

PIPUNCULIDAE (DIPTERA) FROM THE CALDERA DE TABURIENTE NATIONAL PARK, LA PALMA (CANARY ISLANDS, SPAIN) — INVESTIGATING THE MORPHOLOGICAL AND MOLECULAR VARIABILITY IN A NEW SPECIES OF BIG-HEADED FLIES

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ABSTRACT

The present paper is a result of the project "Inventory and study of the invertebrate fauna of the Caldera de Taburiente National Park on La Palma, Canary Islands. Among the four species of Pipunculidae recorded, *Chalarus guanche* Kehlmaier **sp. nov.** is described and also recorded from Madeira, whereas *Tomosvaryella freidbergi* De Meyer, 1995 and *T. parakuthyi* De Meyer, 1995 are first records for La Palma. The morphological and molecular variability of *C. guanche* **sp. nov.** is studied and the presence of intragenomic variation in ITS2 rDNA is discussed.

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Key words: Diptera; Pipunculidae; *Chalarus guanche* sp. nov.; Caldera de Taburiente National Park; La Palma; Canary Islands; new species; DNA-barcoding; intragenomic variation; COI; ITS2.

RESUMEN

Pipunculidae (Diptera) del Parque Nacional de la Caldera de Taburiente, La Palma (Islas Canarias, España) — Investigando la variabilidad morfológica y molecular de una nueva especie de moscas cabezonas

Este trabajo es el resultado del proyecto "Inventario y estudio de la fauna invertebrada del Parque Nacional de la Caldera de Taburiente" en la isla de La Palma, Islas Canarias. De las cuatro especies recogidas, se describe *Chalarus guanche* **sp. nov.** que se registra asimismo de Madeira, y *Tomosvaryella freidbergi* De Meyer, 1995 y *T. parakuthyi* De Meyer, 1995 son nuevos registros para La Palma. Se estudia la variabilidad morfológica y molecular de *C. guanche* **sp. nov.** y se discute la presencia de variación intragenómica en el ADN ITS2.

Palabras clave: Diptera; Pipunculidae; *Chalarus guanche* sp. nov.; Parque Nacional de Caldera de Taburiente; La Palma; Islas Canarias; nueva especie; DNA-barcoding; variabilidad intragenómica; COI; ITS2.

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Introduction

Slightly more than 1,400 species of Pipunculidae are known from all around the world (Rafael & Skevington, 2010). Their common name ‘big-headed flies’ refers to the globular head that is almost entirely covered by their large compound eyes. This panorama view enables them to precisely navigate in dense vegetation, and helps the females to detect suitable hosts for their endoparasitic larvae, which develop mainly within larval and adult Auchenorrhyncha, but have specialised on adult Tipulidae in *Nephrocera* Zetterstedt, 1838 (see Rafael & Skevington, 2010 for a brief review of the family’s biology).

De Meyer *et al.* (2001) present a faunistic overview of the pipunculid fauna known from the Macaronesian archipelagos of Canary Islands (Spain) and Madeira (Portugal), listing 13 species for the Canary Islands, including five for La Palma, and three species known from Madeira. With the current paper, we complement this listing by studying material mainly resulting from a two year trapping survey conducted in the Caldera de Taburiente National Park on La Palma (Domingo-Quero *et al.*, 2003).

Material and methods

The material studied originates from the project “Inventory and study of the invertebrate fauna of the Caldera de Taburiente National Park”. The trapping setup covered six sites (Table 1), ranging from 750–2,345 m above sea level, each equipped with a Malaise trap (MT) and a yellow pan trap (YT) placed under the middle wall of the Malaise trap. The traps were emptied at intervals between 4 and 15 days (average of 7.75 days) from August 1999 to July 2001 by T. Domingo-Quero and A. Sánchez-Ruiz. For further details see Domingo-Quero *et al.* (2003). The material is currently ethanol preserved and in the collections of the Museo Nacional de Ciencias Naturales in Madrid (MNCN), Senckenberg Deutsches Entomologisches Institut in Müncheberg (SDEI) and Senckenberg Museum für Tierkunde Dresden (SMTD).

Morphological terminology follows Kehlmaier & Assmann (2008), including the following abbreviations: LW—length of wing; MWW—maximum width of wing; LS—length of pterostigma; LSC—length of second costal section of wing; LTC—length of third costal section of wing; LFC—length of fourth costal section of wing; psr—posterior setal row of front femur; pvsr—posteroventral setal row of mid femur; aasr—anterior/anterodorsal setal row of hind femur; pdsr—posterodorsal/dorsal setal row of hind femur; LT35—maximum length of tergites 3–5; WT2—maximum width of tergite 2; Lmtdp—length of membranous tip of the distiphallus; Ltdp—length of tip of distiphallus.

Morphometric measurements and resulting ratios were investigated in a series of up to 25 ethanol preserved females. The following aspects were investigated: diameter of largest frontal facet (DFF) (n=25); width of frons at its narrowest point (FN) (n=23); width of frons at level of anterior (median) ocellus (FMO) (n=25); FN:FMO (n=23); DFF:FN (n=23); length of tergite 9 (piercer) in lateral view (n=24); wing length in dorsal view (n=25).

Molecular work was carried out at MNCN and SMTD according to well established standard procedures outlined in Kehlmaier & Assmann (2010). A fragment of the 5’ end of the mitochondrial coding gene cytochrome oxidase subunit I (COI) was obtained using the primer pair LCO1490-JJ (5’-CHACWAAY CATAAAGATATYGG-3’) and HCO2198-JJ (5’-AWACTTCVGGRTGVCCAAARAATCA-3’) (Astrin & Stüben, 2008), whereas part of the nuclear nontranscribed ribosomal Internal Transcribed Spacer Region 2 (ITS2) was generated with the primer pair ITS2A (5’-TGTGAACTGCAGGACACAT-3’) and ITS2B (5’-TATGCTTAAATTCAGGGGGT-3’) (Beebe & Saul, 1995). The resulting sequences were checked by eye for base-calling errors and manually aligned with the software BioEdit (Hall, 1999). Molecular analyses were computed with MEGA6 (Tamura *et al.*, 2013), resulting in unrooted neighbour-joining (NJ) trees of uncorrected pairwise genetic distance (p-dist), using all sites and pairwise deletion for missing data. The sequence data is

Table 1.— List of trapping localities in order of ascending altitudes.

Tabla 1.— Lista de localidades de trampeo por orden de altitud ascendente.

Locality	Altitude	UTM coordinates
Playa del Rio Taburiente	750 m	UTM 28RBS1980-1
Barranco de Las Traves	1,068 m	UTM 28RBS1780-2
Lomo de las Chozas	1,299 m in 1999; 1,277 m in 2000 & 2001	UTM 28RBS2077-3
Roque de la Cumbrecita	1,377 m	UTM 28RBS2177-1
Roque de los Muchachos	2,250 m	UTM 28RBS2084-3
Pico de la Cruz	2,345 m	UTM 28RBS2183-1

deposited at the European Nucleotide Archive (ENA) under LT715904–38 (COI) and LT715939–81 (ITS2), the individual accession numbers being listed in Appendix 1.

Results

Systematic account of big headed flies of La Palma

The 78 specimens collected at the Caldera de Taburiente National Park comprise four species. With 62 individuals, the genus *Chalarus* was dominant. All trapping localities yielded specimens except “Pico de la Cruz”.

PIPUNCULINAE

Dasydorylas setosus (Becker, 1908)

MATERIAL (MT: 2♂♂ 5♀♀; YT: 3♀♀). SPAIN, Canary Islands, La Palma, Caldera de Taburiente National Park: Playa del Rio Taburiente: 15.V.2000, 1♀ (MNCN_Ent 202044, YT), coll. MNCN; 30.V.2000, 1♂ (MT), coll. SMTD; 19.VI.2000, 1♀ (MNCN_Ent 202045, MT), coll. MNCN.— Barranco de las Traves: 5.VI.2000, 1♀ (MNCN_Ent 202042, YT), coll. MNCN; 19.VI.2000, 1♀ (YT), coll. SMTD; 27.VI.2000, 1♂ (MNCN_Ent 202043, MT), coll. MNCN.— Lomo de las Chozas: 1.VI.2000, 1♀ (MNCN_Ent 202047, MT), coll. MNCN.— Roque de la Cumbrecita: 1.VI.2000, 1♀ (MNCN_Ent 202041, MT), coll. MNCN; 21.VI.2000, 1♀ (MNCN_Ent 202046, MT), coll. MNCN.— Roque de los Muchachos: 20.VI.2001, 1♀ (MT), coll. SMTD.

REMARKS. *Dasydorylas setosus* was originally described from specimens collected on Gran Canaria, La Palma and Tenerife, and was redescribed by Kehlmaier (2005). Its current distribution comprises Canary Islands (La Gomera, El Hierro, La Palma, Tenerife, Gran Canaria), mainland Spain, Madeira and Morocco. Here, the species was recorded from 15th May to 27th June. Literature records indicate that it can be found all year round on Canary Islands.

Tomosvaryella freidbergi De Meyer, 1995

MATERIAL (MT: 2♀♀). SPAIN, Canary Islands, La Palma, Caldera de Taburiente National Park: Roque de la Cumbrecita: 1.VI.2000, 1♀ (MNCN_Ent 202050, MT), coll. MNCN.— Roque de los Muchachos: 4.VII.2001, 1♀ (MT), coll. SMTD.

REMARKS. Representing the first record for La Palma, *T. freidbergi* was previously recorded from Canary Islands (Fuerteventura, La Gomera, Tenerife) by De Meyer *et al.* (2001). For species recognition see De Meyer (1995) and Földvári & De Meyer (1999). *Tomosvaryella freidbergi* has been recorded from Canary Islands,

mainland Portugal, mainland Spain, mainland France, Israel, Hungary, Egypt, Iran, Kazakhstan and Kyrgyz Republic. The material at hand was collected from 1st June to 4th July. Literature records for Canary Islands range from February to July and September.

Tomosvaryella parakuthyi De Meyer, 1995

MATERIAL (MT: 2♀♀; YT: 2♂♂). SPAIN, Canary Islands, La Palma, Caldera de Taburiente National Park: Barranco de las Traves: 19.VI.2000, 1♂ (MNCN_Ent 202048, YT), coll. MNCN.— Roque de los Muchachos: 31.V.2001, 1♀ (MNCN_Ent 202049, MT), coll. MNCN; 15.VII.2001, 1♀ (MT), coll. SMTD; 17.VII.2001, 1♂ (YT), coll. SMTD.

REMARKS. First record for La Palma, yet previously cited from Gran Canaria by De Meyer *et al.* (2001). The present material has been recorded between 31st May and 17th July. The specimen from Gran Canaria was collected at the end of February. So far, *T. parakuthyi* is known from Canary Islands, Egypt, Iran and Israel.

CHALARINAE

Chalarus guanche Kehlmaier sp. nov.

Chalarus perplexus in De Meyer *et al.* (2001)

Chalarus sp. near *zyginae* in Kehlmaier & Assmann (2008)

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Figs. 1–3

HOLOTYPE. 13.VI.2000, ♂ (#29, YT), SPAIN, Canary Islands, La Palma, Caldera de Taburiente National Park, Barranco de las Traves, leg. T. Domingo-Quero, coll. MNCN (MNCN_Ent 160689).

PARATYPES. SPAIN, Canary Islands, La Palma, Caldera de Taburiente National Park: Playa del Rio Taburiente: 6.III.2000, 1♀ (#25, MT).— Barranco de las Traves: 22.VIII.2000, 1♀ (#2, MNCN_Ent 160706, MT).— Lomo de las Chozas: 8.III.2000, 1♀ (#21, MNCN_Ent 160705, MT); 22.III.2000, 1♂ (#48, MNCN_Ent 160690, YT); 26.IV.2000, 1♀ (#15, MNCN_Ent 160702, MT); 21.VI.2000, 5♂♂ (#43–47¹, MT), 1♀ (#14, MNCN_Ent 160695, MT); 6.VII.2000, 2♀♀ (#19–20², MT); 19.VII.2000, 3♂♂ (#40–42³, MT); 4♀♀ (#10–13⁴, MT); 26.VII.2000, 1♀ (#17, MT); 16.VIII.2000, 2♂♂ (#38–39, MT); 6.IX.2000, 1♀ (#18, MT).— Roque de los Muchachos:

¹ #44 MNCN_Ent 160692; #45 MNCN_Ent 160693; #46 MNCN_Ent 160694; #47 MNCN_Ent 160691

² #19 MNCN_Ent 160703; #20 MNCN_Ent 160704

³ #40 MNCN_Ent 160700; #41 MNCN_Ent 160701

⁴ #10 MNCN_Ent 160696; #11 MNCN_Ent 160697; #12 MNCN_Ent 160698; #13 MNCN_Ent 160699

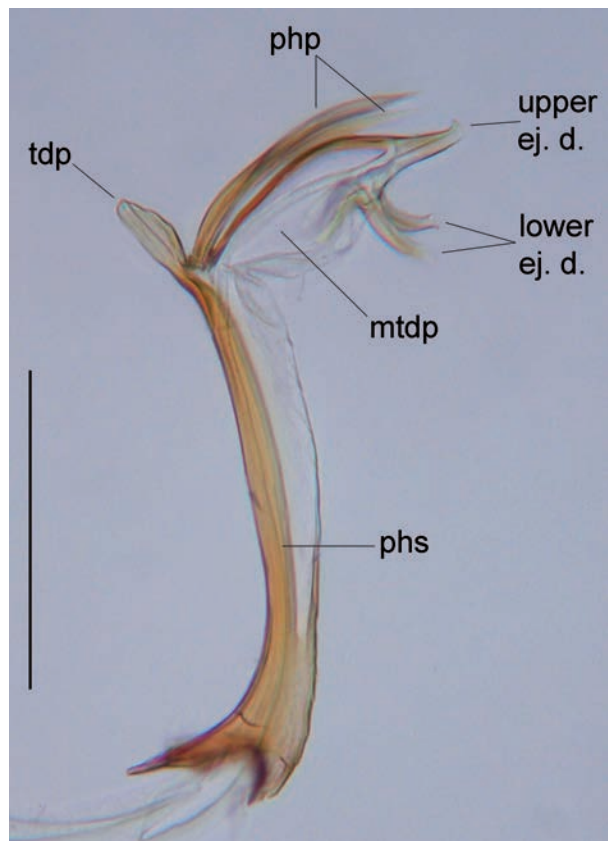


Fig. 1.— Male genitalia of *C. guanche* **sp. nov.** in lateral view. Abbreviations: lower ej. d., lower ejaculatory ductuli; mtdp, membranous tip of distiphallus; php, phallic processes; phs, phallic shaft; tdp, tip of distiphallus; upper ej. d., upper ejaculatory duct. Scale bar: 0.1 mm.

Fig. 1.— Genitalia masculina de *C. guanche* **sp. nov.** en vista lateral. Abreviaturas: lower ej. d., túbulos eyaculadores inferiores; mtdp, ápice membranoso del distifalo; php, procesos fálicos; phs, asta fálica; tdp, ápice del distifalo; upper ej. d., tubo eyaculador superior. Escala: 0,1 mm.

25.VIII.2000, 1♀ (#27, MNCN_Ent 160707, MT). Paratypes in coll. SMTD: #17, #18, #25, #38, #39, #42, #43. Others in coll. MNCN.

NON-TYPE MATERIAL. SPAIN, Canary Islands, La Palma, Caldera de Taburiente National Park: Playa del Rio Taburiente: 17.IV.2000, 1♂ (#55, MT), 1♀ (#23, MNCN_Ent 202027, MT); 15.V.2000, 1♀ (#22, MT); 19.VI.2000, 1♂ (#57, MNCN_Ent 202029, YT); 3.VIII.2000, 4♂♂ (#58–61⁵, YT), 1♀ (#24, MNCN_Ent 202034, YT); 7.VIII.2000, 1♀ (#26, MNCN_Ent 202028, MT); 18.X.1999, 1♂ (#56, MT).— Barranco de las Traves: 8.V.2000, 1♀ (#8, MT); 30.V.2000, 1♀ (#6, MNCN_Ent 160708, MT); 5.VI.2000, 1♂ (#35, MT); 13.VI.2000, 1♂ (#30, MNCN_Ent 202018, MT); 19.VI.2000, 1♀ (#1, MNCN_Ent 202019, MT); 27.VI.2000, 1♂ (#33, MNCN_Ent 202020, MT); 4.VII.2000, 1♂ (#34, MNCN_Ent 202021, MT);

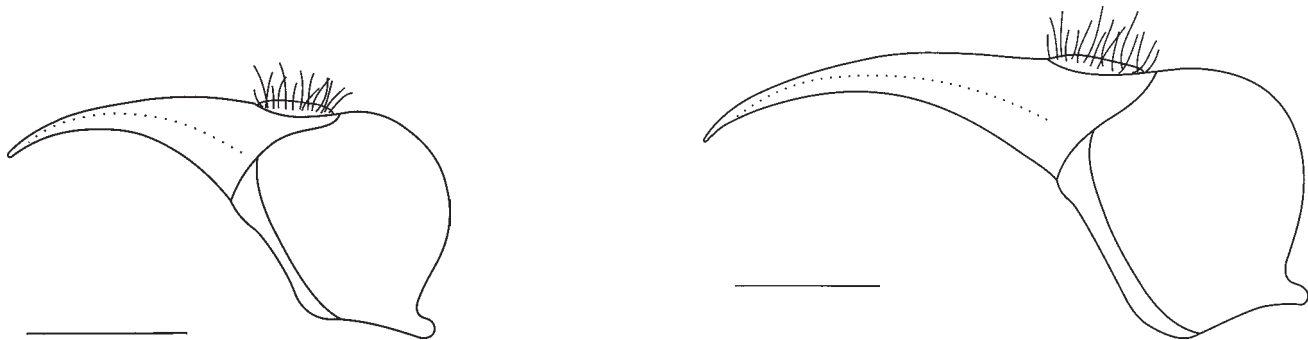
⁵ #58 MNCN_Ent 202030; #59 MNCN_Ent 202031; #60 MNCN_Ent 202032; # 61 MNCN_Ent 202033

10.VII.2000, 1♂ (#28, MNCN_Ent 202023, YT); 24.VII.2000, 1♂ (#32, YT), 1♀ (#3, MNCN_Ent 202022, MT); 3.VIII.2000, 2♀♀ (#4, MNCN_Ent 202024, #5, MNCN_Ent 202025, YT); 7.VIII.2000, 1♂ (#36, MT), 1♀ (#7, MT); 18.IX.2000, 1♂ (#31, MNCN_Ent 202026, YT).— Lomo de las Chozas: 5.I.2000, 1♂ (#51, MNCN_Ent 202035, MT); 26.IV.2000, 1♂ (#49, MT), 1♀ (#16, MT); 1.VI.2000, 1♂ (#37, MNCN_Ent 202036, MT), 1♀ (#9, MT); 6.VII.2000, 3♂♂ (#52–54⁶, MT); 26.VII.2000, 1♂ (#50, MNCN_Ent 202039, MT).— Roque de los Muchachos: 27.VII.2000, 1♂ (#62, MNCN_Ent 202040, YT). Specimens in coll. SMTD: #7, #8, #22, #32, #35, #36, #49, #54, #55, #56. Others in coll. MNCN.— SPAIN, Canary Islands, La Palma, south of Barlovento, laurisilva, 28°39'N 17°52'W, 800 m, 29.X.2002, 3♂♂ (DNA CK145, CK146, CK417), 1♀, leg. et coll. C. Kehlmaier.— PORTUGAL, Madeira, path between Boca de Encumeada and Boca dos Corgos, S of Ribeiro do Póco, 1 km W of Fenda do Ferreiro, moist steep slope, 1200–1250 m, 30.V.1987, 1♂ (M1, X572), leg. W. Barkemeyer, coll. SDEI.— PORTUGAL, Madeira, Fanal, laurel on pasture, 12.IX.1986, 3♂♂ (M2–M4, L1583), leg. P. Ohm, coll. SDEI.— PORTUGAL, Madeira, Pico Facho, near Machico, herbaceous vegetation, 300 m, 19.IX.1986, 1♂ (M5, L1578), leg. P. Ohm, coll. SDEI.

NOTE. The material at hand consists of 71 specimens. Sixty-two from the Caldera de Taburiente National Park (35♂♂, 24 MT, 11YT; 27♀♀, 24MT, 3YT)—unfortunately, specimens #9 and #16 were misplaced, and of specimens #37 and #49 only the genitalia remain—, three males and one female from Barlovento (NE La Palma), and five male specimens from three localities on Madeira. Only specimens attributed to clade A via ITS2 were included in the type series in order to prevent confusion if clade B or C might be considered as a distinct species one day.

DIFFERENTIAL DIAGNOSIS. *Chalarus guanche* **sp. nov.** is part of the *holosericeus* species group as outlined in Kehlmaier & Assmann (2008, 2010). The taxonomic structure of this flock of closely allied taxa is still not satisfactorily resolved. Morphologically, females are closest to *C. zyginae* Jervis, 1992 being slightly larger in size and having their frons not as strongly narrowed (at narrowest point ~1.5 times largest frontal facet instead of 1.0), whereas males should be addressed as *C. holosericeus* agg. (aggregate species), as the genitalia of all taxa of this species group are virtually identical and cannot be used for species separation except in the case of *C. zyginae*. Because of the cryptic nature of this species, only the holotype is described in detail, whereas the observed variability of the females is characterised in Tables 2–3 and Figs 2–4.

⁶ #52 MNCN_Ent 202037; #53 MNCN_Ent 202038



Figs. 2–3.— Female terminalia of *C. guanche* **sp. nov.** Scale bars: 0.1 mm. Fig. 2: Representative of clade A (no. 18). Fig. 3: Representative of clade B (no. 8).

Figs. 2–3.— Terminalia femenina de *C. guanche* **sp. nov.** Escalas: 0,1 mm. Fig. 2: Representante del clado A (n° 18). Fig. 3: Representante del clado B (n° 8).

Table 2.— Morphometric measurements and ratios in female *C. guanche* **sp. nov.** from La Palma.

Tabla 2.— Medidas morfométricas y proporciones en las hembras de *C. guanche* **sp. nov.** de La Palma.

DFF: Diameter of largest frontal facet in mm (n=25).													
0.035	0.04	0.045	0.05									average	median
1	17	4	3									0.0418	0.04
FN: Width of frons at its narrowest point in mm (n=23) — 2 specimens with distorted frons excluded (0.025 mm and 0.035 mm).													
0.04	0.045	0.05	0.055									average	median
4	7	11	1									0.0452	0.05
FMO: Width of frons at level of anterior (median) ocellus in mm (n=25).													
0.07	0.09	0.095	0.1									average	median
1	15	6	3									0.0916	0.09
FN:FMO (n=23).													
0.42	0.44	0.47	0.50	0.53	0.56	0.57	0.58					average	median
1	2	3	7	1	7	1	1					0.51	0.50
DFF:FN (n=23).													
0.73	0.80	0.88	0.89	0.90	1.00	1.11	1.25					average	median
1	7	1	5	3	4	1	1					0.90	0.89
Length of tergite 9 (piercer) in lateral view in mm (n=24)—1 specimen with lost ovipositor excluded.													
0.15	0.17	0.18	0.19	0.20	0.21	0.22	0.24					average	median
1	2	5	2	6	2	4	2					0.20	0.20
Wing length in mm (n=25).													
1.5	1.55	1.7	1.75	1.8	1.85	1.9	1.95	2.05	2.1	2.15	2.25	average	median
1	2	1	3	2	5	1	2	1	4	1	2	1.89	1.85

DESCRIPTION OF HOLOTYPE. Male. Body length 1.9 mm. Head. Face black, silver-grey pollinose. Eyes separated, ommatidial facets slightly enlarged towards the front. Frons black, silver-grey pollinose in lower quarter. At its narrowest point, width of 2 accompanying ommatidial facets. Antenna dark brown. Pedicel with 2 short upper and 2 lower bristles, one of the latter longer than flagellum which is of an ovoid-kidney shape and is slightly longer than wide. Vertex black.

Ocellar triangle with 1 pair of long and 2 pairs of short ocellar bristles. Occiput black, hardly visible in lateral view. Thorax. Entirely dark brown to black. Dorsal surface of prescutum and scutum covered with rather widely spaced black setae, as in other species of the genus, the longest ones towards the lateral and posterior margins (notopleural, supraalar and postalar bristles). Scutellum with 2 pairs of long black marginal bristles, dorsally with 2 pairs of short setae. Wing and halter.

Table 3.— Morphometric measurements and ratios in female *C. guanche* **sp. nov.** from La Palma corresponding to ITS2 clade A and B.

Tabla 3.— Medidas morfométricas y proporciones de las hembras de *C. guanche* **sp. nov.** de La Palma correspondientes a los clados A y B del ITS2.

	♀♀ ITS2 clade A	♀♀ ITS2 clade B
DFF (diameter of largest frontal facet)	0.035–0.045 mm (n=14)	0.04–0.05 mm (n=6)
FN (width of frons at narrowest point)	0.04–0.05 mm (n=13)	0.04–0.05 mm (n=5)
FMO (width of frons at level of anterior/median ocellus)	0.07–0.95 mm (n=14)	0.09–0.1 mm (n=6)
FN:FMO	0.80–1.00 (n=13)	0.90–1.25 (n=5)
DFF:FN	0.44–0.57 (n=13)	0.42–0.56 (n=5)
Tergite 9	0.15–0.21 mm (n=13)	0.22–0.24 mm (n=7)
Wing length	1.5–2.05 mm (n=14)	2.1–2.25 mm (n=7)

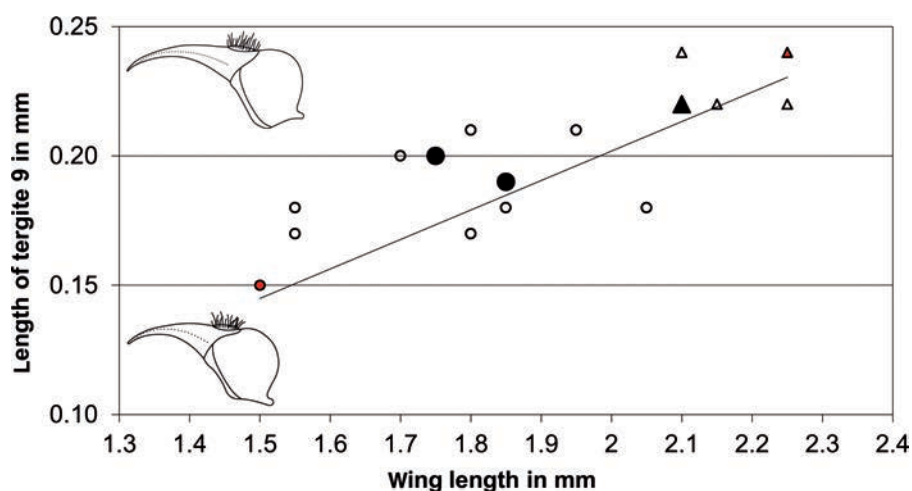


Fig. 4.— Relationship between wing length and length of tergite 9 in female *C. guanche* **sp. nov.** Circles represent clade A (n=13; small open circle: 1 specimen; large full circle: 2 specimens; small red circle: figured specimen #18 (Fig. 2)); triangles represent clade B (n=7; small open triangle: 1 specimen; large full triangle: 3 specimens; small red triangle: figured specimen #8 (Fig. 3)).

Fig. 4.— Relación entre la longitud del ala y la del terguito 9 de las hembras de *C. guanche* **sp. nov.** Los círculos representan el clado A (n=13; círculo pequeño vacío: 1 ejemplar; círculo grande relleno: 2 ejemplares; círculo pequeño rojo: ejemplar figurado n° 18 (Fig. 2)); los triángulos representan el clado B (n=7; triángulo pequeño vacío: 1 ejemplar; triángulo grande relleno: 3 ejemplares; triángulo pequeño rojo: ejemplar figurado n° 8 (Fig.3)).

Length: 1.9 mm. LW:MWW=2.8. Wing surface with a weak brownish tinge and covered with microtrichia except near base. Pterostigma brown and incomplete (LS:LTC=0.8). LSC:LTC:LFC=6.8:5.5:1.0. Wing venation incomplete, as in other members of *Chalarus*. Halter dark brown with stem narrowly white. Leg. Entirely dark brown except tarsal segments slightly paler (mid brown). psr ~13 setae, mid brown; pvsr ~16 setae, light brown, apical one not extending beyond apex; aasr ~9 short setae, mid brown, apical ones extending as far as apex; pdsr ~8 dark brown setae, apical ones extending as far as apex. Pulvilli shorter than distitarsus. Abdomen. Gently ovate in dorsal view, LT35:WT2=1.1. Entirely dark brown. Setae mid to dark brown. Dorsally and ventrally sparse and short, long and dense along lateral margins. Terminalia as

in other members of the *holosericeus* species group (Fig. 1; see also figures in Kehlmaier & Assmann, 2008); surstyli symmetrical, with pronounced medial protuberances, best seen in dorsal view; gonopods symmetrical, broadened in distal half; phallus with straight shaft; tip of distiphallus short and broad; Lmtdp:Ltdp slightly more than 3.0; phallic processes symmetrical, slightly shorter than membranous tip of distiphallus and parallel to the latter; all three ejaculatory ducts placed distally on membranous tip of distiphallus; ejaculatory apodeme parasol-shaped.

Female differs from male by the usual sexual dimorphisms. For morphometric measurements and ratios see Tables 2–3. Bristles of pedicel shorter; none longer than flagellum. Frontal facets moderately to greatly enlarged. Frons at narrowest point about

diameter of largest frontal facet. Frons with pairs of fronto-orbital setae. Tergites 2–5 laterally with shorter setae. Ovipositor as in Figs. 2–3; one and a half to twice length of base and tergite 9 gently curved towards sternites. Base dark brown; piercer somewhat paler towards apex.

ETYMOLOGY. The species is named after the native inhabitants of the Canary Islands. It is believed that the Guanches were of Berber origin (North West African) (Fregel *et al.*, 2009) and migrated to the Canary Islands around 1,000 BC or perhaps earlier.

DISTRIBUTION. The species has been recorded from La Palma (Canary Islands) and Madeira.

MORPHOLOGICAL VARIABILITY. The observed morphological variability in the material of *Chalarus* studied (Tables 2–3) raised the suspicion as to whether it actually contained several undescribed species. The degree of enlargement of the frontal ommatidial facets (DFF) varies by ~40% (0.035–0.05 mm). Wing length, used to assess the size of the fly, by ~50% (1.5–2.25 mm). And the length of tergite 9 by ~60% (0.15–0.24 mm). In the latter, one can also observe that the longer tergite 9 is, the stronger is its curvature (Figs. 2–3). When scoring length of tergite 9 against wing length, a positive correlation between these two morphological traits can be observed (Fig. 4). However, although the extrema clearly differ, there does not seem to exist a precise division.

MOLECULAR ANALYSES. The Internal Transcribed Spacer Region 2 (ITS2) is a nontranscribed fragment of the ribosomal DNA (rDNA). Being nested between the slow evolving 5.8S and 28S rDNA loci, ITS2 is renowned for its high evolutionary rate and has been successfully applied for species delimitation and phylogenetic studies in Pipunculidae and other taxa (Kehlmaier & Assmann, 2010). The aligned ITS2 dataset for the *Chalarus exiguus* species group

is 352bp long and consists of 51 specimens representing six species. In this study, the fragment had approximately 370bp and was sequenced in mostly excellent quality for 41 specimens. The NJ analysis of the ITS2 dataset, divides *C. guanche* **sp. nov.** into three clades, with two present on La Palma (clade A & B) and a third one on Madeira (clade C) (Fig. 5). With 0.6%, the uncorrected pairwise genetic distance is identical between all three clades (Table 4). Each clade has a single private (apomorphic) mutation. In addition, clade A has a 3bp long indel (Fig. 6). The minimum interspecific genetic distance between the species of the *exiguus* group ranges from 1.1% to 3.8% (Table 4). The 41 specimens fall into 26 clade A, 11 clade B and 4 clade C.

In four specimens of clade A (#2, #17, #20, #21), a second fragment of about 220bp amplified with

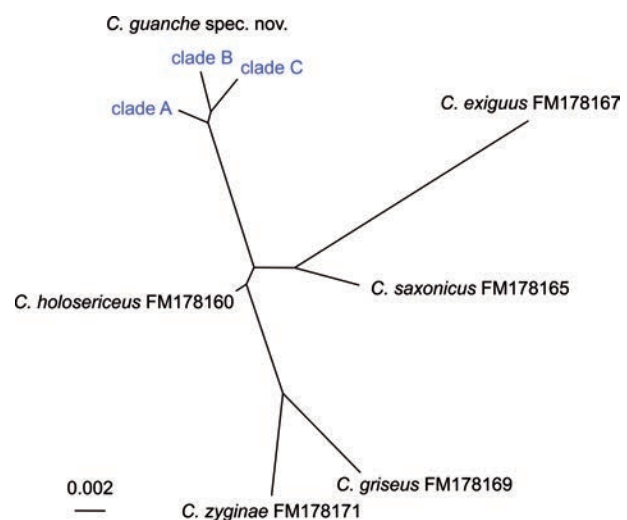


Fig 5.— Unrooted NJ-phenogram of genetic divergence of ITS2 genotypes. Scale bar indicates number of substitutions per amino acid position.

Fig 5.— Fenograma NJ sin raíz de la divergencia genética de los genotipos del ITS2. La escala indica el número de sustituciones por cada posición aminoacídica.

Table 4.— Uncorrected minimum interspecific genetic distance in % of *Chalarus exiguus* species group for ITS2 dataset.

Tabla 4.— Distancia genética interespecifica mínima no corregida en tanto por ciento del grupo de especies de *Chalarus exiguus* para el conjunto de datos del ITS2.

	FM178160	FM178167	FM178169	FM178165	FM178171	clade A	clade B	clade C
<i>C. holosericeus</i> FM178160	—							
<i>C. exiguus</i> FM178167	2.3	—						
<i>C. griseus</i> FM178169	1.7	3.2	—					
<i>C. saxonicus</i> FM178165	1.1	2.3	2.3	—				
<i>C. zyginae</i> FM178171	1.4	3.8	1.4	2.6	—			
<i>C. guanche</i> clade A	1.4	3.8	3.2	1.4	2.9	—		
<i>C. guanche</i> clade B	1.4	3.8	3.2	2.0	2.9	0.6	—	
<i>C. guanche</i> clade C	1.4	3.8	3.2	2.0	2.9	0.6	0.6	—

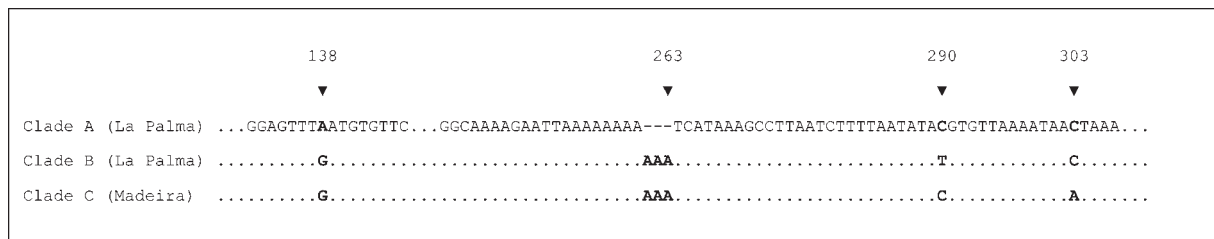


Fig. 6.— Excerpt of ITS2 alignment illustrating the intraspecific variability in *C. guanche* **sp. nov.**

Fig. 6.— Extracto del alineamiento del ITS2 mostrando la variabilidad intraespecífica de *C. guanche* **sp. nov.**

somewhat lower signal strength. This sequence is identical to ITS2 but exhibit a 151bp long deletion from alignment position 167 to 318, resulting in an interference of 75bp in the chromatograms around positions 170 to 240. This shorter sequence was disregarded in the analyses performed but might be an indication for intragenomic variation. An example of this shorter sequence was sent to ENA for specimen #20 (LT715963). Of clade B and C, all 15 sequences show a weak n-1 sequence from alignment position 263 onwards after a 11-fold adenine repeat. In clade A, the homologous section consists of a 8-fold adenine repeat and did not pose any sequencing problems. Although intragenomic variation cannot be ruled out here either, such artefacts may also result from a slippage of the enzyme during PCR amplification. For the present analyses, the low signal of the underlying n-1 sequence was disregarded. Interestingly, all *C. guanche* samples have an additional 20bp prior to the reverse primer (ACCTCAACTCATATGGGATT). In a previous study (Kehlmaier & Assmann, 2010), this motive emerged in only few samples of Chalarinae (FM212652, FM212653, FM212655, FM213119, LN879371), and, slightly modified, also in one Pipunculinae used as outgroup (FM212650). As this oligonucleotide has not been detected in representatives of the *C. exiguus* species group before and its origin is most likely attributed to intragenomic variation as well, it has been excluded from the present analyses, yet included in ENA submissions.

The 5' end of the mitochondrial cytochrome oxidase subunit I (COI) is the classical DNA barcoding fragment sequenced for a multitude of taxa (Hebert *et al.*, 2003). The aligned COI dataset for the *Chalarus exiguus* species group is 658bp long and consists of 40 specimens representing six species. Thirty specimens from La Palma (24 clade A and 6 clade B) and five from Madeira (all clade C) yielded a DNA barcode with moderately to very good sequence quality. The observed genetic divergence (p-distance) between the individual clades ranges from 1.2–3.1% between clades A and B, 1.2–1.8% between clades A and C, and 0.6–1.8% between clades B and C. The maximum intraspecific variability is 0.8% in clade A, 1.2% in clade B, and 0% in clade C. The minimum

interspecific genetic distance between the species of the *exiguus* group ranges from 2.6% (*C. guanche* **sp. nov.** to *C. griseus* and *C. saxonicus* respectively) to 6.8% (*C. exiguus* to *C. holosericeus*) (Table 5). Interestingly, two specimens (#10, #21) with a clade A ITS2 genotype cluster with the specimens that have a clade B ITS2 genotype, providing evidence for hybridisation between both clades (Fig. 7).

Discussion

One of the great challenges of taxonomy is the identification and differentiation between closely allied species. Over the past decades, and with the development of new taxonomic tools, integrative and iterative approaches have been advocated that combine multiple lines of evidence, resulting in a multidimensional synthesis of known facts (Padiál *et al.*, 2010; Schlick-Steiner *et al.*, 2010; Yeates *et al.*, 2011). Here, the data at hand bring up the question whether the observed morphological and molecular heterogeneity in studied *Chalarus* might justify the naming of additional species other than *C. guanche* **sp. nov.**

Morphological characters for distinguishing between species of *Chalarus* are scarce. Whereas male species diagnosis is almost exclusively based on the genitalia, females have to be identified via an array of outer morphological features that can be hard to assess: shape of ovipositor, length of pulvilli, chaetotaxy of legs, size of frontal facets and width of frons. However, deformation of the exoskeleton frequently takes place once the insect is pinned and dried, especially if it was formerly preserved in a liquid. Bristles can break off, the pulvilli may shrivel, the frons can experience a narrowing or the head may partly collapse or fall off. In addition, these artefacts meet with a natural degree of morphological variability within a species. The 25 female specimens suitable for morphometric measurements (Table 2) display a large morphological variability, as seen in the diameter of largest frontal facet (DFF), width of frons at level of anterior/median ocellus (FMO), wing length and length of tergite 9. When the morphometric results are separated by clades A and B (Table 3) only wing length and length of tergite 9 show a weak/marginal

Table 5.— Uncorrected minimum interspecific genetic distance in % of *Chalarus exiguus* species group for COI dataset. Roman numerals i)–xiii) are place holders for the individual haplotypes of *C. guanche* **sp. nov.**. The individual clades of COI are colour coded.

Tabla 5.— Distancia genética interespecifica mínima no corregida en tanto por ciento del grupo de especies de *Chalarus exiguus* para el conjunto de datos de la COI. Los numerales romanos i)–xiii) son marcadores para los haplotipos individuales de *C. guanche* **sp. nov.**. Se han codificado con colores los clados individuales de COI.

	FM178136	FM178127	FM178120	FM178131	FM178122	i)	ii)	iii)	iv)	v)	vi)	vii)	viii)	ix)	x)	xi)	xii)	
ITS2 clade	C																	
<i>C. holosericeus</i> FM178136	—																	
<i>C. saxonicus</i> FM178127	4.0	—																
<i>C. exiguus</i> FM178120	6.8	5.3	—															
<i>C. griseus</i> FM178131	3.5	3.5	6.2	—														
<i>C. zyginae</i> FM178122	4.2	4.8	6.1	4.4	—													
i) M1–5	3.0	2.6	5.5	2.6	3.7	—												
ii) #3	4.3	3.4	5.8	3.7	4.1	1.8	—											
iii) #1	3.8	3.2	5.8	3.3	4.2	1.5	1.1	—										
iv) #8	4.1	3.2	5.8	3.3	4.4	1.5	1.1	0.3	—									
v) #7, 32	4.1	3.1	5.8	3.4	4.2	1.5	0.0	0.8	0.8	—								
vi) #36	3.2	2.8	5.4	2.8	3.6	0.6	0.6	0.6	0.6	0.3	—							
vii) #10	3.5	2.9	5.3	2.9	3.8	1.1	0.8	0.0	0.2	0.6	0.3	—						
viii) #21	4.0	3.0	5.9	3.5	4.4	1.7	1.2	0.2	0.5	0.9	0.8	0.2	—					
ix) #11, 18, 29, 38, 39, 41–43, 45–48	3.3	3.2	5.8	3.0	4.0	1.2	2.4	2.1	2.1	2.1	1.2	1.7	2.3	—				
x) #27, 40, 44	3.5	3.0	5.8	3.2	4.0	1.2	2.5	2.1	2.1	2.1	1.2	1.7	2.3	0.2	—			
xi) #2, 15, CK417	3.8	3.3	5.9	3.5	4.2	1.4	2.6	2.3	2.3	2.3	1.4	1.8	2.4	0.5	0.2	—		
xii) #12, 14, 19	3.8	3.3	5.9	3.5	4.5	1.7	2.9	2.6	2.6	2.6	1.7	2.1	2.7	0.5	0.6	0.6	—	
xiii) #20	4.0	3.5	6.1	3.3	4.7	1.8	3.1	2.7	2.7	2.7	1.8	2.3	2.9	0.6	0.8	0.8	0.2	

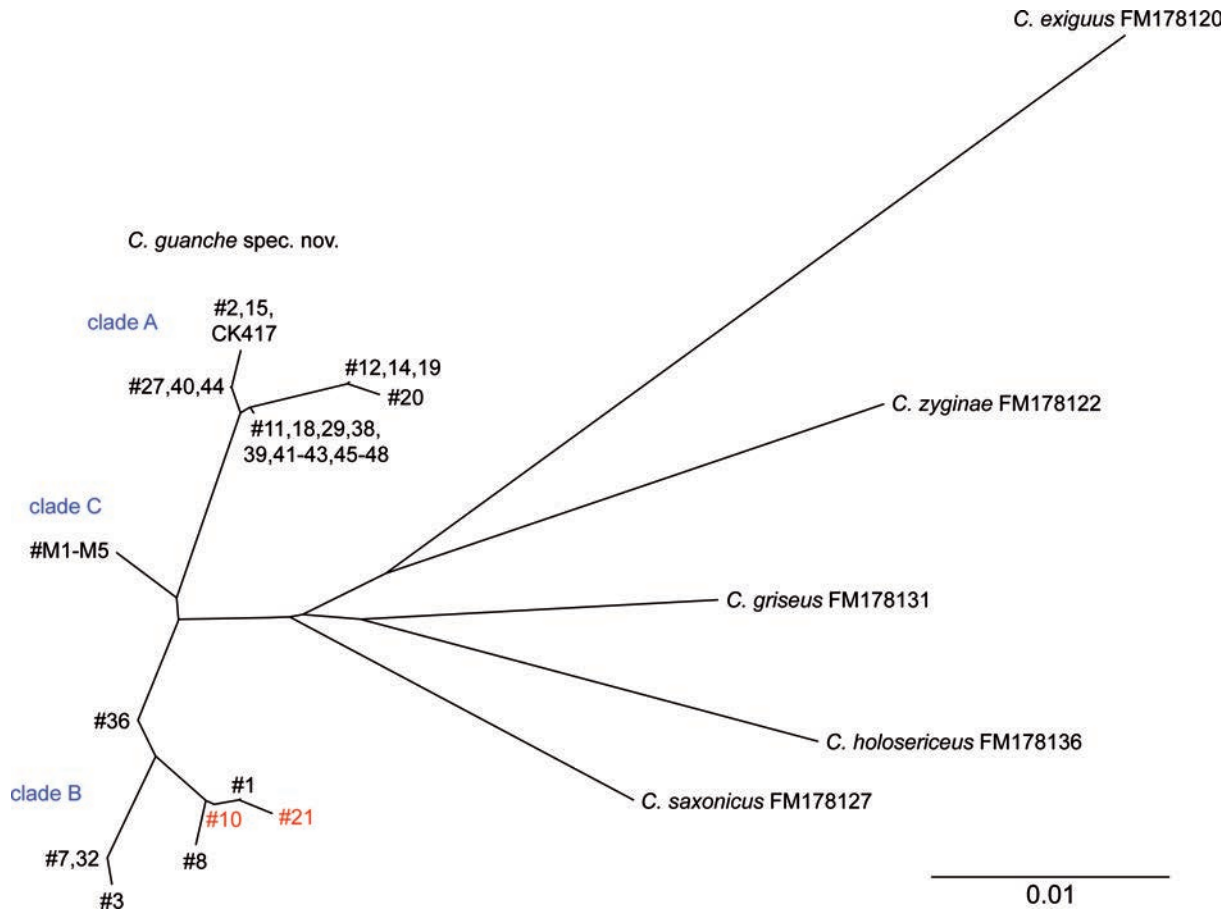


Fig. 7.— Unrooted NJ-phenogram of genetic divergence of COI haplotypes. Highlighted in red are the two specimens that indicate hybridisation by sharing a clade A ITS2 genotype. Scale bar indicates number of substitutions per amino acid position.

Fig. 7.— Fenograma NJ sin raíz de la divergencia genética de los haplotipos de la COI. Resaltados en rojo, los dos ejemplares que indican hibridación al compartir un genotipo del ITS2 del clado A. La escala indica el número de sustituciones por posición aminoacídica.

separation between the two lineages. All other morphological traits and ratios do at least overlap slightly.

The results of the molecular analyses also display an ambiguous picture. On the one hand, haplotype divergence (maximum intraspecific genetic distance) of COI between the specimens of *C. guanche* reaches 3.1%, and thus is higher than the minimum interspecific genetic distance between the species pairs *C. guanche*/*C. saxonicus* and *C. guanche*/*C. holosericeus* (2.6%) and almost as high as *C. holosericeus*/*C. griseus* and *C. saxonicus*/*C. griseus* (3.5%). On the other hand, genotype divergence between the three clades of *C. guanche* for the ITS2 dataset is 0.6%, and thus about half the smallest minimum interspecific genetic distance observed in this species group (1.1% between *C. holosericeus*/*C. saxonicus*). Also, hybridisation between clades A and B could be detected in two cases.

A parenthesis has to be made in regard to the possible presence of intragenomic variability in ITS2. In eukaryotic cells, ITS2, as part of the rDNA gene cluster, is arranged in multiple (typically several

hundred) tandem repeats, which are known to exhibit a certain degree of genetic variability. Several studies have investigated this phenomenon for ITS2 for different taxa, e.g., bacteria (Stewart & Cavanaugh, 2007), fungi (Lindner & Banik, 2011; O’Donnell & Cigelnik, 1997), plants (Weitemier *et al.*, 2015), corals (Odorico & Miller, 1997), sponges (Wörheide *et al.*, 2004), crayfish (Harris & Crandall, 2000), molluscs (Hoy & Rodriguez, 2013), butterflies (Shapoval & Lukhtanov, 2015) and mosquitos (Bourke *et al.*, 2013; Li & Wilkerson, 2007; Vesgueiro *et al.*, 2011). The degree of intragenomic variation is mostly low due to concerted evolution (Brown *et al.*, 1972). However, whenever concerted evolution is outrun by speciation, divergent paralogs, with up to 5% p-distance and more, have been recorded in a single genome (e.g., Wörheide *et al.*, 2004). Such paralogs even exceed interspecific variability (Li & Wilkerson, 2007) and ultimately lead to confound tree topology if included in phylogenetic analyses (Lindner & Banik, 2011). In the present study, the observed artefacts point towards this phenomenon, although a final proof can only be achieved

by cloning and the subsequent sequencing of multiple clones from a single organism.

In a nutshell, both loci support the validity of *C. guanche* **sp. nov.** as a separate taxon, but also leave room as to whether there are additional species present on Macaronesia. Extra insights might be obtained through more intense sampling on all Macaronesian islands, especially in respect to the vertical distribution and possible host associations of the individual clades. The former can currently be characterised by the presence of clade B restricted to the two lower most localities (750–1,068 m), whereas clade A is distributed between 750–2,250 m. Knowledge of the larval host species of the individual lineages of *C. guanche* might also be enlightening, and can be achieved by sampling and dissecting Typhlocybinae leafhoppers—the only known hosts of *Chalarus*—in order to extract any larvae which can then be identified via DNA barcodes.

At present, the known Pipunculidae fauna of La Palma comprise the following seven species: *Chalarus guanche*, *Dasydorylas setosus*, *Eudorylas fluviatilis*, *Tomosvaryella brachybasis*, *T. freidbergi*, *T. geniculata* and *T. parakuthyi*.

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Appendix 1.— Specimen, locality and collecting data of studied material of *C. guanche* **sp. nov.**, including GenBank accession numbers and clade affiliation. Abbreviations: MT, Malaise trap; YT, yellow pan trap. *Specimen misplaced. **Only genitalia left.

Apéndice 1.— Ejemplar, localidad y datos de colecta del material estudiado de *C. guanche* **sp. nov.**, incluyendo los números de registro en GenBank y la afiliación a clados. Abreviaturas: MT, trampa Malaise; YT, trampa Moericke amarilla. *Ejemplar extraviado. **Sólo se conserva la genitalia.

sex	date	trap	specimen no.	ITS2 clade	ITS2	COI
Spain, Canary Islands, La Palma, Playa del Río Taburiente.						
♀	6.III.2000	MT	#25	A	LT715939	—
♂	17.IV.2000	MT	#55	—	—	—
♀	17.IV.2000	MT	#23	B	LT715940	—
♀	15.V.2000	MT	#22	—	—	—
♂	19.VI.2000	YT	#57	—	—	—
♂	3.VIII.2000	YT	#58	—	—	—
♂	3.VIII.2000	YT	#59	—	—	—
♂	3.VIII.2000	YT	#60	—	—	—
♂	3.VIII.2000	YT	#61	—	—	—
♀	3.VIII.2000	YT	#24	—	—	—
♀	7.VIII.2000	MT	#26	B	LT715941	—
♂	18.X.1999	MT	#56	—	—	—
Spain, Canary Islands, La Palma, Barranco de las Traves.						
♀	8.V.2000	MT	#8	B	LT715942	LT715904
♀	30.V.2000	MT	#6	B	LT715943	—
♂	5.VI.2000	MT	#35	—	—	—
♂	13.VI.2000	YT	#29	A	LT715944	LT715905
♂	13.VI.2000	MT	#30	—	—	—
♀	19.VI.2000	MT	#1	B	LT715945	LT715906
♂	27.VI.2000	MT	#33	—	—	—
♂	4.VII.2000	MT	#34	B	LT715946	—
♂	10.VII.2000	YT	#28	—	—	—
♂	24.VII.2000	YT	#32	B	LT715947	LT715907
♀	24.VII.2000	MT	#3	B	LT715948	LT715908
♀	3.VIII.2000	YT	#4	—	—	—
♀	3.VIII.2000	YT	#5	—	—	—
♂	7.VIII.2000	MT	#36	B	LT715949	LT715909
♀	7.VIII.2000	MT	#7	B	LT715950	LT715910
♀	22.VIII.2000	MT	#2	A	LT715951	LT715911
♂	18.IX.2000	YT	#31	—	—	—
Spain, Canary Islands, La Palma, Lomo de las Chozas.						
♂	5.I.2000	MT	#51	—	—	—
♀	8.III.2000	MT	#21	A	LT715952	LT715912
♂	22.III.2000	YT	#48	A	LT715953	LT715913
♂	26.IV.2000	MT	#49**	—	—	—
♀	26.IV.2000	MT	#15	A	LT715954	LT715914
♀	26.IV.2000	MT	#16*	—	—	—
♂	1.VI.2000	MT	#37**	—	—	—
♀	1.VI.2000	MT	#9*	—	—	—
♂	21.VI.2000	MT	#43	A	LT715955	LT715915
♂	21.VI.2000	MT	#44	A	LT715956	LT715916
♂	21.VI.2000	MT	#45	A	LT715957	LT715917
♂	21.VI.2000	MT	#46	A	LT715958	LT715918
♂	21.VI.2000	MT	#47	A	LT715959	LT715919
♀	21.VI.2000	MT	#14	A	LT715960	LT715920

Appendix 1.— (Continued)

sex	date	trap	specimen no.	ITS2 clade	ITS2	COI
♂	6.VII.2000	MT	#52	—	—	—
♂	6.VII.2000	MT	#53	—	—	—
♂	6.VII.2000	MT	#54	—	—	—
♀	6.VII.2000	MT	#19	A	LT715961	LT715921
♀	6.VII.2000	MT	#20	A	LT715962	LT715922
♀	6.VII.2000	MT	#20	A short	LT715963	
♂	19.VII.2000	MT	#40	A	LT715964	LT715923
♂	19.VII.2000	MT	#41	A	LT715965	LT715924
♂	19.VII.2000	MT	#42	A	LT715966	LT715925
♀	19.VII.2000	MT	#10	A	LT715967	LT715926
♀	19.VII.2000	MT	#11	A	LT715968	LT715927
♀	19.VII.2000	MT	#12	A	LT715969	LT715928
♀	19.VII.2000	MT	#13	A	LT715970	—
♂	26.VII.2000	MT	#50	—	—	—
♀	26.VII.2000	MT	#17	A	LT715971	—
♂	16.VIII.2000	MT	#38	A	LT715972	LT715929
♂	16.VIII.2000	MT	#39	A	LT715973	LT715930
♀	6.IX.2000	MT	#18	A	LT715974	LT715931
Spain, Canary Islands, La Palma, Roque de los Muchachos.						
♂	27.VII.2000	YT	#62	—	—	—
♀	25.VIII.2000	MT	#27	A	LT715975	LT715932
Spain, Canary Islands, La Palma, south of Barlovento, laurisilva, 28°39'N 17°52'W, 800 m, leg. et coll. C. Kehlmaier						
♂	29.X.2002	net	DNA CK145	—	—	—
♂	29.X.2002	net	DNA CK146	—	—	—
♂	29.X.2002	net	DNA CK417	B	LT715976	LT715933
♀	29.X.2002	net		—	—	—
Portugal, Madeira, path between Boca de Encumeada and Boca dos Corgos, S of Ribeiro do Póco, 1 km W of Fenda do Ferreiro, moist steep slope, 1,200–1,250 m, X572, leg. W. Barkemeyer, coll. SDEI						
♂	30.V.1987		M1	C	LT715977	LT715934
Portugal, Madeira, Fanal, laurel on pasture, 12.IX.1986, L1583, leg. P. Ohm, coll. SDEI						
♂	12.IX.1986		M2	C	LT715978	LT715935
♂	12.IX.1986		M3	C	LT715979	LT715936
♂	12.IX.1986		M4	C	LT715980	LT715937
Portugal, Madeira, Pico Facho, near Machico, herbaceous vegetation, 300 m, L1578, leg. P. Ohm, coll. SDEI						
♂	19.IX.1986		M5	C	LT715981	LT715938