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Cryptic Biodiversity and Phylogeographic Patterns of Seychellois *Ligia* Isopods

Carlos A. Santamaria
University of South Florida, csantamaria@usf.edu

Joanna K. Bluemel
Marine Conservation Society Seychelles

Nancy Bunbury
Seychelles Islands Foundation

Melinda Curran
Island Conservation Society

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Cryptic biodiversity and phylogeographic patterns of Seychellois *Ligia* isopods

Carlos A Santamaria Corresp., [1, 2] , **Joanna K Bluemel** [3] , **Nancy Bunbury** [4] , **Melinda Curran** [5]

[1] Biology Faculty, College of Science and Mathematics, University of South Florida Sarasota-Manatee, Sarasota, FL, United States
[2] Department of Biological Sciences, Sam Houston State University, Huntsville, Texas, USA
[3] Marine Conservation Society Seychelles, Mahé, Seychelles
[4] Seychelles Islands Foundation, Mahé, Seychelles
[5] Island Conservation Society, Mahé, Seychelles

Corresponding Author: Carlos A Santamaria
Email address: csantamaria@sar.usf.edu

Ligia isopods are conspicuous inhabitants of rocky intertidal habitats exhibiting several biological traits that severely limit their dispersal potential. Their presence in patchy habitats and low vagility may lead to long term isolation, allopatric isolation and possible cryptic speciation. Indeed, various species of *Ligia* have been suggested to represent instead cryptic species complexes. Past studies; however, have largely focused in Eastern Pacific and Atlantic species of *Ligia*, leaving in doubt whether cryptic diversity occurs in other highly biodiverse areas. The Seychelles consists of 115 islands of different ages and geological origins spread across the western Indian Ocean. They are well known for their rich biodiversity with recent reports of cryptic species in terrestrial Seychellois organisms. Despite these studies, it is unclear whether coastal invertebrates from the Seychelles harbor any cryptic diversity. In this study, we examined patterns of genetic diversity and isolation within *Ligia* isopods across the Seychelles archipelago by characterizing individuals from locations across both inner and outer islands of the Seychelles using mitochondrial and nuclear markers. We report the presence of highly divergent lineages of independent origin. At Aldabra Atoll, we uncovered a lineage closely related to the *Ligia vitiensis* cryptic species complex. Within the inner islands of Cousine, Silhouette, and Mahé we detected the presence of two moderately divergent and geographically disjunct lineages most closely related to *Ligia dentipes*. Our findings suggest that the Seychelles may harbor at least three novel species of *Ligia* in need of description and that these species may have originated independently.

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Carlos A. Santamaria[1,2], Joanna K. Bluemel[3], Nancy Bunbury[4], Melinda Curran[5]

[1]: Biology Faculty, College of Science and Mathematics, University of South Florida Sarasota-Manatee, Sarasota, Florida 34243, USA

[2]: Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341, USA

[3]: Marine Conservation Society Seychelles (MCSS), Mahé, Seychelles

[4]: Seychelles Islands Foundation, La Ciotat Building, Mont Fleuri, Mahé, Seychelles

[5]: Island Conservation Society, Pointe Larue, Mahé, Seychelles

Corresponding Author:

Carlos A. Santamaria

Email address: santamaria.carlos.a@gmail.com

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ABSTRACT

Ligia isopods are conspicuous inhabitants of rocky intertidal habitats exhibiting several biological traits that severely limit their dispersal potential. Their presence in patchy habitats and low vagility may lead to long term isolation, allopatric isolation and possible cryptic speciation. Indeed, various species of *Ligia* have been suggested to represent instead cryptic species complexes. Past studies; however, have largely focused in Eastern Pacific and Atlantic species of *Ligia*, leaving in doubt whether cryptic diversity occurs in other highly biodiverse areas. The Seychelles consists of 115 islands of different ages and geological origins spread across the western Indian Ocean. They are well known for their rich biodiversity with recent reports of cryptic species in terrestrial Seychellois organisms. Despite these studies, it is unclear whether coastal invertebrates from the Seychelles harbor any cryptic diversity. In this study, we examined patterns of genetic diversity and isolation within *Ligia* isopods across the Seychelles archipelago by characterizing individuals from locations across both inner and outer islands of the Seychelles using mitochondrial and nuclear markers. We report the presence of highly divergent lineages of independent origin. At Aldabra Atoll, we uncovered a lineage closely related to the *Ligia vitiensis* cryptic species complex. Within the inner islands of Cousine, Silhouette, and Mahé we detected the presence of two moderately divergent and geographically disjunct lineages most closely related to *Ligia dentipes*. Our findings suggest that the Seychelles may harbor at least three novel species of *Ligia* in need of description and that these species may have originated independently.

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INTRODUCTION

The Seychelles encompass some 115 islands (Stoddart, 1984) spread across a large swath of the western Indian Ocean. These islands can be broadly divided into outer and inner islands based on their geological origin, geographic locality, and unique geological histories (Braithwaite 1984). The "outer" islands are low-lying coralline islands that vary in size, age and geographic distribution, with some closer to Africa than to other Seychelles islands (e.g. Aldabra Atoll), and others closer to the "inner" islands. The inner islands, on the other hand, consist of granitic islands thought to have formed as the Indian and Madagascar Plates separated during the breakup of Gondwana some 65 million years ago (Mya) (Plummer & Belle 1995). The inner islands comprise of some 40 islands, including major ones such as Mahé, Praslin and Silhouette as well as smaller islands encircling them (Fig 1). The complex geological history of the Seychelles islands coupled with their remoteness may help explain a richly biodiverse fauna characterized by high rates of endemism and for the presence of highly divergent cryptic lineages recently reported across a variety of Seychellois terrestrial (e.g. Rocha et al. 2011; Rocha et al. 2013; Silva et al. 2010) and freshwater (e.g. Daniels 2011) organisms. These recent reports indicate that our understanding of the Seychelles fauna may be incomplete and that cryptic species may exist in poorly studied Seychellois organisms. This is particularly true for organisms in which cryptic lineages have been reported from other highly isolated archipelagos.

Ligia isopods have been reported to exhibit high levels of cryptic diversity in several regions of the world, including highly isolated habitats such as the Hawaiian islands (Santamaria et al. 2013; Taiti et al. 2003). In this remote and isolated archipelago, *Ligia hawaiensis* was thought to be the only intertidal species of this genus to occupy this habitat (Schmalfuss 2003). This species, however, appears to represent a paraphyletic taxon composed of several highly divergent (>10% COI K2P) lineages (Santamaria et al. 2013). These results indicate that this species may be actually several cryptic species. The high levels of divergence observed in *L. hawaiensis* match those reported in other *Ligia* species (Eberl et al. 2013; Hurtado et al. 2010; Jung 2008; Santamaria et al. 2014; Santamaria et al. 2013; Taiti et al. 2003) and are thought to be a product of their biological traits that severely restrict dispersal abilities: poor desiccation resistance, poor ability to swim, direct development, and inhabitance of patchy rocky habitats. Such traits, when combined with the isolation of oceanic archipelagos can easily disrupt gene flow leading to local cryptic speciation in populations. This leads to the question of whether *Ligia* populations in isolated and remote islands such as those of the Seychelles may also harbor cryptic lineages.

In the Seychelles, *Ligia* isopods have been reported from both inner (Taiti 2014) and outer islands (Ferrara & Taiti 1985). The earliest published record from the Seychelles archipelago indicates the presence of *Ligia exotica* at Aldabra (Ferrara & Taiti 1985). This record is based on of a single 8-mm long juvenile specimen collected at Aldabra Atoll in 1983. More recently, the presence of *Ligia dentipes*, a species redescribed and illustrated by Ferrara and Taiti (1982), from the island of Silhouette was reported (Taiti 2014). These reports are based on morphological identification and in some cases of partial or incomplete organisms, casting doubt on some of the identifications (e.g. *L. exotica* from Aldabra) and leaving unanswered whether Seychelles *Ligia* harbor highly divergent lineages. In this study, we apply molecular and morphological approaches to *Ligia* individuals collected in both the inner and outer islands to determine which *Ligia* species inhabit the Seychelles and whether they harbor any highly divergent cryptic lineages that may present putative cryptic species.

MATERIALS AND METHODS

We collected *Ligia* individuals by hand in locations in the inner and outer islands of the Seychelles Archipelago, Zanzibar and mainland Tanzania, and Sri Lanka. All specimens were collected during 2014, field-preserved in 70% Ethanol, and frozen upon arrival at the laboratory. Collections in the Seychelles were carried out under Seychelles Bureau of Standards permit A0157. The data produced from these samples was complemented using publicly available sequences for four *Ligia* species: *L. occidentalis* (Mexico), *L. exotica* (Mexico), *L. dentipes* (Thailand), and *L. vitiensis* (Indonesia, Timor-Leste, Madagascar). Detailed information for all localities is provided in Table 1.

We extracted total genomic DNA from pereopods/pleopods for 10 individuals per locality following the solid tissue protocol of the ZR Quick-gDNA Miniprep Kit (Zymo Research). During dissections, male specimens were identified to species by visually inspecting the appendix masculina of the second pleopod and comparing it to illustrations from species descriptions and re-illustrations (Ferrara & Taiti 1982; Khalaji-Pirbalouty & Wägele 2010; Taiti et al. 2003; Taiti et al. 1992). For *Ligia* collected in the Seychelles, we PCR amplified a 710-bp segment of the Cytochrome Oxidase I mitochondrial gene (hereafter COI) and a 710-bp fragment of the α-subunit of the Sodium-Potassium ATPase gene (hereafter NaK) for 1–10 individuals per locality using previously published primers and conditions (COI: Folmer et al. 1994; NaK: Tsang et al. 2008). We PCR-amplified an additional three mitochondrial genes for a subset of individuals from each locality in the Seychelles (see Fig 1, Table 1): (a) ~490-bp of the 16S rDNA gene (primers 16Sar/16Sbr; Palumbi 1996); ~495-bp of 12S rDNA (primers crust-12Sf/crust-12Sr; Podsiadlowski & Bartolomaeus 2005); and a 361-bp fragment of the Cytochrome-b (Cytb) gene (primers 144F/151F and 270R/272R; Merritt et al. 1998). For *Ligia*

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from localities outside the Seychelles, we amplified all four mitochondrial genes mentioned above for 1–2 individuals per locality (see Table 1). Positive PCR amplicons were identified using gel electrophoresis prior to cleaning and sequencing at the University of Arizona Genetics Core (UAGC). Sequences were assembled and edited (i.e. primers removed) using Geneious R8.0.2. No evidence indicative of pseudo-genes (i.e. no gaps, indels, early stop codons) was observed in any of the protein coding genes used in this study (i.e., NaK, cyt-b, and COI).

We used TCS v1.21 (Clement et al. 2000) to visualize the relationships between COI haplotypes recovered from *Ligia* from all Seychelles localities to both determine the geographic distribution of COI haplotypes recovered within the archipelago and the levels of fine scale divergence within the archipelago. We calculated the 95% most parsimonious branch connections between haplotypes under the cladogram estimation algorithm of Templeton et al. (1992) with all other settings as default. We also calculated Kimura 2-Parameter distances (K2P) within and between localities for COI in MEGA v7.0.7 (Kumar et al. 2016). We estimated relationships between NaK alleles using a similar approach.

We combined the four mitochondrial gene fragments produced in this study with publicly available sequences for other *L. vitiensis* from the Indo-Pacific and for two other *Ligia* species to be used as outgroups (i.e. *Ligia occidentalis* and *Ligia exotica*). Locality and sequence information for these sequences is presented in Table 1. Since ribosomal genes used in this study exhibit secondary structure that may lead to ambiguous alignments, we aligned each gene dataset separately using MAFFT v.7.0 (Katoh & Standley 2013) as implemented under the GUIDANCE2 algorithm (Sela et al. 2015) in the GUIDANCE server (http://mafft.cbrc.jp/alignment/server/) using 100 bootstrap replicates and all other settings as default. This approach produces confidence scores for each nucleotide position and sequence in the alignment that aid in the non-biased removal of misaligned nucleotide positions. We excluded nucleotide positions from our final alignment if they: (a) produced a confidence score below 1.00 in the Guidance alignments, and/or (b) if they exhibited obvious misalignments. We used MEGA v7.0.7 (Kumar et al. 2016) to estimate pairwise K2P genetic distances for COI and for the 16S rDNA genes, after excluding ambiguously aligned sites.

We used jModeltest v2.1 (Darriba et al. 2012) to determine the most appropriate model of nucleotide evolution for the final concatenated mitochondrial alignment as well as for each mitochondrial gene. We selected from 1,624 models by evaluating their likelihoods on a fixed BioNJ-JC tree under the Bayesian Information Criterion (BIC). We used the most likely model of nucleotide evolution chosen by jModeltest in our phylogenetic reconstructions with two general exceptions. When the chosen model was not implemented by the software, we applied the next more complex model implemented. When the chosen model implemented the joint estimation of Γ and I, we used a simpler +Γ model as the joint estimation of Γ and I can be problematic (see RAxML manual; and pages 113-114 of Yang 2006).

We carried out Maximum Likelihood phylogenetic reconstructions in RAxML v8.2.6 (Stamatakis 2014; Stamatakis et al. 2008) and GARLI v2.0 (Zwickl 2006). In RAxML, we used the Rapid Bootstrap Algorithm to carry out 1,000 bootstrap replicate searches followed by a thorough ML search under the GTR +Γ model. All other settings were as default. Searches in GARLI consisted of 1,000 bootstrap replicates using the appropriate model of evolution identified by jModeltest, with all other settings as default. For each search, we produced a majority-rule consensus tree of all bootstrap replicates using the SumTrees command of DendroPy v3.10.1 (Sukumaran & Holder 2010).

We also carried out phylogenetic reconstructions under Bayesian Inference. Parameters used in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) searches are presented in Table 2. All other parameters used were as default. We also carried out Bayesian searches implementing polytomy priors (Lewis et al. 2005) under Phycas v2.2.0 (Lewis et al. 2015) as to ensure that support values produced by MrBayes were not overestimated (i.e., "star-tree paradox") (Suzuki et al. 2002). We determined if Bayesian analyses had reached stationarity prior to estimating the posterior probability for each node by building majority-rule consensus trees of the stationary stage of each run using the SumTrees command (Sukumaran & Holder 2010). Samples prior to stationarity were discarded as "burnin."

We repeated all phylogenetic searches under three partitioning schemes: (a) all positions within a single partition; (b) positions partitioned by gene; and (c) the best partitioning scheme according to the BIC implemented in PartitionFinder v1.0.0 (Lanfear et al. 2012). PartitionFinder searches were run using the following settings: branch lengths = linked; models = all; model selection = BIC; search = greedy; and an *a priori* partitioning scheme accounting for codon positions and genes.

RESULTS

We sequenced COI and NaK for a total of 50 individuals from across five locations in the Seychelles archipelago. All individuals from the inner islands of the Seychelles were putatively identified as *L. dentipes* based on the gonopod morphology, while those from Aldabra were putatively assigned to *L. vitiensis*. All sequences produced in this study have been deposited in GenBank under accession numbers: XXXXXX-XXXXXXX.

COI and NaK haplotype networks

For COI, we observed a total of 16 haplotypes divided into three networks (Fig 3). Two haplotypes separated by two mutational steps were found solely in individuals collected at Aldabra. A second haplotype network contained all COI individuals collected from Cousine island (E1) and consisted of seven haplotypes separated by 2–9 steps. A third and final network consisted of the seven COI haplotypes recovered from Silhouette (E2) and Mahé (E3, E4) which were separated by 2–15 steps. We recovered two alleles for the NaK gene, with all *Ligia* individuals from the inner islands (E2–E4) sharing a single allele that diverged by 27 steps from an allele found in all the individuals from Aldabra (E1).

Phylogenetic reconstructions

Our concatenated mitochondrial dataset included a total of 16 *Ligia* individuals: five from localities within the Seychelles, nine from other Indo-Pacific localities, and the two outgroups. The final concatenated alignment included 2019 nucleotides, of which 292 positions could not be confidently aligned and were excluded for the phylogenetic analyses (16S rDNA: 138; 12S rDNA: 154). Of the resulting 1733 nucleotides positions, 579 were parsimony informative. jModeltest identified a model consisting of three substitution rates (rate matrix: 012010; see jModeltest manual) as well as +F, +I, and +Γ parameters for our final concatenated mitochondrial dataset under the BIC, AIC, and AICc. We applied this model in GARLI analyses; however, the more complex GTR +Γ model was applied in all other searches as the chosen model is not available in the other software packages (e.g. RAxML, Phycas). The use of GTR was justified as it was included in the 99% cumulative weight interval under all three selection criteria.

Mitochondrial phylogenetic reconstructions (Fig 2) recovered a well-supported split [100 Bootstrap Support (BS) and Posterior Probability (PP)] between the *Ligia* specimens from the Indo-Pacific and the two outgroup taxa. Within the Indo-Pacific *Ligia*, we observed a split between two highly divergent lineages: an 'East African' clade (*Clade ABC*; BS: 71–96; PP: 91–99) and an 'Asian' clade (*Clade DEF*; BS: 100; PP: 100). COI K2P divergences between these two clades ranged from 21.8–27.8% with a mean divergence of 25.2%.

The 'East African' clade (*Clade ABC*) contained all specimens identified as *L. vitiensis* and included the localities of Aldabra Atoll in the Seychelles, Nosy Bay in Madagascar, three localities in Tanzania (i.e., Dar-Es-Salaam, Kilwa Masoko, and Stone Town), and two localities from the Indonesian Archipelago (Komodo, Indonesia and Dili, Timor-Leste). These localities formed three distinct lineages: (a) *Ligia* from Madagascar, mainland Tanzania, and Aldabra (Seychelles) were placed in the well-supported *Clade A* (light orange in all figures; BS: 100; PP: 100); (b) Indonesian archipelago *Ligia* were placed in *Clade B* (cyan in all figures; BS: 100; PP: 100); and (c) *Clade C* was composed of *Ligia* from Stone Town, Zanzibar, Tanzania (light grey in all figures). Although some analyses suggested a sister relationship between clades *A* and *B*, supports for this relationship was low in ML analyses (BS: < 60) and highly variable in BI (PP: 71–100). COI K2P divergences between the three main lineages in the East African clade ranged from 7.12–9.09% (Table 3).

The 'Asian' clade (*DEF*) included all individuals identified as *L. dentipes* which were separated into three main lineages: (a) *Clade D* (red in all figures; BS: 91–100, PP:100) included *Ligia* from Galle (Sri Lanka) and Phuket (Thailand); (b) *Clade E* (blues in all figures; BS: 88–100; PP: 100) consisting of *Ligia* from the inner islands of Seychelles; and (c) *Clade F* (green in all figures) represented solely from *Ligia* collected in Trincomalee, Sri Lanka. As in *Clade ABC*, relationships within the 'Asian' clade were not well resolved. We observed a sister relationship between clades *E* and *F*; however, support values for this relationship were often low (BS: <60–74; PP: <60–65). COI K2P divergences between the three main lineages in the Asian clade ranged from 12.07–14.83% (Table 3). Divergences within the inner Seychelles locations (i.e. *Clade E*) ranged from 0.5–5.2% with all comparisons to the island of Cousine being above 4.5% (Table 4).

DISCUSSION

To date, two species of *Ligia* have been reported to inhabit intertidal habitats in the Seychelles archipelago: *L. dentipes* (Taiti 2014) and *L. exotica* (Ferrara & Taiti 1985). The former is considered to be endemic to coastlines in the north-eastern Indian Ocean, with confirmed records from the Nicobar and Andaman islands, the Maldives, Sri Lanka, and the Seychelles (Taiti 2014 and references therein). *Ligia exotica*, on the other hand, is a species of possible East-Asian origin (Hurtado in preparation) thought to have a cosmopolitan distribution due to human-aided introductions. These records; however, are based on morphological identifications, leaving in doubt their validity and whether they represent highly divergent genetic lineages as reported for *Ligia* species in other regions (Eberl et al. 2013; Hurtado et al. 2010; Santamaria et al. 2014; Santamaria et al. 2013; Taiti et al. 2003). By applying molecular approaches to *Ligia* individuals from both inner and outer islands in the Seychelles, we have determined that *Ligia* diversity in the Seychelles archipelago is underreported. Our phylogenetic reconstructions and morphological identifications suggest the presence in the Seychelles of highly divergent lineages belonging to at least two species complexes: *L. vitiensis* and *L. dilatata*. *Ligia* from the outer island of Aldabra represent a highly divergent lineage within a clade composed of *L. vitiensis* individuals from around the Indo-Pacific, while those from the inner islands of Cousin, Mahé, and Silhouette represent two highly divergent lineages within a clade composed of *L. dentipes* individuals from Asia. These findings are supported by male gonopod morphology and suggest that *Ligia* have colonized the Seychelles archipelago on at least independent two occasions.

Phylogenetic reconstructions place *Ligia* individuals collected at Aldabra Atoll, in *Clade ABC* with *L. vitiensis* individuals collected from East Africa and Madagascar. Despite the poorly resolved relationships within this clade, the geological history of Aldabra, oceanographic patterns in the region, and previous phylogeographic findings suggest a Malagasy or East African origin to be the most likely source of the *Ligia* populations in Aldabra. This outer island is a low lying atoll that originated some 20 Mya from coral reefs growing on volcanic seamounts (Plummer 1995). The atoll does not appear to have experienced any connection to continental landmasses or other nearby-islands, but has undergone several periods of submersion and emergence, including what is thought to have been a complete submersion some 125,000 years ago (Braithwaite et al. 1973). Such geological history would seem to preclude Aldabra as the place of origin for *Ligia* in the *ABC clade*. Colonizers instead appear more likely to have originated in either Madagascar or East Africa given the proximity of Aldabra to these continental landmasses (~420km from the coastline of Madagascar and ~650km from the East African coastline).

Ligia individuals collected from the inner Seychelles islands of Cousin, Mahé, and Silhouette were placed in *Clade DEF* with *L. dentipes* individuals from Asia indicating either an Asian origin for these populations, or a Seychellois origin for *L. dentipes*. Although the relationships within *Clade DEF* are not well resolved, our findings suggest the Seychelles lineage to have evolved from an Asian ancestor: phylogenetic reconstructions place *Ligia* from the inner islands in *Clade E*, which is suggested to have split from an Asian ancestor (though poorly supported). Furthermore, the COI K2P divergences observed between *Ligia* from the inner islands and other *Ligia* from the region range from 12.07 to 27.62% (*L. dentipes*: 12.07–14.83% COI K2P; *L. vitiensis*: 21.85–27.62% COI K2P). These divergences would require mutation rates that are about six times lower than the mutation rate for COI reported for other marine isopods (2.5%/My; Ketmaier et al. 2003) in order to match the age of the inner Seychelles islands (~65 My; Plummer & Belle 1995). These findings, though not conclusive, would suggest an Asian origin for *Ligia* from the inner islands.

The finding that *Ligia* from the inner islands are most closely related to those from Asia represent a rare, yet not exceptional, occurrence for Seychellois flora and fauna. Overall, fauna endemic to the Seychelles appears to largely be of an East African or Malagasy origin (Agnarsson & Kuntner 2012 and references therein). Such findings are consistent with the closer proximity of these islands to East African and Madagascar than to Asian coastlines, and the predominant oceanic currents in the area. Nonetheless, an Asian affinity has been reported for several Seychellois organisms such as the Sooglossidae and Nasikabatrachidae anuran families (Biju & Bossuyt 2003), coleopterans (Gerlach 2009), and *Nepenthes* pitcher plants (Meimberg et al. 2001). Such affinity may be explained by Gondwanan organisms rafting on the Indian continent until its collision with Asia (Karanth 2006) or by the over-water dispersal from Asia to the Seychelles or vice-versa. This latter mechanism has been suggested as the origin for a few terrestrial organisms from the Seychelles (Agnarsson & Kuntner 2012 and references therein) and may explain the origin of *Ligia* in the inner islands. Our phylogeographic findings and morphological identifications coupled with the occurrence of *L. dentipes* across Indian Ocean archipelagos such as the Maldives (Taiti 2014) indicate the possibility of a colonization of the Seychelles archipelago after rafting or "island-hopping" throughout the Indian Ocean; however, the poor support for relationships within *Clade DEF* indicates additional research remains needed to determine the origin and *Ligia* from the inner islands of the Seychelles.

Additional work is also required to determine whether *Ligia* from the Seychelles represent yet to be described cryptic species. We report levels of genetic divergence between sampled individuals from the Seychelles and other members of their clades that exceed those proposed for species-level divergences (COI K2P > 3%; Hebert et al. 2003) and largely match or exceed those reported from comparisons between valid *Ligia* species (Hurtado et al. 2010; Santamaria et al. 2014; Santamaria et al. 2013; Taiti et al. 2003). *Ligia* from Aldabra exhibit COI K2P divergences of ~27% when compared to other *L. vitiensis* individuals from *Clade ABC*, while individuals from the inner islands show COI K2P divergences of ~15% from *L. dentipes* individuals in *Clade DEF*. Lastly, individuals from Cousine Island exhibit COI K2P divergences of 4.5–5.3% when compared to other localities (Mahé and Silhouette) within the inner islands. Interestingly, this pattern is similar to those previously reported for Seychellois herpetofauna from the inner islands (Rocha et al. 2011; Rocha et al. 2013; Silva et al. 2010). The sum of our findings thus suggest the presence of 2–3 putative cryptic species of *Ligia* in the Seychelles: Aldabra *Ligia* being one, and those of Cousine and Silhouette+Mahé representing one or two putative species.

Morphological inspections are needed to determine whether diagnostic characters exist that differentiate between these highly divergent lineages. Uncovering such differences may prove difficult, as previous morphological comparisons of highly divergent *Ligia* lineages within the same species complex suggest lineages to be nearly morphologically identical (Santamaria et al. 2016; Santamaria et al. 2013; Taiti et al. 2003). Our findings also suggest that additional lineages may occur in other *Ligia* populations across the Seychelles, particularly in yet to be sampled outer islands.

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Figure 1(on next page)

Map of sampled localities across the Seychelles

Localities are A1-Basin Cabri, Aldabra Atoll, Seychelles; E1-Cousine Island, Seychelles; E2-Silhouette Island, Seychelles; E3-L'Islette, Western Mahé, Seychelles; E4-Anse Parnel, South-eastern Mahé, Seychelles. Colors and labels for each locality correspond with other figures and tables. Detailed information for each locality is presented in Table 1.

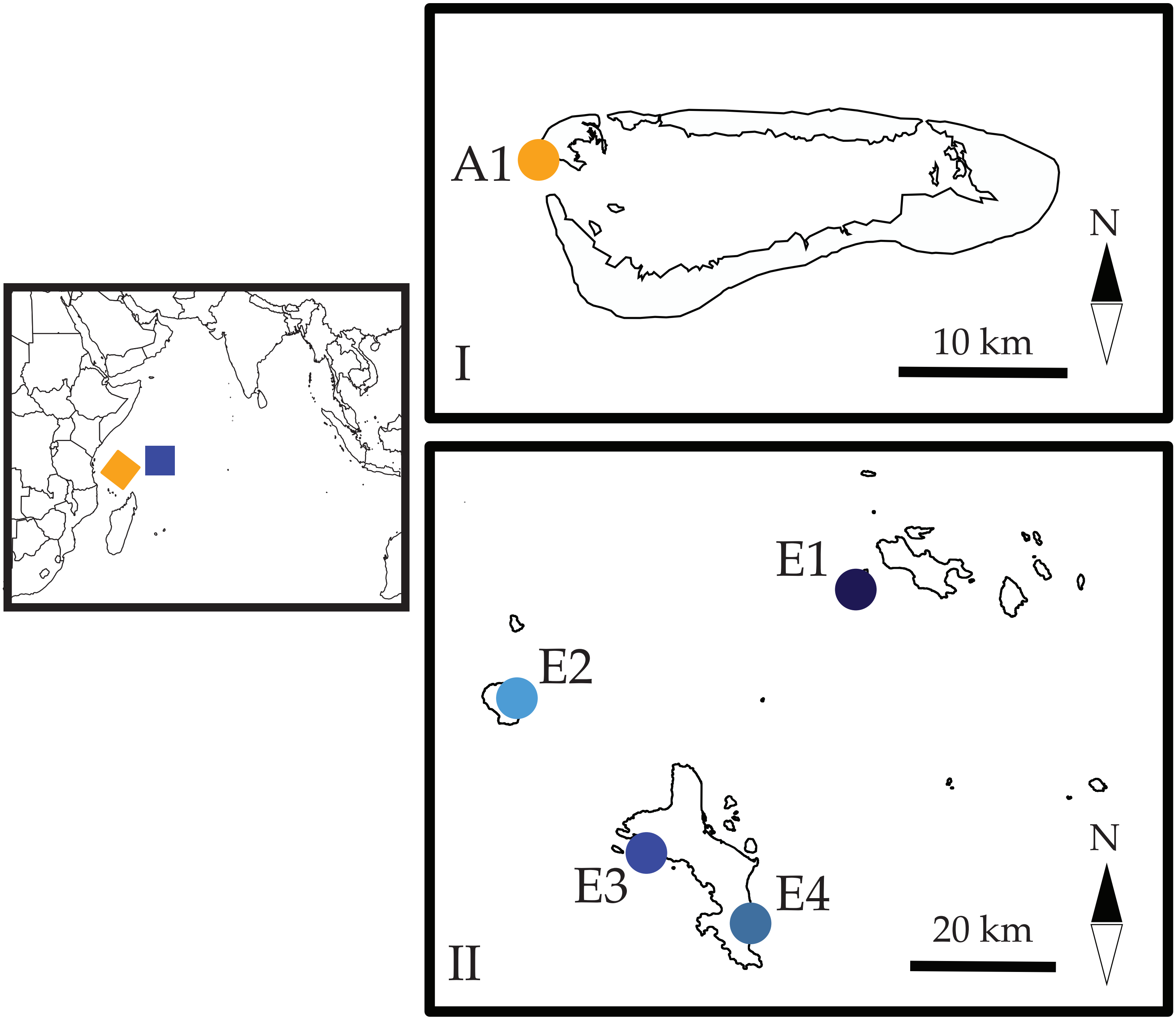
A1
I
10 km
N
E1
E2
E3
E4
II
20 km
N

Figure 2(on next page)

Majority rule consensus tree produced by Bayesian Analysis (GTR +Γ, unparitioned, Phycas) of the concatenated mitochondrial dataset of *Ligia* samples and outgroups included in this study

Numbers by nodes indicate the corresponding range of percent Bootstrap Support (BS; top) for Maximum likelihood; and Posterior Probabilities (PP; bottom) for Bayesian inference methods. Nodes receiving 100% for all methods are denoted with an * while an NS indicates less than 50% node support. Colors represent major lineages as discussed in text.

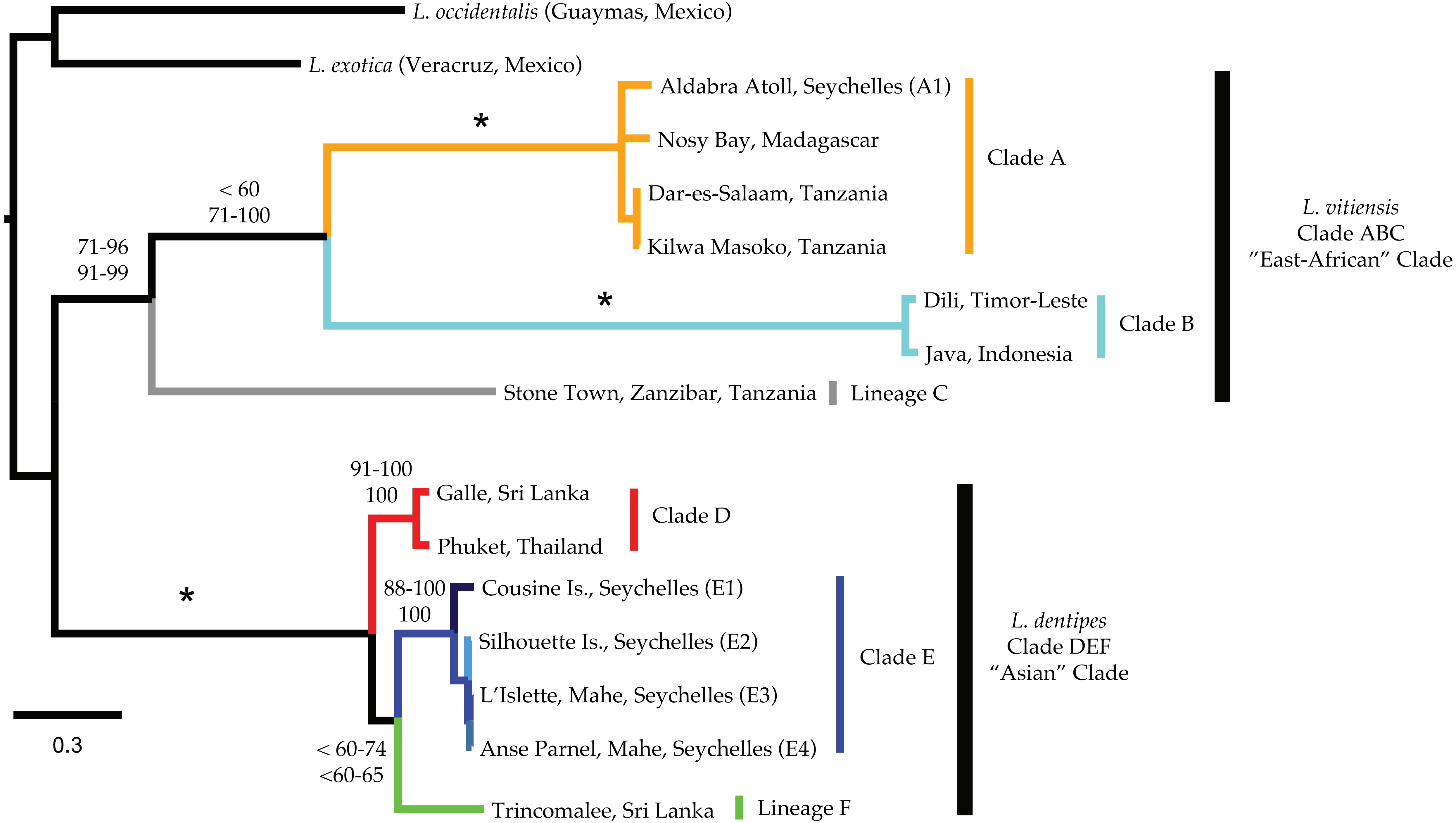

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Figure 3(on next page)

COI haplotype networks for Seychellois *Ligia*

Colors and Locality IDs correspond with those use in all other Figures in Figure 1. Empty circles and hash marks represent unsampled (i.e., missing) haplotypes, while the size of circles is proportional to the frequency at which each haplotype was recovered.

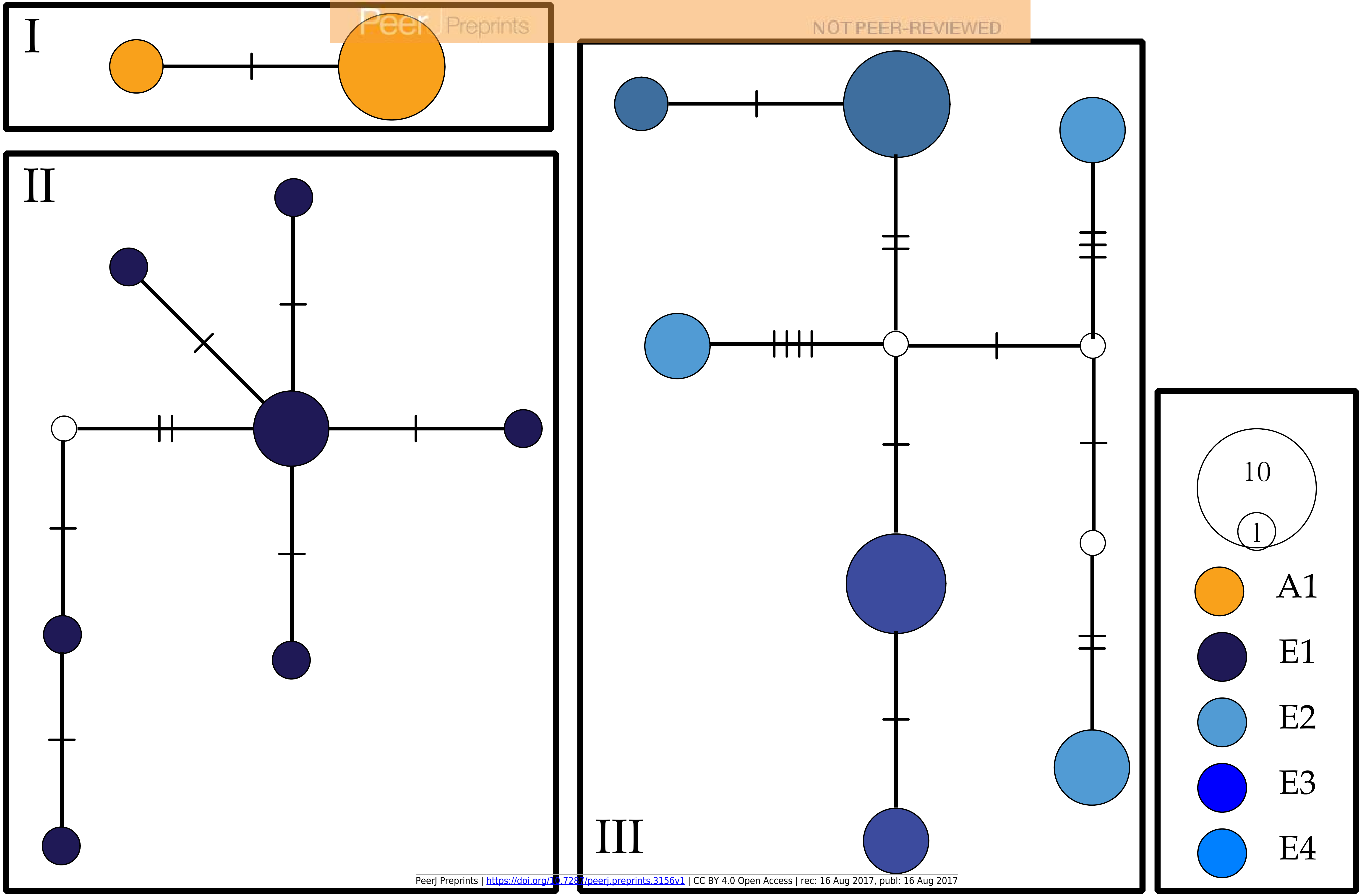

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Table 1(on next page)

Localities included and corresponding GenBank Accession Numbers for all genetic markers used, latitude, and longitude

Map labels correspond with other figures and tables

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Species	**Locality**	**Map Label**	**16S Acc. No.**	**12S Acc. No.**	**COI Acc. No.**	**Cytb Acc. No.**	**NaK acc no**	**Latitude**	**Longitude**
L. vitiensis	Basin Cabri, Aldabra Atoll, Seychelles	A1	XXXXX	XXXXX	XXXXX	XXXXX	XXXXXXX	N/A	N/A
L. vitiensis	Nosy Be, Madgascar		AY051342	N/A	AY051323	N/A	N/A	N/A	N/A
L. vitiensis	The Slipway, Dar-Es-Salaam, Tanzania		XXXXX	XXXXX	XXXXX	XXXXX	N/A	6°45'06.9"S	39°16'18.6"E
L. vitiensis	Kilwa Masoko, Tanzania		XXXXX	XXXXX	XXXXX	XXXXX	N/A	8°55'42.7"S	39°31'17.9"E
L. vitiensis	Dili, Timor-Leste		KF546556	KF546581	KF546662	KF546725	N/A	N/A	N/A
L. vitiensis	Parangtritis, Java, Indonesia		KF546554	KF546582	KF546665	KF546727	N/A	8°01'46.4"S	110°20'29.8"E
L. vitiensis	Stone Town, Zanzibar, Tanzania		XXXXX	XXXXX	XXXXX	XXXXX	N/A	6°09'33.8"S	39°11'26.4"E
L. dentipes	Galle, Sri Lanka		XXXXX	XXXXX	XXXXX	XXXXX	N/A	6°01'49.9"N	80°13'03.3"E
L. dentipes	Patong Bay, Phuket, Thailand		KF555801	KF555838	KF555841	KF555754	N/A	7°53'11.0"N	98°17'10.3"E
L. dentipes	Cousine Island, Seychelles	E1	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	4°20'55.3"S	55°38'41.9"E
L. dentipes	Silhouette Island, Seychelles	E2	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	4°29'08.4"S	55°15'12.4"E
L. dentipes	L'Islette, W. Mahé, Seychelles	E3	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	4°39'46.7"S	55°24'35.0"E
L. dentipes	Anse Parnel, S.E. Mahé, Seychelles	E4	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	4°46'01.1"S	55°31'19.3"E
L. dentipes	Dutch Bay, Trincomalee, Sri Lanka		XXXXX	XXXXX	XXXXX	XXXXX	XXXXX	8°33'52.6"N	81°14'27.8"E
L. occidentalis	Guaymas, Mexico		KF546553	KF546583	KF546666	KF546728	N/A	27°54'44.3"N	110°56'49.6"W
L. exotica	Veracruz, Mexico		KF546552	KF546584	KF546664	KF546726	N/A	19°11'40.2"N	96°07'24.4"W

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Table 2(on next page)

Settings for Maximum Likelihood and Bayesian analyses for the concatenated mitochondrial dataset

Software	Model & Priors[A]	Part Scheme[B]	iterations gen./bootstrap replicates	Sample Freq	Runs/Chain	Burnin	ASDSF[C]	Bayes Factor/ ML Scores (-lnL) [D]	ESS >200[E]	PSRF[F]
RAXML	GTR + Γ	Unpart	1,000	n/a	n/a	n/a	n/a	-9006.5300	n/a	n/a
RAXML	GTR + Γ	Gene	1,000	n/a	n/a	n/a	n/a	-8865.7562	n/a	n/a
RAXML	GTR + Γ	BP	1,000	n/a	n/a	n/a	n/a	-8334.7701	n/a	n/a
Garli	012010 + Γ + F	Unpart	1,000	n/a	n/a	n/a	n/a	-8986.6239	n/a	n/a
Garli	Mixed Model	Gene	1,000	n/a	n/a	n/a	n/a	-8972.0488	n/a	n/a
Garli	Mixed Model	BP	1,000	n/a	n/a	n/a	n/a	-8212.4919	n/a	n/a
MrBayes	GTR + Γ	Unpart	2 x 10^8	5,000	4	25%	0.000724	-8959.8260	Yes	1
MrBayes	GTR + Γ	Gene	2 x 10^8	5,000	4	25%	0.001157	-8928.4492	Yes	1
MrBayes	GTR + Γ	BP	2 x 10^8	5,000	4	25%	0.001170	-8608.5042	Yes	1
Phycas	GTR + Γ	Unpart	1*10^6	50	n/a	25%	n/a	-8959.6281	Yes	n/a
Phycas	GTR + Γ	Gene	1*10^6	50	n/a	25%	n/a	-8879.1040	Yes	n/a
Phycas	GTR + Γ	BP	1*10^6	50	n/a	25%	n/a	-8324.1730	Yes	n/a

[A]: All others default; [B]: BP as indidcated by PartitionFinder (1: 16S+12S+Cytb 2nd codons; 2: COI 1st codons+Cytb 1st codons; 3: COI 2nd codons; 4: Cytb 3rd codons + COI 3rd codons); [C]: Average Standard Deviation of Split Frequencies; [D]: Estimated in Tracer v.1.5.; [E]: Effective Sample Size; [F]: Potential Scale Reduction Factor for all parameters.

Table 3(on next page)

Estimates of evolutionary divergence, as measured by Kimura 2-parameter distances, for main *Ligia* lineages from the study area and outgroups

When applicable minimum and maximum (top values) as well as average divergences (values in parentheses) are provided

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	Clade A	*Clade B*	*Clade C*	*Clade D*	*Clade E*	*Clade F*	*L. exotica*	*L. occidentalis*
Clade A	7.1–9.1% (4.3%)							
Clade B	22.9–24.7% (24.0%)	2.50%						
Clade C	25.8–28.1% (26.8%)	25.9–26.6% (26.2%)	N/A					
Clade D	25.3–27.1% (26.3%)	22.0–23.8% (22.8%)	25.9–27.2% (26.6%)	4.70%				
Clade E	24.3–27.6% (26.3%)	21.9–23.7% (22.1%)	24.7–25.4% (25.2%)	12.4–14.8% (13.7%)	0.5–4.7% (2.9%)			
Clade F	26.3–27.8% (27.3%)	23.3–23.75% (23.5%)	27.7%	13.3–14.2% (13.8%)	12.1–12.7% (12.3%)	N/A		
L. exotica	22.6–25.4% (23.4%)	25.7–25.7% (25.7%)	25.7%	25.9–28.1% (27.0%)	24.9–26.9% (25.9%)	26.3%	N/A	
L. occidentalis	27.5–28.3% (28.0%)	23.8–24.9% (24.3%)	24.6%	24.9%	19.0–20.9% (19.8%)	20.8%	25.6%	N/A

Table 4(on next page)

Estimates of evolutionary divergence, as measured by Kimura 2-parameter distances, for *Ligia* localities from Seychelles inner island localities

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	E1	**E2**	**E3**	**E4**
E1	-----			
E2	4.9%	-----		
E3	5.1%	0.8%	-----	
E4	5.2%	0.9%	0.5%	-----

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