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Revalidation and redescription of *Bungona illiesi* (Lugo-Ortiz & McCafferty) (Ephemeroptera: Baetidae) from Australia, based on mitochondrial and morphological evidence

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Abstract

Bungona illiesi (Lugo-Ortiz & McCafferty) n.comb. was described from a single specimen collected in northern Queensland, Australia, as a species of *Cloeodes* Traver. The distinguishing characteristics of a minute maxillary palp, the absence of spination on the mandibles between the mola and prostheca, narrow-elongate gills, and medially expanded labial palps were later found to occur in *Bungona narilla* Harker, and the two species were synonymized. Extensive collecting in northern Queensland yielded larvae and adults of *Bungona* that differ from *B. narilla* in all life stages. We believe our specimens are equivalent to *B. illiesi* because they were collected only a few kilometres from the type locality, at least one specimen had both minute and normally sized maxillary palps, nearly all specimens had medially expanded labial palps, and the dorsal abdominal colour pattern was similar. Sequences of a 657-bp fragment of the mitochondrial gene cytochrome oxidase I supported the recognition of two species and confirmed the association of larvae and adults of *B. illiesi*. Interspecific p-distances ranged from 18.8–19.4% and the maximum intraspecific divergence observed was 1.7%. Based on the combination of morphological, mitochondrial, and biogeographical data, we show *B. illiesi* should be recognised as a valid species distinct from *B. narilla*. Larvae of *B. illiesi* differ from those of *B. narilla* by having fewer robust setae on the femora and a small lateral tooth on the outer incisor of both mandibles. Male and female adults differ from those of *B. narilla* by having less extensive red colouration on the abdominal terga.

Key words: mayfly, Australia, mtDNA, cytochrome oxidase I, taxonomy

Introduction

The Australian Baetidae remain poorly known, with only 20 species described to date, although unpublished data indicate the occurrence of approximately 60 species. *Bungona* Harker was established for *B. narilla* Harker, 1957 and described from larvae, males, and female subimagos from near Sydney, New South Wales. Adults of *Bungona* are distinguished from those of other Australian baetids by the absence of hindwings, turbinate eyes of the male with parallel margins, double marginal intercalaries in the forewing, a distinctive colour pattern, and the lack of a dorsal projection on the metascutellum. The larvae are differentiated from those of all other Australian Baetidae by the presence of transverse rows of fine setae on abdominal sterna 4–6, an arc of very long, fine setae on the tibiae, and an oblique row of very long, fine setae on the tarsi.

Since its initial description, *Bungona* has been found to be widespread in eastern Australia from northern Queensland to Victoria, and one specimen is known from Tasmania (Suter and Pearson 2001). In their redescription of *Bungona*, Suter and Pearson (2001) synonymized two species that had been described in *Cloeodes* Traver by Lugo-Ortiz and McCafferty (1998) with *B. narilla*. *Cloeodes fustipalpus* Lugo-Ortiz & McCafferty, 1998 was described from a small series of larvae and exuviae from northern New South Wales, and *C. illiesi* Lugo-Ortiz & McCafferty, 1998 was based on a single specimen from near Cairns in northern Queensland.

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Suter and Pearson (2001) clearly showed that *C. fustipalpus* and *B. narilla* were conspecific based on morphological characters and biogeography. Because *C. illiesi* was described from a single specimen, it is difficult to ascertain whether it represents an aberrant specimen of *B. narilla* or is a distinct species. The main diagnostic characteristics for *C. illiesi* identified by Lugo-Ortiz and McCafferty (1998) include a minute second segment of the maxillary palp, the absence of minute spines between the mola and prostheca on the mandibles, bulbous labial palp segment 3, and narrow-elongate gills. As all of these characteristics were found to also occur in some specimens of *B. narilla*, Suter and Pearson (2001) placed *C. illiesi* as a junior synonym of *B. narilla*. The type locality of *C. illiesi*, however, is in northern Queensland north of the Burdekin Gap, a well known biogeographic division in aquatic insects (i.e. Watson and Theischinger 1984; Christidis and Dean 2008), and *C. illiesi* may be a distinct species from *B. narilla*.

In this paper, we show that all *Bungona* from northern Queensland have fixed morphological and mitochondrial character states and extensive mitochondrial sequence divergence from those occurring elsewhere in Australia. Based on morphological characters and proximity to the type locality, we conclude that the northern specimens are equivalent to *C. illiesi* and that it should be placed in *Bungona*. The larvae are redescribed and the adult stages described for the first time.

Methods

Larvae and adults of *Bungona* were collected from throughout its known range except Tasmania. All recently collected material was fixed and preserved in 95–100% ethanol, and some specimens were temporarily stored in 100% propylene glycol. Larvae were collected with kick nets, and adults were collected using black lights. Slides preparations were made using Euparal; prior to being placed in Euparal, parts were soaked briefly in cellosolve (ethylene glycol monoethyl ether) to remove any water.

Twelve specimens were used for molecular analyses; eight specimens were from two localities in northern Queensland and the remaining four specimens were from southeast Queensland, northern New South Wales and southern New South Wales. Total genomic DNA was extracted using a proteinase-K/Chelex solution. A whole specimen or a small amount of thoracic muscle was placed in 100µl of Chelex solution (containing 5% Chelex (weight/volume) , 0.2% SDS, 10mM Tris pH 8, 0.5mM EDTA) and 10µl of 20mg/mL proteinase-K, incubated overnight at 55°C, centrifuged for 5 minutes at 1500rpm and incubated for 5 min at 95°C to deactivate the proteinase-K. Prior to centrifuging, exuviae were removed and returned to alcohol or mounted on a microscope slide with Euparal and cellosolve. A portion of the DNA extraction was diluted 1 in 5 with 1X TE and used for subsequent analyses to reduce the number of freeze/thaw cycles on the original extraction. Undiluted extractions were stored at -80°C and diluted extractions were stored at -20°C.

A 658-bp fragment of the mitochondrial gene cytochrome oxidase I (COI) was amplified using the primers LCO149 and HCO2198 (Folmer *et al*., 1994); primers were M13-tailed to facilitate sequencing. PCR for COI reactions consisted of 3.5µl buffer, 17.5µl 10% w/v trehalose, 0.7µl dNTPs, 1.75µl 50mM $MgCl_2$, 0.7µl of each primer, 0.125µl taq polymerase (Invitrogen), 0.01-5µl of DNA template, and water to 35µl. PCR conditions consisted of 1 min at 94 °C; 5 cycles of 1 min at 94 °C, 1.5 min at 45 °C, 1.5 min at 72 °C; 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C; and 4 min at 72 °C.

PCR products were purified and sequenced in both directions by Macrogen Inc (Seoul, Korea). Contigs were assembled in DNABaser 2.75 (www.DnaBaser.com) and aligned with default settings in Clustal X as implemented in MEGA4 (Tamura *et al*. 2007). All sequences were examined for the presence of double peaks, frame shifts and stop codons. Prior to analysis the first base was removed so all sequences started on the first codon position. Sequences were submitted to GenBank under accession numbers HM017831–HM017842. Pairwise p-distances were calculated in MEGA4 and used to calculate mean, minimum, and maximum inter- and intraspecific divergence. A Neighbour-Joining (NJ) analysis was performed in MEGA4 using Kimura 2 Parameter distance. Maximum Parsimony (MP) analyses were performed in PAUP* 4.0b10 (Swofford 2002) with 10 random sequence addition replicates and tree-bisection-reconnection (TBR) branch

TABLE 1. Material examined

Species	State	Locality	Latitude	Longitude	Date	Elevation (m)	Collector(s)	Accession number	# Specimens	Lifestage	Gender	Storage Method	GenBank Accession #
B. illiesi	QLD	Emerald Creek, downstream of Emerald Creek Falls	-17.05	145.54	13 Oct, 2009		JM Webb, JH Hawking	JWA1952	3	Adult	1 Male 2 Females		
B. illiesi	QLD	Emerald Creek, downstream of Emerald Creek Falls	-17.05	145.54	5 Oct, 2009		JM Webb & JH Hawking	JWA2465	1	Subimago	female		HM017842
B. illiesi	QLD	Emerald Creek, downstream of Emerald Creek Falls	-17.05	145.54	5 Oct, 2009		JM Webb & JH Hawking	JWA1901	1	larvae			
B. illiesi	QLD	Emerald Creek, downstream of Emerald Creek Falls	-17.05	145.54	13 Oct, 2009		JM Webb, JH Hawking	JWA2082	1	larvae		whole specimen on slide	HM017839
B. illiesi	QLD	Emerald Creek, downstream of Emerald Creek Falls	-17.05	145.54	13 Oct, 2009		JM Webb, JH Hawking	JWA1928	3	larvae			
B. illiesi	QLD	Emerald Creek, downstream of Emerald Creek Falls	-17.05	145.54	5 Oct, 2009		JM Webb & JH Hawking	JWA2081	1	Adult	male		HM017838
B. illiesi	QLD	Emerald Creek, downstream of Emerald Creek Falls	-17.05	145.54	5 Oct, 2009		JM Webb & JH Hawking	JWA1836	4	1 Adult, 3 Subimagos	male	Parts of one on slide	
B. illiesi	QLD	Endeavour Falls, Endeavour R, at Endeavour Falls Caravan Park	-15.37	145.03	9 Oct, 2009		JM Webb & JH Hawking	JWA1835	1	Adult	female		
B. illiesi	QLD	Fishers Creek at Palmerston Highway	-17.57	145.9	25 Jun, 2008	80	JM Webb, JH Hawking, S Moore, P Suter	JWA729	3	larvae			
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	24 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA2083	1	larvae		whole specimen on slide	HM017840
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	24 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA2084	1	larvae		whole specimen on slide	HM017841
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	3 Jul, 2008	28	JM Webb, JH Hawking, S Moore	JWA711	1	Subimago	female		
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	26 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA1125	1	Subimago	female		HM017837
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	26 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA812	7	Subimago	2 Males, 5 Females		
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	26 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA650	5	Subimago	2 Males, 3 Females		
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	24 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA793	1	larvae		whole specimen on slide	HM017833
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	24 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA794	1	larvae			HM017834

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TABLE 1.(continued)

Species	State	Locality	Latitude	Longitude	Date	Elevation (m)	Collector(s)	Accession number	# Specimens	Lifestage	Gender	Storage Method	GenBank Accession #
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	24 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA584	13	larvae			
B. illiesi	QLD	Freshwater Creek by Crystal Cascades Caravan Park, off Redlynch Intake Road	-16.93	145.7	24 Jun, 2008	28	JM Webb, JH Hawking, S Moore, P Suter	JWA732	12	larvae			
B. illiesi	QLD	Koolmoon Creek Km4 Tully			29 Nov, 1990		No Collector Indicated	PS634	1	Larvae		whole specimen on slide	
B. illiesi	QLD	Koolmoon Creek Km4 Tully			29 Nov, 1990		No Collector Indicated	PS632	1	larvae		whole specimen on slide	
B. illiesi	QLD	Koolmoon Creek, Km3, Tully			31 Jul, 1990		S.Bunn & M.Gray	PS377	1	larvae		whole specimen on slide	
B. illiesi	QLD	Koombooloomba Creek. Tully Site TR2	-17.86	145.6	27 Jul, 1990		No Collector Indicated	PS371	1	larvae		whole specimen on slide	
B. illiesi	QLD	South Johnstone River upstream Central Mill, at railway bridge	-17.6	145.98	25 Jun, 2008		JM Webb, JH Hawking, S Moore, P Suter	JWA800	1	larvae		parts on slide	
B. narilla	ACT	Cotter River at reserve	-34.32	148.94	18 Mar, 2008		P Suter & J Hawking	JWA216	1	larvae			
B. narilla	NSW	4 Mile Cr at New England Highway	-29.38	151.89	1 Dec, 2007		JM Webb & D Black	JWA355	1	larvae			
B. narilla	NSW	Berrico Creek, Berrico	-32.07	151.82	2 Dec, 2007		A Glaister, J Dean, R St Clair	JWA420	25	larvae			
B. narilla	NSW	Cedar Brush Creek at Brush Creek Rd	-33.15	151.26	10 Mar, 2009	80	JM Webb & JH Hawking	JWA1300	1	larvae			HM017836
B. narilla	NSW	Cedar Brush Creek at Brush Creek Rd	-33.15	151.26	10 Mar, 2009	80	JM Webb & JH Hawking	JWA1301	1	larvae			
B. narilla	NSW	Doudles Folly Creek at Tourist Rd	-34.52	150.52	11 Mar, 2009	611	JM Webb & JH Hawking	JWA1335	5	larvae			
B. narilla	NSW	Gara River at Thalgarrah Field Study Site. Site 6	-30.43	151.48	25 Nov, 2008		P Suter & J Dean	JWA1387	2	larvae			
B. narilla	NSW	Goorudee Rivulet, Upper Murrumbidgee R, North of Adaminaby	-35.98	148.77	11 Mar, 2000		P Suter, T Curmi, R Brown	JWA383	1	Adult	male		
B. narilla	NSW	Kangaroo River, Upper Kangaroo Valley			23 Sep, 1972		J Dean	PS351	1	larvae	female	whole specimen on slide	

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TABLE 1. (continued)

Species	State	Locality	Latitude	Longitude	Date	Elevation (m)	Collector(s)	Accession number	# Specimens	Lifestage	Gender	Storage Method	GenBank Accession #
B. narilla	NSW	Kangaroo River, Upper Kangaroo Valley			23 Sep, 1972		J Dean	PS350	1	larvae	female	whole specimen on slide	
B. narilla	NSW	Murray River @ Tom Groggin Camp Ground	-36.54	148.13	2 Dec, 2009	522	JM Webb, J Mynott, M Shackleton, S Moore	JWA2097	1	larvae			
B. narilla	NSW	Murrumbidgee River at Bobeyan Rd., south of Bolaro	-35.98	148.84	12 Mar, 2009	980	JM Webb & JH Hawking	JWA1318	9	larvae			
B. narilla	NSW	Swampy Plain River at Geehi on Alpine Way, Kosciuszko National Park	-36.38	148.18	9 Nov, 2008	430	JM Webb & SE McCartney	JWA1245	1	larvae		parts on slide	HM017835
B. narilla	NSW	Swampy Plain River at Geehi on Alpine Way, Kosciuszko National Park	-36.38	148.18	9 Nov, 2008	430	JM Webb & SE McCartney	JWA1048	2	larvae			
B. narilla	NSW	Wilson R at Wilson River Road	-31.2	152.48	4 Dec, 2007	437	A Glaister, J Dean, R St Clair	JWA636	1	larvae			
B. narilla	NSW	Woolindilly River, Murphy's Flat			16 Nov, 1990		No Collector Indicated	PS192	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Booloumba Creek, Canondale Ranges; Site3			4 May, 1993		No Collector Indicated	PS601	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Booloumba Creek, Site2, Conondale Ranges			4 May, 1993		No Collector Indicated	PS598	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Booloumba Creek, Site2, Conondale Ranges			4 May, 1993		No Collector Indicated	PS600	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Bundaroo Creek, Canondale Ranges			20 May, 1993		No Collector Indicated	PS628	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Bundaroo Creek, Canondale Ranges			20 May, 1993		No Collector Indicated	PS629	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Harvey Range. Two Mile Creek, Nth QLD			16 Apr, 1979		A.Wells	PS878	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Logan River at Mt. Barney Lodge	-28.28	152.74	7 Dec, 2007		JM Webb & D Black	JWA205	1	Adult	female		HM017831
B. narilla	QLD	Logan River at Mt. Barney Lodge	-28.28	152.74	7 Dec, 2007		JM Webb & D Black	JWA573	2	Adult	female		
B. narilla	QLD	Logan River at Mt. Barney Lodge	-28.28	152.74	2 Dec, 2007		JM Webb & D Black	JWA555	1	larvae			HM017832

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TABLE 1.(continued)

Species	State	Locality	Latitude	Longitude	Date	Elevation (m)	Collector(s)	Accession number	# Specimens	Lifestage	Gender	Storage Method	GenBank Accession #
B. narilla	QLD	Logan River at Mt. Barney Lodge	-28.28	152.74	2 Dec, 2007		JM Webb & D Black	JWA556	1	larvae			
B. narilla	QLD	Logan River at Mt. Barney Lodge	-28.28	152.74	2 Dec, 2007		JM Webb & D Black	JWA354	1				
B. narilla	QLD	Logan River at Mt. Barney Lodge	-28.28	152.74	2 Dec, 2007		JM Webb & D Black	JWA356	0	larvae			
B. narilla	QLD	Stony Creek A. Canondale Ranges			15 Nov, 1993		No Collector Indicated	PS649	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Stony Creek A. Canondale Ranges			15 Nov, 1993		No Collector Indicated	PS647	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Stony Creek A. Canondale Ranges			15 Nov, 1993		No Collector Indicated	PS648	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Stony Creek B, Canondale Ranges			15 Jul, 1993		No Collector Indicated	PS650	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Stony Creek, Cannondale Ranges			15 Mar, 1993		No Collector Indicated	PS635	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Stony Creek, Cannondale Ranges			15 Mar, 1993		No Collector Indicated	PS636	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Sunday Creek, Site5, Canondale Ranges			30 May, 1992		No Collector Indicated	PS627	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Sunday Creek, Site5, Canondale Ranges			30 May, 1992		No Collector Indicated	PS626	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Unnamed Creek, Lower Canondale Ranges			20 May, 1993		No Collector Indicated	PS642	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Unnamed Creek, Lower Canondale Ranges			20 May, 1993		No Collector Indicated	PS637	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Unnamed Creek, Lower Canondale Ranges			20 May, 1993		No Collector Indicated	PS380	1	larvae	female	whole specimen on slide	

continued next page.

TABLE 1. (continued)

Species	State	Locality	Latitude	Longitude	Date	Elevation (m)	Collector(s)	Accession number	# Specimens	Lifestage	Gender	Storage Method	GenBank Accession #
B. narilla	QLD	Unnamed Creek, Lower Canondale Ranges			20 May, 1993		No Collector Indicated	PS640	1	larvae	female	whole specimen on slide	
B. narilla	QLD	Unnamed Creek, Lower Canondale Ranges			20 May, 1993		No Collector Indicated	PS639	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Unnamed Creek, Lower Canondale Ranges			20 May, 1993		No Collector Indicated	PS641	1	larvae	male	whole specimen on slide	
B. narilla	QLD	Unnamed Creek, Lower Canondale Ranges			20 May, 1993		No Collector Indicated	PS638	1	larvae		whole specimen on slide	
B. narilla	TAS	Wilmot/Spellmans Rd C6			13 Oct, 1994		No Collector Indicated	PS521	11	larvae	female	whole specimen on slide	
B. narilla	VIC	Eurobin Creek off CR535, near Mt. Buffalo Nat'l Park Entrance	-36.97	146.86	1 Mar, 2009	300	JM Webb	JWA1293	16	larvae		parts of one on slide	
B. narilla	VIC	Eurobin Creek off CR535, near Mt. Buffalo Nat'l Park Entrance	-36.97	146.86	11 Nov, 2008	300	JM Webb & SE McCartney	JWA998	2	larvae			
B. narilla	VIC	King River, Upstream of L.William-Hovell-Ovens Catchment			12 Nov, 1991		No Collector Indicated	PS352	1	larvae	male	whole specimen on slide	
Centroptilum elongatum	VIC	Eumerella River near Bessiebelle	-38.16	141.95	9 July, 2008		No Collector Indicated	JWA766	1	larvae			HM017830

swapping. Bootstrap support was calculated with 1000 replicates with 10 random sequence addition replicates and TBR. Trees were rooted using a sequence of *Centroptilum elongatum* Suter, 1986 (GenBank accession number HM017830). Unique, fixed bases for each species were identified manually.

In order to obtain a better view of variation within *B. narilla*, 33 haplotypes of a 397-bp fragment of COI of *B. narilla* were obtained from McLean *et al*. (2008) (GenBank accession numbers EU789591–EU789623). A NJ analysis using the 397-bp fragment of all specimens was produced and p-distances recalculated.

Results

There were no double peaks in the chromatograms and no frame shifts, indels, or stop codons were observed in any of the 12 sequences. Two haplotypes were observed in northern specimens, and four in southern specimens. Specimens of *Bungona* from northern Queensland had two haplotypes and were strongly supported as being distinct from southern populations in both NJ (Fig. 21) and MP (not shown) reconstructions. Between group divergence (p-distance) was 18.8–19.4% (mean 19.0% n=12) and within group divergence ranged from 0.0–0.2% (mean 0.04% n=8) in northern populations, and from 0.31–1.7% (mean 1.0% n=4) in southern populations. Of the 657 base positions, there were 129 variable sites. The northern populations had 115 fixed differences from the southern populations (Table 2).

All southern specimens clustered within the 33 haplotypes of a 397-bp fragment of COI from *B. narilla* identified by McLean *et al*. (2008) (Fig. 22). All of the haplotypes in our southern specimens differed from those of McLean *et al*. (2008), except JWA205 from southeast Queensland which was equivalent to "haplotype 6"/EU789594. Using the 357-bp fragment, mean within group divergence was 1.3% in *B. narilla* and 0.1% in northern populations, and mean intergroup divergence was 20.1%.

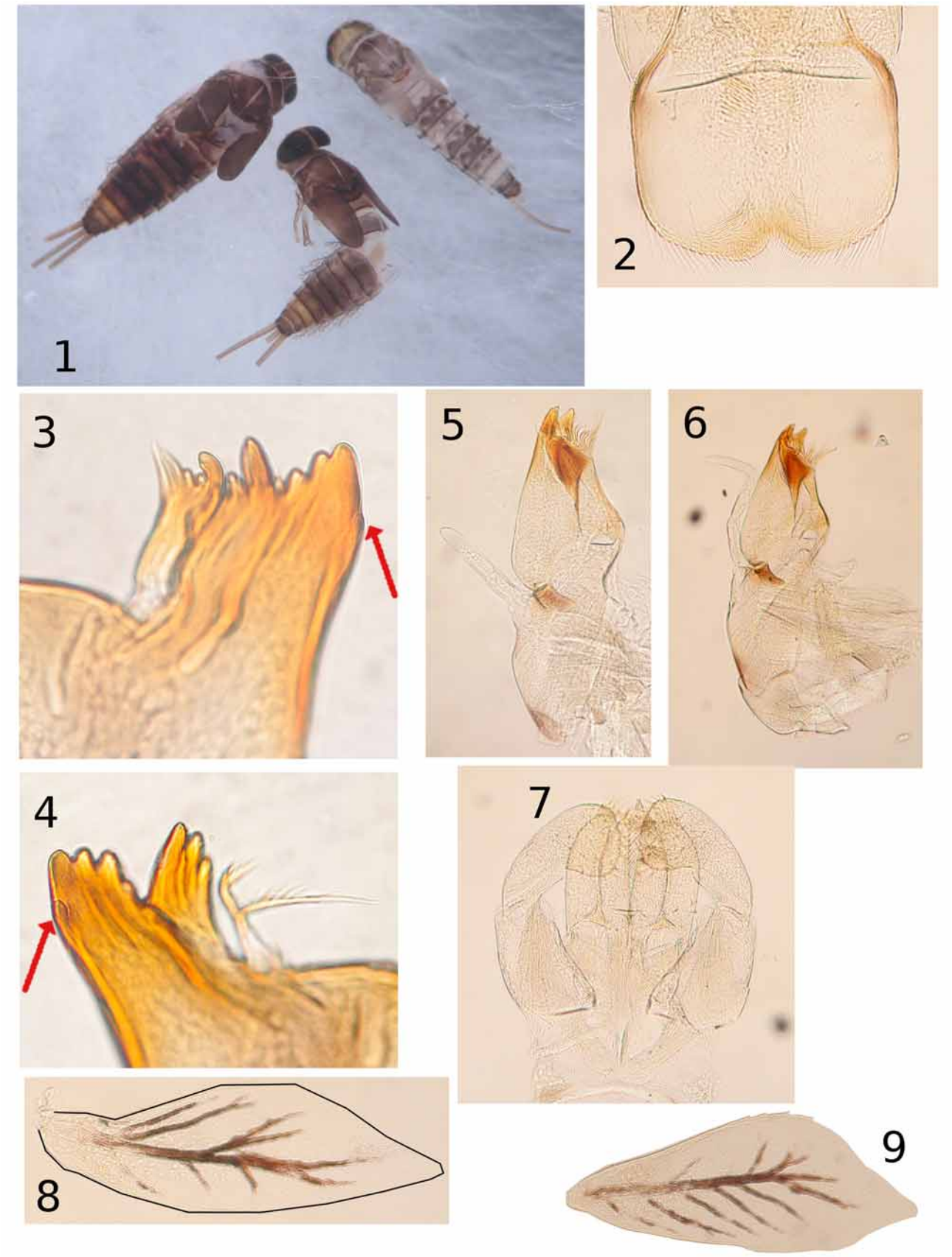
TABLE 2. List of unique fixed character states in a 657-bp fragment of COI for *B. illiesi* and *B. narilla*.

Species	Position:Base
B. illiesi	6:A;30:T;33:A;39:G;45:A;51:G;54:A;55:C;57:T;58:G;60:T;66:G;75:A;78:A;93:C;108:G;114:T;121:G;129:T;135:T;144:C;153:T;156:C;168:T;180:A;183:T;189:A;192:C;205:T;207:G;211:T;213:A;216:T;222:G;228:G;231:T;234:T;240:A;243:A;249:C;258:C;261:A;265:C;276:T;283:C;285:T;286:C;289:A;291:T;294:A;300:T;303:A;306:A;309:T;310:A;315:G;318:T;324:A;327:A;333:T;336:C;342:A;345:G;346:C;348:T;351:G;363:T;369:A;378:G;381:T;405:C;411:T;414:T;417:T;420:A;438:C;450:T;453:A;456:A;459:T;462:T;465:A;471:A;474:T;480:G;481:A;492:A;495:A;501:A;507:G;513:A;516:T;519:G;531:C;534:A;541:T;543:G;552:A;555:T;558:G;561:G;564:A;570:T;577:C;579:T;582:T;588:T;595:T;597:A;603:C;606:T;615:T;618:T;621:C;627:C
B. narilla	6:G;30:A;33:T;39:T;45:T;51:A;54:T;55:T;57:G;58:T;60:A;66:T;75:G;78:T;93:T;108:A;114:C;121:A;129:A;135:C;144:T;153:C;156:T;168:A;180:T;183:G;189:T;192:T;205:C;207:T;211:C;213:T;216:G;222:A;228:A;231:C;234:C;240:T;243:G;249:T;258:T;261:G;265:T;276:A;283:T;285:A;286:T;289:G;291:A;294:T;300:A;303:G;306:T;309:C;310:G;315:A;318:G;324:G;327:G;333:A;336:G;342:T;345:T;346:T;348:A;351:T;363:C;378:T;381:A;405:T;411:A;414:G;417:G;420:T;438:A;450:C;453:T;456:T;459:C;462:C;465:G;471:C;474:A;480:A;481:T;492:T;495:T;501:G;507:C;513:G;516:A;519:T;531:A;534:G;541:C;543:A;552:T;555:G;558:A;564:T;570:C;577:T;579:G;582:A;588:C;595:C;603:T;606:A;615:C;618:G;621:T;627:A

Taxonomy

Bungona illiesi (Lugo-Ortiz & McCafferty, 1998)

Cloeodes illiesi; Lugo-Ortiz & McCafferty 1998: 124 (orig.)
Bungona narilla; Suter and Pearson 2001: 249 (=*C. illiesi*)



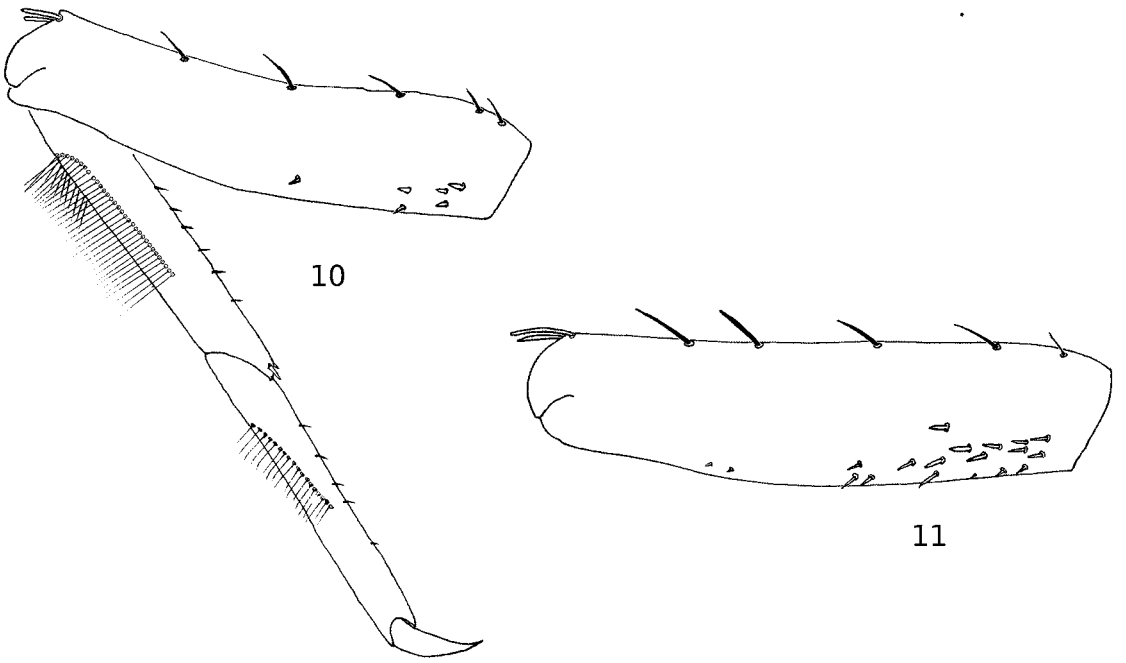
FIGURES 1–9. *Bungona illiesi*. 1. dorsal habitus of three larvae, 2. labrum, dorsal view, 3. left mandible with small lateral tooth indicated, 4. right mandible with small lateral tooth indicated, 5. maxilla showing aberrant minute palp, 6. maxilla from same specimen as Fig. 5 showing normally developed palp, 7. labium, 8. gill 1 with margins outlined, 9. gill 7.

Description

Larva: Body length: 3.1–3.8 mm, female larvae slightly larger than males. Caudal filaments ~ 0.5 x body length.

Head mostly brown; scape and pedicel subequal in length. Labrum (Fig. 2) with length subequal to width; marginal fringe of setae highly branched apically; submarginal fringe with 1+4–5 long simple setae. Right mandible (Fig. 4) with incisors separated apically, outer incisor with 1+3–4 teeth, inner incisor with 3 teeth; prostheca forked; mola with a single fimbriate seta on medial margin; area between prostheca and mola with or without small spines. Left mandible (Fig. 3) with incisors fused to apex, outer incisor with 1+4 teeth, inner incisor with 3–4 teeth; prostheca unforked and robust with apical denticles; area between prostheca and mola with or without small spines. Hypopharynx with superlingua densely covered with fine setae apically, with stout setae laterally becoming stouter basally; lingua with small median lobe covered with fine setae (Lugo-Ortiz and McCafferty 1998, Fig. 11). Maxillae with two segmented palps reaching the apex of the galealacinia, first segment approximately 0.3 times length of galealacinia, second segment sometimes appearing three segmented or may be minute. Galealacinia with 1+3–4 setae basally and single seta near apex. Labium with palp segment 3 slightly expanded medially.

Thorax mostly brown, median line pale. Femora (Fig. 10) with 5–7 long, blunt robust setae and subapical pair of long, blunt robust setae on dorsal margin; apex slightly sinuate and with short fine setae; anterior surface with scale bases, ventrally with 5–9 sharply pointed robust setae basally; posterior surface with scattered fine and short pointed robust setae near ventral margin and many circular chloride cells. Tibiae (Fig. 10) with dense arc of very long, fine setae on outer surface, continuing over outer margin near base onto posterior surface; inner margin with 7–9 short, pointed robust setae. Tarsi (Fig. 10) with 5–9 short, robust setae all of approximately the same length on inner margin; outer margin without robust setae; outer surface with dense row of very long, fine setae.

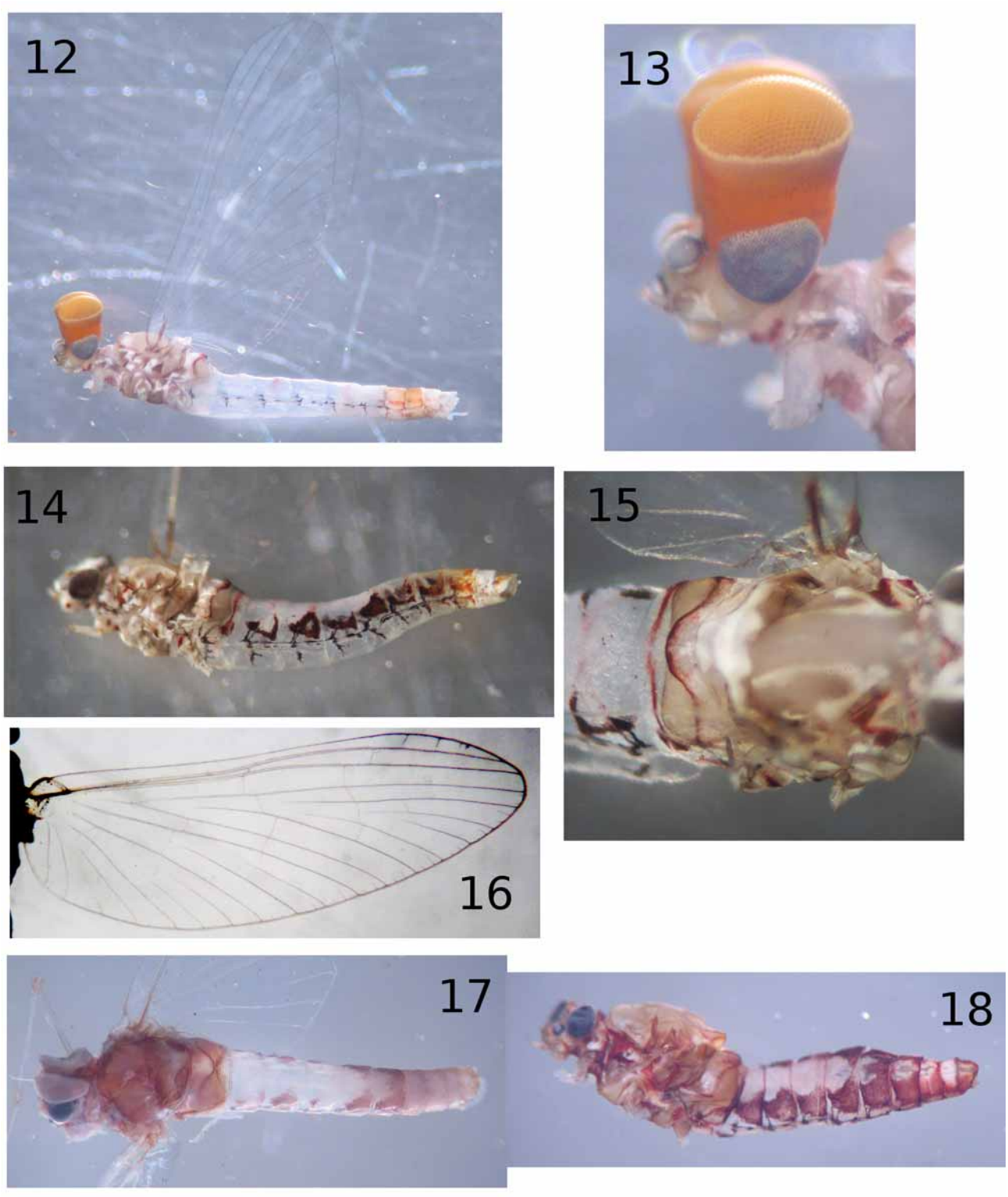


FIGURES 10–11. 10. foreleg of *Bungona illiesi*, 11. forefemur of *Bungona narilla*.

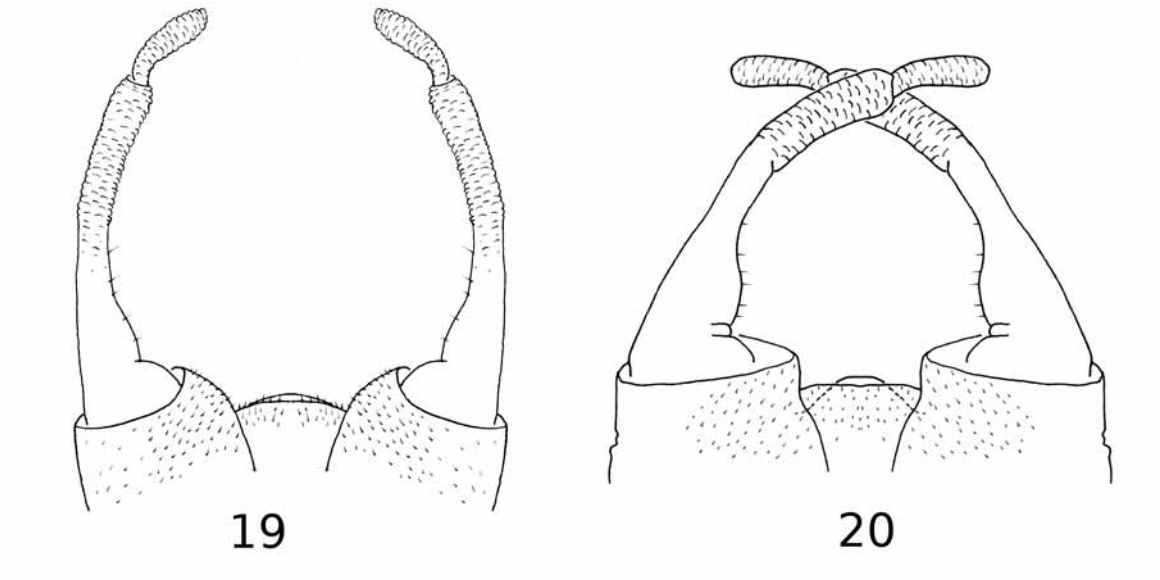
Abdomen with gills present on segments 1–7; gill 1 narrow-elongate (Fig. 8), margin smooth; gills 2–7 ovate to narrow elongate, margins serrate (Fig. 9). Colour pattern variable (Fig. 1), usually mostly brown with segments 4 and 8 paler. Terga 2–10 with sharply pointed spines on middle 2/3 of posterior margins; surface with many triangular scales, scale bases, and sparse fine setae; tergum 1 without spines on posterior margin. Sterna pale; posterior margins of sterna 5–9 with long sharp spines approximately 1.5 times longer than wide

in middle 1/3. Paraprocts with many scale bases and fine setae, margins with 11–13 sharp spines. Caudal filaments unbanded, cerci subequal in length to terminal filament.

Male Adult (Fig. 12): Body length: 4.2–4.7 mm. Forewing 3.5–4.0 mm. Caudal filaments broken and missing.



FIGURES 12–18. *Bungona illiesi*. 12. lateral view of male imago, 13. lateral view of head of male imago, 14. lateral view of female imago, 15. metascutellum of female imago with broadly rounded posterior margin, 16. wing of male imago; *Bungona narilla*. 17. dorsal view of male imago, 18. lateral view of female imago.



FIGURES 19–20. male genitalia. 19. *Bungona illiesi*, 20. *Bungona narilla*.

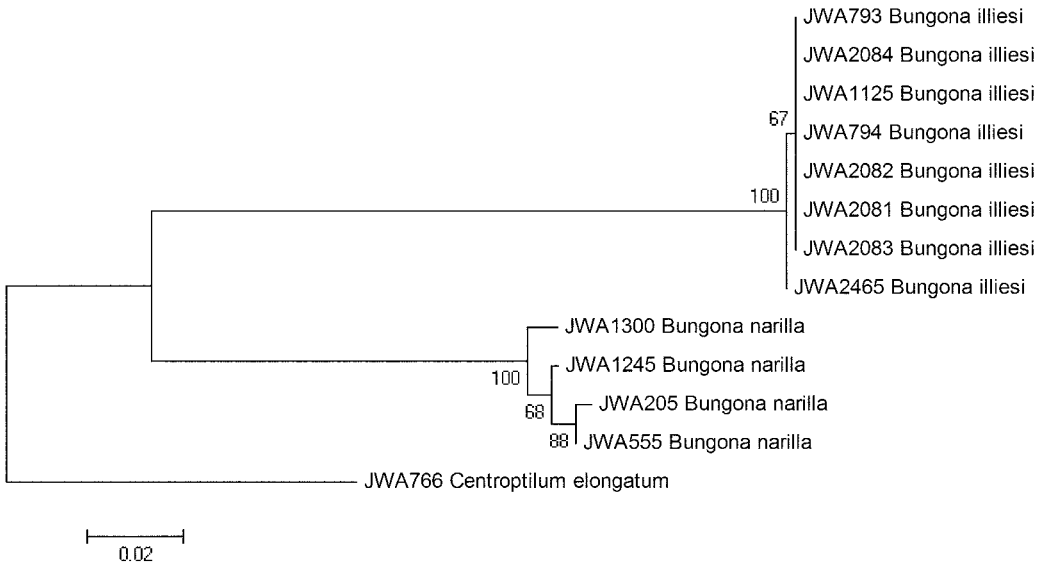


FIGURE 21. Neighbour-Joining tree of 657-bp fragment of COI of 12 specimens of *Bungona* rooted on *Centroptilum elongatum*, Kimura 2 parameter distance. Bootstrap values are indicated on branches.

Head yellow brown, turbinate eyes orange, tall and only slightly expanded distally (Fig. 13), contiguous basally and separated by distance subequal to width of lateral ocellus distally; compound eyes uniformly black. Pronotum and mesonotum cream to pale brown; mesoscutellum white. Forewings colourless; longitudinal veins slightly brown coloured; pterostigma whitish; intercalaries double distal of MP, single basally of MP; MA_2 not attached to crossvein between MA and MP; area between C and Sc without crossveins basal of pterostigma. Hindwings absent. Posterior margin of metanotum concave. Metascutellum cream to pale brown, posterior margin red and broadly rounded, without dorsal projection. Thoracic sterna with sclerites pale brown to brown. Legs missing. Abdominal terga 1–6 white, terga 1, 3, 5 with faint transverse red stripe medially (Fig. 12); tergum 6 with distinct red marking laterally; terga 7–10 with faint pair of submedian red lines; terga 7 and 8 yellowish brown with red longitudinal streak laterally, terga 9 and 10 cream to yellowish brown. Forceps (Fig. 19) white, 3 segmented; separation between segments 1 and 2 indistinct, third segment approximately 3 times longer than wide, segments 2 and 3 covered with scale-like scalloping, segment 1 with small fine setae medially; forceps base and area between bases densely covered

with small sharply pointed stout setae; small somewhat truncate projection dorsally between bases of forceps.

Female Adult (Fig. 14): Body length: 3.8–4.6 mm. Forewing 3.8–4.7 mm. Caudal filaments broken and missing.

Forewings (Fig. 16) similar to those of male except MA_2 attached to crossvein between MA and MP Posterior margin of metanotum only slightly concave (Fig. 15); metascutellum cream with posterior margin red and broadly rounded, without dorsal projection. Abdominal terga (Fig. 14) white with reddish brown markings laterally and posterior margins with faint red transverse band.

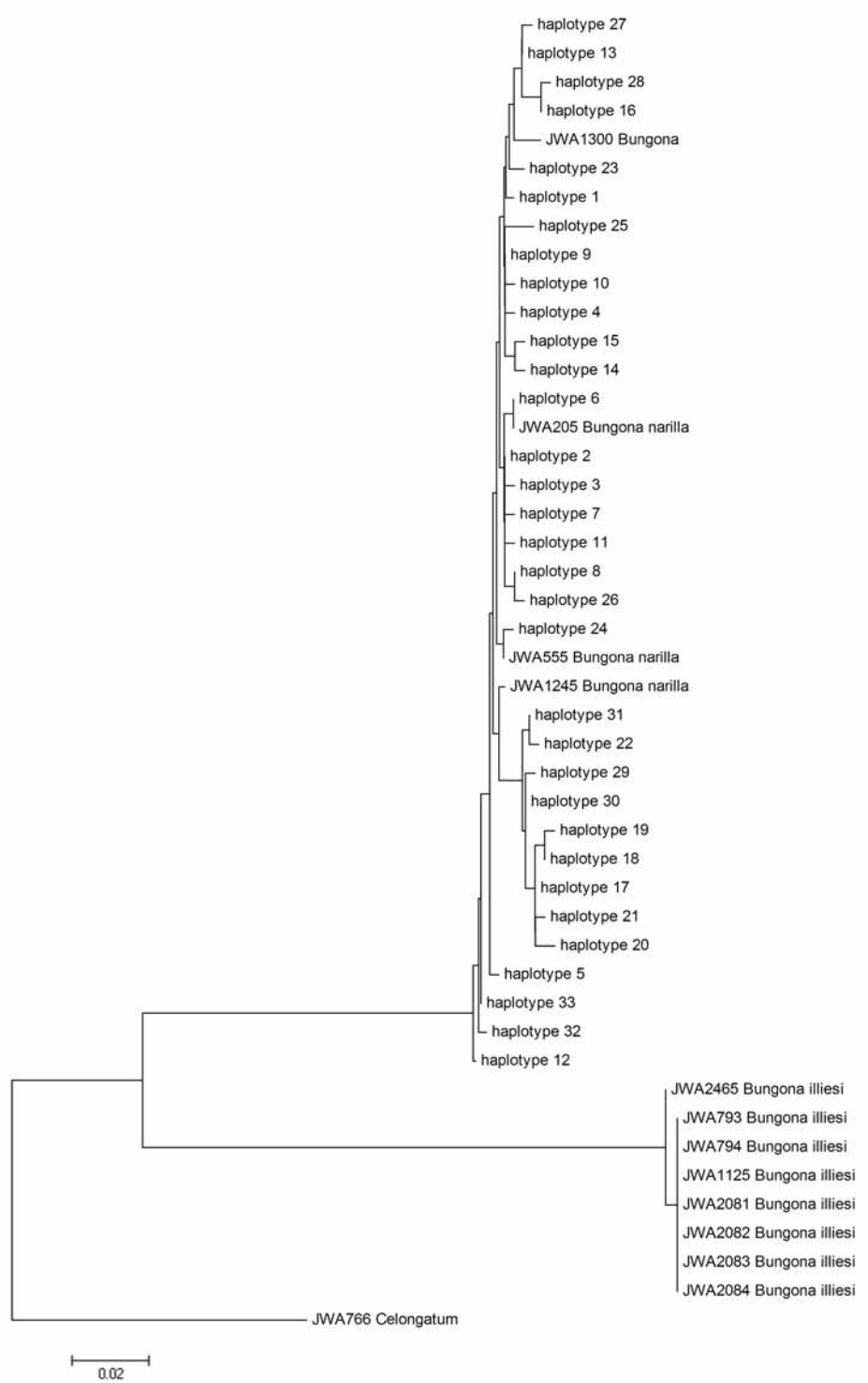


FIGURE 22. Neighbour-Joining tree of 397-bp fragment of COI of 12 specimens of *Bungona* together with 31 haplotypes (GenBank accession numbers EU789591–EU789623) of *B. narilla* from McLean *et al* (2008), rooted on *Centroptilum elongatum,* Kimura 2 parameter distance.

Subimagos: Similar to adults in colouration.

Distribution: wet tropics and Atherton Tablelands of northern Queensland. The southernmost record is from near Tully, QLD. *Bungona narilla* occurs from Tasmania to southeast Queensland, but is expected to occur as far north as the Burdekin Gap north of Townsville, QLD, a recognised biogeographic division for aquatic insects (i.e. Christidis and Dean 2008, Watson and Theischinger 1984).

Diagnosis: Larvae of *B. illiesi* can be differentiated from those of *B. narilla* by a northern Queensland distribution, the presence of a small tooth on the outer margin of the set of incisors (Figs 3,4), and shorter and less dense robust setae at the base of the ventral margin of the fore femur (Fig.10). Previously identified diagnostic characteristics such as the absence of small spines on the mandibles between the mola and incisors, minute maxillary palps, the medial expansion of the labial palps, and narrow-elongate gills (Lugo-Ortiz and McCafferty 1998: Figs 12–15,17) were all found to be variable within populations of both *B. illiesi* and *B. narilla*.

Males and females of *B. illiesi* are easily differentiated from those of *B. narilla* by less extensive red patches on the abdomen (Figs. 12,14) and a northern Queensland distribution. Additionally, in the males of *B. illiesi*, the forceps are slightly more slender, the setae covering the bases of the forceps are longer, and MA_2 is not attached to the crossvein between MA and MP.

Material examined: see Table 1.

Discussion

Bungona shares many similarities with *Cloeodes*, including small transverse rows of setae on the abdominal sterna, a subproximal arc of fine setae on the tibae, edentate claws, two segmented maxillary palps with the second segment having a constriction, right mandible with apically separated incisors and a forked prostheca, left mandible with apically fused incisors and a robust prostheca with apical denticles. Some of the diagnostic characters for *Cloeodes* are known to vary, however. For example, *C. pseudogladius* Gattolliat, 2001 has highly modified, completely fused incisors (Gattolliat 2001) and *C. bicoloratus* Gattolliat, 2001 and *C. freitagae* Gattolliat, 2001 have an unforked right prostheca (Gattolliat 2001). Larvae of *Bungona* differ from *Cloeodes* by having the ventral sternal setae only on segments 4–6, rather than 2–6. The incisors of the left mandibles of *Bungona* are nearly always fused to the apex or near the apex, but at least one specimen of *B. narilla* has elongate teeth on the outer incisor that make it appear that the two incisors are apically separate. Many specimens of *B. narilla* appear to have three segmented maxillary palps, but it is unclear if this is due to the second segment folding or collapsing during slide mounting or if they are actually three segmented.

Adults of *Bungona* differ from *Cloeodes* by having the third segment of the forceps elongate rather than rounded, although *C. inzingae* (Crass, 1947) from South Africa also has an elongate third segment (Waltz and McCafferty 1994), and by the absence of a dorsal projection on the metascutellum. Suter and Pearson (2001) indicated *Bungona* differed from the known species of *Cloeodes* by having MA_2 attached to the crossvein between MA and MP, but in at least *B. illiesi* this varies between males and females. The recently described *C. barituensis* Nieto & Richard, 2008 from Argentina shows the same sexual dimorphism in MA_2 (Nieto and Richard 2008: Figs. 2,4).

A global revision is needed to clarify the relationships of *Cloeodes*, *Bungona*, and related genera as it is possible that *Bungona* is a specialised lineage within *Cloeodes*. Preliminary analyses of fragments of 18S and 16S rDNA (Webb unpublished) from numerous Baetidae genera indicate *Bungona* and Afrotropical *Cloeodes* are relatively distantly related, despite their morphological similarity. Further analyses including Neotropical and Oriental *Cloeodes* and related genera, sequences of protein coding genes, and morphology are required, however, before the relationships among these groups can be inferred. Until these analyses are completed, we believe *Bungona* should be continued to be treated as a valid genus separate from *Cloeodes*.

Acknowledgements

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