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# **Inter-island differentiation of *Leptotes (Cyclyrius) webbianus* (Brullé, 1839) in the Canary Islands (Spain), with description of two new subspecies from La Palma and Gran Canaria (Lepidoptera: Lycaenidae)**

X. Mérit, L. Manil, R. Vila & M. Wiemers

## **Summary**

Phenotypic and molecular genetic analyses of the Canary Islands' endemic *Leptotes (Cyclyrius) webbianus* (Brullé, 1839) reveal new subspecies in La Palma and in Gran Canaria, both differentiated from the nominotypical subspecies found in Tenerife and La Gomera. On El Hierro, the current population turned out to be a recent reintroduction from Tenerife or La Gomera, whereas the original population reported more than a hundred years ago seems to have become extinct.

KEY WORDS: Lepidoptera, Lycaenidae, *Leptotes (Cyclyrius) webbianus*, new subspecies, Canary Islands, Spain.

**Diferenciación inter-islas de *Leptotes (Cyclyrius) webbianus* (Brullé, 1839) en las Islas Canarias (España), con la descripción de dos nuevas subespecies de La Palma y Gran Canaria (Lepidoptera: Lycaenidae)**

## **Resumen**

El análisis fenotípico y genético del endemismo de las Islas Canarias *Leptotes (Cyclyrius) webbianus* (Brullé, 1839) revela nuevas subespecies en La Palma y en Gran Canaria; ambas se diferencian de la subespecie nominotípica encontrada en Tenerife y La Gomera. En El Hierro, la actual población ha resultado ser una reintroducción desde Tenerife o La Gomera, mientras que la población original, documentada hace más de cien años, parece haberse extinguido.

PALABRAS CLAVE: Lepidoptera, Lycaenidae, *Leptotes (Cyclyrius) webbianus*, nuevas subespecies, Islas Canarias, España.

## **Introduction**

A large series of *Leptotes (Cyclyrius) webbianus* (Brullé, 1839) was collected in La Palma and Tenerife by Luc Manil in the early 1980s (December 1981 and July 1983) along with a lower number of male and female specimens from Gomera and Gran Canaria. Luc Manil suspected at that time that the La Palma *L. webbianus* [collected at the same place where *Hipparchia tilosi* Manil, 1984 was described] might represent a different subspecies. More recently, in July/August 2014, Xavier Mérit visited the islands of Tenerife, La Palma, La Gomera and El Hierro to collect fresh specimens of *L. webbianus* for DNA analysis.

# Abbreviations

The following abbreviations are used in the text:

DNA	Desoxyribonucleic acid, a molecule that carries the genetic information for all organisms
Ma	million years
NHMW	Naturhistorisches Museum, Wien
MNCN	Museo Nacional de Ciencias Naturales, Madrid

# Material & methods

The following material was used for phenotypic analyses: Tenerife (57 individuals: 21 ♂♂, 36 ♀♀), La Gomera (3 individuals: 2 ♂♂, 1 ♀), Gran Canaria (13 individuals: 10 ♂♂, 3 ♀♀), La Palma (44 individuals: 25 ♂♂, 19 ♀♀), and El Hierro (5 individuals: 5 ♂♂).

The width of the black margin was measured against a paper ruled in millimetre squares at space n° 2 of the males' hindwings and at space n° 4 of the forewings.

DNA barcodes (a 658 bp fragment of the mitochondrial gene cytochrome *c* oxidase I) were obtained from specimens of all inhabited islands in the Canaries (Tab. 1) using standard laboratory methods (see WIEMERS & FIEDLER, 2007; DINCĂ *et al.*, 2011; RITTER *et al.*, 2013) using the primer pairs LepF (5'- ATT CAA CCA ATC ATA AAG ATA TTG GAA C-3') and LepR (5'- TAA ACT TCT GGA TGT CCA AAA AAT C A-3'), or UniLepF1/UniLepR1. The latter primers are identical to LepF/LepR but have a universal T7 tail (5' - TAA TAC GAC TCA CTA TAG GG - 3') or T3 tail (5' - ATT AAC CCT CAC TAA AG - 3'), respectively, attached to their 5' end. In addition, the single available sequence from GenBank was included in the analysis.

**Table 1.**— Material of *Leptotes (Cyclurius) webbianus* used for DNA analysis of the mitochondrial gene COI

Voucher	Sex	Island	Location	Altitude	UTM	Date	Remarks	GenBank
14V523	♂	El Hierro	La Frontera, Las Lapas, El Luchón	400 m	28 RBR 0474	22-VII-2014	X. Mérit leg.	KU648399
BA09001	♂	Gran Canaria	Cruz de Tejeda	1560 m	28 RDR 4199	10-V-2009	B. Acosta leg.	KU648391
BA09002	♂	Gran Canaria	Cruz de Tejeda	1560 m	28 RDR 4199	10-V-2009	B. Acosta leg.	KU648392
PR02001	♂	La Gomera	Garajonay	1200 m	28 RBS 7911*	29-VIII-2002	e.o. J.E. Pateman leg.	KU648395
PR02002	♀	La Gomera	Garajonay	1200 m	28 RBS 7911*	31-VIII-2002	e.o. J.E. Pateman leg.	KU648396
BA09005	♀	La Palma	Cubo de la Galga, Puntallana	341 m	28 RBS 2985	29-VII-2009	B. Acosta leg.	KU648393
14H868	♂	La Palma	Bosque de los Tilos, San Andrés y Los Sauces	370 m	28 RBS 2687	1-VIII-2014	X. Mérit leg.	KU648398
MW15001	♀	Tenerife	Aeropuerto de Tenerife Sur, Granadilla de Abona	70 m	28 RCS 4503	23-I-2015	M. Wiemers leg.	KU648394
14H867	♂	Tenerife	Monte Izaña, Pico de Las Flores	1455 m	28 RCS 6143	6-VIII-2014	X. Mérit leg.	KU648397
		Tenerife	Los Gigantes		28 RCS 2026* * approximate	30-III-2015	K. Hermansen leg.	KU310889

# Results

## PHENOTYPIC ANALYSIS

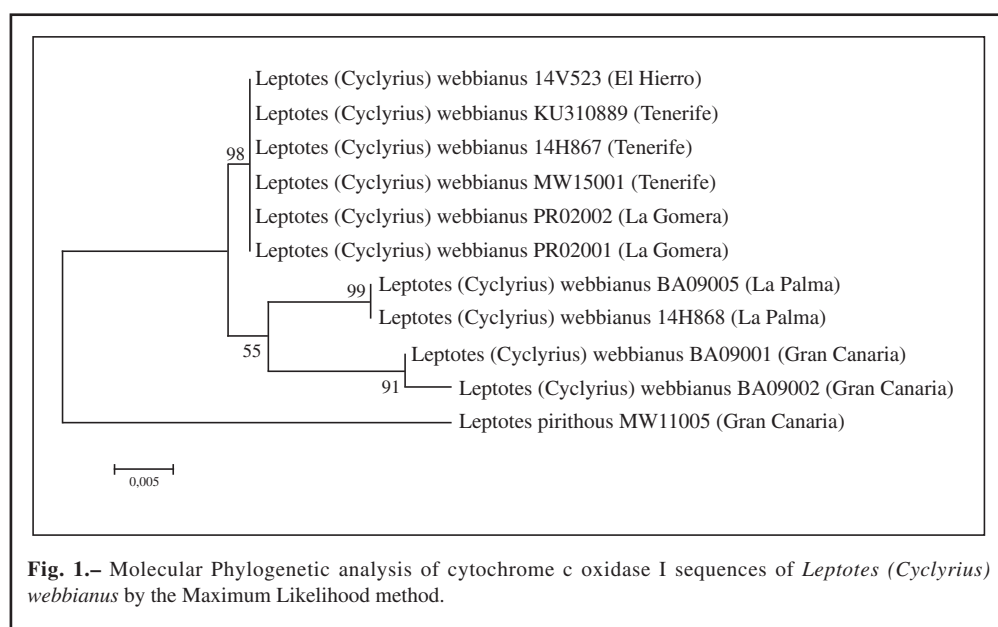
The extension of the black margin on the dorsal side of forewings and hindwings varies between the islands. In the male specimens from Tenerife, La Gomera and El Hierro (butterflies collected by X.

Mérit in 2014), the black margin of both wings typically measures less than 1 mm (0.7-1.1 mm) in width, whereas it measures approximately 1.5 mm in width in specimens from Gran Canaria and approximately 2 mm (1.8-2.3 mm) in width in the specimens from La Palma.

The postdiscal white band between veins 1 and 5 on the hindwing underside measures less than 1 mm (min: 0.8 mm - max: 0.9 mm) in width in specimens from Tenerife, La Gomera, Gran Canaria and El Hierro, compared to a width of about 1.5 mm (min: 1.3 mm – max 1.6 mm) in specimens from La Palma.

#### MOLECULAR ANALYSIS

The DNA analysis of the mitochondrial gene cytochrome *c* oxidase I (COI) confirms the differentiation of the population from La Palma with respect to the Tenerife/La Gomera lineage (minimum genetic p-distance = 1.2%, which corresponds to an age of ca. 0.5-0.8 Ma ago assuming typical substitution rates of 1.5–2.3% per Ma), as well as from the Gran Canaria population (minimum genetic p-distance = 1.5%, which corresponds to ca. 0.7-1.0 Ma ago) (Fig. 1). All analysed specimens from the other islands (Tenerife, La Gomera, and El Hierro), however, have identical COI haplotypes.



**Fig. 1.**— Molecular Phylogenetic analysis of cytochrome *c* oxidase I sequences of *Leptotes (Cyclyrus) webbianus* by the Maximum Likelihood method.

The phylogenetic relationships were inferred by using the Maximum Likelihood method based on the General Time Reversible model. The tree with the highest log likelihood (-1688.5823) is shown. The percentage of trees in which the associated taxa clustered together in a 500 replicates-bootstrap analysis is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 11 nucleotide sequences. There were a total of 1220 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (KUMAR *et al.*, 2016).

## DESCRIPTIONS OF TWO NEW SUBSPECIES

***Leptotes (Cyclirius) webbianus palmae* Mérit, Manil, Vila & Wiemers, ssp. n.**

Material: Holotype ♂, SPAIN, La Palma, San Andrés y Los Sauces, Los Tilos, LP105, 370 m, 1-VIII-2014, X. Mérit leg., deposited in MNCN (fig. 2). Paratypes 1 ♂, 1 ♀, La Palma, near Los Tilos, 400 m, 25-26-VII-1983, L. Manil leg., deposited in Coll. MNCN; 6 ♂♂, 5 ♀♀, La Palma, near Los Tilos, 400 m, 25-26-VII-1983, L. Manil leg., Coll. L. Manil; 2 ♂♂, 5 ♀♀, La Palma, San Andrés y Los Sauces, Los Tilos, LP105, altitude 370 m, 1-VIII-2014, X. Mérit leg., Coll. X. Mérit; 1 ♂, 1 ♀, La Palma, Llano Molino, Barranco de la Galga, N 28° 45' 55"-W 17° 46' 20", 400 m, 1-VIII-2014, X. Mérit leg., Coll. X. Mérit; 8 ♂♂, La Palma, Las Nieves, Barranco de la Madera, N 28° 42' 14"-W 17° 47' 20", altitude 410 m, 31-VII-2014 / 2-VIII-2014, X. Mérit leg., Coll. X. Mérit; 1 ♀, La Palma, Barranco de Agua, altitude 200-700 m, 31-VII-1988, M. Wiemers leg., Coll. M. Wiemers; 2 ♀♀, La Palma, San Isidro, altitude 1000 m, 1-VIII-1988, M. Wiemers leg., Coll. M. Wiemers; 3 ♂♂, 1 ♀, La Palma, Barranco de la Galga, altitude 400-800 m, 3-VIII-1988, M. Wiemers leg., Coll. M. Wiemers; 1 ♂, 1 ♀, La Palma, Barranco de la Galga, altitude 400-800 m, ex-larva, 1-IX-1988, M. Wiemers cult., Coll. M. Wiemers. 1 ♂. used for DNA analysis, La Palma, San Andrés y Los Sauces, Los Tilos, LP105, 370 m, 1-VIII-2014, X. Mérit leg., Coll. R. Vila, code 14H868.

Description and diagnosis: The specimens from La Palma (fig. 2) differ from those from Gran Canaria, Tenerife, La Gomera and El Hierro (specimens collected in 2014) by:

1. the extension of the black margin of both forewings and hindwings upperside,
  2. the larger extension of all the white markings, particularly the larger width of the postdiscal white band of the underside of the hindwings.
  3. their larger size (mean wingspan approximately 1-2 mm larger than on the other occidental islands, 1-3 mm larger than in Gran Canaria). This character is not constant.
- (1) In the male specimens from Tenerife, La Gomera and El Hierro (specimens collected in 2014), the black margin of both wings typically measures less than 1 mm (0.7-1.1 mm) in width, whereas it measures approximately 2 mm (1.8-2.3 mm) in width in all specimens from La Palma (red arrow).
  - (2) The white submarginal spot near the tip of the forewing underside extends along the submarginal area till vein V5 or V4 in most specimens (green ellipse on the plate). The spot in the discal area of spaces 7-8 (black arrows) is whitish rather than brown as in the other islands. The postdiscal white band between veins 1 and 5 on the hindwing underside measures less than 1 mm in width in specimens from Tenerife, La Gomera, Gran Canaria and El Hierro (specimens collected in 2014) compared to a width of about 1.5 mm in specimens from La Palma.
  - (3) In the female, the upperside is often fulvous rather than dark brown as in the other islands, but with a well-contrasted 2 mm dark brown marginal strip in both fore- and hindwings (red arrow).
  - (4) The reverse side of the forewing shows often more contrasted ocelli than in most specimens originating from the other islands (blue arrows).

Distribution: This new subspecies is only known from the island of La Palma. Historical *L. webbianus* (extinct) collected in El Hierro more than a century ago (see discussion below and the picture on the plate) were probably closely related to ssp. *palmae*.

Derivatio nominis: The name of the new subspecies is derived from La Palma island where the subspecies is distributed.

***Leptotes (Cyclirius) webbianus grancanariensis* Mérit, Manil, Vila & Wiemers, ssp. n.**

Material: Holotype ♂, SPAIN, Gran Canaria, Cruz de Tejeda, altitude 1450 m, 8-VII-1983, L. Manil

leg., deposited in MNCN (fig. 3) - Paratypes 1 ♂, Gran Canaria, Cruz de Tejada, altitude 1450 m, 8-VII-1983, L. Manil leg., deposited in Coll. MNCN; 1 ♂♂, 1 ♀, Gran Canaria, Cruz de Tejada, altitude 1450 m, 8-VII-1983, L. Manil leg., Coll. L. Manil; 1 ♂, 1 ♀, Gran Canaria, Pozo de la Nieves, altitude 1850 m, 09-VII-1983, L. Manil leg., Coll. L. Manil; 2 ♂♂, Gran Canaria, Fontanales, altitude 1400 m, 19-VII-1983, L. Manil leg., Coll. L. Manil; 1 ♂, Gran Canaria, Arines, altitude 1300 m, 8-VIII-1990, B. Turlin leg., Coll. X. Mérit; 2 ♂♂, used for DNA analysis, Gran Canaria, Cruz de Tejada, altitude 1560 m, 10-V-2009, B. Acosta leg., Coll. B. Acosta; codes BA09001 and BA09002.

Description and diagnosis: The specimens from Gran Canaria (fig. 3) differ slightly from those from Tenerife, La Gomera and El Hierro (specimens collected in 2014) by the extension of the black margin of both forewings and hindwings of the dorsal face and by the width of the white line of the ventral side of the hindwings.

In the male specimens from Tenerife, La Gomera and El Hierro, the black margin of both wings typically measures less than 1 mm in width, whereas it measures approximately 2 mm in width in all specimens from La Palma and 1.5 mm in width in all specimens from Gran Canaria.

The postdiscal white band between veins 1 and 5 in specimens from Gran Canaria does not exhibit significant differences from the specimens from Tenerife and La Gomera.

The mean wingspan is slightly smaller than in the other islands, but very small specimens are particularly frequent in Gran Canaria.

Distribution: This new subspecies is known only from Gran Canaria.

Derivatio nominis: The name of the new subspecies is derived from Gran Canaria island where the subspecies is distributed.

#### SYSTEMATIC PLACEMENT AND SYNONYMY

##### Original description:

*Polyommatus webbianus* Brullé, 1839. *Hist. Nat. Can. Ent.*, **1839**: p. 93-94, pl. 4, fig. 1, 1a.

Locus typicus: Tenerife island: Las Cañadas (above 1,400 m)

##### Junior synonyms:

*Lycaena fortunata* Staudinger, 1870. *Berl. Ent. Z.*, **14**: 99-100.

Locus typicus: Tenerife island

*Polyommatus webbianus* f. *brunnea* Nordman, 1935. *Commentat. Biol.* 6: 6-7. Female form.

Locus typicus: La Palma island: La Caldera

*Cyclirius* Butler, 1897. *Proc. zool. Soc. Lond.*, **1896**: 830.

Type species of *Cyclirius* Butler, 1897 is *Polyommatus webbianus* Brullé

FOX *et al.*, (1965) synonymized *Cyclirius* with *Leptotes* Scudder, 1876, but this suggestion was not followed by most subsequent authors who continued to use *Cyclirius* or the misspelling *Cyclirius* (but see VIVES MORENO, 2014). We provisionally use *Cyclirius* as subgenus of *Leptotes*, pending further study on the phylogeny of *Leptotes*, and consider the following subspecies:

*Leptotes* (*Cyclirius*) *webbianus webbianus* (Brullé, 1839), from Tenerife (fig. 4), La Gomera and El Hierro (current population)

*Leptotes* (*Cyclirius*) *webbianus palmae* Mérit, Manil, Vila & Wiemers, ssp. n., from La Palma

*Leptotes* (*Cyclirius*) *webbianus grancanariensis* Mérit, Manil, Vila & Wiemers, ssp. n., from Gran Canaria

## Discussion

### INTER-ISLAND DIFFERENTIATION OF *LEPTOTES* (*CYCLYRIUS*) *WEBBIANUS*

According to VAN DEN BOGAARD (2013), the Canary Islands formed between 23 Ma (Fuerteventura) and 1.1 Ma (El Hierro), and lie on the oldest hotspot track in the Atlantic Ocean, which dates back to the Late Jurassic. With 15 Ma, Gran Canaria is the oldest of the western islands, followed by Tenerife and La Gomera (12 and 11 Ma, respectively). With an age of 1.7 Ma, La Palma is only slightly older than El Hierro. Our genetic analysis does not rule out any sequence of colonization events, but is consistent with the scenario that one of the older islands (such as Gran Canaria, Tenerife or La Gomera) was colonized first, and the species then spread to the other islands in a stepwise fashion. The current spread of *Leptotes pirithous* in the Canary Islands (WIEMERS *et al.*, 2013) shows that such colonization can happen within a few years. The lack of differentiation between populations on Tenerife and La Gomera probably indicates persistent gene flow between these islands. This is unsurprising considering the relatively small distance between them (less than 30 km). Additionally, it seems plausible that specimens of *Leptotes* (*Cyclyrus*) *webbianus* can frequently be blown over by trade winds to La Gomera from the high altitude plateau of Las Cañadas del Teide, where the species can be extremely abundant (WIEMERS 1995b).

### *LEPTOTES* (*CYCLYRIUS*) *WEBBIANUS* ON EL HIERRO - AN INTERESTING CASE OF LOCAL EXTINCTION AND RECOLONIZATION

*Leptotes* (*Cyclyrus*) *webbianus* was discovered on El Hierro in 1889 by Simony (REBEL & ROGENHOFER, 1894) by at least three male samples deposited in the NHMW (Fig. 5). The collected specimens show a black margin on the forewings and hindwings upperside with a very similar width as the specimens from La Palma. Moreover, the white line on the verso of the hindwings also shows a similar pattern to those from La Palma. WIEMERS (1995b) reported the existence of these specimens as the only records known from El Hierro, but was unfortunately unable to see them at that time. No further records are known from El Hierro until MÉRIT (2015) 'rediscovered' it in 2014. Interestingly, the newly found specimens display a black margin and white line similar to the specimens from Tenerife and La Gomera. The DNA analysis also supports the hypothesis of a recent recolonization of El Hierro by specimens from Tenerife or La Gomera. It is very likely that the former population discovered by Simony disappeared for unknown reasons and that a new population originating from Tenerife or La Gomera recolonized the island. Unfortunately, the samples collected in 1889 are probably too old for DNA analyses, hence we cannot conclude whether the former population could have been close or identical to the newly described *Leptotes* (*Cyclyrus*) *webbianus palmae*.

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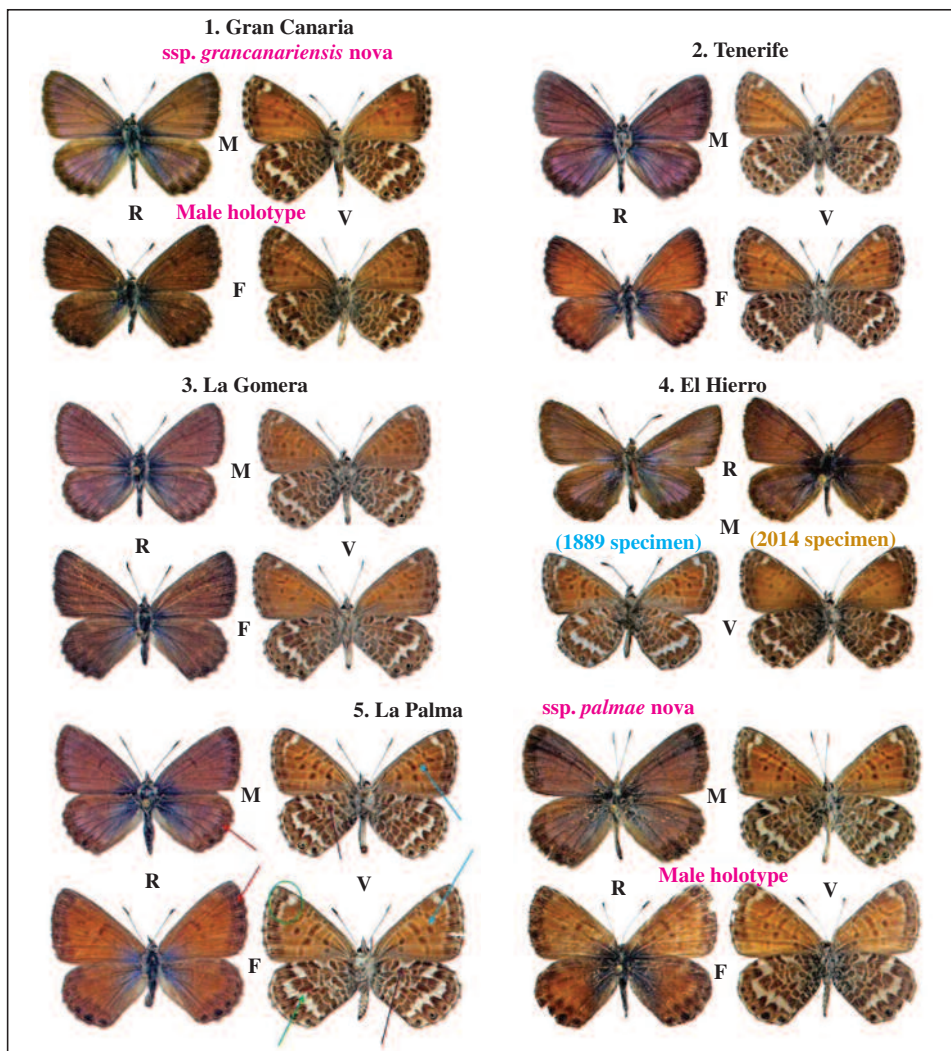
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**Figs. 1-5.**— *Leptotes (Cyclurius) webbianus grancanariensis* ssp. n. Male holotype R/V (first row) and female paratype R/V (second row): Gran Canaria, Cruz de Tejeda, 1450 m, 8-VII-1983, L. Manil leg. **2.** *Leptotes (Cyclurius) webbianus webbianus* from Tenerife. Male R/V (first row): Las Cañadas, 2300 m, 13-VII-1983, and female R/V (second row): Taganana, 20 m, 17-XII-1981. L. Manil leg. **3.** *Leptotes (Cyclurius) webbianus webbianus* from La Gomera. Male R/V (first row): Chipude, 1200 m, 21-VII-1983 and female R/V (second row): Las Rosas, 600 m, 20-VII-1983. L. Manil leg. (Photos L. Manil). **4.** *Leptotes (Cyclurius) webbianus* from El Hierro. Left: male R/V: El Hierro: Montaña Tenezedra, 700 m, 29-VIII-1889, Simony leg., in coll. NHMW (Photos M. Wiemers). Right: male R/V: El Hierro: El Lunchón, 22-VII-2014, X. Mérit leg. (Photos L. Manil); *Leptotes (Cyclurius) webbianus palmae* ssp. n. The 4 pictures on the left: Male paratype R/V (first row) and female paratype R/V (second row). Los Tilos, La Palma, 26.VII.1983, L. Manil leg. (Photos L. Manil). The 4 pictures on the right: Male holotype (first row) and female allotype (second row) (San Andrés y Los Sauces, Los Tilos, LP105, 370 m, 1-VIII-2014. X. Mérit leg. (Photos L. Manil). M: Male. F: female. R: recto (upperside). V: verso (underside).