

**OUT OF ASIA: EVOLUTIONARY HISTORY OF THE INVASIVE
SUPRALITTORAL ISOPOD *LIGIA EXOTICA***

A Thesis

by

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ABSTRACT

The supralittoral isopod *Ligia exotica* Roux, 1828 is an invasive species with a tropical and temperate cosmopolitan distribution, frequently found in harbors and ports. In the New World, this isopod has a broad distribution along the Atlantic coast, being particularly common in the US Gulf of Mexico, where it inhabits rocky artificial substrates. Although it has been suggested that *L. exotica* has an Old World origin, from where it was introduced to other regions via wooden ships and solid ballast, the native range of this isopod remains uncertain. Recent molecular work in East Asia uncovered the presence of two highly divergent lineages of *L. exotica*, and suggests that this region is the source of the nonindigenous US populations of Georgia and O'ahu, Hawaii. The goal of the present study was to better understand the evolution and invasion history of this isopod, based on phylogenetic analyses of a fragment of the mitochondrial 16S ribosomal rDNA gene. The dataset examined included publicly available sequences associated with published and unpublished work, as well as newly generated sequences from the Gulf of Mexico, South America, Hawaii, Africa, and Asia. Different Maximum Likelihood (ML) and Bayesian Inference (BI) programs were implemented to reconstruct the phylogeny of *L. exotica*.

Ligia exotica was comprised of several highly genetically divergent lineages, probably corresponding to a cryptic species complex. Most of the genetic diversity was detected in the region spanning Southeast to East Asia, which appears to constitute the native range of *L. exotica*. Temperature appears to influence the distribution and levels

of genetic diversity of *L. exotica* clades. Greater opportunities for diversification of *L. exotica* appear to have occurred in the warmer waters.

Phylogenetic patterns suggest that multiple independent invasions of *L. exotica* have occurred around the world. Haplotypes observed in nonnative populations belong only to two sister clades, suggesting that the potential to become invasive may be phylogenetically constrained. In Asia, these clades akin to the nonnative populations are distributed in warmer regions; thus, environmental similarity between donor and recipient regions might have increased the chance of a successful invasion.

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NOMENCLATURE

ACCTRAN	Accelerated transformation
AIC	Akaike information criterion
AICc	Corrected Akaike information criterion
BI	Bayesian inference
BIC	Bayesian information criterion
BS	Bootstrap support value
ESS	Effective sample size
K2P	Kimura-2-parameter
MCMC	Markov chain Monte Carlo
ML	Maximum likelihood
PP	Bayesian posterior probability
PSRF	Potential scale reduction factor
rDNA	Ribosomal DNA
SST	Sea surface temperature

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1. INTRODUCTION

Numerous marine invasive species have dispersed extensively throughout the world via marine vessels over the past several centuries (Banks et al., 2015; Carlton, 1987). Native ranges for a large number of them, however, remain elusive (i.e., they are cryptogenic), hampering our understanding of their invasion histories (Carlton, 1996). Use of molecular data can greatly aid in the identification of their native ranges, cryptic diversity, and of the source and recipient regions (Geller et al., 2010).

The supralittoral isopod *Ligia exotica* Roux, 1828, commonly known as wharf roach, has been considered an invasive species with a tropical and temperate cosmopolitan distribution, frequently found in harbors and ports (Schmalfuss, 2003; Taiti et al., 2003; Van Name, 1936; Yin et al., 2013). Numerous aspects of this isopod have been studied, including its ecology (Cha et al., 2013; Christofolletti et al., 2011; Lopes-Leitzke et al., 2009; Lopes-Leitzke et al., 2011; Sazima, 1970), physiology (Miyamoto et al., 2006; Miyamoto et al., 2007; Sakurai et al., 1999a; b; Tsai and Dai, 2001; Tsai et al., 1998; Yamagishi, 2007; Yamagishi et al., 2004), biomimetic properties (Tani et al., 2014), and its potential use as a source of food (Chai et al., 2011), natural products (Kim et al., 2000), and as a biomarker for coastal toxicology (Matsunaga et al., 2015; Qiu et al., 2016; Undap et al., 2013). Yet, the native range of *L. exotica* remains elusive.

An Old World origin has been proposed for *L. exotica*, from where it was unintentionally introduced around the world on wooden ships and solid ballast (Griffiths

et al., 2011; Van Name, 1936). The distribution of this isopod in the New World is broad, ranging in the Atlantic from New Jersey (US) to Montevideo (Uruguay), including the Gulf of Mexico (Mulaik, 1960; Schultz, 1977). Collections of *L. exotica* in the US Atlantic, eastern Gulf of Mexico, Brazil, and Uruguay date back to the 1880's; whereas records in the western Gulf of Mexico date back to the first half of the 20th century (Richardson, 1905; Van Name, 1936). Jetties and other man-made structures have provided suitable habitats for this isopod around the Gulf of Mexico (Schultz and Johnson, 1984). In the Pacific coast of the Americas, *L. exotica* has been reported from the Gulf of California, Mexico, to Punta Arenas, Chile (Van Name, 1936), but some records may be dubious (see below).

Similarly to the other coastal members of *Ligia*, *L. exotica* is a direct developer (i.e., lacks a planktonic larval stage; a feature of peracarids) that occupies a narrow vertical range between the supralittoral and the water line, mainly occurring on rocky substrates, man-made or natural (Hurtado et al., 2010; Santamaria et al., 2013). The broad distribution of *L. exotica* suggests that it possesses unique invasive capabilities. With the exception of *L. oceanica*, an endemic of the Atlantic coast of Europe that has been introduced to some localities in the northern Atlantic coast of the US (Richardson, 1905), all other coastal species of *Ligia* do not appear to have been introduced outside their range, or at least not to as many geographically distant places as *L. exotica* (Schmalzfuss, 2003). A set of physiological characteristics that may have contributed to its ability to travel to (on watercraft), and establish thriving populations at, distant

localities includes superior osmoregulatory ability and desiccation resistance (Tsai et al., 1997; 1998).

The taxonomy of *L. exotica* has been problematic. It was originally described from Marseille (France), within the range of its congener *L. italica*, which is broadly distributed throughout the Mediterranean basin (Schmalfuss, 2003). The invasive nature of *L. exotica* was recognized in its original description, as Roux (1828) considered that its origin was not France, but proposed that a ship had transported this isopod from Cayenne, French Guiana. He suggested this isopod might have survived such a voyage at the bottom of the hold, where small amounts of water could have provided the required moisture. Several species have been synonymized with *L. exotica* (*L. gaudichaudii*, *L. grandis*, and *L. olfersii*), whereas in the Indian and Pacific Ocean a number of very similar species occur, which have been identified as *L. exotica*, but may correspond to different species (Schmalfuss, 2003; Van Name, 1936). The Caribbean-endemic *Ligia baudiniana* Milne Edwards, 1840 appears to have been described based on individuals of *L. exotica* collected in Veracruz, Mexico (Santamaria et al., 2014). *Ligia exotica* may have also been confused with *L. occidentalis*, a native species from the Gulf of California and the Pacific region between the Baja Peninsula and southern Oregon. Despite being reported in the Gulf of California (Mulaik, 1960; Richardson, 1905), *L. exotica* was not found in a thorough collecting effort of *Ligia* along the shores of this basin and adjacent regions (Hurtado et al., 2010).

Recent molecular studies in East Asia (Jung et al., 2008; Yin et al., 2013) uncovered the presence of two highly divergent lineages of *L. exotica*, and indicated the

occurrence of a closely related species in this region (*L. cinerascens* Budde-Lund, 1885). Jung et al. (2008) re-assessed the previously reported (Kwon, 1993) occurrence of *L. exotica* in South Korea. They conducted molecular phylogenetic analyses of a fragment of the mitochondrial 16S ribosomal rDNA gene from individuals sampled along the South Korean coast, including previously reported sequences of *L. exotica* from two non-native US populations (i.e., Georgia and the Hawaiian island of O’ahu). They found two highly divergent clusters: the “eastern group”, which includes haplotypes occurring mainly along the eastern and southeastern coastlines of South Korea; and the “western group”, which includes haplotypes occurring mainly along the western and southwestern coastlines of South Korea. These two lineages were in turn highly divergent from the lineage comprised of the US haplotypes. Jung et al. (2008) suggested that the “western group”, “eastern group”, and the *L. exotica* lineage from the US, each represents a distinct species, and that *L. exotica* appeared to be absent from South Korea. Their understanding on the phylogenetic relationships of the three lineages was limited, however, due to the lack of outgroups in their dataset. Yin et al. (2013) conducted morphological and phylogenetic analyses of *Ligia* specimens sampled throughout the northeastern coastline of China. Their phylogenetic analyses also included the sequences examined by Jung et al. (2008), and used several distant taxa as outgroups. They found two highly genetically divergent lineages, and examination of traditional morphological characters indicated that one corresponded to *L. exotica* and the other to *L. cinerascens*. The “eastern group” sequences of South Korea, and those of Georgia and O’ahu, clustered within the *L. exotica* clade, whereas the “western group” sequences

of South Korea clustered within the *L. cinerascens* clade. Within the *L. exotica* clade, two highly divergent lineages were observed, one of which was comprised of the samples from Georgia and O’ahu, as well as the South Yellow Sea, leading Yin et al. (2013) to suggest that East Asia was a source of invasive *L. exotica* populations.

The presence of multiple divergent lineages in East Asia may indicate that the native range of *L. exotica* includes this region, which is supported by other lines of evidence. First, phylogenetic analyses examining the relationships within the genus *Ligia* (unpublished) indicate that the sister taxon of *L. exotica* is *L. cinerascens*, which also occurs in East Asia. Second, in other regions of the world where *L. exotica* is present, other more broadly distributed presumably native *Ligia* species occur. Whether the *L. exotica* clade extends further south in Asia and shows additional cryptic diversity, which will further support this region as the native range of this isopod, needs to be determined.

Sequences for the 16S rDNA gene identified as *Ligia* sp. from Southeast to East Asia have been released in GenBank, but phylogenetic analyses have not been published yet. Herein, we report phylogenetic analyses of a dataset that includes the above sequences, the ones reported for *L. exotica* and *L. cinerascens* from published studies, and new sequences we obtained from specimens of these isopods in the Gulf of Mexico, South America, Hawaii, Africa, and Asia. We tested whether the new sequences from Asia belong to the *L. exotica* or *L. cinerascens* lineages and show further cryptic diversity. We expected to find higher genetic diversity in *L. exotica* populations from Asia, as it seems to represent the native range of this isopod, than in presumed nonnative

populations (e.g. the New World). Phylogenetic analyses can also shed light on the invasion history of *L. exotica*. We conducted a thorough sampling of *L. exotica* in the Gulf of Mexico, to determine whether signatures of multiple independent invasion events are detectable in this basin. Its subtropical location, high volume of maritime traffic, and presence of numerous artificial substrates that provide suitable habitats for non-indigenous species (e.g. jetties), renders the Gulf of Mexico very vulnerable to marine invasions, which is reflected in a high occurrence of non-indigenous marine species (Tunnell et al., 2009). Examination of other introduced populations from around the world is expected to provide a global understanding on the invasive history of *L. exotica*.

2. MATERIAL AND METHODS

2.1 Sampling

Specimens of *L. exotica* were obtained from 40 localities around the world (Figure 1; Appendix 1), mainly from the Gulf of Mexico and East Asia. We also obtained specimens of *L. cinerascens* (from East Asia), which was used as outgroup in the phylogenetic reconstructions. Phylogenetic analyses including most *Ligia* species (unpublished) indicate that *L. cinerascens* is sister to the *L. exotica* clade. Yin et al. (2013) also found a sister relationship between *L. exotica* and *L. cinerascens*, in a dataset that also included *L. occidentalis*, and used *L. oceanica* and *Idotea baltica* (Idoteidae) as outgroups. Thus, the reciprocal monophyly of *L. cinerascens* and *L. exotica* is well-supported. The use of *L. cinerascens* as the only outgroup enabled the retention of more confidently-aligned characters and less homoplasy, which should enhance resolution within the *L. exotica* clade. Specimens were preserved in 70-100% ethanol.

2.2 DNA extraction, PCR, and sequencing

Total genomic DNA was isolated from pleopods or legs of *Ligia* specimens with the DNeasy Blood & Tissue kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocol. A ~490-bp 16S rDNA region was amplified using published primers 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi, 1996). Each PCR reaction contained 1-3 µl DNA template, 0.5 µl each primer (10 pmol), 0.1 µl Taq DNA polymerase, 0.5 µl

(a)



Figure 1. Sampled localities in (a) the global range and (b) Asia. Shapes denote different species: dots represent *L. exotica*; squares represent *L. cinerascens*. Colors correspond to clades in Figure 2.

(b)

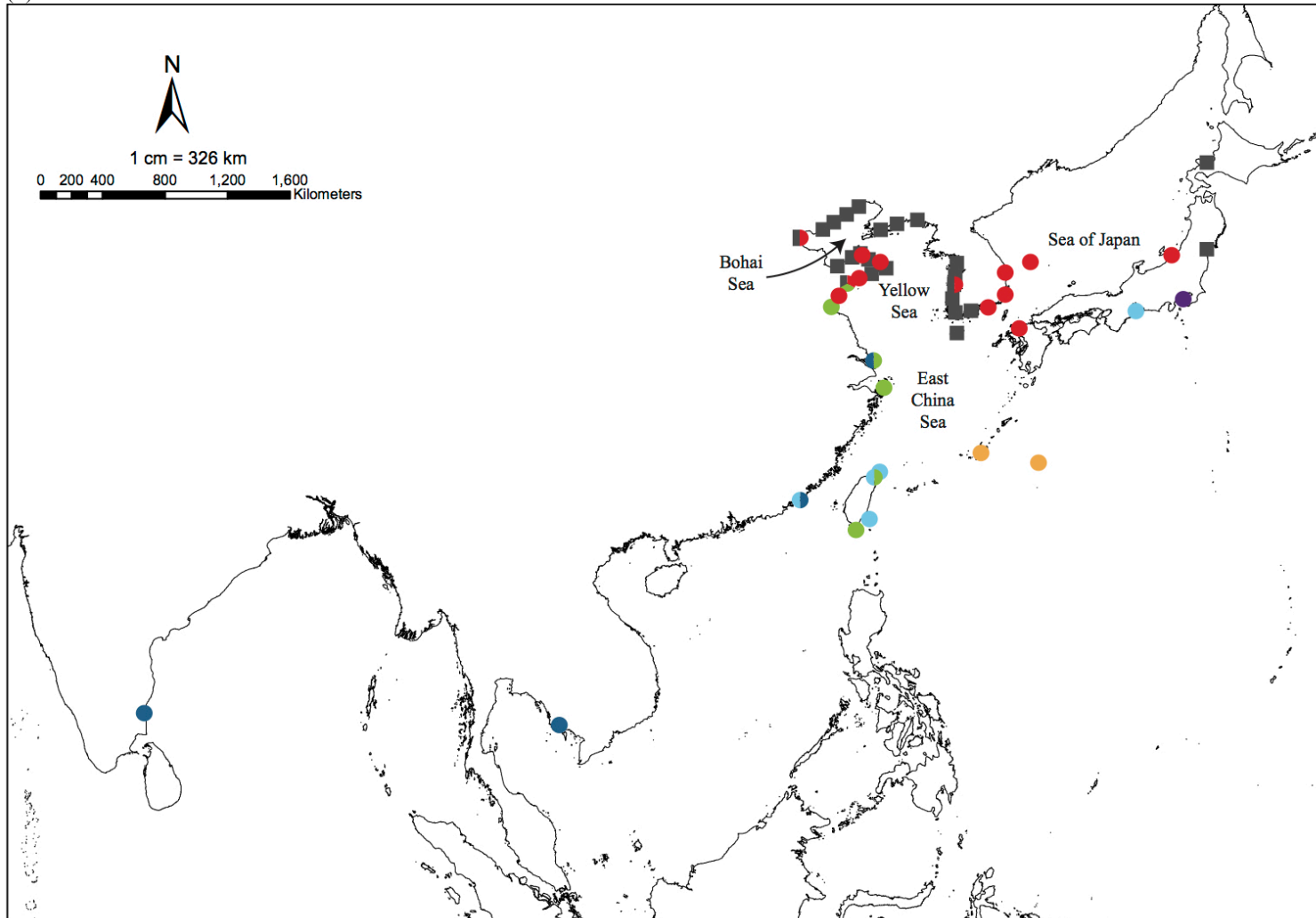


Figure 1 Continued.

dNTPs (10 mM), and 2.5 μ l 10 \times PCR buffer (15 mM MgCl₂, 500 mM KCl, 100 mM Tris-HCl, pH 8.3). PCR conditions used were: 4 min at 94°C followed by 30 cycles of 1 min at 94°C; 30 s at 49°C, 1.5 min at 72°C; and a final extension at 72°C for 4 min. PCR products were cycle sequenced at the University of Arizona Genetics Core (UAGC).

2.3 Dataset and sequence alignment

Sequencher 4.8 (Genecodes, Ann Arbor, MI) was used to assemble the new sequences and trim the primer regions. We also included all 16S rDNA sequences of *L. exotica* and *L. cinerascens* reported in Jung et al. (2008) and Yin et al. (2013), as well as 16S rDNA sequences of *Ligia* sp. from Asia available in GenBank, but not incorporated into a published study (Appendix 1). When present, primer regions were also removed from GenBank sequences.

All sequences were aligned in MAFFT v.7 (Katoh, 2013) online using the Q-INS-I strategy, which considers the secondary structure of RNA, with default parameters (e.g., gap opening penalty = 1.53). Unique haplotypes were identified on the basis of absolute pairwise distances calculated with PAUP v.4.0b10 (Swofford, 2002), and redundant sequences were removed from analyses. Gblocks 0.91b (Castresana, 2000; Talavera and Castresana, 2007) was used to identify positions with questionable homology that were removed prior to phylogenetic analyses. The following GBlocks parameters were used: “Minimum Number Of Sequences For A Conserved Position” = 50% of the number of sequences + 1 (i.e., 42); “Minimum Number Of Sequences For A

Flank Position” = 85% of the number of sequences (i.e., 70); “Maximum Number Of Contiguous Nonconserved Positions” = 4 or 8; “Minimum Length Of A Block” = 5 or 10; and “Allowed Gap Positions” = half.

2.4 Phylogenetic analyses

To determine the most appropriate model of DNA substitution, jModelTest v.2.1.4 (Darriba et al., 2012) was used to calculate likelihood scores among 88 candidate models for 16S rDNA gene, based on the fixed BIONJ-JC tree under the Akaike Information Criterion (AIC), corrected AIC (AICc), and the Bayesian Information Criterion (BIC). The best model selected by the BIC was employed in phylogenetic analyses, except in the following two cases. First, if the selected model was not available in the specific Maximum Likelihood (ML) or Bayesian Inference (BI) program, the next most complex model was implemented. Second, considering the potential problems associated with using two parameters, a proportion of invariable sites (I) and a Gamma distribution of rates among sites (Γ), simultaneously in the model [see RAxML manual and (Yang, 2006)], we chose the simpler Γ if the best model included both I and Γ parameters.

For the ML analyses, RAxML v.8.0.14 (Stamatakis, 2014) and GARLI v.0.951GUI (Zwickl, 2006) were used. RAxML executed 1,000 bootstrap replicates with a thorough ML search under the standard non-parametric bootstrap algorithm and the GTR+ Γ model, whereas GARLI implemented 1,000 bootstrap replicates, the BIC selected model, and all other settings as default. The majority-rule consensus trees for

each analysis were calculated using the SumTrees command of DendroPy v.3.10.1 (Sukumaran and Holder, 2010).

For Bayesian Inference (BI), MrBayes v.3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012) and Phycas v.1.2.0 (Lewis et al., 2005a) were employed. To alleviate the unpredictable behavior in Bayesian analysis when dealing with hard polytomies (i.e., “star-tree paradox”), which can lead to arbitrary resolutions and overestimation of posterior probabilities (Alfaro and Holder, 2006; Kolaczowski and Thornton, 2006; Lewis et al., 2005b; Suzuki et al., 2002; Yang and Rannala, 2005), an analysis employing a polytomy prior was implemented in Phycas [see Phycas manual and (Lewis et al., 2005b)]. The following criteria were used to determine if the Bayesian analyses had reached convergence, and if an adequate sample of the posterior had been generated: (a) the posterior probability values tended to be stable; (b) AWTY (Nylander et al., 2008; Wilgenbusch et al., 2004) exhibited a high correlation between the split frequencies of independent runs; (c) the average standard deviation of the split frequencies of independent runs became stable and approached zero; (d) Potential Scale Reduction Factor (PSRF), a convergence diagnostic obtained after summarizing the sampled parameter values in MrBayes, was close to one; and (e) the Effective Sample Size (ESS) for the posterior probabilities evaluated in Tracer v.1.6 (Rambaut et al., 2014) exceeded 200. Samples prior to reaching stationarity were eliminated as “burn-in”. The posterior probability for each node was estimated by computing a majority-rule consensus of post-burnin tree samples using the SumTrees command (Sukumaran and Holder, 2010).

Given the low number of alleles and shallow genetic divergences found within the clade involving the “Gulf of Mexico” haplotypes (see Results), we also conducted a maximum parsimony branch and bound search in PAUP* v.4.0a149 (Swofford, 2002) for this subset of specimens. Ambiguous character optimization was achieved by the accelerated transformation (ACCTRAN) algorithm. The conservative estimate of pairwise genetic distances with Kimura-2-parameter (K2P) correction was calculated with PAUP* v.4.0a149 (Swofford, 2002).

3. RESULTS

3.1 Model selection

A total of 97 sequences of the *L. exotica* clade and 41 of the *L. cinerascens* clade were examined (Appendix 1). The final 16S rDNA gene excluding redundant sequences consisted of 81 taxa (51 in the *L. exotica* clade and 30 in the *L. cinerascens* clade). After alignment, a total of 454 characters (out of 488) were retained, for which homology was reliable, and 97 of these were parsimony informative. Information about identical sequences and their corresponding groups is presented in Appendix 2. jModelTest selected a complex model (i.e., TPM2uf) with five substitution parameters (see jModelTest manual), +I, and + Γ according to the AIC (weight = 0.2607) and AICc (weight = 0.3509), and a relatively simple model (i.e., HKY) with two substitution parameters (see jModelTest manual), +I, and + Γ according to the BIC (weight = 0.3183). When applicable in different programs, the exact models selected by the three criteria were implemented. In addition, we implemented the GTR+ Γ model, which was included in the 99.9% cumulative weight interval of all selection criteria, in all of the methods, to assess the sensitivity of clade support values to variations in the substitution model (Appendix 3).

3.2 Phylogenetic results

In general, the use of different substitution models or priors yielded similar overall topologies of phylogenetic trees, although some discrepancies, reflected in node

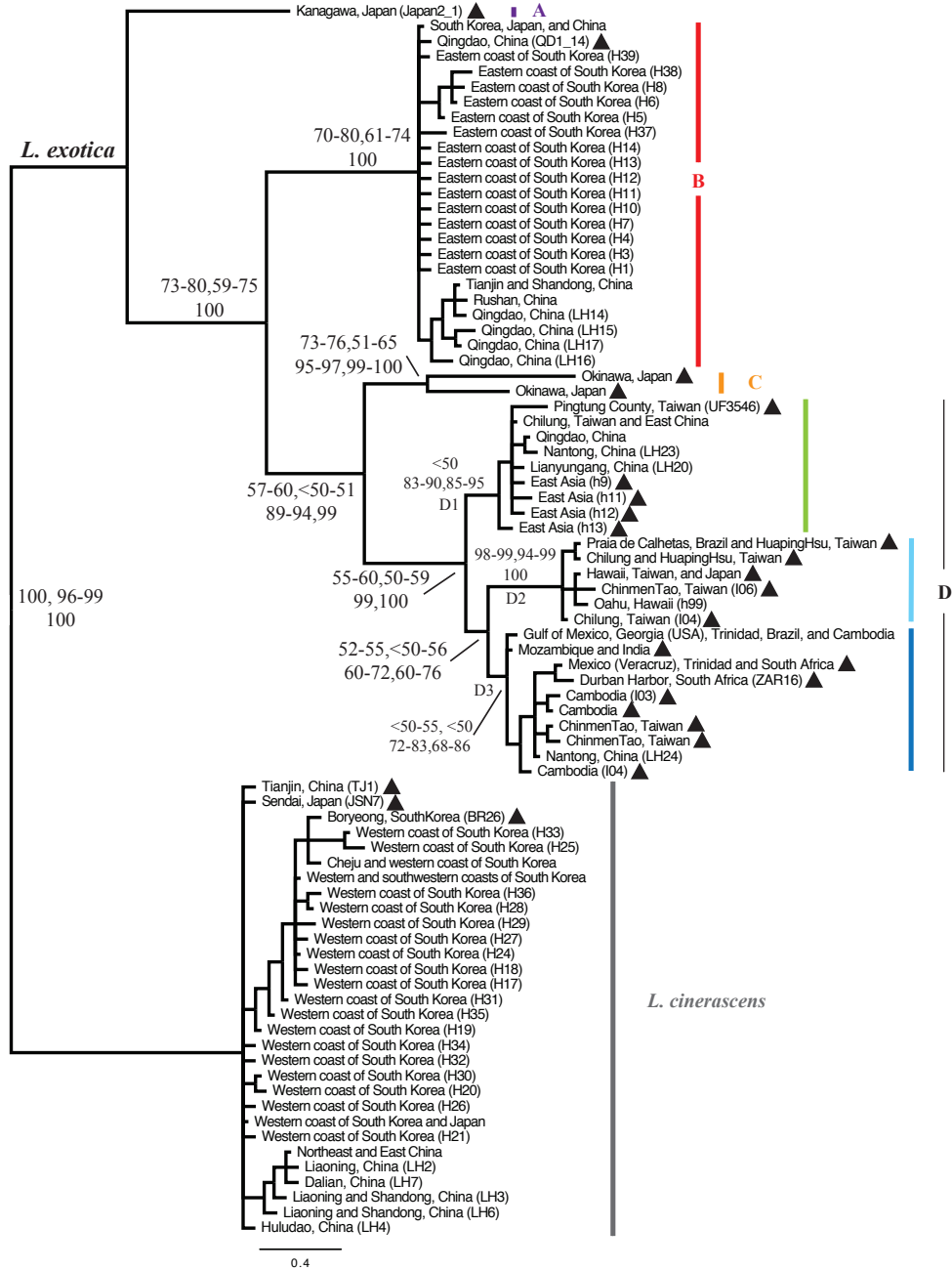


Figure 2. Bayesian majority consensus tree of *Ligia* samples from localities in Figure 1. The tree was obtained by MrBayes for 16S rDNA (model GTR+ Γ), and rooted with *L. cinerascens*. Letters denote four major lineages (i.e., A, B, C, and D) of *L. exotica* and three clades (i.e., D1, D2, and D3) of the lineage D. Clade colors correspond to Figure 1. Numbers by nodes indicate the corresponding range for Bootstrap Support (BS; top) for Maximum Likelihood [RAxML (left) and GARLI (right)] and Posterior Probabilities (PP; bottom) for Bayesian Inference [MrBayes (left) and Phycas (right)]. Each range reflects pooled values obtained under different substitution models (i.e., GTR+ Γ , HKY+I+ Γ , and TPM2uf+I+ Γ) in corresponding program. The triangles denote new haplotypes that haven't been reported in the previous studies of Jung et al. (2008) and (Yin et al., 2013).

support values (Appendix 3), were observed among different approaches (Figure 2; new haplotypes denoted with a triangle). Our phylogenetic reconstruction recovered a highly supported split [Bootstrap Support (BS): 96–100; Posterior Probability (PP): 100] between *L. exotica* and *L. cinerascens*. The *L. cinerascens* clade is restricted to the northern part of East Asia, in the western coast of South Korea, Honshu and Hokkaido in Japan, and northeastern China. Maximum K2P divergence observed within this clade was 2.9% (Table 1).

Table 1. Conservative estimates of evolutionary divergence among major lineages within *L. exotica* and *L. cinerascens*, as measured by percent Kimura-2-parameter distances. Lower matrix: distance range. Upper matrix: average distance. Values on diagonal show minimum and maximum within-clade divergence. Empty cells: no ranges available because selected clade was represented by a single sample.

	<i>L. exotica</i> clade A	<i>L. exotica</i> clade B	<i>L. exotica</i> clade C	<i>L. exotica</i> clade D	<i>L. cinerascens</i>
<i>L. exotica</i> clade A	-	11.5	12.5	10.5	10.4
<i>L. exotica</i> clade B	11.1-12.1	0.2-2.0	8.8	10.0	11.7
<i>L. exotica</i> clade C	11.9-13.2	7.3-10.8	6.3	7.6	13.6
<i>L. exotica</i> clade D	9.8-11.1	8.3-11.6	6.7-9.2	0.2-4.6	13.0
<i>L. cinerascens</i>	9.4-11.0	10.8-13.1	12.3-15.0	11.6-14.9	0.1-2.9

Our analyses revealed 22 new 16S rDNA haplotypes within the *L. exotica* clade (shown with triangles in Figure 2) that were not reported in the previous studies of Jung et al. (2008) and Yin et al. (2013). The *L. exotica* clade was divided into four main lineages (named A, B, C, and D). Node support for different analyses and substitution

models is shown in Appendix 3. Divergences between and within main lineages are shown in Table 1. At the base of the *L. exotica* clade, a relatively distant (K2P divergence = 10.0–13.2%) lineage from Japan (A) diverged from a clade that contains the remaining lineages (BS: 59–80; PP: 100; Appendix 3). Within the latter clade, a basal split (K2P divergence = 7.3–11.6%) is observed between a lineage consisting mainly of samples from temperate regions in East Asia (B; BS: 61–80; PP: 100; Appendix 3; maximum within-clade K2P divergence = 2.0%) and a clade containing the remaining lineages. Some of the populations in Clade B have overlapping distributions with *L. cinerascens* in China (e.g. Tianjin and Shandong) and the western coastline of South Korea (e.g. Boryeong) (Figure 1). Within the other clade, a basal divergence (K2P divergence = 6.7–9.2%) is observed between a lineage from Okinawa, Japan (C; BS: 51–76; PP: 95–100; Appendix 3), which contains two highly divergent lineages from this island (6.3% K2P divergence), and a clade (D) with the remaining samples (BS: 50–60; PP: 99–100; Appendix 3; maximum within-clade K2P divergence = 4.6%). Within clade D, three main lineages are distinguished. The first (D1 in tree) is restricted to East Asia localities (BS: <50; PP: 83–95; Appendix 3; maximum within-clade K2P divergence = 1.3%). The second (D2) has haplotypes found in East Asia, but also in invasive populations from Hawaii and Brazil (BS: 94–99; PP: 100; Appendix 3; maximum within-clade K2P divergence = 0.9%). The third (D3), a weakly supported lineage (BS: <50–55; PP: 68–86; Appendix 3; maximum within-clade K2P divergence = 1.1%), has haplotypes observed in invasive populations from the Gulf of Mexico, Trinidad, Brazil, South Africa, Mozambique, and is also found in South to East Asia.

Figure 3 shows a strict consensus unrooted parsimony tree (made of the 18 most parsimonious trees; CI excluding uninformative characters = 0.8421; RI = 0.9552) for clade D (i.e., the only clade found to contain haplotypes found in invasive populations). The three previously described main lineages within this clade are represented by different haplotype colors (i.e., D1 gray circles, D2 black circles, and D3 white circles). Seven haplotypes were observed in invasive populations, three within D2 and four within D3 (denoted by stars). D3 contains the haplotype that was most common in invasive populations of the Gulf of Mexico, and was also found in the US Atlantic coast (Georgia), Trinidad (Chaguaramas Bay), Brazil (Ilha Grande), and Cambodia. Another D3 haplotype was found in Veracruz, Mexico, Trinidad (Chaguaramas Bay), and South Africa, but was not observed in Asia. A third haplotype was observed in Mozambique, which likely represents another invasive population, and in India. The fourth putatively invasive D3 haplotype was only observed in South Africa. Within D2, a haplotype was found in Oahu (Pearl Harbor) and Hawaii Island, which was also observed in Japan and Taiwan. Another D2 haplotype was found exclusively in Oahu (Honolulu harbor). Finally, a third D2 haplotype was observed in Brazil (Praia de Calhetas, Cabo de Santo Agostinho) and also in Taiwan. Overall, we observed higher levels of genetic diversity in the putatively native range (maximum K2P divergence = 15.0%) of *L. exotica* than in putatively invasive populations. A higher number of haplotypes and clades was detected in native populations. Four of the seven haplotypes found in invasive populations were not found in native populations (assuming the India population is non-native), but they

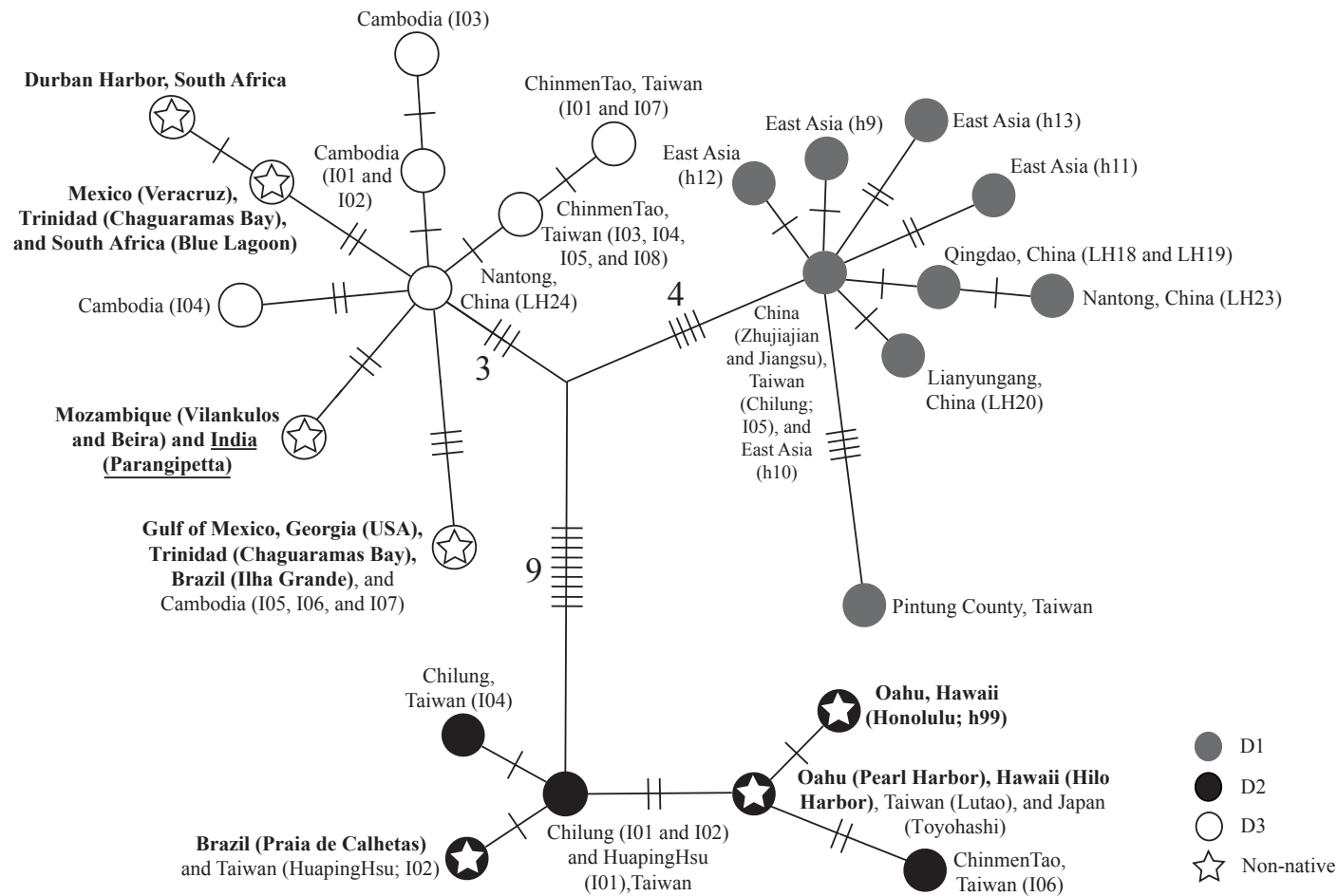


Figure 3. A strict (unrooted) consensus of the 18 most parsimonious trees depicting the relationships among haplotypes in the lineage D of *L. exotica*. Ambiguous character optimization was achieved by the accelerated transformation (ACCTRAN) algorithm. Slashes indicate the number of parsimony steps. The branch lengths within each clade (i.e., D1, D2, and D3) reflect the number of base substitutions. The numbers near the slashes correspond to the number of parsimony steps. Localities where each haplotype was found are listed next to the circles. Localities in bold are those outside the putative native range. Underlined locality label denotes uncertainty regarding its native vs. non-native status (see text).

were only 1–3 mutational steps away from haplotypes found in native populations (the maximum number of steps observed among members of clade D was 21).

4. DISCUSSION

The *L. exotica* clade is comprised of highly divergent lineages, probably corresponding to a cryptic species complex. The region between Southeast and East Asia contains most of the genetic diversity found within this clade. In contrast, only seven of the 51 haplotypes found in the *L. exotica* clade were observed in the putative nonnative populations (i.e., those in the Americas, Hawaii, and Africa), four of which were also represented in Asia, and all are closely related to haplotypes observed in Asia. Furthermore, the haplotypes observed in nonnative populations belong only to two closely related clades, suggesting that the potential to become invasive may be phylogenetically constrained.

As its name implies, since its description based on specimens from the Mediterranean Sea, *L. exotica* was considered a nonnative of this basin (Roux, 1828). Several lines of evidence suggest that the region spanning Southeast to East Asia constitutes its native range, although the exact boundaries are yet to be determined. First, outside of this range, *L. exotica* tends to occur as isolated populations within the range of other more broadly distributed and genetically diverse clades of *Ligia*. One notable exception is in most of the Gulf of Mexico (a mostly sandy coastline), where *L. exotica* is common in many artificial rocky substrates in the absence of other *Ligia* species. Nonetheless, *L. exotica* exhibits very low genetic diversity in this region; with a single haplotype observed, except for Veracruz, where a different closely related haplotype was observed. Another notable exception is the Atlantic coast between Brazil

and Argentina, where *L. exotica* is found and no other *Ligia* species are reported (Schmalzfuss, 2003). We sampled two geographically distant localities in this region; one had the most common haplotype of the Gulf of Mexico and the other had a haplotype also found in Taiwan. Secondly, *L. exotica* exhibits much higher genetic diversity within its putative native range than in all other sampled populations. Whereas the native range is home to four major clades of *L. exotica* (clades A–D), non-native populations are only represented by two of the three subclades of clade D. Thirdly, the sister taxon of *L. exotica* (i.e., *L. cinerascens*) is also distributed in East Asia, suggesting that their ancestor occupied, and diversified within, this region. Although Southeast and East Asia likely constitute the native range of *L. exotica*, identification of its entire native range will require more comprehensive surveys of this and adjacent regions. For example, it is possible that India constitutes part of its native range.

Occurrence of multiple genetically divergent clades within the native range of *L. exotica* is similar to the phylogeographic patterns observed in the following recognized species of *Ligia*, whose distribution includes or is limited to tropical and/or subtropical coasts of other regions: *L. occidentalis*, whose range spans the Pacific coast between central Mexico and southern Oregon, including the Gulf of California (Eberl et al., 2013; Hurtado et al., 2010); *L. baudiniana* in the Caribbean and a small Pacific region between Central and South America (Santamaria et al., 2014); *L. hawaiiensis* in the Hawaiian archipelago (Santamaria et al., 2013); and *L. italica* in the Mediterranean basin (Hurtado et al. unpublished). Interestingly, despite reports of the occurrence of *L. exotica* in the Gulf of California (Mulaik, 1960; Richardson, 1905), we failed to find it during

extensive surveys of this and the adjacent regions (Eberl et al., 2013; Hurtado et al., 2010). Although it is possible that *L. exotica* occurs in hitherto unsampled Pacific coast localities of the New World, it is likely that past records of this species were misidentifications of the morphologically similar species *L. occidentalis*.

The relatively high genetic diversity of *L. exotica* in its native range contrasts with the low diversity observed in its sister lineage *L. cinerascens* (maximum K2P divergence within this species = 2.9%), suggesting different evolutionary histories. One evident difference between the two species is their geographic distributions. Within our study area alone, *L. cinerascens* was generally found in relatively colder (mostly temperate) regions, including the northern Yellow Sea, Bohai Sea, Korean Peninsula, and the Japanese archipelago. Other records indicate that the range of *L. cinerascens* extends further north into the Kuril Islands (Yin et al., 2013) and the Peter de Great Gulf (i.e., the southernmost part of Russia in the Sea of Japan; Zenkevich, 1963). Although the ranges of *L. exotica* and *L. cinerascens* overlap (Figure 1), *L. exotica* is generally found in warmer (mostly tropical and subtropical) regions. Due to its distribution at higher latitudes, the lower genetic diversity of *L. cinerascens* may reflect a history of recent extinction-expansion events associated with glacial and postglacial cycles. A similar pattern of recognized species of *Ligia* from high latitudes (at least in the northern hemisphere) harboring low genetic diversity occurs in *L. pallasii* (Eberl, 2013) and *L. oceanica* (Raupach et al., 2014).

Within *L. exotica*, Clade B, which is mostly restricted to temperate areas, exhibits comparatively lower genetic diversity (maximum K2P divergence = 2.0%) than

clades C and D, which occur in warmer regions. The range and genetic diversity of Clade A (found at Kanagawa, Japan) has not been adequately assessed. The pattern of comparatively lower diversity within Clade B, whose distribution overlaps with part of the range of *L. cinerascens*, may also be explained by a history of recent extinction-recolonization events associated with glacial cycles. A similar pattern of reduced genetic diversity at higher latitudes within a recognized coastal isopod species occurs in the northernmost clade of *L. occidentalis* in California (Eberl et al., 2013) and of *Tylos punctatus* in the northernmost range, between Southern California and the Baja Peninsula (Hurtado et al., 2014).

Temperature also appears to be an important factor determining the distribution of the other *L. exotica* lineages, which are found in warmer waters. Although the northern distribution of *L. exotica* Clade D1 overlaps with the southern range of Clade B in the Yellow Sea, Clade D1 was detected as far south as Taiwan. Clade D2 was found in warmer waters. A haplotype of this clade was observed in the southern coast of Honshu, Japan, which is in a region with warmer water, and was also found in Taiwan and Hawaii. The only locality where lineage A was found is also in the southern coast of Honshu. Clade D3 was restricted to warmer waters and reached the southernmost areas (i.e., Cambodia, possibly India) in what appears to be the native range of *L. exotica*. Sea surface temperature (SST) has been shown to be an important factor determining the distribution of lineages in *L. occidentalis*. In this isopod, the geographical limit between two main clades largely reflects the changes in SST that define the Point Conception biogeographical boundary in California (Eberl et al., 2013).

Although coastal *Ligia* are essentially terrestrial and do not venture into open water, SST influences abiotic factors likely important to their survival and reproduction, such as air temperature, sea and land breezes, atmospheric humidity and coastal fog (Eberl et al., 2013).

A dynamic past geological history in the Southeast-East Asia region (Ni et al., 2014; Wang, 1999) may have contributed to divergences within the *L. exotica* clade. However, we cannot identify specific events. Opportunities for long-standing isolation and differentiation appear to have occurred in the Japanese archipelago, such as the basal differentiation of lineage A, to date found at a single locality in Honshu, Japan, and the presence of the two highly divergent lineages from Okinawa observed within Clade C. It is likely that the Japanese archipelago harbors additional morphologically cryptic diversity. Large divergences among the three main lineages in Clade D also suggest greater opportunities for diversification have occurred in the warmer waters. The island of Taiwan also exhibits high levels of genetic diversity, with the presence of multiple divergent lineages, as observed in the present study and in a previous study based on the Cytochrome Oxidase I (COI) gene (Chang, 2013).

Phylogenetic patterns suggest multiple independent invasions of *L. exotica* have occurred around the world. Haplotypes found at invasive populations, however, belong only to two closely related lineages (D2 and D3). Of the seven haplotypes observed in invasive populations, three were also found in Southeast-East Asia and one in India, which may be part of the native range. The other three, albeit not found in the presumed native range, were only separated by a few substitutions from haplotypes found in the

native range. It is thus possible that we failed to sample them in the native range, as in situ substitution is highly unlikely, given the evolutionarily short period since invasion. An Asian origin of *L. exotica* invasive populations, probably through ship transport, seems undisputable. Once out of Asia, it is likely that individuals from nonnative populations colonized other new localities. This may have occurred in the New World, for example, where the same haplotype is present in the Atlantic US coast, Gulf of Mexico, and Brazil.

In Asia, lineages D2 and D3 are distributed in warmer waters, akin to the ones occupied by the nonnative populations examined. Environmental similarity between donor and recipient regions might increase the chance of a successful invasion (Seebens et al., 2013). For example, the red imported fire ant *Solenopsis invicta* initially established in regions with similar environments (Fitzpatrick et al., 2007). It is remarkable, however, that the closely related lineage D1 does not harbor haplotypes found in invasive populations, despite being found also in Asian localities with warm waters (e.g. Taiwan). Therefore, a similar environmental temperature does not appear to be the only factor contributing to the invasiveness of *L. exotica* D2 and D3 lineages.

The presence of invasive haplotypes only in clades D2 and D3 may also suggest that some characteristics conferring invasive advantages arose only in these lineages, and not in lineage D1. Because lineages D2 and D3 appear to be sisters, notwithstanding the low clade support values, the “invasiveness” traits were most likely present in their common ancestor. Such characteristics may have allowed invasive individuals to endure long journeys in the holds of wooden ships, into which they were likely unintentionally

loaded along with the ballast stones that were commonly used during the 18th and 19th centuries (Griffiths et al., 2011; Van Name, 1936). This rocky cargo was usually dumped once a ship reached its destination. Surviving in the holds of ships, however, would have been challenging to *Ligia* isopods due to limited access to seawater. Low desiccation resistance is a feature of these isopods, constituting one of the factors that constrains their distribution to a very narrow vertical range between the supralittoral and the water line (Carefoot and Taylor, 1995). A superior desiccation resistance and osmoregulation ability, which would enhance survival of such journeys, has been reported in *L. exotica* from Taiwan (Tsai et al., 1997; 1998), where clades D2 and D3, along with D1, indeed occur. Once in a new harbor, the rather homogeneous rocky habitat occupied by *Ligia* (Santamaria et al., 2016), similar temperatures to source localities, and high reproductive rates would have contributed to their successful establishment. Indeed, high reproductive rates have been reported for *L. exotica* in a nonnative Brazilian population (Lopes et al., 2006). Comparable to *L. exotica*, all invasive populations of the leafmining fly pest *Liriomyza sativae*, distributed at a global scale, cluster in a single clade (Scheffer and Lewis, 2005).

5. CONCLUSIONS

Ligia exotica was comprised of several highly genetically divergent lineages, probably corresponding to a cryptic species complex. Most of the genetic diversity was detected in the region spanning Southeast to East Asia, which appears to constitute the native range of *L. exotica*. In contrast, very little genetic diversity was found outside the above region. Temperature appears to influence the distribution and levels of genetic diversity of *L. exotica* clades. A clade that is mostly restricted to temperate areas, exhibits comparatively lower genetic diversity than clades restricted to warmer areas. Similarly, the sister lineage of *L. exotica*, *L. cinerascens*, whose range partly overlaps with that of *L. exotica* in a relatively cold region, exhibits comparatively lower genetic diversity. Greater opportunities for diversification of *L. exotica* appear to have occurred in the warmer waters, and the Japanese archipelago harbors several highly divergent endemic lineages.

Phylogenetic patterns suggest that multiple independent invasions of *L. exotica* have occurred around the world. An Asian origin of *L. exotica* invasive populations, probably through ship transport, seems indisputable. Once out of Asia, it is likely that individuals from nonnative populations colonized other new localities, which appears to have occurred in the New World. Haplotypes observed in nonnative populations belong only to two sister clades, suggesting that the potential to become invasive may be phylogenetically constrained. In Asia, these lineages are distributed in warmer regions, akin to the ones occupied by the nonnative populations examined; thus, environmental

similarity between donor and recipient regions might have increased the chance of a successful invasion.

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APPENDIX 1

Localities included in this study, with corresponding GenBank accession numbers, and geographic information. ID labels correspond with those used in other figures.

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>L. exotica</i>	Goodland, FL, USA	Goodland_Florida_Goodland1	KX447715	This study	25°55'57"N	81°39'21"W
<i>L. exotica</i>	Palacios, TX, USA	Palacios_Texas_PAL1	KX447716	This study	28°44'18"N	96°24'6"W
<i>L. exotica</i>	Cedar Key, FL, USA	CedarKey_Florida_Cedar2	KX447717	This study	29°8'8"N	83°2'11"W
<i>L. exotica</i>	Biloxi Small Craft Harbor, Biloxi, MS, USA	Biloxi_Mississippi_Biloxi1	KX447718	This study	30°23'31"N	88°53'8"W
<i>L. exotica</i>	Sunshine Skyway Bridge North Rest Area, St. Petersburg, FL, USA	StPetersburg_Florida_Tampa2	KX447719	This study	27°39'14"N	82°40'41"W
<i>L. exotica</i>	Municipal Harbor, Port Aransas, TX, USA	PortAransas_Texas_Aransas1	KX447720	This study	27°50'24"N	97°3'50"W
<i>L. exotica</i>	Long Beach Harbor, Biloxi, MS, USA	Biloxi_Mississippi_LBeachFL2	KX447721	This study	30°20'41"N	89°8'42"W
<i>L. exotica</i>	Pensacola, FL, USA	Pensacola_Florida_Pensacola1	KX447722	This study	30°25'11"N	87°11'36"W
<i>L. exotica</i>	South Padre Island, TX, USA	SouthPadreIsland_Texas_SPI1	KX447723	This study	26°4'44"N	97°10'9"W
<i>L. exotica</i>	Parangipetta, India	Parangipetta_India_India1_1	KX447724	This study	11°29'24"N	79°45'36"E
<i>L. exotica</i>	Praia de Calhetas, Cabo de Santo Agostinho, Brazil	CalhetasBeach_Brazil_CAR34_1	KX447725	This study	8°20'38"S	34°56'43"W
<i>L. exotica</i>	Lagoa Azul, Ilha Grande, Costa Verde, Brazil	IlhaGrande_Brazil_LEB1	KX447726	This study	23°11'S	44°18'W
<i>L. exotica</i>	Kanagawa, Japan	Kanagawa_Japan_Japan2_1	KX447727	This study	35°9'25"N	139°36'43"E
<i>L. exotica</i>	Hilo Harbor, Hawai'i, HI, USA	HiloHarbor_Hawaii_LexHilo1	KX447728	This study	19°43'57"N	155°3'26"W
<i>L. exotica</i>	Pearl Harbor, O'ahu, HI, USA	PearlHarbor_Hawaii_LexOah1	KX447729	This study	21°21'50"N	W
<i>L. exotica</i>	Avery Island, LA, USA	AveryIsland_Louisiana_AV11	KX447730	This study	29°54'57"N	91°54'14"W
<i>L. exotica</i>	San Juan de Ulúa Fort, Veracruz, Mexico	SanJuandeUlúaFort_Veracruz_Mexico_CAR30_1	KF546552	Santamaria et al. 2013	19°12'34"N	96°7'51"W

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>L. exotica</i>	Jetty by Adolfo Ruiz Cortines statue, Veracruz, Mexico	Jetty_Veracruz_Mexico_CAR31_1	KX447731	This study	19°11'40"N	96°7'24"W
<i>L. exotica</i>	Chaguaramas Bay, Trinidad, Trinidad and Tobago	ChaguaramasBay_Trinidad_CAR6_1	KX447732	This study	10°40'57"N	61°37'21"W
<i>L. exotica</i>	Chaguaramas Bay, Trinidad, Trinidad and Tobago	ChaguaramasBay_Trinidad_CAR6_2	KX447733	This study	10°40'57"N	61°37'21"W
<i>L. exotica</i>	Eastpoint, FL, USA	Eastpoint_Florida_EastPt1	KX447734	This study	29°44'21"N	84°52'25"W
<i>L. exotica</i>	Lutao, Taitung, Taiwan	Lutao_Taiwan_tait_1	KX447735	This study	N/A	N/A
<i>L. exotica</i>	Vilankulos, Mozambique	Vilankulos_Mozambique_EA_L1_1	KX447736	This study	21°59'52"S	35°19'30"E
<i>L. exotica</i>	Beira, Mozambique	Beira_Mozambique_EA_L2_3	KX447737	This study	19°50'53"S	34°53'35"E
<i>L. exotica</i>	Kitadaito son, Okinawa, Japan	Okinawa_Japan_TOYA4	KX447738	This study	25°56'45"N	131°17'56"E
<i>L. exotica</i>	Zhujiajian Island, Zhejiang, China	Zhujiajian_China_ZH2_5	KX447739	This study	29°54'N	122°53'E
<i>L. exotica</i>	Pingtung County, Taiwan	PingtungCounty_Taiwan_UF3546	KX447740	This study	22°29'44"N	120°36'52"E
<i>L. exotica</i>	Toyohashi, Japan	Toyohashi_Japan_JTO2	KX447741	This study	N/A	N/A
<i>L. exotica</i>	Okinawa, Japan	Okinawa_Japan_OK2_7	KX447742	This study	26°28'46"N	127°55'40"E
<i>L. exotica</i>	Ulleungdo Island, South Korea	Ulleungdo_SouthKorea_UL13	KX447743	This study	37°30'6"N	130°51'11"E
<i>L. exotica</i>	Boryeong, South Korea	Boryeong_SouthKorea_Daechen1	KX447744	This study	38°4'53"N	127°38'16"E
<i>L. exotica</i>	Woojuk, South Korea	Woojuk_SouthKorea_Woojuk1	KX447745	This study	N/A	N/A
<i>L. exotica</i>	Fukuoka, Japan	Fukuoka_Japan_JFU11	KX447746	This study	33°35'N	130°24'E
<i>L. exotica</i>	Niigata, Japan	Niigata_Japan_JNI17	KX447747	This study	37°54'58"N	139°2'11"E
<i>L. exotica</i>	Qingdao, Shandong, China	Qingdao_China_QD1_14	KX447748	This study	36°3'58"N	120°22'10"E
<i>L. exotica</i>	Durban Harbor, KwaZulu-Natal, South Africa	DurbanHarbor_SouthAfrica_ZAR16	KX447749	This study	N/A	N/A
<i>L. exotica</i>	Blue Lagoon, Umgeni River Mouth, KwaZulu-Natal, South Africa	BlueLagoon_SouthAfrica_ZAR17	KX447750	This study	29°48'36"S	31°2'8"E
<i>L. cinerascens</i>	Otaru, Japan	Otaru_Japan_JOR1	KX447751	This study	43°11'N	141°E

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>L. cinerascens</i>	Tianjin, China	Tianjin_China_TJ1	KX447752	This study	39°08'N	117°11'E
<i>L. cinerascens</i>	Sendai, Japan	Sendai_Japan_JSN7	KX447753	This study	38°16'N	140°52'E
<i>L. cinerascens</i>	Boryeong, South Korea	Boryeong_SouthKorea_BR26	KX447754	This study	38°4'53"N	127°38'16"E
<i>L. cinerascens</i>	Boseong, South Korea	Boseong_SouthKorea_GH11	KX447755	This study	34°48'28"N	127°8'15"E
<i>L. cinerascens</i>	Cheju, South Korea	Cheju_SouthKorea_Cheju1	KX447756	This study	33°22'N	126°32'E
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H36	AY545635	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H35	AY545634	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H34	AY545633	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H33	AY545632	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H32	AY545631	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H31	AY545630	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H30	AY545629	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H29	AY545628	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H28	AY545627	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H27	AY545626	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H26	AY545625	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H25	AY545624	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H24	AY545623	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H23	AY545622	Jung et al. 2008	N/A	N/A

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H22	AY545621	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H21	AY545620	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H20	AY545619	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H19	AY545618	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H18	AY545617	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H17	AY545616	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H16	AY545615	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Western Group, South Korea	Western_SouthKorea_H15	AY545614	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H39	EU213044	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H38	EU213043	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H37	EU213042	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H14	AY545613	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H13	AY545612	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H12	AY545611	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H11	AY545610	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H10	AY545609	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H9	AY545608	Jung et al. 2008	N/A	N/A

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H8	AY545607	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H7	AY545606	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H6	AY545605	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H5	AY545604	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H4	AY545603	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H3	AY545602	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H2	AY545601	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Eastern Group, South Korea	Eastern_SouthKorea_H1	AY545600	Jung et al. 2008	N/A	N/A
<i>Ligia sp.</i>	Cambodia	Cambodia_I07	AY606092	GenBank	N/A	N/A
<i>Ligia sp.</i>	Cambodia	Cambodia_I06	AY606091	GenBank	N/A	N/A
<i>Ligia sp.</i>	Cambodia	Cambodia_I05	AY606090	GenBank	N/A	N/A
<i>Ligia sp.</i>	Cambodia	Cambodia_I04	AY606089	GenBank	N/A	N/A
<i>Ligia sp.</i>	Cambodia	Cambodia_I03	AY606088	GenBank	N/A	N/A
<i>Ligia sp.</i>	Cambodia	Cambodia_I02	AY606087	GenBank	N/A	N/A
<i>Ligia sp.</i>	Cambodia	Cambodia_I01	AY606086	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chinmen Tao, Taiwan	ChinmenTao_Taiwan_I08	AY606099	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chinmen Tao, Taiwan	ChinmenTao_Taiwan_I07	AY606098	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chinmen Tao, Taiwan	ChinmenTao_Taiwan_I06	AY606097	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chinmen Tao, Taiwan	ChinmenTao_Taiwan_I05	AY606096	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chinmen Tao, Taiwan	ChinmenTao_Taiwan_I04	AY606095	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chinmen Tao, Taiwan	ChinmenTao_Taiwan_I03	AY606094	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chinmen Tao, Taiwan	ChinmenTao_Taiwan_I01	AY606093	GenBank	N/A	N/A

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>Ligia sp.</i>	Okinawa, Japan	Okinawa_Japan_I06	AY606110	GenBank	N/A	N/A
<i>Ligia sp.</i>	Okinawa, Japan	Okinawa_Japan_I05	AY606109	GenBank	N/A	N/A
<i>Ligia sp.</i>	Okinawa, Japan	Okinawa_Japan_I04	AY606108	GenBank	N/A	N/A
<i>Ligia sp.</i>	Okinawa, Japan	Okinawa_Japan_I02	AY606107	GenBank	N/A	N/A
<i>Ligia sp.</i>	Okinawa, Japan	Okinawa_Japan_I01	AY606106	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chilung, Taiwan	Chilung_Taiwan_I05	AY606105	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chilung, Taiwan	Chilung_Taiwan_I04	AY606104	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chilung, Taiwan	Chilung_Taiwan_I02	AY606103	GenBank	N/A	N/A
<i>Ligia sp.</i>	Chilung, Taiwan	Chilung_Taiwan_I01	AY606102	GenBank	N/A	N/A
<i>Ligia sp.</i>	HuapingHsu, Taiwan	HuapingHsu_Taiwan_I02	AY606101	GenBank	N/A	N/A
<i>Ligia sp.</i>	HuapingHsu, Taiwan	HuapingHsu_Taiwan_I01	AY606100	GenBank	N/A	N/A
<i>L. exotica</i>	Tianjin and Shandong, China	TianjinAndShandong_China_LH8	JX414122	Yin et al. 2013	N/A	N/A
<i>L. exotica</i>	Rushan, Shandong, China	Rushan_China_LH9	JX414123	Yin et al. 2013	36°50'59"N	121°36'50"E
<i>L. exotica</i>	Rushan, Shandong, China	Rushan_China_LH10	JX414124	Yin et al. 2013	36°50'59"N	121°36'50"E
<i>L. exotica</i>	Rushan, Shandong, China	Rushan_China_LH11	JX414125	Yin et al. 2013	36°50'59"N	121°36'50"E
<i>L. exotica</i>	Shandong, China	Shandong_China_LH12	JX414126	Yin et al. 2013	N/A	N/A
<i>L. exotica</i>	Weihai, Shandong, China	Weihai_China_LH13	JX414127	Yin et al. 2013	37°26'14"N	122°9'42"E
<i>L. exotica</i>	Qingdao-Zhanqiao, Shandong, China	Qingdao_China_LH14	JX414128	Yin et al. 2013	36°3'41"N	120°19'10"E
<i>L. exotica</i>	Qingdao-Zhanqiao, Shandong, China	Qingdao_China_LH15	JX414129	Yin et al. 2013	36°3'41"N	120°19'10"E
<i>L. exotica</i>	Qingdao-Zhanqiao, Shandong, China	Qingdao_China_LH16	JX414130	Yin et al. 2013	36°3'41"N	120°19'10"E
<i>L. exotica</i>	Qingdao-Hongdao, Shandong, China	Qingdao_China_LH17	JX414131	Yin et al. 2013	36°10'58"N	120°16'57"E

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>L. exotica</i>	Qingdao-Hongdao, Shandong, China	Qingdao_China_LH18	JX414132	Yin et al. 2013	36°10'58"N	120°16'57"E
<i>L. exotica</i>	Qingdao-Hongdao, Shandong, China	Qingdao_China_LH19	JX414133	Yin et al. 2013	36°10'58"N	120°16'57"E
<i>L. exotica</i>	Lianyungang, Jiangsu, China	Lianyungang_China_LH20	JX414134	Yin et al. 2013	34°46'32"N	119°26'34"E
<i>L. exotica</i>	Jiangsu, China	Jiangsu_China_LH21	JX414135	Yin et al. 2013	N/A	N/A
<i>L. exotica</i>	Nantong, Jiangsu, China	Nantong_China_LH22	JX414136	Yin et al. 2013	32°5'7"N	121°35'51"E
<i>L. exotica</i>	Nantong, Jiangsu, China	Nantong_China_LH23	JX414137	Yin et al. 2013	32°5'7"N	121°35'51"E
<i>L. exotica</i>	Nantong, Jiangsu, China	Nantong_China_LH24	JX414138	Yin et al. 2013	32°5'7"N	121°35'51"E
<i>L. exotica</i>	East Asia	EastAsia_h9	KJ802850	GenBank	N/A	N/A
<i>L. exotica</i>	East Asia	EastAsia_h10	KJ802851	GenBank	N/A	N/A
<i>L. exotica</i>	East Asia	EastAsia_h11	KJ802852	GenBank	N/A	N/A
<i>L. exotica</i>	East Asia	EastAsia_h12	KJ802853	GenBank	N/A	N/A
<i>L. exotica</i>	East Asia	EastAsia_h13	KJ802854	GenBank	N/A	N/A
<i>L. exotica</i>	Honolulu Harbor, O'ahu, HI, USA	Oahu_Hawaii_h99	AY051339	Yin et al. 2013	N/A	N/A
<i>L. exotica</i>	Cumberland Island, GA, USA	CumberlandIsland_Georgia_USA	AF260861	Yin et al. 2013	30°51'N	81°27'W
<i>L. cinerascens</i>	Northeast and East China	NortheastAndEastChina_LH1	JX414115	Yin et al. 2013	N/A	N/A
<i>L. cinerascens</i>	Liaoning, China	Liaoning_China_LH2	JX414116	Yin et al. 2013	N/A	N/A
<i>L. cinerascens</i>	Liaoning and Shandong, China	LiaoningAndShandong_China_LH3	JX414117	Yin et al. 2013	N/A	N/A
<i>L. cinerascens</i>	Huludao, Liaoning, China	Huludao_China_LH4	JX414118	Yin et al. 2013	40°40'30"N	120°49'33"E
<i>L. cinerascens</i>	Tianjin and Shandong, China	TianjinAndShandong_China_LH5	JX414119	Yin et al. 2013	N/A	N/A

Species	Locality Names	ID	Accession Numbers	Sources	Lat	Long
<i>L. cinerascens</i>	Liaoning and Shandong, China	LiaoningAndShandong_China_LH6	JX414120	Yin et al. 2013	N/A	N/A
<i>L. cinerascens</i>	Dalian-Heishijiao, Liaoning, China	Dalian_China_LH7	JX414121	Yin et al. 2013	38°57'53"N	121°18'53"E

APPENDIX 2

Haplotype information about identical sequences and corresponding representatives preserved in the phylogenetic analyses.

Haplotype	Representative	Members	The number of members
1	Goodland_Florida_Goodland_1	Palacios_Texas_PAL1 CedarKey_Florida_Cedar2 Biloxi_Mississippi_Biloxi1 StPetersburg_Florida_Tampa2 PortAransas_Texas_Aransas1 Biloxi_Mississippi_LBeachFL2 Pensacola_Florida_Pensacola_1 SouthPadreIsland_Texas_SPI1 AveryIsland_Louisiana_AVII Chaguaramas_Bay_Trinidad_CAR6_2 Eastpoint_Florida_EastPt_1 IlhaGrande_Brazil_LEB_1 Cambodia_I07 Cambodia_Cambodia_I06 Cambodia_I05 CumberlandIsland_Georgia_USA	16
2	HiloHarbor_Hawaii_LexHilo_1	PearlHarbor_Hawaii_LexOah1 Lutao_Taiwan_tait_1 Toyohashi_Japan_JTO2	3
3	SanJuandeUluaFort_Veracruz_Mexico_CAR30_1	Jetty_Veracruz_Mexico_CAR31_1 Chaguaramas_Bay_Trinidad_CAR6_1 BlueLagoon_SouthAfrica_ZAR17	3

Haplotype	Representative	Members							The number of members
4	Parangipetta_India_India1_1	Vilankulos_Mozambique_EA_L1_1	Beira_Mozambique_EA_L2_3						2
5	Western_SouthKorea_H23	Otaru_Japan_JOR1	Western_SouthKorea_H15						2
6	Ulleungdo_Korea_UL13	Boryeong_Korea_Daechen1	Woojuk_Korea_Woojuk1	Fukuoka_Japan_JFU11	Niigata_Japan_JNI17	Eastern_SouthKorea_H2	Weihai_China_LH13	Eastern_SouthKorea_H9	7
7	Cambodia_I01	Cambodia_I02							1
8	ChinmenTao_Taiwan_I03	ChinmenTao_Taiwan_I08	ChinmenTao_Taiwan_I05	ChinmenTao_Taiwan_I04					3
9	ChinmenTao_Taiwan_I01	ChinmenTao_Taiwan_I07							1
10	Okinawa_Japan_OK2_7	Okinawa_Japan_I05	Okinawa_Japan_I02	Okinawa_Japan_I01	Okinawa_Japan_TOYA4				4
11	Okinawa_Japan_I04	Okinawa_Japan_I06							1

Haplotype	Representative	Members	The number of members			
12	Chilung_Taiwan_I05	Zhujiajian_China_ZH2_5	Jiangsu_China_LH21	Nantong_China_LH22	EastAsia_h10	4
13	Chilung_Taiwan_I01	Chilung_Taiwan_I02	HuapingHsu_Taiwan_I01			2
14	CalhetasBeach_Brazil_CAR34_1	HuapingHsu_Taiwan_I02				1
15	Rushan_China_LH10	Rushan_China_LH9	Rushan_China_LH11			2
16	TianjinAndShandong_China_LH8	Shandong_China_LH12				1
17	Qingdao_China_LH19	Qingdao_China_LH18				1
18	NortheastAndEastChina_LH1	TianjinAndShandong_China_LH5				1
19	Western_SouthKorea_H22	Cheju_Korea_Chejul				1
20	Boseong_Korea_GH11	Western_SouthKorea_H16				1

APPENDIX 3

Clade support obtained from different methods under different substitution model (i.e., GTR+ Γ , TPM2uf+I+ Γ , and HKY+I+ Γ) with the dataset that only included unique sequences (Appendix 6).

Clade	ML					BI				
	RAxML - GTR+ Γ	RAxML - HKY+I+ Γ	GARLI - GTR+ Γ	GARLI - TPM2uf+I+ Γ	GARLI - HKY+I+ Γ	MrBayes - GTR+ Γ	MrBayes - TPM2uf+I+ Γ	MrBayes - HKY+I+ Γ	Phycas - GTR+ Γ	Phycas - HKY+I+ Γ
A	100	100	99	96	99	100	100	100	100	100
B	80	70	61	74	61	100	100	100	100	100
C	76	73	65	51	65	95	96	97	99	100
D	60	55	52	59	50	99	99	99	100	100
D1	n/a	n/a	n/a	n/a	n/a	90	87	83	95	85
D2	99	98	94	99	96	100	100	100	100	100
D3	55	n/a	n/a	n/a	n/a	83	81	72	86	68
D2+D3	52	55	n/a	56	n/a	60	63	72	60	76
C+D	57	60	n/a	51	n/a	89	94	94	99	99
B+C+D	80	73	60	75	59	100	100	100	100	100

APPENDIX 4

Settings for Maximum Likelihood and Bayesian analyses. ^A All others default; ^B Average Standard Deviation of Split Frequencies; ^C estimated in Tracer v.1.6; ^D Effective Sample Size; ^E Potential Scale Reduction Factor for all parameters.

Method	Model and Priors ^A	Iterations Generations / Bootstrap Replicates	Sample Frequency	Runs/Chains	Burn-in	ASDSF ^B	Bayes Factors / ML Scores (-lLn) ^C	ESS>200 ^D	PSRF ^E
RAxML	GTR+ Γ	1,000	n/a	n/a	n/a	n/a	-1936.5887	n/a	n/a
RAxML	HKY+I+ Γ	1,000	n/a	n/a	n/a	n/a	-1946.8587	n/a	n/a
GARLI	GTR+ Γ	1,000	n/a	n/a	n/a	n/a	-1802.5711	n/a	n/a
GARLI	TPM2uf+I+ Γ	1,000	n/a	n/a	n/a	n/a	-2055.0212	n/a	n/a
GARLI	HKY+I+ Γ	1,000	n/a	n/a	n/a	n/a	-1650.6554	n/a	n/a
MrBayes	GTR+ Γ	100,000,000	10,000	4	10%	0.003743	-2213.7510	Yes	1
MrBayes	TPM2uf+I+ Γ	100,000,000	10,000	4	10%	0.003915	-2218.5443	Yes	1
MrBayes	HKY+I+ Γ	100,000,000	10,000	4	10%	0.003395	-2217.5341	Yes	1
Phycas	GTR+ Γ	1,000,000	10	1/1	10%	n/a	-2193.3224	Yes	n/a
Phycas	HKY+I+ Γ	1,000,000	10	1/1	10%	n/a	-2201.1564	Yes	n/a

APPENDIX 5

Sequences alignment in Nexus format (a separate file).

APPENDIX 6

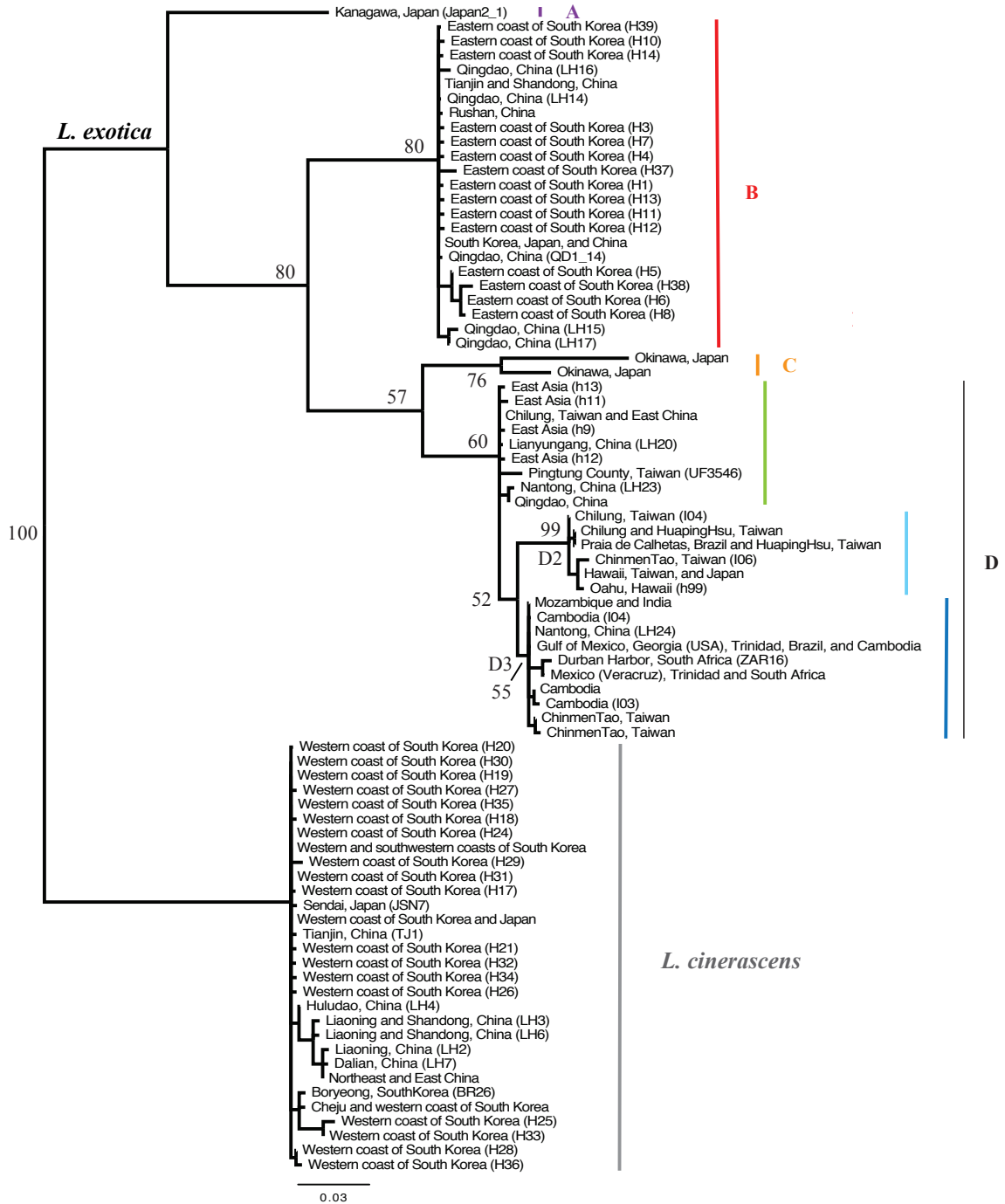
Phylogenetic trees of 81 unique samples reconstructed by RAxML, GARLI, MrBayes, and Phycas, and that of 138 samples reconstructed by RAxML.

A to I. Majority-rule consensus trees of 81 samples summarized the results from RAxML (A and B), GARLI (C, D, and E), MrBayes (F and G) and Phycas (H and I) under different substitution models (i.e., GTR+ Γ , HKY+I+ Γ and TPM2uf+I+ Γ). Colors correspond to clades in Figure 2. Numbers by the nodes indicate the corresponding Bootstrap Support (BS) for Maximum Likelihood (RAxML and GARLI) analyses or Posterior Probabilities (PP) for Bayesian Inference (MrBayes and Phycas). **J.** Majority-rule consensus tree (RAxML bootstrap) of 138 samples under GTR+ Γ model. Colors correspond to clades in Figure 2. Numbers by the nodes indicate the corresponding Bootstrap Support (BS) for Maximum Likelihood (RAxML) analysis.

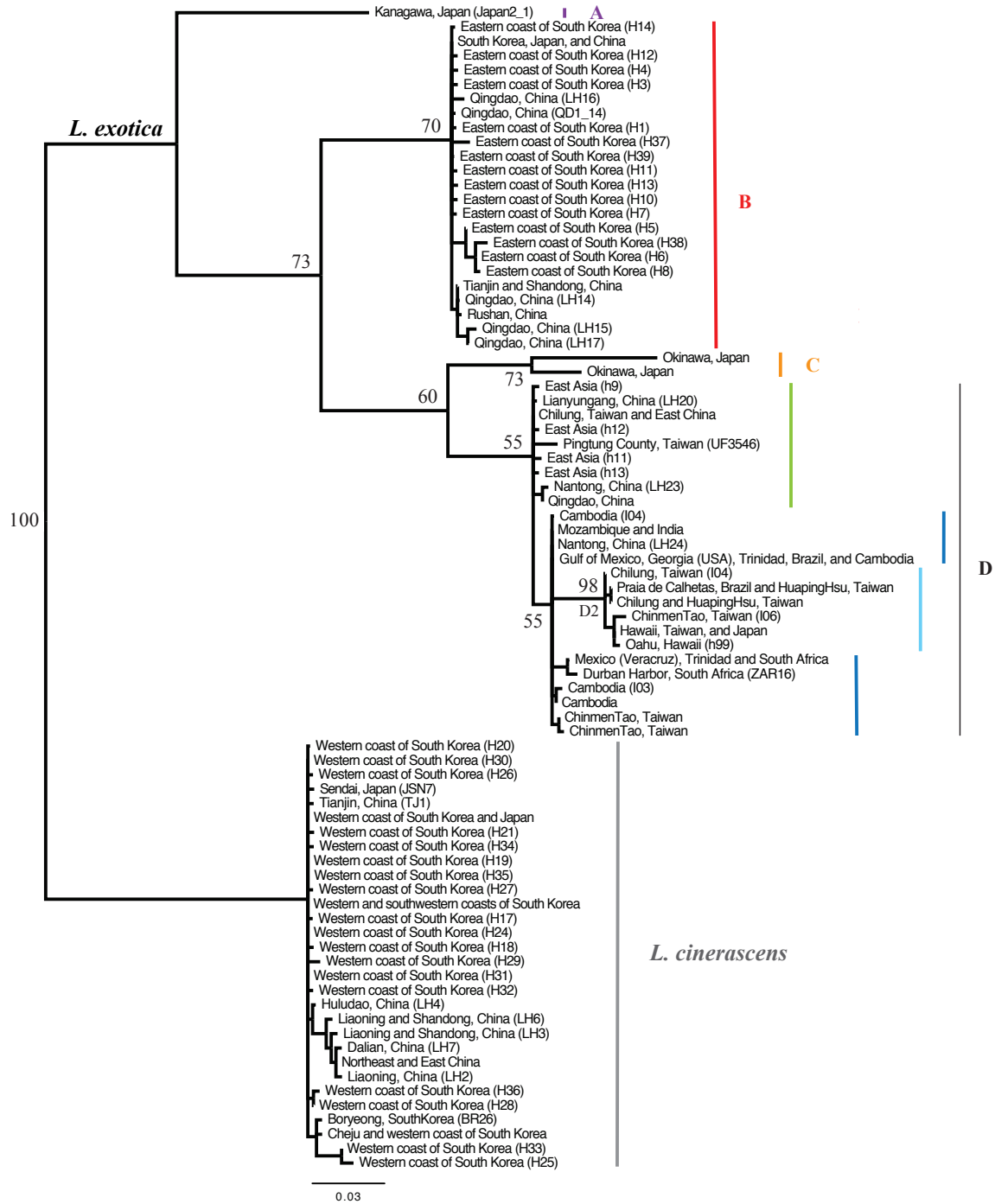
- A. Majority-rule consensus tree of 81 samples reconstructed by RAxML under GTR+ Γ model.
- B. Majority-rule consensus tree of 81 samples reconstructed by RAxML under HKY+I+ Γ model.
- C. Majority-rule consensus tree of 81 samples reconstructed by GARLI under GTR+ Γ model.
- D. Majority-rule consensus tree of 81 samples reconstructed by GARLI under HKY+I+ Γ model.
- E. Majority-rule consensus tree of 81 samples reconstructed by GARLI under TPM2uf+I+ Γ model.
- F. Majority-rule consensus tree of 81 samples reconstructed by MrBayes under HKY+I+ Γ model.
- G. Majority-rule consensus tree of 81 samples reconstructed by MrBayes under TPM2uf+I+ Γ model.
- H. Majority-rule consensus tree of 81 samples reconstructed by Phycas under GTR+ Γ model.
- I. Majority-rule consensus tree of 81 samples reconstructed by Phycas under HKY+I+ Γ model.
- J. Majority-rule consensus tree of 138 samples reconstructed by RAxML under GTR+ Γ model.

Appendix 6 Continued.

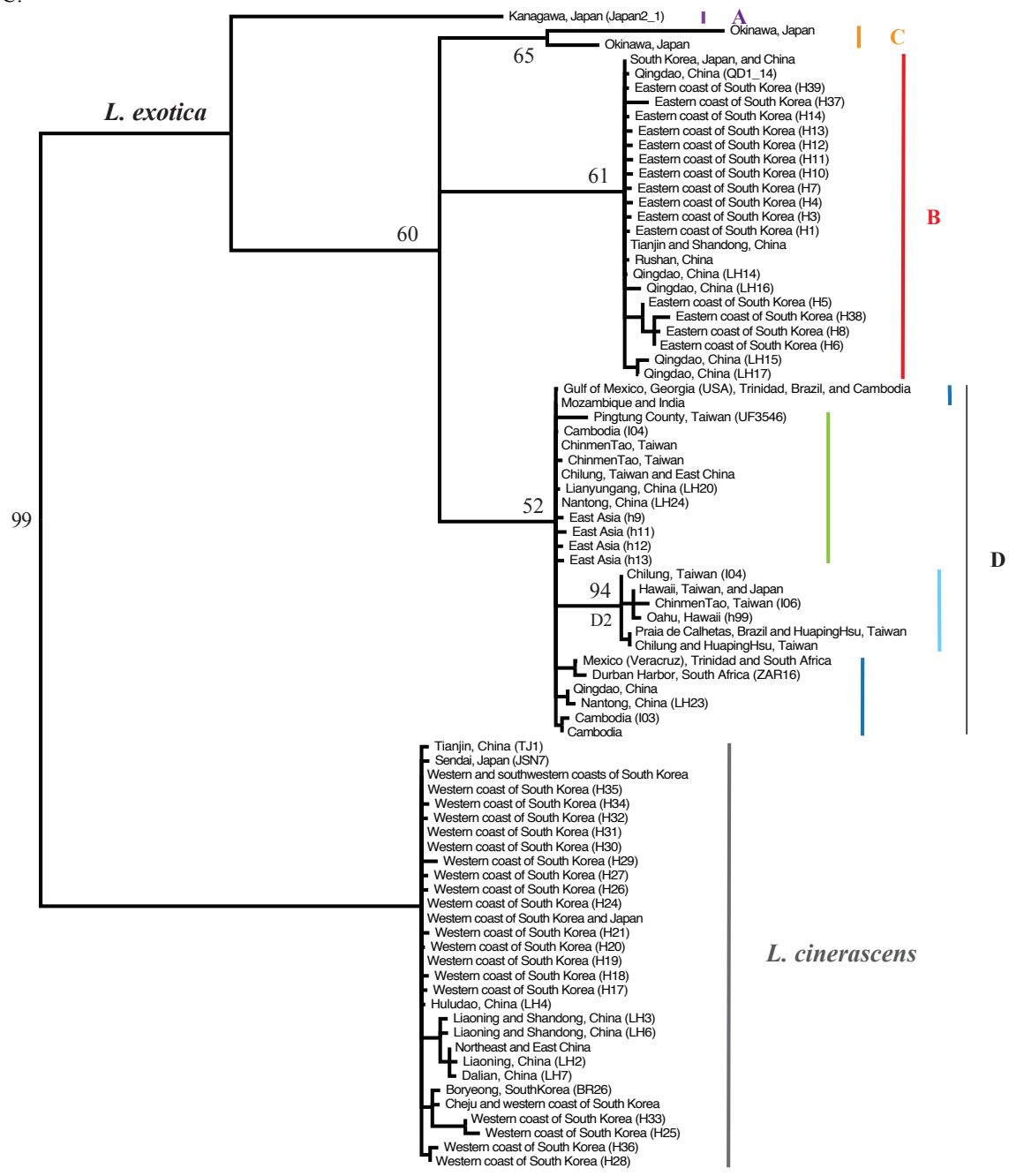
A.



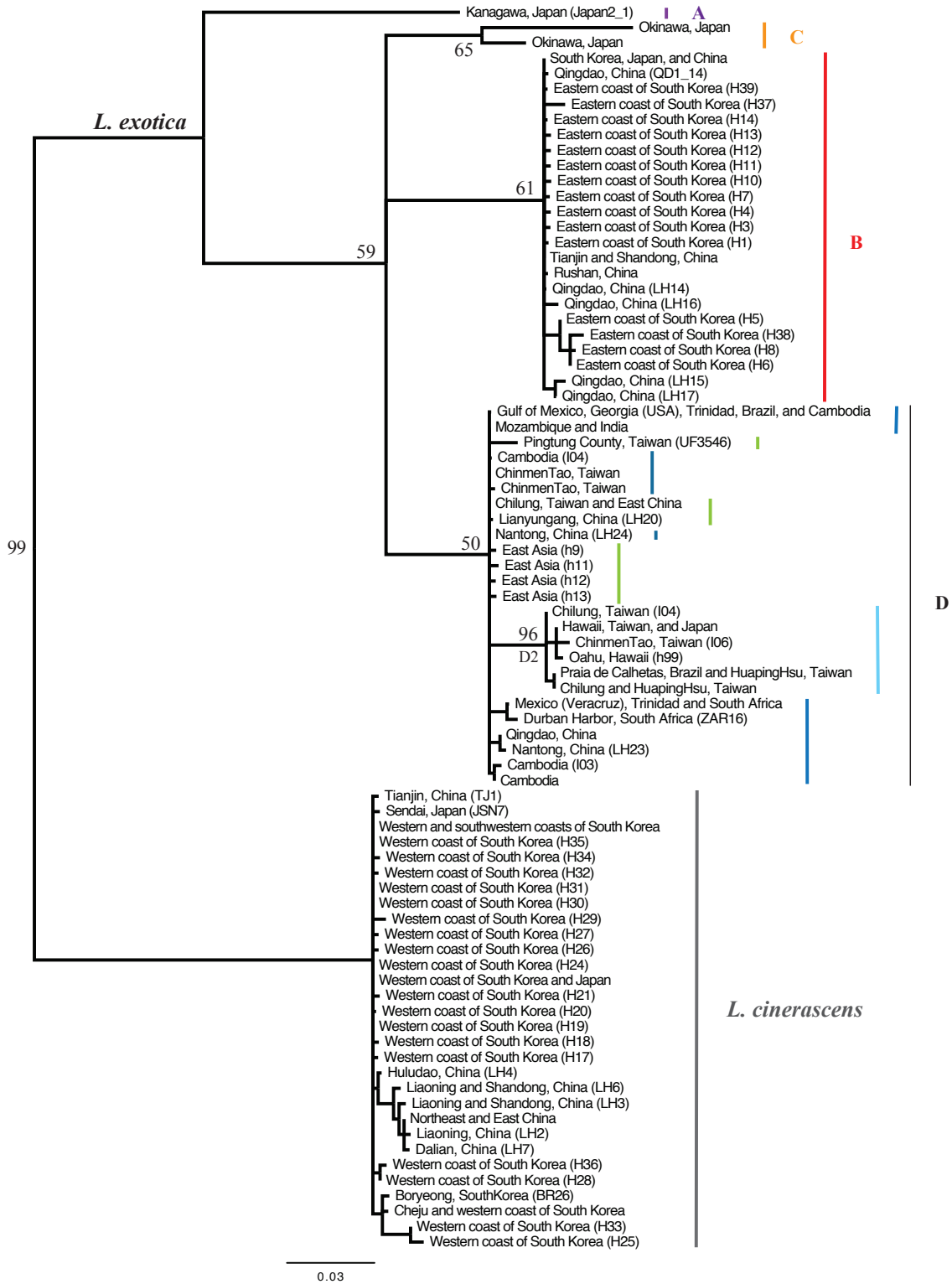
B.



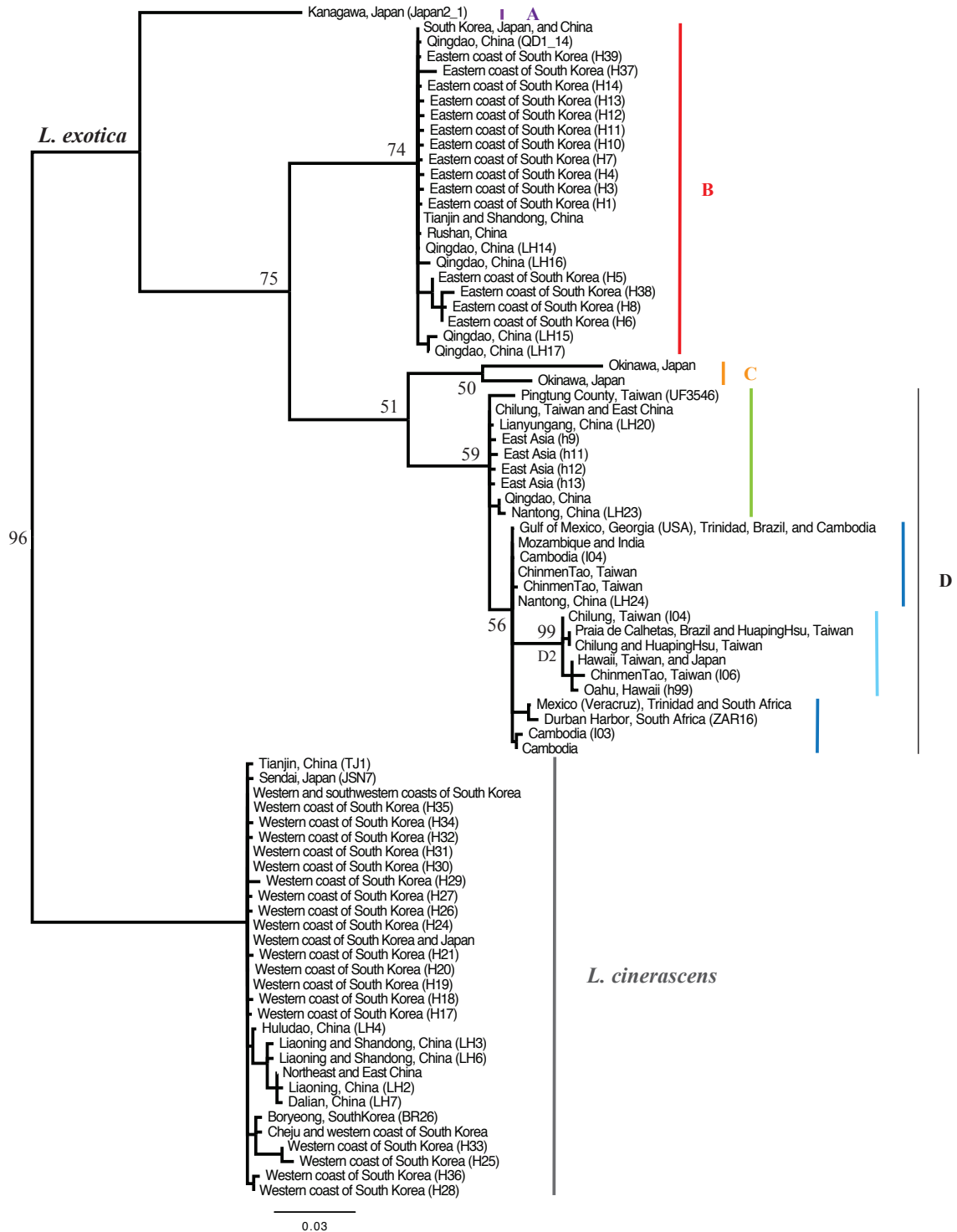
C.



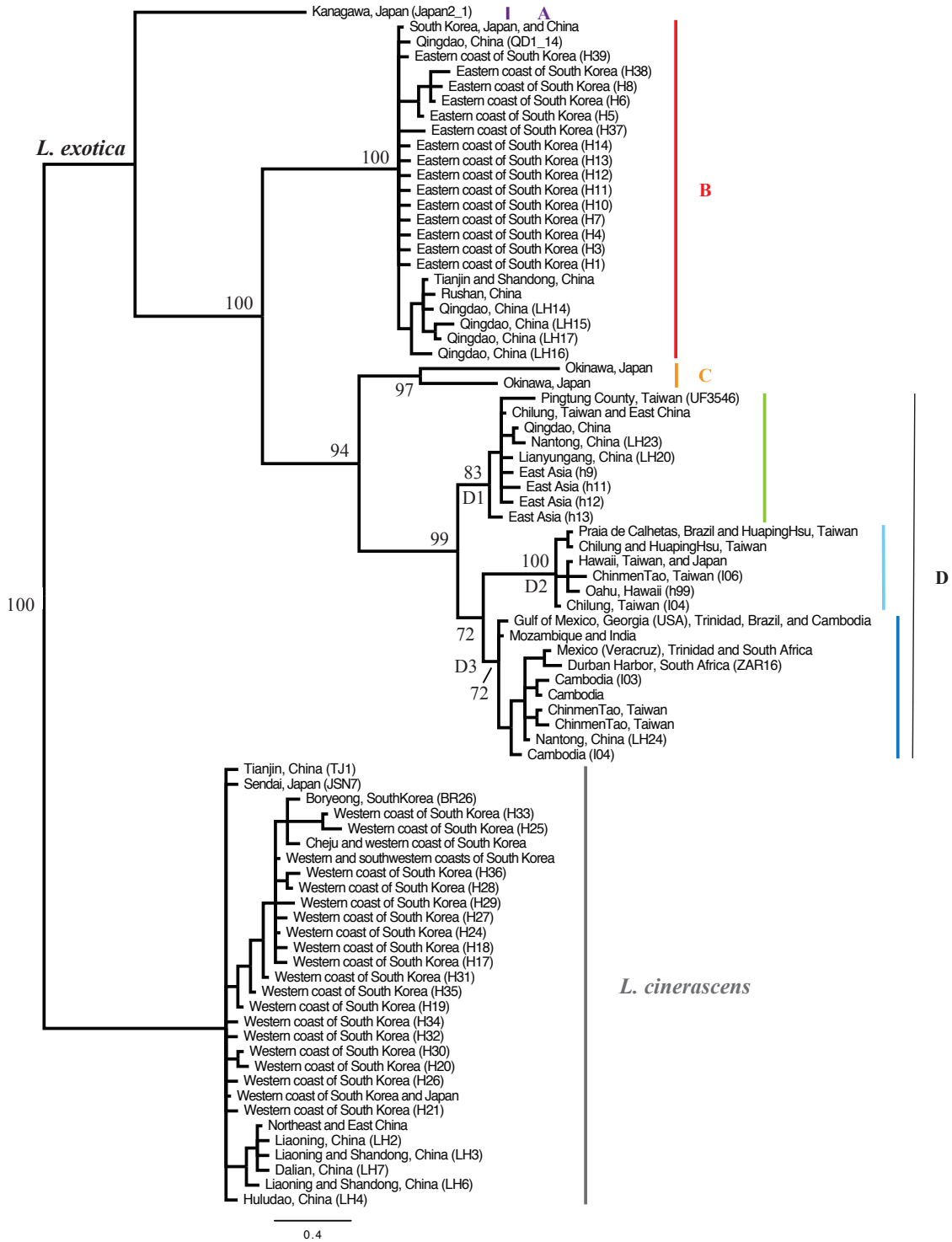
D.



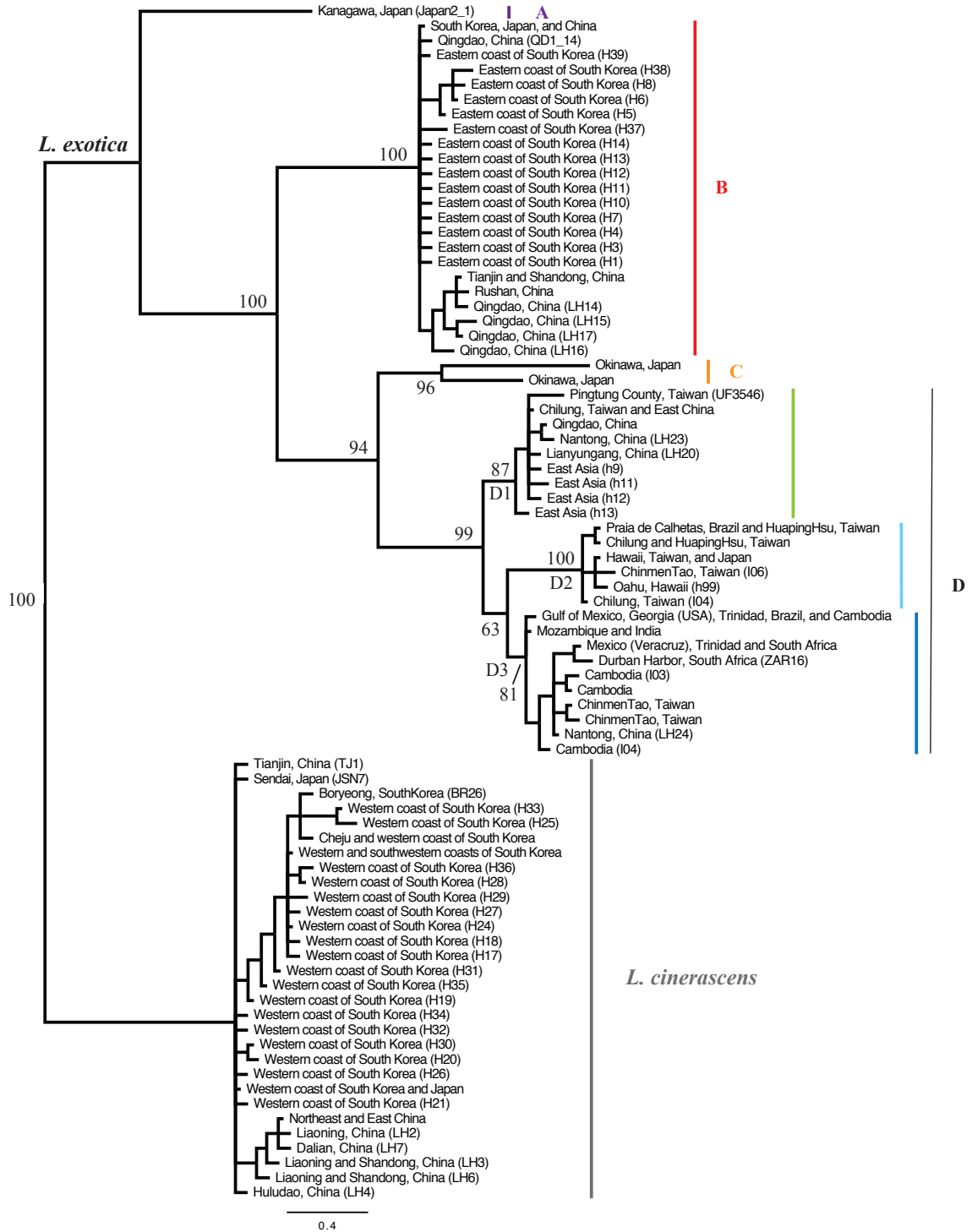
E.



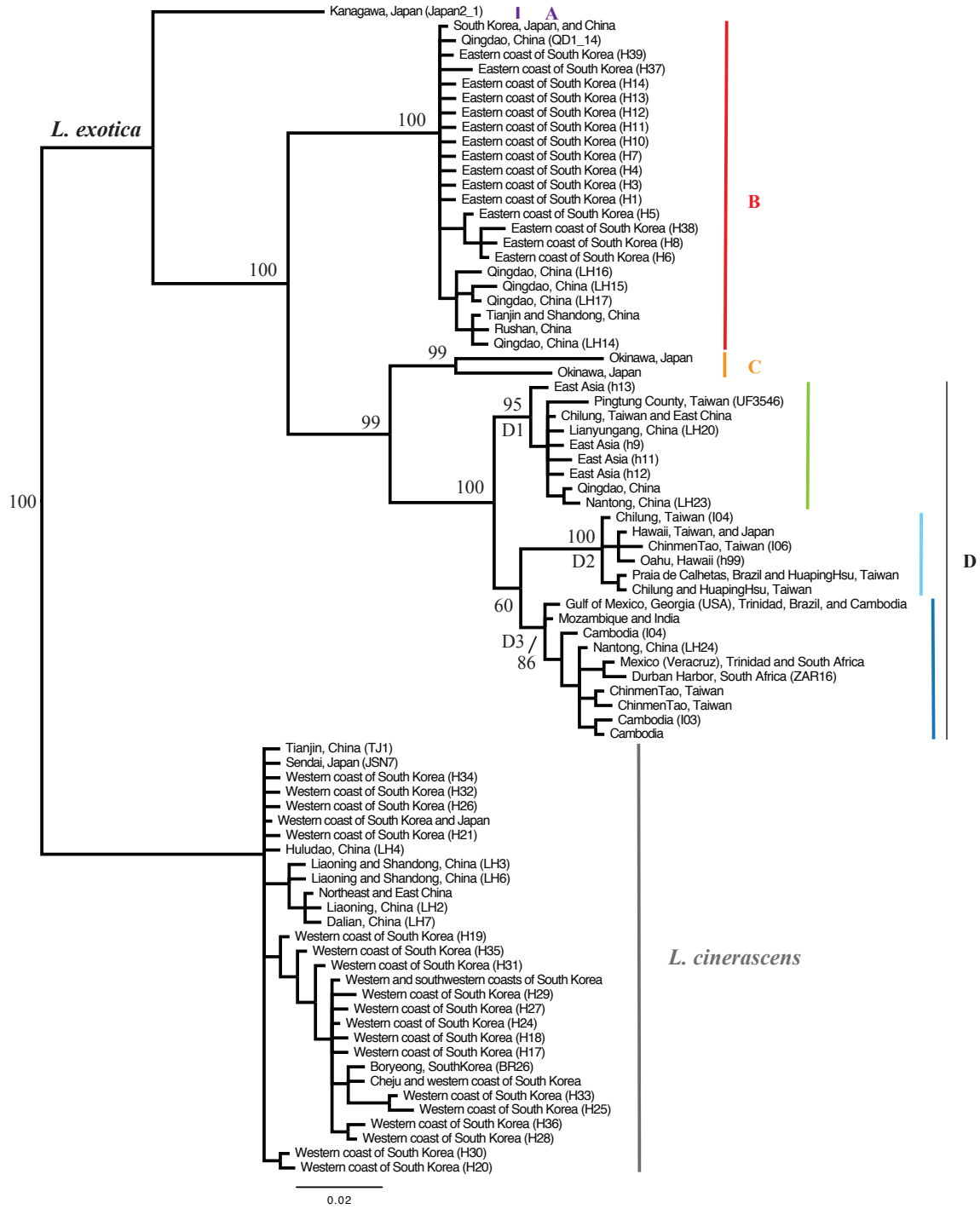
F.



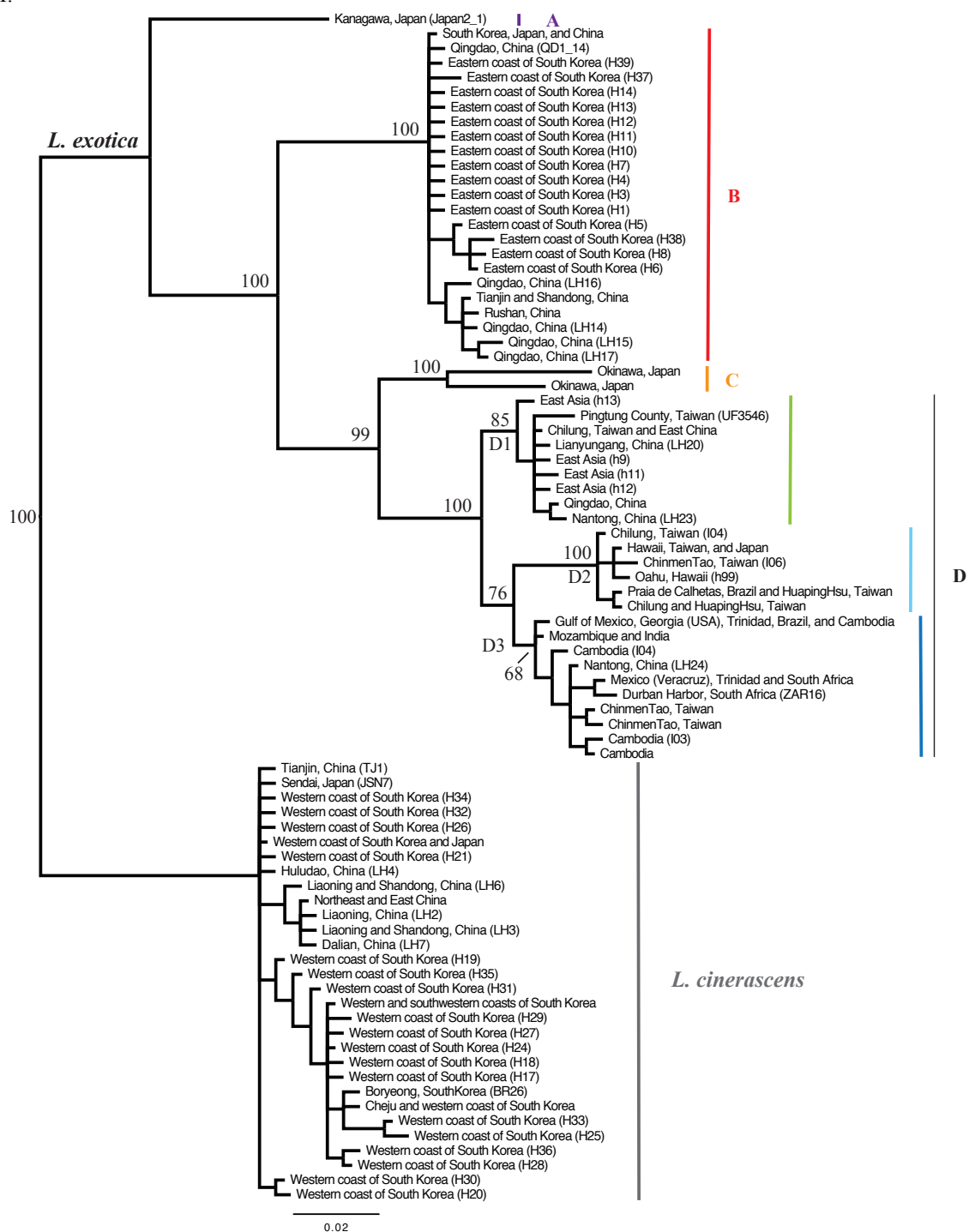
G.



H.



I.



J.

