Fiji and its location in the South Pacific; numbers indicate sampling stations (see Table 1).

the same time, Vanua Levu was formed (Neall & Trewick, 2008); however, the island emerged only between 4 and 3 Mya, with the east and south-eastern peninsula having an even younger subaerial history of at most 2 Myr or considerably less (Rodda, 1994).

To date there are ten species of truncatelloidean gastropods attributed to the genus *Fluviopupa* Pilsbry, 1911 known from Fiji (Haase *et al*., 2006), but no systematic survey has been conducted.

The aims of our work were: (1) to systematically survey the main islands of Fiji for tateid gastropods in continuation of the taxonomic account of Haase *et al*. (2006); (2) to reconstruct their phylogenetic relationships; and (3) to reconstruct their colonization history across the main islands of the archipelago.

MATERIAL AND METHODS

Material

Most specimens examined in this study were collected in June 2012 (for more information see Fig. 1 and Table 1). They were preserved in 70% ethanol in the field and transferred to propylene glycol for shipment. Upon arrival in our laboratory, snails were returned to 96% ethanol. Museum collection numbers of known species not described in the results section are listed in Table 2. As out-group taxa we selected two tateid species from New Zealand (*Opacuincola delira* Haase, 2008 and *Potamopyrgus estuarinus* Winterbourn 1971), as well as one each from New Caledonia (*Hemistomia winstonefi* Haase & Bouchet, 1998), Australia (*Tatea huonensis* Tenison-Woods, 1876), and Brazil (*Potamolithus ribeirensis* Pilsbry, 1911), based on the latest phylogeny of truncatelloidean gastropods (Wilke *et al*., 2013). Assuming that long-distance dispersal events are very rare, and therefore the radiations across archipelagos are reciprocally monophyletic (Haase *et al*. 2010a), we here focus solely on Fiji and do not include the taxa from Vanuatu (Zielske & Haase, in press). A test of this assumption and synthesis of the phylogenetic and biogeographic history of the entire family Tateidae, including representatives of all major clades, is in preparation (S. Zielske, W. F. Ponder, M. Haase, unpubl. data). Some of the sequence data of out-group taxa was taken from GenBank (Appendix 1).

Morphology

Our methods essentially follow those described in Zielske & Haase (in press). Digital photographs of up to 20 shells were taken under a dissecting stereomicroscope (Zeiss, SteREO Discovery.V20) with a Zeiss Axio Cam MR3 to measure five shell dimensions – shell height (sh) and width, aperture height (ah) and width, and width of the body whorl (bww) (parallel and perpendicular to the coiling axis, respectively) – using the program AxioVision 4.8 (Zeiss). Whorls were counted to the nearest eighth of a whorl. Statistical comparisons, including the multivariate techniques of canonical variates analysis (CVA), multivariate analysis of variance (MANOVA), and Hotelling's T^2-tests, and

Table 1. Locality data

Station	Species	GPS
1	***Fluviopupa tasmani* sp. nov.**	18°15′03.5″S, 177°57′43.8″E
2	***Fluviopupa uka* sp. nov.**	18°08′23.8″S, 178°12′40.1″E
3	***Fluviopupa uka* sp. nov.**	18°08′01.5″S, 178°12′16.5″E
4	***Fluviopupa moolae* sp. nov.**	18°06′44.5″S, 178°11′10.8″E
5	*Fluviopupa vakamalolo*	ca. 17°45′41.2″S, 178°24′25.1″E
	***Fluviopupa bula* sp. nov.**	
6	***Fluviopupa bula* sp. nov.**	ca. 17°45′41.2″S, 178°24′25.1″E
7	***Fluviopupa bula* sp. nov.**	ca. 17°45′41.2″S, 178°24′25.1″E
8	***Fluviopupa bula* sp. nov.**	ca. 17°45′41.2″S, 178°24′25.1″E
	Fluviopupa derua	
9	*Fluviopupa seasea*	18°05′46.1″S, 178°23′39.5″E
10	***Fluviopupa bula* sp. nov.**	17°44′01.8″S, 178°25′50.0″E
11	***Fluviopupa bula* sp. nov.**	17°42′17.3″S, 178°22′07.0″E
12	*Fluviopupa mekeniyaqona*	17°45′10.4″S, 178°08′09.7″E
13	*Fluviopupa mekeniyaqona*	17°45′07.9″S, 178°08′15.4″E
	Fluviopupa mekewesi	
14	***Fluviopupa vulavula* sp. nov.**	17°46′42.8″S, 178°07′09.9″E
15	***Fluviopupa moolae* sp. nov.**	18°04′35.6″S, 178°09′47.2″E
16	***Fluviopupa namosi* sp. nov.**	18°04′18.1″S, 178°09′35.5″E
17	***Fluviopupa namosi* sp. nov.**	18°03′13.1″S, 178°09′27.9″E
18	***Fluviopupa moolae* sp. nov.**	18°02′15.9″S, 178°09′13.2″E
	***Fluviopupa namosi* sp. nov.**	
19	***Fluviopupa moolae* sp. nov.**	18°00′59.6″S, 178°15′13.0″E
20	***Fluviopupa dromodromo* sp. nov.**	18°00′31.4″S, 178°17′10.4″E
21	***Fluviopupa dumontdurvilli* sp. nov.**	17°57′50.1″S, 178°22′55.2″E
22	***Fluviopupa drau* sp. nov.**	17°34′50.6″S, 178°14′38.5″E
23	***Fluviopupa drau* sp. nov.**	17°39′08.7″S, 178°15′40.0″E
	***Fluviopupa forsteri* sp. nov.**	
24	***Fluviopupa tunuloa* sp. nov.**	16°40′48.2″S, 179°47′30.0″E
25	***Fluviopupa tunuloa* sp. nov.**	16°41′00.1″S, 179°42′17.0″E
	***Fluviopupa raradamu* sp. nov.**	
26	***Fluviopupa vatukuca* sp. nov.**	16°34′29.5″S, 179°30′50.4″E
27	***Fluviopupa vatukuca* sp. nov.**	16°34′19.1″S, 179°30′31.8″E
28	***Fluviopupa vakalevu* sp. nov.**	16°37′20.4″S, 179°09′48.6″E
29	*Fluviopupa* cf. *pupoidea*	17°47′22.4″S, 177°45′23.5″E
	Fluviopupa irinimeke	
30	*Fluviopupa* cf. *pupoidea*	17°48′00.9″S, 177°44′40.8″E
31	*Fluviopupa irinimeke*	17°52′41.7″S, 177°42′16.4″E
32	***Fluviopupa nagodro* sp. nov.**	17°45′32.9″S, 177°43′00.0″E
33	***Fluviopupa nagodro* sp. nov.**	17°40′02.1″S, 177°31′29.8″E
	Fluviopupa savuione	
34	***Fluviopupa nagodro* sp. nov.**	17°40′24.1″S,177°33′05.7″E
	***Fluviopupa savuione* sp. nov.**	
35	***Fluviopupa lailai* sp. nov.**	17°43′51.9″S, 177°33′04.0″E
	***Fluviopupa korobebe* sp. nov.**	
36	*Fluviopupa daunivucu*	17°43′17.1″S, 177°33′59.0″E
37	*Fluviopupa daunivucu*	17°45′53.7″S, 177°35′59.0″E
38	*Fluviopupa daunivucu*	17°46′23.2″S, 177°37′15.7″E

univariate Student's *t*-tests were performed using PAST 2.0 (Hammer, Harper & Ryan, 2001). Shells of each three males and females were dissolved in 1 N HCl, dissected, and anatomies drawn from digital images taken with the same microscope. In preparation for scanning electron microscopy each three shells, radulae, and opercula were cleaned using a 5% sodium hypochlorite solution and cephalopodia were dried using

Table 2. Museum collection numbers of material not included in the species descriptions

Species	Station	Collection number
Fluviopupa daunivucu	36	USP12220 (*N* = 10)
Fluviopupa daunivucu	37	AMS C483429 (*N* = 5), MNHN-IM-2012-29797 (*N* = 5), USP12221 (*N* = 8), ZMB121022 (*N* = 8)
Fluviopupa daunivucu	38	AMS C483430 (*N* = 10), MNHN-IM-2012-29798 (*N* = 10), USP12222 (*N* = 32), ZMB121023 (*N* = 33)
Fluviopupa derua	8	AMS C483431 (*N* = 10), MNHN-IM-2012-29799 (*N* = 10), USP12223 (*N* = 14), ZMB121024 (*N* = 15)
Fluviopupa irinimeke	31	USP12224 (*N* = 8), ZMB121025 (*N* = 9)
Fluviopupa mekeniaquona	12	AMS C483427 (*N* = 10), MNHN-IM-2012-29795 (*N* = 10), USP12218 (*N* = 12), ZMB121020 (*N* = 12)
Fluviopupa mekeniaquona	13	AMS C483428 (*N* = 5), MNHN-IM-2012-29796 (*N* = 5), USP12219 (*N* = 13), ZMB121021 (*N* = 13)
Fluviopupa mekewesi	13	USP12227 (*N* = 1)
Fluviopupa cf. *pupoidea*	29	USP12225 (*N* = 7)
Fluviopupa cf. *pupoidea*	30	AMS C483432 (*N* = 10), MNHN-IM-2012-29800 (*N* = 10), USP12226 (*N* = 24), ZMB121026 (*N* = 24)
Fluviopupa seasea	9	AMS C483426 (*N* = 5), MNHN-IM-2012-29794 (*N* = 5), USP12217 (*N* = 5), ZMB121019 (*N* = 5)

Abbreviations: AMS, Australian Museum Sydney; MNHN, Museum national d'Histoire naturelle, Paris; USP, museum and scientific collection of the University of the South Pacific, Suva; ZMB, Zoologisches Museum Berlin.

hexamethyldisilazane (Nation, 1983). The objects were coated with gold and investigated in a Zeiss EVO LS10 scanning microscope.

DNA ISOLATION AND SEQUENCING

DNA was isolated from two entire snails of each sample using the DNeasy Blood and Tissue Kit (QIAGEN). The primers 16Sar, introduced by Palumbi *et al.* (1991), and 16Sr (Zielske & Haase, in press) were used to amplify a ~700-bp fragment of the *16S* rRNA, and primers L1460 and H1298, with H1298 modified at position 12 (G → A), developed by Folmer *et al.* (1994), amplified a 658-bp fragment of the cytochrome *c* oxidase subunit I (COI) gene. Polymerase chain reactions were performed using standard protocols (e.g. Zielske & Haase, in press). Products were purified enzymatically using exonuclease and shrimp alkaline phosphatase, and were sequenced using Big Dye Terminator Ready Reaction Mix v3.1 (ABI) and PCR primers on an ABI 3130xl Genetic Analyzer. All Sequences are available from GenBank, with accession numbers listed in Appendix 1.

Attempts to sequence additional nuclear genes for a better resolution of deeper nodes failed; however, this problem is not uncommon in gastropod studies (Dayrat *et al.*, 2011), and even the internal transcribed spacer 2 (ITS2) primers established for the same genus from Vanuatu (Zielske & Haase, in press) failed to work.

PHYLOGENETIC ANALYSES

Sequences were edited using BioEdit (Hall, 1999). Because of the lack of indels the protein-coding mitochondrial *COI* gene could be aligned by eye. The mitochondrial *16S* rRNA was initially aligned using ClustalW (Thompson, Higgins & Gibson, 1994). This alignment was then refined in RNAsalsa (Stocsits *et al.*, 2009) using secondary structure information from *Cacozeliana lacertina* Gould, 1861 (http://www.rna.icmb.utexas.edu/SIM/4D/Mollusk/; AF101007; Cannone *et al.*, 2002), with a final manual edit in BioEdit (Hall, 1999). Partition Finder (Lanfear *et al.*, 2012) was used to decide whether stem and loop structures in *16S* rRNA and codon positions in *COI* should be treated as partitions with individual substitution models, and to find the best-fitting models according to the corrected Akaike information criterion. A test for substitution saturation was performed in Dambe (Xia & Xie, 2001), treating gaps as unknown states. Phylogenetic analyses were performed in a maximum likelihood (ML) and Bayesian (BA) framework. ML analyses were run using Garli (Zwickl, 2006), with 500 independent search replicates and a bootstrap analysis with 1000 replicates, and MrBayes (Ronquist *et al.*, 2012) was run for 10 million generations, with a burn-in of 10%, considering the standard deviation of split frequencies of independent runs and convergence of parameter estimates, which were monitored in Tracer (Rambaut & Drummond, 2007) and Mr Bayes.

Ancestral ranges were reconstructed using the Bayesian Binary Method implemented in RASP (Yu, Harris & He, 2013) with ten chains and 50 000 cycles based on the most likely tree calculated in Garli defining either the ranges Viti Levu and Vanua Levu or south, west, central, and east Viti Levu, as well as Vanua Levu (see map inset in Fig. 2).

RESULTS

PHYLOGENETIC ANALYSES

According to DAMBE the data set showed no substitution saturation. Following partition finder, stem (HKY + G) and loop (TVM + I + G) structures in *16S* rRNA and codon positions in *COI* (positions I–III: TIM + I, F 81 + I, and TVM + G) were defined as separate partitions in both BA and ML analyses. As a measure of variance in the in-group, pairwise distances reached 7.92% for *COI* and 2.81% for *16S* rRNA.

The trees resulting from BA and ML analyses were practically identical (Fig. 2). The Fijian taxa built a very well supported monophyletic group of three larger clades plus *Fluviopupa seasea* Haase, Ponder & Bouchet, 2006 as sister species to the remaining species and *Fluviopupa dromodromo* sp. nov. as sister species to clade II. The monophyly of all three clades was well supported, with bootstrap values larger than 85 and BA posterior probabilities larger than 0.95. The oldest clade (clade I) comprised only and all samples found in the southern part of Viti Levu, except for *F. seasea* and *F. dromodromo* sp. nov. Clade III comprised all taxa from Vanua Levu and eastern Viti Levu, and clade II comprised all samples found in central and western Viti Levu. The position of the very distinct *F. dromodromo* sp. nov. as a sister taxon of clade II as well as the relationship between clades II and III were well supported only in the BA analysis, and therefore need to be discussed carefully. The same held true for *F. seasea*. sp. nov.

Relationships between different species in clades I and II were practically unresolved. If sister relationships within those clades were supported by bootstrapping or posterior probabilities, then the branch lengths were in general negligibly small. The same held for most taxa attributed to clade III. *Fluviopupa derua* Haase, Ponder & Bouchet, 2006, *Fluviopupa drau* sp. nov., *Fluviopupa bula* sp. nov., *Fluviopupa dumontdurvilli* sp. nov., and *Fluviopupa vatukuca* sp. nov. could not be distinguished based on the phylogenetic tree. Only *Fluviopupa tunuloa* sp. nov. from Vanua Levu and *Fluviopupa forsteri* sp. nov. from Viti Levu as one subclade, as well as *Fluviopupa vakalevu* sp. nov. and *Fluviopupa raradamu* sp. nov. as another subclade, were differentiated with good support and notable branch lengths.

The ancestral range reconstruction (Fig. 2) distinguishing only the two islands showed that the whole radiation across Fiji is most likely to have originated in Viti Levu, and that Vanua Levu was colonized three times independently, with one subsequent return to Viti Levu. If the island of Viti Levu is devided into subranges the common ancestor of the whole radiation is reconstructed in southern Viti Levu. The same held true for the common ancestors of clade I, clades II and III, and clade II and *F. dromodromo* sp. nov. The ancestor of clade II was reconstructed in western Viti Levu, with a subsequent dispersal to central and eastern Viti Levu. In conflict with the first ancestral range reconstruction, the ancestor of clade III is inferred to have been from Vanua Levu, with two subsequent colonizations of eastern Viti Levu and a return to Vanua Levu.

Out-group taxa from New Zealand, *Opacuincola delira* Haase, 2008 and *Potamopyrgus estuarinus* (Winterbourn, 1971), turned out to be more closely related to the Fijian taxa than *Tatea huonensis* from Australia and *Hemistomia winstonefi* from New Caledonia. This sister relationship of New Zealand and Fijian species was highly supported (Fig. 2).

SYSTEMATIC DESCRIPTIONS

The phylogenetic analysis revealed a few well-defined species and three clades of genetically cryptic species that only differ in morphology or anatomy. In the following we only introduce new species if the evidence is unambiguous, referring to the 'pragmatic species concept' of Haase & Bouchet (2006).

Apart from *Fluviopupa korobebe* sp.nov., which could not be analysed genetically, descriptions are ordered according to the three main clades. In order to facilitate species discrimination, the delineation of cryptic species is summarized for each clade following the respective descriptions. A comprehensive comparison based on the most informative features, also including the ten previously described species, is provided in Appendix 2.

Museum acronyms: AMS, Australian Museum Sydney; MNHN, Museum National d'Histoire Naturelle, Paris; USP, museum and scientific collection of the University of the South Pacific, Suva; ZMB, Zoologisches Museum Berlin.

Genus: Fluviopupa

For a general description of the genus *Fluviopupa* from Fiji see Haase *et al*. (2006). Characteristics attributed to the whole genus are not repeated in species descriptions, to avoid reiteration. Shell measurements are summarized in Appendix 3.

***Fluviopupa korobebe* sp. nov.**

Type material: Holotype USP12167; paratypes USP12185 (N = 4).

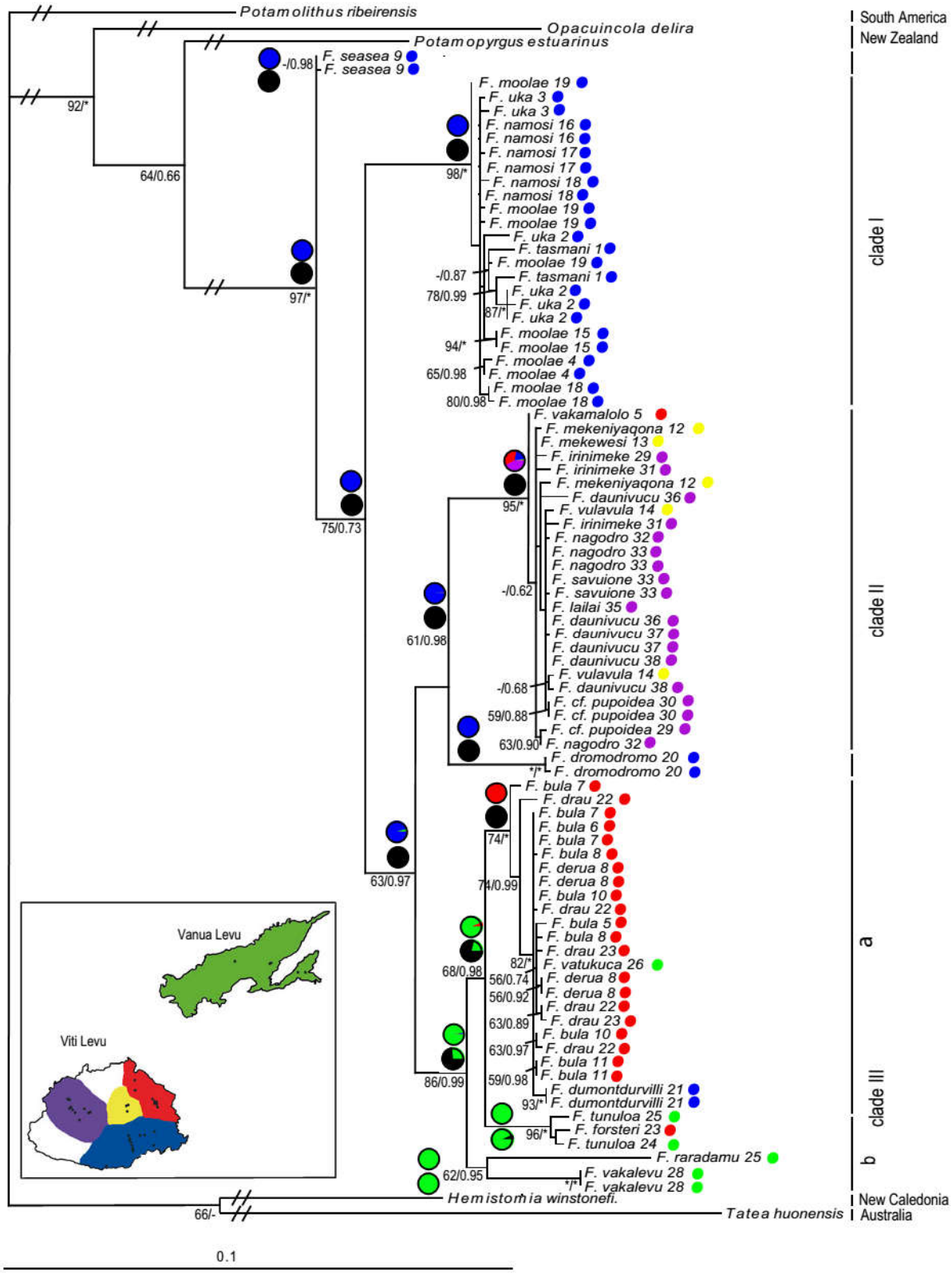
Potamolithus ribeirensis
Opacuincola delira
Potamopyrgus estuarinus
F. seasea 9
F. seasea 9
F. moolae 19
F. uka 3
F. uka 3
F. namosi 16
F. namosi 16
F. namosi 17
F. namosi 17
F. namosi 18
F. namosi 18
F. moolae 19
F. moolae 19
F. uka 2
F. tasmani 1
F. moolae 19
F. tasmani 1
F. uka 2
F. uka 2
F. uka 2
F. moolae 15
F. moolae 15
F. moolae 4
F. moolae 4
F. moolae 18
F. moolae 18
F. vakamalolo 5
F. mekeniyaqona 12
F. mekewesi 13
F. irinimeke 29
F. irinimeke 31
F. mekeniyaqona 12
F. daunivucu 36
F. vulavula 14
F. irinimeke 31
F. nagodro 32
F. nagodro 33
F. nagodro 33
F. savuione 33
F. savuione 33
F. lailai 35
F. daunivucu 36
F. daunivucu 37
F. daunivucu 37
F. daunivucu 38
F. vulavula 14
F. daunivucu 38
F. cf. pupoidea 30
F. cf. pupoidea 30
F. cf. pupoidea 29
F. nagodro 32
F. dromodromo 20
F. dromodromo 20
F. bula 7
F. drau 22
F. bula 7
F. bula 6
F. bula 7
F. bula 8
F. derua 8
F. derua 8
F. bula 10
F. drau 22
F. bula 5
F. bula 8
F. drau 23
F. vatukuca 26
F. derua 8
F. derua 8
F. drau 22
F. drau 23
F. bula 10
F. drau 22
F. bula 11
F. bula 11
F. dumontdurvilli 21
F. dumontdurvilli 21
F. tunuloa 25
F. forsteri 23
F. tunuloa 24
F. raradamu 25
F. vakalevu 28
F. vakalevu 28
Hemistomia winstonefi.
Tatea huonensis
-/0.98
92/*
64/0.66
97/*
75/0.73
98/*
-/0.87
78/0.99
87/*
94/*
65/0.98
80/0.98
95/*
-/0.62
61/0.98
-/0.68
59/0.88
63/0.90
/
74/*
74/0.99
63/0.97
68/0.98
82/*
56/0.74
56/0.92
63/0.89
63/0.97
59/0.98
86/0.99
93/*
96/*
62/0.95
/
66/-
Vanua Levu
Viti Levu
South America
New Zealand
clade I
clade II
a
clade III
b
New Caledonia
Australia
0.1

Figure 2. Maximum-likelihood tree (best of 500 replicates) of the concatenated data set; vertical lines indicate that branches have been shortened by 75%; values are maximum-likelihood bootstrap values/Bayesian posterior probabilities; */* = 100/1; scale bar, substitutions per site; numbers indicate sampling sites; pie charts at nodes show results of ancestral range reconstructions – lower only Viti Levu/Vanua Levu, upper with Viti Levu subranges, colour code indicated in the map, black Viti Levu ($P < 0.05$ not shown).

Type locality: Spring, a few 100 m west of Korobebe/Nandele, beside road (station 35: 17°43′51.9″S, 177°33′04.0″E), Viti Levu, Fiji.

Etymology: This species is named after the Village of Korobebe, formerly known as Nandele, near which it was found.

Diagnosis: This species is characterized by its relatively small and slender shell. Species of similar size, *F. seasea* and *Fluviopupa savuione* sp. nov., are significantly burlier (Student's *t*-test of sh/shell width (sw): $P < 0.01$).

Description
Shell (Fig. 3A): Slender conic, nearly white to brown; 1.6–2 mm high, ~1.9 times higher than wide, convex whorls, aperture ovate pyriform.

Operculum: With minute or without smear.

External features: Partly reduced black epidermal pigmentation.

Mantle cavity: Ctenidium with 14–16 filaments, connected with pericardium by a short duct; osphradium behind middle of ctenidium reaching quarter of its length; kidney protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females but making a U-shaped loop at anterior end of prostate in males; radula (Fig. 5A) R, 5 1 5/3 3; L, 4–5 1 4–5; M1, 16–20; M2, 28–32; small fan-shaped caecum (Fig. 8D).

Female genitalia: Ovary starting 0.75–1.0 whorls below apex, comprising up to 1.0 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia (N = 1): Testis lobate, starting 1.5 whorls below apex, comprising 1.0 whorls, reaching posterior chamber of stomach; seminal vesicle leaving testis 0.33 whorls proximal to anterior end; penis (Fig. 7A) tapering continuously from broad base to blunt end.

Remarks: Only a few individuals of this species were found, which is why we had to refrain from genetic analyses.

Clade I

***Fluviopupa tasmani* sp. nov.**

Type material: Holotype USP12168; paratypes AMS C483408 ($N = 10$), MNHN-IM-2012-2705 ($N = 10$), USP12186 ($N = 39$), and ZMB121000 ($N = 39$).

Type locality: Stream over rock besides Queen's Road, Korovou (station 1: 18°15′03.5″S, 177°57′43.8″E), Viti Levu, Fiji.

Etymology: This species is dedicated to the Dutch explorer Abel Janszoon Tasman (1603–1659), who in 1643 was the first to discover the islands of Fiji.

Diagnosis: Fluviopupa tasmani sp. nov. is most similar to *Fluviopupa uca* sp. nov., but has a larger and overall burlier shell (Hotellings T^2-test and Student's *t*-tests of all five shell parameters, $P < 0.05$; sh/sw and ah/aperture width (aw) ratios, $P < 0.01$), with a sinuate aperture. *Fluviopupa moolae* sp. nov., which is comparably burly, has less convex whorls. Other Fijian species of similar size or shape, like *Fluviopupa irinimeke* Haase, Ponder & Bouchet 2006 or *Fluviopupa lali* Haase, Ponder & Bouchet 2006 do not share the terminal penis lappet.

Description
Shell (Figs 3B, 4A): Conic, light brown or white; larger than 2 mm, ~1.8 times higher than wide, moderately convex whorls; protoconch comprising 1–1.1 whorls; aperture ovate pyriform, outer lip sinuate.

Operculum: With small white smear.

External features: Variably intense black pigmentation, area above distal genitalia unpigmented.

Mantle cavity: Ctenidium with 20–23 filaments, abutting directly on pericardium; osphradium behind middle

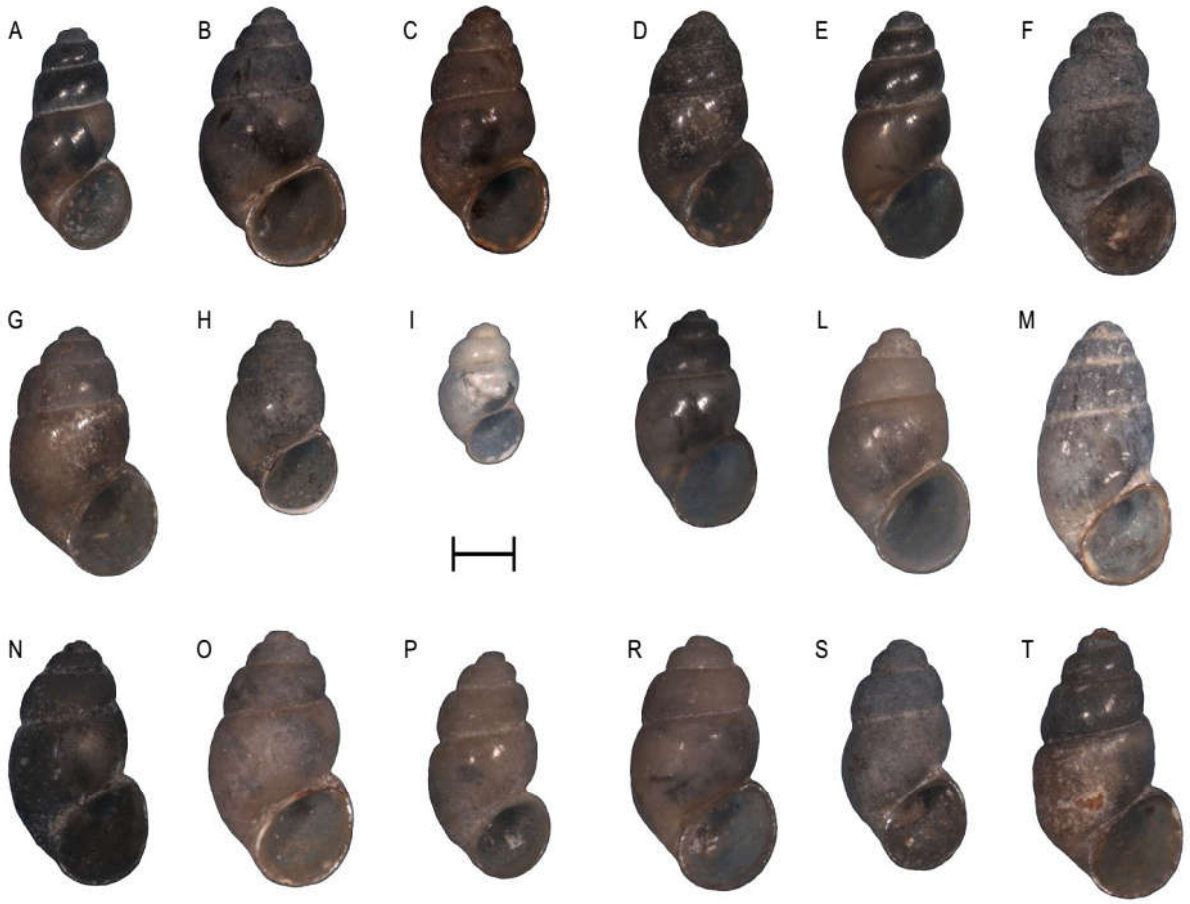

Figure 3. Holotypes: A, ***Fluviopupa korobebe* sp. nov.**; B, ***Fluviopupa tasmani* sp. nov.**; C, ***Fluviopupa uka* sp. nov.**; D, ***Fluviopupa moolae* sp. nov.**; E, ***Fluviopupa namosi* sp. nov.**; F, ***Fluviopupa nagodro* sp. nov.**; G, ***Fluviopupa savuione* sp. nov.;** H, ***Fluviopupa lailai* sp. nov.**; I, ***Fluviopupa vulavula* sp. nov.**; K, ***Fluviopupa dromodromo* sp. nov.**; L, ***Fluviopupa bula* sp. nov.**; M, ***Fluviopupa dumontdurvilli* sp. nov.**; N, ***Fluviopupa drau* sp. nov.**; O, ***Fluviopupa vatukuca* sp. nov.**; P, ***Fluviopupa forsteri* sp. nov.**; R, ***Fluviopupa tonuloa* sp. nov.**; S, ***Fluviopupa raradamu* sp. nov.**; T, ***Fluviopupa vakalevu* sp. nov.** Scale bar: 500 μm.

of ctenidium reaching third of its length; kidney protruding into roof of mantle cavity; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females but making U-shaped loop at anterior end of prostate in males; radula (Fig. 5B) R, 4–5 1 4–5/4 4; L, 4–5 1 4–5; M1, 24–26; M2, 30–31; well-developed fan-shaped caecum (Fig. 8A).

Female genitalia: Ovary starting 1.0 whorls below apex, comprising up to 1.25 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland translucent white, posterior section opaque white, albumen gland translucent white; genital opening terminal.

Male genitalia: Testis lobate, starting 0.75–1.5 whorls below apex, comprising 1–1.5 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.25 whorls proximal to anterior end; penis (Fig. 7B) slender, tapering continuously, left lappet forming small papilla with terminal genital opening, blunt end.

Remarks: Males and females of this species differ significantly (Hotellings T^2-test, $P < 0.05$) with males being more slender in sw, bww, and aw (Student's t-test, $P < 0.01$).

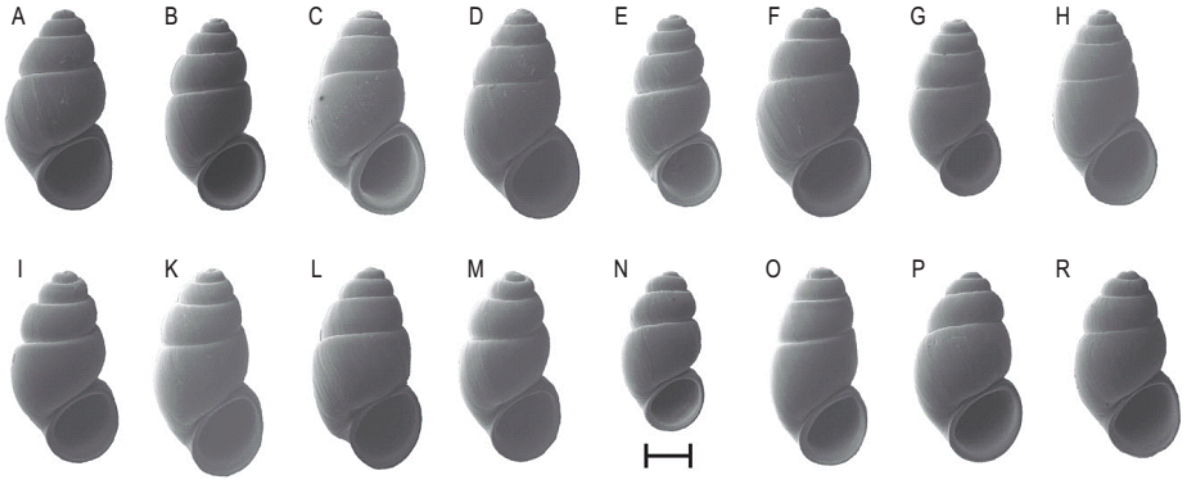

Figure 4. Scanning electron microscopy (SEM) of shells: A, ***Fluviopupa tasmani*** **sp. nov.**; B, ***Fluviopupa uka*** **sp. nov.**; C, ***Fluviopupa moolae*** **sp. nov.**; D, ***Fluviopupa moolae*** **sp. nov.** station 18; E, ***Fluviopupa namosi*** **sp. nov.**; F, ***Fluviopupa nagodro*** **sp. nov.** station 33; G, ***Fluviopupa savuione*** **sp. nov.**; H, ***Fluviopupa vulavula*** **sp. nov.**; I, ***Fluviopupa dromodromo*** **sp. nov.**; K, ***Fluviopupa bula*** **sp. nov.**; L, ***Fluviopupa dumontdurvilli*** **sp. nov.**; M, ***Fluviopupa drau*** **sp. nov.**; N, ***Fluviopupa vatukuca*** **sp. nov.**; O, ***Fluviopupa forsteri*** **sp. nov.**; P, ***Fluviopupa tonuloa*** **sp. nov.**; R, ***Fluviopupa vakalevu*** **sp. nov.** If not indicated, shells were taken from type localities. Scale bar: 500 μm.

Fluviopupa uka **sp. nov.**

Type material: Holotype USP12169; paratypes AMS C483409 (N = 10), MNHN-IM-2012-2706 (N = 10), USP12187 (N = 25), and ZMB121001 (N = 22).

Type locality: Trickle over rock beside Namosi Road (station 3: 18°08′01.5″S, 178°12′16.5″E), Viti Levu, Fiji.

Additional material: Trickle over rock beside Namosi Road (station 2: 18°08′23.8″S, 178°12′40.1″E; AMS C483416 N = 10, MNHN-IM-2012-29784 N = 10, USP12203 N = 17, ZMB121008 N = 17), Viti Levu, Fiji.

Etymology: Uka is the Fijian word for rain and refers to the heavy rainfall we had to endure the day we collected this species.

Diagnosis: The overall shell size and shape of this species with convex whorls is very similar to *Fluviopupa namosi* sp. nov. (see CVA, Fig. 10), but its aperture is more ovate and it has a terminal penis lappet. The latter also distinguishes it from previously described species of similar size, such as *Fluviopupa daunivucu* Haase, Ponder & Bouchet 2006. *Fluviopupa moolae* sp. nov., which has an overlapping range, has hardly convex whorls and is stouter (Hotellings T^2-test and Student's t-test sh/sw, $P < 0.01$).

Description

Shell (Figs 3C, 4B): Slender conic, colour varying from almost white to light brown, shell variable in size and shape but smaller than 2.4 mm, convex whorls; protoconch comprising 0.85–1.0 whorls; aperture ovate pyriform.

Operculum: Without white smear.

External features: Pigmentation variable, with some individuals being nearly unpigmented, area above distal genitalia and head in front of the eyes usually unpigmented.

Mantle cavity: Ctenidium with 18–22 filaments, abutting directly on pericardium; osphradium behind middle of ctenidium, reaching a quarter of its length; kidney only slightly or not protruding into roof of mantle cavity; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females, but making a weak U-shaped loop at anterior end of prostate in males; radula (Fig. 5C) R, 4–5 1 4–5/4 4; L, 5 1 4–5; M1, 23–24; M2, 28–31; short to well-developed fan-shaped caecum.

Female genitalia: Ovary starting 0.75–1.5 whorls below apex, comprising up to 1.5 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds to same as of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland translucent white; genital opening terminal.

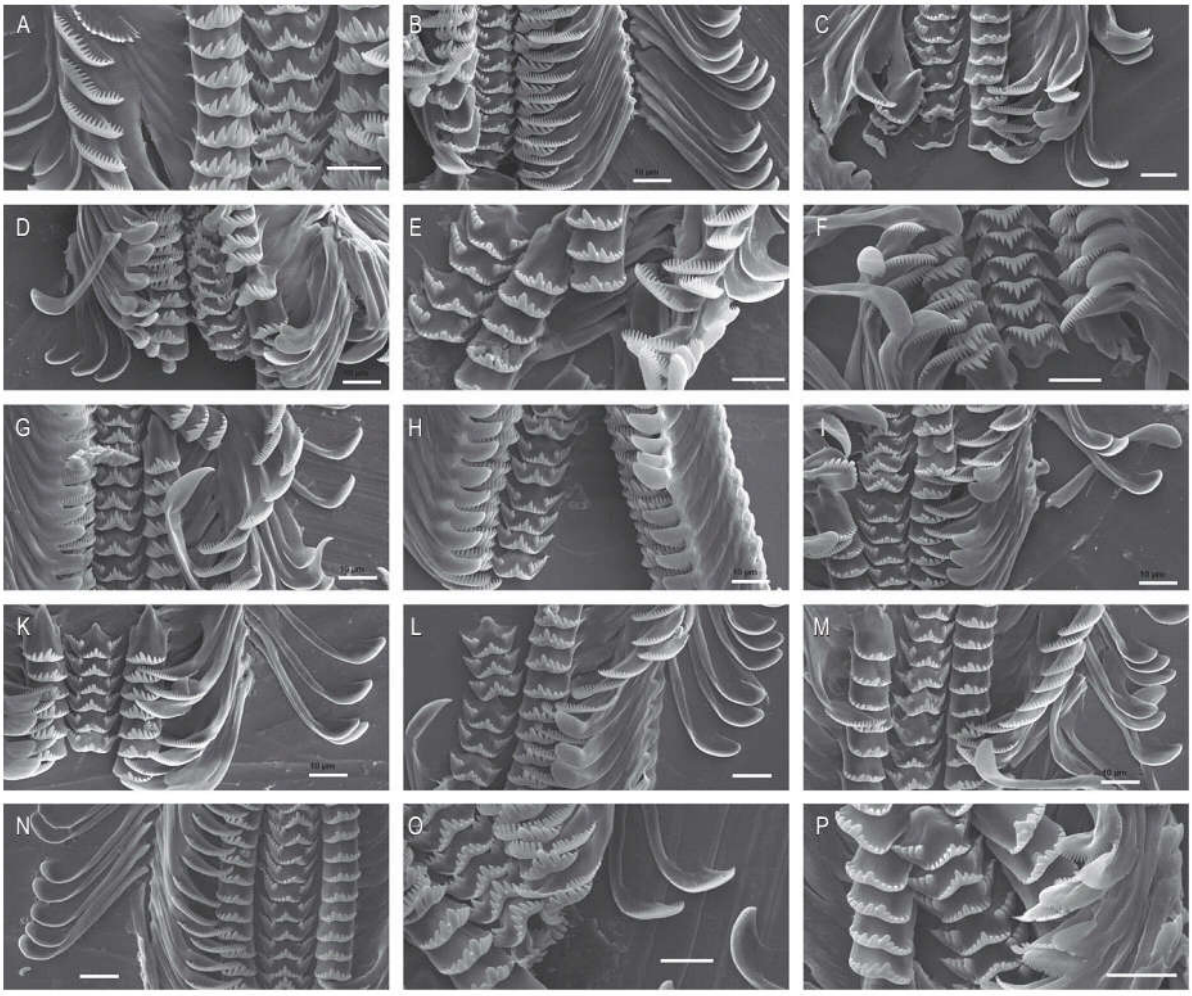

Figure 5. Scanning electron microscopy (SEM) of radula: A, ***Fluviopupa korobebe*** **sp. nov.**; B, ***Fluviopupa tasmani*** **sp. nov.**; C, ***Fluviopupa uka*** **sp. nov.**; D, ***Fluviopupa moolae*** **sp. nov.**; E, ***Fluviopupa namosi*** **sp. nov.**; F, ***Fluviopupa nagodro*** **sp. nov.**; G, ***Fluviopupa savuione*** **sp. nov.**; H, ***Fluviopupa vulavula*** **sp. nov.**; I, ***Fluviopupa dromodromo*** **sp. nov.**; K, ***Fluviopupa bula*** **sp. nov.**; L, ***Fluviopupa dumontdurvilli*** **sp. nov.**; M, ***Fluviopupa drau*** **sp. nov.**; N, ***Fluviopupa vatukuca*** **sp. nov.**; O, ***Fluviopupa tunuloa*** **sp. nov.**; P, ***Fluviopupa vakalevu*** **sp. nov.** Scale bar: 10 μm.

Male genitalia: Testis lobate, starting 0.75–1.25 whorls below apex, comprising 1–1.5 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.33 whorls proximal to anterior end; penis (Fig. 7C, D) slender, tapering continuously from a broad base, left lappet forming papilla with terminal genital opening, blunt end.

Fluviopupa moolae **sp. nov.**

Type material: Holotype USP12170; paratypes AMSC 483410 (N = 10), MNHN-IM-2012-2707 (N = 10), USP12188 (N = 18), and ZMB121002 (N = 18).

Type locality: Trickle beside waterfall near Namosi Road (station 4: 18°06′44.5″S, 178°11′10.8″E), Viti Levu, Fiji.

Additional material: Stream near Namosi Road (station 15: 18°04′35.6″S, 178°09′47.2″E; AMS C483417 N = 10, MNHN-IM-2012-29785 N = 10, USP12204 N = 22, ZMB121009 N = 22), spring at Narukutua (station 18: 18°02′15.9″S, 178°09′13.2″E; AMS C483418 N = 10, MNHN-IM-2012-29786 N = 10, USP12205 N = 17, ZMB121010 N = 18), spring at Waidina Road (station 19: 18°00′59.6″S, 178°15′13.0″E; AMS C483419 N = 10, MNHN-IM-2012-29787 N = 10, USP12106 N = 24, ZMB121011 N = 24), all Viti Leu, Fiji.

Etymology: Moola is the name of the lucky charm that accompanied us on our fieldtrip through Fiji.

Diagnosis: Fluviopupa moolae sp. nov. is recognized by its burly shell with hardly convex whorls and the presence of the hypobranchial gland. *Fluviopupa* cf. *pupoidea* Pilsbry, 1911, which has similar whorls, is much larger (Haase *et al.*, 2006). Compared with the sympatric *F. namosi* sp. nov., this species is significantly less slender and has notably less convex whorls.

Description
Shell (Figs 3D, 4C, D): Conic, very light brown to brown, smaller than 2.2 mm, hardly convex whorls; protoconch comprising 0.9–1.1 whorls, aperture D-shaped to pyriform.

Operculum: With small white smear.

External features: Variable pigmentation, area above distal genitalia hardly or not pigmented.

Mantle cavity: Ctenidium with 16–23 filaments, abutting on or connected with pericardium by a very short vessel; osphradium behind middle of ctenidium reaching quarter to third of its length; kidney hardly protruding into pallial roof; hypobranchial gland occasionally apparent in dissections, reaching up to seventh gill filament.

Digestive system: Rectum close to pallial oviduct in females but making a weak U-shaped loop at anterior end of prostate in males; radula (Fig. 5D) R, 5–6 1 5–6/3–4 3–4; L, 4–6 1 4–6; M1, 20–26; M2, 27–33; well-developed fan-shaped caecum.

Female genitalia: Ovary starting 0.75–1.5 whorls below apex, comprising up to 1.5 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland three-quarters of capsule gland, about two-thirds to three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland translucent milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 0.75–1.5 whorls below apex, comprising 0.75–1.5 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.25–0.5 whorls proximal to anterior end; penis (Fig. 7E, F) slender, tapering continuously from a broad base, left lappet forming papilla with terminal genital opening, blunt end.

Remarks: Specimens found at station 18 are larger than those from the other localities, but no further distinguishing anatomical or morphological features were present. As *F. moolae* sp. nov. and *F. namosi* sp. nov. coexist at this site, the larger size might result from character displacement.

***Fluviopupa namosi* sp. nov.**
Type material: Holotype USP12171; paratypes AMS C483411 ($N = 5$), USP12189 ($N = 5$), and ZMB121003 ($N = 5$).

Type locality: Stream near Namosi Road (station 16: 18°04′18.1″S, 178°09′35.5″E), Viti Levu, Fiji.

Additional material: Trickle at Namosi Road (station 17: 18°03′13.1″S, 178°09′27.9″E; USP12207 $N = 13$), spring at Narukutua (station 18: 18°02′15.9″S, 178°09′13.2″E; AMS C483420 $N = 10$, MNHN-IM-2012-29788 $N = 10$, USP12208 $N = 40$, ZMB121012 $N = 40$), both Viti Levu, Fiji.

Etymology: Named after the Province Namosi.

Diagnosis: Fluviopupa namosi sp. nov. is the only species found in southern Viti Levu with a blunt penis tip lacking a terminal penis lappet. For comparison with the sympatric *F. moolae* sp. nov., see above. Regarding shell size this species is most similar to *F. drau* sp. nov., *F. tunuloa* sp. nov., and *F. vatukuca* sp. nov., from which it is genetically very distinct. Furthermore, it is significantly less burly (Student's *t*-test sh/sw, $P < 0.01$). The same holds true for the previously described *F. lali* and *F. irinimeke*. *Fluviopupa daunivucu* is similarly slender, but has fewer whorls (with a maximum of 4.25 versus a minimum of 4.125 in this species) and has a more pyriform aperture.

Description
Shell (Figs 3E, 4E): Slender conic, light brown, shell 1.8–2.1 times higher than wide, convex whorls; protoconch comprising 0.9–1.0 whorls; aperture pyriform to nearly round.

Operculum: Without or with very small white smear.

External features: Variably intense black epidermal pigmentation; rim, area above distal genitalia, and tentacles usually only sparsely or not pigmented.

Mantle cavity: Ctenidium with 15–19 filaments, abutting directly to pericardium; osphradium behind middle of ctenidium reaching third of its length; kidney hardly protruding into pallial roof; hypobranchial gland not apparent in dissections.

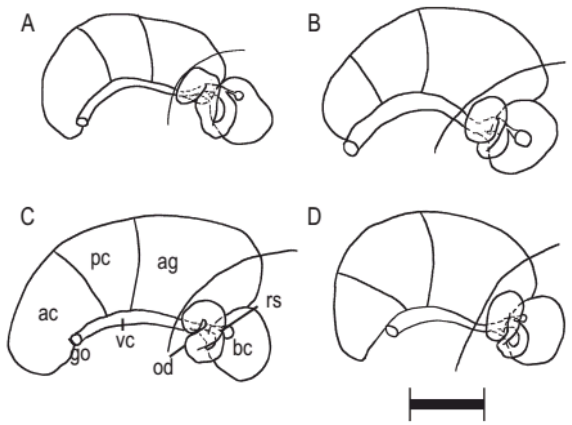

Figure 6. Distal female genitalia: examples for terminal (A and B) and subterminal (C and D) genital openings; (B) receptaculum seminis lying against central part of bursa. A, ***Fluviopupa nagodro* sp. nov.**; B, ***Fluviopupa bula* sp. nov.**; C, ***Fluviopupa namosi* sp. nov.**; D, ***Fluviopupa dromodromo* sp. nov.** Abbreviations: ac, anterior capsule gland; ag, albumen gland; bc, bursa copulatrix; go, genital opening; od, oviduct; pc, posterior capsule gland; rs, receptaculum seminis; vc, ventral channel. Scale bar: 200 μm.

Digestive system: Rectum close to pallial oviduct in females but making a weak broad U-shaped loop at anterior end of prostate in males; radula (Fig. 5E) R, 5–6 1 5–6/4 4; L, 5–6 1 4–6; M1, 22–23; M2, 32–34; short to well-developed fan-shaped caecum.

Female genitalia (Fig. 6C): Ovary starting 1.0–1.5 whorls below apex, comprising up to 1.0 whorls; receptaculum seminis lying against anterior or central third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland three-quarters of capsule gland, about two-thirds to three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland translucent milky white; genital opening subterminal to terminal.

Male genitalia: Testis lobate, starting 1.0–1.5 whorls below apex, comprising 1.0 to 1.25 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.33–0.5 whorls proximal to anterior end; penis (Fig. 7G) slender, tapering continuously, blunt end.

Species distinction within clade I
Fluviopupa uka sp. nov. and *F. namosi* sp. nov. are more slender than *F. tasmani* sp. nov. and *F. moola*. sp. nov. The latter is recognized by barely convex whorls and by the presence of the hypobranchial gland. *Fluviopupa uka* sp. nov. is the only species of this clade lacking the opercular smear, and *F. namosi* sp. nov. lacks the terminal penis lappet.

Clade II

***Fluviopupa nagodro* sp. nov.**

Type material: Holotype USP12172; paratypes AMS C483412 (N = 5), MNHN-IM-2012-2708 (N = 5), USP12190 (N = 10), and ZMB121004 (N = 10).

Type locality: Stream near Nausori Highlands Road, ~10 km west of Bukuya (station 32: 17°45′32.9″S, 177°43′00.0″E), Viti Levu, Fiji.

Additional material: Stream at 9.1 km on road to Abaca (station 33: 17°40′02.1″S, 177°31′29.8″E; AMS C483421 N = 5, MNHN-IM-2012-29789 N = 5, USP12109 N = 13, ZMB121013 N = 13) and trickle besides Savuione falls in Koroyanitu National Heritage Park (station 34: 17°40′24.1″S, 177°33′05.7″E; USP12210 N = 2), both Viti Levu, Fiji.

Etymology: Named after Nagodro, the district where this species was collected and the homonymous mountain, which is the type locality.

Diagnosis: With a size of more than 2 mm, this species is significantly larger than the co-occurring *F. savuione* sp. nov. (Hotellings T^2-test, $P < 0.01$; Student's t-test of all five shell parameters, $P < 0.01$). Furthermore, it has a higher number of whorls and more denticles at the central and inner marginal radula teeth. *Fluviopupa mekewesi* Haase, Ponder & Bouchet 2006, the only other species with a terminal penis lappet previously described from the same area, is smaller and has fewer denticles at the inner and outer marginal radula teeth. In the canonical variates analysis (CVA; Fig. 10) and genetic analysis, *Fluviopupa mekeniyaqona* Haase, Ponder & Bouchet 2006 and *Fluviopupa daunivucu* Haase, Ponder & Bouchet 2006 are not distinguishable, but they lack a terminal penis lappet.

Description
Shell (Figs 3F, 4F): Ovate conic, very light brown to brown; rarely smaller than 2 mm, 1.75–1.8 times higher than wide, convex whorls; protoconch comprising 0.9–1.1 whorls; aperture ovate pyriform, outer lip sinuate.

Operculum: With small smear.

External features: Black epidermal pigmentation, area above distal genitalia only sparsely pigmented and mantle rim unpigmented.

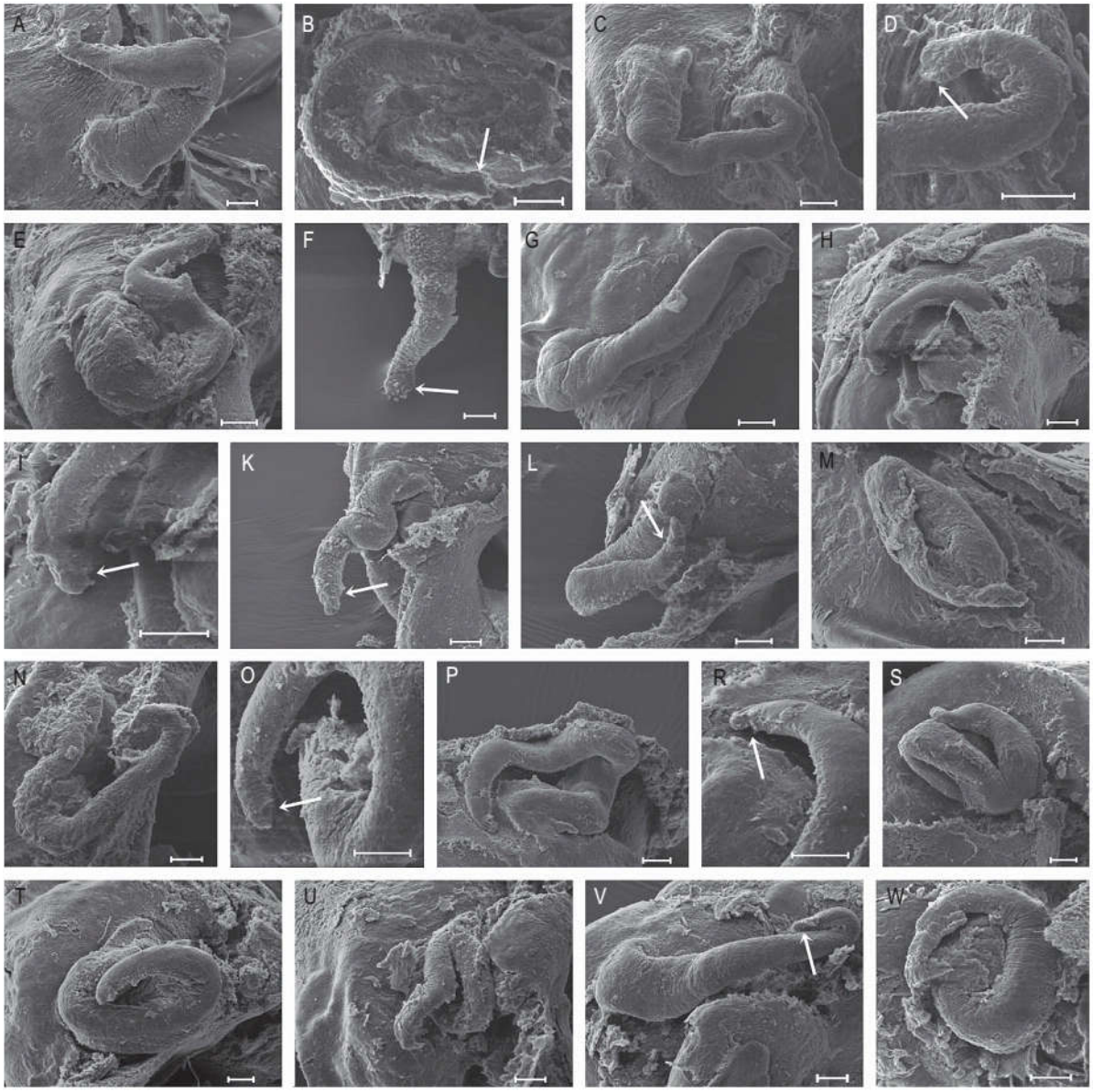

Figure 7. Scanning electron microscopy (SEM) of penis: A, ***Fluviopupa korobebe*** **sp. nov.**; B, ***Fluviopupa tasmani*** **sp. nov.**; C, D, ***Fluviopupa uka*** **sp. nov.**; E, F, ***Fluviopupa moolae*** **sp. nov.**; G, ***Fluviopupa namosi*** **sp. nov.**; H, I, ***Fluviopupa nagodro*** **sp. nov.**; K, ***Fluviopupa savuione*** **sp. nov.**; L, ***Fluviopupa vulavula*** **sp. nov.**; M, ***Fluviopupa dromodromo*** **sp. nov.**; N, O, ***Fluviopupa bula*** **sp. nov.**; P, R, ***Fluviopupa dumontdurvilli*** **sp. nov.**; S, ***Fluviopupa drau*** **sp. nov.**; T, ***Fluviopupa vatukuca*** **sp. nov.**; U, ***Fluviopupa forsteri*** **sp. nov.**; V, W, ***Fluviopupa vakalevu*** **sp. nov.** Scale bar: 400 μm.

Mantle cavity: Ctenidium with 17–23 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium reaching third of its length; kidney protruding into pallial roof; hypobranchial gland occasionally apparent in dissections, reaching fifth filament (counted from anterior).

Digestive system: Rectum close to pallial oviduct in females but making a U-shaped loop at anterior end of prostate in males; radula (Fig. 5F) R, 5–6 1 5–6/3–4 3–4; L, 4–5 1 4–5; M1, 23–26; M2, 29–31; well-developed fan-shaped caecum.

Female genitalia (Fig. 6A): Ovary starting 0.75–1.25 whorls below apex, comprising up to 1.25 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland 75–100% of capsule gland, about three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 1.0–1.5 whorls below apex, comprising 1.0–1.5 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.5 whorls proximal to anterior end; penis (Fig. 7H, I) slender, tapering continuously, left terminal penis lappet forming very small papilla with terminal genital opening, blunt end.

***Fluviopupa savuione* sp. nov.**
Type material: Holotype USP12173; paratypes USP12191 ($N = 9$).

Type locality: Stream 9.1 km on road to Abaca (station 33: 17°40′02.1″S, 177°31′29.8″E), Viti Levu, Fiji.

Additional material: Trickle beside Savuione Falls in Koroyanitu National Heritage Park (station 34: 17°40′24.1″S, 177°33′05.7″E; USP12229 $N = 2$), Viti Levu, Fiji.

Etymology: This species is named after the Savuione Falls in the Koroyanitu national park, one of two localities in which we found the new species.

Diagnosis: This species is characterized as being one of the smallest found on Fiji. *Fluviopupa korobebe* sp. nov. and *F. mekewesi*, the only species of comparable height, are more slender (for *F. mekewesi* sh/sw > 2; for *F. korobebe* sp. nov. Student's *t*-test of sh/sw: $P < 0.01$).

Description
Shell (Figs 3G, 4G): Ovate conic, very light to light brown; always smaller than 2 mm, ~1.7 times higher than wide, moderately convex whorls, aperture ovate pyriform.

Operculum: Without or with very small smear.

External features: Sparse black epidermal pigmentation, area above distal genitalia and tentacles always unpigmented.

Mantle cavity: Ctenidium with 16–19 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium reaching third of its length; kidney hardly protruding into pallial roof; hypobranchial gland reaching sixth filament of gill.

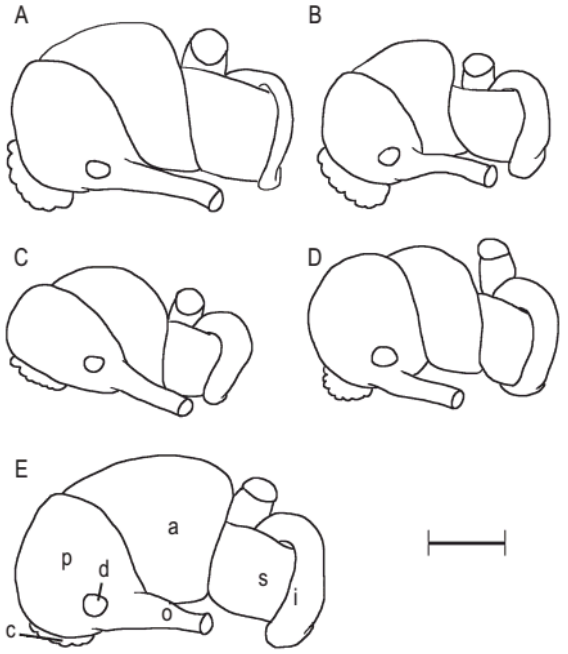

Figure 8. Stomachs: examples for well-developed (A/B), short/not protruding very far (C/E), and small (D/E) fan-shaped caeca. A, ***Fluviopupa tasmani* sp. nov.**; B, ***Fluviopupa vakalevu* sp. nov.**; C, ***Fluviopupa savuione* sp. nov.**; D, ***Fluviopupa korobebe* sp. nov.**; E, ***Fluviopupa dumontdurvilli* sp. nov.** Abbreviations: a, anterior chamber of stomach; c, caecum; d, digestive gland opening; i, intestine; o, oesophagus; p, posterior chamber of stomach; s, style sac. Scale bar: 200 μm.

Digestive system: Rectum close to pallial oviduct in females but making a U-shaped loop at anterior end of prostate in males; radula (Fig. 5G) R, 4 1 4/3 3; L, 4–5 1 4–5; M1, 20–24; M2, 28–31; short fan-shaped caecum (Fig. 8C).

Female genitalia: Ovary starting 0.75–1.25 whorls below apex, comprising up to 1.25 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds to same as length of capsule gland, about three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 0.5 whorls below apex, comprising 1.0 whorls, reaching posterior chamber of stomach; seminal vesicle leaving testis 0.5 whorls proximal to anterior end; penis (Fig. 7K) slender, tapering continuously, left terminal penis lappet forming very small papilla with terminal genital opening, pointed end.

***Fluviopupa lailai* sp. nov.**
Type material: Holotype USP12174; paratypes USP12192 ($N = 2$).

Type locality: Spring a few 100 m west of Korobebe/Nandele, beside road (station 35: 17°43′51.9″S, 177°33′04.0″E), Viti Levu, Fiji.

Etymology: Lailai is the Fijian word for small, and refers to the fact that this is the smallest *Fluviopupa* species found in Fiji so far.

Diagnosis: With a shell height of less than 1.5 mm this is by far the smallest species found in Fiji. *Fluviopupa mekewesi*, which is also quite small, is differentiated by its slender, turriform-shaped shell.

Description
Shell (Fig. 3H): Ovoid conic, very light brown or white; rarely larger than 1.5 mm, ~1.7 times higher than wide, convex whorls, aperture ovate pyriform.

External features: Sparse black pigmentation.

Mantle cavity: Ctenidium with 14 filaments, connected with pericardium by a short vessel; osphradium behind middle of ctenidium; kidney protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females; short fan-shaped caecum.

Female genitalia ($N = 1$): Ovary starting 1.25 whorls below apex, comprising 0.75 whorls; receptaculum seminis lying against central third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Remarks: As only a few individuals of this species were found, only one specimen was dissected.

***Fluviopupa vulavula* sp. nov.**
Type material: Holotype USP12175; paratypes USP12193 ($N = 3$).

Type locality: Found 6 km along the road south of Laselevu (station 14: 17°46′42.8″S, 178°07′09.9″E), Viti Levu, Fij.

Etymology: This species is named after its shell colour. Vula or vulavula is the Fijian word for white.

Diagnosis: This small- to medium-sized species with a minute terminal penis lappet is characterized by its nearly white shell, with barely convex whorls. The last two characteristics also delineate it from the equally sized *F. mekeniyaqona*, which occurs in the same area.

Description
Shell (Figs 3I, 4H): Conic, light brown to nearly white, ~2 mm high, barely convex whorls; protoconch comprising 1.0 whorls; aperture ovate to D-shaped.

Operculum: With small weak white smear.

External features: Variably intense black epidermal pigmentation, mantle rim and area above distal genitalia unpigmented.

Mantle cavity: Ctenidium with 16–21 filaments, abutting directly on pericardium; osphradium behind middle of ctenidium reaching third of its length; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in both sexes; radula (Fig. 5H) R, 4–5 1 4–5/4 4; L, 4 1 4; M1, 19; M2, 26–27; well-developed fan-shaped caecum.

Female genitalia: Ovary starting 1.5–1.75 whorls below apex, comprising up to 1.0 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland three-quarters of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 1.0 whorls below apex, comprising 1.0–1.5 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.33 whorls proximal to anterior end; penis slender, tapering continuously from a broad base, minute

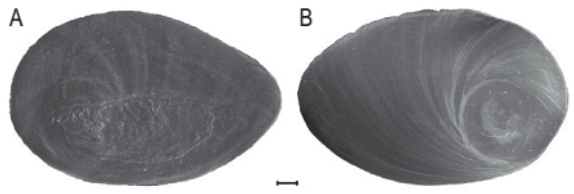

Figure 9. Operculum. ***Fluviopupa dromodromo* sp. nov.**, left interior, right exterior. Scale bar: 50 µm.

terminal penis (Fig. 7L) lappet forming papilla with terminal genital opening, pointed end.

***Fluviopupa dromodromo* sp. nov.**

Type material: Holotype USP12176; paratypes USP12194 ($N = 6$).

Type locality: Spring at Waidina Road (station 20: 18°00′31.4″S, 178°17′10.4″E), Viti Levu, Fiji.

Etymology: Dromodromo is the Fijian word for the colour yellow, and refers to the colour of the posterior section of the capsule gland.

Diagnosis: This slender and medium-sized species is genetically well differentiated from its congeners and characterized by its intense brown shell, with marked convex whorls and a nearly round aperture. Those features also separate *F. dromodromo* sp. nov. from *F. tasmani* sp. nov. and *F. nagodro* sp. nov., which have a similar shell height. The similar and previously described *F. daunivucu* sp. nov. has fewer whorls and a more slender shell with an ovate–pyriform aperture, and *F. irinimeke* is larger with a more ovate aperture. Furthermore, it lacks the terminal penis lappet.

Description

Shell (Figs 3K, 4I): Slender conic, brown, rarely smaller than 2 mm, ~1.9 times higher than wide, convex whorls; protoconch comprising 0.8–0.9 whorls; aperture ovate to round.

Operculum (Fig. 9): With small white smear.

External features: Black epidermal pigmentation, rim and area above distal genitalia sparsely or not pigmented.

Mantle cavity: Ctenidium with 18–22 filaments, connected with pericardium by a short vessel; osphradium behind middle of ctenidium reaching quarter of its length; kidney protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females but making a U-shaped loop at anterior end of prostate in males; radula (Fig. 5I) R, 4–5 1 4–5/3–4 3–4; L, 5 1 4; M1, 22–24, M2, 31–34; well-developed fan-shaped caecum.

Female genitalia (Fig. 6D): Ovary starting 1.0–1.5 whorls below apex, comprising up to 1.0 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland same as capsule gland, about three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque to yellowish white, albumen gland translucent milky white; genital opening subterminal.

Male genitalia: Testis lobate, starting 0.75–1.0 whorls below apex, comprising 1.0 whorls, reaching posterior chamber of stomach; seminal vesicle leaving testis less than 0.25 whorls proximal to anterior end; penis (Fig. 7M) slender, tapering continuously, left lappet forming papilla with terminal genital opening, blunt end.

Species distinction within clade II

Fluviopupa pupoidea is by far the largest species of this clade. By far the smallest is *F. lailai* sp. nov., and the similarly small *F. mekewesi* is further recognized by being unpigmented and having a terminal penis lappet. The latter character is shared with *F. nagodro* sp. nov, *F. sauvione* sp. nov., *F. vulavula* sp. nov., *F. dromodromo* sp. nov., and *F. pupoidea*. *Fluviopupa vulavula* sp. nov. is the only species with barely convex whorls and lacking a hypobranchial gland. *Fluviopupa nagodro* sp. nov. and *F. sauvione* sp. nov., both with distinct hypobranchial glands, can be separated by the extent of the gastric caecum and opercular smear. Both are smaller in *F. sauvione* sp. nov., which also has the smaller shell. *Fluviopupa irinimeke*, beside *F. dromodromo* sp. nov., the only species with a slender conic shell, is lacking the penis lappet and can be recognized by having a hypobranchial gland and a small caecum. *Fluviopupa mekeniyaquona*, *Fluviopupa vakamalolo* Haase, Ponder & Bouchet 2006, and *F. daunivucu* are of similar size and lack the penis lappet. Besides the differing size of the caecum, *F. daunivucu* has an opercular smear and is the only species of the whole clade with a D-shaped aperture. Furthermore, the species differ in the course of the rectum, which forms a loop in the males of *F. nagodro* sp. nov., *F. sauvione* sp. nov., *F. dromodromo* sp. nov., and *F. vakamalolo*.

Clade IIIa

***Fluviopupa bula* sp. nov.**

Type material: Holotype USP12177; paratypes AMS C483413 ($N = 10$), MNHN-IM-2012-2709 ($N = 10$), USP12195 ($N = 24$), and ZMB121005 ($N = 25$).

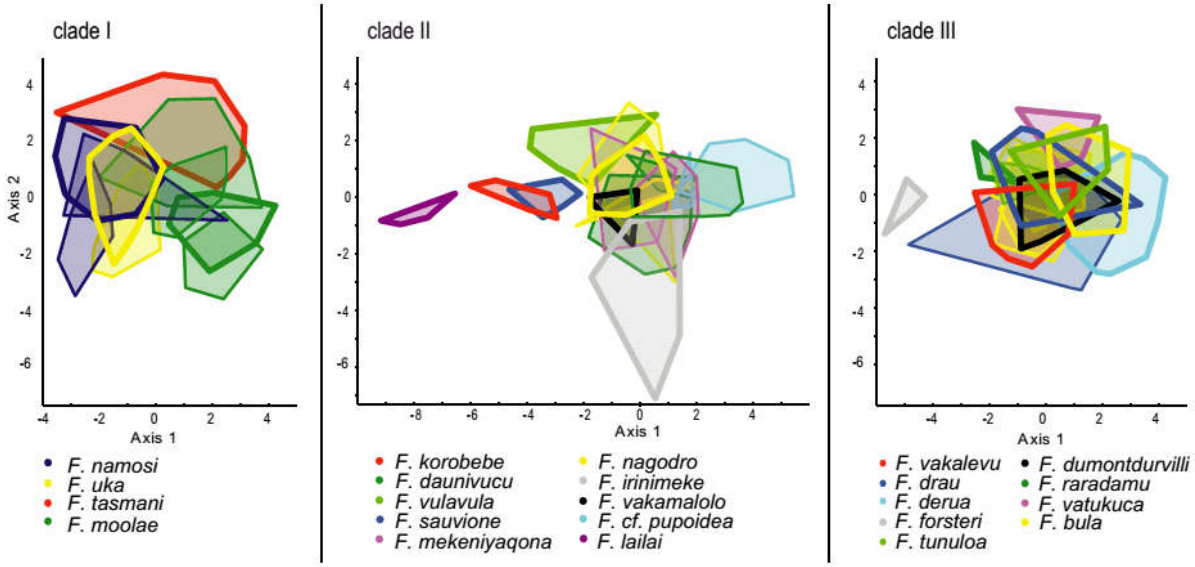

Figure 10. Canonical variates analysis of species attributed to clades I–III, respectively, based on shell measurements of up to 20 individuals per sample; samples from type localities with bold lines.

Type locality: Waidagaruru Stream (station 10: 17°44′01.8″S, 178°25′50.0″E), Viti Levu, Fiji.

Additional material: Wailotua Cave, ~20 m before western exit (station 5: ~17°45′41.2″S, 178°24′25.1″E; USP12211 $N = 6$, ZMB121014 $N = 6$); Wailotua, left tributary above cave, small basin under small waterfall (station 6: ~17°45′41.2″S, 178°24′25.1″E; USP12212 $N = 7$); Wailotua, bay in river, a few metres below station 6 (station 7: ~17°45′41.2″S, 178°24′25.1″E; AMS C483422 $N = 5$, MNHN-IM-2012-29790 $N = 5$, USP12213 $N = 5$, ZMB121015 $N = 5$); Wailotua, right tributary above cave (station 8: ~17°45′41.2″S, 178°24′25.1″E; AMS C483423 $N = 10$, MNHN-IM-2012-29791 $N = 10$, USP12214 $N = 14$, ZMB121016 $N = 15$); spring in Wainarogosavu (station 11: 17°42′17.3″S, 178°22′07.0″E; AMS C483424 $N = 10$, MNHN-IM-2012-29792 $N = 10$, USP12215 $N = 20$, ZMB121017 $N = 20$), all Viti Levu, Fiji.

Etymology: The Fijian word bula means life and is usually used as a greeting, meaning 'Hallo'.

Diagnosis: Fluviopupa bula sp. nov. is recognized by a very light-brown shell colour and a high number of denticles at the outer marginal radular teeth. It is much smaller and has a more robust operculum with a white smear, compared with *F. derua*, which occurs in the same area and is genetically not distinguishable. Concerning shell size and shape, it is most similar to *F. drau* sp. nov., whose kidney does not protrude into the pallial roof and has fewer denticles at the outer and inner marginal teeth, and *F. tunuloa* sp. nov., from Vanua Levu, which is genetically distinct, has fewer denticles at both marginal teeth, and a ctenidium with fewer filaments. In the CVA (Fig. 10), *F. vakalevu* sp. nov. is well differentiated only at the type locality, but it has more convex whorls, a ctenidium with fewer filaments, and only a small caecum. For differentiation, see the diagnosis of *F. dumontdurvilli* sp. nov.

Description

Shell (Figs 3L, 4K): Conic, very light to light brown, ~2 mm high, moderately convex whorls; protoconch comprising 0.9–1.0 whorls; aperture ovate–pyriform, outer lip slightly sinuate.

Operculum: With white smear.

External features: Black epidermal pigmentation, except for area above distal genitalia.

Mantle cavity: Ctenidium with 18–22 filaments, abutting directly on pericardium; osphradium behind middle of ctenidium reaching third to quarter of its length; kidney protruding into roof of mantle cavity; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females, but making a U-shaped loop at anterior end of prostate in males; radula (Fig. 5K) R, 4–5 1 4–5/3–4 3–4; L, 5–6 1 5–6; M1, 24–27; M2, 34–37; well-developed fan-shaped caecum.

Female genitalia (Fig. 6B): Ovary starting 1.0–1.5 whorls below apex, comprising up to 1.5 whorls; receptaculum seminis lying against central third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland same as capsule gland, about two-thirds to three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland translucent milky white, posterior section opaque to yellowish white, albumen gland milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 0.75–1.25 whorls below apex, comprising 1.0–1.25 whorls, reaching posterior chamber of stomach; seminal vesicle leaving testis 0.33 whorls proximal to anterior end; penis (Fig. 7N, O) slender, tapering continuously, left lappet forming small papilla with terminal genital opening, with pointed end.

***Fluviopupa dumontdurvilli* sp. nov.**

Type material: Holotype USP12178; paratypes USP12196 ($N = 4$).

Type locality: Spring east of Navurevure (station 21: 17°57′50.1″S, 178°22′55.2″E), Viti Levu, Fiji.

Etymology: This new species is dedicated to the French explorer Jules-Sébastien-César Dumont d'Urville, who especially in his work as a cartographer made important contributions to the exploration of Fiji.

Diagnosis: This new species is characterized by its barely convex whorls, a short vessel connecting pericardium and ctenidium, and the presence of a hypobranchial gland. Furthermore, it is one of few species with a small and short gastric caecum.

Description

Shell (Figs 3M, 4L): Ovate conic, light brown; rarely smaller than 2 mm, ~1.8 times higher than wide, rarely convex whorls; protoconch comprising 1.0–1.1 whorls; aperture ovate pyriform.

Operculum: With very small smear.

External features: Black epidermal pigmentation, mantle rim unpigmented.

Mantle cavity: Ctenidium with 19–23 filaments, connected with pericardium by a short duct; osphradium behind middle of ctenidium reaching quarter to third of its length; kidney protruding into pallial roof; hypobranchial gland reaching fifth filament of gill.

Digestive system: Rectum close to pallial oviduct in females but making a weak broad U-shaped loop at anterior end of prostate in males; radula (Fig. 5L) R, 4 1 4/4 4; L, 4 1 5; M1, 23–24; M2, 30–31; small and very short fan-shaped caecum (Fig. 8E).

Female genitalia: Ovary starting 1.5 whorls below apex, comprising up to 1.0 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds to three-quarters of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland translucent white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 1.0–1.5 whorls below apex, comprising 1.25–1.5 whorls, reaching posterior chamber of stomach; seminal vesicle leaving testis 0.33 whorls proximal to anterior end; penis (Fig. 7P, R) slender, tapering continuously, left lappet forming small papilla with terminal genital opening, with pointed end.

***Fluviopupa drau* sp. nov.**

Type material: Holotype USP12179; paratypes AMS C483414 ($N = 5$), MNHN-IM-2012-2710 ($N = 5$), USP12197 ($N = 12$), and ZMB121006 ($N = 13$).

Type locality: Trickle near King's Road, south of Savusavu (station 22: 17°34′50.6″S, 178°14′38.5″E), Viti Levu, Fiji.

Additional material: Stream over rock near King's Road, south of Rokovuaka (station 23: 17°39′08.7″S, 178°15′40.0″E; AMS C483425 $N = 10$, MNHN-IM-2012-29793 $N = 10$, USP12216 $N = 40$, ZMB121018 $N = 40$), Viti Levu, Fiji.

Etymology: Drau is the Fijian word for leaf and refers to the fact that the snails examined in this study are often found sitting on decaying leaves in small streams.

Diagnosis: This species is most similar to species occurring on Vanua Levu, for comparison with similar species from Viti Levu see the description of *F. dumontdurvilli* sp. nov. above. In comparison with the co-occurring *F. forsteri* sp. nov. it is significantly larger (Hotelling's T^2-test, $P < 0.01$; Student's t-test of all five shell parameters, $P < 0.01$) and has a more D-shaped aperture. Species from Vafua Levu in general have a ctenidium with fewer filaments and fewer denticles at the outer marginal teeth. Furthermore,

F. tunuloa sp. nov., which is genetically well differentiated but most similar in overall shell shape and size, is less pigmented.

Description
Shell (Figs 3N, 4M): Conic, light brown to brown; variable in size, moderately convex whorls; protoconch comprising 0.9–1.0 whorls; apex blunt; aperture D-shaped, outer lip slightly sinuate.

Operculum: Eventually with minute smear.

External features: Variably intense black pigmentation, rim and area above distal genitalia usually only sparsely or not pigmented.

Mantle cavity: Ctenidium with 18–23 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium reaching quarter to third of its length; kidney not protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females but making a broad U-shaped loop at anterior end of prostate in males; radula (Fig. 5M) R, 4 1 4/3–4 3–4; L, 4–5 1 4–5; M1, 23–24; M2, 29–32; small and short fan-shaped caecum.

Female genitalia: Ovary starting 1.0 to 1.25 whorls below apex, comprising up to 1.0 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland 75–100% of capsule gland, about two-thirds to three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland translucent white, posterior section translucent milky white, albumen gland milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 0.75–1.25 whorls below apex, comprising 0.75–1.25 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.25–0.33 whorls proximal to anterior end; penis (Fig. 7S) slender, tapering continuously, blunt end.

***Fluviopupa vatukuca* sp. nov.**
Type material: Holotype USP12180; paratypes USP12198 ($N = 2$).

Type locality: Trickle and small stream near Vatukuca village (station 26: 16°34′29.5″S, 179°30′50.4″E), Vanua Levu, Fiji.

Additional material: Stream near Vatukuca village (station 27: 16°34′19.1″S, 179°30′31.8″E; USP12228, $N = 1$), Vanua Levu, Fiji.

Etymology: Vatukuca is the name of the village where we found this species.

Diagnosis: This newly described species is genetically well separated from the other species occurring on the island of Vanua Levu. Furthermore, it is burlier than the other species occurring on the same peninsula (Student's *t*-test for sh/sw at type localities, $P < 0.05$). The previously described *F. lali* is more slender, with fewer protoconch whorls, and has a broader and shorter, more pointed penis. For delimitation from species found on Viti Levu see the description of *F. drau* sp. nov., *F. dumontdurvilli* sp. nov., *F. bula* sp. nov., and *F. vulavula* sp. nov. above.

Description
Shell (Figs 3O, 4N): Ovate conic, light brown; ~2 mm high and 1.6–1.65 times higher than wide, moderately convex whorls; protoconch comprising 0.9 whorls ($N = 1$), aperture ovate pyriform.

Operculum: Eventually with very small white smear.

External features: Black epidermal pigmentation; tentacles, mantle rim, and area above distal genitalia unpigmented.

Mantle cavity: Ctenidium with 16–18 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium, reaching third of its length; kidney hardly protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females but making a broad U-shaped loop at anterior end of prostate in males; radula (Fig. 5N) R, 5–6 1 5–6/3–4 3–4; L, 5 1 5; M1, 24–25; M2, 29–30; short to well-developed fan-shaped caecum.

Female genitalia ($N = 1$): Ovary starting 1.25 whorls below apex, comprising 1.0 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland three-quarters of capsule gland, about three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia ($N = 2$): Testis lobate, starting 1.0–1.25 whorls below apex, comprising 1.0–1.25 whorls, overlapping posterior chamber of stomach; seminal

vesicle leaving testis 0.5 whorls proximal to anterior end; penis (Fig. 7T) tapering continuously from broad base to pointed end.

Species distinction within clade IIIa
In this clade, *F. dumontdurvilli* sp. nov. is the only species with a terminal penis lappet and a hypobranchial gland. *Fluviopupa derua* has the smallest and most slender shell. *Fluviopupa drau* sp. nov. is the only species with a D-shaped aperture and *F. vatukuca* sp. nov. has the ctenidium with the smallest number of filaments. Further differences can be found in the extent of the caecum, which is small and short in *F. durmontdurvilli* sp. nov. and *F. drau* sp. nov., but small in *F. derua*, and short to well-developed in *F. vatukuca* sp. nov.

Clade IIIb

***Fluviopupa forsteri* sp. nov.**
Type material: Holotype USP12181; paratypes USP12199 ($N = 2$).

Type locality: Stream over rock near King's Road south of Rokovuaka (station 23: 17°39′08.7″S, 178°15′40.0″E), Viti Levu, Fiji.

Etymology: This species is dedicated to the German explorer Johann Georg Adam Forster (1754-1794), a naturalist particularly renowned for his ethnological research. He was progressive for his time and approached South Pacific indigenous people free of the typical Western or Christian prejudices. He is renowned as a founder of modern travel literature.

Diagnosis: This small species is characterized by its relatively small aperture and convex whorls. This makes it look most similar to *F. seasea*, from which it is genetically well differentiated. Furthermore, *F. seasea* is burlier (mean of sh/sw 1.77 versus 1.88) and has a relatively larger aperture (mean of sh/ah 2.73 versus 2.96).

Description
Shell (Figs 3P, 4O): Slender conic, light brown, rarely larger than 2 mm, ~1.9 times higher than wide, convex whorls; protoconch comprising 0.9 whorls ($N = 1$); aperture ovate to nearly round.

Operculum: Eventually with minute smear.

External features: Usually only sparsely pigmented, pigmentation, if apparent, concentrated on the ventral parts of the visceral sac.

Mantle cavity: Ctenidium with 16–19 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium reaching quarter of its length; kidney protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females but making a U-shaped loop at anterior end of prostate in males, radula R, 4 1 4/3 3; L, 4 1 4; M1, 18–20; M2, 24–26; small to well-developed fan-shaped caecum.

Female genitalia: Ovary starting 1.0 whorls below apex, comprising up to 1.25 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia: Testis lobate, starting 1.0–1.25 whorls below apex, comprising 1.0–1.25 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.33 whorls proximal to anterior end; penis (Fig. 7U) slender, tapering continuously, blunt end.

***Fluviopupa tunuloa* sp. nov.**
Type material: Holotype USP12182; paratypes USP12200 ($N = 3$).

Type locality: Waterfall next to Buca Road, a few kilometres west of Loa (station 24: 16°40′48.2″S, 179°47°30.0″E), Tunuloa Peninsula, Vanua Levu, Fiji.

Additional material: Seepage near roadside of Drekeniwai Road (station 25: 16°41′00.1″S, 179°42′17.0″E), Tunuloa Peninsula, Vanua Levu, Fiji.

Etymology: Tunuloa refers to the name of the peninsula where this species was found.

Diagnosis: This species is genetically very distinct, compared with most of other Fijian taxa. For distinction from species of the island of Viti Levu see above under the description of *F. drau* sp. nov. and *F. dumontdurvilli* sp. nov. *Fluviopupa vatukuca* sp. nov. found on the same island and with a similar shell height is significantly burlier (Student's t-test for sh/sw, $P < 0.05$), and has a less pointy shell shape. The previously described *F. lali* is more pigmented and has a higher number of denticles at the central, lateral, and inner marginal radula teeth (Haase *et al.*, 2006).

Description
Shell (Figs 3R, 4P): Ovoid conic, light brown to brown; about 2 mm high and 1.75 times higher than wide,

moderately convex whorls; protoconch comprising 1.0 whorls ($N = 1$); aperture ovate pyriform, outer lip hardly sinuate.

Operculum: Eventually with minute smear.

External features: Only sparsely pigmented, pigmentation concentrated on ventral parts of visceral sac but area above distal genitalia unpigmented.

Mantle cavity: Ctenidium with 15–18 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium reaching quarter to third of its length; kidney slightly protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in both sexes; radula (Fig. 5O) R, 4 1 4/3 3; L, 5 1 5; M1, 21–23; M2, 32–34; short to well-developed fan-shaped caecum.

Female genitalia: Ovary starting 0.75–1.25 whorls below apex, comprising up to 1.25 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland two-thirds of capsule gland, about three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland translucent white; genital opening terminal.

Male genitalia ($N = 2$): Testis lobate, starting 1.0 whorls below apex, comprising 1.0–1.25 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.25–0.33 whorls proximal to anterior end.

Remarks: As we only found a few male specimens, not all anatomical features, like penis shape, could be examined.

***Fluviopupa raradamu* sp. nov.**

Type material: Holotype USP12183; paratypes USP12201 ($N = 4$).

Type locality: Seepage near roadside of Drekeniwai Road (station 25: 16°41′00.1″S, 179°42′17.0″E), Tunuloa Peninsula, Vanua Levu, Fiji.

Etymology: Raradamu is the Fijian word for the colour brown and refers to the shell colour of this species.

Diagnosis: This new species is genetically well differentiated from all other congeners, and is one of only a few species on Fiji were the rectum is close to the pallial oviduct in both sexes. Furthermore, it is significantly smaller than the sympatric *F. tunuloa* sp. nov. in all five shell parameters (Hotellings T^2-test, $P < 0.01$; Student's t-tests, $P < 0.01$, at type localities), and is slightly smaller (Hotellings T^2-test, $P < 0.05$; Student's t-test for sw, ah, aw, bww, $P < 0.05$, at type localities) but significantly burlier than *F. vatukuca* sp. nov. (Student's t-test for sh/sw, $P < 0.01$), occurring on the same island. For delimitation from species found on Viti Levu see the description of *F. drau* sp. nov., *F. bula* sp. nov., and *F. dumontdurvilli* sp. nov. above.

Description

Shell (Fig. 3S): Conic, brown, less than 2 mm high, moderately convex whorls, aperture D-shaped to ovate.

External features: Unpigmented except for roof of mantle cavity and head.

Mantle cavity: Ctenidium with 15–17 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium reaching quarter to third of its length; kidney protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in both sexes; small fan-shaped caecum.

Female genitalia ($N = 1$): Ovary starting 0.75 whorls below apex, comprising 1.0 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland same as capsule gland, about three-quarters of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland milky white; genital opening terminal.

Male genitalia ($N = 1$): Testis lobate, starting 1.0 whorls below apex, comprising 1.0 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.33 whorls proximal to anterior end.

Remarks: As we found only a few specimens not all anatomical features, like penis shape, could be examined.

***Fluviopupa vakalevu* sp. nov.**

Type material: Holotype USP12184; paratypes AMS C483415 ($N = 10$), MNHN-IM-2012-2711 ($N = 10$), USP12202 ($N = 40$), and ZMB121007 ($N = 40$).

Type locality: Stream next to Savusavu–Labasa Highway, eastern roadside ~5 km south of Dama Road (station 28: 16°37′20.4″S, 179°09′48.6″E), Vanua Levu, Fiji.

Etymology: Vaka levu is the Fijian expression for plenty, and refers to the fact that this was one of the few

species, especially on the island of Vanua Levu, that occurred as a large population so that we could collect a larger sample.

Diagnosis: Fluviopupa vakalevu sp. nov. with its unique turriform shell shape is genetically well separated. Compared with the species co-occurring on Vanua Levu it has a high number of denticles at the outer marginal teeth and is the only species with a small terminal penis lappet. Regarding the shell size it has in general more whorls and is more slender (Student's *t*-tests for sh/sw at type localitiess, $P < 0.01$) than *F. tunuloa* sp. nov. and *F.vatukuca*. sp. nov. *Fluviopupa raradamu* sp. nov., which is comparably slender, is smaller overall (Student's *t*-tests for sh, sw, ah, and aw, $P < 0.05$). In comparison with *F. lalinimeke* and *F. lali* it has a more slender bww. For delimitation from species found on Viti Levu, see the description of *F. bula* sp. nov., *F. dromodromo* sp. nov., and *F. dumontdurvilli* sp. nov. above.

Description
Shell (Figs 3T, 4R): Conic turriform, very light brown; ~2.1 mm high and 1.8–1.85 times higher than wide, hardly convex whorls; protoconch comprising 0.9 whorls; aperture ovate pyriform.

Operculum: Without smear.

External features: Variably intense black pigmentation, rim and area above distal genitalia always unpigmented.

Mantle cavity: Ctenidium with 15–19 filaments, directly abutting to pericardium; osphradium behind middle of ctenidium reaching third of its length; kidney rarely protruding into pallial roof; hypobranchial gland not apparent in dissections.

Digestive system: Rectum close to pallial oviduct in females, but making a U-shaped loop at anterior end of prostate in males; radula (Fig. 5P) R, 5 1 5/4 4; L, 5 1 5; M1, 25; M2, 33–35; well-developed fan-shaped caecum (Fig. 8B).

Female genitalia: Ovary starting 0.75–1.0 whorls below apex, comprising up to 0.75 whorls; receptaculum seminis lying against anterior third of bursa copulatrix, bursal duct entering below anterior edge of bursa; length of albumen gland three-quarters of capsule gland, about two-thirds of albumen gland extending into pallial roof, anterior section of capsule gland milky white, posterior section opaque white, albumen gland translucent white; genital opening terminal.

Male genitalia: Testis lobate, starting 1.0–1.25 whorls below apex, comprising 1.0 whorls, overlapping posterior chamber of stomach; seminal vesicle leaving testis 0.33–0.5 whorls proximal to anterior end; penis (Fig. 7V, W) tapering continuously, small left terminal penis lappet, blunt end.

Remarks: Sexes differ significantly (Hotellings T^2-test, $P < 0.01$), with males being smaller in all five shell dimensions (Student's *t*-tests: $P < 0.01$).

Species distinction within clade IIIb
The only further genetically cryptic species of clade III are *F. tunuloa* sp. nov. and *F. forsteri* sp. nov., which besides shell shape are separated by the higher number of denticles of the marginal radular teeth in *F. tunuloa* sp. nov.

MORPHOMETRICS

To delimitate genetically inseparable species we performed a CVA of each of the three clades (Fig. 10). In clade I some of the samples, especially at type localities, are well separated, but there are also large overlapping areas especially regarding the type localities of *F. uka* sp. nov. and *F. namosi* sp. nov. Most species of clade II were well separated. Focusing on the newly described species *F. nagodro* sp. nov. and *F. lailai* sp. nov. largely overlap with each other and *F. mekeniyaqona* and *F. daunivucu*. Regarding clade III, *F. forsteri* sp. nov., *F. raradamu* sp. nov., *F. vatukuca* sp. nov., and also *F. derua* are well delimitated, whereas the type localitys of *F. drau* sp. nov., *F. dumontdurvilli* sp. nov., *F. tunuloa* sp. nov., *F. bula* sp. nov., and *F. vakalevu* sp. nov. largely overlap, but are well separated in terms of genetics, locality, and/or anatomy.

DISCUSSION

The phylogenetic analyses revealed five deeper genetic lineages – clades I–III, *F. seasea*, and *F. dromodromo* – and three young radiations – the terminals of clades I–III. Together with the ancestral range reconstruction, this tree shape is in congruence with the geological history of the islands. The common ancestor of the Fijian taxa colonized the South of Viti Levu, which was part of the first significant land mass to emerge in the Late Miocene (Rodda, 1994; Neall & Trewick, 2008). From there, the younger areas of western and central Viti Levu – clade II – and eastern Viti Levu and Vanua Levu – clade I – were colonized. Those areas only emerged in the Pliocene or even later (Rodda, 1994). In the Late Pliocene and Pleistocene various tectonic events and volcanic activity made new land available, which may be the cause for the young radiations of clades I–III and for the more recent dispersal

events among regions within those clades. The ancestral range reconstructions were somewhat ambiguous concerning the number and/or order of dispersal events. For the interchange between eastern Viti Levu and Vanua Levu, the reconstruction distinguishing only the two islands indicated three independent colonizations of Vanua Levu from eastern Viti Levu, and one subsequent return. With Viti Levu subdivided into regions the analysis resulted in a prior colonization of Vanua Levu. How often snails dispersed from there to eastern Viti Levu remained unclear because incomplete lineage sorting probably obscured relationships among species. This also holds true for clade II, considering specimens from central Viti Levu, and in general is typical for young radiations (Funk & Omland, 2003). Incomplete lineage sorting is probably also the reason for the incongruence of several of the morphologically well-defined species lacking any genetic differentiation, a picture frequently encountered among tateids (Haase, 2008; Zielske *et al*., 2011; Zielske & Haase, in press).

Another problem reconstructing ancestral ranges might be that samples were unevenly distributed, and therefore some ranges are presumably underrepresented. Despite the inaccessibility of boondocks (especially in the centre of the islands) this problem is mainly caused by anthropogenic environmental changes, for example through land use for spacious pine and sugarcane plantations, as well as cattle farming, on Vanua Levu, and in the northern and western parts of Viti Levu (Anderson, 2002; Dadhich & Nadaoka, 2012). This may also have resulted in the loss of (type) localities of recently described species, e.g. the type localities of *F. vakamalolo* Haase *et al*., 2006 and *F. lali* Haase *et al*., 2006.

The colonization history outlined above is not necessarily linked temporally with the geological history of Fiji. A rough calibration based on the average corrected genetic distance among the three clades (5.80%) and the substitution rate of cytochrome *c* oxidase subunit I (*COI*) estimated for New Zealand species (3.26 ± 0.14%/Myr; Haase, Marshall & Hogg, 2007) would indeed suggest that the radiation over Fiji is younger (less than 2 Myr) and thus uncoupled from the geological history. An ultrametric, time-calibrated tree generated in BEAST (Drummond *et al*., 2012), based solely on the above substitution rate (see Zielske & Haase, in press), was topologically inconsistent with all other analyses, so that we refrained from its presentation here. We postpone the temporal estimation of the colonization of Fiji to a comprehensive analysis across the entire family based on more genetic loci and geological calibration points (S. Zielske, W. F. Ponder, M. Haase, unpubl. data). In the same account a comprehensive analysis of the tateid biogeographic history across the entire South Pacific region will be provided; however, our phylogenetic analyses showed with high support that species from Fiji are much closer related to tateids from New Zealand, which represent a much older radiation (Haase *et al*., 2007), than to those from less distant New Caledonia or Australia. This fits into the scenario of the stepping stone-like dispersal of tateids across the South Pacific out of New Zealand, similar to the suggestion of Haase *et al*. (2010a), apart from the exclusion of New Caledonia. The vast majority of the flora of Fiji is of Indo-Malesian origin (Ryan, 2009). For animals, comprehensive data do not exist but several analyses point in the same direction (Monaghan *et al*., 2006; Balke *et al*., 2007; Lucky & Sarnat, 2010). Thus, the New Zealand origin of Fijian tateid gastropods – no matter whether direct or via stepping stones – is exceptional.

Also, anatomical features (operculum without pegs, radula, fan-shaped caecum of variable extent, usually blunt penis, occasionally with a small left lappet) support a direct or indirect New Zealand origin of the tateid radiation across the South Pacific (compare Haase *et al*., 2010a). The variation of those features is not necessarily appropriate to examine relationships within the genus *Fluviopupa*, however, and subtle morphological and anatomical differentiation of genetically distinct species, and even genera, is quite typical for the family and related taxa studied (e.g. Liu, Heshler & Clift, 2003; Wilke, 2003; Haase, 2008). The apparent mosaic-like evolution and the high degree of homoplasy pose additional problems for truncatelloidean systematics (see, Falniowski & Szarowska, 1995; Hershler & Ponder, 1998; Colgan *et al*., 2007; Wilke *et al*., 2013). This exceptional situation is reflected by the impossibility to separate the three main lineages or several of the genetically distinct species found on Fiji using morphological or anatomical features.

As discussed recently (Haase, Naser & Wilke, 2010b), the most plausible vectors for long-distance dispersal of truncatelloidean gastropods are birds. This is further supported by more recent studies demonstrating that small snails, aquatic as well as terrestrial, can even survive passage through the gut of birds (van Leeuwen *et al*., 2012; Wada, Kawakami & Chiba, 2012).

ACKNOWLEDGEMENTS

We are indebted to Gillianne Brodie, Thomas Dunn, and Marika Tuiwawa, all from the University of the South Pacific in Suva, for invaluable logistic support and an uncounted number of field guides throughout the islands. At the University of Greifswald we thank Rabea Schlüter, head of the Laboratory of Electron Microscopy, and our technician Christel Meibauer, who helped with DNA work. Furthermore, we thank an anonymous reviewer whose comments helped to improve

an earlier version of this article. Financial support was received from the Deutsche Forschungsgemeinschaft (grant HA4752/2-1).

REFERENCES

Anderson A. 2002. Faunal collapse, landscape change and settlement history in remote Oceania. *World Archaeology* **33:** 375–390.

Balke M, Wewalka G, Alarie Y, Ribera I. 2007. Molecular phylogeny of pacific island colymbetinae: radiation of New Caledonian and Fijian species (Coleoptera, dytiscidae). *Zoologica Scripta* **36:** 173–200.

Bernasconi R. 1995. Two new cave prosobranch snails from Papua New Guinea: *Selmistomia beroni* n. gen. n. sp. (Caenogastropoda: Hydrobiidae) and *Georissa papuana* n. sp. (Archaeogastropoda: Hydrocenidae). (Zoological results of the British Speleological Expedition to Papua New Guinea 1975.). *Revue Suisse de Zoologie* **102:** 373–386.

Cannone J, Subramanian S, Schnare M, Collett J, D'Souza L, Du Y, Feng B, Lin N, Madabusi L, Muller K, Pande N, Shang Z, Yu N, Gutell R. 2002. The Comparative RNA Web (CRW) Site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BMC Bioinformatics* **3:** 15.

Colgan DJ, Ponder WF, Beacham E, Macaranas J. 2007. Molecular phylogenetics of Caenogastropoda (Gastropoda: Mollusca). *Molecular Phylogenetics and Evolution* **42:** 717–737.

Dadhich AP, Nadaoka K. 2012. Analysis of terrestrial discharge from agricultural watersheds and its impact on nearshore and offshore reefs in Fiji. *Journal of Coastal Research* **28:** 1225–1235.

Dayrat B, Conrad M, Balayan S, White TR, Albrecht C, Golding R, Gomes SR, Harasewych MG, de Frias Martins AM. 2011. Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): new insights from increased taxon sampling. *Molecular Phylogenetics and Evolution* **59:** 425–437.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Falniowski A, Szarowska M. 1995. Can poorly understood new characters support a poorly understood phylogeny? Shell-structure data in Hydrobiid systematics (Mollusca: Gastropoda: Prosobranchia: Hydrobiidae). *Journal of Zoological Systematics and Evolutionary Research* **33:** 133–144.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3:** 294–299.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics* **34:** 397–423.

Haase M. 2005. Rapid and convergent evolution of parental care in hydrobiid gastropods from New Zealand. *Journal of Evolutionary Biology* **18:** 1076–1086.

Haase M. 2008. The radiation of hydrobiid hastropods in New Zealand: a revision including the description of new species based on morphology and mtDNA sequence information. *Systematics and Biodiversity* **6:** 99–159.

Haase M, Bouchet P. 1998. Radiation of crenobiontic gastropods on an ancient continental island: the *Hemistomia*-clade in New-Caledonia (Gastropoda: Hydrobiidae). *Hydrobiologia* **367:** 43–129.

Haase M, Bouchet P. 2006. The radiation of hydrobioid gastropods (Caenogastropoda, Rissooidea) in Ancient Lake Poso, Sulawesi. *Hydrobiologia* **556:** 17–46.

Haase M, Fontaine B, Gargominy O. 2010a. Rissooidean freshwater gastropods from the Vanuatu archipelago. *Hydrobiologia* **637:** 53–71.

Haase M, Gargominy O, Fontaine B. 2005. Rissooidean freshwater gastropods from the middle of the Pacific: the genus *Fluviopupa* on the Austral Islands (Caenogastropoda). *Molluscan Research* **25:** 145–163.

Haase M, Marshall B, Hogg I. 2007. Disentangling causes of disjunction on the South Island of New Zealand: the Alpine fault hypothesis of vicariance revisited. *Biological Journal of the Linnean Society* **91:** 361–374.

Haase M, Naser MD, Wilke T. 2010b. Ecrobia grimmi in brackish Lake Sawa, Iraq: indirect evidence for long-distance dispersal of hydrobiid gastropods (Caenogastropoda: Rissooidea) by birds. *Journal of Molluscan Studies* **76:** 101–105.

Haase M, Ponder WF, Bouchet P. 2006. The genus Fluviopupa Pilsbry, 1911 from Fiji (Caenogastropoda, Rissooidea). *Journal of Molluscan Studies* **72:** 119–136.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41:** 95–98.

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4:** 1–9.

Hershler R, Ponder WF. 1998. *A review of morphological characters of hydrobioid snails*. Washington, DC: Smithonian Institution Press.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29:** 1695–1701.

Liu HP, Heshler R, Clift K. 2003. Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Molecular Ecology* **12:** 2771–2782.

Lucky A, Sarnat EM. 2010. Biogeography and diversification of the Pacific ant genus *Lordomyrma* Emery. *Journal of Biogeography* **37:** 624–634.

Monaghan MT, Balke M, Pons J, Vogler AP. 2006. Beyond barcodes: complex DNA taxonomy of a south pacific island radiation. *Proceedings of the Royal Society B-Biological Sciences* **273:** 887–893.

Nation JL. 1983. A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. *Biotechnic and Histochemistry* **6:** 347–351.

Neall VE, Trewick SA. 2008. The age and origin of the Pacific islands: a geological overview. *Philosophical Transactions of the Royal Society B: Biological Sciences* **363:** 3293–3308.

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Gabowski G. 1991. *The simple fool's guide to PCR*. Honolulu: Dept. of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Ponder WF. 1981. *Posticobia norfolkensis* (Sykes), an apparently-extinct, fresh-water snail from Norfolk Island (Gastropoda: Hydrobiidae). *Proceedings of the Linnean Society of New South Wales* **105:** 17–21.

Ponder WF. 1982. Hydrobiidae of Lord Howe Island (Mollusca: Gastropoda: Prosobranchia). *Australian Journal of Marine and Freshwater Research* **33:** 89–159.

Ponder WF, Clark GA. 1988. A morphological and electrophoretic examination of '*Hydrobia buccinoides*', a variable brackish-water gastropod from temperate Australia (Mollusca: Hydrobiidae). *Australian Journal of Zoology* **36:** 661–689.

Rambaut A, Drummond AJ. 2007. Tracer v1.5. Available at: http://beast.bio.ed.ac.uk/Tracer

Rodda P. 1994. Geology of Fiji. In: *Geology and submarine resources of the Tonga-Lau-Fiji region*, SOPAC Technical Bulletin. 131–151.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61:** 539–542.

Ryan P. 2009. Fiji, biology. In: Gillespie RG, Clague DA, eds. *Encyclopedia of islands*. Berkley, CA: University of California Press, 298–305.

Stocsits RR, Letsch H, Hertel J, Misof B, Stadler PF. 2009. Accurate and efficient reconstruction of deep phylogenies from structured RNAs. *Nucleic Acids Research* **37:** 6184–6193.

Thompson J, Higgins D, Gibson T. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Van Leeuwen CHA, van der Velde G, van Groenendael JM, Klaassen M. 2012. Gut travellers: internal dispersal of aquatic organisms by waterfowl. *Journal of Biogeography* **39:** 2031–2040.

Wada S, Kawakami K, Chiba S. 2012. Snails can survive passage through a bird's digestive system. *Journal of Biogeography* **39:** 69–73.

Wilke T. 2003. *Salenthydrobia* n.gen. (Rissooidea: Hydrobiidae): a potential relict of the Messinian salinity crisis. *Zoological Journal of the Linnean Society* **137:** 319–336.

Wilke T, Haase M, Hershler R, Liu H-P, Misof B, Ponder W. 2013. Pushing short DNA fragments to the limit: phylogenetic relationships of 'hydrobioid' gastropods (Caenogastropoda: Rissooidea). *Molecular Phylogenetics and Evolution* **66:** 715–736.

Xia X, Xie Z. 2001. DAMBE: software package for data analysis in molecular biology and evolution. *Journal of Heredity* **92:** 371–373.

Yu J, Harris AJ, He X-J. 2013. RASP (reconstruct ancestral state in phylogenies) 2.1 beta. Available at: http://mnh.scu.edu.cn/soft/blog/RASP

Zielske S, Glaubrecht M, Haase M. 2011. Origin and radiation of rissooidean gastropods (Caenogastropoda) in ancient lakes of Sulawesi. *Zoologica Scripta* **40:** 221–237.

Zielske S, Haase M. in press. When snails inform about geology: pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda: Tateidae). *Journal of Zoological Systematics and Evolutionary Research*. doi: 10.1111/jzs.12053.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Dissertation, University of Texas, Austin.

APPENDIX 1

GenBank accession numbers

Sample	*COI*	*16S* rRNA
Fluviopupa bula 5	KF939744	KF939661
Fluviopupa bula 6	KF939746, KF939747	KF939663, KF939664
Fluviopupa bula 7	KF939748, KF939749	KF939665, KF939666
Fluviopupa bula 8	KF939750, KF939751, KF939752, KF939753	KF939667, KF939668, KF939669, KF939670
Fluviopupa bula 10	KF939758, KF939759	KF939675, KF939676
Fluviopupa bula 11	KF939760, KF939761	KF939677, KF939678
Fluviopupa daunivucu 36	KF939811, KF939812	KF939728, KF939729
Fluviopupa daunivucu 37	KF939813, KF939814	KF939730, KF939731
Fluviopupa daunivucu 38	KF939815, KF939816	KF939732, KF939733
Fluviopupa derua 8	KF939754, KF939755	KF939671, KF939672
Fluviopupa drau 22	KF939785, KF939786, KF939787, KF939788	KF939702, KF939703, KF939704, KF939705
Fluviopupa drau 23	KF939789, KF939790	KF939706, KF939707
Fluviopupa dromodromo 20	KF939781, KF939782	KF939698, KF939699
Fluviopupa dumontdurvilli 21	KF939783, KF939784	KF939700, KF939701
Fluviopupa forsteri 23	KF939791	KF939708
Fluviopupa irinimeke 29	KF939798	KF939715
Fluviopupa irinimeke 31	KF939802, KF939803	KF939719, KF939720
Fluviopupa lailai 35	KF939810	KF939727
Fluviopupa mekeniaquona 12	KF939762, KF939763	KF939679, KF939680
Fluviopupa mekewesi 13	KF939764	KF939681
Fluviopupa moolae 4	KF939742, KF939743	KF939659, KF939660
Fluviopupa moolae 15	KF939767, KF939768	KF939684, KF939685
Fluviopupa moolae 18	KF939775, KF939776	KF939692, KF939693
Fluviopupa moolae 19	KF939777, KF939778, KF939779, KF939780	KF939694, KF939695, KF939696, KF939697
Fluviopupa nagodro 32	KF939804, KF939805	KF939721, KF939722
Fluviopupa nagodro 33	KF939806, KF939807	KF939723, KF939724
Fluviopupa namosi 16	KF939769, KF939770	KF939686, KF939687
Fluviopupa namosi 17	KF939771, KF939772	KF939688, KF939689
Fluviopupa namosi 18	KF939773, KF939774	KF939690, KF939691
Fluviopupa cf. *pupoidea* 29	KF939799	KF939716
Fluviopupa cf. *pupoidea* 30	KF939800, KF939801	KF939717, KF939718
Fluviopupa raradamu 25	KF939794	KF939711
Fluviopupa savuione 33	KF939808, KF939809	KF939725, KF939726
Fluviopupa seasea 9	KF939756, KF939757	KF939673, KF939674
Fluviopupa tasmani 1	KF939734, KF939735	KF939651, KF939652
Fluviopupa tunuloa 24	KF939792	KF939709
Fluviopupa tunuloa 25	KF939793	KF939710
Fluviopupa uka 2	KF939736, KF939737, KF939738, KF939739	KF939653, KF939654, KF939655, KF939656
Fluviopupa uka 3	KF939740, KF939741	KF939657, KF939658
Fluviopupa vakalevu 28	KF939796, KF939797	KF939713, KF939714
Fluviopupa vakamalolo 5	KF939745	KF939662
Fluviopupa vatucuca 26	KF939795	KF939712
Fluviopupa vulavula 14	KF939765, KF939766	KF939682, KF939683
*Hemistomia winstoneft**	JX970617	JX 970548
Opacuincola delira	AY631090	AY634068
Potamolithus ribeirensis	JX970618	JX970549
Potamopyrgus estuarinus	AY631103	AY634081
Tatea huonensis	JX970619	JX970550

*Listed as *Hemistomia* sp.

APPENDIX 2

Comparison of diagnostic morphological and anatomical features

		Shell					Mantle cavity				Digestive system			
		Height in mm (range)	Shape	Whorls	Aperture	Opercular smear	Pigmentation	Ctenidial filaments	Length of osphradium in relation to ctenidium	Hypobranchial gland	Rectal loop	Caecum	Radula: number of denticles of marginal teeth M1, M2	Penis lappet
Clade I	*Fluviopupa korobebe*	1.6–2.0	slender conic,	2	0, 1	0, 1	2	14–16	¼	0	1	1	16–20, 28–32	0
	Fluviopupa tasmani	2.0–2.5	conic	1	0, 1	2	1, 2	20–23	⅕	0	1	3	24–26, 30–31	1
	Fluviopupa uka	1.5–2.4	slender conic	2	0, 1	0	1, 2	18–22	¼	0	2	2, 3	23–24, 28–31	1
	Fluviopupa maolae	1.5–2.5	conic	0	1, 3	2	1, 2	16–23	¼	1	2	3	20–26, 27–33	1
	Fluviopupa namosi	1.5–2.5	slender conic	2	1, 2	0, 1	1, 2	15–19	⅕	0	2	2, 3	22–23, 32–34	0
Clade II	*Fluviopupa nagodro*	1.8–2.5	ovate conic	2	0, 1	1	2	17–23	⅕	1	1	3	23–26, 29–31	1
	Fluviopupa sauvione	1.5–1.9	ovate conic	1	0, 1	0, 1	1	16–19	⅕	1	1	2	20–24, 28–31	1
	Fluviopupa lailai	1.1–1.5	ovoid to conic	2	0, 1	–	1	14	–	0	–	2	–	–
	Fluviopupa vulavula	1.7–2.3	conic	0	0, 3	2	1, 2	16–21	⅕	0	0	3	19, 26–27	1
	Fluviopupa dromodromo	1.9–2.5	slender conic	2	0, 2	2	2	18–22	¼	0	1	3	22–24, 31–34	1
	*Fluviopupa irinimeke**	2.0–2.5	slender conic	1	0	2	2	20–24	⅕	2	0	1	21–26, 27–37	0
	*Fluviopupa mekeniyaquona**	1.7–2.3	ovate conic	1	0, 1	0	1	15–17	⅕	0	0	2	22–24, 24–28	0
	*Fluviopupa mekewesi**	1.3–1.7	slender conic, turriform	1	0, 1	0	0	12–14	⅕	0	0	3	20–22, 18–24	1
	*Fluviopupa pupoidea**	3.3–3.2	ovoid, slender conic	1	0, 1	1, 2	2	25	⅕	2	0	3	25–28, 33–35	0
	*Fluviopupa vakamaloko**	1.5–2.2	conic	2	1	0	1	15–20	⅕	0	1	1	26–34, 26–41	0
	*Fluviopupa daunivucu**	1.8–2.3	ovate conic, slender pupiform	1	3	2	2	14–18	⅕	0	0	3	18–23, 25–32	0
Clade IIIa	*Fluviopupa bula*	1.8–2.5	conic	1	0, 1	2	2	18–22	⅕ – ¼	0	1	3	24–27, 34–37	1
	Fluviopupa durmontdurvilli	2.0–2.5	ovate conic	0	0, 1	1	2	19–23	⅕ – ¼	2	2	0	23–24, 30–31	1
	Fluviopupa drau	1.8–2.5	conic	1	3	0, 1	1, 2	18–23	⅕ – ¼	0	1	0	23–24, 29–32	0
	Fluviopupa vatukuca	1.8–2.2	ovate conic	1	0, 1	0, 1	2	16–18	⅕	0	1	2, 3	24–25, 29–30	0
	*Fluviopupa derua**	1.6–2.1	slender conic	0	0, 1	0	2	18–23	⅕	0	2	1	26–30, 27–34	0
Clade IIIb	*Fluviopupa forsteri*	1.7–2.1	slender conic	2	0, 2	0, 1	1	16–19	¼	0	1	2, 3	18–20, 24–26	0
	Fluviopupa tunulua	1.9–2.3	ovoid conic	1	0, 1	0, 1	1	15–18	⅕ – ¼	0	0	2, 3	21–23, 32–34	–
	Fluviopupa raradamu	1.7–2.0	conic	1	0, 3	–	1	15–17	⅕ – ¼	0	0	1	–	–
	Fluviopupa vakaleu	1.8–2.4	conic, turriform	0	0, 1	0	1, 2	15–19	⅕	0	1	3	25, 33–35	1
	*Fluviopupa seasea**	1.5–2.0	conic	2	0	2	3	16–20	⅕	0	3	3	22–24, 27–32	0
	*Fluviopupa lalinimeke**	1.8–2.2	conic	2	0	0, 1, 2	3	17–21	¼	0	0	3	23–26, 26–30	0
	*Fluviopupa lali**	1.8–2.4	conic	1	0, 1	2	3	17–19	⅕	0	1, 2	2	25–27, 27–32	0

Key: aperture – 0, ovate; 1, pyriform; 2, round; 3, D-shaped; caecum – 0, small and short; 1, small; 2, short; 3, well developed; course of rectum – 0, close to pallial genital glands in males (m) and females (f); 1, close to pallial genital glands in f, loop in males; 2, close to pallial genital glands in f, weak loop in m; 3, variable; hypobranchial gland – 0, not apparent in dissections; 1, weakly developed; 2, well developed; left penis lappet, 0, not apparent; 1, distinct; opercular smear – 0, absent; 1, minute; 2, distinct; pigmentation – 0, unpigmented; 1, pigmentation reduced; 2, largely pigmented; whorls, 0, hardly convex; 1, moderately convex; 2, convex. If more than one state is listed, species were variable.

–, missing data.

*Data taken from Haase *et al.*, 2006 (using a different method of shell measurement).

APPENDIX 3

Shell morphometry and sex ratio

Species/locality/*N*/sex ratio		sh	sw	ah	aw	bww	sh/sw	ah/aw	sw/bww	sh/ah	sw/aw	w
Fluviopupa korobebe	Holotype	1.94	0.96	0.71	0.66	0.83	2.03	1.07	1.16	2.74	1.45	4.375
Station 35	Mean	1.81	0.94	0.70	0.63	0.83	1.92	1.10	1.13	2.59	1.49	4.2
N = 8	Median	1.79	0.95	0.69	0.64	0.84	1.90	1.10	1.13	2.57	1.49	4.25
3 f/1 m	Min	1.68	0.86	0.65	0.57	0.72	1.87	1.05	1.09	2.54	1.38	3.75
	Max	1.94	1.03	0.76	0.66	0.90	2.03	1.17	1.19	2.74	1.57	4.5
	SD	0.11	0.06	0.04	0.03	0.06	0.05	0.04	0.04	0.07	0.06	0.24
	Cv	6.32	6.76	5.73	5.08	6.95	2.94	4.04	3.33	2.60	4.20	5.90
Fluviopupa tasmani	Holotype	2.25	1.27	0.86	0.85	1.11	1.77	1.01	1.14	2.62	1.49	4.625
Station 1	Mean	2.19	1.21	0.85	0.81	1.07	1.81	1.05	1.13	2.57	1.49	4.3
N = 20	Median	2.18	1.21	0.86	0.82	1.07	1.82	1.04	1.12	2.59	1.48	4.31
13 f/7 m	Min	2.00	1.11	0.79	0.75	0.97	1.67	1.00	1.08	2.45	1.39	4
	Max	2.41	1.30	0.90	0.89	1.19	1.88	1.14	1.21	2.74	1.62	4.5
	SD	0.10	1.05	0.03	0.04	0.06	0.06	0.04	0.04	0.09	0.06	0.14
	Cv	4.82	4.46	3.88	4.79	5.29	3.48	3.86	3.60	3.46	4.31	3.22
Fluviopupa uka	Holotype	2.17	1.12	0.80	0.74	1.00	1.94	1.08	1.12	2.71	1.51	4.625
Station 3	Mean	2.06	1.10	0.77	0.71	0.95	1.87	1.08	1.16	2.67	1.55	4.333
N = 20	Median	2.06	1.12	0.77	0.72	0.95	1.88	1.07	1.16	2.69	1.55	4.375
13 f/7 m	Min	1.65	0.95	0.65	0.62	0.81	1.73	1.02	1.08	2.52	1.43	3.875
	Max	2.35	1.22	0.88	0.80	1.03	1.97	1.15	1.29	2.84	1.71	4.75
	SD	0.21	0.09	0.07	0.05	0.06	0.08	0.04	0.05	0.11	0.06	0.25
	cv	10.09	8.18	8.83	7.71	6.85	4.26	3.45	4.73	4.03	4.07	5.71
Fluviopupa uka	Mean	1.86	1.01	0.72	0.67	0.89	1.85	1.06	1.13	2.60	1.50	4.143
Station 2	Median	1.87	1.00	0.71	0.67	0.90	1.84	1.06	1.12	2.62	1.51	4.125
N = 20	Min	1.56	0.88	0.60	0.58	0.78	1.75	0.96	1.00	2.38	1.30	3.75
15 f/5 m	Max	2.19	1.16	0.84	0.76	1.00	2.11	1.12	1.21	2.70	1.59	4.375
	SD	0.17	0.09	0.07	0.05	0.05	0.08	0.04	0.05	0.08	0.07	0.19
	cv	9.24	9.01	9.24	6.97	6.16	4.17	3.89	4.56	3.07	4.84	4.57
Fluviopupa moolae	Holotype	2.03	1.16	0.85	0.75	0.94	1.75	1.13	1.23	2.39	1.55	4.5
Station 4	Mean	2.06	1.18	0.88	0.78	0.98	1.75	1.12	1.21	2.35	1.51	4.33
N = 20	Median	2.08	1.19	0.88	0.80	0.99	1.77	1.12	1.20	2.36	1.49	4.375
10 f/10 m	Min	1.82	1.06	0.79	0.69	0.89	1.60	1.03	1.16	2.18	1.40	4.125
	Max	2.23	1.28	0.95	0.85	1.04	1.86	1.22	1.27	2.49	1.64	4.5
	SD	0.10	0.05	0.04	0.05	0.04	0.06	0.04	0.03	0.08	0.06	0.11
	cv	4.73	4.54	5.14	5.89	4.35	3.74	3.76	2.53	3.45	4.11	2.65
Fluviopupa moolae	Mean	1.83	1.10	0.82	0.72	0.90	1.66	1.15	1.22	2.22	1.54	4.222
Station 15	Median	1.85	1.11	0.84	0.73	0.92	1.67	1.15	1.22	2.23	1.53	4.25
N = 20	Min	1.58	0.97	0.73	0.64	0.81	1.46	1.11	1.15	2.04	1.44	4.0
12 f/8 m	Max	1.99	1.18	0.89	0.76	0.96	1.79	1.19	1.34	2.33	1.65	4.5
	SD	0.11	0.06	0.04	0.04	0.05	0.08	0.03	0.05	0.07	0.05	0.13
	cv	6.08	5.05	4.86	5.13	5.34	4.84	2.26	4.07	3.31	3.56	3.01
Fluviopupa moolae	Mean	2.26	1.26	0.92	0.79	1.05	1.79	1.16	1.20	2.46	1.60	4.667
Station 18	Median	2.27	1.27	0.93	0.79	1.07	1.76	1.15	1.21	2.44	1.62	4.625
N = 20	Min	2.11	1.06	0.78	0.72	0.95	1.66	1.03	1.07	2.20	1.47	4.25
12 f/8 m	Max	2.44	1.34	0.99	0.83	1.16	2.07	1.30	1.38	2.82	1.82	5.125
	SD	0.10	0.07	0.05	0.03	0.06	0.10	0.07	0.08	0.14	0.10	0.21
	cv	4.31	5.34	5.61	4.04	5.53	5.48	5.96	6.56	5.62	6.18	4.63

APPENDIX 3 *Continued*

Species/locality/*N*/sex ratio		sh	sw	ah	aw	bww	sh/ sw	ah/ aw	sw/ bww	sh/ ah	sw/ aw	w
Fluviopupa moolae	Mean	1.98	1.14	0.82	0.71	0.95	1.75	1.15	1.19	2.42	1.59	4.5
Station 19	Min	1.77	1.02	0.74	0.65	0.87	1.61	1.09	1.08	2.31	1.49	4.5
N = 16	Max	2.21	1.29	0.91	0.80	1.08	1.85	1.28	1.30	2.54	1.73	4.25
5 f/10 m	SD	0.14	0.08	0.06	0.05	0.05	0.07	0.04	0.05	0.07	0.06	5
	Median	1.98	1.12	0.80	0.70	0.95	1.75	1.15	1.19	2.41	1.58	0.19
	cv	7.10	6.96	7.00	6.49	5.67	3.87	3.73	4.19	2.94	3.93	4.23
Fluviopupa namosi	Holotype	2.14	1.04	0.78	0.72	0.95	2.06	1.08	1.10	2.74	1.44	4.625
Station 16	Mean	2.13	1.09	0.75	0.70	0.93	1.95	1.07	1.17	2.84	1.55	4.571
N = 20	Median	2.12	1.10	0.75	0.70	0.93	1.97	1.07	1.16	2.88	1.55	4.571
14 f/6 m	Min	1.93	1.00	0.68	0.63	0.86	1.85	1.01	1.11	2.56	1.46	4.25
	Max	2.32	1.18	0.82	0.76	1.06	2.09	1.17	1.28	3.02	1.67	4.875
	SD	0.11	0.05	0.04	0.03	0.05	0.08	0.04	0.05	0.14	0.05	0.14
	cv	5.22	4.59	4.85	4.88	5.19	3.97	3.83	3.91	4.90	3.49	3.01
Fluviopupa namosi	Mean	2.01	1.06	0.75	0.69	0.94	1.89	1.08	1.14	2.69	1.54	4.444
Station 17	Median	1.96	1.07	0.76	0.70	0.93	1.92	1.07	1.13	2.73	1.51	4.5
N = 14	Min	1.85	0.94	0.66	0.61	0.83	1.61	1.04	1.08	2.22	1.47	4.125
7 f/7 m	Max	2.24	1.15	0.84	0.76	1.03	2.06	1.19	1.24	2.91	1.64	4.75
	SD	0.13	0.06	0.05	0.04	0.05	0.11	0.04	0.04	0.17	0.06	0.16
	cv	6.53	5.43	6.68	6.02	5.54	5.70	3.80	3.96	6.35	3.79	3.69
Fluviopupa namosi	Mean	1.91	0.99	0.70	0.64	0.83	1.92	1.10	1.19	2.72	1.56	4.444
Station 18	Median	1.94	0.98	0.71	0.64	0.85	1.91	1.10	1.20	2.72	1.56	4.5
N = 20	Min	1.56	0.86	0.59	0.54	0.69	1.80	1.01	1.11	2.60	1.44	4.125
8 f/12 m	Max	2.22	1.07	0.78	0.73	0.95	2.07	1.15	1.27	2.84	1.67	4.625
	SD	0.16	0.06	0.05	0.05	0.07	0.08	0.04	0.05	0.07	0.07	0.15
	cv	8.24	5.82	7.02	8.04	7.97	4.09	3.41	4.07	2.46	4.41	3.40
Fluviopupa nagodro	Holotype	2.18	1.28	0.91	0.79	1.05	1.70	1.15	1.22	2.40	1.62	4.375
Station 32	Mean	2.09	1.21	0.87	0.74	0.99	1.74	1.18	1.22	2.40	1.63	4.5
N = 20	Median	2.09	1.21	0.88	0.75	0.99	1.72	1.19	1.22	2.38	1.63	4.5
7 f/13 m	Min	1.89	1.10	0.80	0.64	0.91	1.64	1.10	1.11	2.29	1.46	4.125
	Max	2.43	1.32	0.95	0.80	1.06	1.87	1.34	1.33	2.59	1.84	5.125
	SD	0.11	0.05	0.04	0.04	0.04	0.06	0.06	0.06	0.08	0.08	0.2
	cv	5.46	4.54	4.68	5.57	4.12	3.60	4.77	4.88	3.30	5.10	4.45
Fluviopupa nagodro	Mean	2.12	1.20	0.88	0.76	1.01	1.77	1.16	1.19	2.42	1.58	4.49
Station33	Median	2.14	1.18	0.88	0.76	1.01	1.80	1.15	1.19	2.42	1.59	4.5
N = 18	Min	1.87	1.10	0.80	0.66	0.86	1.63	1.07	1.11	2.23	1.48	4.25
8 f/3 m	Max	2.30	1.30	0.98	0.83	1.14	1.92	1.28	1.33	2.71	1.73	4.625
	SD	0.12	0.05	0.05	0.04	0.06	0.07	0.05	0.06	0.13	0.07	0.12
	cv	5.88	4.08	5.41	5.34	6.54	4.23	4.77	5.15	5.38	4.49	2.72
Fluviopupa nagodro	Mean	2.19	1.18	0.87	0.79	1.06	1.86	1.10	1.11	2.54	1.50	4.5
Station 34	Median	2.29	1.20	0.90	0.82	1.08	1.86	1.09	1.12	2.53	1.51	4.5
N = 3	Min	1.95	1.05	0.75	0.69	0.96	1.81	1.09	1.09	2.49	1.47	4.25
	Max	2.34	1.30	0.94	0.86	1.14	1.90	1.11	1.14	2.59	1.52	4.625
	SD	0.21	0.13	0.10	0.09	0.09	0.05	0.01	0.03	0.05	0.03	0.19
	cv	10.55	11.58	12.40	12.30	9.02	2.75	0.66	2.63	2.03	1.83	4.64
Fluviopupa savuione	Holotype	1.72	1.00	0.68	0.66	0.87	1.72	1.04	1.15	2.51	1.52	3.875
Station 33	Mean	1.75	1.03	0.73	0.65	0.87	1.71	1.12	1.18	2.41	1.59	4.125
N = 10	Median	1.77	1.02	0.74	0.65	0.87	1.73	1.13	1.18	2.41	1.61	4.063
2 f/3 m	Min	1.55	0.96	0.68	0.58	0.81	1.62	1.04	1.14	2.29	1.52	3.875
	Max	1.85	1.12	0.76	0.69	0.91	1.80	1.20	1.26	2.53	1.65	4.375
	SD	0.10	0.05	0.03	0.03	0.03	0.06	0.05	0.04	0.09	0.06	0.20
	cv	5.77	4.73	4.72	5.49	3.71	3.87	4.80	3.56	3.95	3.67	5.07

APPENDIX 3 *Continued*

Species/locality/*N*/sex ratio		sh	sw	ah	aw	bww	sh/ sw	ah/ aw	sw/ bww	sh/ ah	sw/ aw	w
Fluviopupa savuione Station 34; *N* = 1		1.49	0.89	0.65	0.57	0.77	1.67	1.15	1.16	2.28	1.56	3.875
Fluviopupa lailai	Holotype	1.23	0.75	0.52	0.50	0.71	1.63	1.04	1.07	2.37	1.51	3.875
Station 35	Mean	1.30	0.76	0.53	0.49	0.67	1.71	1.10	1.14	2.44	1.57	3.75
N = 5	Median	1.23	0.75	0.52	0.50	0.68	1.71	1.11	1.12	2.45	1.57	3.75
1f	Min	1.18	0.67	0.48	0.43	0.61	1.63	1.04	1.07	2.37	1.51	3.375
	Max	1.47	0.84	0.60	0.54	0.71	1.79	1.14	1.25	2.51	1.63	4
	SD	0.14	0.07	0.05	0.04	0.04	0.07	0.04	0.07	0.05	0.05	0.23
	cv	11.15	10.05	10.79	9.65	6.58	4.28	3.77	6.49	2.16	3.06	6.55
Fluviopupa vulavula	Holotype	1.86	1.04	0.80	0.71	0.89	1.79	1.13	1.19	2.33	1.47	4.25
Station 14	Mean	1.97	1.07	0.82	0.70	0.89	1.85	1.17	1.20	2.41	1.53	4.5
N = 9	Median	2.02	1.05	0.80	0.72	0.90	1.85	1.17	1.20	2.36	1.54	4.5
2 f/3 m	Min	1.74	0.99	0.76	0.62	0.80	1.71	1.10	1.12	2.30	1.40	4.25
	Max	2.23	1.29	0.97	0.79	0.99	1.96	1.23	1.30	2.61	1.64	4.875
	SD	0.18	0.09	0.07	0.06	0.07	0.09	0.05	0.06	0.11	0.08	0.24
	cv	9.18	8.92	8.38	8.76	7.91	4.94	4.18	5.24	4.69	5.64	5.37
Fluviopupa dromodromo	Holotype	2.29	1.21	0.87	0.81	1.08	1.89	1.07	1.12	2.63	1.49	4.625
	Mean	2.23	1.18	0.82	0.77	1.02	1.89	1.07	1.16	2.70	1.54	4.5
Station 20	Median	2.21	1.18	0.83	0.77	1.02	1.87	1.07	1.16	2.71	1.53	4.5
N = 20	Min	1.95	1.06	0.72	0.69	0.89	1.74	1.01	1.09	2.46	1.40	4.25
4 f/12 m	Max	2.50	1.29	0.88	0.85	1.12	2.07	1.17	1.21	2.97	1.71	4.875
	SD	0.14	0.05	0.04	0.04	0.05	0.09	0.04	0.03	0.12	0.07	0.14
	cv	6.40	4.64	5.15	5.18	5.30	4.61	3.83	2.98	4.37	4.90	3.83
Fluviopupa bula	Holotype	2.09	1.20	0.90	0.81	1.05	1.74	1.11	1.14	2.32	1.48	4.375
Station 10	Mean	2.14	1.23	0.90	0.80	1.03	1.74	1.13	1.19	2.39	1.55	4.429
N = 20	Median	2.14	1.24	0.91	0.79	1.03	1.74	1.12	1.18	2.39	1.54	4.444
9 f/11 m	Min	1.83	1.09	0.79	0.73	0.94	1.59	1.05	1.15	2.28	1.45	4.125
	Max	2.43	1.35	1.03	0.87	1.10	1.85	1.21	1.32	2.55	1.68	4.75
	SD	0.14	0.07	0.06	0.04	0.05	0.07	0.04	0.04	0.06	0.06	0.16
	cv	6.83	5.91	6.20	5.23	5.10	3.87	3.74	3.53	2.55	4.15	3.76
Fluviopupa bula	Mean	2.23	1.15	0.78	0.69	1.00	1.95	1.13	1.15	2.85	1.65	–
Station 5	Median	2.17	1.13	0.77	0.69	1.00	1.92	1.11	1.12	2.86	1.65	–
N = 5	Min	2.10	1.05	0.73	0.66	0.92	1.90	1.07	1.09	2.78	1.53	–
-/2 m	Max	2.44	1.29	0.88	0.73	1.09	2.01	1.21	1.28	2.94	1.77	–
	SD	0.15	0.11	0.06	0.03	0.06	0.05	0.05	0.08	0.06	0.10	–
	cv	7.10	9.73	8.09	4.11	6.74	2.97	4.70	7.08	2.32	6.11	–
Fluviopupa bula	Mean	2.14	1.19	0.86	0.76	1.00	1.80	1.12	1.19	2.50	1.55	4.5
Station 6	Median	2.15	1.20	0.87	0.77	1.00	1.80	1.11	1.19	2.50	1.55	4.5
N = 18	Min	1.85	1.07	0.77	0.70	0.90	1.65	1.04	1.10	2.39	1.43	4.125
3 f/7 m	Max	2.36	1.29	0.94	0.83	1.11	1.91	1.22	1.29	2.63	1.64	4.75
	SD	0.14	0.06	0.06	0.04	0.05	0.06	0.04	0.05	0.06	0.05	0.20
	cv	6.87	5.49	6.56	5.13	5.51	3.62	3.59	4.17	2.64	3.56	4.43
Fluviopupa bula	Mean	2.17	1.20	0.87	0.77	1.02	1.80	1.14	1.18	2.48	1.56	4.5
Station 7	Median	2.14	1.20	0.87	0.77	1.02	1.80	1.14	1.18	2.48	1.57	4.5
N = 20	Min	2.00	1.10	0.78	0.71	0.95	1.72	1.06	1.11	2.28	1.45	4.125
5 f/10 m	Max	2.35	1.29	0.91	0.85	1.11	1.89	1.21	1.26	2.65	1.69	4.785
	SD	0.11	0.05	0.03	0.03	0.04	0.06	0.05	0.04	0.09	0.06	0.2
	cv	5.02	4.31	3.52	4.58	4.12	3.11	4.15	3.56	3.86	4.00	4.57

APPENDIX 3 *Continued*

Species/locality/*N*/sex ratio		sh	sw	ah	aw	bww	sh/ sw	ah/ aw	sw/ bww	sh/ ah	sw/ aw	w
Fluviopupa bula	Mean	2.00	1.12	0.81	0.74	0.98	1.79	1.09	1.15	2.48	1.51	4.333
Station 8	Median	2.02	1.12	0.80	0.74	0.97	1.80	1.10	1.14	2.49	1.51	4.333
N = 20	Min	1.87	1.05	0.75	0.69	0.91	1.68	1.03	1.10	2.33	1.45	4.125
8 f/12 m	Max	2.19	1.18	0.88	0.79	1.04	1.86	1.14	1.21	2.63	1.62	4.75
	SD	0.09	0.03	0.04	0.03	0.03	0.06	0.03	0.03	0.08	0.05	0.17
	cv	4.56	2.88	4.61	3.93	3.09	3.16	2.79	2.70	3.39	3.07	4.03
Fluviopupa bula	Mean	2.02	1.15	0.82	0.74	0.98	1.76	1.12	1.18	2.46	1.56	4.167
Station 11	Median	1.99	1.15	0.82	0.74	0.99	1.75	1.11	1.17	2.48	1.56	4.25
13 f/7 m	Min	1.81	1.05	0.74	0.67	0.89	1.65	1.03	1.10	2.32	1.47	4
	Max	2.25	1.27	0.90	0.81	1.05	1.88	1.21	1.26	2.60	1.70	4.375
	SD	0.13	0.05	0.04	0.04	0.04	0.06	0.04	0.04	0.08	0.05	0.11
	cv	6.40	4.58	5.27	5.52	4.30	3.59	3.87	3.31	3.34	3.44	2.68
Fluviopupa dumontdurvilli	Holotype	2.28	1.17	0.86	0.77	1.03	1.95	1.12	1.14	2.66	1.52	4.75
	Mean	2.22	1.23	0.89	0.77	1.05	1.81	1.16	1.18	2.49	1.60	4.778
Station 21	Median	2.21	1.22	0.88	0.77	1.04	1.78	1.15	1.17	2.48	1.61	4.75
N = 9	Min	2.05	1.15	0.85	0.71	0.94	1.73	1.11	1.12	2.34	1.52	4.5
2 f/4 m	Max	2.44	1.36	1.02	0.84	1.13	1.95	1.23	1.28	2.66	1.71	5
	SD	0.14	0.07	0.06	0.04	0.06	0.06	0.04	0.05	0.11	0.07	0.17
	cv	6.25	5.85	6.36	5.61	6.24	3.62	3.90	4.72	4.33	4.20	3.69
Fluviopupa drau	Holotype	2.10	1.15	0.87	0.77	1.02	1.83	1.13	1.13	2.41	1.49	4.25
Station 22	Mean	2.10	1.20	0.85	0.74	1.02	1.75	1.14	1.17	2.48	1.61	4.2
N = 20	Median	2.10	1.18	0.83	0.75	1.00	1.76	1.14	1.17	2.49	1.63	4.25
11 f/9 m	Min	1.82	1.08	0.76	0.65	0.93	1.61	1.04	1.10	2.30	1.47	3.875
	Max	2.47	1.44	1.08	0.85	1.15	1.93	1.27	1.25	2.65	1.82	4.5
	SD	0.18	0.10	0.08	0.04	0.07	0.08	0.06	0.05	0.11	0.09	0.2
	cv	8.74	8.49	8.99	5.58	6.69	4.83	5.59	3.93	4.30	5.70	4.91
Fluviopupa drau	Mean	2.31	1.27	0.92	0.80	1.07	1.82	1.15	1.19	2.52	1.59	4.625
Station 23	Median	2.26	1.26	0.92	0.80	1.06	1.81	1.15	1.18	2.51	1.58	4.625
N = 20	Min	2.07	1.14	0.71	0.66	0.96	1.70	1.06	1.12	2.31	1.47	4.25
12 f/8 m	Max	2.55	1.39	1.01	0.88	1.16	2.00	1.28	1.27	3.02	1.72	5.125
	SD	0.16	0.07	0.07	0.05	0.05	0.07	0.05	0.04	0.15	0.07	0.23
	cv	6.89	5.58	7.84	6.61	4.73	4.12	4.48	3.12	5.97	4.25	5.14
Fluviopupa vatukuca	Holotype	2.17	1.24	0.86	0.82	1.10	1.75	1.06	1.13	2.52	1.52	4.25
Station 26	Mean	2.01	1.23	0.86	0.78	1.04	1.63	1.10	1.19	2.35	1.58	4.167
N = 6	Median	2.02	1.23	0.87	0.78	1.04	1.62	1.10	1.20	2.30	1.56	4.2
1 f/2 m	Min	1.89	1.16	0.76	0.74	1.00	1.55	1.02	1.12	2.17	1.52	4
	Max	2.17	1.30	0.93	0.82	1.10	1.75	1.22	1.26	2.52	1.71	4.25
	SD	0.10	0.05	0.06	0.03	0.04	0.07	0.07	0.05	0.13	0.07	0.10
	cv	4.99	4.64	6.94	3.67	3.77	4.59	6.32	4.71	5.92	4.43	2.55
Fluviopupa vatukuca Station 27; *N* = 1		1.73	1.07	0.73	0.70	0.99	1.61	1.03	1.08	2.39	1.52	3.875
Fluviopupa forsteri	Holotype	1.93	1.03	0.65	0.63	0.93	1.87	1.03	1.11	2.98	1.63	4.25
Station 23	Mean	1.92	1.02	0.65	0.63	0.92	1.88	1.04	1.11	2.96	1.62	4.333
N = 7	Median	1.93	1.03	0.65	0.62	0.91	1.87	1.03	1.12	2.89	1.63	4.375
2 f/2 m	Min	1.74	0.94	0.60	0.61	0.86	1.84	0.98	1.08	2.87	1.52	4.125
	Max	2.06	1.07	0.68	0.66	0.97	1.97	1.10	1.13	3.18	1.70	4.5
	SD	0.10	0.04	0.02	0.02	0.03	0.04	0.04	0.02	0.11	0.06	0.10
	cv	5.04	3.98	3.78	2.90	3.80	2.36	3.95	2.01	3.84	3.43	3.73

APPENDIX 3 *Continued*

Species/locality/*N*/sex ratio		sh	sw	ah	aw	bww	sh/sw	ah/aw	sw/bww	sh/ah	sw/aw	w
Fluviopupa tunuloa	Holotype	2.15	1.30	0.92	0.82	1.09	1.65	1.12	1.19	2.35	1.60	4.25
Station 24	Mean	2.13	1.24	0.90	0.78	1.05	1.72	1.14	1.18	2.38	1.58	4.286
N = 11	Median	2.15	1.23	0.91	0.80	1.05	1.70	1.14	1.18	2.37	1.59	4.25
6f	Min	1.92	1.16	0.81	0.67	0.95	1.64	1.07	1.13	2.26	1.50	4.125
	Max	2.28	1.32	0.96	0.82	1.09	1.85	1.21	1.22	2.57	1.73	4.5
	SD	0.10	0.05	0.04	0.04	0.04	0.07	0.05	0.03	0.09	0.07	0.14
	cv	4.58	4.01	4.93	5.62	4.25	3.86	4.16	2.90	3.85	4.37	3.26
Fluviopupa tunuloa	Mean	2.07	1.17	0.82	0.74	1.01	1.78	1.11	1.15	2.52	1.57	4.2
Station 25	Median	2.08	1.18	0.83	0.74	1.01	1.77	1.10	1.15	2.51	1.58	4.25
N = 8	Min	1.97	1.11	0.76	0.70	0.96	1.72	1.06	1.13	2.45	1.51	3.75
2 f/2 m	Max	2.14	1.22	0.87	0.77	1.06	1.84	1.17	1.18	2.61	1.67	4.375
	SD	0.05	0.04	0.04	0.03	0.04	0.04	0.04	0.02	0.05	0.05	0.21
	cv	2.62	3.87	4.55	4.06	3.62	2.26	3.95	1.71	2.25	3.23	5.20
Fluviopupa raradamu	Holotype	1.94	1.10	0.81	0.68	0.92	1.77	1.18	1.19	2.40	1.61	4.125
Station 25	Mean	1.90	1.07	0.76	0.69	0.94	1.77	1.10	1.15	2.51	1.55	4.2
N = 4	Median	1.93	1.08	0.76	0.69	0.93	1.79	1.07	1.13	2.52	1.55	4.2
1 f/2 m	Min	1.77	1.04	0.70	0.66	0.91	1.71	1.06	1.13	2.40	1.50	4.125
	Max	1.97	1.10	0.81	0.73	0.96	1.81	1.18	1.19	2.60	1.61	4.25
	SD	0.09	0.03	0.05	0.03	0.02	0.04	0.06	0.03	0.08	0.04	0.07
	cv	5.02	2.82	6.61	4.10	2.65	2.69	5.66	2.93	3.45	2.97	1.83
Fluviopupa vakalevu	Holotype	2.33	1.27	0.87	0.82	1.06	1.84	1.06	1.20	2.68	1.55	4.75
Station 28	Mean	2.13	1.16	0.83	0.74	0.97	1.83	1.12	1.20	2.57	1.57	4.5
N = 20	Median	2.15	1.17	0.83	0.74	0.98	1.82	1.12	1.19	2.58	1.58	4.5
10 f/10 m	Min	1.83	1.02	0.70	0.64	0.84	1.65	1.01	1.12	2.36	1.45	4.25
	Max	2.33	1.33	0.93	0.83	1.06	1.97	1.20	1.31	2.69	1.72	4.625
	SD	0.14	0.08	0.06	0.04	0.06	0.06	0.05	0.05	0.08	0.07	0.11
	cv	6.84	7.12	7.21	5.97	5.82	3.55	4.44	4.32	3.26	4.65	2.49

Abbreviations: ah, aperture height; aw, aperture width; bww, body whorl width; cv, coefficient of variation, adjusted for sample size; f, females; m, males; max, maximum; min, minimum; SD, standard deviation; sh, shell height; sw, shell width; w, whorls.
All measurements are given in mm.

2.3. Molecular Phylogeny and a modified approach of character based barcoding

Molecular Phylogenetics and Evolution 89 (2015) 171–181

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny and a modified approach of character-based barcoding refining the taxonomy of New Caledonian freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae) ☆

Susan Zielske *, Martin Haase

Vogelwarte, Zoological Institute and Museum, Greifswald University, Soldmannstr. 23, 17489 Greifswald, Germany

ARTICLE INFO

Article history:
Received 31 October 2014
Revised 11 April 2015
Accepted 21 April 2015
Available online 28 April 2015

Keywords:
CAOS
Dated phylogeny
Hybridization
Introgression
Rissooidea
South Pacific

ABSTRACT

The islands of New Caledonia represent one of the world's biodiversity hotspots with many endemic species including freshwater gastropods of the family Tateidae. A phylogenetic analysis based on the mitochondrial COI and 16S rRNA and the nuclear ITS2 genes revealed two cryptic genera, *Crosseana* gen. n. and *Novacaledonia* gen. n. In order to provide character-based diagnoses we modified a DNA barcoding approach identifying strings of pairwise diagnostic characters, i.e. alignment positions, at which two genera are alternatively fixed for different nucleotides. The combination or string of all pairwise diagnostic characters was unique for each genus. Inconsistent mitochondrial and nuclear topologies suggest that *Hemistomia cockerelli* Haase and Bouchet, 1998 and *H. fabrorum* Haase and Bouchet, 1998, two morphologically well-defined species, hybridize. The age of the most recent common ancestor of the New Caledonian radiation of Tateidae was estimated at 24.6 ± 9.5 MY. These findings are in line with the notion that New Caledonia is rather a Darwinian island that was colonized after an extended phase of submergence – in case of the tateids probably from Australia – despite being a fragment of Gondwanaland.

1. Introduction

New Caledonia is one of the world's biodiversity hotspots (Myers et al., 2000). It has a high degree of endemism, which holds especially for the flora with more than 74% endemics, but also for reptiles and invertebrates (Chazeau, 1993; Jaffré et al., 1994; Bauer et al., 2006; Oláh et al., 2006; Espeland and Johanson, 2010). Most of the endemic species have narrow ranges occurring only in very restricted areas on the islands (Grandcolas et al., 2008; Wulff et al., 2013). This situation is attributed to a long history of isolation as well as the very heterogeneous topography and geochemistry including ultramafic bed-rock or, in short, to the unique geological history (Grandcolas et al., 2008; Murienne, 2009). New Caledonia includes the main island (Grande Terre) of more than 16.000 km^2 comprising more than 85% of the whole landmass, the Loyalty Islands in the East, the Isle of Pines in the South and the Belep archipelago in the North. Grande Terre, divided in a humid east coast and a drier west coast by a central chain of mountains, is a fragment of the continental crust of Zealandia which began to separate from the rest of Gondwanaland about 65 MYA (Schellart et al., 2006; Neall and Trewick, 2008). The current position of the main island was reached about 50 MYA during the Eocene, but further severe geological changes occurred as a consequence of the collision with the Loyalty arc including the main phase of uplift of the main island during the Oligocene (Grandcolas et al., 2008; Neall and Trewick, 2008). Subsequent quaternary uplift in the context of the subduction of the Vanuatu trench resulted in the emergence of the Loyalty Islands (Neall and Trewick, 2008) which are of volcanic origin.

Most recently published biological studies support the theory that the main island of New Caledonia was entirely submerged between 65 and 45 MYA and re-colonized in a stepping stone like fashion out of South East Asia or directly from Australia (Espeland and Murienne, 2011; Cruaud et al., 2012; Swenson et al., 2013). Consequently, New Caledonia is a Darwinian island rather than a Gondwanan refuge as originally thought (Holloway, 1979; Morat et al., 1986; Lowry, 1998). However, the potential existence of ephemeral islands acting as refuge-habitats in the region of New Caledonia during times of submergence of Grande Terre, supported by the existence of relictual groups (Ladiges and Cantrill, 2007; Heads, 2008), has not been finally ruled out (Grandcolas et al., 2008; Murienne, 2009).

☆ This paper has been recommended for acceptance by Jan Strugnell.

* Corresponding author.
E-mail addresses: susan.zielske@googlemail.com (S. Zielske), martin.haase@uni-greifswald.de (M. Haase).

http://dx.doi.org/10.1016/j.ympev.2015.04.020
1055-7903/

Currently there are more than 50 species of truncatelloidean freshwater gastropods related to the genus *Hemistomia* described from and all endemic to New Caledonia (Haase and Bouchet, 1998). This revision was based on morphology and anatomy. Due to the small number of characters suitable for cladistic analyses and their often high degree of homoplasy the five genera of this putative clade are poorly defined and in need of corroboration, which is typical for such small snails (e.g. Colgan et al., 2007). A number of species, in particular larger ones, were only tentatively allocated to *Hemistomia* or *Kanakyella*, respectively (Haase and Bouchet, 1998). Our present molecular analyses indeed revealed morphologically cryptic genera. In order to provide character-based diagnoses (cf. DeSalle et al., 2005; Zou et al., 2011; Jörger and Schrödl, 2013) we included molecular data introducing a modified approach of character-based barcoding (Rach et al., 2008; Reid et al., 2011; Bergmann et al., 2013).

During an expedition to New Caledonia in 2012 we collected species belonging to *Hemistomia*, *Kanakyella* and *Leiorhagium*. We were not able to re-collect *Pidaconomus hybrida* Haase and Bouchet, 1998, the only species of the genus and a single site endemic, probably because the locality data in Haase and Bouchet (1998) were not precise enough to find the site again. *Caledoconcha* occurs with two species in a mountainous area difficult to access without local guides, who were not available when we visited the area. Therefore, also *Caledoconcha* is lacking in our analyses.

The aim of our work was threefold: (1) reconstruct a phylogeny based on genetic data in order to establish the monophyly of the *Hemistomia*-clade sensu Haase and Bouchet (1998), (2) to solve the taxonomic problems outlined above, and (3) to establish whether these truncatelloidean gastropods support the Gondwanan vicariance rather than the Darwinian island concept of biogeography based on a dated phylogeny.

2. Materials and methods

2.1. Material

Specimens were collected at 60 sites across Grande Terre, in June 2012 (Fig. 1, Appendix A). Snails were preserved in 70% ethanol in the field and transferred to propylene glycol for shipment. Upon arrival in our lab, snails were returned to 96% ethanol. Based on the latest phylogeny of rissooidean/truncatelloidean gastropods (Wilke et al., 2013) we selected two tateid species from New Zealand (*Opacuincola delira* Haase, 2008, *Potamopyrgus estuarinus* Winterbourn, 1971), as well as one each from Australia (*Tatea huonensis* Tenison Woods, 1876) and Brazil (*Potamolithus ribeirensis* Pilsbry, 1911) as outgroup taxa. Sequence data of outgroup taxa were taken from Genbank (Appendix A).

2.2. DNA isolation and sequencing

DNA was isolated using QIAGEN's DNeasy Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany) from two whole snails of each sample. The primers used to amplify a ca.540 bp long fragment of the mitochondrial (mt) 16S rRNA were 16Sar and 16Sbr introduced by Palumbi et al. (1991). A 658 bp long fragment of the mt cytochrome oxidase subunit I (COI) gene was amplified using the primers L1460 and H1298 (modified at postion 12: G → A) introduced by Folmer et al. (1994) and a ca. 430 bp long fragment of the nuclear (nc) internal transcribed spacer 2 (ITS2) with the primers ITSfn (Zielske and Haase, 2014) and ITSr4 (Oliverio and Mariottini, 2001). Polymerase chain reactions were performed using standard protocols (Zielske and Haase, 2014).

PCR-products were purified enzymatically using exonuclease and shrimp alkaline phosphatase and sequenced on an ABI

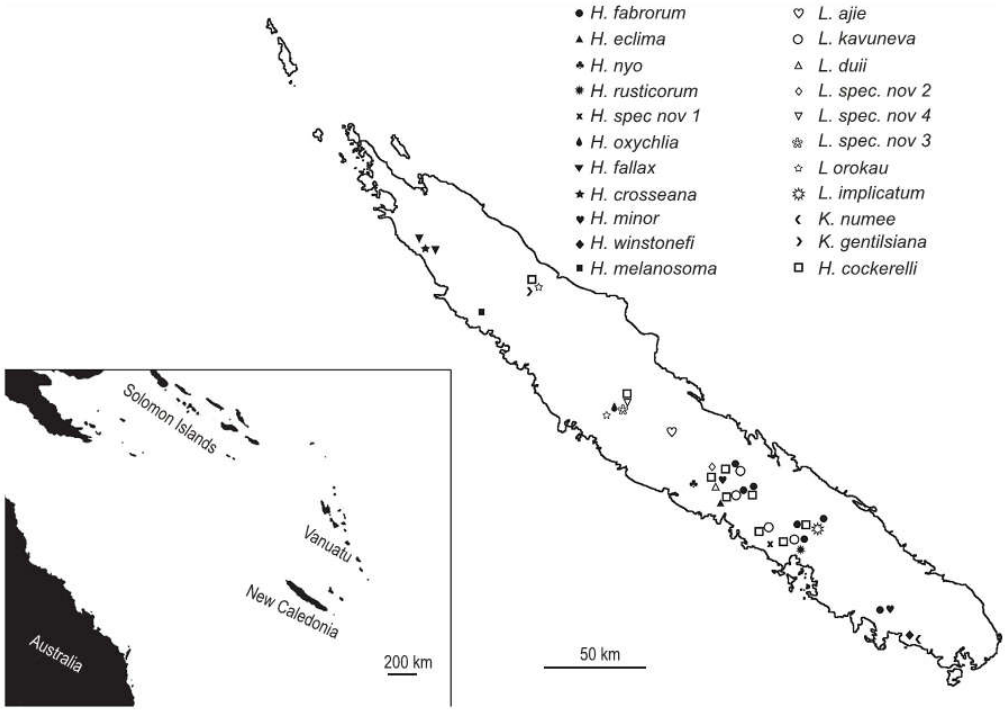

Fig. 1. Map of Grande Terre, the main island of New Caledonia, its position in the South Pacific region and distribution of the sampled species.

3130xl Genetic Analyzer using ABI's Big Dye Terminator Ready Reaction Mix v3.1 (Carlsbad, California, USA) and the PCR primers. All sequences are available from GenBank (see Appendix A).

2.3. Phylogenetic analyses

Sequences were initially edited using BioEdit 7.0.5.3 (Hall, 1999). Due to the lack of indels the protein coding mt COI gene could be aligned by eye. The mt 16SrRNA and nc ITS2 sequences were initially aligned using ClustalW (Thompson et al., 1994). The resulting alignments were refined in RNAsalsa 0.8.1 (Stocsits et al., 2009) using secondary structure information of *Cacozeliana lacertina* Gould 1861 [16S rRNA, http://www.rna.icmb.utexas.edu/SIM/4D/Mollusk/ (Cannone et al., 2002), AF101007] and *Haliotis discus* Reeve 1846 [ITS2, http://its2.bioapps.biozentrum.uni-wuerzburg.de (Koetschan et al., 2010), GI 28627842] and finally manually edited in BioEdit. Aliscore v.2.0 (Misof and Misof, 2009) was used to check for ambiguous and randomly similar sites.

Partition Finder (Lanfear et al., 2012) was used to decide whether stem- and loop-structures in 16SrRNA and ITS2 and codon positions in COI should be treated as separate partitions with individual substitution models and to find the best fitting models according to the corrected Akaike Information Criterion. Tests for substitution saturation and validation of the molecular clock were performed in DAMBE 5.3.54 (Xia and Xie, 2001) treating gaps as unknown states. Phylogenetic analyses were conducted in maximum likelihood (ML) and Bayesian frameworks. Maximum likelihood analyses were performed with 500 replicates in Garli 2.0 (Zwickl, 2006) for mt and nc genes separately and for the concatenated dataset. Confidence was assessed by 500 bootstrap replicates. Bayesian analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) with a burn-in of 10% of 7 M generations for ITS2, 15 M generations for the mt data, and 50 M generations for the concatenated dataset. Lengths of the runs were determined using the evaluation methods provided by MrBayes and Tracer 5.1 (Rambaut and Drummond, 2007). Pair-wise distances were calculated in PAUP* 4b10 (Swofford, 2003) and Neighbor Networks in SplitsTree4 (Huson and Bryant, 2006) to help clarify the species affiliation of some cryptic samples. Because the new genera are difficult to define based on morphology and anatomy, we used the CAOS (Characteristic Attribute Organization System) workbench (Sarkar et al., 2008) to identify molecular diagnostic character states for the different genera. The program found very few simple pure characters (unique character states that occur in all investigated specimens of a particular genus but not in any specimen of the other genera), which are usually used for DNA-taxonomy (Reid et al., 2011; Bergmann et al., 2013). Therefore, we searched for characters with alternatively fixed states among pairs of genera based on topologically constrained trees. The combinations or strings of all pair-wise diagnostic characters were then unique for each genus and included in the diagnoses.

Divergence times were estimated in BEAST 1.7.5 (Drummond et al., 2012). These analyses involved only New Caledonian taxa. Due to the limited variability after exclusion of the outgroup taxa, genes were no longer partitioned in stem and loop structures or codon positions, respectively. The molecular clock was calibrated using the COI substitution rate for New Zealand taxa [3.26 ± 0.14% sequence divergence/MY, (Haase et al., 2007)] resulting in a clock rate of 0.0162 ± 0.0002 substitutions per site per MY. We tested three different clock constraints: (1) the strict molecular clock for all three partitions, setting the prior for the COI clock rate; (2) the log normal relaxed clock for COI, due to the fact that the strict molecular clock could not be confirmed unambiguously; (3) the log normal relaxed clock for all three genes. For the two latter analyses we set the priors assuming a normally distributed COI clock rate (mean = 0.0162, stdev = 0.025) and a stdev prior of 0.00083 which allowed for a more progressive rate variation than the narrow original confidence interval. For each analysis two runs of 20 M replicates after a burn-in of 10% were combined examining parameter estimation with Tracer v.1.5 (Rambaut and Drummond, 2007). Finally we performed a Bayes factor test using the same program to identify the best fitting model.

3. Results

According to Aliscore no sites had to be excluded from the alignment. DAMBE showed no substantial substitution saturation and partition finder suggested analyzing stem and loop structures as well as codon positions of COI separately. The best fitting substitution models are listed in Table 1. *P*-distances as a measure of genetic variation reached up to 11.03% in 16S rRNA, 20.43% in COI, and 32.20% in ITS2 considering only the New Caledonian taxa (see also Table 2).

The strict molecular clock was not rejected by the least squares method in DAMBE. Using the likelihood ratio test in the same program the strict molecular clock was rejected for COI ($P < 0.05$), but not for 16SrRNA and ITS2 ($P > 0.44$).

The monophyly of the New Caledonian taxa was supported only in the analysis of the concatenated and mt datasets (Fig. 2). The analysis based only on ITS 2 failed to resolve the relationships between the larger clades. Furthermore, none of the analyses could estimate with certainty whether the New Caledonian taxa are closer to their Australian or New Zealand relatives. The ML analysis of the concatenated dataset was the only one resulting in resolved basal relationships with the Australian *Tatea* as sister group to the ingroup. However, this result was not supported by bootstrapping.

Considering the New Caledonian taxa the analyses resulted in three clades of which the two larger ones were well supported in both Bayesian and ML frameworks. Clade I comprised species of the genus *Hemistomia* and clade II *Leiorhagium*. The third clade consisted of species originally, in part tentatively, attributed to the genera *Hemistomia* and *Kanakyella*. This clade was monophyletic only in the analyses of the concatenated sequences and of ITS2.

Relationships between and within the clades partly differed across the analyses (Fig. 3). However, the following relationships were consistent within clade I: except for *H. cockerelli*, *H. fabrorum* and *H. minor* all species were monophyletic and well supported in the Bayesian and ML analyses. *H. eclima* was always identified as closest relative of *H. minor*. *H. fabrorum*, *H. cockerelli*, *H. nyo*, and *H.* sp. n. 1 formed a well-supported clade, however, *H. cockerelli* was always paraphyletic, and *H. fabrorum* was paraphyletic in the analyses based on mt genes. The allocation of samples to either *H. cockerelli* or *H. fabrorum* was unambiguous based on morphology except for sample 46, which was intermediate. The conflicting positions of specimens from samples 9A (morphologically *H. cockerelli*), 39 (morphologically *H. fabrorum*) – and 46 are illustrated by neighbor-nets (Fig. 3).

Relationships among species of *Leiorhagium* (clade II) inferred from either mt or nc sequences were only weakly supported (Fig. 3). The resolution improved considerably analyzing the

Table 1
Substitution models.

	Best model	Best MrBayes model	Unpartitioned
16SrRNA stem	TVM + I	HKY + I	TIM + I + G
16SrRNA loop	GTR + I + G	GTR + I + G	
ITS stem	K81uf + G	HKY + I	SYM + I + G
ITS loop	TIM + I + G	SYM + I + G	
COI p1	TIM + G	GTR + G	TIM + I + G
COI p2	HKY + I	HKY + I	
COI p3	TVM + G	GTR + G	

Table 2
Corrected genetic distances of unpartitioned genes within and between species occurring at different localities; *H. cockerelli* I only includes specimens that formed a clade across all analyses.

		COI		16S		ITS	
Species		Mean	Max	Mean	Max	Mean	Max
H. fallax	–	2.55	5.11	0.77	1.30	1.85	2.84
L. orokau	–	0.10	0.15	0.24	0.42	0.44	0.66
L. kavuneva	–	0.36	1.46	0.05	0.20	1.08	2.04
L. sp. n. 3	–	2.62	3.94	0.55	0.82	0.46	0.71
H. minor	–	3.46	5.11	–	–	7.37	11.46
H. fabrorum	–	3.46	6.42	1.04	1.07	1.96	6.38
H. cockerelli	–	9.40	20.15	1.84	4.90	5.27	12.38
H. cockerelli I	–	4.60	10.68	0.57	1.79	3.69	7.78
H. cockerelli	*H. fabrorum*	15.08	19.67	2.95	4.57	8.32	13.72
H. cockerelli I	*H. fabrorum*	16.13	19.67	2.90	4.57	8.70	13.72
H. fabrorum	*H. cf. fabrorum* 9	7.14	9.90	1.73	2.07	6.56	9.59
	H. cf. fabrorum 39	8.29	14.75	2.60	5.05	1.81	4.58
	H. cf. fabrorum 39-1	13.60	14.75	4.27	5.05	1.61	4.17
	H. cf. fabrorum 39-2	2.98	5.83	0.92	1.79	2.00	4.59
	H. cf. cockerelli 46	13.53	15.27	4.45	5.49	9.41	12.26
H. cockerelli	*H. cf. fabrorum* 9	15.66	19.88	3.31	4.15	6.30	8.44
	H. cf. fabrorum 39	15.41	19.49	3.81	5.40	8.54	11.59
	H. cf. fabrorum 39-1	14.65	19.49	4.51	5.39	8.46	11.42
	H. cf. fabrorum 39-2	16.28	18.40	3.11	3.83	8.61	11.59
	H. cf. cockerelli 46	12.93	16.59	3.48	5.49	9.12	16.25
H. cockerelli I	*H. cf. fabrorum* 9	16.41	19.88	3.34	4.15	6.76	8.44
	H. cf. fabrorum 39	17.09	19.49	4.06	5.40	8.99	11.59
	H. cf. fabrorum 39-1	17.01	19.49	5.01	5.39	8.92	11.42
	H. cf. fabrorum 39-2	17.47	18.40	3.11	3.83	9.05	11.59
	H. cf. cockerelli 46	12.19	16.59	2.96	3.46	8.46	10.93

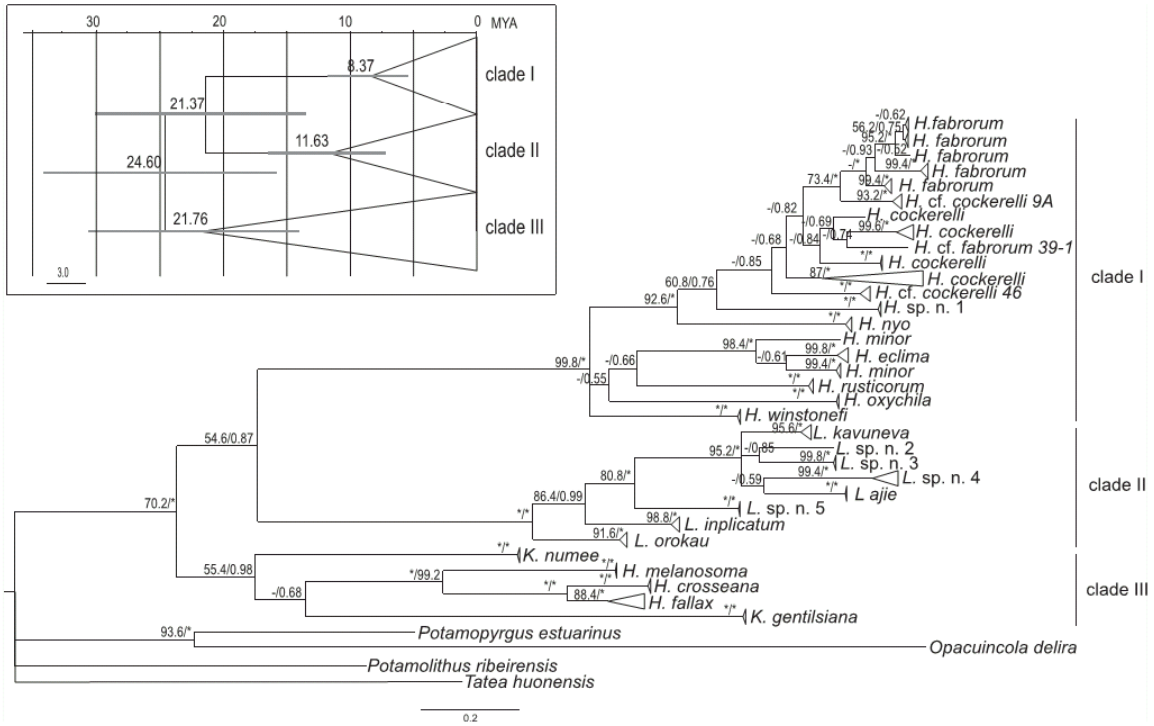

Fig. 2. Bayesian tree of the concatenated dataset; node values are: bootstrap support values/Bayesian posterior probabilities; monophyletic species clades with support values >80/0.8 were collapsed; embedded: Beast results, presented nodes had posterior probabilities >80.

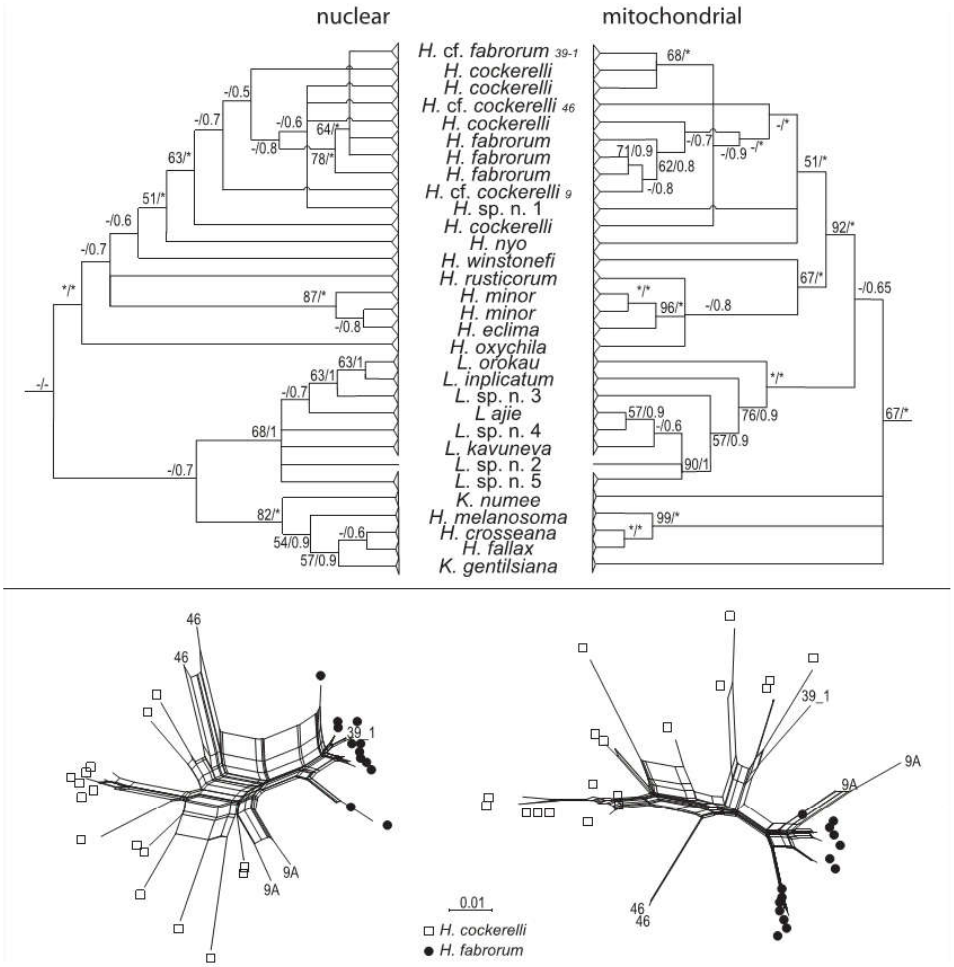

Fig. 3. Comparison of phylogenetic reconstructions resulting from analyses of mt and nc genes; upper panel: cladograms redrawn from Bayesian and ML analyses; node values are bootstrap support values/Bayesian posterior probabilities; species clades with support values >80/0.8 were collapsed; lower panel: Splits Tree analysis of the *H. cockerelli–H. fabrorum* clade.

Table 3
Number of molecular diagnostic characters distinguishing Tateid genera from New Caledonia; Abbreviations: *H. Hemistomia, L. Leiorhagium; K. Kanakyella; N. Novacaledonia; C. Crosseana.*

	H.	L.	K.	N.
L.	1			
K.	12	32		
N.	13	25	151	
C.	2	17	65	64

concatenated data. Only the relationships between *L. ajie*, *L. kavuneva*, and the new species 2, 3 and 4 remained ambiguous (Fig. 2).

H. crosseana, *H. fallax* and *H. melanosoma* formed a well-supported monophyletic group within clade III based on the mt and concatenated data. The affiliation of *K. gentilsiana* and *K. numee* to clade III was well supported only by the nc data and the Bayesian analysis of the concatenated dataset. In no analyses were these species sister taxa (Figs 2 and 3). The taxonomic consequences of these phylogenetic results are two new genera for *H. crosseana*, *H. fallax* and *H. melanosoma* on the one hand, and *K. numee* on the other hand introduced at the end of the Results section. The four new species will be described in another paper (Haase and Zielske, unpublished data).

The CAOS analysis based on the ML phylogeny of the concatenated dataset presented herein resulted in three simple pure characters identifying only two of the five genera. Relaxing the standards for diagnosability as explained in the Methods section we found 180 characters that can be used to separate one genus from at least one other genus. The number of diagnostic character states separating each pair of genera based on molecular data and the characters themselves are listed in Table 3 and Appendix B, respectively. The length of the strings of pair-wise diagnostic characters was negatively correlated with the number of species contained in each genus (Spearman's rank correlation, $rs = -0.98$, $p = 0.03$).

The Bayes factor test comparing different combinations of clock constraints in BEAST identified the clock relaxed for all three genes as best fitting model (Table 4). The tree topology concerning the larger clades was the same as described above for the concatenated dataset with good support for the monophyly of clades I and II. The runs assuming a strict clock for 16S rRNA and ITS2 or for all three

Table 4
Log_{10} Bayes factors of likelihoods.

Model	ln *P* (model | data)	S.E.	Log_{10} Bf		
			All relaxed	COI relaxed	All strict
All relaxed	−12,875.831	±0.234	–	60.917	60.849
COI relaxed	−13,016.089	±0.181	−60.917	–	−0.068
All strict	−13,015.941	±0.168	−60.849	0.068	–

genes resulted in a different tree topology with good support for a sister relationship of clades II and III as in the nc analysis in MrBayes. In all three analyses relationships within the clades differed only for taxa that were inconsistently resolved also in the preceding reconstructions.

Assuming the relaxed clock for the three partitions resulted in the following ages (Fig. 2) for the most recent common ancestor of clades I to III: 11.63 MY (95% HPD interval: 7.24–16.45 MY), 8.37 MY (5.48–11.71 MY), 21.76 MY (14.08–30.56 MY). The origin of all New Caledonian taxa was estimated at 24.60 MYA (15.84–34.14 MYA). Age estimates based on the other BEAST analyses were very similar despite the different topologies.

4. Systematic descriptions

Family TATEIDAE Thiele, 1925

4.1. Genus *Novacaledonia* gen. n.

Type species: *Kanakyella numee* Haase and Bouchet, 1998, by monotypy.

Etymology: Nova Caledonia is the latinized name of New Caledonia.

Diagnosis: *Novaceledonia* is well differentiated from the other genera of Tateidae on New Caledonia by the diagnostic string of molecular states given in Appendix B. This holds especially for the differentiation from *Kanakyella* from which the new genus was separated. Morphologically, *Novacaledonia* is characterized by the following combination of characters: relatively large pupoid shell lacking the palatal denticle, 5 opercular pegs, the hypobranchial gland almost reaching the tip of the capsule gland, renal oviduct with wide loop of 270° and distally positioned seminal receptacle. Compared to the genus *Kanakyella* it has smaller numbers of opercular pegs and denticles on all radular teeth, a larger hypobranchial gland and a penis bearing a lobe on the right side.

Remarks: Because of the similarity of the female genitalia the type species of this genus was originally allocated to the genus *Kanakyella*, albeit tentatively because of the geographic distance between the species (Haase and Bouchet, 1998). The present phylogenetic analysis suggests that this similarity is due to convergence.

4.2. Genus *Crosseana* gen. n.

Type species: *Hydrobia crosseana*, Gassies 1874

Etymology: This genus is named after Joseph Charles Hippolyte Crosse (1826–1898) who was one of the first scientists describing hydrobioid species from New Caledonia.

Diagnosis: Diagnostic molecular states are given in Appendix B. Morphologically and anatomically, *Crosseana* is characterized by a pupoid to broad-conical shell lacking a palatal denticle, 3–5 opercular pegs, the hypobranchial gland always apparent but of variable extent, a median line of cilia on the cephalic tentacles and the penis bearing a lobe at the right side.

Remarks: The species allocated to this genus – the type species as well as *C. fallax* and *C. melanosoma* – were originally allocated to the genus *Hemistomia* because of the similar female genitalia.

5. Discussion

The monophyly of the group of genera defining the *Hemistomia*-clade *sensu* Haase and Bouchet (1998) was well supported only by the Bayesian analysis of the concatenated data. To achieve an unambiguous resolution probably requires the addition of further, more conservatively evolving genes, which is supported by preliminary analyses across the entire family Tateidae (Zielske et al., unpublished data). Among the three genera included in our analyses only *Leiorhagium* was monophyletic. Not surprisingly, the larger species tentatively attributed to *Hemistomia* and *Kanakyella*, respectively, rendered these genera para- or polyphyletic. Therefore, two new genera, *Crosseana* and *Novacaledonica*, were introduced for *H. crosseana*, *H. fallax*, *H. melanosoma* and *K. numee*, respectively. Defining species or even higher taxa of Rissooidea and Truncatelloidea based on unique combinations of morphological and anatomical character states is often difficult due to convergence and morphostasis (Falniowski and Szarowska, 1995; Hershler and Ponder, 1998; Liu et al., 2003; Wilke, 2003; Colgan et al., 2007; Haase, 2008; Wilke et al., 2013). Therefore, we tried to identify also combinations of diagnostic molecular characters for the delimitation and definition of our genera, an approach successfully employed e.g. for Odonata (Rach et al., 2008). In several cases, character-based bar coding has proved to be advantageous over the widely applied distance based approach (e.g., Rach et al., 2008; Bergmann et al., 2013), because the former does not rely on an arbitrarily defined threshold and the often lacking barcoding-gap of the latter, which has also a number of other shortcomings (e.g., DeSalle et al., 2005; Fregin et al., 2012; Sauer and Hausdorf, 2012). However, although we have analyzed three gene fragments and not only the barcoding sequence of COI (cf. DeSalle et al., 2005; Jörger and Schrödl, 2013) we identified only three single-pure characters which defined only two of the five genera. Therefore, we modified the original CAOS-approach (Sarkar et al., 2008) and included strings of all pair-wise diagnostic characters, i.e. combinations of all characters which are fixed for alternative bases in pairs of genera, in the diagnoses of the genera. Each combination of pair-wise diagnostic characters is now diagnostic for a particular genus. Ranging from 25 (for *Hemistomia*) to 180 (for *Kanakyella*), the lengths of the strings were of course negatively correlated with the number of species and specimens the genera contained (in general and in our analyses): The number of pair-wise diagnostic characters decreased with increasing genetic variation within a genus. The number may further decrease with the addition of species. However, this problem is not unique to molecular characters. Thus, our modified character-based barcoding approach retained significantly more characters appropriate for DNA-taxonomy than the original one and will probably be useful in many cases with similar distributions of genetic variation among taxa compared.

Any single-gene based approach would have failed to reveal the topological discrepancies concerning *H. cockerelli* and *H. fabrorum*. In general, incongruence of gene trees and species trees may have a number of causes including incomplete lineage sorting and introgression by hybridization (Maddison, 1997). Both phenomena, widely observed across the animal kingdom (e.g. Bachtrog et al., 2006; Koblmüller et al., 2007; Smith et al., 2013; Yamazaki et al.,

2008), are also known from truncatelloidean gastropods (e.g. Haase, 2005; Prie and Bichain, 2009; Zielske and Haase, 2014). Incomplete lineage sorting is often observed in recent speciation events indicated by short branch lengths. Since in our case branch lengths are rather long, we assume that the observed incongruence is due to hybridization. In a forthcoming paper we will analyse this case in more detail based on additional sequences and morphological investigations.

The age estimates revealed that the most recent common ancestor of our sample of the New Caledonian radiation is not older than 24.6 ± 9.5 MY. This estimate may be an underestimate of the age of the entire radiation since non-sampled (*Pidaconomus*, *Caledochoncha* or unknown taxa) or possibly extinct lineages could not be taken into account. In any case, our findings are in line with the notion that New Caledonia is rather a Darwinian island that was colonized after a long phase of submergence despite being a fragment of Gondwanaland (Espeland and Murienne, 2011; Cruaud et al., 2012). Our analyses only vaguely indicated that the New Caledonian species were closer related to Australian than to New Zealand taxa. Divergence estimates for New Zealand tateids were slightly younger (Haase et al., 2007), though, confirming this hypothesis. Furthermore the vast majority of New Caledonia's flora and fauna is of Australian or Malesian origin (e.g. Balke et al., 2007; Duangjai et al., 2009; Espeland and Johanson, 2010; Swenson et al., 2013).

Acknowledgments

We thank Christine Poellabauer who supported us in Noumea/New Caledonia and all locals who helped us during field work. At the University of Greifswald we thank our technician Christel Meibauer, who helped with DNA work. The manuscript was improved by the comments of anonymous reviewers. Financial support was received from the Deutsche Forschungsgemeinschaft (Grant HA4752/2-1).

Appendix A

Locality data, GenBank accession numbers and voucher material (MNHN, Muséum National d'Histoire Naturelle, Paris; NHMW, Naturhistorisches Museum Wien).

Station	GPS	Species	COI	16S rRNA	ITS	Collection number
NeCa 1	22°08′59.0″'S′, 166°29'10.6″E	*H. fabrorum*	KJ490829	KJ490749	KJ490670	NHMW
			KJ490830	KJ490750	KJ490671	110185
		H. minor	KJ490831		KJ490672	NHMW
						110186
NeCa 3	22°15'42.4″S′, 166°34'08.7″E	*N. numee*	KJ490832	KJ490751	KJ490673	NHMW
			KJ490833	KJ490752	KJ490674	110187
		H. winstonefi	KJ490834	KJ490753	KJ490675	NHMW
			KJ490835	KJ490754	KJ490676	110188
NeCa 6	21°48'08.0″S′, 166°04'14.6″E	*H. rusticorum*	KJ490836	KJ490755	KJ490677	NHMW
			KJ490837	KJ490756	KJ490678	110189
		L. kavuneva	KJ490838	KJ490757		NHMW
						110190
		H. fabrorum	KJ490839	KJ490758	KJ490679	NHMW
						110191
NeCa 7	21°44'47.4″S′, 166°05'31.3″E	*H. cockerelli*	KJ490840		KJ490680	NHMW
						110192
NeCa 8	21°44'32.1″S′, 166°05'20′6″E	*H. fabrorum*	KJ490841	KJ490759	KJ490681	NHMW
			KJ490842	KJ490760	KJ490682	110193
NeCa 9	21°44'30.9″S′, 166°05'57.9″E	*H. fabrorum*	KJ490843	KJ490761	KJ490683	NHMW
			KJ490844		KJ490684	110194
		L. inplicatum	KJ490845	KJ490762	KJ490685	NHMW
			KJ490846	KJ490763	KJ490686	110195
NeCa 10	21°42'55.4″S′, 166°07'21.1″E	*H. fabrorum*	KJ490847	KJ490764	KJ490687	NHMW
			KJ490848	KJ490765	KJ490688	110196
NeCa 11	21°48'16.8″S′, 166°00'00.8″E	*H. cockerelli*	KJ490849	KJ490766	KJ490689	NHMW
			KJ490850		KJ490690	110197
NeCa 12	21°49'46.9″S′, 165°56'42.9″E	*H. sp. n. 1*	KJ490851	KJ490767	KJ490691	NHMW
			KJ490852			110181
NeCa 13	21°47'30.8″S′, 165°54'31.6″E	*H. cockerelli*	KJ490853	KJ490768	KJ490692	NHMW
			KJ490854	KJ490769	KJ490693	110198
NeCa 15	21°47'24.4″S′, 165°54'51.2″E	*L. kavuneva*	KJ490855	KJ490770	KJ490694	NHMW
			KJ490856	KJ490771	KJ490695	110199
NeCa 17	21°39'52.8″S′, 165°43'10.3″E	*H. cockerelli*	KJ490857	KJ490772	KJ490696	NHMW
						110200
NeCa 19	21°39'58.4″S′, 165°43'08.2″E	*H. eclima*	KJ490858	KJ490773	KJ490697	NHMW
			KJ490859	KJ490774	KJ490698	110201

(*continued on next page*)

Appendix A (*continued*)

Station	GPS	Species	COI	16S rRNA	ITS	Collection number
NeCa 20	21°38′38.4″S, 165°46′52.1″E	*L. kavuneva*	KJ490860	KJ490775	KJ490699	NHMW
			KJ490861	KJ490776	KJ490700	110202
NeCa 21	21°38′11.9″S, 165°46′36.6″E	*H. cockerelli*	KJ490862	KJ490777	KJ490701	NHMW
			KJ490863		KJ490702	110203
		L. kavuneva	KJ490864	KJ490778		NHMW
						110204
NeCa 22	21°38′11.4″S, 165°49′37.3″E	*L. kavuneva*	KJ490865	KJ490779	KJ490703	NHMW
			KJ490866	KJ490780		110205
NeCa 25	21°34′15.7″S, 165°49′41.2″E	*H. fabrorum*	KJ490867	KJ490781	KJ490704	NHMW
			KJ490868	KJ490782	KJ490705	110206
NeCa 27	21°30′52.2″S, 165°48′05.0″E	*L. kavuneva*	KJ490869	KJ490783	KJ490706	NHMW
						110207
NeCa 28	21°31′07.4″S, 165°48′20.0″E	*H. fabrorum*	KJ490870	KJ490784	KJ490707	NHMW
			KJ490871	KJ490785	KJ490708	110208
NeCa 30	21°34′21.6″S, 165°41′02.5″E	*H. minor*	KJ490872	KJ490786	KJ490709	NHMW
			KJ490873	KJ490787	KJ490710	110209
		L. sp. n. 2	KJ490874		KJ490711	MNHN
						IM
						2000–27866
		H. cockerelli	KJ490875		KJ490712	NHMW
						110210
NeCa 31	21°33′33.5″S, 165°42′11.3″E	*H. cockerelli*	KJ490876	KJ490788		NHMW
						110211
NeCa 32	21°34′55.9″S, 165°40′16.7″E	*H. cockerelli*	KJ490877		KJ490713	NHMW
						110212
NeCa 33	21°35′04.8″S, 165°39′07.5″E	*L. sp. n. 3*	KJ490878	KJ490789	KJ490714	MNHN
			KJ490879	KJ490790	KJ490715	IM
						2000–27863;
						NHMW
						110184
NeCa 35	21°36′50.3″S, 165°35′31.5″E	*H. nyo*	KJ490880	KJ490791	KJ490716	NHMW
			KJ490881	KJ490792	KJ490717	110213
NeCa 36	21°38′22.1″S, 165°51′37.5″E	*H. cockerelli*	KJ490882	KJ490793	KJ490719	NHMW
			KJ490883	KJ490794	KJ490719	110214
NeCa 38	21°38′09.3″S, 165°51′52.7″E	*H. fabrorum*	KJ490884	KJ490795		NHMW
			KJ490885	KJ490796		110215
		H. cockerelli	KJ490886	KJ490797	KJ490720	NHMW
			KJ490887	KJ490798	KJ490721	110216
NeCa 39	21°37′56.1″S, 165°51′54.4″E	*H. c.f. fabrorum*	KJ490888	KJ490799	KJ490722	NHMW
			KJ490889	KJ490800	KJ490723	110217
NeCa 41	21°38′12.3″S, 165°51′34.1″E	*H. cockerelli*	KJ490890	KJ490801		NHMW
						110218
NeCa 42	21°16′32.2″S, 165°12′17.6″E	*L. orokau*	KJ490891	KJ490802	KJ490724	NHMW
			KJ490892	KJ490803	KJ490725	110219
NeCa 43	21°16′06.0″S, 165°14′32.0″E	*H. oxychila*	KJ490893	KJ490804	KJ490726	NHMW
			KJ490894	KJ490805	KJ490727	110220
		L. sp. n. 4	KJ490895	KJ490806	KJ490728	MNHN
			KJ490896	KJ490807	KJ490729	IM
						2012–36075;
						NHMW
						110183
NeCa 44	21°14′47.9″S, 165°15′45.0″E	*L. sp. n. 5*	KJ490897	KJ490808	KJ490730	MNHN
			KJ490898	KJ490809	KJ490731	IM
						2000–27868;
NeCa 46	21°14′30.2″S, 165°16′30.8″E	*H. c.f. cockerelli*	KJ490899	KJ490810	KJ490732	NHMW
			KJ490900	KJ490811	KJ490733	110221
NeCa 49	21°15′24.4″S, 165°14′46.4″E	*L. sp. n. 4*	KJ490901	KJ490812	KJ490734	MNHN
						IM
						2000–27861;
						NHMW
						110182
NeCa 50	20°49′13.6″S, 164°36′56.4″E	*C. melanosoma*	KJ490902	KJ490813	KJ490735	NHMW
			KJ490903	KJ490814	KJ490736	110222

Appendix A (*continued*)

Station	GPS	Species	COI	16S rRNA	ITS	Collection number
NeCa 51	20°32'32.2″S', 164°18'33.0″E	*C. crosseana*	KJ490904	KJ490815	KJ490737	NHMW
			KJ490905	KJ490816	KJ490738	110223
NeCa 52	20°32'52.1″S', 164°20'38.7″E	*C. fallax*	KJ490906	KJ490817	KJ490739	NHMW
			KJ490907	KJ490818	KJ490740	110224
NeCa 53	20°29'28.7″S', 164°15'24.9″E	*C. fallax*	KJ490908	KJ490819	KJ490741	NHMW
			KJ490909	KJ490820		110225
NeCa 56	20°42'44.2″S', 164°48'31.2″E	*H. cockerelli*	KJ490910	KJ490821	KJ490742	NHMW
			KJ490911	KJ490822	KJ490743	110226
NeCa 57	20°42'43.9″S', 164°47'47.5″E	*L. orokau*	KJ490912	KJ490823	KJ490744	NHMW
			KJ490913	KJ490824	KJ490745	110227
NeCa 58	20°42'22.4″S', 164°47'20.0″E	*K. gentilsiana*	KJ490914	KJ490825	KJ490746	NHMW
			KJ490915	KJ490826		110228
NeCa 60	21°22'26.6″S', 165°26'08.1″E	*L. c.f. ajie*	KJ490916	KJ490827	KJ490747	NHMW
			KJ490917	KJ490828	KJ490748	110229
–	–	*Opacuincola delira*	AY631090	AY634068	–	
–	–	*Potamolithus ribeirensis*	JX970618	JX970549	–	
–	–	*Potamopyrgus estuarinus*	AY631103	AY634081	KC875220	
–	–	*Tatea huonensis*	JX970619	JX970550	–	

Appendix B

Diagnostic molecular characters. These characters distinguish one particular genus from at least one other. If no state is listed at a particular position the genus is ambiguous in this position. Alignment positions are based on the alignment deposited in treebase (http://purl.org/phylo/treebase/phylows/study/TB2:S15532/; for review see: http://purl.org/phylo/treebase/phylows/study/TB2:S15532?x-access-code=32d9fc8c4e235f37ca68e4fc76d9c70f&format=html.).Genera and bases are abbreviated by their respective initials. Character states unique for one genus are given in bold. Reading example, position 247: *Kanakyella* has G and thus can be separated from *Novacaledonia* and *Crosseana* which are fixed for A at this position while species of *Hemistomia* and *Leiorhagium* have more than one state.

	16S	11	15	16	17	18	23	30	31	32	48	50	53	98	110	135	140	153	180	193	198	231	243	247
H																								
L														T	T	C	A	T			A			
K		T	A	C	A	G	A	G	A	G	C	C	G	T	C	C	G	C	A	T	G	T	G	G
C			T			A	A	A		A	T	T	A	T	T	T	A	C		T				A
N		A	A	A	T	A	G	G	G	A	T	A	A	C	T	C	G	C	G	C	A	A	C	A
	249	254	255	261	271	272	279	282	283	303	312	327	328	331	337	338	341	345	348	349	364	375	413	433
H																								
L		A	G		G		A	T		A	A	T				G			C	T		A		T
K	C	G	T	T	A	C	G	A	T	A	A	C	A	A	T	A	A	A	T	C	C	A	A	A
C	T					T	A	A	G	T	T		A			A	T		T	T		A		A
N	T	G	A	C	G	T	A	A	G	A	A		T	G	A	A	A	C	T	T	T	G	G	A
	472	473	482	483	508	516	COI	540	549	553	564	570	579	582	585	588	589	591	597	606	609	612	613	615
H																								
L	G	A	T	C		C															A			
K	G	G	C	C	C	C		T	T	C	A	C	T	T	G	C	C	G	G	A	G	A	C	T
C	A	A	T	T	T	T		T		T	G	T		A		T			A	T	A	A	T	
N	G	A	T	C	C	T		A	C	T	G	T	G	G	A	A	T	A	A	A	A	A	G	T	A
	621	624	630	631	633	634	636	639	645	660	669	684	696	697	702	705	708	711	726	735	736	738	744	747
H						T				T								A						T
L				T				T	T	T	A	T	T						T		C	T		T
K	G	A	C	C	A	C	T	G	T	A	T	C	C	C	G	G	C	G	T	G	C	A	A	A
C	A		T	T					T		T		T	T	A			G	T		T	A	T	
N	A	G	T	T	G	T	G	A	C	T	C	A	T	T	G	A	T	A	C	A	T	A	T	T

(*continued on next page*)

Appendix B (*continued*)

	759	762	765	768	780	786	789	801	802	805	810	813	816	817	819	822	823	825	826	828	837	840	846	849
H			T			T			C										C	T				A
L					T	T	T	T	C			T				A						A		A
K	T	C	C	A	A	T	C	A	T	T	C	T	A	C	A	G	C	T	T	A	A	T	G	A
C	T			A	T	T	T	A	T	T	A	T		T			T	G			A	T		A
N	C	A	T	G	T	C	T	G	T	C	T	A	G	C	T	A	T	A	C	T	G	T	A	G

	851	855	864	867	870	873	879	882	885	886	888	898	900	906	930	942	945	951	954	969	981	990	993	1005
H	G		T											T	T									
L	G	T																						
K	A	G	T	A	A	A	C	T	C	C	A	A	C	T	T	G	C	T	T	G	T	A	A	G
C	G	A	T			T	C		T				T	T	T			T	T	A	T	A	T	
N	G	A	C	C	G	T	T	C	T	T	G	G	G	C	C	A	T	G	G	A	C	G	A	A

	1017	1020	1023	1024	1026	1029	1032	1038	1039	1041	1050	1053	1075	1078	1080	1081	1083	1087	1089	1092	1104	1113	1116	1119
H				T					T			T				C	T	T						
L																								
K	C	G	G	T	T	A	T	T	C	T	G	T	C	C	A	C	T	C	A	G	T	T	G	G
C	A	A	A	T	A	A	A	T	C	T		T				C	T	T				A	A	A
N	A	G	G	C	T	G	T	C	T	A	A	C	T	T	G	T	A	C	T	A	G	A	A	A

	1125	1137	1144	1155	1158	1164	1167	1170	1173	1176	1179	ITS	1180	1191	1192	Sum of diagnostic characters
H	T	T	G								T				T	25
L																47
K	A	T	G	C	A	A	C	G	T	A	T		C	T	C	180
C		T	G							A	T		T			112
N	T	C	T	T	G	G	T	A	C	C	C		T	C	T	179

References

Bachtrog, D., Thornton, K., Clark, A., Andolfatto, P., 2006. Extensive introgression of mitochondrial DNA relative to nuclear genes in the *Drosophila yakuba* species group. Evolution 60, 292–302.

Balke, M., Wewalka, G., Alarie, Y., Ribera, I., 2007. Molecular phylogeny of Pacific Island Colymbetinae: radiation of New Caledonian and Fijian species (Coleoptera, Dytiscidae). Zool. Scripta 36, 173–200.

Bauer, A.M., Jackman, T., Sadlier, R.A., Whitaker, A.H., 2006. A revision of the Bavayia validiclavis group (Squamata: Gekkota: Diplodactylidae), a clade of New Caledonian geckos exhibiting microendemism. Proc. Cali.f Acad. Sci. 57, 503–547.

Bergmann, T., Rach, J., Damm, S., DeSalle, R., Schierwater, B., Hadrys, H., 2013. The potential of distance- based thresholds and character-based DNA barcoding for defining problematic taxonomic entities by COI and ND1. Mol. Ecol. Resour. 13, 1069–1081.

Cannone, J., Subramanian, S., Schnare, M., Collett, J., D'Souza, L., Du, Y., Feng, B., Lin, N., Madabusi, L., Muller, K., Pande, N., Shang, Z., Yu, N., Gutell, R., 2002. The Comparative RNA Web (CRW) Site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinform. 3 (2) [Correction: BMC Bioinformatics. 3:15.].

Chazeau, J., 1993. Research on New Caledonian terrestrial fauna: achievements and prospects. Biodiver. Lett. 1, 123–129.

Colgan, D.J., Ponder, W.F., Beacham, E., Macaranas, J., 2007. Molecular phylogenetics of Caenogastropoda (Gastropoda: Mollusca). Mol. Phylogenet. Evol. 42, 717–737.

Cruaud, A., Jabbour-Zahab, R., Genson, G., Ungricht, S., Rasplus, J.-Y., 2012. Testing the emergence of New Caledonia: fig wasp mutualism as a case study and a review of evidence. PLoS One 7, e30941.

DeSalle, R., Egan, M.G., Siddall, M., 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philos. Trans. Roy. Soc. B 360, 1905–1916.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Duangjai, S., Samuel, R., Munzinger, J., Forest, F., Wallnöfer, B., Barfuss, M.H.J., Fischer, G., Chase, M.W., 2009. A multi-locus plastid phylogenetic analysis of the pantropical genus Diospyros (Ebenaceae), with an emphasis on the radiation and biogeographic origins of the New Caledonian endemic species. Mol. Phylogenet. Evol. 52, 602–620.

Espeland, M., Johanson, K.A., 2010. The diversity and radiation of the largest monophyletic animal group on New Caledonia (Trichoptera: Ecnomidae: Agmina). J. Evolut. Biol. 23, 2112–2122.

Espeland, M., Murienne, J., 2011. Diversity dynamics in New Caledonia: towards the end of the museum model? BMC Evol. Biol. 11, 254.

Falniowski, A., Szarowska, M., 1995. Can poorly understood new characters support a poorly understood phylogeny? Shell-structure data in Hydrobiid systematics (Mollusca: Gastropoda: Prosobranchia: Hydrobiidae). J. Zool. Syst. Evol. Res. 33, 133–144.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3, 294–299.

Fregin, S., Haase, M., Olsson, U., Alström, P., 2012. Pitfalls in comparison of genetic distances: A case study of the avian family Acrocephalidae. Mol. Phylogenet. Evol. 62, 319–328.

Grandcolas, P., Murienne, J., Robillard, T., Desutter-Grandcolas, L., Jourdan, H., Guilbert, E., Deharveng, L., 2008. New Caledonia: a very old Darwinian island? Philos. Trans. Roy. Soc B 363, 3309–3317.

Haase, M., 2005. Rapid and convergent evolution of parental care in hydrobiid gastropods from New Zealand. J. Evolut. Biol. 18, 1076–1086.

Haase, M., 2008. The radiation of hydroid gastropods in New Zealand: a revision including the description of new species based on morphology and mt DNA sequence information. Syst. Biodivers. 6, 99–159.

Haase, M., Bouchet, P., 1998. Radiation of crenobiontic gastropods on an ancient continental island: the Hemistomia-clade in New-Caledonia (Gastropoda: Hydrobiidae). Hydrobiologia 367, 43–129.

Haase, M., Marshall, B., Hogg, I., 2007. Disentangling causes of disjunction on the South Island of New Zealand: the Alpine fault hypothesis of vicariance revisited. Biol. J. Linn. Soc. 91, 361–374.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Heads, M., 2008. Panbiogeography of New Caledonia, south-west Pacific: basal angiosperms on basement terranes, ultramafic endemics inherited from volcanic island arcs and old taxa endemic to young islands. J. Biogeogr. 35, 2153–2175.

Hershler, R., Ponder, W.F., 1998. A Review of Morphological Characters of Hydrobioid Snails. Smithonian Institution Press, Washington, DC.

Holloway, J.D., 1979. A Survey of the Lepidoptera, Biogeography, and Ecology of New Caledonia, vol. 15. W. Junk, The Hague (Series entomologica).

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

Jaffré, T., Morat, P., Veillon, J.-M., 1994. La flore: caractéristiques et composition floristique des principales formations végétales. Bois forét des tropique 242, 7–30.

Jörger, K.M., Schrödl, M., 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. Front. Zool. 10, 1–27.

Koblmüller, S., Duftner, N., Sefc, K., Aibara, M., Stipacek, M., Blanc, M., Egger, B., Sturmbauer, C., 2007. Reticulate phylogeny of gastropod-shell-breeding cichlids

from Lake Tanganyika – the result of repeated introgressive hybridization. BMC Evol. Biol. 7, 1–13.
Koetschan, C., Förster, F., Keller, A., Schleicher, T., Ruderisch, B., Schwarz, R., Müller, T., Wolf, M., Schultz, J., 2010. The ITS2 Database III—sequences and structures for phylogeny. Nucleic Acids Res. 38, D275–D279.
Ladiges, P.Y., Cantrill, D., 2007. New Caledonia–Australian connections: biogeographic patterns and geology. Aust. Syst. Bot. 20, 383–389.
Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.
Liu, H.P., Heshler, R., Clift, K., 2003. Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. Mol. Ecol. 12, 2771–2782.
Lowry, P., 1998. Diversity, endemism and extinction in the flora of New Caledonia: a review. In: Peng, C.-I., Lowry, P. (Eds.). Rare, Threatened and Endangered Floras of the Pacific Rim. Monograph Series No 16. Institute of Botany, Academica Sinica, Taipei, pp. 181–206.
Maddison, W.P., 1997. Gene trees in species trees. Syst. Biol. 46, 523–536.
Misof, B., Misof, K., 2009. A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: a more objective means of data exclusion. Syst. Biol. 58, 21–34.
Morat, P., Veillon, J.-M., MacKee, H.S., 1986. Floristic relationships of New Caledonian rainforest phanerogams. Telopea 2, 631–679.
Murienne, J., 2009. New caledonia, biology. In: Encyclopedia of Islands. University of California Press, Berkley, California, pp. 643–645.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.
Neall, V.E., Trewick, S.A., 2008. The age and origin of the Pacific islands: a geological overview. Philos, Trans. Roy. Soc. B 363, 3293–3308.
Oláh, J., Johanson, K.A., Barnard, P.C., 2006. Revision of the South Pacific endemic genera *Orthopsyche* McFarlane 1976, *Abacaria* Mosely 1941 and *Caledopsyche* Kimmins 1953 with the description of 29 new species (Trichoptera: Hydropsychidae). Zootaxa 1356, 1–78.
Oliverio, M., Mariottini, P., 2001. Contrasting morphological and molecular variation in *Coralliophila meyendorffii* (Muricidae, Coralliophilinae). J. Mollus Stud. 67, 243–246.
Palumbi, S., Martin, A., Romano, S., McMillan, W., Stice, L., Gabowski, G., 1991. The simple fool's guide to PCR. Kewalo Marine Laboratory and Univ. of Hawaii.
Prie, V., Bichain, J.-M., 2009. Phylogenetic relationships and description of a new stygobite species of *Bythinella* (Mollusca, Gastropoda, Caenogastropoda, Amnicolidae) from southern France. Zoosystema 31, 987–1000.
Rach, J., DeSalle, R., Sarker, I.N., Schierwater, B., Hadrys, H., 2008. Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. Proc. Roy. Soc. B Biol. Sci. 275, 237–247.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.5. <http://beast.bio.ed.ac.uk/Tracer>.
Reid, B., Le, M., McCord, W., Iverson, J., Georges, A., Bergmann, T., Amato, G., Desalle, R., Naro-Maciel, E., 2011. Comparing and combining distance-based and character-based approaches for barcoding turtles. Mol. Ecol. Resour. 11, 956–967.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Sarkar, I.N., Planet, P.J., Desalle, R., 2008. CAOS software for use in character-based DNA barcoding. Mol. Ecol. Resour. 8, 1256–1259.
Sauer, J., Hausdorf, B., 2012. A comparison of DNA-based methods for delimiting species in a Cretan land snail radiation reveals shortcomings of exclusively molecular taxonomy. Cladistics 28, 300–316.
Schellart, W.P., Lister, G.S., Toy, V.G., 2006. A Late Cretaceous and Cenozoic reconstruction of the Southwest Pacific region: tectonics controlled by subduction and slab rollback processes. Earth Sci. Rev. 76, 191–233.
Smith, K.L., Hale, J.M., Kearney, M.R., Austin, J.J., Melville, J., 2013. Molecular patterns of introgression in a classic hybrid zone between the Australian tree frogs, *Litoria ewingii* and *L. paraewingi*: evidence of a tension zone. Mol. Ecol. 22, 1869–1883.
Stocsits, R.R., Letsch, H., Hertel, J., Misof, B., Stadler, P.F., 2009. Accurate and efficient reconstruction of deep phylogenies from structured RNAs. Nucleic Acids Res. 37, 6184–6193.
Swenson, U., Nylinder, S., Munzinger, J., 2013. Sapotaceae biogeography supports New Caledonia being an old Darwinian island. J. Biogeogr. http://dx.doi.org/10.1111/jbi.12246.
Swofford, D., 2003. PAUP*. Phylogenetic Analysis using Parsimony (*and other methods). Sinnauer Associates, Sunderland, Massachusetts.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Wilke, T., 2003. Salenthydrobia n.gen. (Rissooidea: Hydrobiidae): a potential relict of the Messinian salinity crisis. Zool. J. Linn. Soc. Lond. 137, 319–336.
Wilke, T., Haase, M., Hershler, R., Liu, H.-P., Misof, B., Ponder, W., 2013. Pushing short DNA fragments to the limit: Phylogenetic relationships of "hydrobioid" gastropods (Caenogastropoda: Rissooidea). Mol. Phylogenet. Evol. 66, 715–736.
Wulff, A.S., Hollingsworth, P.M., Ahrends, A., Jaffré, T., Veillon, J.-M., L'Huillier, L., Fogliani, B., 2013. Conservation priorities in a biodiversity hotspot: analysis of narrow endemic plant species in new caledonia. PLoS One 8. http://dx.doi.org/10.1371/journal.pone.0073371.
Xia, X., Xie, Z., 2001. DAMBE: software package for data analysis in molecular biology and evolution. J. Hered. 92, 371–373.
Yamazaki, Y., Kouketsu, S., Fukuda, T., Araki, Y., Nambu, H., 2008. Natural hybridization and directional introgression of two species of Japanese toads *Bufo japonicusformosus* and *Bufo torrenticola* (Anura: Bufonidae) resulting from changes in their spawning habitat. J. Herpetol. 42, 427–436.
Zielske, S., Haase, M., 2014. When snails inform about geology: pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda: Tateidae). J. Zool. Syst. Evol. Res. 52, 217–236.
Zou, S., Li, Q., Kong, L., Yu, H., Zheng, X., 2011. Comparing the usefulness of distance, monophyly and character-based DNA barcoding methods in species identification: a case study of Neogastropoda. PLoS One 6. http://dx.doi.org/10.1371/journal.pone.0026619.
Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. (Ph.D. dissertation). University of Texas, Austin.

2.4. Long distance dispersal of freshwater gastropods

Journal of Biogeography (J. Biogeogr.) (2016)

ORIGINAL ARTICLE

The enigmatic pattern of long-distance dispersal of minute freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae) across the South Pacific

Susan Zielske[1,*], Winston F. Ponder[2] and Martin Haase[1]

[1]*Vogelwarte, Zoological Institute and Museum, Greifswald University, Soldmannstr. 23, 17489 Greifswald, Germany,* [2]*Australian Museum Research Institute, 1 William Street, Sydney 2010, NSW, Australia*

*Correspondence: Susan Zielske, Vogelwarte, Zoological Institute and Museum, Greifswald University, Soldmannstr. 23, 17489 Greifswald, Germany.
E-mail: susan.zielske@googlemail.com

ABSTRACT

Aim Tateid freshwater gastropods have an enigmatic distribution in the South Pacific (SP) region. We reconstructed their diversification and dispersal pathways and estimated a timeframe for their radiations.

Location New Caledonia, Vanuatu, Fiji, the Austral Islands, further species from Sulawesi, Australia, New Zealand and Lord Howe Island.

Methods Bayesian and maximum likelihood methods were used to calculate dated phylogenies based on two mitochondrial and three nuclear gene fragments, test hypotheses and perform ancestral range reconstructions. We compared two calibration strategies based on the COI substitution rate and two sets of island ages selected (1) independent of the ancestral range reconstruction and (2) using information from the latter.

Results The common ancestor of the SP Tateidae occurred in Australia. Its descendents colonized Sulawesi and Lord Howe Island and gave rise to the radiations on New Caledonia and New Zealand. The more remote archipelagos harbouring the genus *Fluviopupa* Pilsbry, 1911 were colonized out of New Zealand. *Fluviopupa* evolved on the Austral Islands and colonized the archipelago against the progression rule from east to west. Vanuatu was colonized twice independently from the Austral Islands and served as hub for dispersal events to Fiji and Lord Howe Island. The second strategy for calibrating the time trees resulted in considerable younger colonization estimates, was generally well in accordance with the geological record and required fewer *ad hoc* hypotheses to explain the diversification and dispersal pattern.

Main conclusions Our analyses of the evolutionary history of Tateidae across the SP revealed several enigmatic aspects not in accordance with the dominant pattern published for other taxa. In particular, the complex westward dispersal pathways of *Fluviopupa*, which originated on the Austral Islands but had an ancestor in New Zealand, are unique. Geographical distance was not an appropriate predictor of relationships.

Keywords
ancestral range reconstruction, dated phylogeny, long-distance dispersal, passive dispersal, radiation, South Pacific islands, Truncatelloidea

INTRODUCTION

The South Pacific (SP) region between South-east Asia/Australia and South America south of the equator covers more than 40 M km^2 of which < 1% is above sea level. The major islands and archipelagos are: Solomon Islands, Bismarck Archipelago, New Caledonia (NC), Vanuatu, New Zealand (NZ), Fiji, Tonga, Samoa, Cook Islands and the islands and archipelagos of French Polynesia. Except NC and NZ, which are continental fragments, these islands are of oceanic origin.

However, although intensely debated, NZ and NC may also be considered oceanic regarding the evolution of their biota as both have probably experienced long phases of significant submergence until the late Eocene and late Oligocene respectively (Waters & Craw, 2006; Murienne, 2009; Carr *et al.*, 2015). The dominant sources of terrestrial and freshwater biota for colonization of the SP islands are Malesia and South-east Asia, as well as Australia. In some cases, NC and NZ are also likely sources for the oceanic islands, whereas America is the more likely source for colonization of the North Pacific Hawaiian Islands (Keppel *et al.*, 2009). In general, species diversity in the SP islands decreases from the west to the east, apparently the result of isolation and area effects (Keppel *et al.*, 2009). Colonization patterns are diverse with unique or multiple colonization events and followed by more or less rapid *in situ* speciation or adaptive radiations (e.g. Muellner *et al.*, 2008; Keppel *et al.*, 2009; Murienne *et al.*, 2011; Birch & Keeley, 2013).

The colonization and evolution of freshwater organisms on oceanic islands are particularly under-studied. Here, we focus on small (few mm in length) freshwater gastropods of the family Tateidae, which probably originated in Australia (Wilke *et al.*, 2013) and have a peculiar SP distribution, being known from five islands/archipelagos: (1) Lord Howe Island (LHI) harbours 15 species-group taxa in three genera (Ponder, 1982), its volcanic rocks are maximally 6.9 Myr old (McDougall *et al.*, 1981); (2) NC harbours seven genera with more than 50 species (Haase & Bouchet, 1998; Zielske & Haase, 2015) and was presumably colonized after a phase of submergence 37 Ma (e.g. Swenson *et al.*, 2014); (3) Vanuatu with 20 species all attributed to *Fluviopupa* (Haase *et al.*, 2010a; Zielske & Haase, 2014a) and continuously emergent probably only since the latest Pliocene (Robin *et al.*, 1993; Greene *et al.*, 1994); (4) Fiji with 28 known *Fluviopupa* species (Haase *et al.*, 2006; Zielske & Haase, 2014b) and a complex geological history (Rodda, 1994); and (5) the Austral Islands (AI) in French Polynesia harbouring six *Fluviopupa* species (Haase *et al.*, 2005) extend over 1200 km from the McDonald Seamount volcano in the east to the island of Rimatara in the west and are formed by three volcanic hotspot chains with the oldest island emergent since maximally 12.1 Myr (Bonneville, 2009; Maury *et al.*, 2014). Apart from the Australian mainland and Tasmania, where the estuarine genus *Tatea* T. Woods, 1879 (Ponder *et al.*, 1991) occurs, tateids are known from brackish water habitats only in NZ, where they invaded freshwater three times independently (Haase, 2005). One tateid from Norfolk Island is probably extinct (Ponder, 1981). In the Malesian region, Tateidae have colonized the ancient lakes of Sulawesi (Haase & Bouchet, 2006; Zielske *et al.*, 2011) and there are a few records from New Guinea (Bernasconi, 1995). Taxa from South America are probably the sister group of these Australasian and Pacific island taxa (Wilke *et al.*, 2013) and, due to the lack of data, are not further considered.

The aim of our work was to reconstruct the evolutionary history of the Tateidae across the SP region including the establishment of a time frame. We particularly focused on the island radiations, testing their respective monophyly and two dispersal scenarios based on geographical considerations against the unconstrained tree. We further asked whether the radiation on NC was informative with regard to this island being considered a Gondwanan relict or a Darwinian island (Espeland & Murienne, 2011 and literature cited therein). We also tested the prediction that the age of the lineage from Sulawesi would match the age of the ancient lakes where it occurs (Hall, 2009; Zielske *et al.*, 2011).

MATERIALS AND METHODS

Material

The material used in this study was collected during 2011 and 2012. Material from Australia and NZ was from the Australian Museum, Sydney, and the Museum of New Zealand (Te Papa Tongarewa), respectively, and some material from Vanuatu was from the Natural History Museum in Paris. Finally, some sequences were taken from GenBank (see Appendix S1 in Supporting Information). Outgroup taxa were chosen based on the phylogeny of Wilke *et al.* (2013). Taxonomic authorities for all taxa are given in Appendix S1.

DNA isolation and sequencing

DNA was isolated from one or two whole snails of each sample using QIAGEN's (Hilden, Germany) DNeasy Blood and Tissue Kit. The primers used to amplify two mitochondrial (mt) and three nuclear (nr) gene fragments, are listed in Appendix S2. Polymerase chain reactions (PCRs) were performed using standard protocols and annealing temperatures listed in Appendix S2. PCR-products were purified enzymatically using exonuclease I and shrimp alkaline phosphatase and sequenced on an ABI 3130xl Genetic Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA) using the PCR primers and ABI's Big Dye Terminator Ready Reaction Mix 3.1. All sequences are available from GenBank with accession numbers listed in Appendix S1.

Phylogenetic analyses

Sequences were edited using BioEdit 7.0.5.3 (Hall, 1999). Due to the lack of indels the protein coding COI and Histone 3 genes could be aligned by eye. The 16S, 18S and 28S rRNAs were initially aligned using ClustalW 2.1 (Thompson *et al.*, 1994). These alignments were then refined in RNAsalsa 0.8.1 (Stocsits *et al.*, 2009) using secondary structure information of *Cacozeliana lacertina* (Gould, 1861) (http://www.rna.icmb.utexas.edu/SIM/4D/Mollusk/, AF101007) for 16S rRNA, *Littorina obtusata* Menke 1845 (http://www.bioinformatics.psb.ugent.be/webtools/RNA, X94274) for 18S rRNA and the 28S rRNA structure estimated for Pulmonata (provided by Harald Letsch, see Letsch & Kjer, 2011). The alignments were finally manually edited in

BioEdit 7.0.5.3 (Hall, 1999). Aliscore 2.0 (Misof & Misof, 2009) with a window size of four (more sensitive than the default setting of six) was used to identify randomly similar positions in the loop structures of the rRNAs' to be excluded from the subsequent analyses. A test for substitution saturation was performed in Dambe 5.3.54 (Xia & Xie, 2001) treating gaps as unknown states. Partition Finder 1.1.1 (Lanfear *et al.*, 2012) was used to decide whether stem- and loop structures in the RNAs' and codon positions in COI and Histone 3 should be treated as partitions with individual substitution models and to find the best fitting models according to the corrected Akaike's information criterion. Phylogenetic analyses for each gene and the mt, nr and concatenated data sets were performed in maximum likelihood (ML) and Bayesian frameworks. Maximum likelihood analyses were performed using the program Garli 2.0 (Zwickl, 2006). Confidence was assessed by bootstrapping with 500 replicates for the concatenated data set and 300 replicates for single gene analyses. Bayesian analyses were performed using MrBayes 3.2.2 (Ronquist & Huelsenbeck, 2003). Lengths of runs and burnin were determined using the evaluation methods provided by MrBayes and Tracer 1.6 (Rambaut & Drummond, 2007). To more quickly reach convergence in the concatenated data set, we increased the temperature of the heated chains and the number of chains. To check for possible effects of the taxon sampling on the branch lengths and phylogeny of putative members of the genus *Fluviopupa* all analyses were repeated with reduced alignments comprising *Fluviopupa*, NZ taxa and two of the most closely related Australian taxa, *Trochidrobia punicea* and *Caldicochlea globosa*, as outgroups.

Divergence time estimations were performed using beast 1.7.5 (Drummond *et al.*, 2012). Outgroup taxa were excluded except for one, *Beddomeia krybetes*. Emergence times of islands (Table 1) were used as calibration points, if they were continuously above sea level subsequent to emergence, together with the COI-substitution rate of NZ tateids [3.26 ± 0.14% sequence divergence/Myr, (Haase *et al.*, 2007)] resulting in a clock rate of 0.0162 ± 0.0002 substitutions per site per Myr. We assumed the lognormal relaxed clock for all partitions and set the priors assuming a normally distributed COI clock rate (mean = 0.0162, SD = 0.025) with a SD prior of 0.00083 which allowed for a more progressive rate variation than the narrow original confidence interval of Haase *et al.* (2007). We combined the results of nine independent analyses, each comprising 30 Mio generations, logging every 10,000th in order to obtain effective sample sizes of > 200 for all parameters and setting a burn in of 10%. Considering the results of the phylogenetic analyses and the ancestral range reconstructions (see below) we repeated the analysis using only the COI rate and the age of the southern-most, that is, youngest of the AI, Rapa Iti, for calibration (see Discussion for justification). Omitting NC as calibration point allowed us to test its potential Gondwanan refuge status in contrast to the first approach, using all available emergence times as calibrators.

Table 1 Calibration points used in the beast analyses.

Island	Emergence	References
Lord Howe Island	6.9 Ma	McDougall *et al.* (1981)
New Caledonia	37 Ma	Espeland & Murienne (2011)
Rurutu	12 Ma	Duncan & McDougall (1976)
Tubuai	10.4 Ma	Duncan & McDougall (1976)
Raivavae	6.8 Ma	Duncan & McDougall (1976)
Rapa Iti	5.1 Ma	Krummenacher & Noetzlin (1966)

We tested two hypotheses of island colonization for the species attributed to *Fluviopupa* repeating the ML analysis of the reduced concatenated data set in Garli using constraint files. The hypotheses were: (1) The radiations on each island/archipelago are monophyletic; (2) the genus dispersed in a stepping-stone fashion according to the minimum spanning tree based on geographical distances in Fig. 1c (see also Appendix S3). The resulting trees were tested against each other and the unconstrained tree using the approximately unbiased test implemented in Consel 0.20 (Shimodaira & Hasegawa, 2001) which is based on a multiscale bootstrap correcting for the selection bias of other methods (Shimodaira & Hasegawa, 2001).

Ancestral range reconstructions were performed using the Bayesian binary method implemented in Rasp 2.1 (Yu *et al.*, 2015) allowing for the maximum number of areas at each node, not defining the root distribution and using the F81+G model. In addition, the likelihood reconstruction method implemented in Mesquite 2.75 (Maddison & Maddison, 2011) was applied using default settings. These analyses were based on the ML trees of both the complete and reduced alignments. Ranges were defined at the archipelago level: Sulawesi, Australia, Lord Howe Island, NZ, NC, Vanuatu, Fiji and AI.

RESULTS

According to Aliscore, 31 sites of the 18S rRNA alignment and 49 sites of the 28S rRNA alignment, all of them in loops, were excluded. The partitioning schemes and models suggested by Partition Finder are listed in Appendix S4. The phylogenetic analyses of the different data sets resulted in largely identical phylogenies. Therefore, we present only ML and Bayesian analyses of the concatenated data (Fig. 2). One lineage was formed by taxa of the *Beddomeia* group (Ponder *et al.*, 1993; Wilke *et al.*, 2013) and *Oncomelania minima* (Pomatiopsidae). The affiliation of the latter to this clade was only weakly supported. The monophyly of the family Tateidae was well supported only in the analyses of mt genes and ML analysis of the concatenated data set, while in the Bayesian analysis of the concatenated data set and analyses of only nr genes it was not supported at all.

The Tateidae were subdivided in three large clades, as follows.

1. The Australian genera *Austropyrgus*, *Posticobia* and *Tatea* (Aus I) as well as *Hemistomia gemma gemma* from LHI. The sister relationship of the Indonesian genus *Sulawesidrobia* to

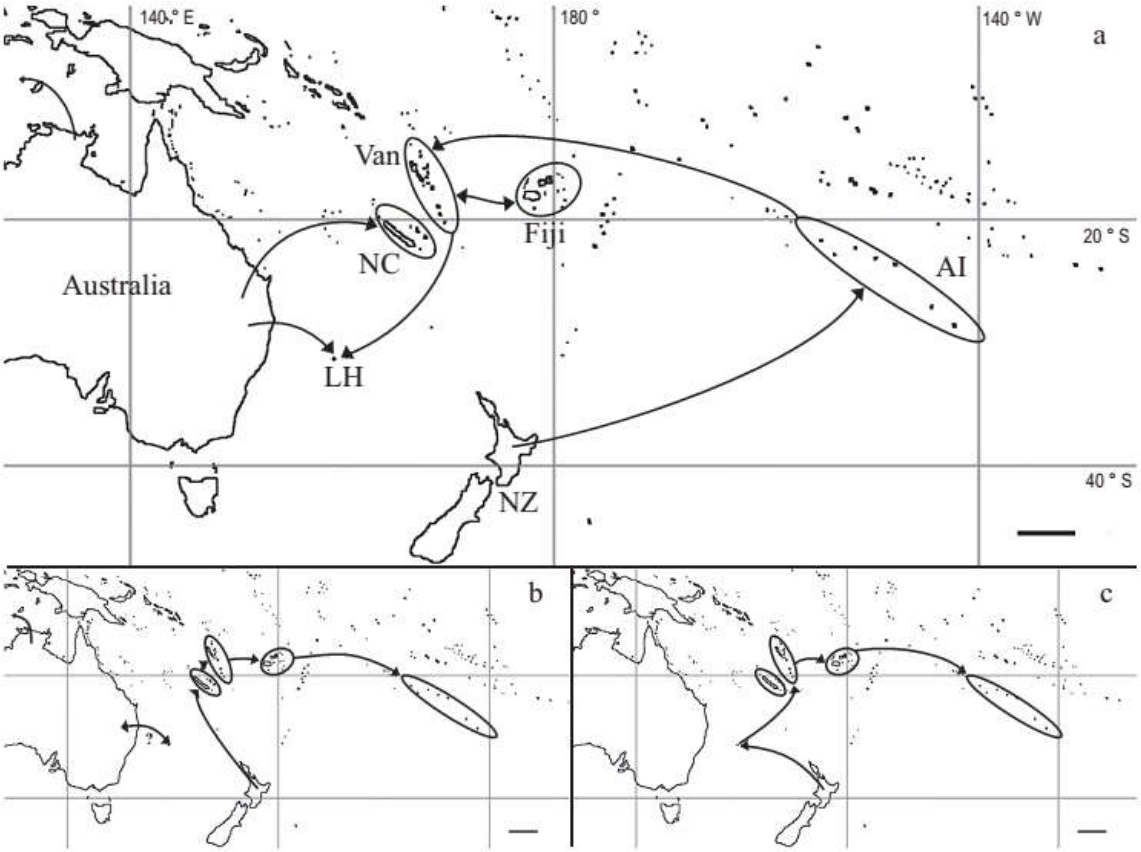

Figure 1 Map of the South Pacific (Mercator Projection) and hypotheses of diversification and dispersal of truncetelloidean gastropods. Arrows reflect primarily ancestor-descendent relationships and not necessarily dispersal. (a) Most-likely scenario based on results of this study; (b) anatomy-based hypothesis based on Haase *et al.* (2010a); (c) hypothesis based on a minimum spanning tree using geographical data; scale bars = 500 km.

this clade was not supported and the positions of *Tatea* and *Posticobia* were well supported only in the mitochondrial analysis.

2. The well-supported second clade was subdivided into two well-supported lineages, one comprising all species from NC and the other with the Australian genera *Fonscochlea*, *Caldicochlea* and *Trochidrobia* (Aus II).

3. A well-supported clade comprised three smaller clades of NZ taxa (NZ I – III) and a consistently well-supported clade encompassing all *Fluviopupa* species. This NZ and *Fluviopupa* clade was the allopatric sister group to clade 2. Together, the NZ taxa were paraphyletic with respect to *Fluviopupa*. Their interrelationships remain unresolved but their composition was basically in concordance with the mitochondrial phylogenies of Haase (2005, 2008).

The genus *Hemistomia* with representatives on NC and LHI was polyphyletic in all analyses. The relationships within the genus *Fluviopupa* were almost identically resolved in all analyses. AI species were in a well-supported clade with two subclades, one comprising the two species from Raivavae and the second comprising those from Tubuai and Rurutu and two specimens from Santo (Vanuatu). The monophyly of the AI' sister clade was only weakly supported but the analyses resulted in compatible phylogenies. It included as sister-taxa the LHI species and a clade of Fiji and Vanuatu species. The latter was divided into three well-supported subclades of Fijian species, two species from the island of Erromango (Vanuatu) and one subclade comprising the remaining species from Vanuatu.

Figure 2 Maximum likelihood tree of the concatenated data set; values are bootstrap support values/posterior probabilities, **-/-** < 60/0.6, * 100/1; diagonal lines indicate that the branch has been shortened by75%; scale bar = substitutions per site; circles behind species names indicate origin and at nodes results of ancestral range reconstructions (colour code: black/grey-Australia; purple-Indonesia; cyan-New Zealand; green-New Caledonia; red-Lord Howe Island; yellow-Vanuatu; blue-Fiji; orange-Austral Islands); for a detailed analysis of the *Fluviopupa* clade shaded in dark grey see Fig. 3.

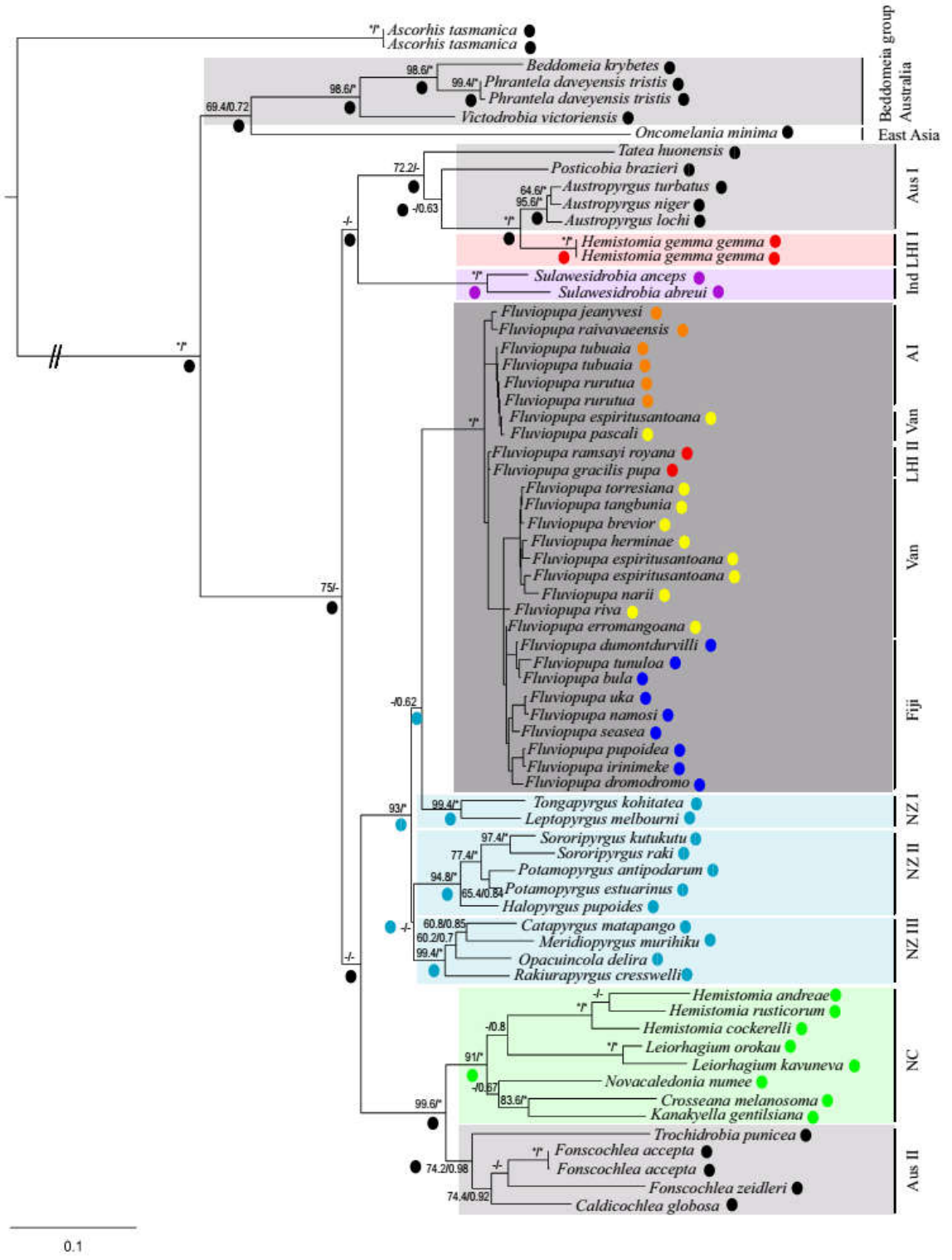

Table 2 Comparison of ancestral range reconstructions (Bayesian binary and likelihood methods) for the genus *Fluviopupa* and node ages; node numbers see Fig. 3; left/right entire data set/reduced data set; abbreviations: AI = Austral Islands, LHI = Lord Howe Island, NC = New Caledonia, NZ = New Zealand, Van = Vanuatu; only states with likelihood/probability > 5 are shown, *value larger than 95; in brackets 95% highest posterior density interval, ages of nodes with Bayesian posterior densities < 55 are not defined.

Node	RASP: Bayesian probability		Mesquite: proportional likelihood		Node ages in Myr	Node ages in Myr Rapa Iti calibrated
1	AI	59.02/45.68	AI	45.58/47.00	8.61 (6.86–10.5)	4.95 (4.71–5)
	NZ	31.37/43.17	NZ	7.93/6.62		
	Van	5.4/7.63	Van	27.31/26.15		
			LHI	18.25/18.14		
2	AI	*/*	AI	*/*	3.99 (2.78–5.21)	2.71 (2.06–3.38)
3	AI	*/*	AI	*/*	1.57 (0.86–2.26)	1.12 (0.66–1.62)
4	AI	*/*	AI	*/*	0.62 (0.32–0.89)	0.46 (0.25–0.67)
5	AI	*/*	AI	*/*	0.51 (0.27–0.74)	0.38 (0.24–0.55)
6	AI	*/*	AI	*/*	0.36 (0.16–0.53)	0.27 (0.13–0.41)
7	AI	*/*	AI	*/*	0.25 (0.1–0.38)	0.19 (0.08–0.29)
8	Van	*/*	Van	*/*	0.18 (0.06–0.27)	0.13 (0.05–0.21)
9	Van	61.10/57.40	Van	36.42/35.22	Not defined	Not defined
	LHI	33.86/36.38	AI	32.59/33.29		
			LHI	24.32/24.34		
			NZ	5.70/4.83		
10	LHI	*/*	LHI	*/*	0.69 (0.29–1.11)	0.48 (0.21–0.79)
11	Van	*/*	Van	*/*	5.14 (4.11–6.33)	3.56 (3.01–4.08)
12	Van	*/*	Van	*/*	1.99 (1.16–3.34)	1.4 (0.89–2.18)
13	Van	*/*	Fiji	66.07/70.06	4.74 (3.77–5.84)	Not defined
			Van	33.93/39.93		
14	Fiji	*/*	Fiji	*/*	1.82 (1.11–2.61)	1.42 (0.88–2.06)
15	Fiji	*/*	Fiji	*/*	4.28 (3.35–5.17)	2.77 (2.19–3.35)
16	Fiji	*/*	Fiji	*/*	2.75 (1.79–3.7)	2.02 (1.41–2.7)
17	Fiji	*/*	Fiji	*/*	2.93 (2.06–3.91)	2.17 (1.57–2.83)

The comparison of different hypotheses of island colonization (Fig. 1) showed that none of the alternative hypotheses was at least as likely as the unconstrained tree (AU-test, $P < 0.01$).

Bayesian and ML approaches identified identical ancestral ranges (Fig. 2, Table 2). Based on these trees, the common ancestor of the SP Tateidae occurred in Australia. Its descendents colonized Sulawesi and LHI and gave rise to the radiations of NC and NZ. New Zealand harboured the ancestor of *Fluviopupa*. The origin of the most recent common ancestor of *Fluviopupa* was reconstructed ambiguously, with the most-likely ancestral area being the AI with all methods used. Bayesian and likelihood analyses only weighed the support for the alternative areas, viz. NZ and Vanuatu, differently. Thus, in the most-likely scenario, *Fluviopupa* colonized Vanuatu independently twice from the AI. And from Vanuatu, it dispersed further to LHI. How often the islands of Fiji were reached from Vanuatu and if there was a subsequent back-colonization event could not be unambiguously determined. The Bayesian binary method indicated two independent radiations on Fiji from Vanuatu; and using the likelihood method of Mesquite, one radiation on Fiji with a subsequent return to Vanuatu were inferred. Testing alternative phylogenies considering the inconsistencies in the relationships between the larger clades or the NZ clades did not influence these results significantly (not shown).

The dated phylogeny calculated in BEAST supported the phylogenetic tree found using the other programs. The age estimates are given in Fig. 4 and Table 2. In the second analysis, using only the age of Rapa Iti for the entire genus *Fluviopupa* and the COI-substitution rate as calibrators, divergence times were in general 20–30%, and for the genus *Fluviopupa* up to 43% younger. In the following these dates are given in brackets. The most recent common ancestor of the radiation on NC was dated to 35.5 [95% highest posterior density (HPD): 32.6–37] [28.5 (22.7–34.9)] Ma and that of the Sulawesian species to 15.1 (10.5–20) [10.8 (7.4–14.7)] Ma. The genus *Fluviopupa* originated 8.6 (5.0) Ma with the nodes 1, 2, 11 and 15 (Fig. 4) showing the largest differences between the two analyses. The LHI clade was estimated as 0.7 (0.5) Myr, that of the Fiji/Vanuatu clade as 5.1 (3.6) Myr, and the AI clade estimated to be 4.0 (2.7) Myr old. Figure 4 and Table 2 give the 95% highest posterior density HPD intervals for all estimates.

DISCUSSION

Radiation across the Pacific islands

Our analyses of the evolutionary history of Tateidae across the SP revealed several enigmatic aspects not in accordance with the pattern published for many other taxa (Keppel *et al.*, 2009). In particular, the complex and largely westward

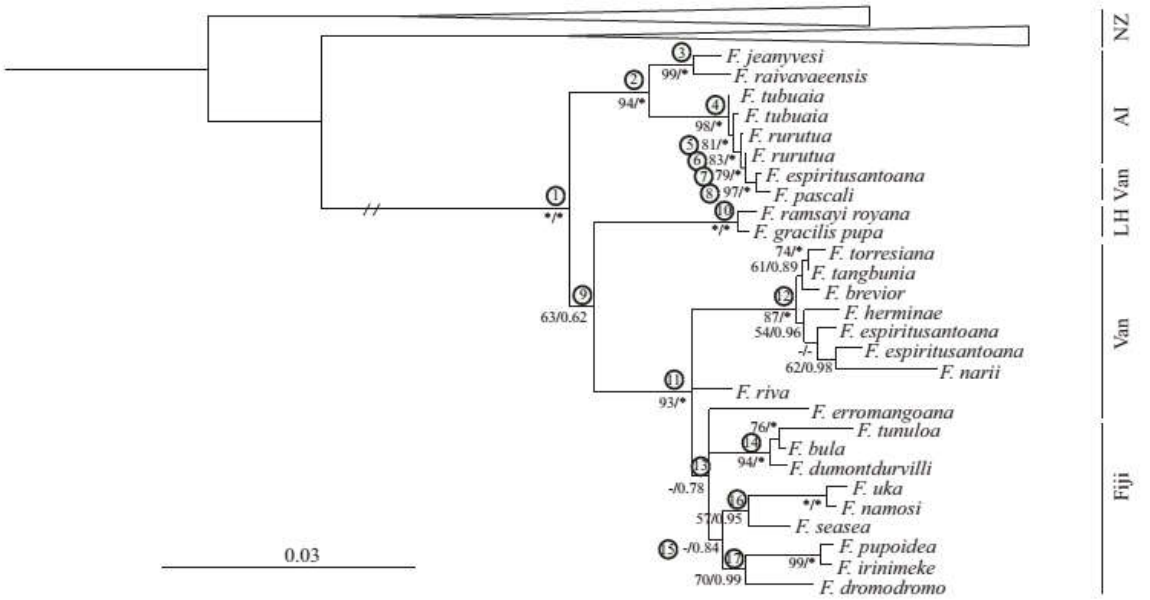

Figure 3 Maximum likelihood tree of the concatenated reduced data set; values are bootstrap support values/posterior probabilities, **-/-** < 50/0.5, * 100/1; diagonal lines indicate that the branch has been shortened by 50%; scale bar = substitutions per site; for the ancestral range reconstruction of numbered nodes see Table 2.

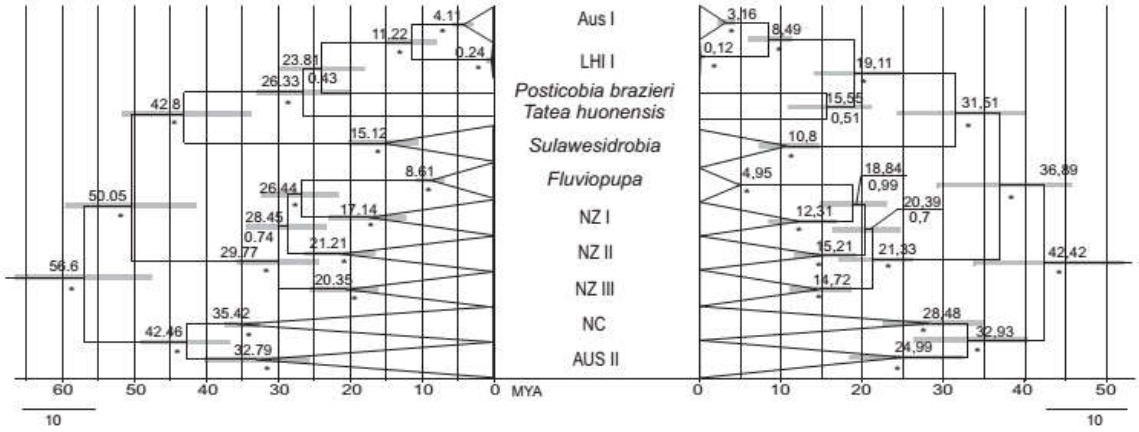

Figure 4 Dated phylogeny; left side calibrated using all available emergence times for islands; right side calibrated using only the age of Rapa Iti; bars indicate 95% highest posterior density intervals; * indicates posterior probabilities > 95; for detailed results of the *Fluviopupa* clade see Table 2; scale bar = substitutions per site.

dispersal pathways of *Fluviopupa* are unique. Geographical distance apparently is not an appropriate predictor of relatedness.

The ancestral range reconstructions show the radiation of *Fluviopupa* in the AI originated from an ancestor in NZ, which subsequently colonized Vanuatu, Fiji and LHI. This radiation is rather exceptional with only a few other studies, mainly plant genera, with a similar pattern (Berry *et al.*, 2004; Birch & Keeley, 2013). Such patterns are presumably rare because dispersal from NZ involves long distances and survival under different climatic conditions (Keppel *et al.*, 2009).

The likelihood and probability values for the AI as the range of the common ancestor of *Fluviopupa* were rather low, but still higher than for Vanuatu or NZ. This ambiguity was probably a consequence of the limited phylogenetic resolution due to the young age of this genus. The origin of *Fluviopupa* on the AI is supported by their emergence predating that of Vanuatu (up to 12 Myr) and that a stepping-stone-like dispersal across the different islands is more likely than assuming a now extinct ancestral population of *Fluviopupa* in NZ and several dispersal events from there. Our results suggest that the AI were settled from south-east to north-

west, that is, from the youngest to the oldest parts of the archipelago (Maury *et al.*, 2014) against the expected progression rule of stepping-stone dispersal from the oldest to the youngest island well known for insects, for example, crickets and drosophilids from Hawaii (see Cowie & Holland, 2008, also for exceptions to this rule). Unfortunately, the two Rapa Iti species were not available for this study to complete the picture.

The next dispersal step was the archipelago of Vanuatu, which was apparently reached twice independently. Hence, the colonization of Vanuatu by *Fluviopupa* was more complex than assumed by Zielske & Haase (2014a), who considered oceanic dispersal events sufficiently rare to assume monophyly of the Vanuatu species. In addition, a third lineage may have originated on Fiji (see below). The polyphyly of *F. espiritusantoana* and *F. pascali* from Vanuatu is confusing (the latter here represented only once, but see Zielske & Haase, 2014a) and these taxa appear, surprisingly, in the AI clade (see Zielske & Haase, 2014a). The recently described effect of ancient lineage fusion (Garric *et al.*, 2014) might be a plausible explanation for this finding.

The radiation on Fiji was divided into three clades but its monophyly was not supported (contra Zielske & Haase, 2014b) suggesting that Fiji was colonized possibly more than once from Vanuatu. However, according to the likelihood approach, this latter route may have been taken also in the opposite direction (see also below). This westerly orientated dispersal picture supports the increasing evidence (e.g. Bellemain & Ricklefs, 2008; Lapoint *et al.*, 2013) against the hypothesis that the colonization of remote islands is a dead end journey (Mayr & Diamond, 2001). A recently described species from mainland Queensland attributed to *Fluviopupa* (Ponder & Shea, 2014) may lend further support to these observations.

In contrast to the island radiations of *Fluviopupa*, the radiation of the *Hemistomia*-clade (Haase & Bouchet, 1998) on NC with a single origin in Australia was well supported by all analyses. This colonization pattern represents a common dispersal route for NC's biota (e.g. Espeland & Johanson, 2010; Swenson *et al.*, 2014). The high support for the monophyly of the NC taxa was in contrast to our recent analysis based on only three gene fragments (Zielske & Haase, 2015). Although NC is reported to be one of the main sources for dispersal across the SP (van Balgooy, 1971), we found no indication of tateid gastropods having used this route.

Lord Howe Island has been reached by tateid gastropods not only from Vanuatu, but a second time by species formally attributed to *Hemistomia* (Ponder, 1982) from Australia. This fits the general description of this island's biota representing a mosaic of geographical affinities (Hickman, 2009). As *Hemistomia* is endemic to NC, the taxonomy of the species attributed to that genus from LHI must be revised.

Age estimates

Both calibration strategies are based on the ages of different sets of continuously emergent islands as well as the COI rate and have been guided by the phylogenetic reconstructions showing the monophyly of the genera from NC and the paraphyly and south-east–north-west colonization history of the *Fluviopupa* species on the AI. Calibrations based on island ages have been criticized for not taking into account that the flora and fauna of currently emergent islands may have evolved earlier on nearby, now submerged islands (Heads, 2011). Tectonically, the SP has been an extremely active area and the geological history of NC and its surroundings is complex (Pelletier, 2007). However, assuming that the most recent common ancestor of the NC tateids pre-dates the re-emergence of NC also requires the assumption that the descendent lineages have colonized NC independently. The AI are at the southern edge of the SP superswell which generated several volcanic chains (Adam & Bonneville, 2005). However, considering (1) the direction of colonization from the younger to the older islands, (2) the absence of older, submerged islands east of the AI, and (3) the absence of tateids from all nearby archipelagos and islands lying between NZ and the AI justifies the assumption of evolution *in situ*.

The calibration strategies resulted in significantly different age estimates. Using the emergence times of five islands as maximum age estimates of the respective species/lineages occurring there consistently produced older dates than the alternative approach constraining the origin of *Fluviopupa* to the age of Rapa Iti, the youngest of the AI harbouring two species, which could not be included in our analyses. The latter approach took into account (1) the ancestral state reconstruction suggesting that *Fluviopupa* originated on the AI (2) the south-east–north-west colonization pattern of the AI assuming that Rapa Iti was colonized accordingly, that is, prior to Raivavae. Using the age of Raivavae, whose species were included, would have given very similar estimates since Raivavae is not much older than Rapa Iti (Table 1). Although using several calibration points is theoretically preferable (Hillis *et al.*, 1996), our second approach resulted in more plausible age estimates in accordance with geological evidence. In addition, the 'older scenario' would require many more *ad hoc* hypotheses in order to explain the inferred pattern. For example, *Fluviopupa* would have evolved on one of the older, western AI, spread to the east, than gone extinct in the west to give room for the colonization of the ancestors of the species we see today from the east. Also Vanuatu with its complex history of repeated sea level changes would have been colonized more than a million years earlier than assumed by Zielske & Haase (2014a), which is hard to match with the geological evidence (Taylor, 1992; Robin *et al.*, 1993). Similarly, the onset of the radiation in NZ would predate the re-emergence of all or at least much of NZ by several millions of years (Trewick *et al.*, 2007; but see Mildenhall *et al.*, 2014; Carr *et al.*, 2015). Therefore, we base the following discussion on the age estimates of the Rapa Iti/COI rate calibration if not otherwise stated.

The onset of the radiation on NC was dated at 28.5 (22.7–34.9) Ma and is similar to our previous inference based

solely on NC species, fewer outgroup taxa, and fewer genes (Zielske & Haase, 2015). Since also the Australian ancestor in common with its Australian sister clade post-dates the phase of submergence of NC 65–45 Ma (Pelletier, 2007), the NC tateids are inconclusive regarding the question whether NC was in fact a Gondwanan refuge or a Darwinian island (Espeland & Murienne, 2011). Considering currently submerged islands as possible refuges (e.g. Meffre *et al.*, 2007; Pelletier, 2007) does not resolve this ambiguity (see above).

Our analyses indicated an origin of NZ tateids at 21.3 (17.2–26.2) Ma which is in accordance with the geological evidence of a main phase of uplift in the early Miocene. This resulted in a radiation via dispersal–vicariance (e.g. Trewick *et al.*, 2007; Giribet & Boyer, 2010). However, the NZ tateid freshwater species evolved autochthonously from brackish water species rather than having dispersed from Australia (Haase, 2005).

The islands of Vanuatu were initially colonized *c.* 3.56 (3.0–4.1) Ma a figure generally in accordance with our previous results, although this dating now applies to a higher node, then estimated almost 1.5 Myr younger (Zielske & Haase, 2014a; node II in Fig. 3). The main radiation across Vanuatu started only 1.4 (0.9–2.2) Ma. Although colonized from Vanuatu, the clades of Fiji are somewhat older than this main radiation, viz. 1.4 (0.9–2.1) to 2.8 (2.2–3.4) Myr. This is presumably due to the complex patterns of phases of repeated emergence and (partial) submergence of both archipelagos' islands (Taylor, 1992; Robin, 1993; Rodda, 1994) resulting in bottlenecks and subsequent explosive radiations. Given the complex geological history of these archipelagos, the phylogenetic pattern would also be consistent with an initial metapopulation of a wide-spread ancestral species (Heads, 2011).

Both radiations on LHI were estimated as being younger than 1 Myr. Due to the small number of species available from this island the reliability of this dating is questionable, but considering the islands' age of 6.9 Myr (McDougall *et al.*, 1981) probably unproblematic for the reconstruction of the gross picture.

The two species included from Sulawesi resulting in an age of 10.8 (7.4–14.7) Myr for their most recent common ancestor, significantly older than the lakes from which those species were described (Hall, 2009; Zielske *et al.*, 2011), suggesting a more complex evolutionary pattern than assumed for other ancient lake species in that region (Stelbrink *et al.*, 2014; Von Rintelen *et al.*, 2014).

The likely vectors for dispersal of small freshwater snails across thousands of kilometres of open ocean are usually assumed to be birds, as suggested for gastropods in general (Rees, 1965; Van Leeuwen *et al.*, 2012) and truncatelloideans, in particular (e.g. Liu *et al.*, 2003; Haase *et al.*, 2010b). Several species of extant and recently extinct water birds have wide distributions across the SP (Steadman, 2006). This assumption also holds against the deep time frame set in our analyses as all major bird lineages were established already in the Palaeogene (Mayr, 2009). Whether seamounts and ridges (e.g. Mortimer *et al.*, 2010; Maury *et al.*, 2014) represent submerged islands that could have served as stepping stones facilitating the spread, remains speculative. The exceptional and predominant westward direction of dispersal of *Fluviopupa* coincides with the direction of the trade winds. Cyclones moving in the same direction may have also facilitated dispersal across these impressive distances across open water (Vagvolgyi, 1975; Gillespie *et al.*, 2012). However, our reconstruction of the evolutionary history of tateid freshwater gastropods still does not fully explain the peculiar pattern of occurrence, in particular the apparent absence of the family from island groups such as Tonga, Samoa or the Cook Islands interspersed between archipelagos where these snails are well established.

ACKNOWLEDGEMENTS

We are indebted to numerous local guides who supported our fieldwork. At the University of Greifswald, Christel Meibauer helped with DNA work. The manuscript was improved by the comments of anonymous referees. Financial support was provided by the Deutsche Forschungsgemeinschaft (grant HA4752/2-1).

REFERENCES

Adam, C. & Bonneville, A. (2005) Extent of the South Pacific superswell. *Journal of Geophysical Research*, **110**, B09408.

Bellemain, E. & Ricklefs, R.E. (2008) Are islands the end of the colonization road. *Trends in Ecology and Evolution*, **23**, 461–468.

Bernasconi, R. (1995) Two new cave prosobranch snails from Papua New Guinea: *Selmistomia beroni* n. gen. n. sp. (Caenogastropoda: Hydrobiidae) and *Georissa papuana* n. sp. (Archaeogastropoda: Hydrocenidae). (Zoological results of the British Speleological Expedition to Papua New Guinea 1975.). *Revue suisse de zoologie*, **102**, 373–386.

Berry, P.E., Hahn, W.J., Systma, K.J., Hall, J.C. & Mast, A. (2004) Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. *American Journal of Botany*, **91**, 601–614.

Birch, J. & Keeley, S.C. (2013) Dispersal pathways across the Pacific: the historical biogeography of *Astelia* s.l. (Asteliaceae, Asparagales). *Journal of Biogeography*, **40**, 1914–1927.

Bonneville, A. (2009) French Polynesia, Geology. *Encyclopedia of islands* (ed. by R.G. Gillespie and D.A. Clague), pp. 338–343. University of California press, Berkley, CA.

Carr, L.M., McLenachan, P.A., Waddell, P.J., Gemmell, N.J. & Penny, D. (2015) Analyses of the mitochondrial genome of *Leiopelma hochstetteri* argues against the full drowning of New Zealand. *Journal of Biogeography*, **42**, 1066–1076.

Cowie, R.H. & Holland, B.S. (2008) Molecular biogeography and diversification of the endemic terrestrial fauna of the Hawaiian Islands. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 3363–3376.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Duncan, R.A. & McDougall, I. (1976) Linear volcanism in French Polynesia. *Journal of Volcanology and Geothermal Research*, **1**, 197–227.

Espeland, M. & Johanson, K.A. (2010) The diversity and radiation of the largest monophyletic animal group on New Caledonia (Trichoptera: Ecnomidae: Agmina). *Journal of Evolutionary Biology*, **23**, 2112–2122.

Espeland, M. & Murienne, J. (2011) Diversity dynamics in New Caledonia: towards the end of the museum model? *BMC Evolutionary Biology*, **11**, 254.

Garric, R.C., Benavides, E., Russello, M.A., Hyseni, C., Edwards, D.L., Gibbs, J.P., Tapia, W., Ciofi, C. & Caccone, A. (2014) Lineage fusion in Galapagos giant tortoises. *Molecular Ecology*, **23**, 5276–5290.

Gillespie, R.G., Baldwin, B.G., Waters, J.M., Fraser, C.I., Nikula, R. & Roderick, G.K. (2012) Long-distance dispersal: a framework for hypothesis testing. *Trends in Ecology and Evolution*, **27**, 47–56.

Giribet, G. & Boyer, S.L. (2010) 'Moa's Ark' or 'Goodbye Gondwana': is the origin of New Zealand's terrestrial invertebrate fauna ancient, recent, or both? *Invertebrate Systematics*, **24**, 1–8.

Greene, H.G., Collot, J.-Y., Fisher, M.A. & Crawford, A.J. (1994) Neogene tectonic evolution of the New Hebrides Island arc: a review incorporating ODP drilling results. *Proceedings of the Ocean Drilling Program, Scientific Results* 134 (ed. by H.G. Greene, J.-Y. Collot and L.B. Stokking *et al.*), pp. 19–46. College Station, TX (Ocean Drilling Program).

Haase, M. (2005) Rapid and convergent evolution of parental care in hydrobiid gastropods from New Zealand. *Journal of Evolutionary Biology*, **18**, 1076–1086.

Haase, M. (2008) The radiation of hydrobiid gastropods in New Zealand: a revision including the description of new species based on morphology and mtDNA sequence information. *Systematics and Biodiversity*, **6**, 99–159.

Haase, M. & Bouchet, P. (1998) Radiation of crenobiontic gastropods on an ancient continental island: the *Hemistomia*-clade in New Caledonia (Gastropoda: Hydrobiidae). *Hydrobiologia*, **367**, 43–129.

Haase, M. & Bouchet, P. (2006) The radiation of hydrobioid gastropods (Caenogastropoda, Rissooidea) in ancient Lake Poso, Sulawesi. *Hydrobiologia*, **556**, 17–46.

Haase, M., Gargominy, O. & Fontaine, B. (2005) Rissooidean freshwater gastropods from the middle of the Pacific: the genus *Fluviopupa* on the Austral Islands (Caenogastropoda). *Molluscan Research*, **25**, 145–163.

Haase, M., Ponder, W.F. & Bouchet, P. (2006) The genus *Fluviopupa* Pilsbry, 1911 from Fiji (Caenogastropoda, Rissooidea). *Journal of Molluscan Studies*, **72**, 119–136.

Haase, M., Marshall, B. & Hogg, I. (2007) Disentangling causes of disjunction on the South Island of New Zealand: the Alpine fault hypothesis of vicariance revisited. *Biological Journal of the Linnean Society*, **91**, 361–374.

Haase, M., Fontaine, B. & Gargominy, O. (2010a) Rissooidean freshwater gastropods from the Vanuatu archipelago. *Hydrobiologia*, **637**, 53–71.

Haase, M., Naser, M.D. & Wilke, T. (2010b) *Ecrobia grimmi* in brackish Lake Sawa, Iraq: indirect evidence for long-distance dispersal of hydrobiid gastropods (Caenogastropoda: Rissooidea) by birds. *Journal of Molluscan Studies*, **76**, 101–105.

Hall, R. (2009) Southeast Asia's changing palaeogeography. *Blumea*, **54**, 148–161.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Heads, M. (2011) Old taxa on young islands: a critique of the use of island age to date island-endemic clades and calibrate phylogenies. *Systematic Biology*, **60**, 204–218.

Hickman, C.S. (2009) Lord Howe Island. *Encyclopedia of islands* (ed. by R.G. Gillespie and D.A. Clague), pp. 568–572. University of California press, Berkeley, CA.

Hillis, D., Mable, B.K. & Moritz, C. (1996) Applications of molecular systematics. *Molecular systematics*, 2nd edn (ed. by D.M. Hillis, C. Moritz and B.K. Mable), pp. 515–544. Sinauer Associates, Sunderland, MA.

Keppel, G., Lowe, A.J. & Possingham, H.P. (2009) Changing perspectives on the biogeography of the tropical South Pacific: influences of dispersal, vicariance and extinction. *Journal of Biogeography*, **36**, 1035–1054.

Krummenacher, D. & Noetzlin, J. (1966) Ages isotopiques K/AR de roches prélevées dans les possessions francaise du Pacifique. *Bulletin de la Societe Geologique de France*, **8**, 173–175.

Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**, 1695–1701.

Lapoint, R.T., O'Grady, P.M. & Whiteman, N.K. (2013) Diversification and dispersal of the Hawaiian Drosophilidae: the evolution of Scaptomyza. *Molecular Phylogenetics and Evolution*, **69**, 95–108.

Letsch, H.O. & Kjer, K.M. (2011) Potential pitfalls of modelling ribosomal RNA data in phylogenetic tree reconstruction: evidence from case studies in the Metazoa. *BMC Evolutionary Biology*, **11**, 146.

Liu, H.-P., Hershler, R. & Clift, K. (2003) Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Molecular Ecology*, **12**, 2771–2782.

Maddison, W.P. & Maddison, D.R. (2011) *Mesquite: a modular system for evolutionary analysis*. Version 2.75. Available at: http://mesquiteproject.org.

Maury, R., Legendre, C., Chauvel, C., Guille, G., Blais, S., Giullou, H. & Rossi, P. (2014) Geology an atypical hotspot chain. *Biodiversity of the Austral Islands, French Polynesia* (ed. by J.-Y. Meyer and E.M. Claridge), pp. 21–38. Publications scientifiques du MNHN, Paris.

Mayr, E. & Diamond, J. (2001) *The Birds of Northern Melanesia Speciation, ecology and biogeography*. Oxford University Press, New York.

Mayr, G. (2009) *Paleogene fossil birds*. Springer-Verlag, Berlin.

McDougall, I., Embleton, B.J.J. & Stone, D.B. (1981) Origin and evolution of Lord Howe Island, Southwest Pacific Ocean. *Australian Journal of Earth Sciences*, **28**, 155–176.

Meffre, S., Crawford, A.J. & Quilty, P.G. (2007) Arc-continent collision forming a large island between New Caledonia and New Zealand in the Oligocene. Extended Abstracts, Australian Earth Sciences Convention 2006, Melbourne, 3pp.

Mildenhall, D.C., Mortimer, N., Bassett, K.N. & Kennedy, E.M. (2014) Oligocene paleogeography of New Zealand: maximum marine transgression. *New Zealand Journal of Geology and Geophysics*, **57**, 107–109.

Misof, B. & Misof, K. (2009) A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: as more objective means of data exclusion. *Systematic Biology*, **58**, 21–34.

Mortimer, N., Gans, P.B., Palin, J.M., Meffre, S., Herzer, R.H. & Skinner, D.N.B. (2010) Location and migration of Miocene-Quaternary volcanic arcs in the SW Pacific region. *Journal of Volcanology and Geothermal Research*, **190**, 1–10.

Muellner, A.N., Pannell, C.M., Coleman, A. & Chase, M.W. (2008) The origin and evolution of Indomalesian, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography*, **35**, 1769–1789.

Murienne, J. (2009) New Caledonia, Biology. *Encyclopedia of Islands*, (eds. R. Gillespie & D. Clague), pp. 643–645. University of California Press, Berkley, CA.

Murienne, J., Edgecombe, G.D. & Giribet, G. (2011) Comparative phylogeography of the centipedes *Cryptops pictus* and *C. niuensis* (Chilopoda) in New Caledonia, Fiji and Vanuatu. *Organsims, Diversity & Evolution*, **11**, 61–74.

Pelletier, B. (2007) Geology of the New Caledonia region and its implication for the study of the New Caledonian biodiversity. *Compendium of marine species of New Caledonia* (ed. by C.E. Payri and B. Richer de Forgess), pp. 19–32. Documents Scientifiques et Techniques. 117, seconde édition, IRD, Nouméa.

Ponder, W.F. (1981) *Posticobia norfolkensis* (Sykes), an apparently-extinct, fresh-water snail from Norfolk Island (Gastropoda: Hydrobiidae). *Proceedings of the Linnean Society of New South Wales*, **105**, 17–21.

Ponder, W.F. (1982) Hydrobiidae of Lord Howe Island (Mollusca: Gastropoda: Prosobranchia). *Australian Journal of Marine and Freshwater Research*, **33**, 89–159.

Ponder, W.F. & Shea, M.E. (2014) A new species of the *Fluviopupa* group (Caenogastropoda: Tateidae) from north-east Queensland, Australia. *Molluscan Research*, **34**, 71–78.

Ponder, W.F., Colgan, D.J. & Clark, G.A. (1991) The morphology, taxonomy and genetic structure of Tatea (Mollusca: Gastropoda: Hydrobiidae), estuarine snails from temperate Australia. *Australian Journal of Zoology*, **39**, 447–497.

Ponder, W.F., Clark, G.A., Miller, A.C. & Toluzzi, A. (1993) On a major radiation of freshwater snails in Tasmania and eastern Victoria: a preliminary overview of the Beddomeia group (Mollusca: Gastropoda: Hydrobiidae). *Invertebrate Taxonomy*, **7**, 501–750.

Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.5*. Available at: http://beast.bio.ed.ac.uk/Tracer.

Rees, W.J. (1965) The aerial dispersal of Mollusca. *Proceedings of the Malacological Society of London*, **36**, 269–282.

Robin, C., Mozier, M., Crawford, A.J. & Eggins, S.M. (1993) *The geology, volcanology, petrology-geochemistry, and tectonic evolution of the New Hebrides Island Arc, Vanuatu: IAVCEI, Canberra 1993: Excursion Guide*. Record 1993/059. Australian Geological Survey Organisation, Canberra.

Rodda, P. (1994) Geology of Fiji. *Geology and submarine resources of the Tonga-Lau-Fiji region*. SOPAC Technical Bulletin, (eds. A.J. Stevenson, R.H. Herzer & P.F. Ballance) pp. 131–151. SOPAC Secretariat, Suva, Fiji.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Shimodaira, H. & Hasegawa, M. (2001) CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics*, **17**, 1246–1247.

Steadman, D.E. (2006) *Extinction and biogeography of tropical pacific birds*. University of Chicago Press, Chicago, IL.

Stelbrink, B., Stöger, I., Hadiaty, R.K., Schliewen, U.K. & Herder, F. (2014) Age estimates for an adaptive lake fish radiation, its mitochondrial introgression, and an unexpected sister group: Sailfin silversides of the Malili Lakes system in Sulawesi. *BMC Evolutionary Biology*, **14**, 1471–2148.

Stocsits, R.R., Letsch, H., Hertel, J., Misof, B. & Stadler, P.F. (2009) Accurate and efficient reconstruction of deep phylogenies from structured RNAs. *Nucleic Acids Research*, **37**, 6184–6193.

Swenson, U., Nylinder, S. & Munzinger, J. (2014) Sapotaceae biogeography supports New Caledonia being an old Darwinian island. *Journal of Biogeography*, **41**, 797–809.

Taylor, F.W. (1992) Quaternary vertical tectonics of the central New Hebrides Island arc. *Proceedings of the Ocean Drilling Program Initial Reports*, 134 (ed. by J.-Y. Collot, H.G. Greene and L.B. Stokking *et al.*), College Station, TX (Ocean Drilling Program), 33–42.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

Trewick, S.A., Paterson, A.M. & Campbell, H.J. (2007) Hello New Zealand. *Journal of Biogeography*, **34**, 1–6.

Vagvolgyi, J. (1975) Body size, aerial dispersal, and origin of the Pacific land snail fauna. *Systematic Zoology*, **24**, 465–488.

van Balgooy, M.M.J. (1971) Plant-geography of the Pacific as based on a census of phanerogam genera. *Blumea*, **6** (Suppl.), 1–122.

Van Leeuwen, C.H.A., van der Velde, G., van Groenendael, J.M. & Klaassen, M. (2012) Gut travellers: internal dispersal of aquatic organisms by waterfowl. *Journal of Biogeography*, **39**, 2031–2040.

Von Rintelen, T., Stelbrink, B., Marwoto, R.M. & Glaubrecht, M. (2014) A snail perspective on the biogeography of Sulawesi, Indonesia: origin and intra-island dispersal of the viviparous freshwater gastropod *Tylomelania*. *PLoS ONE*, **9**, e09017.

Waters, J.M. & Craw, D. (2006) Goodbye Gondwana? New Zealand biogeography, geology, and the problem of circularity. *Systematic Biology*, **55**, 351–356.

Wilke, T., Haase, M., Hershler, R., Liu, H.-P., Misof, B. & Ponder, W. (2013) Pushing short DNA fragments to the limit: pshylogenetic relationships of 'hydrobioid' gastropods (Caenogastropoda: Rissooidea). *Molecular Phylogenetics and Evolution*, **66**, 715–736.

Xia, X. & Xie, Z. (2001) DAMBE: software package for data analysis in molecular biology and evolution. *Journal of Heredity*, **92**, 371–373.

Yu, Y., Harris, A.J., Blair, C. & He, X.-J. (2015) RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution*, **87**, 46–49.

Zielske, S. & Haase, M. (2014a) When snails inform about geology: Pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda: Tateidae). *Journal of Zoological Systematics and Evolutionary Research*, **52**, 217–236.

Zielske, S. & Haase, M. (2014b) New insights into tateid gastropods and their radiation on Fiji based on anatomical and molecular methods (Caenogastropoda: Truncatelloidea). *Zoological Journal of the Linnean Society*, **172**, 71–102.

Zielske, S. & Haase, M. (2015) Molecular phylogeny and a modified approach of character-based barcoding refining the taxonomy of New Caledonian freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae). *Molecular Phylogenetics and Evolution*, **89**, 171–181.

Zielske, S., Glaubrecht, M. & Haase, M. (2011) Origin and radiation of rissooidean gastropods (Caenogastropoda) in ancient lakes of Sulawesi. *Zoologica Scripta*, **40**, 221–237.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Dissertation, The University of Texas, Austin, TX.

SUPPORTING INFORMATION

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

Appendix S1 Collection numbers of vouchers and GenBank accession numbers.
Appendix S2 Primers.
Appendix S3 Matrix of geographical distances.
Appendix S4 Partitioning schemes and substitution models used for complete and reduced data sets.

BIOSKETCHES

S. Zielske is a doctoral candidate at the University of Greifswald, Germany. This study is a component of her thesis. Her research interest focuses on the taxonomy and in particular molecular phylogeny of tateids in the South- and Indo-Pacific region.

M. Haase is an evolutionary biologist with strong roots in taxonomy. His main interest is the diversification of freshwater and land snails in space and time. Another focus of his research is the evolutionary and functional significance of shell shape. Methodologically, he is integrating morphological, anatomical and molecular approaches.

W. Ponder is a Senior Fellow of the Australian Museum. His interests include the systematics, diversity and conservation of freshwater gastropods and he has published a number of papers on Australian Tateidae.

Author contributions: M.H. conceived the project; all authors did fieldwork; S.Z. collected and analysed the data, the latter with contributions from M.H.; W.P provided Australian material; S.Z. wrote the manuscript and M.H. and W.P. contributed to the text.

Editor: Malte Ebach

The enigmatic pattern of long distance dispersal of minute freshwater gastropods (Caenogastropoda, Tateidae) across the South Pacific.

Zielske *et al.*

SUPPORTING INFORMATION

Appendix S1: Collection numbers of vouchers and GenBank accession numbers

specimen	Collection number	COI	16S rRNA	Histon	18S rRNA	28S rRNA
Ascorhis tasmanica (Martens, 1858)	AM C.302374	KT313287	KT313130	KT313316	KT313163	KT313225
Ascorhis tasmanica (Martens, 1858)	AM C.302374	KT313288	KT313131	KT313317	KT313164	KT313226
Austropyrgus lochi Clark, Miller & Ponder, 2003	AM C.201237	KT313289	KT313132	KT313318	KT313165	KT313227
Austropyrgus niger (Quoy & Gaimard, 1834)	AM C.201044	KT313290	KT313133	KT313319	KT313166	KT313228
Austropyrgus turbatus Ponder, Colgan, Clark & Miller, 1994	AM C.202468	KT313291	KT313134	KT313320	KT313167	KT313229
Beddomeia krybetes Ponder & Clark, 1993	AM C.165636	KT313292	KT313135	KT313321	KT313168	KT313230
Caldicochlea globosa Ponder, Colgan, Terzis, Clark & Miller, 1996	AM C.186834	KT313293	KT313136	-	KT313169	KT313231
Catapyrgus matapango Haase, 2008	MNZ M174169	KT313294	KT313137	KT313322	KT313170	KT313232
Crosseana melanosoma (Haase & Bouchet 1998)	NHMW 110222	KJ490902	KJ490813	-	KT313206	KT313266
Fluviopupa brevior (Ancey, 1905)	MNHN	KC875084	KC875004	KT313323	KT313171	KT313233
Fluviopupa erromangoana Zielske & Haase 2014	ZMB 117883	KC875088	KC875008	KT313324	KT313172	-
Fluviopupa espiritusantoana Haase, Fontaine & Gargominy 2010	MNHN 199	KC875095	KC875018	KT313327	KT313175	KT313236
Fluviopupa espiritusantoana Haase, Fontaine & Gargominy 2010	MNHN 162	KC875090	KC875010	KT313325	KT313173	KT313234
Fluviopupa espiritusantoana Haase, Fontaine & Gargominy 2010	MNHN 173	KC875091	KC875011	KT313326	KT313174	KT313235
Fluviopupa gracilis pupa (Iredale, 1944)	AM C.396495	KT313295	KT313138	KT313328	KT313176	KT313237
Fluviopupa herminae Zielske & Haase 2014	ZMB 117895	KC875113	KC875042	KT313329	KT313177	KT313238
Fluviopupa irinimeke Haase, Ponder & Bouchet, 2006	USP 12224	KF939798	KF939715	KT313330	KT313178	KT313239
Fluviopupa jeanyvesi Haase, Gargominy & Fontaine 2005	NHMW 110407	KT313296	KT313139	KT313331	KT313179	KT313240
Fluviopupa narii Haase, Fontaine & Gargominy 2010	MNHN 201	-	KC875021	KT313332	KT313180	KT313241
Fluviopupa pascali Haase, Fontaine & Gargominy 2010	MNHN 163	KC875097	KC875022	KT313333	KT313181	KT313242
Fluviopupa pupoidea Pillsbry, 1911	USP 12226	KF939717	KF939800	KT313334	KT313182	KT313243
Fluviopupa raivavaeensis Haase, Gargominy & Fontaine 2005	NHMW 110408	KT313297	KT313140	KT313335	KT313183	KT313244
Fluviopupa ramsayi royana (Iredale, 1944)	AM C.478049	KT313298	KT313141	KT313336	KT313184	KT313245
Fluviopupa riva Zielske & Haase 2014	ZMB 117892	KC875086	KC875006	KT313337	KT313185	KT313246
Fluviopupa rurutua Haase, Gargominy & Fontaine 2005	NHMW 110409	KT313299	KT313142	KT313338	KT313186	KT313247
Fluviopupa rurutua Haase, Gargominy & Fontaine 2005	NHMW 110410	KT313300	KT313143	KT313339	KT313187	KT313248
Fluviopupa seasea Haase, Ponder & Bouchet, 2006	USP12217	KF939756	KF939673	KT313340	KT313188	KT313249
Fluviopupa namosi Zielske & Haase 2014	USP 12208	KF939774	KF939691	KT313343	KT313191	KT313251
Fluviopupa tunuloa Zielske & Haase 2014	USP 12200	KF939793	KF939710	KT313346	KT313194	KT313254
Fluviopupa bula Zielske & Haase 2014	USP 12215	KF939760	KF939677	KT313342	KT313190	KT313250
Fluviopupa dromodromo Zielske & Haase 2014	USP 12176	KF939781	KF939698	KT313344	KT313192	KT313252
Fluviopupa uka Zielske & Haase 2014	USP 12203	KF939736	KF939653	KT313341	KT313189	-
Fluviopupa durmontdurvilli Zielske & Haase 2014	USP 12178	KF939783	KF939700	KT313345	KT313193	KT313253
Fluviopupa tangbunia Zielske & Haase 2014	AM C.477751	KC875137	KC875065	KT313347	KT313195	KT313255
Fluviopupa torresiana Haase, Fontaine & Gargominy 2010	MNHN	KC875101	KC875029	KT313348	KT313196	KT313256

specimen	Collection number	COI	16S rRNA	Histon	18S rRNA	28S rRNA
Fluviopupa tubuaia Haase, Gargominy & Fontaine 2005	NHMW 110411	KT313301	KT313144	KT313349	KT313197	KT313257
Fluviopupa tubuaia Haase, Gargominy & Fontaine 2005	NHMW 110412	KT313302	KT313145	KT313350	KT313198	KT313258
Fonscochlea accepta Ponder, Hershler & Jenkins, 1989	AM C.202412	KT313303	KT313146	KT313351	KT313199	KT313259
Fonscochlea accepta Ponder, Hershler & Jenkins, 1989	AM C.202412	KT313304	KT313147	-	KT313200	KT313260
Fonscochlea zeidleri Ponder, Hershler & Jenkins, 1989	AM C.202424	AY622460	KT313148	KT313352	KT313201	KT313261
Halopyrgus pupoides (Hutton, 1882)	M174068	JX970616	KT313149	KT313353	KT313202	KT313262
Hemistomia andreae Haase & Zielske 2015	NHMW 110183	KJ490893	KJ490804	-	KT313205	KT313265
Hemistomia cockerelli. Haase & Bouchet, 1998	NHMW 110198	KJ490853	KJ490768	-	KT313208	KT313268
Hemistomia gemma gemma Ponder, 1982	AM C.478052	KT313305	KT313150	KT313354	KT313203	KT313263
Hemistomia gemma gemma Ponder, 1982	AM C.478052	-	KT313151	KT313355	KT313204	KT313264
Hemistomia rusticorum Haase & Bouchet, 1998	NHMW 110189	KJ490836	KJ490755	-	KT313207	KT313267
Kanakyella gentilsiana (Crosse, 1874)	NHMW 110228	KJ490914	KJ490825	KT313356	KT313209	KT313269
Leiorhagium kavuneva Haase & Bouchet, 1998	NHMW 110202	KJ490860	KJ490775	-	KT313211	KT313271
Leiorhagium orokau Haase & Bouchet, 1998	NHMW 110219	KJ490891	KJ490802	-	-	KT313272
Leptopyrgus melbourni Haase, 2008	M174092	AY631075	AY634053	KT313357	JX970588	-
Meridiopyrgus murihiku Haase, 2008	M174204	AY631086	KT313152	KT313358	KT313212	KT313273
Novacaledonia numee Haase & Bouchet, 1998	NHMW 110187	KJ490832	KJ490751	-	KT313210	KT313270
Oncomelania minima Bartsch, 1936	-	AB611791	AB611790	-	AB611788	AB611789
Opacuincola delira Haase, 2008	MNZ M174126	KT313306	KT313154	KT313359	KT313214	KT313275
Phrantela daveyensis tristis Ponder & Clark, 1993	AM C.395054	KT313307	KT313155	KT313360	KT313215	KT313276
Phrantela daveyensis tristis Ponder & Clark, 1993	AM C.395054	KT313308	KT313156	KT313361	KT313216	KT313277
Posticobia brazieri (E.A. Smith, 1882)	AM C.201405	KT313309	KT313157	KT313362	KT313217	KT313278
Potamopyrgus antipodarum (Gray, 1843)	-	EU573983	EU573989	-	EU573997	JF960417
Potamopyrgus estuarinus Winterbourn, 1971	MNZ M174052	AY631104	KT313158	KT313363	KT313218	KT313279
Rakiurapyrgus cresswelli (Climo, 1974)	MNZ M158224	KT313310	KT313159	KT313364	KT313219	KT313280
Sororipyrgus kutukutu Haase, 2008	MNZ M174085	AY631109	AY634087	KT313365	KT313220	KT313281
Sororipyrgus raki Haase, 2008	MNZ M174079	KT313311	KT313160	-	KT313221	KT313282
Sulawesidrobia abreui Zielske, Glaubrecht & Haase, 2011	ZMB 191643	HM587351	HM587394	KT313366	HM587420	KT313283
Sulawesidrobia anceps Zielske, Glaubrecht & Haase, 2011	ZMB 191640	HM587346	HM587388	KT313367	HM587417	KT313284
Tatea huonensis (Tenison-Woods, 1876)	AM C.159230	KT313312	JX970550	KT313368	KT313222	-
Tongapyrgus kohitatea Haase, 2008	MNZ M174176	AY631124	AY634102	KT313369	-	-
Trochidrobia punicea Ponder, Hershler & Jenkins, 1989	AM C.186918	KT313313	KT313161	KT313370	KT313223	KT313285
Victodrobia victoriensis Ponder & Clark, 1993	AM C.166267	KT313314	KT313162	KT313371	KT313224	KT313286

Appendix S2: Primers.

Gene	Primers	Reference	Annealing temperature	Remarks
COI	LCO1460/HCO1298	Folmer *et al.* (1994)	46° C	HCO modified at position 12 (G → A)
16S rRNA	16S ar/16S br	Palumbi *et al.* (1991)	51° C	Touch-down PCR with the first ten cycles dropping from 60 °C
Histon	H3F/H3R	Colgan *et al.* (2000)	55° C	Touch-down PCR with the first twenty cycles dropping from 64 °C
18S rRNA	18S f/18S r	Holland *et al.* (1991)	42° C	Touch-down PCR with the first twenty cycles dropping from 51 °C
28S rRNA	F63.2/LSU3	Benke *et al.* (2009)	55° C	Touch-down PCR with the first ten cycles dropping from 64 °C
	D2RB/D23F	Park & Foighil (2000)	55° C	for internal sequencing

Appendix S3: Matrix of geographical distances

	Aus	NZ	NC	LHI	Van	Fiji	AI	Sul
Aus	-	2250	1400	580	1800	2700	5700	1650
NZ		-	2190	1730	2460	2340	3690	6930
NC			-	1300	510	1380	4630	5310
LHI				-	1800	2440	5120	5200
Van				-	-	1020	4420	5500
Fiji						-	3440	6510
AI							-	9920
Sul								-

Appendix S4: Partitioning schemes and substitution models used for complete and reduced data sets.

gene	partition	ML		MrBayes		Beast
		complete	reduced	complete	reduced	dataset
COI	codon pos. 1, 2	TrN+I+Γ	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ	TrN+I+Γ
	codon pos. 3	K3P+Γ	TVM+Γ	HKY+Γ	GTR+Γ	TVM+Γ
16S rRNA	stem	TVM+I+Γ	HKY+I+Γ	GTR+I+Γ	HKY+I+Γ	TVM+I+Γ
	loop	TIM+I+Γ	TrN+I+Γ	GTR+I+Γ	HKY+I+Γ	TIM+I+Γ
Histone		TIM+I+Γ	TVM+Γ	GTR+I+Γ	GTR+Γ	TIM+I+Γ
18S rRNA	stem	HKY+I	HKY	HKY+I	HKY+I	K3P+I+Γ
	loop	K3P+I+Γ	K3P+Γ	GTR+I+Γ	F81+Γ	
28S rRNA	stem	TIM+I+Γ	HKY+I+Γ	GTR+I+Γ	HKY+I	TrN+I+Γ
	loop	TrN+I+Γ	TrN+Γ	SYM+I+Γ	GTR+Γ	TrN+Γ

Literature cited herein:

Benke, M., Brändele, M., Albrecht, C. & Wilke, T. (2009) Pleistocene phylogeography and phylogenetic concordance in cold-adapted spring snails (*Bythinella* spp.). *Molecular Ecology*, **18**, 890-903.

Colgan, D.J., Ponder, W.F. & Eggler, P.E. (2000) Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta,* **29**, 29-63.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Holland, P.W., Hacker, A.M. & Williams, N.A. (1991) A molecular analysis of the phylogenetic affinities of Saccoglossus cambrensis Brambell & Cole (Hemicordata). *Philosophical Transactions of the Royal Society of London. Series B*, *Biological Sciences* **332**, 185-189.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Gabowski, G. (1991) The simple fool's guide to PCR. Dept. of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu.

Park, J.-K. & Ó Foighil, D. (2000) Sphaeriid and Corbiculid Clams Represent Separate Heterodont Bivalve Radiations into Freshwater Environments. *Molecular Phylogenetics and Evolution*, **14**, 75-88.

2.5. Contribution to manuscripts

The fieldwork underling all studies was done together with M. Haase. Further material was provided by different museums, for details see the respective manuscripts. The original idea to this project funded by the german science foundation (grant HA4752/2-1) was conceived by M. Haase.

Zielske S., Haase M. (2014) **When snails inform about geology: Pliocene emergence of islands of Vanuatu indicated by a radiation of truncatelloidean freshwater gastropods (Caenogastropoda).** *Journal of Zoological Systematics and Evolutionary Research* **215**: 217-236.

I collected and analysed the data (90%), and I wrote the manuscript (75%) including all species descriptions (90%) and editing of figures (100%). M. Haase contributed to the data analyses, writing and revision of the manuscript.

Zielske S., Haase M. (2014) **New insights into tateid gastropods and their radiation on Fiji based on anatomical and molecular methods (Caenogastropoda: Truncatelloidea).** *Zoological Journal of the Linnean Society* **172**: 71-102.

I collected and analysed the data (90%) and wrote the manuscript (80%) including all species descriptions (100%) and editing of figures (100%). M. Haase contributed to the data analyses, writing and revision of the manuscript.

Zielske S, Haase M (2015) **Molecular phylogeny and a modified approach of character-based barcoding refining the taxonomy of New Caledonian freshwater gastropods (Caenogastropoda, Truncatelloidea, Tateidae).** *Molecular Phylogenetics and Evolution* **89**: 171-181.

I collected and analysed the data (90%) and wrote the manuscript (80%) including all figures (100%). M. Haase contributed to the data analyses, writing and revision of the manuscript.

Zielske S., Ponder W. F., Haase M. (2016) **The enigmatic pattern of long distance dispersal of minute freshwater gastropods (Caenogastropoda, Tateidae) across the South Pacific.** *Journal of Biogeography*, in press.

I collected and analysed the data (80%) and wrote the manuscript (75%) including all figures (100%). M. Haase contributed to the data analyses. M.Haase and W. F. Ponder contributed to the writing and revision of the manuscript.

3. Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.
Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Greifswald, 16.06.2016

Susan Zielske