



Redescription of *Clubiona blesti* Forster, 1979 (Araneae: Clubionidae) with a preliminary molecular phylogeny of New Zealand *Clubiona*

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Abstract

The New Zealand spider *Clubiona blesti* Forster, 1979 is redescribed, with the male described for the first time, and a preliminary molecular phylogenetic analysis of cytochrome *c* oxidase subunit I (COI) mtDNA sequences for eight species of New Zealand *Clubiona* and an outgroup from Tasmania is presented.

There is considerable intraspecific variation in *C. blesti*, both genetic and in the morphology of copulatory organs, which may be explained by its wide distribution. The lack of a geographic structure based on consistent differences between populations may suggest great dispersal ability. Given the limited sample size, further sampling and data on additional genetic markers will be necessary to confirm this.

The phylogenetic analysis of seven more species indicated that *Clubiona cambridgei* is the sister species of *C. blesti* and confirmed the existence of at least two monophyletic groups among the New Zealand *Clubiona*: species with a striped abdomen and with a spotted abdomen.

Key words: Taxonomy, spiders, morphological variation, genetic divergence, COI

Introduction

New Zealand is classified as a biodiversity hotspot (Myers *et al.* 2000) with high levels of endemism in most studied taxa (Kuschel 1975). Spiders are no exception and it is estimated that 93% of all species in the country are endemic (Paquin *et al.* 2010). A large percentage of these species remain undescribed, which hinders morphological identification or classification of specimens in biodiversity studies (Malumbres-Olarte *et al.* in press). More than half of the New Zealand spider species known to date were described by R.R. Forster (Patrick *et al.* 2000). Despite this enormous contribution, a considerable number of species were described from only one sex, and in certain cases, split into separate species due to morphological mismatches (e.g. Vink *et al.* 2011). Genetic information has proved invaluable for correcting such mistakes, as well as for describing new species and studying their phylogenetic relationships (e.g. Vink 2002; Vink & Dupérré 2010; Vink *et al.* 2011).

The genus *Clubiona* Latreille, 1804 has a worldwide distribution, with 461 described species (Platnick 2011) known from all continents except Antarctica. It is the only representative of the family Clubionidae in New Zealand, where it is represented by 14 endemic species, of which two have been described from only female specimens, *Clubiona blesti* Forster, 1979 and *Clubiona torta* Forster, 1979 (Forster 1979; Paquin *et al.* 2010). It is common in native ecosystems, such as forests and tussock grasslands, however, there has been no phylogenetic analysis of the New Zealand species of *Clubiona* since the systematic revision by Forster (1979).

This paper redescribes *C. blesti*, with the first description of the male, and presents the first preliminary molecular phylogeny of New Zealand *Clubiona* species based on the genetic marker cytochrome *c* oxidase subunit I (COI).

Materials and methods

A total of 28 specimens belonging to nine morphologically identified *Clubiona* species were examined in this study (see Table 1); eight New Zealand *Clubiona* species and the Australian species *Clubiona elaphines* Urquhart, 1893. All specimens were preserved in 70% or 95% ethanol and obtained on loan from the Lincoln University Entomology Research Museum (LUNZ), the Museum of New Zealand Te Papa Tongarewa (MONZ) and the Auckland Museum (AMNZ). Most specimens had been collected between 2006 and 2011 and stored at -20°C, which allowed for DNA extraction and amplification (see Vink *et al.* 2005).

Measurements of morphological characters of *C. blesti* were recorded in millimetres, using an ocular micrometer fitted to a Nikon SMZ1500 11.25x stereomicroscope. Five leg measurements were presented in the format: total length (femur, patella, tibia, metatarsus, tarsus). Nine measurements were taken for the eyes: AME (anterior median eyes), ALE (anterior lateral eyes), PME (posterior median eyes), PLE (posterior lateral eyes), and five measurements of distances between them (AME-AME, AME-ALE, ALE-ALE, PME-PME, PME-PLE). Leg spination was not given as it varied between specimens.

Photographs were taken with a QIMAGING MicroPublisher 5.0 RTV Color Digital Camera and Automontage (Synchrosopy). Epigyna and pedipalps were dissected using the end of a syringe needle, cleared with lactic acid, and illustrated using images obtained combining photographs with Automontage to trace and establish correct proportions. The locality map was produced with ArcMap 10.0 (ESRI Inc.).

TABLE 1. Specimens examined

Specimen code	Collection code (LUNZ/MONZ/AMNZ)	Sex	Species	Location, date and collector	GenBank accession number
BLOL1	LUNZ00012640	female	<i>Clubiona blesti</i>	OL, Temple Peak Station (44°47.2'S 168°27.8'E), 27.II.2003, CJV	Not sequenced
BLMC1	LUNZ00012641	female	<i>Clubiona blesti</i>	MC, Ellangowan Scenic Reserve (43°47.9'S 173°01.6'E), 12.XII.2006, JM-O	JN377951
BLMC2	LUNZ00012642	female	<i>Clubiona blesti</i>	MC, Ellangowan Scenic Reserve (43°47.9'S 173°01.6'E), 20.II.2007, JM-O	JN377952
BLCO1	LUNZ00012643	female	<i>Clubiona blesti</i>	CO, Te Papanui Conservation Park (45°39.9'S 169°47'E), 18.I.2008, JM-O	JN377953
BLCO2	LUNZ00012644	female	<i>Clubiona blesti</i>	CO, Te Papanui Conservation Park (45°39.9'S 169°47.1'E), 20.II.2008, JM-O	JN377954
BLCO3	LUNZ00012645	female	<i>Clubiona blesti</i>	CO, Te Papanui Conservation Park (45°40'S 169°46.6'E), 4.III.2008, JM-O	JN377955
BLCO4	LUNZ00012646	female	<i>Clubiona blesti</i>	CO, Te Papanui Conservation Park (45°40.8'S 169°45.3'E), 2.III.2008, JM-O	JN377956
BLCO5	LUNZ00012647	female	<i>Clubiona blesti</i>	CO, Te Papanui Conservation Park (45°40'S 169°46.6'E), 14.XII.2008, JM-O	JN377957
BLCO6	LUNZ00012648	male	<i>Clubiona blesti</i>	CO, Te Papanui Conservation Park (45°40.8'S 169°45.3'E), 2.III.2008, JM-O	JN377958
BLCO7	LUNZ00012649	female	<i>Clubiona blesti</i>	CO, Te Papanui Conservation Park (45°40.8'S 169°45.3'E), 21.XII.2007, JM-O	JN377959
BLDN1	LUNZ00012709	female	<i>Clubiona blesti</i>	DN, Swampy Spur (45°48.08'S 170°29.40'E), 20.X.2012, MW	Not sequenced
CMMC1	LUNZ00012651	female	<i>Clubiona cambridgei</i>	MC, Spencer Park (43°25.8'S 172°42.4'E), 2.IV.2011, CJV	JN377962
CMMC2	LUNZ00012658	male	<i>Clubiona cambridgei</i>	MC, Travis Wetland (43°29.1'S 172°41.4'E), 16.XII.1995, RPM	Not sequenced
CMMC3	LUNZ00012658	female	<i>Clubiona cambridgei</i>	MC, Travis Wetland (43°29.1'S 172°41.4'E), 16.XII.1995, RPM	Not sequenced

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TABLE 1 (continued)

Specimen code	Collection code (LUNZ/MONZ/AMNZ)	Sex	Species	Location, date and collector	GenBank accession number
CMWA1	LUNZ00012661	male	<i>Clubiona cambridgei</i>	WA, Lake Wairarapa (41°12.9'S 175°11.4'E), 2.V.2011, JM-O	JN377967
CMWA2	LUNZ00012662	male	<i>Clubiona cambridgei</i>	WA, Lake Wairarapa (41°12.9'S 175°11.4'E), 2.V.2011, JM-O	JN377968
CMWA3	LUNZ00012660	female	<i>Clubiona cambridgei</i>	WA, Lake Wairarapa (41°12.9'S 175°11.4'E), 2.V.2011, JM-O	JN377969
CLTK1	LUNZ00012652	male	<i>Clubiona clima</i>	TK, Bell Block (39°01.2'S 174°08.7'E), 10.VIII.2010, CJV and SJC	JN377961
CLWA1	LUNZ00012653	male	<i>Clubiona clima</i>	WA, Lake Wairarapa (41°12.9'S 175°11.4'E), 2.V.2011, JM-O	JN377966
CAMC1	LUNZ00012650	male	<i>Clubiona cada</i>	MC, Somerfield (43°33.8'S 172°37.7'E), 23.V.2011, LJHV and CJV	JN377960
CDKE1	AMNZ	male	<i>Clubiona cada</i>	KE, Raoul Island (29°16.04'S, 177°54.47'W), 14.V.2011, WGC	JQ347511
CVWA1	LUNZ00012655	female	<i>Clubiona convoluta</i>	WA, Mangareia Rd. (40°50.6'S 175°50.3'E), 29.IV.2011, JM-O	JN377972
CNWN1	LUNZ00012654	male	<i>Clubiona consensa</i>	WN, Akatarawa Forest (41°3.5'S 175°5.4'E), 22.IV.2011, JM-O	JN377970
CNND1	AS.001721	male	<i>Clubiona consensa</i>	ND, Matthew Reserve (35°09.3'S, 173°17.5'E), 23.III.2010, PJS and BMF	JN377965
PECH1	LUNZ00012707	male	<i>Clubiona peculiaris</i>	CH, Kaingaroa (43°43.8288'S, 176°16.2156'W), 22.XI.2011, IANS	JQ347512
HUSD1	LUNZ00012656	female	<i>Clubiona huttoni</i>	SD, Deep Bay (41°04.2'S 173°46.5'E), 3.X.2010, JMK	JN377964
HUMC1	LUNZ00012657	female	<i>Clubiona huttoni</i>	MC, Somerfield (43°33.8'S 172°37.7'E), 14.VIII.2010, CJV	JN377963
ELTA	LUNZ00012708	male	<i>Clubiona elaphines</i>	Tasmania, Travellers Rest (41°29.783'S, 147°05.283'E), 01.XI.2011, JD	JQ347510

Collection codes: LUNZ=Lincoln University Entomology Research Museum, AS=Museum of New Zealand Te Papa Tongarewa (MONZ), AMNZ=Auckland Museum.

Abbreviations of collectors: BMF=B.M. Fitzgerald, IANS=I.A.N. Stringer, JD=J. Douglas, JMK=J.M. Kean, RPM=R.P. Macfarlane, LJHV=L.J. Hudson Vink, PJS=P.J. Sirvid, SJC=S.J. Crampton, WGC=W.G. Chinn, MW=M. Wakelin.

The two-letter location codes follow Crosby *et al.* (1998).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from two legs per specimen. A ZM Genomic DNA II Kit (Zymo Research) was used for specimens BLMC1, BLMC2, BLCO1-BLCO7. DNA was extracted non-destructively (Paquin & Vink 2009) from the remaining specimens using a ZR Genomic DNA™-Tissue MiniPrep kit (Zymo Research). A fragment of over 1000 base pairs (bp) was amplified from cytochrome *c* oxidase subunit I (COI) by polymerase chain reaction (PCR). The target fragment was selected because of its previous use in molecular phylogenetic and systematic studies (e.g. Hedin & Maddison 2001; Vink *et al.* 2008, 2009; Framenau *et al.* 2010) and as a DNA barcoding region (e.g. Robinson *et al.* 2009). Initially, specimens were amplified with the primers LCO-1490 (5'-GGTCAACAAATCATCATAAAGATATTGG-3') (Folmer *et al.* 1994) and C1-N-2776-spider (5'-GGATAATCAGAATANCGNCGAGG-3') (Vink *et al.* 2005). However, this primer combination did not work for all specimens and the primers LCO-1490 and C1-N-2568 (5'-GCTACAACATAATAAGTATCATG-3') (Hedin & Maddison 2001) were used for the remaining specimens. PCR amplification was carried out using *i-Taq*™ DNA Polymerase (iNtRON Biotechnology) in a Mastercycler® (Eppendorf) thermocycler with a cycling profile of 35

cycles of 94°C denaturation (30 s), 48°C annealing (30 s), 72°C extension (1 min) with initial denaturation of 3 min and final extension of 5 min. Excess primers and salts were removed from PCR products with a DNA Clean & Concentrator™-5 Kit (Zymo Research). Purified PCR products were sequenced in both directions at either the Bio-Protection Centre (Lincoln University, New Zealand) or Macrogen Corporation (Seoul, Korea). The forward and reverse sequences were assembled, compared and edited with Sequencher™ 4.0 (Gene Codes Corporation). No insertions, deletions or stop codons were found, suggesting no amplification of pseudogenes.

Phylogenetic analyses

A total of 24 sequences from eight New Zealand *Clubiona* species (*C. blesti*; *C. cada* Forster, 1979; *C. cambridgei* L. Koch, 1873; *C. clima* Forster, 1979; *C. consensa* Forster, 1979; *C. convoluta* Forster, 1979; *C. huttoni* Forster, 1979; *C. peculiaris* L. Koch, 1873) were analysed. The COI sequence of a specimen of the Tasmanian species *C. elaphines* was used as the outgroup, as there are similarities in its morphology to New Zealand *Clubiona* species. A matrix of uncorrected *p* distances of the 25 sequences was computed; we used the Kimura-2-Parameter (K2P) model (Kimura 1980), which has been used as the standard for constructing genetic distance matrices, only for comparison with previously found intraspecific distances, as there is no evidence that this model is better than simpler metrics such as *p* distance (Collins *et al.* in press). Mean, minimum and maximum values of intra- and interspecific distances between the New Zealand *Clubiona* sequences were then calculated and presented as box plots as they facilitate the interpretation of possible “barcoding gaps” between species (Astrin *et al.* 2006). Matrices and trees were computed using the software R version 2.13.0. (R Development Core Team 2011).

Molecular phylogenetic trees were reconstructed using Bayesian and maximum likelihood approaches. First, the best fitting model for each codon position was selected with jModeltest (Posada 2008) using the corrected Akaike Information Criterion (AICc) (Posada & Buckley 2004). A partitioned Bayesian analysis (Brandley *et al.* 2005) was implemented in MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003), using the models F81 (Felsenstein 1981) for the first codon, TIM3+ γ (Yang 1994; Posada 2003) for the second codon and TIM2+ γ (Yang 1994; Posada 2003) for the third codon positions. Bayesian analyses were conducted by running two simultaneous, completely independent analyses, each with four heated chains, sampling every 1000th tree. The analyses were run for at least 10 million generations, by which time the average standard deviation of split frequencies had dropped below 0.005, which indicated that the two tree samples had converged. MrBayes was used to construct majority rule consensus trees, discarding the first 25% of trees generated as burn-in. For the maximum likelihood analysis, a model average was obtained through a decision theory (DT) framework for the entire set of sequences with jModeltest and the parameters were included in PhyML v3.0 (Guindon & Gascuel 2003), with bootstrap analysis of 1000 replicates. All trees were viewed and edited with FigTree version 1.3.1 (Rambaut 2009).

Results

Taxonomy

Clubionidae Wagner, 1887

Clubiona Latreille, 1804

Clubiona Latreille, 1804: 134. Type species: *Araneus pallidulus* Clerck, 1757, by subsequent designation.

Clubiona blesti Forster, 1979

Figs 1–3, 4a, 5–6, 7c–f

Clubiona blesti Forster, 1979: 78, fig. 309 (description of female).

Type material. NEW ZEALAND: Fiordland: holotype female (not examined), Lyttles Flat, in wet tussock in bog, 17 February 1975, A.D. Blest (Otago Museum).

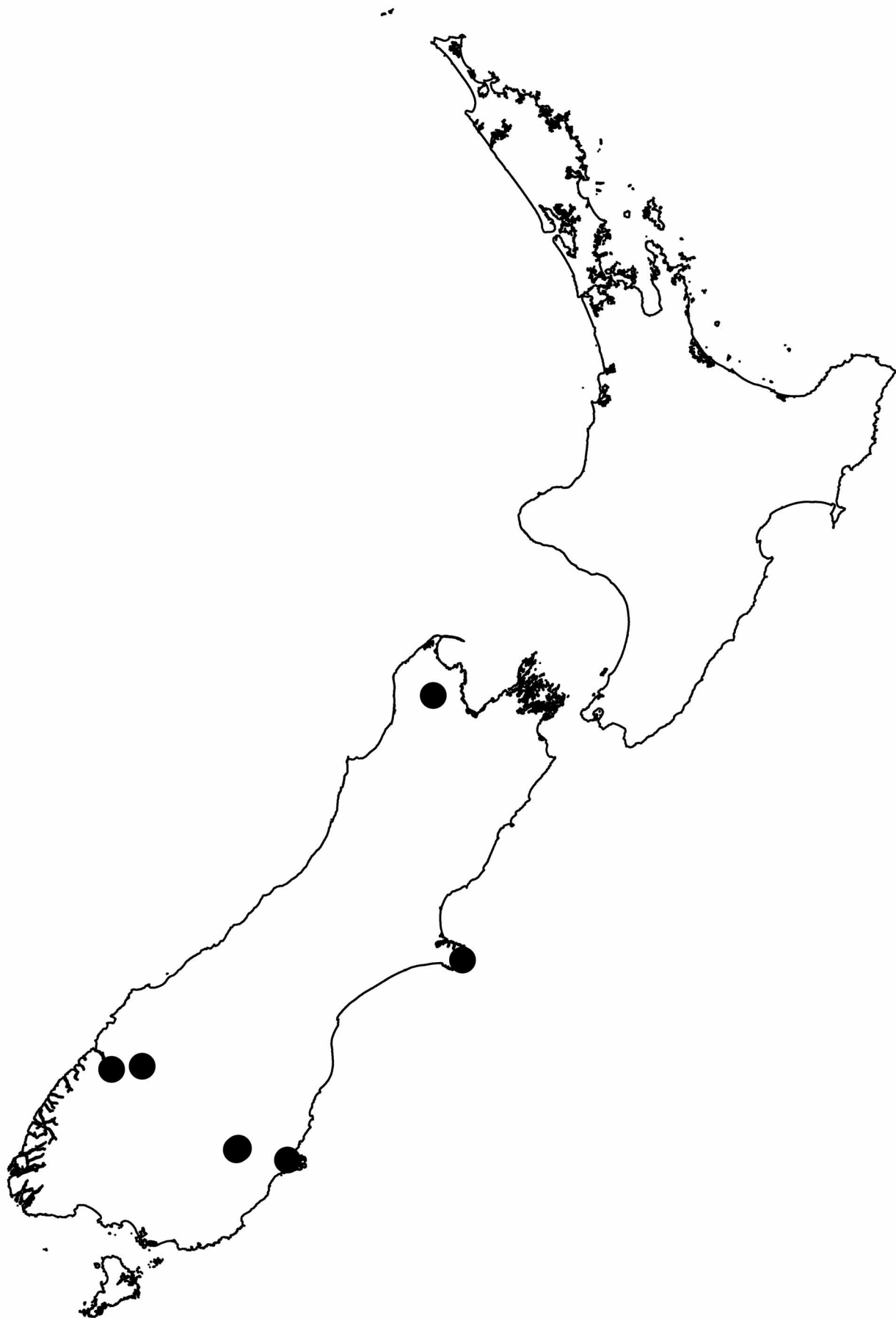


FIGURE 1. Map of collection localities for *Clubiona blesti*.

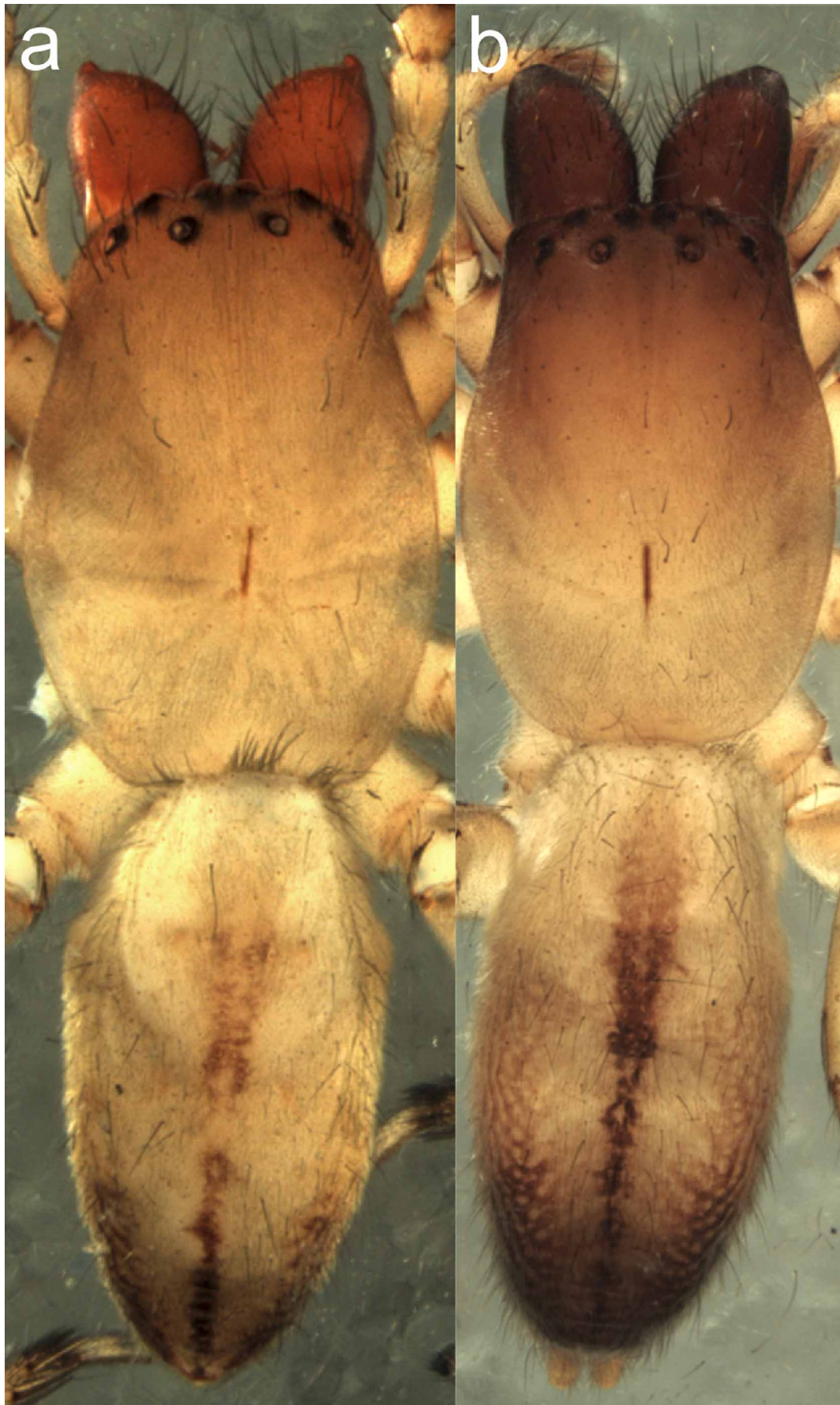


FIGURE 2. *Clubiona blesti*. a male habitus (BLCO6), b female habitus (BLCO2).

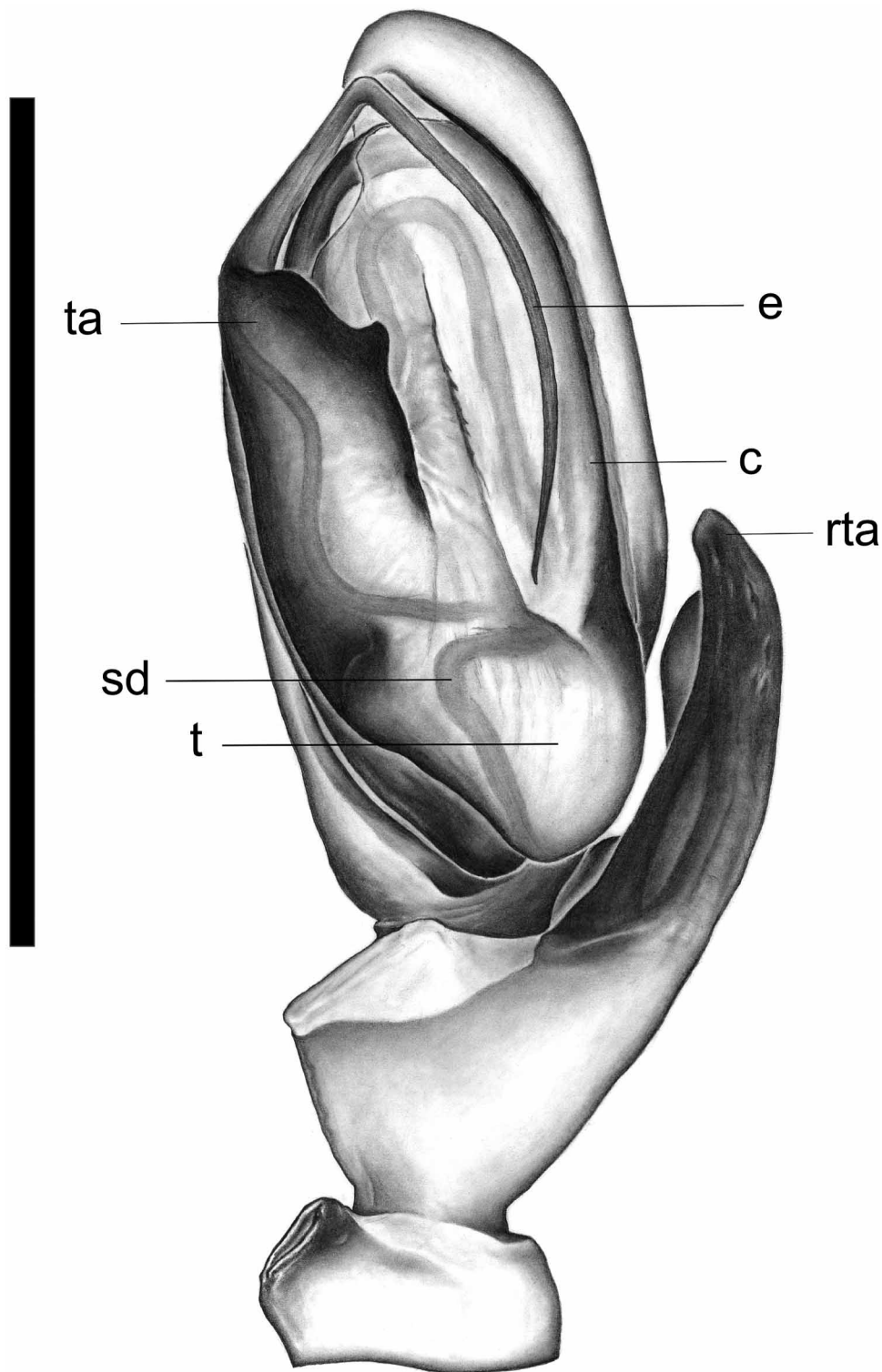


FIGURE 3. *Clubiona blesti*, left pedipalp. c—conductor, e—embolus, rta—retrolateral tibial apophysis, sd—sperm duct, t—tegulum, ta—tegular apophysis. Scale bars = 0.5 mm.

Other material examined. NEW ZEALAND: Otago Lakes: Temple Peak 44°47.158'S 168°27.804'E, 27 February 2003, C.J. Vink, 1 female (LUNZ00012640). **Mid Canterbury:** Ellangowan Scenic Reserve 43°47.9'S 173°01.6'E, 12 December 2006, J. Malumbres-Olarte, 1 female (LUNZ00012641); same data except 20 February 2007, 1 female (LUNZ00012642). **Central Otago:** Te Papanui Conservation Park 45°40.8'S 169°45.3'E, 2 March 2008, J. Malumbres-Olarte, 1 male (LUNZ00012648); same data, 1 female (LUNZ00012646); same data except 21

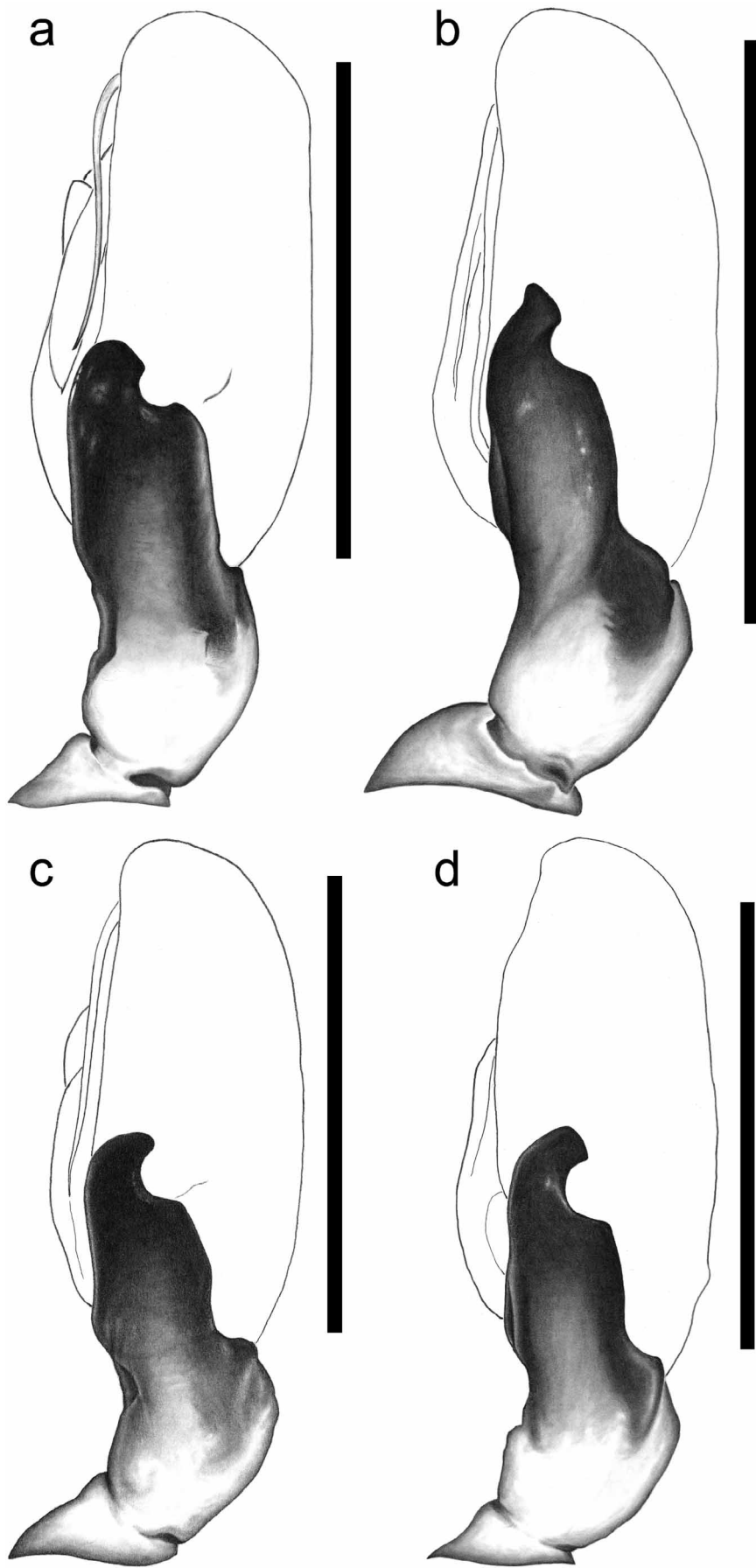


FIGURE 4. Left pedipalps, retrolateral view. a *Clubiona blesti*; b *Clubiona cambridgei*, Travis Wetland (CMMC2); c *Clubiona cambridgei*, Lake Wairarapa (CMWA2); d *Clubiona cambridgei*, Lake Wairarapa (CMWA1).

December 2007, 1 female (LUNZ00012649); same data except 45°39.9'S 169°47'E, 18 January 2008, 1 female (LUNZ00012643); same data except 20 February 2008, 1 female (LUNZ00012644); same data except 45°40'S 169°46.6'E, 4 March 2008, 1 female (LUNZ00012645); same data except 14 December 2008, 1 female (LUNZ00012647). **Dunedin:** Swampy Spur 45°48.08'S 170°29.40'E, 20 October 2011, M. Wakelin, 1 female (LUNZ00012709).

Other locality records. NEW ZEALAND: Nelson: Lake Sylvester, Cobb, 1300 m, tussock, 30 April 1969, J.S. Dugdale (Otago Museum, not examined).

Diagnosis. *Clubiona blesti* is distinguished from other New Zealand *Clubiona* species by an abdominal pattern that consists of only a medium band that extends along the entire length of the dorsal surface (Fig. 2). This band is either absent, partial or accompanied by lateral stripes in other New Zealand *Clubiona* species (see Forster 1979: figs 286–296). Males can be identified by the apically notched retrolateral tibial apophysis, which is broader than in *C. cambridgei* (Fig. 4a–d and see Forster 1979: figs 299–300). Females can be recognised by the oval shape of the copulatory opening, which is constricted somewhat at the anterior end (Figs 5, 6, 7).

Description. Male (LUNZ00012648): Carapace length 3.2, width 2.2, height 1.7; abdomen length 3.7, width 1.8; sternum length 1.7, width 1. AME 0.17, ALE 0.19, PME 0.13, PLE 0.15, AME–AME 0.09, AME–ALE 0.2, ALE–ALE 0.87, PME–PME 0.46, PME–PLE 0.33. Leg I 8.3 (2, 1.5, 2.3, 1.7, 0.8); leg II 8.2 (2.1, 1.5, 2.1, 1.7, 0.8); leg III 6.2 (1.7, 1, 1.3, 1.6, 0.6), leg IV 8.7 (2.2, 1.3, 1.9, 2.5, 0.8).

Chelicerae with five promarginal teeth in a row and no retromarginal teeth.

Pedipalp as in diagnosis. Embolus arising from tegular apophysis, bent 90 degrees ventrally, extending almost to tegulum. Sperm duct with horizontal U-turn visible through tegulum. Tegular apophysis with nob-like projection (Fig. 3).

Colour in ethanol (Fig. 2a). Carapace and sternum yellowish-brown, darker in cephalic area. Abdomen pale grey-brown with dorsal dark-brown medium folium. Legs pale yellow-brown.

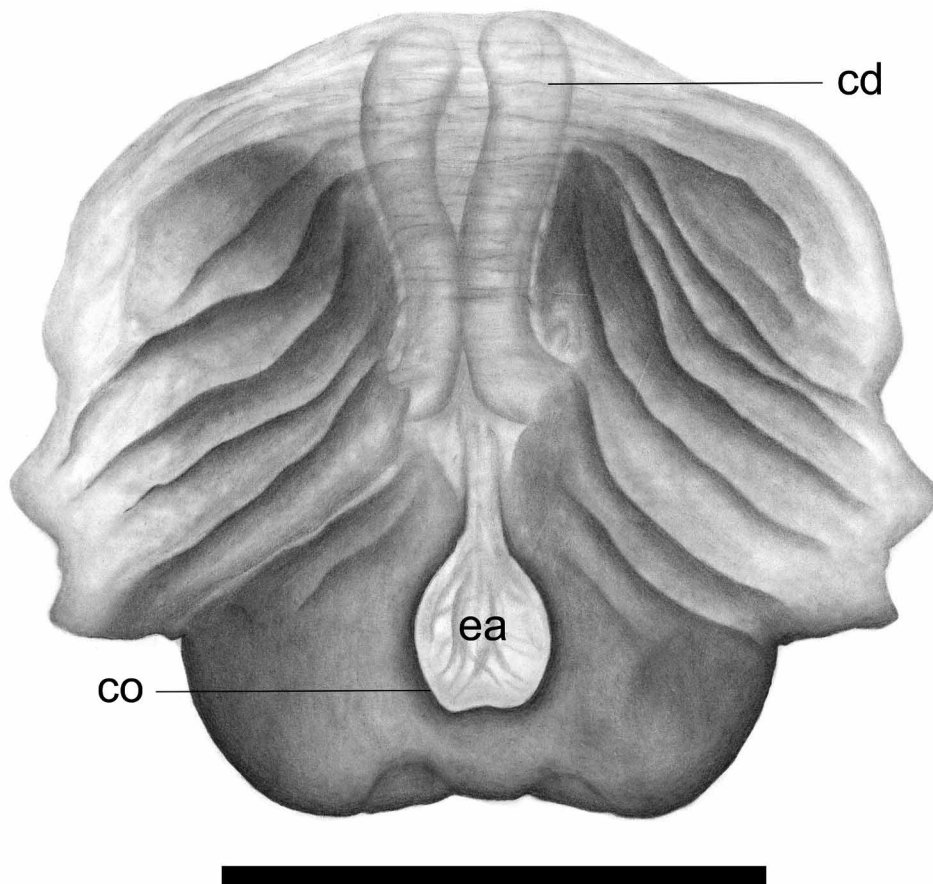


FIGURE 5. *Clubiona blesti*, epigynum, ventral, not cleared. cd—copulatory duct, co—copulatory opening, ea—epigynal atrium. Scale bar = 1.0 mm.

Female (LUNZ00012640): Carapace length 3.9, width 2.9, height 1.8; abdomen length 5.8, width 3; sternum length 2.3, width 1.3. AME 0.2, ALE 0.23, PME 0.13, PLE 0.15, AME-AME 0.16, AME-ALE 0.25, ALE-ALE 1.09, PME-PME 0.63, PME-PLE 0.47. Leg I 9.4 (2.5, 1.7, 2.5, 1.8, 0.9); leg II 9.4 (9.4, 2.5, 1.7, 2.5, 1.8, 0.9); leg III 7.3 (2.1, 1.3, 1.6, 1.7, 0.6), leg IV 10.8 (2.9, 1.5, 2.6, 3, 0.8, 10.8). Size range: carapace length 2.2–4.8 (mean 3.5, n=10), body length 6.0–10.2 (mean 8.1, n=10).

Five promarginal cheliceral teeth, with the second one the largest and the distal one very small, and two or three retromarginal teeth.

Copulatory opening as in diagnosis. Epigynum strongly sclerotised and laterally flat, with rugose ventral surface; posterior margin with a pair of lobes in all examined specimens except for specimen LUNZ00012642, from Central Canterbury, which had a flat margin. Single copulatory opening with slight septum at posterior edge that leads to anteriorly visible copulatory ducts. Copulatory ducts bent vertically and directed ventrally, and connected to spermathecae. Spermathecae separated into two parts with fertilisation ducts arising from ventral side (Figs 5, 6, 7c–f).

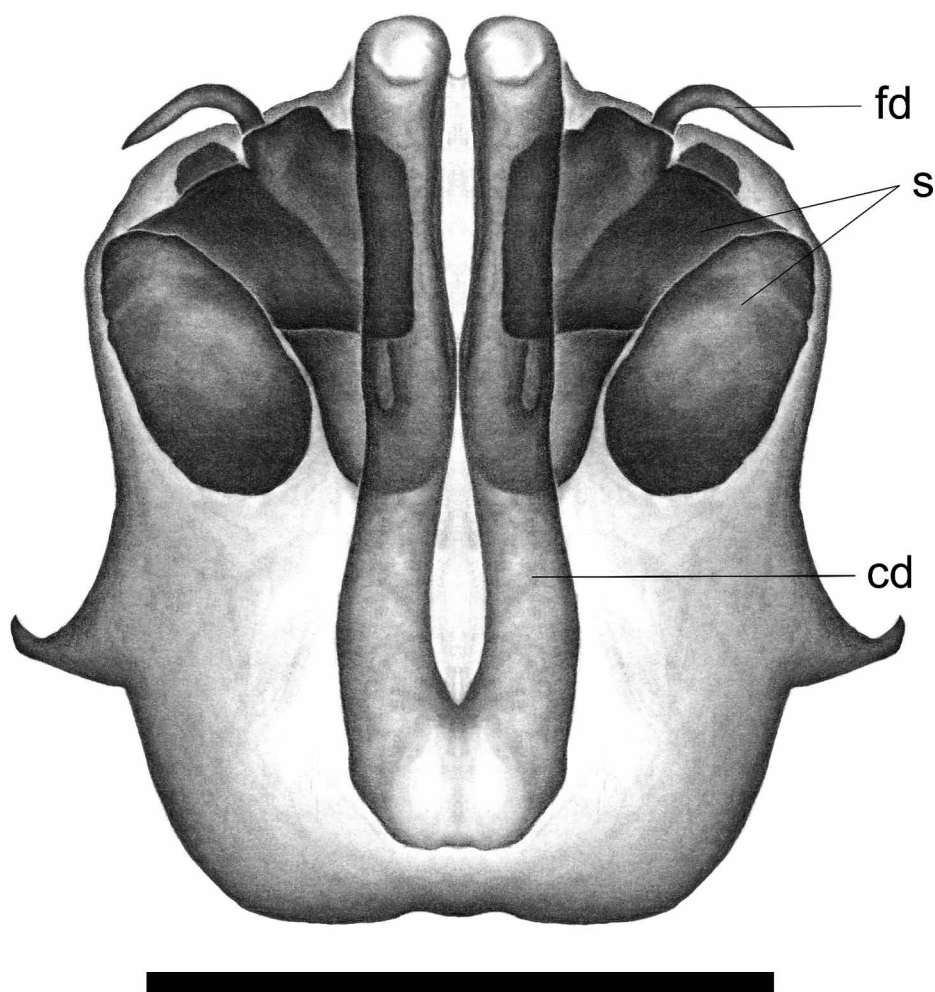


FIGURE 6. *Clubiona blesti*, epigynum, dorsal view, cleared. cd—copulatory duct, fd—fertilisation duct, s—spermatheca. Scale bar = 1.0 mm.

Colour in ethanol (Fig. 2b). As for male.

Biology. Forster (1979) suggested that *C. blesti* is an active hunter associated with tussocks. The examined specimens were collected from the leaves and bases of *Chionochloa rigida* (Raoul) Zotov, an alpine tussock grass species.

Distribution. New Zealand South Island (Fiordland, Dunedin, Central Otago, Otago Lakes, Mid Canterbury, Nelson) (Fig. 1).

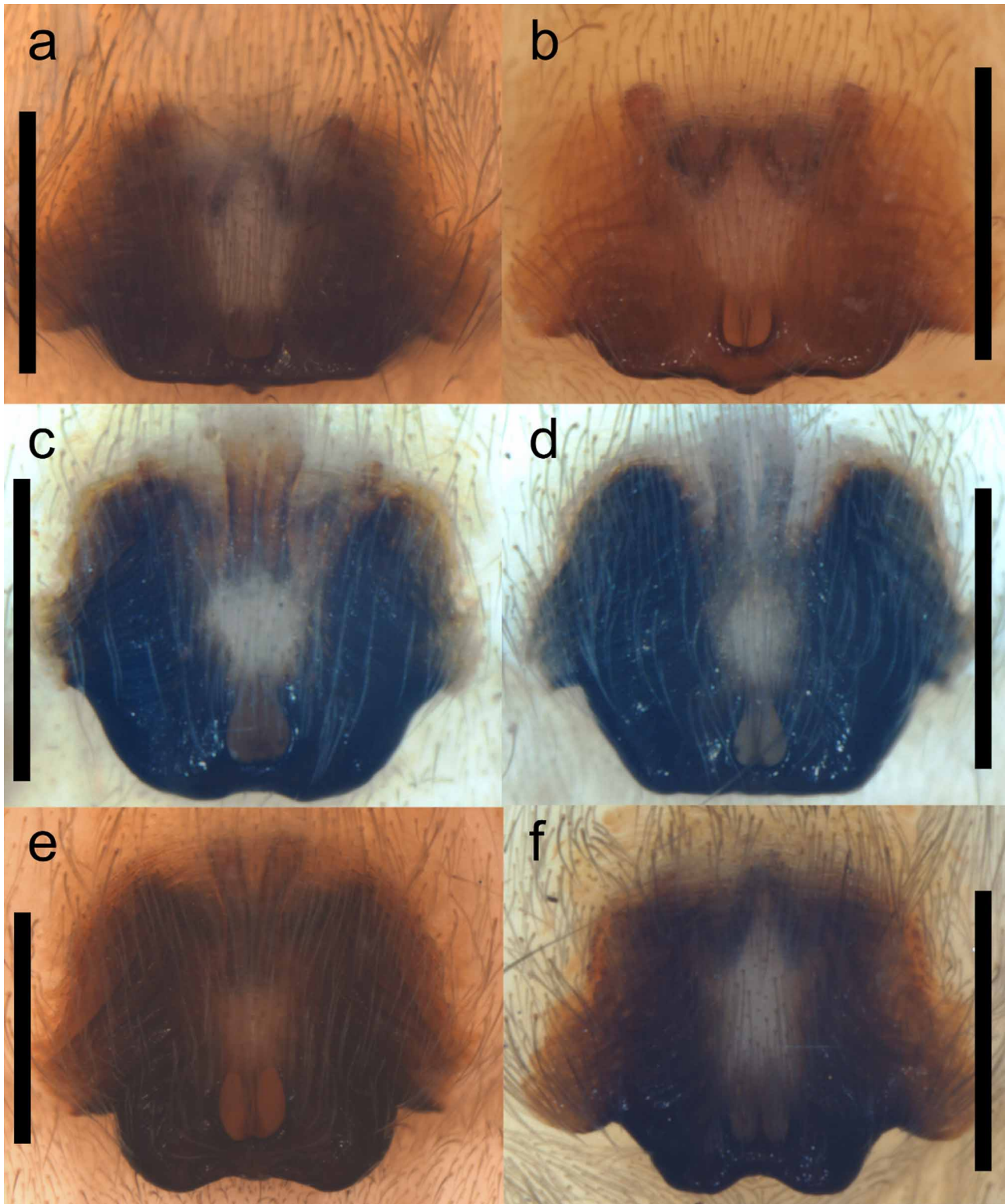


FIGURE 7. Epigyna, ventral view. *Clubiona cambridgei*; a Lake Wairarapa, WA (CMWA3); b Travis Wetland, MC (CMMC3). *Clubiona blesti*; c Ellangowan Scenic Res., MC (BLMC1); d Ellangowan Scenic Reserve, MC (BLMC2); e Temple Peak, OL (BLOL1); f Te Papanui Conservation Park, CO (BLC07). Scale bar = 1.0 mm.

Genetic and phylogenetic analyses

Genetic diversity. Mitochondrial COI (GenBank accession numbers JN377951–JN377972, JQ347510–JQ347512). The intraspecific distance varied between 0–3.5%, with a mean of 2%, and the interspecific distance with respect to other New Zealand *Clubiona* was between 3.6%–10.5%, with a mean of 7.9% (Fig. 8, Table 2).

All COI sequences included in the matrix were 1006 bp long, except for two shorter sequences from the specimens BLCO3 *Clubiona blesti* (860 bp) and CNND1 *Clubiona consensa* (904 bp). The mean interspecific distance between the New Zealand *Clubiona* was 8.5%, with minimum and maximum values of 3.6% and 12.1%, respectively (Fig. 8, Table 2).

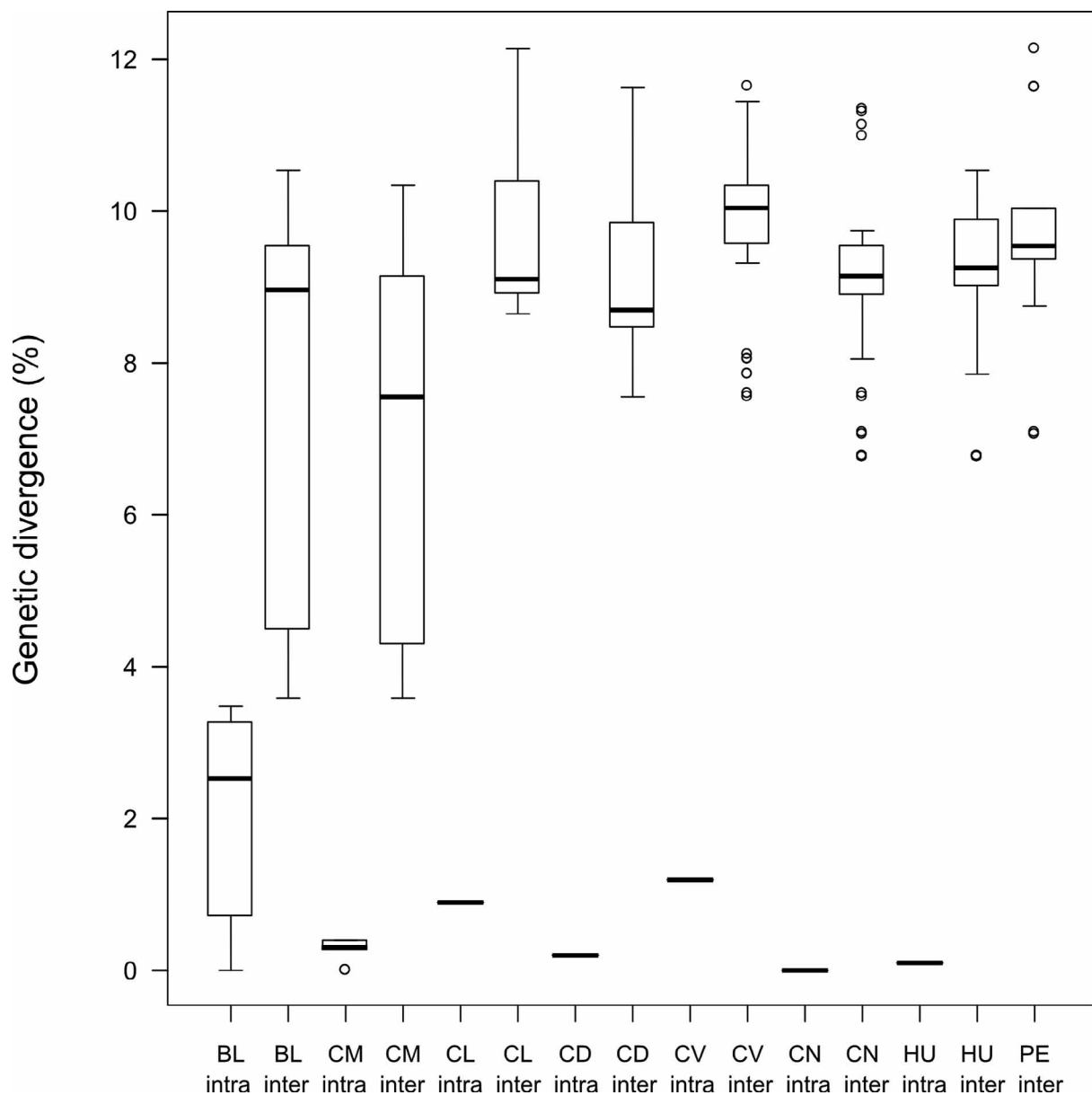


FIGURE 8. Intraspecific (intra) and interspecific (inter) divergence in COI sequences of the analysed New Zealand *Clubiona* species. BL *C. blesti*, CM *C. cambridgei*, CL *C. clima*, CD *C. cada*, CV *C. convoluta*, CN *C. consensa*, HU *C. hutoni*, PE *C. peculiaris*.

Phylogeny of eight New Zealand *Clubiona*. The Bayesian and maximum likelihood analyses resulted in trees with the same topology except for the node connecting the *C. cada* and *C. clima* specimens with the rest of the specimens (Fig. 9). This node was present only in the maximum likelihood tree and had 34% bootstrap support. *Clubiona cambridgei* was identified as the sister species of *C. blesti*.

According to both phylogenetic trees, all 24 COI sequences of New Zealand *Clubiona* specimens formed a monophyletic group, which was first divided into two groups and then further into four clades each. The resulting eight clades corresponded to eight morphologically identified species (Fig. 9). These clades had bootstrap support values above 90% in the maximum likelihood tree and were also highly supported (≥ 0.95) in the Bayesian tree with the only exception of the *C. blesti* clade, which had a posterior probability of 0.63.

TABLE 2. Intraspecific and interspecific genetic distances in cytochrome *c* oxidase subunit I for eight New Zealand *Clubiona* species.

Species	Intraspecific distance (%)			Interspecific distance (%)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
<i>Clubiona blesti</i>	2.0	0.0	3.5	7.9	3.6	10.5
<i>Clubiona cambridgei</i>	0.3	0.0	0.4	6.8	3.6	10.3
<i>Clubiona cada</i>	0.9	0.9	0.9	9.7	8.6	12.1
<i>Clubiona clima</i>	0.2	0.2	0.2	9.1	7.6	11.6
<i>Clubiona convoluta</i>	1.2	1.2	1.2	9.8	7.6	11.6
<i>Clubiona consensa</i>	0.0	0.0	0.0	9.0	6.8	11.3
<i>Clubiona huttoni</i>	0.1	0.1	0.1	9.2	6.8	10.5
<i>Clubiona peculiaris</i>	-	-	-	9.7	9.1	10.9

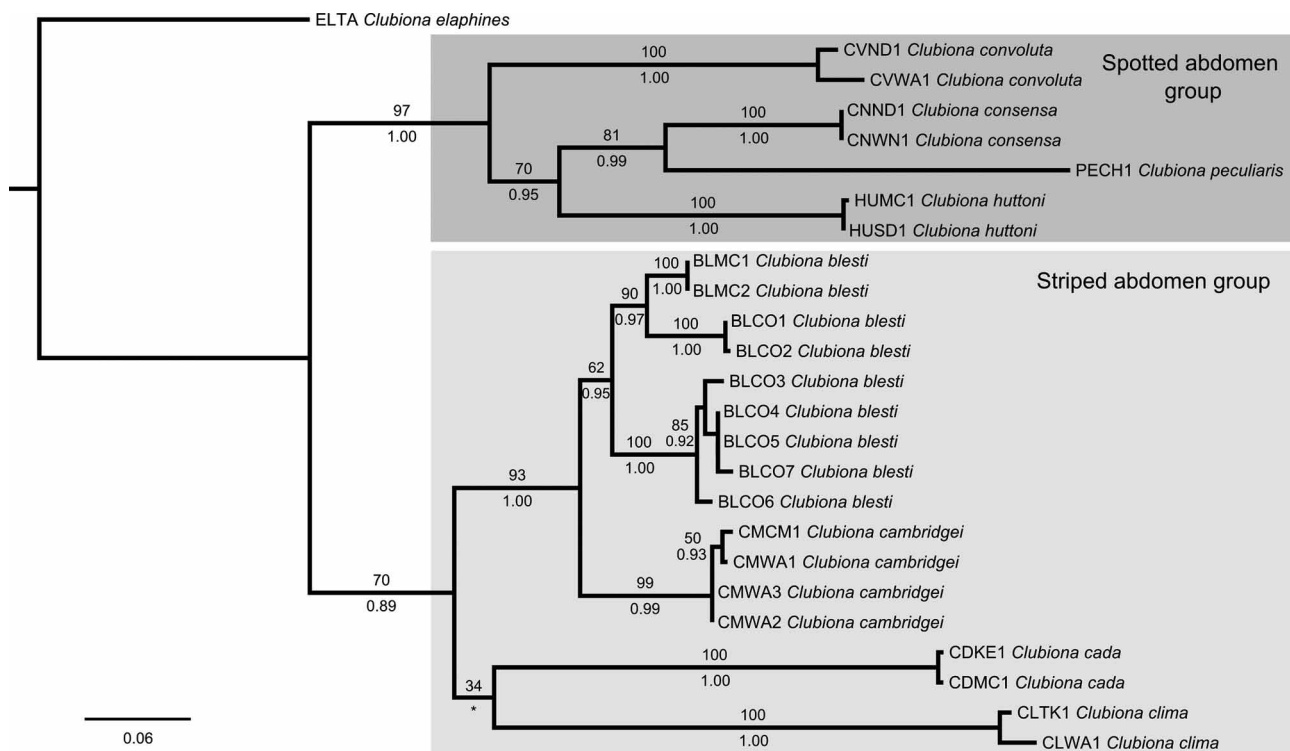


FIGURE 9. Maximum likelihood tree of COI sequences of seven *Clubiona* species. Values above and below the branches represent bootstrap values and posterior probabilities of the Bayesian consensus tree, respectively. Only values above 0.50 are shown, except for the node that was not present in the Bayesian tree, represented by an asterisk (bootstrap value of 0.34).

Discussion

There is considerable morphological and genetic diversity among the examined specimens of *C. blesti* (see Figs 7–8 and Table 2). This morphological diversity is exhibited by the female epigynum, which, along with the male pedipalp, is the most common reliable species diagnostic character for spiders (Coddington *et al.* 1991). Polymorphism in spiders has been described (e.g., Forster 1970; Reillo 1989; Gillespie & Tabashnik 1990; Oxford & Gillespie 1996; Jocque 2002) and there are reports of intraspecific variation in genital morphology (Huber & Astrin 2009; Costa-Schmidt & de Araujo 2010). This morphological (and also genetic) diversity can hinder identification and phylogenetic placement of species and is especially problematic when the variation in copulatory

organs is significant, as it is in some relatively widespread species (e.g. Wang & Qiao 2009), of which *C. blesti* is an example.

Although large, the maximum intraspecific divergence in COI among the examined *C. blesti* specimens (p distance = 3.5%, K2P distance = 3.6%) may not be extremely rare as it is similar to the average maximum K2P value of 3.16% found in the study by Robinson *et al.* (2009), where they used data on a relatively large number of individuals and species from other published studies. The relationship between individuals from different populations does not follow a consistent geographical structure. For instance, two of the specimens from Central Otago (BLCO1 *Clubiona blesti* and BLCO2 *Clubiona blesti*) are more closely placed in the phylogenetic tree to the two specimens from Mid Canterbury (BLMC1 *Clubiona blesti* and BLMC2 *Clubiona blesti*) than to other specimens from the same locality in Central Otago.

High intraspecific diversity and complex phylogeographic patterns have been found in mtDNA of some New Zealand invertebrates (Trewick 2000; Boyer *et al.* 2007; Buckley *et al.* 2009; Pons *et al.* 2011). There is no evidence of such a trend in other widespread New Zealand endemic spider species (Vink *et al.* 2008; Vink & Dupérré 2010; Lattimore *et al.* 2011), and proving that this is the case of *C. blesti* will require the analyses of more specimens from distant locations across the South Island of New Zealand. Furthermore, unravelling the relationships between *C. blesti* specimens from different populations and testing biogeographic hypotheses that could explain them will also require the analysis of faster evolving gene regions. Nevertheless, the available data show that some distant specimens are genetically closer to each other than specimens from nearer locations, which can be explained by the good dispersal ability of *C. blesti*, perhaps, through ballooning.

The COI phylogenetic analyses presented here show that *C. cambridgei* is probably the sister species of *C. blesti* (Fig. 9); a relationship that is also supported by morphological similarities. Although misidentification of *C. blesti* and *C. cambridgei* is possible, especially given the diversity in copulatory organs, the sharper apical area of the retrolateral tibial apophysis of the male *C. cambridgei* (Fig. 4b-d) and the differences in the shape of the copulatory openings (Fig. 5) should be sufficient to distinguish them.

The phylogeny of the most common and widespread New Zealand *Clubiona* species presented here supports previous suggestions about the existence of two main species groups in the country: spotted abdomen species and striped abdomen species (Fig. 9). Differences in the dorsal abdominal patterns of New Zealand *Clubiona* were noted in illustrations by Forster (1979) (figs 286–296). The spotted abdomen species includes *C. blesti*, *C. cambridgei*, *C. cada* and *C. clima*, whereas in the striped abdomen group are *C. convoluta*, *C. consensa*, *C. peculiaris* and *C. huttoni*.

This is the first attempt of a phylogeny for all of the 14 New Zealand *Clubiona* species. Wider sampling and further genetic and morphological analyses will lead to a complete phylogeny and perhaps the confirmation of two monophyletic clades with distinct morphological characters.

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