University of South Bohemia in České Budějovice
Faculty of Science

World travellers: phylogeny and biogeography of the butterfly genus *Leptotes* (Lepidoptera: Lycaenidae)

RNDr. Thesis

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Annotation

In this thesis, we investigated phylogenetic and biogeographical relationships of neglected butterflies of the subtribe Leptotina (Lepidoptera: Lycaenidae), widely distributed in tropical and subtropical areas around the world. We used both mitochondrial and nuclear markers and different molecular analyses to resolve relationships within the subtribe. We also focused on diversification times and biogeographical patterns of Old World taxa and detailed phylogeography of the most widespread species, *Leptotes pirithous*.

Declaration [in Czech]

Prohlašuji, že svoji rigorózní práci jsem vypracoval/a samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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V Českých Budějovicích, 16. září 2019

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Mgr. Jana Marešová

This thesis is based on the following publication:

Contribution of the candidate

Jana Marešová is the second and corresponding author of the paper. Jana carried out the laboratory work, contributed to data analyses and wrote the manuscript with ZFF. She is responsible for the data visualisation. The study is based on her bachelor thesis.

Co-author agreement

Zdeněk Faltýnek Fric, the first author of the publication, fully acknowledges the contribution of Jana Marešová as stated above.

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RNDr. Zdeněk Faltýnek Fric, Ph.D.

World travellers: phylogeny and biogeography of the butterfly genus Leptotes (Lepidoptera: Lycaenidae)

Z D E N E K   F.   F R I C[1], J A N A   M A R E S O V A[1,2], T O M A S   K A D L E C[3],
R O B E R T   T R O P E K[1,4], T O M A S Z   W.   P Y R C Z[5,6]
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Abstract. Leptotina butterflies (Lycaenidae, Polyommatiinae) are found mostly in tropical and subtropical areas around the globe, marginally penetrating into temperate regions. Here, we investigated phylogenetic and biogeographical relationships of most representatives of the subtribe, using both likelihood and Bayesian approaches. We also estimated the timing of their diversification. And lastly, we studied phylogeographic patterns of the most widespread species, *Leptotes pirithous*. DNA sequences from two mitochondrial (COI, COII) and two nuclear genes (wingless, Ef1α) were analysed for 13 species of the genus *Leptotes* Scudder and one species of the genus *Cyclyrius* Butler. Both genera together form a monophyletic clade, and *Cyclyrius* is rooted deep inside *Leptotes*. Therefore, we designate *Cyclyrius* to be a junior synonym of *Leptotes*. According to our study, the genus *Leptotes* originated between the late Eocene and early Oligocene (35–31 Ma). During the Miocene it dispersed to the rest of the southern hemisphere, with further speciation events within the Indo-Australian region, and separate radiations in the Americas and the Afrotropics. *Leptotes webbianus* from the Canary Islands turned out to be sister to the American clade from which it split *c*. 12 Ma. *Leptotes pirithous* originated in Madagascar *c*. 4 Ma and invaded the whole of Africa and southern Europe, including numerous surrounding islands. Populations of *L. pirithous* from Mauritius and Madagascar turned out to represent a distinct species (*Leptotes durrelli* **sp.n.**) and the same applies to the Australasian populations of *Leptotes plinius* (*Leptotes lybas* **stat. rev.**).

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid: zoobank.org:pub:20308930-988B-4327-A35F-CC983D46263B.

Introduction

The butterfly genus *Leptotes* Scudder is distributed in all zoogeographic regions except Antarctica. This distribution led the lepidopterist Bernard D'Abrera (2009: 812) to state, in his highly controversial introduction to his atlas of Afrotropical butterflies, that the genus represents 'more delightful puzzles to test the phase-locked mind of any Darwiniac. The genus has been recorded not only in Africa, but also in the Oriental, Australian, Holarctic and Neotropical Regions, and there is the remarkable (?) presence of a species endemic to the Galapagos Islands'. This statement attracted our attention and we decided to solve this 'puzzle' using evolutionary biology approaches.

Wide cross-continental distribution ranges are known mainly among temperate butterfly genera, e.g. in *Polygonia* Hübner (Wahlberg *et al.*, 2009) or *Erebia* Dalman (Peña *et al.*, 2015).

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However, pantropical or pansubtropical distributions of butterflies are usually known only for those genera with vagrant or migratory species, such as *Danaus* Kluk (Zalucki & Clarke, 2004), *Junonia* Hübner (Kodandaramaiah & Wahlberg, 2007) or *Vanessa* Fabricius (Wahlberg & Rubinoff, 2011). There are numerous studies about evolutionary connections between Old World continents, including butterflies [*Charaxes* Ochsenheimer (Aduse-Poku *et al.*, 2009); *Junonia* (Kodandaramaiah & Wahlberg, 2007)]. On the other hand, the number of tropical butterfly genera occurring on both sides of the Atlantic Ocean is limited, and biogeographical connections between Afrotropical and Neotropical regions are exceptional (Kodandaramaiah & Wahlberg, 2007; Silva-Brandao *et al.*, 2008; Wahlberg & Rubinoff, 2011). In the cited studies, the authors found the almost global distributions to be the result of long-distance dispersal with unknown routes, which happened *c.* 5 Ma in *Junonia*, and unclear but apparently much older (> 15 Ma) in *Vanessa* (Wahlberg & Rubinoff, 2011). In *Acraea* Fabricius s.l., Silva-Brandao *et al.* (2008) show an African origin for an otherwise monophyletic Neotropical branch; unfortunately, this work lacks any estimate of the timing of the events. Among the Hesperiidae, the genus *Celaenorrhinus* Hübner s.l. is the only one that shows a nearly pantropical distribution, with representatives in Neotropical, Oriental and Afrotropical regions. According to Libert (2014), based on morphological and molecular (COI) data, at least South American species segregate with a group of African species in a monophyletic clade; however, there are no radiation timing estimates here either (cf. D.R. Dolibaina, unpublished data; A. Przystałkowska, unpublished data).

Similar examples of large-scale distributions among Lycaenidae are the genera *Zizula* Chapman, *Zizina* Chapman (see Yago *et al.*, 2008) and *Lampides* Hübner (cf. Lohman *et al.*, 2008). Among these, only *Zizula cyna* (Edwards) occurs in the Neotropical region; the other species occur solely in the Old World. The genus *Zizina* is known from two distinct temperate regions (East Asia and New Zealand), with the tropical *Z. otis* (Fabricius) distributed in between. This species seems to have spread across the Afrotropical, Oriental and Australian regions in a massive dispersal event *c.* 2 Ma (Yago *et al.*, 2008). The only member of the genus *Lampides*, the migratory *L. boeticus* (Linnaeus), does not show any clear phylogeographic pattern, which indicates that there is persistent gene flow among vast areas of its Palaeotropical distribution (Lohman *et al.*, 2008).

The genus *Leptotes* comprises small, similar-looking butterflies; many species can only be identified through genitalia dissections. Despite its global distribution, the genus is not species-rich, with only about 30 described species (Table 1). It contains few widespread species, such as *L. plinius* (Fabricius), known from a vast area ranging from India to Australia, *L. pirithous* (Linnaeus) from Africa and Eurasia, and two species from the Americas [*L. cassius* (Cramer) and *L. marina* (Reakirt)]. Several species are sympatric in continental Africa and South America, and the others are endemic to various islands (Table 1). The genus, together with the genus *Cyclyrius* Butler, forms the section Leptotes within the arrangement of Lycaenidae proposed

Table 1. List of *Leptotes* species with their distribution.

African species
Leptotes adamsoni Collins & Larsen, 1991
Leptotes babaulti (Stempffer, 1935**)**
Leptotes brevidentatus (Tite, 1958)
Leptotes casca (Tite, 1958)[a]
***Leptotes durrelli** **n. sp.**[a]
Leptotes jeanneli (Stempffer, 1935**)**
Leptotes mandersi (Druce, 1907)[a]
Leptotes marginalis (Stempffer, 1944)
Leptotes mayottensis (Tite, 1958)[a]
Leptotes pirithous (Linnaeus, 1767) (also in Mediterranean Europe and southwest Asia)
Leptotes pulcher (Murray, 1874)
Leptotes pyrczi Libert, 2011[a]
Leptotes rabefaner (Mabille, 1877)[a]
Leptotes sanctithomae (Sharpe, 1893)[a]
Leptotes socotranus (Ogilvie-Grant, 1899)[a]
Leptotes webbianus (Brullé, 1839)[a]
Asian and Australian species
***Leptotes lybas** (Godart, 1823)**
Leptotes plinius (Fabricius, 1793)
American species
Leptotes andicola (Godman & Salvin, 1891)
Leptotes bathyllos Tessmann, 1928
Leptotes callanga (Dyar, 1913)
Leptotes cassius (Cramer, 1775)
Leptotes delalande Bálint & Johnson, 1995
Leptotes krug Bálint, Johnson, Salazar & Velez, 1995
Leptotes lamasi Bálint & Johnson, 1995
Leptotes marina (Reakirt, 1868)
Leptotes parrhasioides (Wallengren, 1860)[a]
Leptotes perkinsae Kaye, 1931[a]
Leptotes trigemmatus (Butler, 1881)

Species investigated in this study are marked in **bold**. Taxa marked with an asterisk are newly described or elevated to species rank.
[a]Island endemics.

by Eliot (1973), or the subtribe Leptotina according to modern concepts (cf. Talavera *et al.*, 2015).

The generic arrangement of individual species of the subtribe Leptotina has varied over time. Originally, the genus *Leptotes* was erected by Scudder (1876) for American species, whereas Old World species were known under the genus name *Syntarucus* Butler. The similarity between these genera was highlighted by Stempffer (1935), and since then various species have been transferred back and forth between the two genera (Clench, 1963; Fox *et al.*, 1965; Vári & Kroon, 1986; Larsen, 1991a, b).

The situation within *Cyclyrius* is equally complicated, as it was believed to comprise two species (cf. Stempffer, 1967): *C. webbianus* (Brullé) distributed in the Canary Islands, and *C. mandersi* (Druce) in Mauritius. Because of a similar underside pattern, the Neotropical species *Leptotes callanga* (Dyar) was also included in this genus (Larsen, 1991b). However, Bálint & Johnson (1995) associated the latter species with other Neotropical members of the genus *Leptotes*. Finally, Libert (2011) moved *C. mandersi* to the genus *Leptotes*, leaving *Cyclyrius* as monobasic. Furthermore, Merit *et al.* (2017) recently described two subspecies of *C. webbianus*.
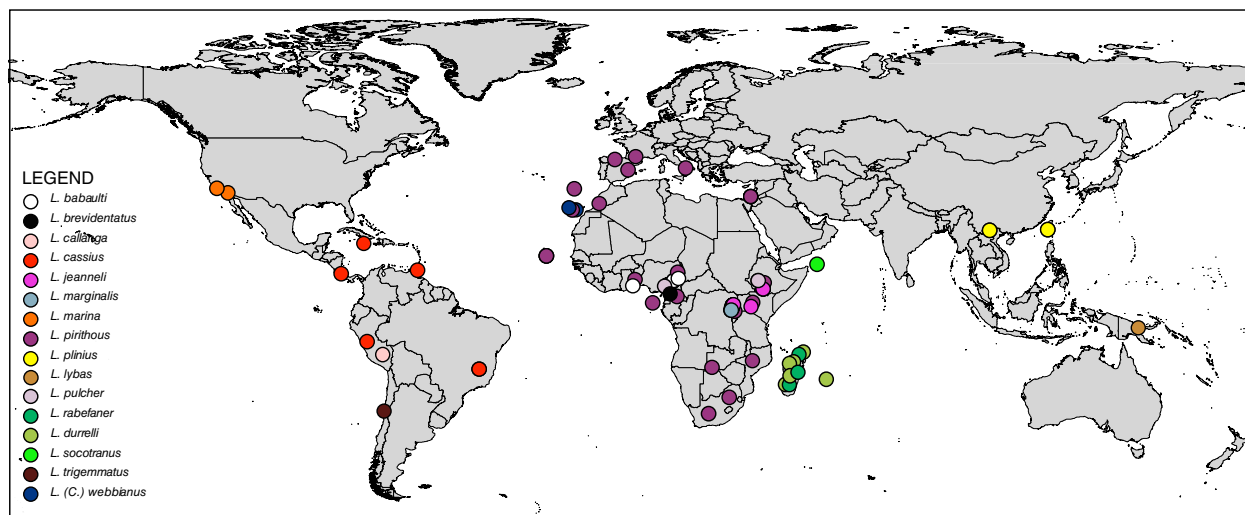
Fig. 1. Map showing an origin of the material of subtribe Leptotina used for phylogenetic analyses in this study. [Colour figure can be viewed at wileyonlinelibrary.com].

In this paper: (i) we present a phylogenetic analysis resolving the relationships within the Leptotina subtribe; (ii) we also focus on diversification times and biogeographic patterns, especially targeting Old World taxa; and (iii) we investigate in detail the phylogeography of the most widespread Old World species, *L. pirithous*, distributed across the whole of Africa and Madagascar, Mediterranean Europe, southwest Asia, and a number of islands throughout the region.

Materials and methods

Material, DNA extraction and sequencing

We dissected genitalia and sequenced 302 individuals of the genus *Leptotes*, from which 103 samples with sequences of at least two gene regions were used for this study. In total, our dataset comprises samples from several different populations of *L. plinius*, four American species, eight African *Leptotes* species and *Cyclyrius webbianus* from the Canary Islands (see Appendix S1 for details and Fig. 1 for distribution of samples in our study). As outgroup taxa, we selected four species – *Azanus mirza*, *Everes comyntas*, *Lampides boeticus* and *Zizula hylax* – all in the lycaenid subfamily Polyommatinae (cf. Stradomsky, 2016). We identified the specimens based on genital morphology following the revisions by Bálint & Johnson (1995) and Libert (2011). Male and female abdomens were soaked in 10% KOH solution for 5–10 min. Subsequently, abdomens were cleaned in water in order to expose the sclerotized genitalia. Female abdomens were stained in chlorazole black in order to identify soft genital parts. Dissected genitalia were cleansed of water using ethanol 90% and 95% solutions. The genitalia were measured using SCION IMAGE v. Alpha 4.0.3.2.

We extracted DNA from two legs or from the anterior part of the abdomen using the Geneaid Blood and Tissue extraction kit (Taiwan). We also used DNA extracted by Monaghan *et al.*

(2009). We sequenced the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and cytochrome *c* oxidase subunit II (COII) and the nuclear genes wingless (wg) and the 3′ part of elongation factor 1-alpha (Ef1α). The primers are listed in Appendix S2. For the PCR, we followed protocols by Monteiro & Pierce (2001), Wahlberg & Wheat (2008) and Vila *et al*. (2011). The PCR products were sequenced by Macrogen Inc. (South Korea) using an ABI 3730XL DNA analyser (Massachusetts). The sequences were aligned with GENEIOUS v.7.1.9. (Kearse *et al*., 2012) and submitted to GenBank (http://www .ncbi.nlm.nih.gov/) (Appendix S1).

We removed ambiguous regions at the start and end positions of each primer region and thus our dataset comprised a total of 2763 bp (1514 bp of COI, 428 bp of COII, 361 bp of wg, and 460 bp of Ef1α). The data were partitioned by gene regions and codon positions. The best partition schemes were tested and selected using PARTITION FINDER v.1.1.1 (Lanfear *et al*., 2012), which was shown in Peña *et al*. (2015) to be superior to traditional partitioning methods.

Phylogenetic analyses

We used maximum likelihood (ML) and Bayesian inference (BI) approaches for the phylogenetic analyses. The ML analysis was carried out in RAXML v.7.7.1 (Stamatakis *et al*., 2008) using the RAXML BLACKBOX computing environment with 100 bootstrap replications. The BI analysis was carried out in MRBAYES v.3.2 (Ronquist *et al*., 2012) with four chains running for 10 million generations and sampling every 1000 generations. We discarded the first 10% of trees as burn-in, which was sufficient according to our control using the 'sump' command. The analyses were run using the computational facilities of MetaCentrum of the Czech National Grid Infrastructure (https:// metavo.metacentrum.cz/).
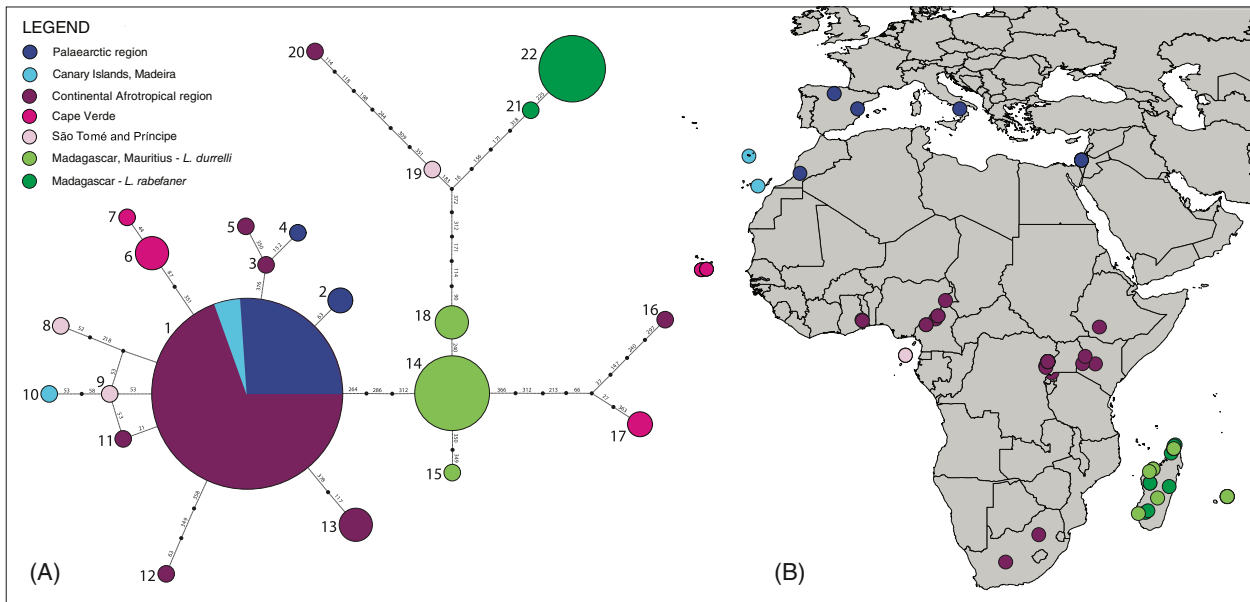
Fig. 2. Phylogeography of the *Leptotes pirithous* complex. (A) Result of the statistical parsimony network analysis; (B) origin of individual samples. The colours between the network and map are congruent. [Colour figure can be viewed at wileyonlinelibrary.com].

Diversification time estimation and biogeography

To calculate estimates of diversification times, we used BEAST v.1.8.4. (Drummond *et al.*, 2012) with unlinked substitution and clock models. We used two secondary calibration points from Chazot *et al.* (2019). The first calibration point was set to the root with an age of 34.52 Ma (± 1.3 Ma, SD), and the second point was set after removal of *A. mirza* to 28.93 Ma (± 1.2 Ma). We used a speciation birth-death model with relaxed lognormal clock. The analyses were run for 50 000 000 generations, sampled every 5000 generations and repeated three times. The parameters of all three runs were compared in TRACER v.1.5 (Drummond & Rambaut, 2007), where we also checked the model convergence (effective sample size > 200). Trees of all three runs were combined by LOGCOMBINER v.1.8.4. (http://beast.bio.ed.ac.uk/logcombiner) (Drummond *et al.*, 2012), 10% of trees were discarded as burn-in. The maximum credibility tree was selected using TREEANNOTATOR v.1.8.4 (http://beast.bio.ed.ac.uk/treeannotator) (Drummond *et al.*, 2012). We ran the analyses in the CIPRES portal (Miller *et al.*, 2010).

We used the maximum credibility tree from the BEAST analysis for the biogeographical analysis. To prevent possible effects of false information due to uncertainty of the outgroup taxa, we removed all the outgroup taxa from the tree. We selected eight distribution areas: America (i.e. both Americas combined, as only two species reached North America), the Canary Islands, continental Africa (including the Mediterranean), the Cape Verde Islands, the Malagasy area (Madagascar + Mauritius), Socotra, the Oriental region (including Taiwan) and the Australian region (see Appendix S3). We used RASP v. 4.0 (Yu *et al.*, 2015), which incorporates the R package BIOGEOBEARS (Matzke,

2012, 2013). The framework compares three alternative models of previous geographic range estimation based on the Akaike information criterion and, for each of them, also a variant with a founder effect (parameter *j*): dispersal-extinction cladogenesis (DEC), dispersal-vicariance analysis (DIVALIKE) and BI for discrete areas (BAYAREALIKE). We allowed for a maximum of five possible areas, based on the proximity of the areas (Appendix S4).

Phylogeographic structure of L. pirithous

For *L. pirithous*, we sequenced the 5′ half of the COI gene (= 'barcode region'). The dataset comprised 58 samples covering the entire species distribution range (Fig. 2B). We also added nine samples of its sister species, *L. rabefaner*. We analysed the data using statistical parsimony networks implemented in TCS v.1.21 (Clement *et al.*, 2000) with a cut-off of 95%.

Results

Sequence partitioning scheme

In total, we had 12 possible data partitions (three for COI, three for COII, three for wg and three for Ef1α). Based on the Bayesian information criterion (BIC), the algorithm selected a partitioning scheme comprising seven partitions. However, as not all substitution models were available in all phylogenetic programs, we used the first available model with the lowest BIC scores for the corresponding analyses (see Appendix S5).

Phylogeny and taxonomic implications

The trees obtained from ML and BI analyses showed congruent results. The genera *Leptotes* and *Cyclyrius* together form a monophyletic clade; however, *Cyclyrius* is placed within *Leptotes*. To avoid making *Leptotes* paraphyletic, we follow the decision of Clench (1963) and consider *Cyclyrius* as a junior synonym of *Leptotes*. Thus, the species name combination should be *Leptotes webbianus* (Brullé, 1839), as proposed by Fox *et al.* (1965). The subtribe Leptotina comprises only a single genus, *Leptotes*.

The first split occurred between *L. plinius* and the other *Leptotes* taxa. The latter then divided into two main branches, the first branch comprising all American species and the second branch all African species (Fig. 3A, B). The position of the species endemic to the Canary Islands, *L. webbianus*, is as sister to the American taxa.

Within the monophyletic *Leptotes plinius* there is a deep split between Asian and Austro-Papuan samples and between continental Asian and Taiwanese populations (Appendix S6). These differentiations are deeper than most splits between species pairs in Africa or the Americas. Based on morphological examination of material from India, Sri Lanka, Thailand, China, Indonesia and Australia, we split *Leptotes plinius* in two distinct species: *L. plinius*, distributed in continental Southeast Asia and Taiwan; and *Leptotes lybas* **stat. rev.**, which used to be classified as a subspecies of *L. plinius*, and is distributed in Australia, New Guinea, islands east of Wallace's Line and Java. The two species have similar shaped male valvae but differ clearly by the shape of the brachiae (= subunci), which are short and bearing a 'comb' of small teeth in *L. plinius*, but long and bifurcated close to their base in *L. lybas* (Fig. 4A–H; adults, Fig. 5A–D).

In the American group, *L. callanga* is sister to all other included American species. *Leptotes marina* is also monophyletic, but *L. cassius*, the most widespread American species, is paraphyletic with respect to *Leptotes trigemmatus*. However, we acknowledge that this result may be an artefact of under-representation of other American species in our dataset. More detailed taxon sampling of the American species is needed.

The African species form two groups. The first group is represented by continental African species (all of them monophyletic). One clade contains *Leptotes brevidentatus* and *Leptotes pulcher*, the other clade contains *Leptotes jeanneli*, and a sister pair of *Leptotes babaulti* and *Leptotes marginalis*. Each of the two species, *L. jeanneli* and *L. babaulti*, comprises two clusters, indicating the possibility of cryptic diversity. However, due to the relatively low number of studied specimens and the lack of clear morphological differences, we refrain from any taxonomic action at this moment.

The second African group represents the island species *L. socotranus*, *L. rabefaner*, and the most widespread species *L. pirithous*. The European individuals of *L. pirithous* are not distinguishable from the other conspecific populations from continental Africa. However, the populations from Madagascar and Mauritius form a unique cluster, basal to all other populations of *L. pirithous*, recognizable by appearance and genitalia

characteristics (Fig. 4I–P). Therefore, we describe these populations as a distinct species (see the section on 'Taxonomic implications').

Diversification time estimation and biogeography

The genus *Leptotes* originated between the late Eocene/early Oligocene and early Miocene, when the ancestor of Oriental *L. plinius* and Australasian *L. lybas* split from the ancestor of the remaining taxa of the genus. The preferred biogeography model is dispersal-vicariance (DIVALIKE) (Fig. 6; Table 2), followed by two models with founder effect (DIVALIKE + J and DEC + J), with similar results but differing in the detail (Appendix S7). The ancestral distribution of older branches is estimated to have been wide, including Afrotropical, Oriental and Neotropical regions, and the current distribution results from vicariant splits. The origin of the *L. plinius* ancestor is Oriental, with dispersal and subsequent vicariance of the Australasian sister taxon *L. lybas*. The split between American and Canarian taxa took place *c*. 12 Ma, and the radiation of American taxa began at least *c*. 8 Ma. The radiation of the African taxa began during the Miocene (*c*. 12 Ma) with a vicariant split of the island taxa (*L. socotranus*, *L. rabefaner*, *L. durrelli* and *L. pirithous*) from the continental species.

The DIVALIKE model also estimates a wide ancestral distribution for the ancestor of *L. webbianus* and the American species. One of the alternative models (Appendix S7) suggests more reliable dispersal to the Americas through the Canary Islands. The species occurring in the Indian Ocean islands and the African species are, according to the model, vicariant (see Fig. 6). However, due to the lack of data (two species occurring in Comoros, unavailable for the study), we are unable to solve this puzzle at this time. Finally, the most widespread species, *L. pirithous*, originated in Madagascar, and later dispersed to Africa and surrounding areas.

Intraspecific genetic structure of Leptotes pirithous

Little can be said about the origin of continental African species because they occur in extended sympatry, but the most widespread species, *L. pirithous*, originated in the Malagasy area *c*. 7–5 Ma (Fig. 6). From there the species dispersed to the African continent, where it diversified into several haplotypes distributed across vast areas of the continent (Fig. 2A). The species also colonized southern parts of Europe and many islands around Africa (e.g. Príncipe, Cape Verde, Canary Islands, and Madeira). Its haplotype network (Fig. 2A) shows a connection between *L. rabefaner* (haplotypes 21 and 22) and populations of *L. durrelli* from Madagascar and Mauritius (haplotypes 14, 15 and 18; but the populations of both islands share haplotypes). The other *L. pirithous* populations are separated into three distinct haplotype lineages. The first lineage (haplotypes 19 and 20), which comprises one specimen from South Africa and one from Príncipe, is derived from the connection between both Malagasy taxa. Another *L. pirithous*
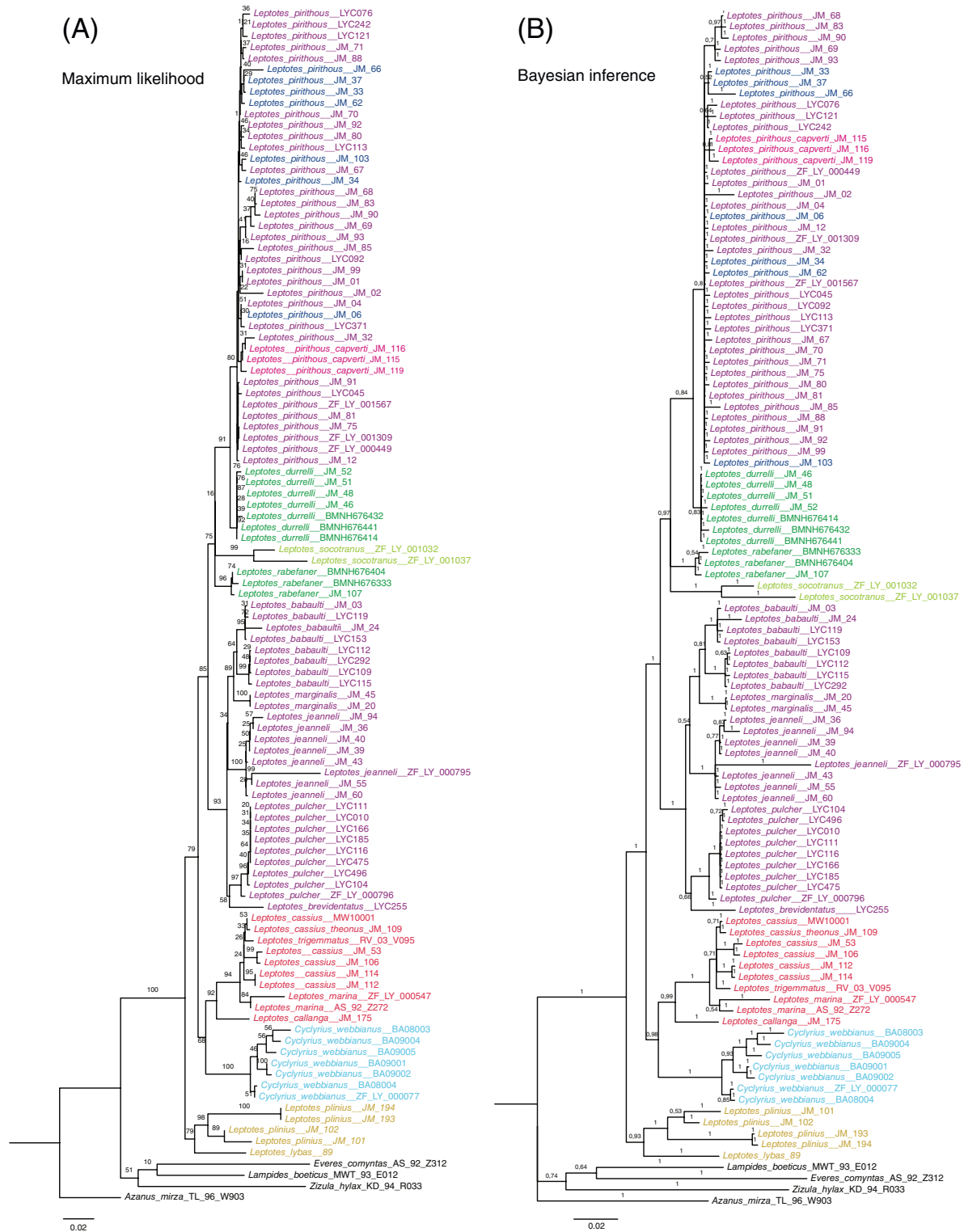
(A)

Maximum likelihood

(B)

Bayesian inference

Fig. 3. Trees obtained from the combined datasets of cytochrome *c* oxidase subunit I (COI), COII, elongation factor 1-alpha (Ef1α) and wingless. (A) maximum likelihood analysis; (B) Bayesian inference. The node values represent bootstrap values (A) and posterior probabilities (B). The colours of samples correspond to the colours of areas in Fig. 6. [Colour figure can be viewed at wileyonlinelibrary.com].
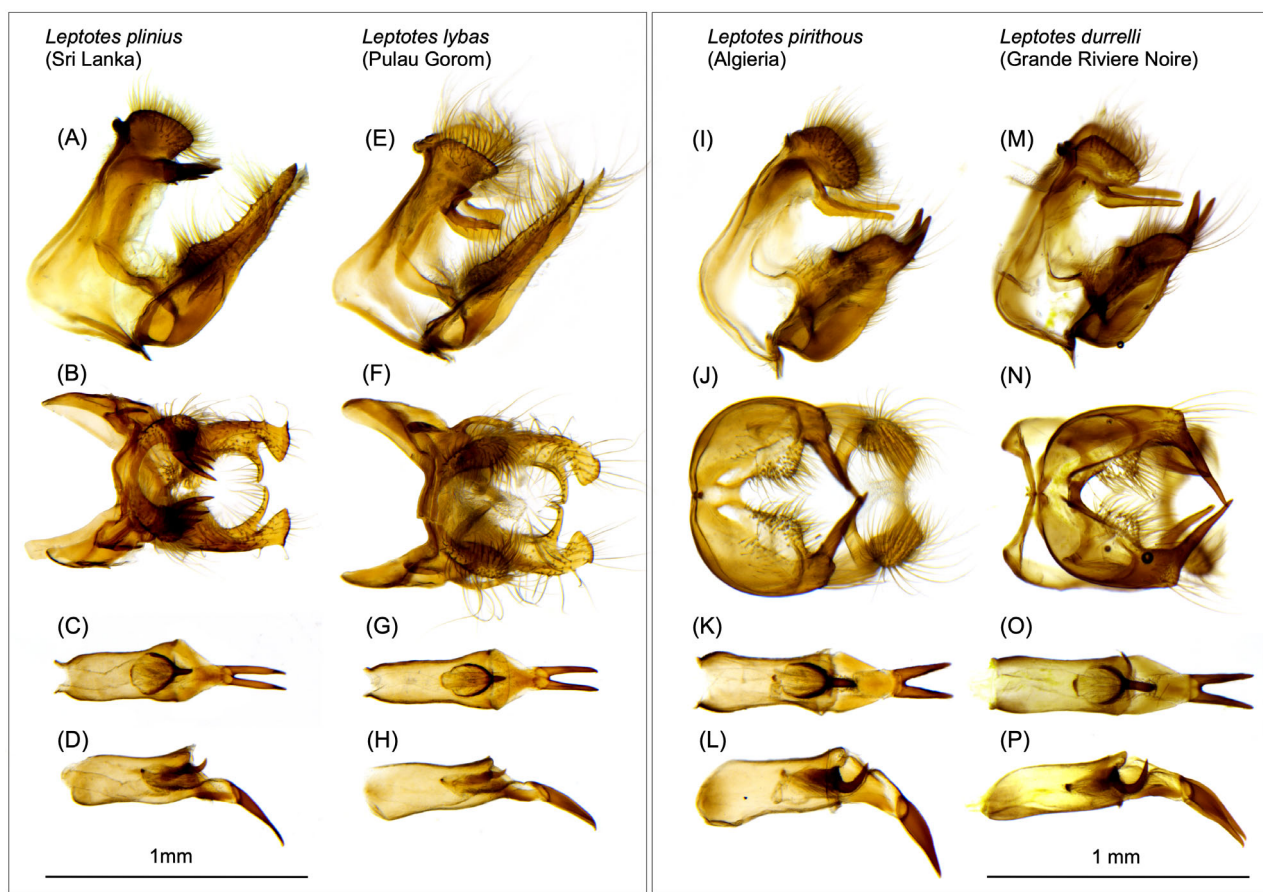
Fig. 4. Male genitalia of revised or newly described species of *Leptotes*: (A−D) *L. plinius*; (E−H) *L. lybas*; (I−L) *L. pirithous* male; (M−P) *L. durrelli*. Top row: lateral view of the entire genitalia. Second row: dorsal (B, F) or ventral (J, N) view. Third row: dorsal view of phallus. Fourth row: lateral view of phallus. [Colour figure can be viewed at wileyonlinelibrary.com].

lineage (haplotypes 16 and 17) is formed by one specimen from Ethiopia and several specimens from the Cape Verde Islands (*L. p. capverti*). The last and most numerous haplotype group is formed by haplotype 1 and derived haplotypes, shared between continental Africa, Europe and the Atlantic islands (Canary Islands, Cape Verde, Príncipe).

Discussion

As we aimed to resolve the phylogenetic relationships within the Leptotina subtribe and its biogeographical pattern, we also found one new undescribed species (*L. durrelli* **sp.n.**) from Mascarenes and Madagascar. Furthermore, we elevated populations of *L. plinius* from the Sunda region as well as from east of Wallace's line to full species status: *L. lybas*. In our study, we confirmed that the genus *Leptotes* is monophyletic if the genus *Cyclyrius* is included (Fig. 3A, B). Thus, subtribe Leptotina consists of a single genus. Although our biogeographical model preferred vicariance to dispersal, similar to the division of Gondwana, we have shown the split of *Leptotes* to be much more recent (< 35 Ma in the Oligocene) (Fig. 6). Therefore, and

contrary to the preferred biogeography model, we are convinced that the origin of the genus in Gondwana before the continental fragmentation can be ruled out, as rifting of the southern supercontinent was initiated in the late Jurassic, well before the subtribe ancestor evolved (Lomolino *et al.*, 2010). Thus, the distribution of the genus *Leptotes* can be explained by long-distance dispersal across continental or oceanic barriers. The same pattern has been observed in other butterfly genera (Monteiro & Pierce, 2001; Braby *et al.*, 2007; Kodandaramaiah & Wahlberg, 2007; Aduse-Poku *et al.*, 2009). The results of other studies show that many classic Gondwanan groups combine an ancient vicariance pattern with relatively recent dispersal events (Waters *et al.*, 2000; Cooper *et al.*, 2001). Distributions of such taxa could not have been much affected by the continental movements, and the divergence events were most probably caused by other factors, such as the changing climate.

Climate effects on the distribution of Leptotes

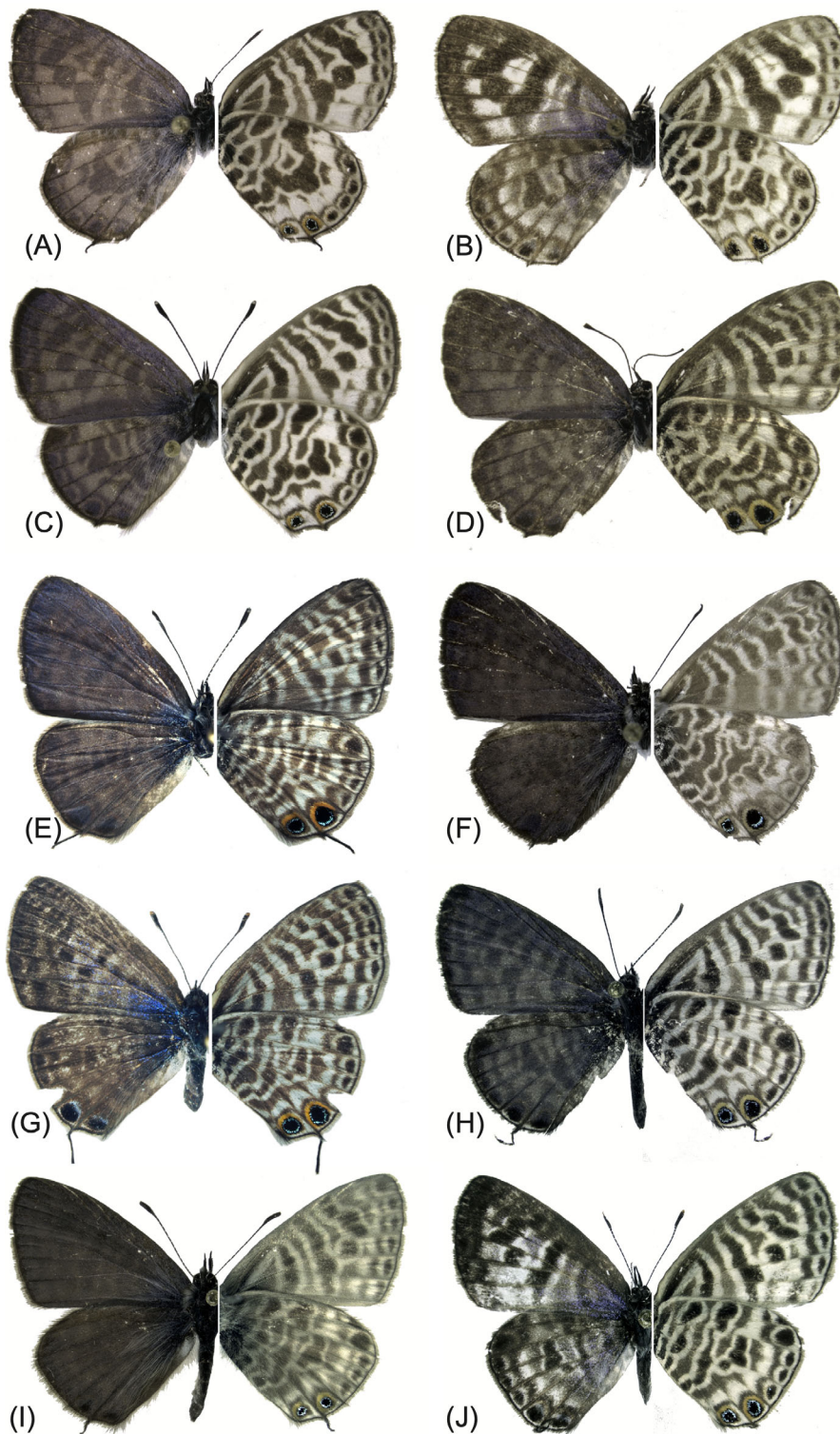The genus *Leptotes* is estimated to have evolved sometime between the late Eocene and Oligocene (33−27 Ma) (Fig. 6),

Fig. 5. Habitus of specimens of species newly described or with changed taxonomic status in the genus *Leptotes*. (A) *Leptotes plinius* (male, specimen JM-102, Taiwan); (B) *L. plinius* (female, specimen JM-101, Taiwan); (C) *L. plinius* (male, specimen ZF-LY-002086, India, West Bengal); (D) *L. lybas* (male, specimen 89, Papua New Guinea); (E) *L. durrelli* **sp.n.** (holotype); (F) *L. durrelli* (male, specimen JM-50); (G) *L. durrelli* (female); (H) *L. pirithous* (male, specimen JM-77, D. R. Congo); (I) *L. pirithous* (male, Morocco); (J) *L. pirithous* (female, specimen JM-67, D. R. Congo). [Colour figure can be viewed at wileyonlinelibrary.com].
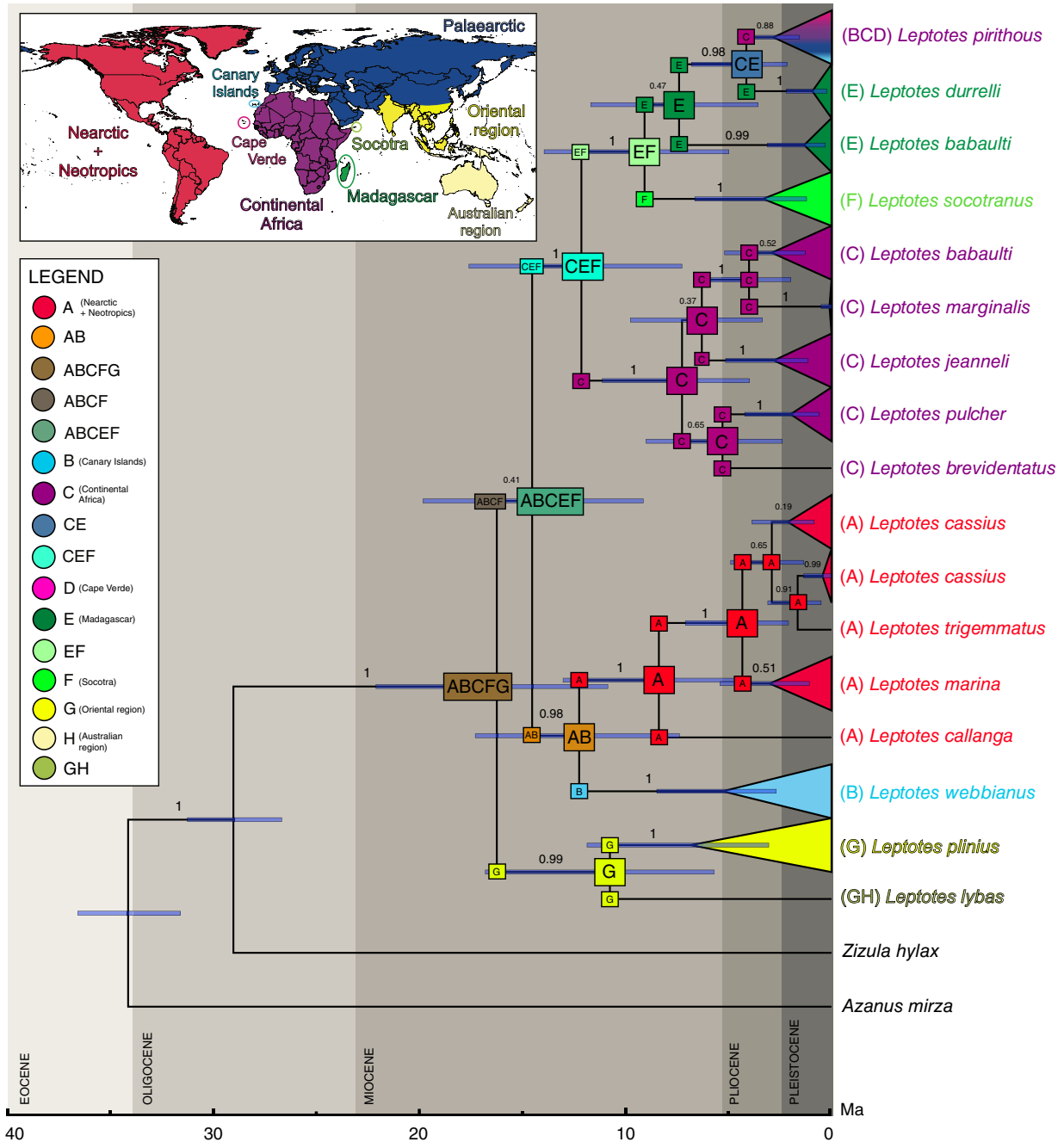
Fig. 6. Historical biogeography of the *Leptotes* butterflies. BIOGEOBEARS reconstruction of ancestral distributions of the *Leptotes* butterflies based on the phylogenetic tree resulting from BEAST analysis. The numbers over branches are the posterior probabilities of the respective nodes. Pie charts on each node depict the relative probabilities of ancestral ranges. A, America; B, Canary Islands; C, continental Africa + Mediterranean region; D, Cape Verde Islands; E, Madagascar; F, Socotra; G, Oriental region; H, Australian region. The colours of samples correspond to the colours of areas as depicted on the map. [Colour figure can be viewed at wileyonlinelibrary.com].

when the world's climate and ecosystems began to undergo significant global cooling, which sharply transformed the warm and humid climate to a relatively dry one (Zachos *et al.*, 2001). The following period from the later part of the Oligocene (27–26 Ma) to the middle Miocene was characterized by a

warmer climate. The genus diversification started in the middle Miocene when the closure of the Tethys Sea caused drastic cooling of global temperatures (Hamon *et al.*, 2013). The climate became drier and the conditions gradually converted the majority of the forested lands into more open habitats.

Table 2. Comparison of biogeography models by BIOGEOBEARS based on corrected Akaike information criterion (AICc), where the lowest value indicates the best suitable model.

Model	Ln *L*	Parameters (no.)	*d*	*e*	*j*	AICc
DEC	−35.06	2	0.0093	0.0074	0.0000	74.99
DEC + J	−29.4	3	0.0023	1.0e−12	0.0084	66.65
DIVALIKE	−28.88	2	0.0048	1.0e−12	0.0000	62.61
DIVALIKE + J	−28.76	3	0.0042	9.7e−10	0.0059	65.37
BAYAREALIKE	−40.38	2	0.0057	0.099	0.0000	85.62
BAYAREALIKE + J	−35.31	3	0.0024	0.0047	0.0510	78.46

D, dispersal parameter; *e*, extinction parameter; *j*, founder effect parameter.

The historical dynamics of dry and wet climate or consequent changes in the dominance of forests and savannahs probably affected the speciation and biogeography of the genus *Leptotes*, as its species occur in various, mostly open, habitats, such as sparse forests and savannahs, and even in desert wadis (Larsen, 2005). Many species are frequently found in agricultural landscapes, gardens and city parks. Also, the endemic *L. mandersi* from Mauritius, regarded as nearly extinct (Lawrence, 2016), seems to prefer open habitats in coastal forests (Florens & Probst, 1995; Pyrcz, 2017).

Africa and Oriental region

The first split within the genus was between the Oriental and Australasian (*L. plinius* and *L. lybas*) and other taxa *c*. 17 Ma (Fig. 6). At the same time, the land connection between Africa and Asia is believed to have been formed (Willis & McElwain, 2002). For other butterfly species, dispersals between Asia and Africa through the Arabian Peninsula have similarly been proposed (Kodandaramaiah & Wahlberg, 2007; Aduse-Poku *et al*., 2009). Another possible scenario for the early diversification of *Leptotes* is similar to the one described in swallowtail butterflies (Condamine *et al*., 2013), i.e. a radiation long after the separation of Africa via long-distance dispersal across the Indian Ocean (Warren *et al*., 2010). The past sea level oscillations enabled the presence of a chain of sizeable islands across the western Indian Ocean, acting as stepping stones through Asia-India-Madagascar-Africa during the Cenozoic. The trans-Indian distribution of many terrestrial groups supports the existence of this chain of islands (Warren *et al*., 2010). In the case of *Leptotes*, genetic data from more specimens inhabiting the Mascarene Islands are needed to support this scenario. Unfortunately, samples from the Seychelles and Comoro Islands were unavailable for this study.

The colonization events were followed by a major diversification of the genus. Within the African and American clades, the ancestors radiated to form several currently sympatric species (Figs 3, 6). In Africa, many *Leptotes* species (*jeanneli*, *babaulti*, *marginalis*, *brevidentatus* and *pulcher*) evolved during the middle and late Miocene (13–5 Ma; Fig. 6) when the grass-dominated savannah biome began to expand and become widespread, as documented by pollen and carbon isotopes from both West and East Africa (Jacobs, 2004).

Americas and the Canary Islands

Whereas our biogeography models (Fig. 6) suggest that vicariant events explain the current distribution, the DEC + J model suggests colonization of America from the Canary Islands, similar to other animals and plants (Grehan, 2017). This possibility seems to be more logical; furthermore, the Canary Islands are volcanic in origin so their fauna cannot be there due to a vicariance event. The route of colonization of the New World could have been through a transatlantic dispersal from Africa, considering the New World clade as a sister group to the African clades, with the position of the Canarian species in between.

The Canary Island endemic *L. webbianus* seems to have originated *c*. 12 Ma (7–17 Ma), which is in concordance with the presumed age of the oldest currently inhabited island, Gran Canaria, formed 15 Ma (van den Bogaard, 2013).

The Leptotes pirithous group

We found that the species with the widest distribution (and probably with the highest dispersal abilities in the Old World), *L. pirithous*, originated in Madagascar *c*. 4 Ma and then dispersed to the whole of Africa, including numerous surrounding islands (Fig. 6). This situation is the opposite of the usual view of island radiations. We have an exceptional case of a younger island species invading a continent already occupied by related species with analogous habitat requirements, instead of young island taxa derived from their continental ancestors of either African (cf. Lamb *et al*., 2011) or Oriental origin (cf. Fabre *et al*., 2012; Federman *et al*., 2015). A similar pattern was found by Bukontaite *et al*. (2015) in several beetle genera. It also corresponds to a review by Samonds *et al*. (2013), who pointed out that after the middle Miocene, ocean currents changed to an east–west direction, enabling dispersion from Madagascar. On the other hand, we did not find any differences between populations from Madagascar and Mauritius. Vences *et al*. (2004) also did not find differences between frogs from Madagascar and Reunion, only a tiny difference between those from Madagascar and Mauritius. Additionally, as members of the genus *Leptotes* prefer open or transitional habitats, we confirmed the findings of Bond *et al*. (2008) that such habitats are really natural and not anthropogenically derived in Madagascar. Similarly, whereas the species seems to have been present in the Cape Verde Islands for at least 100 years (Tennent

& Russell, 2015), the Canary Islands were only colonized about 20 years ago (Wiemers *et al.*, 2013). Thus, non-Malagasy populations of *L. pirithous* are of multiple origins, which could be caused by either incomplete lineage sorting, when several founders simultaneously occupied continental Africa, or an independent multiple colonization from the Malagasy subregion. Some of the lineages co-occur, for instance, on Príncipe.

Our findings in the genus *Leptotes* differ from other studies on butterflies. For instance, the Satyrine subtribe Mycalesina colonized Madagascar twice and radiated there (Kodandaramaiah *et al.*, 2010; Aduse-Poku *et al.*, 2015). Madagascar is known to have been colonized by swallowtails from Asia several times, and, on one occasion, the island provided a stepping-stone between India and Africa (Condamine *et al.*, 2013).

We hope that adding other species endemic to islands around Africa (*L. pyrczi* from Príncipe, *L. sanctithomae* from São Tomé, *L. mandersi* from Mauritius, and *L. casca* and *L. mayottensis* from Comoros) will support our findings and refine the biogeographical details. The South American species, including *L. parrhasioides* from Galapagos or the *Leptotes* populations on various Caribbean islands, deserve a detailed study as well.

Taxonomic implications

Leptotes durrelli sp.n. Fric, Pyrcz & Wiemers

http://zoobank.org/urn:lsid:zoobank.org:pub:20308930-988B-4327-A35F-CC983D46263B

Holotype. ♂, MAURITIUS, Grande Riviere Noire, 1 km east, 10–15 m, 26.xii.2016 (*Pyrcz*) (Zoological Museum of the Jagiellonian University, MZUJ). Labelled 'MZUJ-33/2016', 'prep. genit. 506, 09.03.2017/J. Lorenc' (Fig. 5E).

Paratypes. MAURITIUS: 2 ♂ (Fig. 5F), 1 ♀ Rivière Noire, 18.vi.2012 *(Morrall)* (IECA); 1 ♂, 1 ♀ Rivière Noire, 18.vi.2012 *(Morrall)* (ZSSM) (vouchers ZF-LY-001763 and ZF-LY-001764); 2 ♂ and 1 ♀: MAURITIUS, Palmar, Le Surcouf, 5–10 m, 20.xii.2016 (*Pyrcz*) (CEP-MZUJ); 1♀: MAURITIUS, Palmar, Le Surcouf, 5–10 m, 20.xii.2016 (*Pyrcz*) (CEP-MZUJ); 3♂: MAURITIUS, Trou d'Eau Douce, 1 km South, 35–40 m, 24.xii.2016 (*Pyrcz*) (CEP-MZUJ); 1 ♀: MAURITIUS, Bassin Blanc, 2 Km N Chamouny, 360–370 m, 22.xii.2016 (Fig. 5G) (*Pyrcz*) (CEP-MZUJ); 3 ♂ and 1 ♀: MAURITIUS, Chamarel, 2 Km E, 320–330 m, 22.xii.2016 (*Pyrcz*) (CEP-MZUJ); 5 ♂ and 1 ♀: MAURITIUS, Grande Riviere Noire, 1 Km East, 10–15 m, 26.xii.2016 (*Pyrcz*) (CEP-MZUJ); 1 ♂: MAURITIUS, Grande Riviere Noire, 1 Km East, 10–15 m, 25.xii.2016 (*Pyrcz*) (CEP-MZUJ).

Other material. REUNION: 2 ♂: NE Le Petit Serré, 24.iii.1986 *(Boyer)* (CEP-MZUJ); 1 ♂: Mont-Vert les Hauts, 21.33401°S, 55.57569°E, 17.iv. 2018, (*Fric*) (IECA); MADAGASCAR: 1 ♂, 1 ♀ Mahajanga, 16.0499°S, 45.90773°E, 20 m ASL, 3.xii.2002 (*Cameron*) (vouchers AC0073-8033283,

AC0074-8033282), 16.4666°S, 453509°E, 140 m ASL, 5.xi.2002 (*Cameron*) (voucher AC0075-8002981); 1 ♂ Fianarantsoa, 20.5947°S, 46.5631°E, 1550 m ASL, 25.i.2003 (*Cameron*) (AC0076-8033915); 1 ♂ Toliara, 23.0438°S, 43.6097°E, 30 m ASL (*Cameron*) (AC0077-8034813) (CASC); Ankarana (vouchers BMNH676414, BMNH676431, BMNH676432, BMNH676441) (BMNH); 1 ♂: Fianarantsoa, env. of Setam lodge, 21.249896°S, 47.423815°E, 12.iv.2018 (*Fric*) (IECA).

Diagnosis. The appearance of *L. durrelli* is similar to Mediterranean populations of *L. pirithous* in having a darker blue colour in males and dark females with a blue basal half of the forewings. This pattern contrasts with lighter blue males and females of *L. pirithous* from the Afrotropical region. The underside pattern of *L. durrelli* differs from that of *L. pirithous*, as the latter has almost square spots and also the arrangement of the spots between R, M1, M2 and M3 is almost linear in *L. pirithous*, whereas the spots are arranged in an arch in *L. durrelli*. Also the genitalia are similar in these two species but differ in size and proportions between the phallus and the carinas. The genitalia of another species known from the area, *L. rabefaner*, are very distinct (see Stempffer, 1935) as the end of the valva bears several long teeth similar to *L. jeannelli*.

Description. Forewing 11–12 mm. Males on upper side of wings uniformly dark blue, some specimens with two black submarginal spots between CuA1, CuA2 and A1 veins on hindwings. Basal colour on undersides of wings is greyish-brown with darker spots surrounded by white colour. The pattern of spots is similar to *L. pirithous*, but the spots are generally more rounded, almost circular, especially on hindwings. Females are brown on upper side with bluish basal half of forewing, with large discoidal spot and a series of rounded postdiscal spots. Hindwings are brown with few metallic scales close to their base, a lighter submarginal area and with two distinct submarginal spots between CuA1, CuA2 and A1 veins. Female underside pattern is the same as in males. Hindwings of both sexes end with a long tail at CuA2 (missing due to damage in illustrated specimens). However, the wing pattern traits are generally not sufficient for differentiation from its sister species, *L. pirithous*, which is very variable throughout its range (cf. Fig. 5H–J).

Male genitalia. Male genitalia are very similar to *L. pirithous* in having long ampulla on valva without any tooth, otherwise characteristic for other *Leptotes* species. Curved phallus bears on ventral part of its caudal end two long, sharp, heavily sclerotized points ('carinas' in Libert, 2011) (Fig. 4O, P). Cornutus on vesica ('cuneus' sensu Libert, 2011) in the lateral view curved as in *L. pirithous*. Carinas are rather shorter, the ratio between the length of carina and the sclerotized part of phallus is 0.58 (SD 0.034) for *durrelli*, whereas it is 0.63 (0.037) for *L. pirithous*; the differences are statistically significant (ANOVA, $F = 5.52$, d.f. = 1, 11, $P = 0.039$). Phallus of *durrelli* has similar length to that of *pirithous*, but seen from lateral

side it is much narrower (0.21 mm, SD = 0.011) in comparison with *pirithous* (0.24 mm, SD = 0.024) ($F = 8.63$, d.f. = 1, 11, $P = 0.014$). However, the most significant differences are in the ratio between the length of the sclerotized part of phallus and its width, which is 2.46 (SD = 0.145) in *durrelli*, whereas it is only 2.03 (SD = 0.105) in *pirithous* (ANOVA, $F = 38.7$, d.f. = 1, 11, $P < 0.001$).

Female genitalia. Very similar to *L. pirithous*. Antevaginal lamella is straighter and less curved at its base. Postvaginal sclerite covers *c.* 2/3 of the lamella, whereas in *L. pirithous* it covers less than half of the length of the lamella.

Remarks. The species is not currently endangered; it uses various secondary habitats.

Etymology. We name this taxon after Gerald Durrell (1925–1995), the British naturalist, conservationist and author of numerous books. He recognized and supported the importance of the Malagasy area, not only by founding the Mauritian Wildlife Foundation trust but also by popularizing the area and its species in his book *Golden Bats and Pink Pigeons: A Journey to the Flora and Fauna of a Unique Island*.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Overview of samples of *Leptotes* and outgroup species used for total phylogeny (T) or *Leptotes pirithous* phylogeography pattern (P) studies with GenBank Accession numbers/BOLD Sequence ID (marked by #).

Appendix S2. Primers used for molecular phylogeny reconstruction of the genera *Leptotes* and *Cyclyrius*.

Appendix S3. Distribution of species of the genus *Leptotes* used in biogeographical analysis.

Appendix S4 Dispersal limitation used for biogeography analyses of the genus *Leptotes*. '1', dispersal between these areas allowed; '0', dispersal between these areas not allowed.

Appendix S5 Best substitution models selected by PartitionFinder for all seven partitions in appropriate software. Positions in regions represent the codon positions in a translation to aminoacids not in the dataset position.

Appendix S6 Phylogenetic relationships based on COI calculated by Maximum Likelihood between *Leptotes plinius* and *L. lybas*. Blue branches indicate samples missing in the complete phylogeny (Fig. 3).

Appendix S7 Alternative biogeography models of ancestral distribution of the genus Leptotes, resulting from modelling in BioGeoBears. A) DEC + J, B) DIVALIKE+J.

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