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Phylogeny and classification of *Nesobasis* Selys, 1891 and *Vanuatubasis* Ober & Staniczek, 2009 (Odonata: Coenagrionidae)

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Abstract

Island archipelagos in the South Pacific have relatively high species endemism within the insect order Odonata, specifically damselflies. *Nesobasis* Selys, 1891, an endemic damselfly genus from Fiji, includes over 20 species, but a clear understanding of its evolutionary relationship to other damselflies in the region is lacking. Scientists have questioned the monophyly of *Nesobasis* due to variations within the genus leading to the establishment of three divisions provisionally named as: *comosa-*, *erythrops-* and *longistyla-* groups. However, *Nesobasis* has shown to be monophyletic in previous phylogenetic analyses. Using additional species in this study, we investigate the phylogenetic relationships between *Nesobasis* and other damselflies from the region, specifically the endemic *Vanuatubasis* Ober & Staniczek, 2009 from the neighboring island archipelagos of Vanuatu. The relationship between these taxa has not yet been examined with molecular data. Five genes were used in a maximum likelihood phylogenetic reconstruction and examined morphological data to determine the relationship between these genera. Our results recover three distinct clades overall with *Vanuatubasis* nested within *Nesobasis* (i.e., non-monophyletic). *Vanuatubasis* is sister to the *longistyla* and *erythrops* groups. The third group, *comosa,* was found sister to the clade of *Vanuatubasis* (*longistyla* + *erythrops*). As a result of these findings, we propose the new genus, *Nikoulabasis* gen. nov.

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Keywords
South Pacific; new genus; Zygoptera; damselfly; Fiji; Vanuatu

Introduction

Archipelagos have long been recognized as excellent places to study species diversity (Darwin 1835; Losos & Ricklefs 2009). Islands are estimated to account for 3–5% (Whittaker et al. 2007; Kier et al. 2009; Weigelt et al. 2013; Russell & Kueffer 2019) of the earth's landmass and, when corrected for the surface area, have about 3.6 more species per km^2 than continental areas (Tershy et al. 2015). Islands offer a unique opportunity to study biodiversity as populations are bounded, gene flow is often limited, distances between-island are easily measured, and island communities tend to be less diverse than mainland communities but with increased endemism (Heaney et al. 2005; Gillespie 2007; Whittaker et al. 2007; Kier et al. 2009; Russell & Kueffer 2019). This is especially true in the Pacific ocean, which supports over 10,000 islands (Benstead et al. 2009).

With over 1 million insects described and named (Zhang 2011; Stork 2018), insects offer a unique and highly diverse system to study island biodiversity and biogeography (e.g., O'Grady & Desalle 2008), but are generally understudied compared to other terrestrial organisms such as birds, mammals, and plants (Stork 2007). One example of an insect island biodiversity and biogeography study looked at a genus of Lepidoptera *Polyura* Billberg, 1820, from the Indo-Australian archipelago and found cases of island hoping and long-distant dispersal events throughout *Polyura* evolutionary history (Toussaint & Balke 2016). Biogeographical dispersal events have been seen in other insect studies (Condamine et al. 2013; Clouse et al. 2015), which demonstrates only a small amount of the diversity and awe studying insects can provide over other terrestrial organisms.

Odonates represent an ideal insect system to explore evolutionary questions on islands as they are often highly endemic and diverse on island systems (Marinov 2015). An example of this can be found across the islands of Hawaii, where Polhemus (1997) and Jordan et al. (2003), together, summarize the taxonomy and provide a comprehensive phylogeny of the Hawaiian endemic genus *Megalagrion* McLachlan, 1883, composed of 23 described species. The Hawaiian islands show species richness and a high level of island endemism (Jordan et al. 2003). However, the evolutionary and biogeographic history of how these species were established across the Hawaiian Islands is likely complex (Jordan et al. 2005; Heads 2011). *Megalagrion* has become a notable system to study insect evolution because of its well-established taxonomy and robust phylogeny (Cooper 2010; Cooper et al. 2016; Jones & Jordan 2015; Scales & Butler 2016). Previous work focused on Hawaiian damselflies demonstrates exemplary adaptive radiation on an oceanic archipelago. Studies of other island endemics, especially in the South Pacific, are important to understand more generally how damselflies diversify upon reaching novel environments.

Coenagrionidae, a family of damselflies consisting of over 1300 species (Dijkstra et al. 2014; Schorr & Paulson 2018; Paulson 2019; Paulson et al. 2022) has been studied for over 150 years (O'Grady & May 2003); a solid taxonomic understanding of this family is lacking, but a recent study brings some clarity (Willink et al. 2022). Subjectivity within character states, lack of apparent diagnostic features, and classification schemes have exacerbated the problem, especially when addressing the taxonomy of island damselflies, as these systems are often the most underworked and incomplete. Previous work in the South Pacific has increased understanding in odonate evolution with overviews of the faunas from particular islands groups (Jordan et al. 2003; Marinov 2012; Marinov & Pikacha 2013; Marinov & Waqa-Sakiti 2013; Beatty et al. 2017; Grand et al. 2019; Marinov et al. 2019). An example of this is the radiation of endemic damselflies in Fiji, the genus *Nesobasis* Selys, 1891. *Nesobasis* presently includes 24 species with more to be described (C. Beatty, A. Cordero, N. Donnelly, pers. comm.) and has a highly conserved habitat preference with up to 12 species present at the same locality (Donnelly 1990; Beatty et al. 2007; Van Gossum et al. 2008; Beatty et al. 2017).

A noted taxonomic issue within *Nesobasis* was discussed by Donnelly (1990) when he suggested *Nesobasis* could represent two distinct genera but had difficulty reliably diagnosing genera due to significant variability in morphology and coloration. Despite these issues, Donnelly did propose three groups within *Nesobasis*: *erythrops*, *longistyla,* and *comosa*. The groups share morphological characters from the head, prothorax, legs, genitalia, and appendages at the posterior end, particularly the cerci and paraprocts. The *erythrops* group can be defined by the "L" shape of the cerci in the dorsal view with paraprocts about half the length of the cerci. The *comosa* group has large, rounded cerci with paraprocts about the same length as the cerci. The *longistyla* group has long, forcipate cerci with paraprocts less than half the length of the cerci (Donnelly 1990, 1994). Recent phylogenetic reconstruction also supported the three distinct groupings within *Nesobasis*, which included the first phylogenetic estimate for the group, additional species not yet described, and recovered all three species groups, with the *comosa* clade reconstructed as sister to the *erythrops* + *longistyla* clades (Beatty et al. 2017). The overall grouping still showed *Nesobasis* as monophyletic, and despite this, no taxonomic revisions have been made. Looking at other island-endemic genera in the South Pacific may be insightful in clearing up the taxonomy of *Nesobasis*.

There remain significant gaps in our understanding of the taxonomy of *Nesobasis* due to delayed species descriptions and considerable morphological variation within *Nesobasis*. Further, the relationship of *Nesobasis* to other genera within the South Pacific, particularly close island groups, is unclear. In the 1900s, Kimmins (1936, 1958) erected two new *Nesobasis* taxa from Vanuatu, *N. malekulana* Kimmins, (1936) and *N. bidens* Kimmins, (1958) respectively. These species had unique morphological characteristics, such as the paraprocts being about three times as long as the cerci, which is reversed in *Nesobasis,* with cerci usually longer than paraprocts. Several other researchers noted that their distinct morphology could constitute the description of a new genus (Tillyard 1924; Donnelly 1990). It was not until 2009 that the genus

Vanuatubasis (Ober & Staniczek 2009) was erected with both *V. malekulana* and *V. bidens* being placed within the new genus and the newly described species, *V. santoensis* (Ober & Staniczek 2009). It was hypothesized that *Vanuatubasis,* based on morphology, would be closely related to *Nesobasis* (Ober & Staniczek 2009), but a study comparing the two genera has not previously been done. A more comprehensive sampling of *Nesobasis* and island damselflies is needed to elucidate our understanding of this complex genus, and its relationships within the Pacific Coenagrionidae. We provide the inclusion of the endemic genus, *Vanuatubasis,* in a phylogenetic reconstruction with *Nesobasis,* as its geographic closeness to Fiji could be insightful. Thus, by including *Vanuatubasis* in a phylogenetic reconstruction, we more appropriately test the monophyly of *Nesobasis* and produce a more precise taxonomy to better support both future taxonomic and evolutionary studies.

Material and methods

Taxon sampling

The material used for this study has been collected for more than 50 years by various researchers and deposited in museums and private collections. For this manuscript and phylogenetic analysis, we opted to leave out *Melanesobasis* due to its distant relationship as shown by Beatty et al. (2017). Field trips by some of the authors have been initiated within Fiji's and Vanuatu's islands to obtain more specimens for the analyses outlined in the methodology. These included expeditions between 2009–2018 (in Fiji) and 2017–2019 (in Vanuatu) (Marinov et al. 2019; Saxton et al. 2021; Supplementary Table S1). Mainly adult odonates were collected with aerial nets and either preserved dried or in 95% ethanol and stored at –80°C for molecular analysis. Preservation techniques vary depending on the personal preferences of the researcher at the time of sampling.

Morphological analysis

Phenetic characters used as diagnostic in other studies on *Nesobasis* and *Vanuatubasis* (e.g., Donnelly 1990; Ober & Staniczek 2009) have been utilized to hypothesize a new ergotaxonomy based on structural and color variations of these Pacific taxa. The following body parts, utilized as important diagnostic features in the above mentioned studies (Donnelly 1990; Ober & Staniczek 2009), were considered the most important for the comparative analysis: cerci, paraprocts, and penis (males), ovipositor (females), prothorax (both sexes), frontal part of the head (both sexes).

The morphological description follows the terminology of Garrison et al. (2010); wing venation designation follows Riek & Kukalová-Peck (1984). All measurements are in millimeters (mm).

*Morphology (*Fig. 1*):* AL – abdomen length (appendages excluded); C – cerci; DL – distal lobe of penis; O – ovipositor; P – paraprocts; S1–10 – abdominal segments 1 to 10.

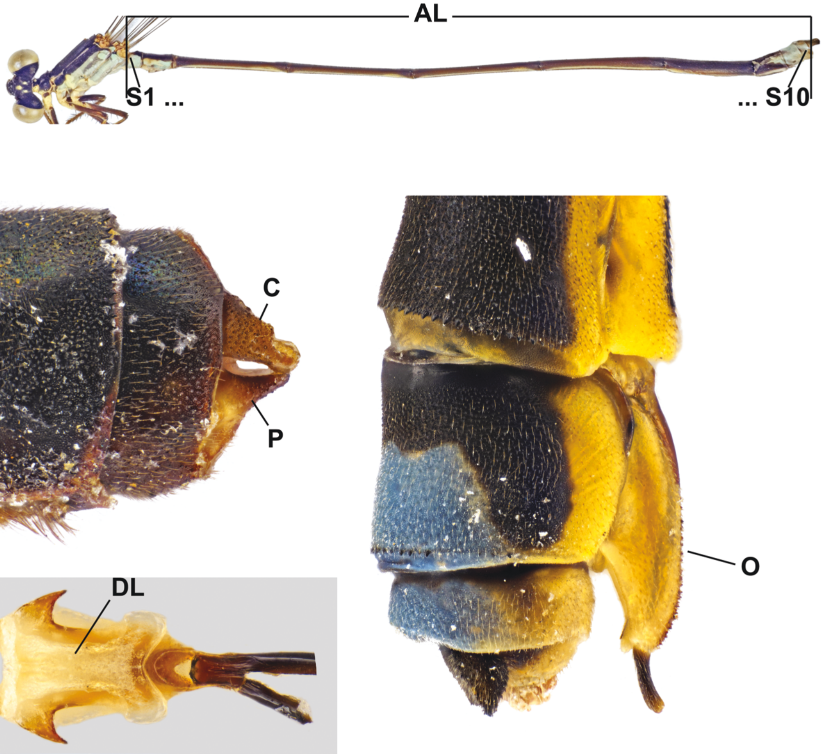

Fig. 1. Morphological features used for idiognoses: AL – abdomen length (appendages excluded); C – cerci; DL – distal lobe of penis; O – ovipositor; P – paraprocts; S1–10 – abdominal segments 1 to 10. Species: *Nesobasis caerulecaudata* (abdomen); *Nikoulabasis malcolmi* (cerci, paraproct); *Nesobasis monika* (penis, ovipositor).

Molecular analysis

Extraction, DNA amplification, and sequencing

Sequences were gathered from previous analyses exploring relationships in South Pacific damselflies, including Beatty et al. (2017) and Lorenzo-Carballa et al. (2019). A total of 52 specimens representing 15 described *Nesobasis* species and 15 undescribed *Nesobasis* from Fiji, three of the described *Vanuatubasis* specimens, and one new *Vanuatubasis* species are included in the phylogenetic reconstruction. We included additional members of Coenagrionidae in our analysis for outgroups: *Ischnura ramburii* (Selys in Sagra, 1857), *Ischnura heterosticta* (Burmeister, 1839), *Stenagrion dubium* (Laidlaw, 1912), *Coenagrion terue* (Asahina, 1949), *Pseudagrion ignifer* (Tillyard, 1906), and *Megalagrion heterogamias* (Perkins, 1899); the outgroup has a diverse selection of specimens that are more basal in the family to account for the size of Coenagrionidae. Outgroups were collected from previous fieldwork and selected from the Brigham Young University frozen tissue bank.

Table 1. The PCR protocol for the genes Cytochrome Oxidase 1 (CO1), Internal Transcribed Spacer (ITS), the Protein Arginine Methyltransferase (PRMT), and the mitochondrial 12S rRNA gene (12S).

Gene	Cycles	Hot Start	Step 1	Step 2	Step 3
CO1	39	94°C 3 min	94°C 1 min	54°C 1 min	72°C 2 min
ITS	30	94°C 3 min	95°C 1 min	52°C 1.5 min	72°C 2 min
PMRT	40	94°C 3 min	94°C 1 min	54°C 1 min	72°C 1 min
12S	35	94°C 3 min	94°C 1 min	54°C 1 min	72°C 1 min

We used thoracic muscle tissue and followed manufacturer protocols to extract DNA using Qiagen QIAamp DNA mini kits. Two regions of Cytochrome Oxidase 1 (CO1, ~757 bp and LCO, ~658 bp), Internal Transcribed Spacer (ITS1, ~572 bp), mitochondrial 12S rRNA gene (12S, ~367 bp), and the Protein Arginine Methyltransferase (PRMT, ~640bp), were sequenced (see Table 1). We performed PCR reactions on an Eppendorf Mastercycler with a total volume of 25ml (1.5ml DNA, 2.0ml primer, 9.0ml dH2O, and 12.5ml Mastermix). We used the ThermoFisher thermoscientific DreamTaq Green PCR MasterMix with a 2X concentrated solution of *Taq* DNA Polymerase (0.05 U/mL), a reaction buffer, 4.0 mM $MgCl_2$, and 0.4 mM of each dNTPs (see PCR protocol in Table 1). Successful PCR reactions were identified using gel electrophoresis, and sequence reactions were conducted at the Brigham Young University DNA Sequencing Center.

Alignment and tree reconstruction

Using Geneious Prime v. 2021.0.3 software (http://www.geneious.com/ Biomatters Ltd., Auckland, New Zealand), we examined the chromatograms and aligned sequences with MAFFT using default parameters (Katoh & Standley 2013). Genes were individually aligned and concatenated with a final nucleotide alignment length of 3,224 bp, 24.2% identical sites, and a pairwise identity of 72%.

Phylogenetic relationships among the taxa were reconstructed using maximum likelihood (ML). We used the IQ-TREE webserver to construct our maximum likelihood tree (Trifinopoulos et al. 2016; Minh et al. 2020). We selected the Model Finder (Kalyaanamoorthy et al. 2017) option to determine the best model for our data. According to the Bayesian Information Criterion (BIC), the best-fit model for our data was TIM2+F+R3, with the number of Parsimony-informative sites = 759 bp (23.5%). We conducted 1000 pseudo-replicates of the ultrafast bootstrap (Hoang et al. 2018) in our ML analysis. We visualized the trees using FigTree v.1.4 (Rambaut 2012).

Nomenclature transcription of the tree

Assigning nomenclature ranks to taxonomic groups of the phylogenetic tree was done following the recommended ranking proposed by the *International Code of Zoological Nomenclature* (hereafter *Code*) (Anonymous 1999). Ranks were given only to monophyletic clades where we observed congruence between the results from both morphological and molecular analyses. Sister taxa were given the same rank as proposed in Dubois & Raffaëlli (2009). Introduction of the new taxa follows the same plan and included idiognoses (sensu Dubois 2017) with: (1) size range (AL), (2) morphology, (3) sex dimorphism: male terminal appendages, penis, female ovipositor, mesostigmal plate; (4) etymology, (5) chorology. Diagnosis is provided only for the distinction of the three genera treated here.

Results

Taxonomic results

Molecular phylogenetic results

The phylogeny is well resolved and has overall high nodal support, particularly along the backbone (Fig. 2). The genus *Nesobasis* has two distinct and well-supported groups with *Vanuatubasis* nested within, rendering *Nesobasis* non-monophyletic. The *comosa* group is recovered as sister to *Vanuatubasis* + *longistyla* group + *erythrops* group with a bootstrap value of 95%. *Vanuatubasis* forms a monophyletic clade, with a bootstrap

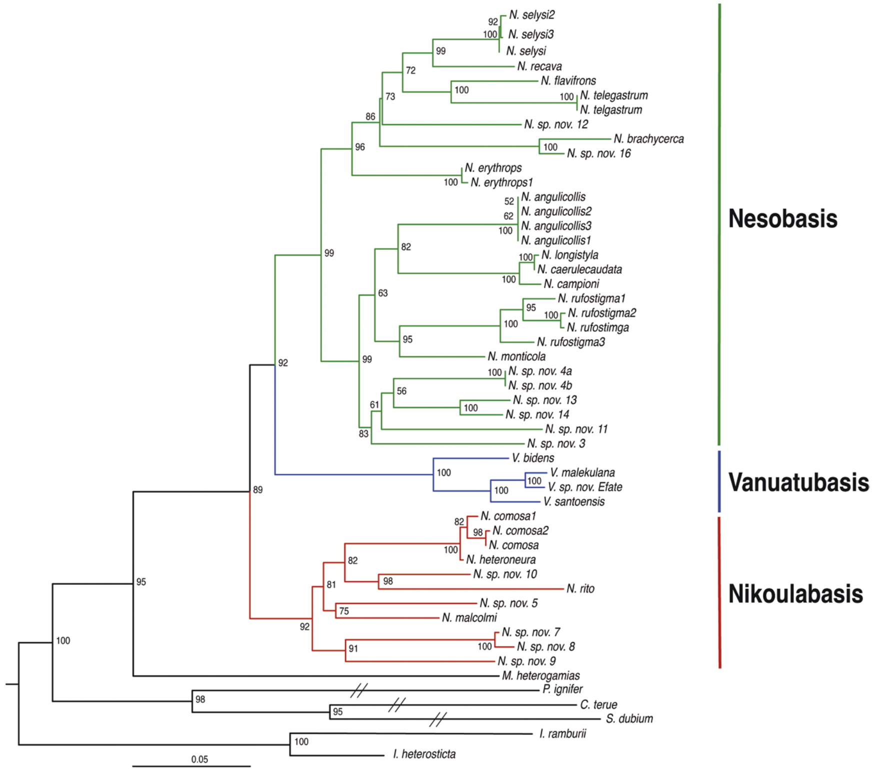

Fig. 2. Resulting phylogeny from ML analysis. Genera branches are highlighted. Support values (bootstrap) are shown prior to nodes.

value of 100%. *Vanuatubasis* is rendered sister to the *longistyla* group + *erythrops* group with 89% nodal support. The nodal support for the *longistyla* group is 98%. The *longistyla* group is sister to the *erythrops* group with 86% nodal support. The erythrops group has a nodal support value of 97%. Within *Vanuatubasis, V. bidens* is sister to the remaining. *Vanuatubasis santoensis* is sister to (*V. malekulana* + *V_sp_nov_Efate*). All relationships have a nodal support value of 100%.

As a result of the phylogenetic relationships, we propose a new ergotaxonomy of the endemic Coenagrionidae to Fiji and Vanuatu (*Melanesobasis* excluded):

- Order Odonata Fabricius, 1793
  - Suborder Zygoptera Selys, 1854
    - Family Coenagrionidae Kirby, 1890
      - Subfamily Teinobasinae Tillyard, 1917
        - Genus *Nesobasis* Selys, 1891
        - Genus *Vanuatubasis* Ober & Staniczek, 2009
        - Genus *Nikoulabasis* Ferguson et al., 2021, gen. new

***Nesobasis* Selys, 1891**

Type species – *Nesobasis erythrops* Selys, 1891
Idiognosis (Donnelly 1990, with a few modifications; Fig. 3). Coenagrionid damselflies ranging in size (AL) from 25 to 53 mm; postero-lateral margin of median lobe of pronotum keeled; posterior lobe of pronotum rounded raised; males: cerci variable in shape and size, longer than paraprocts, the latter expanded at the bases with or without sharply pointed tips; genitalia: penis with the distal lobe narrower than the preceding lobe, parallel sided throughout or with terminal expansions giving it a T-like appearance, ovipositor with venter of the valve finely dentate; mesostigmal plate flat or with a raised tubercule.

Etymology (from Ober & Staniczek 2009). From Greek νησος (nesos = island) and βάση (basi = basis). The suffix “basis” common in many zygopteran genera.

*Distribution***.** Endemic to Fiji where it has been recorded from the islands of: Kadavu, Koro, Ovalau, Qamea, Taveuni, Vanua Levu, Viti Levu (Donnelly 1990; Rashni et al. 2022).

***Vanuatubasis* Ober & Staniczek, 2009**

Type species – *Nesobasis malekulana* Kimmins, 1936
Idiognosis (Fig. 4). Coenagrionid damselflies ranging in size (AL) from 30 to 37.5 mm; postero-lateral margin of the median lobe of pronotum keeled; posterior lobe of pronotum triangular with acutely to obtusely (almost round) the mid-dorsal point which is raised and supported with a vertical ridge; males: cerci about three-times shorter than paraprocts; genitalia: penis with distal lobe narrowing at the middle and expanded at the terminal part, ovipositor with venter of the valve finely dentate; mesostigmal plate flat or with auricle-like expansion at the dorso-posterior side.

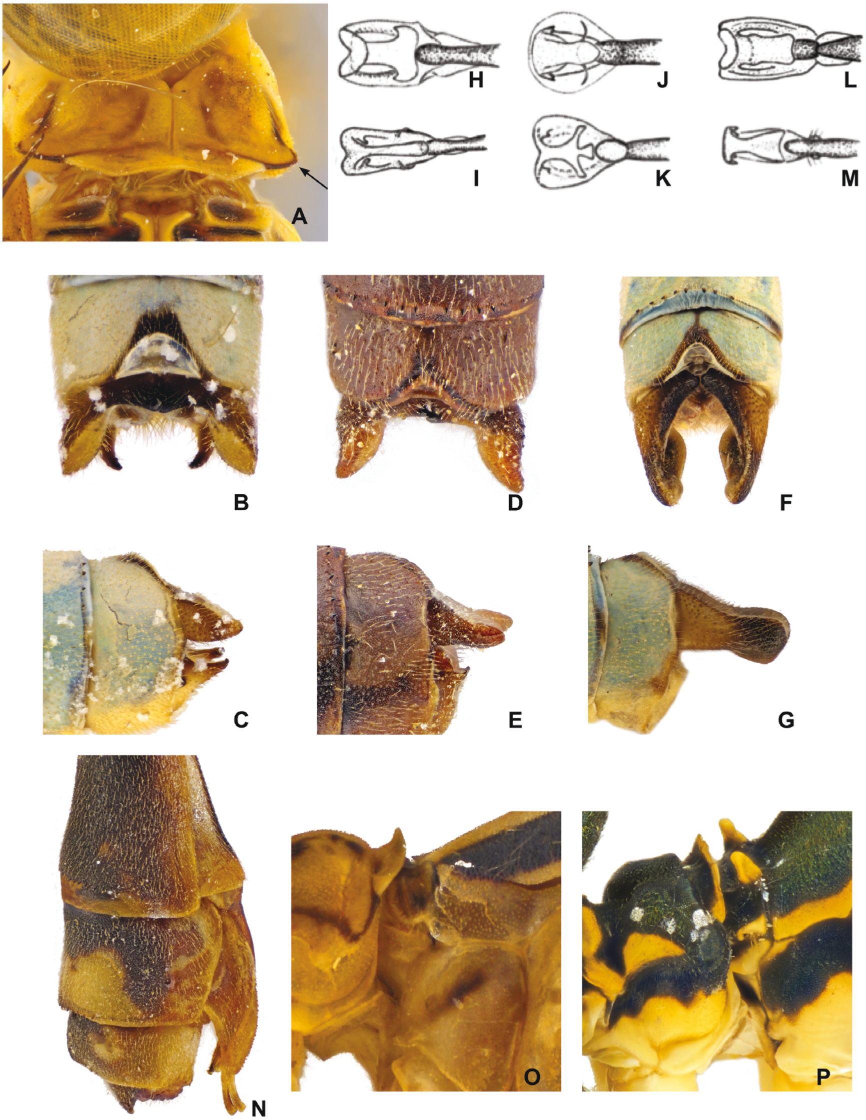

Fig. 3. Diagnostic features of *Nesobasis*: (A) pronotum with keel, *N. flavifrons*; (B–G) cerci and paraprocts of *N. recava* (B–C), *N. rufostigma* (D–E), *N. caerulecaudata* (F–G); (H–M) penises of *N. angulicollis* (H), *N. ingens* (I), *N. erythrops* (J), *N. leveri* (K), *N. longistyla* (L), *N. rufostigma* (M); (N) ovipositor, *N. flavostigma*; (O-P) mesostigmal plate without raised tubercle, *N. flavifrons* (O) and with raised tubercle *N. campioni* (P).

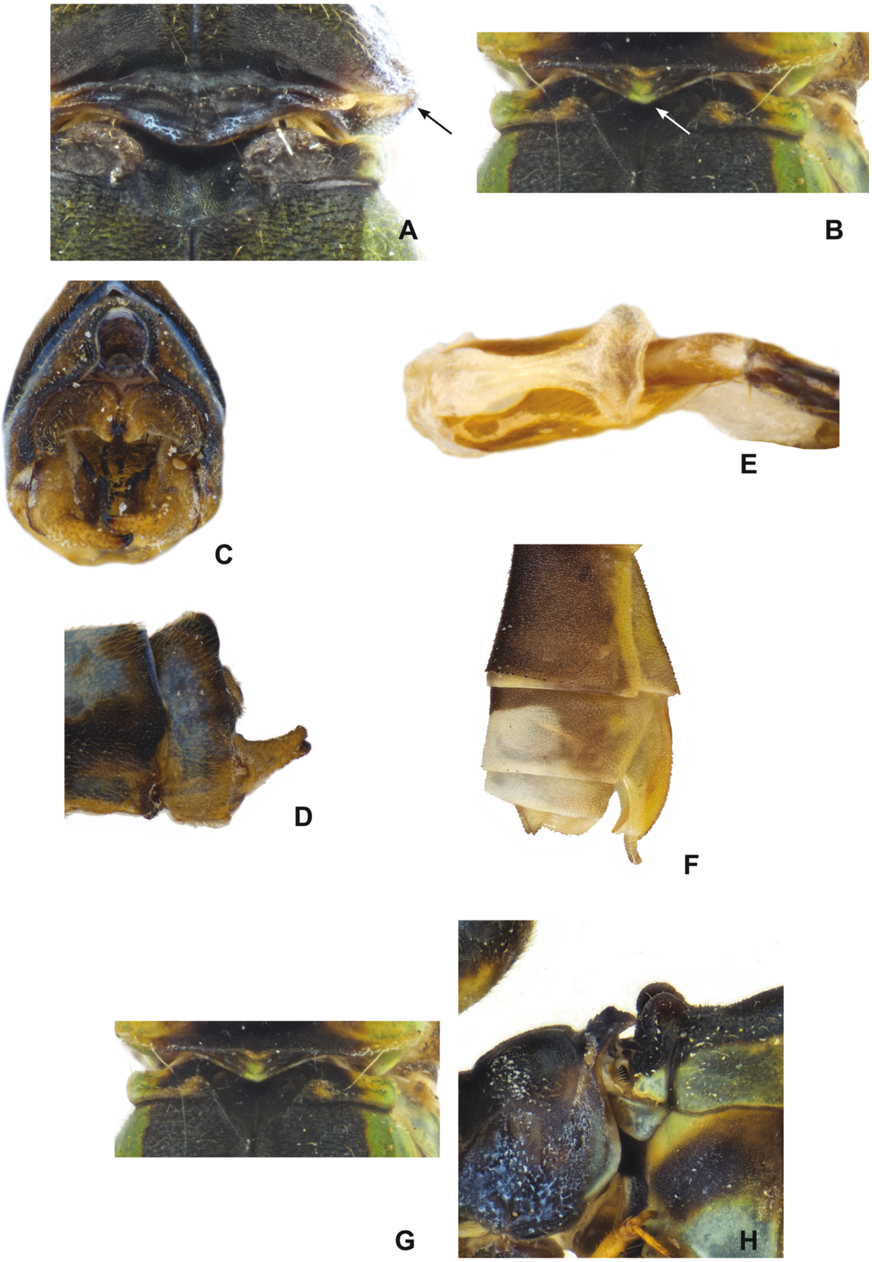

Fig. 4. Diagnostic features of *Vanuatubasis*: (A) pronotum with keel, *V. malekulana*; (B) acute posterior edge of pronotum, *V. santoensis*; (C–D) cerci and paraprocts, *V. malekulana*; (E) penis *V. malekulana*; (F) ovipositor, *V. bidens*; (G–H) mesostigmal plate without raised auricle, *V. santoensis* (G) and with raised auricle *V. malekulana* (H).

Etymology (from Ober & Staniczek 2009). The name is composed of "Vanuatu," which refers to its presently known distribution, and the suffix "basis."

*Distribution. E*ndemic to Vanuatu where it has been recorded from the islands of: Aneityum, Efate, Espiritu Santo, Maewo, Malekula, Pentecost (Ober & Staniczek 2009; Saxton et al. in prep.).

Nikoulabasis **Ferguson et al., 2023**

Type species – *Nesobasis comosa* Tillyard, 1924 (by present designation)
*Idiognosis. C*oenagrionid damselflies ranging in size (AL) from 27 to 36 mm; postero-lateral margin of median lobe of pronotum rounded, with a slightly raised rim, but not keeled; posterior lobe of pronotum flat and rounded and elevated; males: cerci and paraprocts subequal; genitalia: penis with broad distal lobe and terminal lobe lacking internal tooth, ovipositor with venter of the valve coarsely dentate; mesostigmal plate flat to flat with raised inner dorsal corner.

Etymology. The new nomen has three parts: *Nike* (goddess of victory from Greek mythology), *koula* meaning gold in Fijian, and *-basis* shows that the new genus pertains to the Teinobaisini in which that suffix is common. The nomen is to reflect the second Olympic gold medal of the Fijian Rugby Sevens team in Tokyo, 2021. Combined, *Nike* and *koula* reference the English name Nicholas, which is a dedication for Thomas (**Nick**) Donnelly – a scientist who greatly contributed to Odonata studies in Fiji and neighboring islands.

Distribution. Endemic to Fiji where it has been recorded from the islands of: Kadavu, Koro, Ovalau, Taveuni, Vanua Levu, Viti Levu (Donnelly 1990; Donnelly & Marinov in prep.).

Diagnosis (Fig. 5). Members of the three genera can be differentiated in general appearance with *Nikoulabasis* looking stouter than *Nesobasis* and *Vanuatubasis* (Fig. 6A–C). In addition, the following structural features of *Nikoulabasis* are found to be the most important diagnostic (alternatives for *Nesobasis* and *Vanuatubasis* given in brackets); for comparison refer to Figure 6E–F: postero-lateral margin of median lobe of pronotum with rim (keel), penis with broad distal lobe (narrow), ovipositor coarsely dentate (finely), male cerci almost always subequal (not subequal, but see below). Species included in the two genera (*Nesobasis* and *Vanuatubasis*) are very similar in general habitus. The main difference in both sexes is observed in the shape of the posterior lobe of the prothorax: rounded flat or raised (*Nesobasis*) versus triangular with acute to obtuse midpoint (*Vanuatubasis*). Males of *Vanuatubasis* are characterized with cerci which are shorter than paraprocts and in dorsal view hardly projecting out from the posterior end of S10. Cerci in *Nesobasis* are always sticking out of S10 for at least half the length of the segment and usually longer than paraptrocts (may look almost subequal in some specimens of species e.g., *N. selysi* Tillyard, 1924, *N. leveri* Kimmins, 1943, *N. recava* Donnelly, 1990).

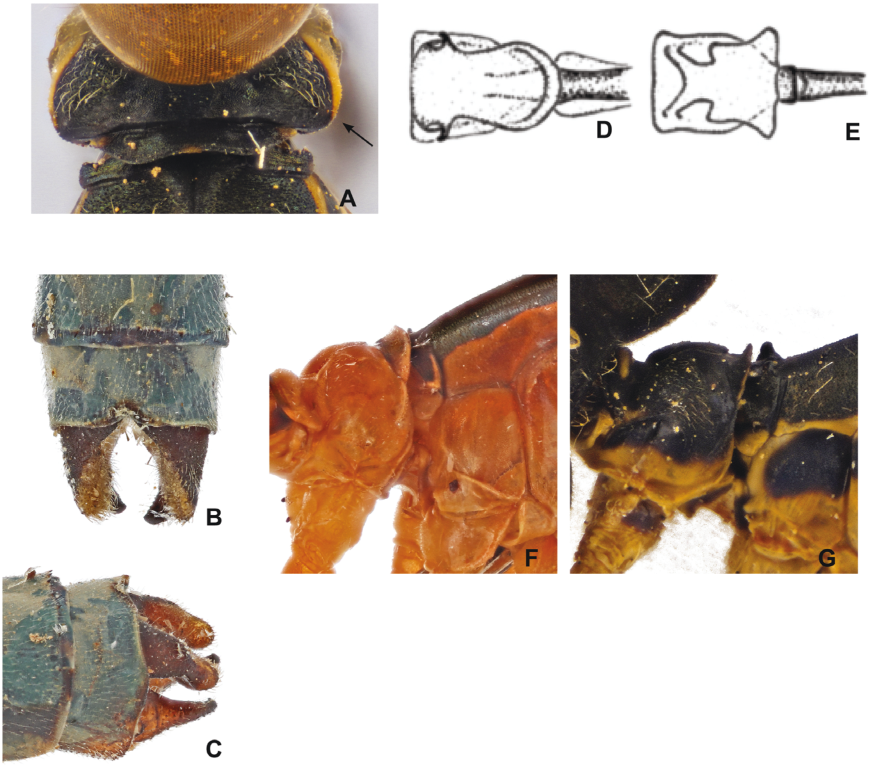

Fig. 5. Diagnostic features of *Nikoulabasis*: (A) pronotum without keel, *N. comosa*; (B–C) cerci and paraprocts, *N. heteroneura*; (D–E) penises of *N. aurantiaca* (D), *N. malcolmi* (E); (F) ovipositor, *N. comosa*; (G–H) mesostigmal plate without raised tubercle, *N. aurantiaca* (G) and with raised auricle *N. martina* (H).

Discussion

The split of the genus *Nesobasis* has been suggested before (Donnelly 1990). Beatty et al. (2017) tested the monophyly of *Nesobasis* with other coenagrionid genera and recovered the genus as monophyletic. However, their study was based only on Fijian damselflies and lacked specimens from other localities; we chose to add more taxa for our phylogenetic analysis. Our phylogeny tested the monophyly of *Nesobasis* by adding four species from *Vanuatubasis* to the phylogeny. Our phylogeny is also the first time that the interspecies relationships of *Vanuatubasis* have been reconstructed. Our results rendered *Nesobasis* as non-monophyletic, an interesting result considering the history of the group and the uncertainty surrounding the extensive morphological characteristics between the major species groups in the genus. The placement of *Vanuatubasis* as sister to the *longistyla* + *erythrops* groups makes a taxonomic adjustment necessary. We suggest species from the *comosa* group be given a new generic status, preserving *Vanuatubasis* and *Nesobasis* (retaining *longistyla* + *erythrops* groups). Another option could be to subsume *Vanuatubasis* into *Nesobasis* and have a large *Nesobasis* genus. We favor a new generic status for the *comosa* group because: 1) the three genera show

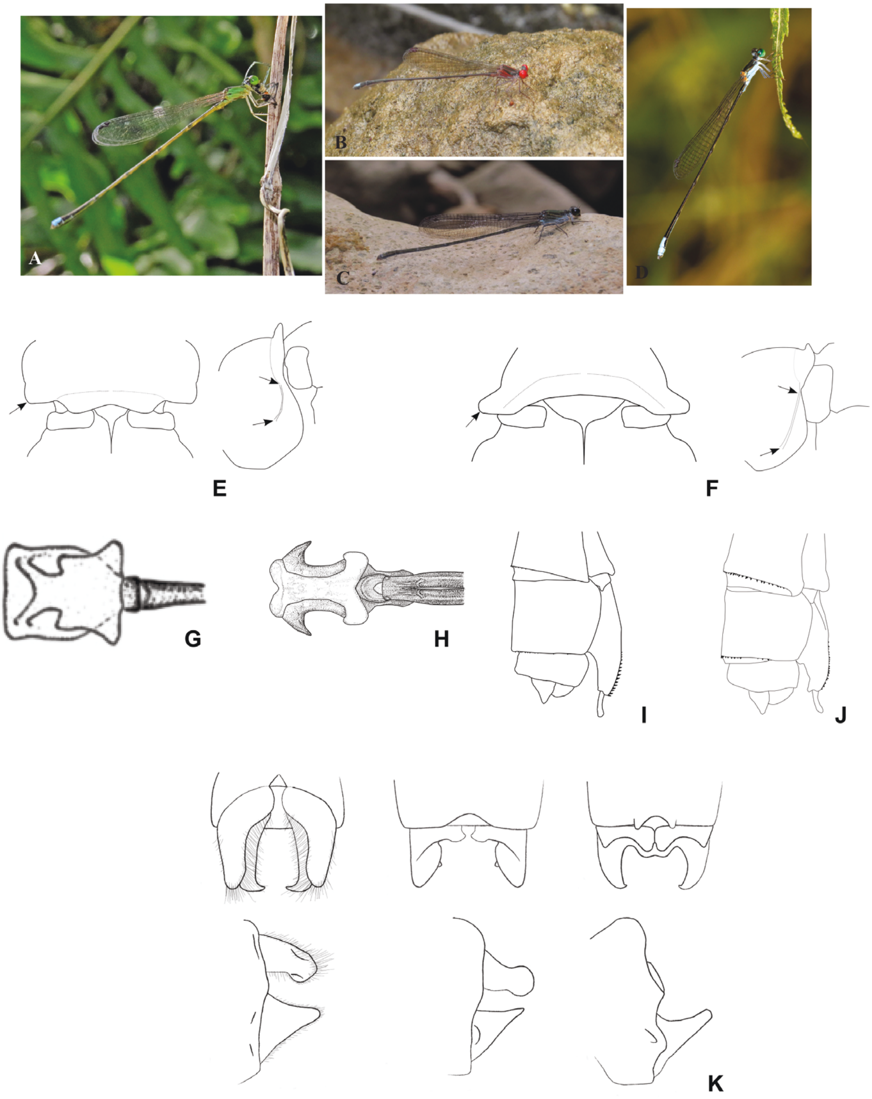

Fig. 6. Diagnosis of *Nikoulabasis* in comparison to *Nesobasis* and *Vanuatubasis*: (A-D) habitus of *Vanuatubasis bidens* (A), *Nesobasis erythrops* (B), *Nikoulabasis comosa* (C), *Nesobasis caerulecaudata* (D); (E–F) postero-lateral corner of pronotum: not-keeled (E), keeled (F); (G–H) penises: with broad distal lobe (G), narrow distal lobe (H); (I–J) ovipositors: coarsely dentated (I), finely dentated (J); (K) male appendages of (left to right): *Nikoulabasis, Nesobasis,* and *Vanuatubasis.*

distinct phylogenetic relationships, 2) the morphology is easily diagnosable, and 3) the biogeography aligns with tectonic analyses.

The species relationships of *Nesobasis* recovered in our phylogenetic reconstruction align with other phylogenies (Beatty et al. 2017; Lorenzo-Carballa et al. 2019). The previous phylogenies recovered the *longistyla* group as sister to the *erythrops* group, with the *comosa* group being sister to the *longistyla* + *erythrops* group. Our phylogeny placed *Vanuatubasis* as sister to the *longistyla* + *erythrops* group and the *comosa* group as sister to *Vanuatubasis* + *longistyla* group + *erythrops* group. The species recovered were supported as monophyletic. However, some of the species' relationships within the groups differed between the phylogenies. Most differences between our species relationships and those of Beatty et al. (2017) and Lorenzo-Carballa et al. (2019) are likely due to an additional gene (PRMT) and additional taxa included in our phylogenetic reconstruction. All generic relationships show high nodal support with three distinct genera, supporting our hypothesis that *Vanuatubasis* not be subsumed into *Nesobasis* and justifying our reasoning for the erection of the new genus.

Comparatively, *Vanuatubasis* resembles *Nesobasis,* and the morphology supports a close relationship, but *Vanuatubasis* differs from *Nesobasis* in the following characters: 1) the cerci of males are broad and short; 2) the paraprocts of males are significantly longer than the cerci, forceps-like, apically curved inwards; and 3) the pronotal hind lobe is raised and medially protruding, tapering to a rounded or acute apex (Ober & Staniczek 2009). However, the relationship between the *Nikoulabasis* and *Nesobasis* is more complicated due to overwhelming differences in the morphology (*see Donnelly 1990), which* seems to support a more distant relationship.

Although our research did not have biogeographical analyses, our data does align with what is currently known about the important biogeographical connection between Fiji and Vanuatu (Heads 2008). Some studies highlight the islands of Fiji and Vanuatu being much closer by about 10 Mya and have only recently been diverging over the past 7 Mya (Taylor, Gascoyne, & Colley 2000; Hall 2002), which may suggest why there is a unique relationship between the genera, *Nikoulabasis* and *Nesobasis*, not being a closely related as initially thought. Since we do not have data, we can only speculate on the potential dispersal events that have led to a unique evolutionary history of these genera. Further analyses, taking into account the geological history, will be critical in pushing our knowledge of the distribution of South Pacific damselflies further.

Currently, there are only three described *Vanuatubasis* species, with at least six more to be described (Marinov et al. 2019; Saxton et al. 2022). However, there remains a general gap in our knowledge of *Vanuatubasis* and Odonata diversity across the Vanuatu archipelago and the South Pacific. Thus, more collecting across South Pacific islands is necessary to understand damselfly evolution better. We also encourage other taxonomists to build future conclusions on large sample sizes. When building phylogenies, for example, when possible, including as much distributional and ecological information and as many of the known taxa as possible will further our ability to better understand the evolution of these extraordinary organisms.

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Supplementary material

Supplementary material is available online at:
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