ZooKeys 330: 1–26 (2013)
doi: 10.3897/zookeys.330.5750
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
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Description of *Longidorus cholevae* sp. n. (Nematoda, Dorylaimida) from a riparian habitat in the Rila Mountains, Bulgaria

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Academic editor: *Sergei Subbotin* | Received 6 July 2013 | Accepted 20 August 2013 | Published 9 September 2013

http://zoobank.org/DC6FB8CC-F362-4734-8A80-F711D144DF06

Citation: Peneva VK, Lazarova SS, De Luca F, Brown DJF (2013) Description of *Longidorus cholevae* sp. n. (Nematoda, Dorylaimida) from a riparian habitat in the Rila Mountains, Bulgaria. ZooKeys 330: 1–26. doi: 10.3897/zookeys.330.5750

Abstract

A description is provided of *Longidorus cholevae* **sp. n.**, a bisexual species associated with wild cherry (*Prunus avium* L.) from the Rila Mountains, Bulgaria. The position of *L. cholevae* sp. n. among other species of the genus was elucidated by using morphological and molecular data. Phylogenetic analyses were performed of D2-D3 expansion domains of the 28S rRNA and the partial ITS1 containing regions by Neighbor-Joining, Maximum Likelihood and Bayesian Inference methods. The species is characterised by a female body length of 6.1–8.1 mm; long odontostyle (106–129 µm); lip region wide (21.5–24 µm) rounded and continuous with the body profile; amphidial pouches short and wide, funnel-shaped; a posteriorly situated guide ring (30–37 µm); normal arrangement of pharyngeal glands, and short bluntly rounded to hemispherical tail. Four juvenile stages indentified, first stage with elongate conoid tail. Males with 2–4 adanal pairs and a row of 11–13 single ventromedian supplements, spicules 96–120 µm long. Based both on morphological and molecular data the new species appearred to be the most similar with a group of species distributed in Europe sharing common charcters such as amphidial fovea, lip region and tail shapes, and having similar odontostyle and body length: *L. poessneckensis, L. caespiticola, L. macrosoma, L. helveticus, L. carniolensis* and *L. pius.* An updated list of *Longidorus* species and a partial polytomous keys to the *Longidorus* species with long odontostyle (code A45) and short tail (code H1) are provided.

Keywords
D2D3, ITS, Longidoridae, morphology, phylogeny

Introduction

Chen et al. (1997) further developed a polytomous key for identification of 103 species known at that time of the genus *Longidorus* Micoletzky, 1925. Subsequently, Loof and Chen (1999) provided codes for another 13 species, two of which were cosidered as junior sysnonyms. Recently, another four species, originally described either as *Paralongidorus* (e.g *P. monegrensis* and *P. milanis*) or *Longidoroides* (*L. spiralis* and *L. boshi*) were transferred to *Longidorus* (Roca 2006, Decraemer and Coomans 2007). Andrássy (2009) provided a list of the species belonging to the genus, noting that 69 species were registered to occur in Europe. To this list eight new species were added which originated from different parts of the world: Ukraine (*L. holovachovi* Peneva, Sususlovsky & Lazarova, 2009), Slovenia (*L. carniolensis* Širca, Urek, Lazarova, Elshishka & Peneva, 2011), Iran (*L. kheirii* Pedram, Niknam, Robbins, Ye & Karegar, 2008 and *L. tabrizicus* Niknam, Pedram, Ghahremani Nejad, Ye, Robbins & Tanha Maafi, 2010), Philippines (*L. mindanaoensis* Coomans, Tandingan De Ley, Angsinco Jimenez & De Ley, 2012 and Spain (*L. baeticus* Gutiérrez- Gutiérrez, Cantalapiedra-Navarrete, Monte-Borrego, Palomares-Rius & Castillo, 2013, *L. oleae* Gutiérrez-Gutiérrez, Cantalapiedra-Navarrete, Monte-Borrego, Palomares-Rius & Castillo, 2013 and *L. andalusicus* Gutiérrez- Gutiérrez, Cantalapiedra-Navarrete, Monte-Borrego, Palomares-Rius & Castillo, 2013). Currently, there are 158 *Longidorus* species and an updated list of the species belonging to this important plant parasitic genus is presented as Appendix 1.

Molecular approaches and phylogenetic studies provide additional tools to the routine identification of plant parasitic nematodes. Further, the ribosomal DNA sequences represent a useful diagnostic aproach in the characterisation and phylogenetic reconstruction within Longidoridae, above all, where morphological characters may led to ambiguous identification (De Luca et al. 2004, 2009, Neilson et al. 2004, He at al. 2005, Palomares-Rius et al. 2008, 2010).

During a study of the longidorid fauna of natural habitats in Bulgaria (2005-2009) several populations of the genus *Longidorus* were recovered from various locations in the Rila Mountains, one of which represented an undescribed species.

The aim of the present study was to characterise morphologically and molecularly this new species and to infer its phylogenetic relationships with other species of the genus *Longidorus* by using the D2-D3 expansion domains of the 28S rDNA and the ITS containing region.

Materials and methods

Nematodes were isolated from soil samples by a decanting and sieving technique. *Longidorus* specimens recovered were heat killed at 55°C for two minutes, fixed in a 4%

formalin/1% glycerol mixture, processed to anhydrous glycerol (Seinhorst 1959), and mounted on glass microscope slides. Drawings were prepared using an Olympus BX51 compound microscope with differential interference contrast (DIC). Photographs were taken using an Axio Imager.M2-Carl Zeiss compound microscope equipped with a digital camera (ProgRes C7) and specialised software (CapturePro Software 2.8). Measurements were made using an Olympus BX41 light microscope, a digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA), and computer Digitrak 1.0f programme, (Philip Smith, Scottish Crop Research Institute, Dundee, UK).

A partial polytomous keys was prepared for the identification of *Longidorus* species with long odontostyle (A45) and short tail (H1). This key, based on that by Chen et al. (1997), but incorporating newly described species after 1997 and the addition of some new characters: J – number of juvenile stages – J1 – 4 stages; J2 – 3 stages; K – shape of tail in J1 – using the same codes as for female tail and introducing K7 – tail digitate or with mucro.

DNA extraction and amplification

Specimens for molecular analysis were kept in DESS solution (Yoder et al. 2006). Genomic DNA was extracted from fifteen individual nematodes as described by De Luca et al. (2004). The crude DNA isolated from each individual nematode was directly amplified. The partial 18S-ITS1-5.8S-ITS2 regions were amplified using the forward primer 18S-Ext (5'-TGATTACGTCCCTGCCTTT-3') and the reverse primer 26S-Ext (5'-TTTCACTCGCCGTTACTAAGG-3') (Vrain et al. 1992) and the D2-D3 expansion segments of 28S rDNA was amplified using the D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (Castillo et al. 2003). PCR cycling conditions used for amplification were: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 50s, annealing at 55°C for 50s and extension at 72°C for 1 min and a final step at 72°C for 7 min. The size of amplification products was determined by comparison with the molecular weight marker ladder 100 (Fermentas, St. Leon-Rot, Germany) following electrophoresis of 10 ml on a 1% agarose gel.

Sequencing and phylogenetic analysis

PCR products of the ITS region from two individual nematodes were purified for cloning and sequencing using the protocol provided by the manufacturer (High Pure PCR elution kit, Roche, Germany). Purified ITS fragments were cloned in TA cloning vector (Invitrogen) and several clones were sequenced using an ABI Prism 377 sequencer (PE Applied Biosystem, Foster City, CA). Similarly, the D2-D3 regions of rDNA from two individual nematodes were purified and used for direct sequencing.

The sequences of the new species have been deposited in GenBank with the accession numbers: FR775757 – FR775760 for the ITS clones; and FR775761, FR775762 for the D2-D3 regions. Additionally, another four sequences (ITS and D2-D3) belonging to a population identified as *Longidorus* cf. *caespiticola* Hooper, 1961 were produced and deposited using the same methodology (see Table 1 for accession numbers and locality). The morphometrics of this population and detailed discussion will be presented in another publication.

Further, a BLAST (Basic Local Alignment Search Tool) search at NCBI (National Center for Biotechnology Information) was performed using the obtained ITS and D2-D3 sequences as queries to confirm their nematode origins and to identify the most closely related nematode sequences. Different *Longidorus* species were used in the phylogenetic analyses of ITS1-5.8S-ITS2 and D2-D3 regions due to sequence availability in the GenBank database (Table 1). The multiple sequence alignments (MSA) of both datasets were performed using MAFFT algorithm (Katoh et al. 2002) with GUIDANCE Web-based program available at http://guidance.tau.ac.il/ (Penn et al. 2010a). The MSA reliability evaluation was based on GUIDANCE alignment, sequence and columns scores (Penn et al. 2010b). Unreliable columns below 0.93 confidence score were removed from the D2-D3 MSA alignment. Subsequently, the MSAs were manually optimised and trimmed using MEGA 5 (Tamura et al. 2011). *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939, *X. index* Thorne & Allen, 1950 and *X. insigne* Loos, 1949 were used as out group taxa for both D2-D3 and ITS sequence datasets, respectively.

Base compositional differences were evaluated using the χ^2-test. Sequence divergences (uncorrected *p* distance) were calculated using MEGA 5.0 (Tamura et al. 2011). The phylogenetic reconstructions of both D2-D3 and partial 18S-ITS1 rDNA datasets were performed using neighbor joining (NJ) and maximum likelihood (ML) algorithms as implemented in MEGA 5.0 (Tamura et al. 2011) as well as the Bayesian inference (BI) using MrBayes v. 3.2.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003, Ronquist et al. 2012). The *NJ phylogenetic inferences* were performed under the following settings: Maximum Composite Likelihood method for computing evolutionary distances; Gamma distributed rates among sites, estimated values set up to 0.3395 (D2-D3) and 0.1127 (18S-ITS1); 2000 bootstrap replications. A total of 640 and 290 positions in the final datasets were used for both analyses, respectively. The most appropriate substitution models were determined using the FindModel web tool (Tao et al. 2005, Posada and Crandall 1998), by comparing the Akaike information criterion (AIC, Akaike 1973) and Maximum Likelihood value (*ln*L) scores of the 28 possible models. *ML analyses* settings as applied in MEGA 5 were General Time Reversible model (GTR), Gamma distribution (G); number of discrete Gamma rates equal to 4; 1000 bootstrap replications for D2-D3 rDNA and Kimura 2 parameter-model (+G, 4 rates and 1000 bootstrap replications) for 18S-ITS1 region. Bayesian MCMC tree searches were conducted using MrBayes 3.2.1. For each analysis, two independent runs were conducted with 4 chains each and default heating parameters (1 cold, 3 heated, *temp* = 0.2). Each analysis was run for 10,000,000 generations

Table 1. Species of fam. Longidoridae used in phylogenetic reconstructions.

Nematode species	Locality	Accession number	Reference
L. caespiticola Hooper, 1961	Brdo, Slovenia	HM447030	Širca and Urek 2009
L. caespiticola	Brussegem, Belgium	AF480079	Rubtsova et al. 2001
L. caespiticola	Gandesbergen, Germany	AF480080	Rubtsova et al. 2001
L. caespiticola	Viermaal, Belgium	AF480081	Rubtsova et al. 2001
L. caespiticola	Scotland, UK	AY601567	He et al. 2005
*L.*cf *caespiticola*	Sokolovo, Bulgaria	HG329719–HG329721	Present study
L. carniolensis	Krmačina, Slovenia	JN631811	Širca et al. 2011
L. carniolensis	Drašiči, Slovenia	JN631812	Širca et al. 2011
L. cholevae sp. n.	Bulgaria	FR775757–FR775762	Present study
L. elongatus (de Man, 1876) Thorne & Swanger, 1936	Scotland, UK	AF511417	Ye et al. 2004
L. helveticus Lamberi, Kunz, Grunder, Molinari, De Luca, Agostinelli & Radicci, 2001	Trška gora, Slovenia	HM447031	Širca and Urek 2009
L. helveticus	Stari Ledinci, Serbia	EF538753 JN627412	Kumari et al. 2009 Kumari and Subbotin 2012
L. helveticus	Camenzuid, Switzerland	AY601566	He et al. 2005
L. helveticus	Chodovlice, Czech Republic	JN627410, JN627414	Kumari and Subbotin 2012
L. helveticus	Silničná, Czech Republic	JN627411, JN627415	Kumari and Subbotin 2012
L. helveticus	Switzerland	AJ549985	De Luca et al. 2004
L. macrosoma Hooper, 1961	Liége, Belgium	AF480082	Rubtsova et al. 2001
L. macrosoma	Austria	EF538752	Kumari et al. 2009
L. macrosoma	unknown	AY580055	unpublished
L. macrosoma	Switzerland	AY601565	He et al. 2005
L. macrosoma	Switzerland	AJ549978, AJ549979	De Luca et al. 2004
L. macrosoma	unknown	AY430184	unpublished
L. pius Barsi & Lamberti, 2000	Republic of Macedonia	AM743178–AM743184	Barsi and De Luca 2008
L. poessneckensis Altherr, 1974	Czech Republic	EF538750	Kumari et al. 2009
L. poessneckensis	Slovakia	EF538751	Kumari et al. 2009
L. raskii Lamberti & Agostinelli, 1993	Switzerland	AJ549983, AJ549984	De Luca et al. 2004
L. diadecturus Eveleigh & Allen, 1982	Elkins, White river, USA	AY601584	He et al. 2005
X. diversicaudatum (Micoletzky, 1927) Thorne, 1939	Slovakia	EF538755	Kumari et al. 2009
Xiphinema index Thorne & Allen, 1950	Argentina	AY601628	He et al. 2005
Xiphinema insigne Loos, 1949	Taiwan	AY563427	Chen et al. 2004

with a sample frequency of 1000 generations. The first 25% of the chains discarded as burning and the remaining 75% trees kept to summarise the tree topology, branch lengths, and posterior probabilities (PP) of branch support. The evolutionary models for nucleotide substitutions were set up as for ML analyses. Convergence diagnostic calculated every 1000 generations with predefined stopvalue equal to 0.01. A single strict consensus tree was visualised using FigTree v1.4.0 graphical viewer (http://tree.bio.ed.ac.uk/software/figtree/). Posterior probabilities values of ³0.8 (BI) and bootstrap values of ³70 (NJ and ML) were considered as credible support values for nodes.

Taxonomy

***Longidorus cholevae* sp. n.**
http://zoobank.org/882B3067-D244-4B8F-9312-B6E0F55B6C90
http://species-id.net/wiki/Longidorus_cholevae
Figures 1–9

Measurements. See Table 2

Description. *Female.* Body plump, assuming a C to open spiral shape. Lip region continuous, anteriorly rounded. Labial papillae prominent. Cuticle 8–10 µm thick at poslabial region, 5–7 µm along the body and 12–14 µm on tail posterior to anus. Guide ring 6–7 µm wide. One lateral pore anterior to guide ring, 2–4 along odontostyle, 1–2 along odontophore, 4–5 in narrow part of the oesophagus and 3–4 in bulb region as well as 3–5 dorsal and 7–10 ventral; numerous lateral body pores observed. Amphidial fovea pouch like, short, almost as wide as long, funnel shape with code E5 according to Chen et al. (1997) and type 4 according to Decraemer and Coomans (2007), amphidial aperture assumed to be a minute pore, hardly visible under light microscope; fusus (sensillium pouch) at 51.6±2.7 (49.5–56) µm, n=7 from anterior end. Odontostyle slender, 2 µm wide at base. Pharyngo-intestinal valve, variable in shape (broadly rounded to heart-shape) and size, slightly wider than long: 19±1.4 (17–20) × 15.4±3.1 (12–19) µm, n=5. Normal arrangement of pharyngeal glands: nuclei of the dorsal and subventral glands situated at 23.6–32.1 % (n=3) and 50.7–58.9 % (n=8) of the distance from anterior end of the bulb. Dorsal gland nuclei 2 µm diam., subventral gland nuclei 3–4 µm diam. Nerve ring surrounding odontophore base, at 222.9±11.3 (203–242.5) µm from anterior end, a second nerve ring situated at a short distance behind the first one. Lateral chord 25–29 µm wide. Vagina extending to *ca.* half corresponding body width. *Pars distalis vaginae* 23–27 µm long; *pars proximalis vaginae* 28–35 µm long, thick walled. Uteri very long, anterior uterus 481.0±105.1 (372.5–662.5), posterior uterus 473.2±114.2 (357.5–660) µm long, respectively; well developed sphincter between uterus and *pars dilatata oviductus*, *pars dilatata* and uteri usually containing numerous sperm cells. Prerectum 426.9±79.7 (310–595) µm long, rectum 45.5±1.6 (43–48) µm or about 0.7–0.8 of body diameter at anus. Tail bluntly conoid, rounded to hemispherical. Two pairs of lateral pores.

Table 2. Measurements of females, males and juvenile stages of *Longidorus cholevae* sp. n. from Bachevo village. All measurements are given in μm (mean ± standard deviation, with range in parentheses).

–	Holo-	Females	Males	J1	J2	J3	J4
n	**type**	**11**	**11**	**9**	**8**	**9**	**11**
L	7199	6788 ± 573 (6127–8083)	6390 ± 594 (5415–7111)	1209 ± 63 (1135–1289)	1874 ± 236 (1554– 2251)	3048 ± 406 (2336–3447)	4798 ± 442 (4148–5666)
a	83.3	72.1 ± 7.4 (61.1–83.3)	70.2 ± 6.2 (63.9–82.0)	47.0 ± 1.9 (43.8–50.3)	51.1 ± 2.4 (49.0–55.3)	56.5 ± 3.8 (50.2–61.3)	63.8 ± 5.9 (54.8–76.6)
b	13.1	14.3 ± 1.5 (12.3–17.9)	12.7 ± 1.2 (10.7–14.7)	4.5 ± 0.4 (3.9–5.1)	5.8 ± 0.9 (4.5–7.2)	7.9 ± 0.9 (7.2–9.9)	10.9 ± 1.5 (9.2–14.1)
c	202.4	199.7 ± 15.4 (171.2–220.4)	199.6 ± 18.3 (171.1–227.8)	29.6 ± 3.8 (26.1–36.6)	48.2 ± 3.6 (43.2–53.9)	78.3 ± 7.2 (66.1–91.3)	136.5 ± 19.9 (115.5–181.1)
c’	0.6	0.6 ± 0.06 (0.5–0.7)	0.6 ± 0.06 (0.6–0.8)	2.1 ± 0.19 (1.8–2.4)	1.4 ± 0.1 (1.2–1.5)	0.9 ± 0.07 (0.8–1.0)	0.7 ± 0.06 (0.6–0.8)
V (%)	52.5	50.5 ± 2.2 (46.7–53.4)	-	-	-	-	-
G_1 (%)	13.0	14.0 ± 2.8 (8.6–17.7)	-	-	-	-	-
G_2 (%)	11.5	14.2 ± 1.6 (11.6–17.1)	-	-	-	-	-
d	1.3	1.3 ± 0.04 (1.2–1.4)	1.3 ± 0.04 (1.3–1.4)	1.7 ± 0.08 (1.6–1.8)	1.6 ± 0.09 (1.5–1.7)	1.6 ± 0.11 (1.4–1.7)	1.5 ± 0.08 (1.3–1.5)
d’	1.5	1.5 ± 0.06 (1.4–1.6)	1.5 ± 0.03 (1.4–1.6)	1.6 ± 0.07 (1.5–1.7)	1.7 ± 0.1 (1.6–1.8)	1.7 ± 0.1 (1.5–1.8)	1.7 ± 0.08 (1.6–1.9)
Odontostyle	121	120.1 ± 7.2 (106–129)	121.2 ± 5.1 (115–131)	61.1 ± 3.5 (56–66)	65.9 ± 2.8 (62–71)	84.7 ± 3.3 (79–90)	99.1 ± 5.3 (88–105)
Replacement odontostyle	-	-	-	65.0 ± 1.8 (61–67)	78.1 ± 4.3 (74–86.5)	101.4 ± 4.5 (96–109)	117.5 ± 7.9 (105.5–131)
Developing gonads	-	-	-	19.9 ± 3.2 (16–25)	28.3 ± 3.2 (24–34)	53.1 ± 8.7 (41–65)	135.5 ± 11.4 (114–147)
Odontophore	88	76.3 ± 3.3 (74 - 81)	73.7 ± 5.0 (69.5 - 81)	41.7 ± 5.4 (36–48)	48.6 ± 3.3 (42 - 52)	62.6 ± 2.5 (60 - 66)	71.1 ± 3.6 (67- 79)
Pharynx	550	481.9 ± 47.8 (439–577)	507.0 ± 45.6 (421–584)	273.6 ± 19.3 (250–302)	318.4 ± 23.6 (277–349)	398.1 ± 47.7 (311.5–450.5)	445.7 ± 38.9 (362–491)
Anterior to guiding ring	36	32.6 ± 2.21 (30–37)	33.5 ± 1.1 (32–36)	16.2 ± 0.7 (15–18)	19.3 ± 0.74 (18–20)	24.2 ± 1.6 (22.5–27)	28.4 ± 1.4 (25.5–31)
Bulb length	139	128 ± 12.5 (114.5–146.5)	124 ± 7.2 (115–137)	60.7 ±5.1 (53–66)	72.5 ± 8.9 (65–90)	100.7 ±5.6 (92–108)	116.8 ± 9.4 (105–128)
Bulb width	34	34.1 ± 2.9 (30–38)	33.0 ± 3.3 (28–38)	15 ± 1.2 (14–17)	20.2 ± 2.0 (18–23)	26.3 ± 1.2 (24–27)	29.8 ± 2.5 (26–34)
Tail	35.5	34.1 ± 2.9 (28.5–38)	32.2 ± 3.3 (29–39)	41.3 ± 4.8 (35–48.5)	38.8 ± 2.8 (34–43)	38.9 ± 3.3 (34–44.5)	35.4 ± 2.6 (31–38)
Length of hyaline part	19	18.1 ± 1.10 (17–20)	14.5 ± 2.2 (12–18)	10.9 ± 1.8 (9–14)	11.8 ± 2.09 (9–15)	13.9 ± 1.6 (12–17)	14.5 ± 1.4 (12–16.5)
Body diameter at: - lip region	22.5	22.8 ± 0.8 (21.5–24)	23.0 ± 0.7 (22–24)	9.5 ± 0.30 (9–10)	12.0 ± 0.6 (11–13)	15.5 ± 1.2 (14–17)	19.5 ± 1.0 (18–21)
- guiding ring	39	37.5 ± 2.5 (35–43)	37.7 ± 1.7 (34– 40)	15.1 ± 0.32 (14–15.5)	20.1 ± 1.09 (18–22)	26.2 ± 1.8 (23.5–28.3)	33.2 ± 1.7 (30 - 35.5)
- base of pharynx	74.5	75.9 ± 8.4 (69–100)	77.5 ± 8.8 (66–90.5)	26.3 ± 2.0 (24–30)	35.2 ± 2.8 (31–38)	49.0 ± 4.60 (41–53)	63.4 ± 5.2 (57–77)
- mid-body/at vulva	86	93.7 ± 9.1 (83–106)	91.7 ± 11.5 (73–111)	25.9 ± 2.1 (23–29.5)	36.6 ± 3.8 (31–41)	54.1 ± 7.2 (43.5–68)	75.6 ± 8.5 (66–94)
- anus	57	54.8 ± 4.4 (48–66)	52.4 ± 4.0 (46–58)	19.5 ± 1.2 (18–22)	28.7 ± 2.3 (25–31)	42.8 ± 4.3 (36–48)	52.2 ± 2.5 (47–56)
- hyaline part	47	42.9 ± 3.8 (37–48)	36.0 ± 4.0 (27–42)	10.9 ± 1.77 (9–14)	17.9 ± 2.4 (14.5–21)	29.0 ± 3.4 (25–37)	37.2 ± 3.0 (33–42)
Spicules	-		105.9 ± 6.9 (96–120)				

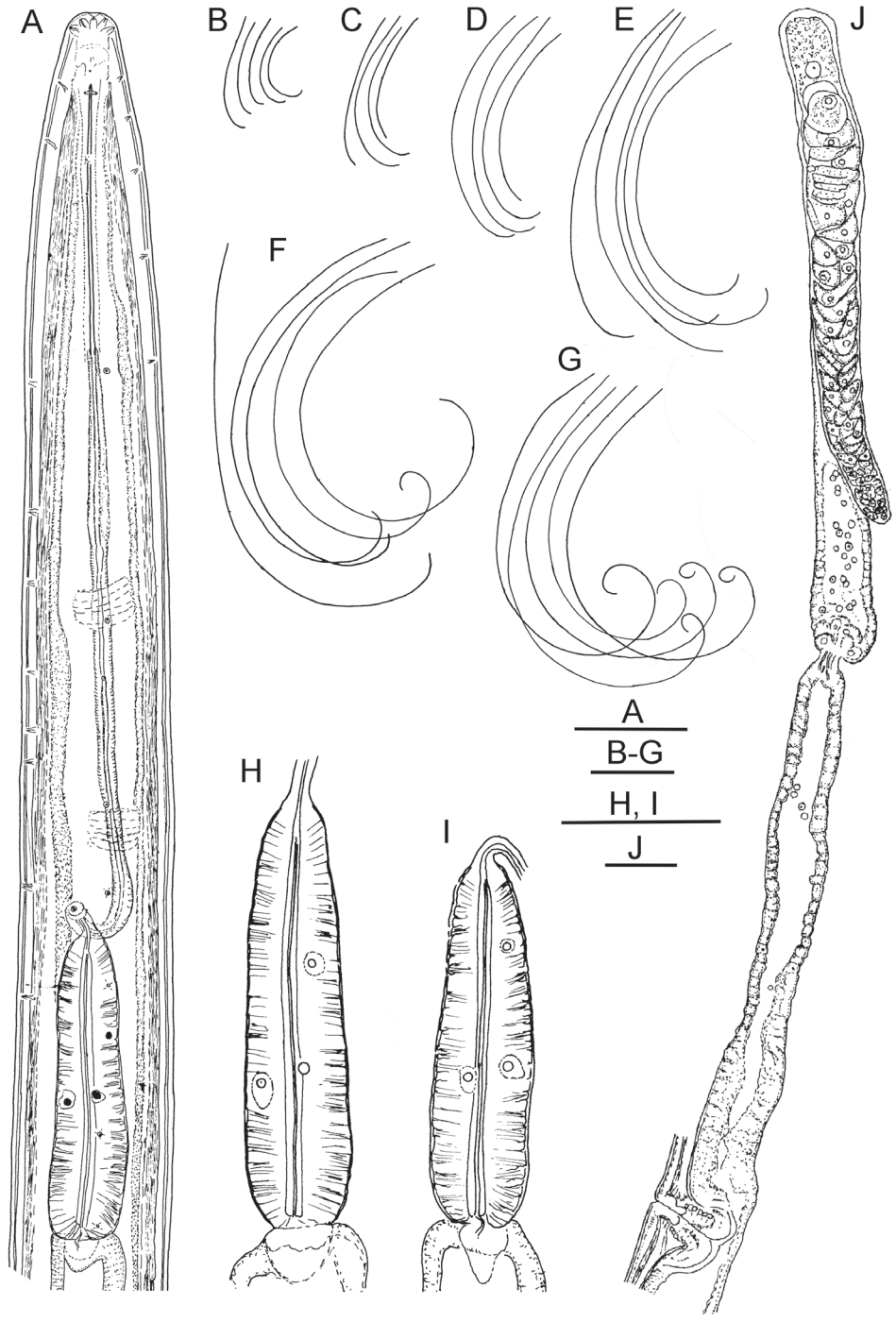

Figure 1. *Longidorus cholevae* sp. n. *Female*: **A** Anterior end **F** Habitus **I** Pharyngeal bulb **J** Anterior genital branch *Male:* **G** Habitus **H** Pharyngeal bulb *Juveniles*: **B–E** Habitus of first-, second-, third- and fourth-stage juveniles. Scale-bars: **A, H, I, J** 50 μm; **B–G** 1 mm.

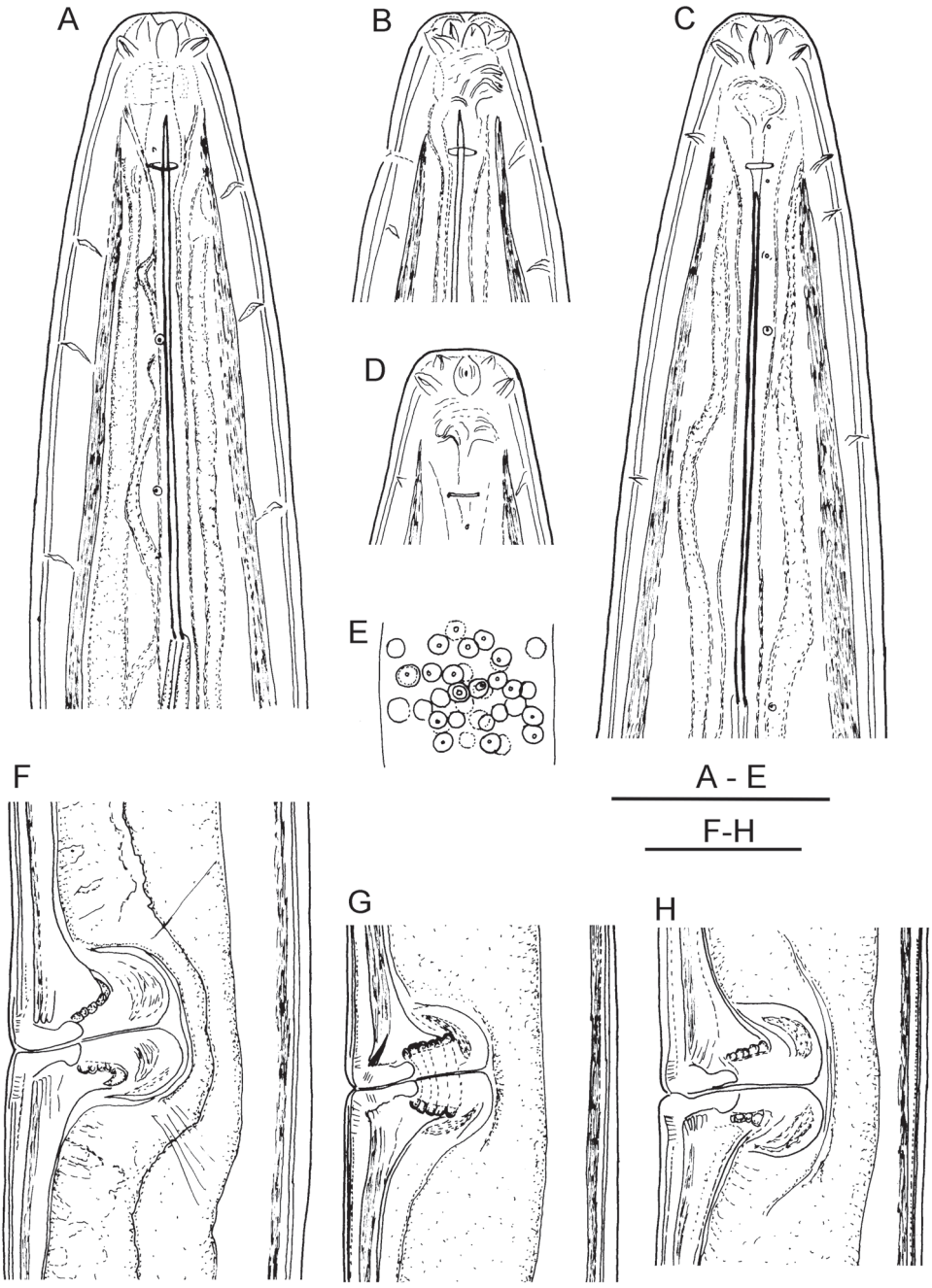

Figure 2. *Longidorus cholevae* sp. n. *Female*: **A** Anterior end **B** Lip region/amphidial fovea **F–H** Variations in vagina shape; *Male*: **C** Anterior end **D** Lip region/amphidial fovea **E** Sperms. Scale-bars: **A–H** 50 μm.

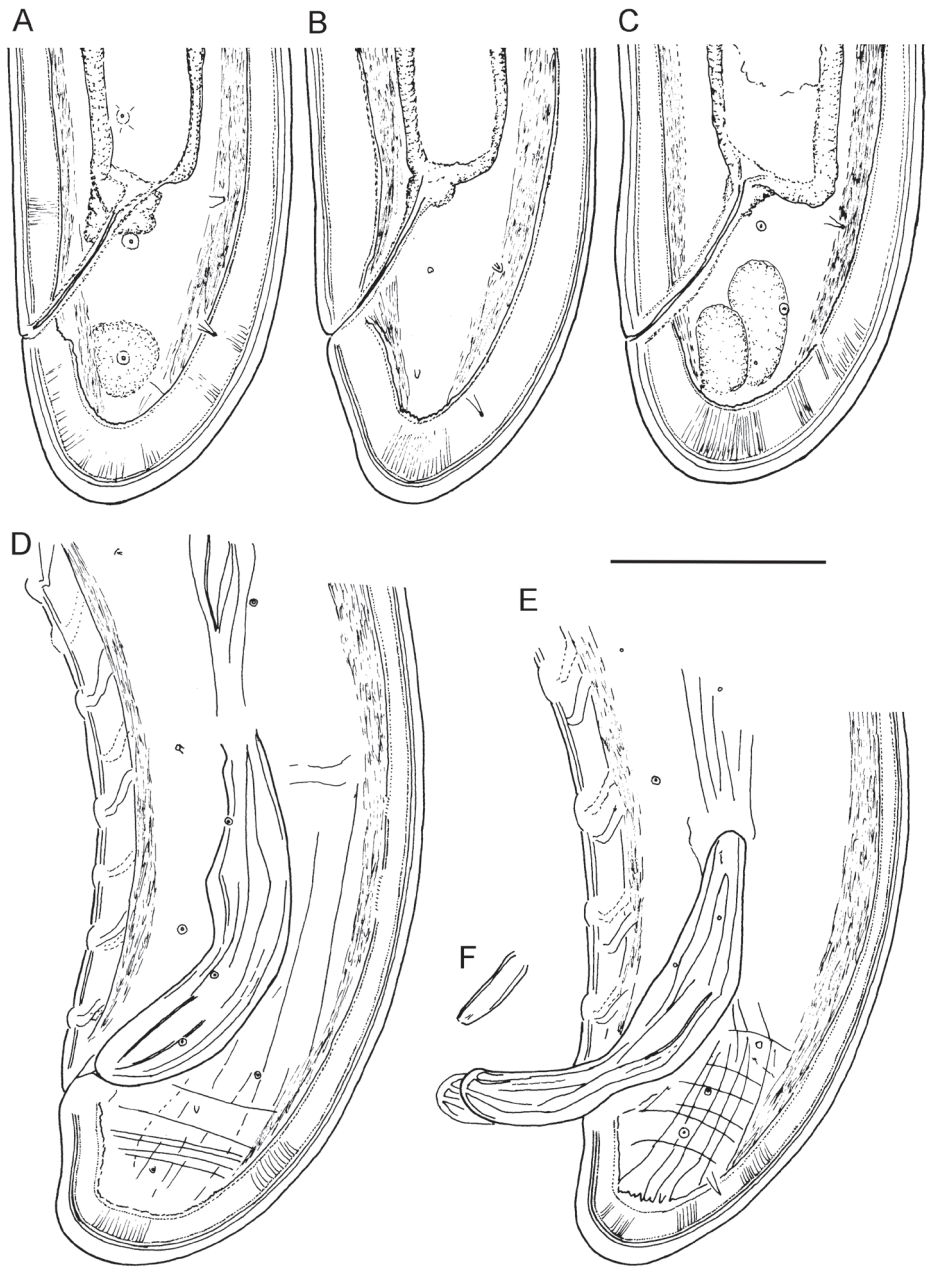

Figure 3. *Longidorus cholevae* sp. n. *Female*: **A–C** Variations in tail shape; *Male*: **D, E** Tail ends with spicules **F** Lateral piece. Scale-bars: 50 μm.

Male. Habitus as in females, posterior part more strongly coiled ventrad. Shape of lip region similar to that in females. Cuticle 5–8 μm thick at poslabial region, 7–9 at guiding ring level, 4–6 μm along the body and 9–13 μm on tail posterior to cloaca.

One lateral pore anterior to guide ring, 2–3 along odontostyle, 1–2 along odontophore, 3–5 in narrow part of the oesophagus and 3–4 in pharyngeal bulb region as 4 dorsal and 7–10 ventral; numerous lateral body pores present. Fusus at 52.3±3.7 (47–57) μm, n=7 from anterior end. Nerve ring surrounding odontophore base, at 231.8±12.2 (217.5–259.5) μm from anterior end, a second nerve ring situated at a short distance behind the first one. Pharyngo-intestinal valve, variable in shape (broadly rounded to heart-shape) and size, almost as long as wide: 16.6±3.2 (13–23) × 18±3.1 (13–22) μm, n=6. Lateral chord 20–25 μm wide. Supplements 3–4 adanal pairs followed by 10–14 arranged irregularly in a single row. Spicules massive, slightly curved ventrally, lateral guiding piece 27–28 μm long. Spermatozoids round small (4–6 μm diam.). Tail short, bluntly conoid, dorsally convex, ventrally slightly concave, three pairs of lateral pores.

Juveniles. Morphometrics obtained from juvenile specimens, and of the relationship between the lengths of their functional and replacement odontostyles and body lengths, confirmed the presence of four juvenile stages (Figure 9). Habitus in the shape of more or less open C, tail of the first stage juvenile conoid elongated whereas in the subsequent developmental stages the tail is conoid (second stage) to bluntly conoid (third and fourth stage).

Type locality and plant association. Bachevo village, Rila Mountains, co-ordinates 41°56'14.97"N, 23°25'15.02"E, 1032 m asl, riparian vegetation; soil around the roots of wild cherry (*Prunus avium* L.), *Juniperus communis* L., *Urtica dioica* L. and grasses.

Type material. Holotype and 1 paratype females, 2 males, and 23 juveniles deposited in the nematode collection of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria. Other paratypes deposited as follows: two females, one male and 8 juveniles in the Nematode collection of the Foodand Environment Research Agency, Sand Hutton, UK (former Rothamsted Nematode Collection); one female, one male and 6 juveniles in the USDA Nematode Collection, Beltsville, Maryland, USA; one female, one male and 8 juveniles in the Riverside Nematode Collection, University of California, Riverside, USA; one female, one male and 5 juveniles in the Nematode Collection of the Institute of Plant Protection, Bari, Italy; one female, one male and 12 juveniles in the Wageningen Nematode Collection (WANECO), Wageningen, the Netherlands.

Diagnosis and relationships. *Longidorus cholevae* sp. n. is a comparatively large bisexual species (6.1–8.1 mm) with odontostyle over 100 μm (106–129 μm) long, lip region wide (21.5–24 μm), continuous, anteriorly rounded, amphidial fovea pouch like, almost as wide as long, posteriorly situated guide ring, short, bluntly rounded to hemispherical tail and normal arrangement of pharyngeal glands.

The alpha-numeric codes for *L. cholevae* sp. n. to be applied to the polytomic identification key for *Longidorus* species by Chen et al. (1997) are, A45, B4, C23, D1, E5, F34, G12, H1, I2. The group of comparatively large *Longidorus* species (code F34) with a long odontostyle (code A45), pouch like amphidial fovea, elongate funnel (E4) or short (funnel or stirrup shaped (E5), normal arrangement of pharyngeal glands and short rounded tail (code H1) consists of a few species: *L. poessneckensis*, *L. caespiticola*, *L. macrosoma*, *L. helveticus*, *L. carniolensis*, *L. macroteromucronatus* Altherr, 1974, *L.*

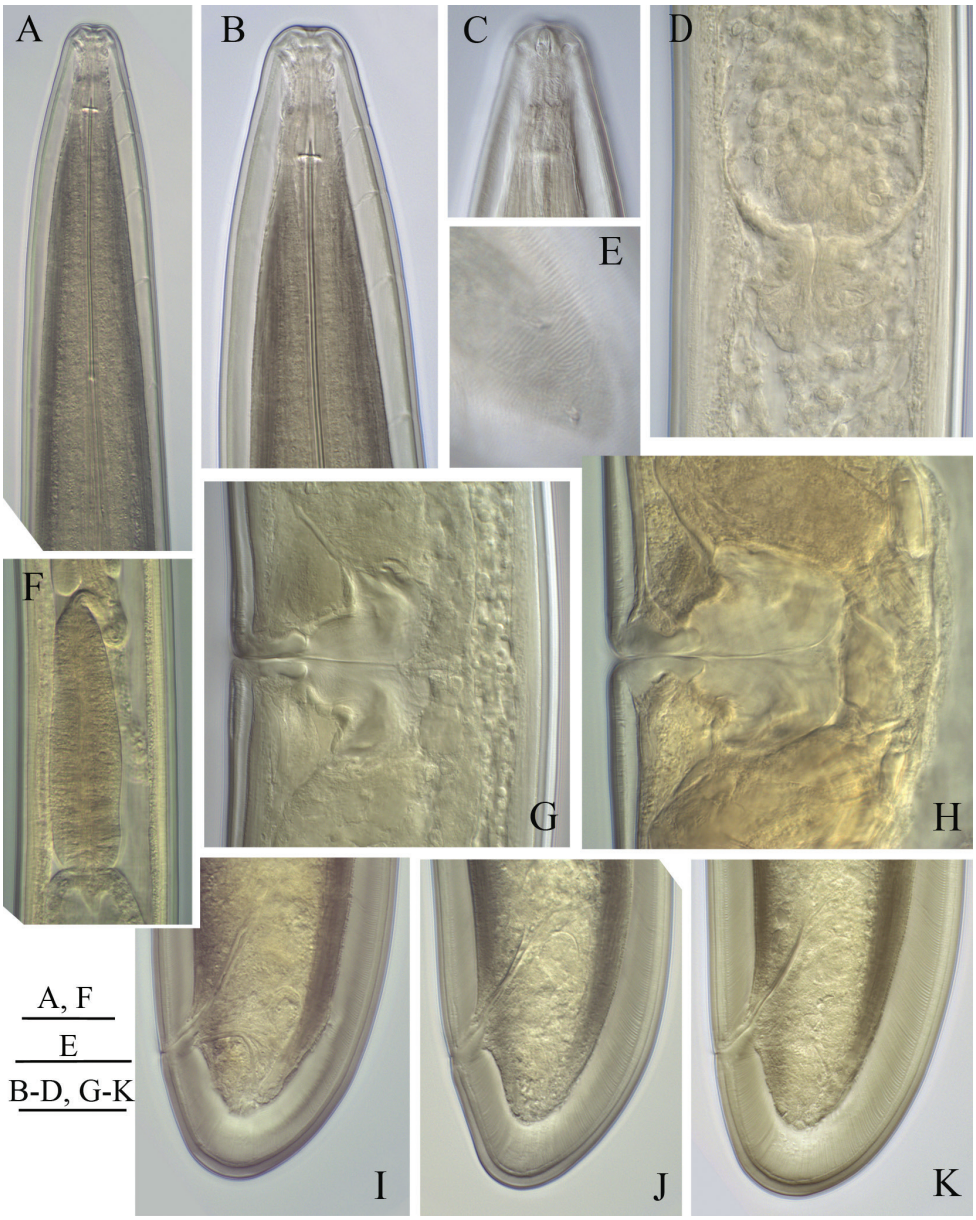

Figure 4. *Longidorus cholevae* sp. n. *Female:* **A** Anterior region of holotype **B** Head end **C** Amphidial fovea of holotype **D** Sphincter between uterus and *pars dilatata oviductus* **E** Caudal pores **F** Pharyngeal bulb **G, H** Variations in vagina shape; **I–K** Variations in tail shape. Scale bars: **A, F** 40 μm; **E** 20 μm; **B–D, G–K** 30 μm.

pseudoelongatus Altherr, 1976, *L. pius*. It differs from all these species except for *L caespiticola* and *L. pseudoelongatus*, by the more anteriorly situated guide ring (ave. 32.6 (30–37) *vs* ave. 40 (36–43) μm in *L. poessneckensis*; 37–48 μm in *L. macrosoma*;

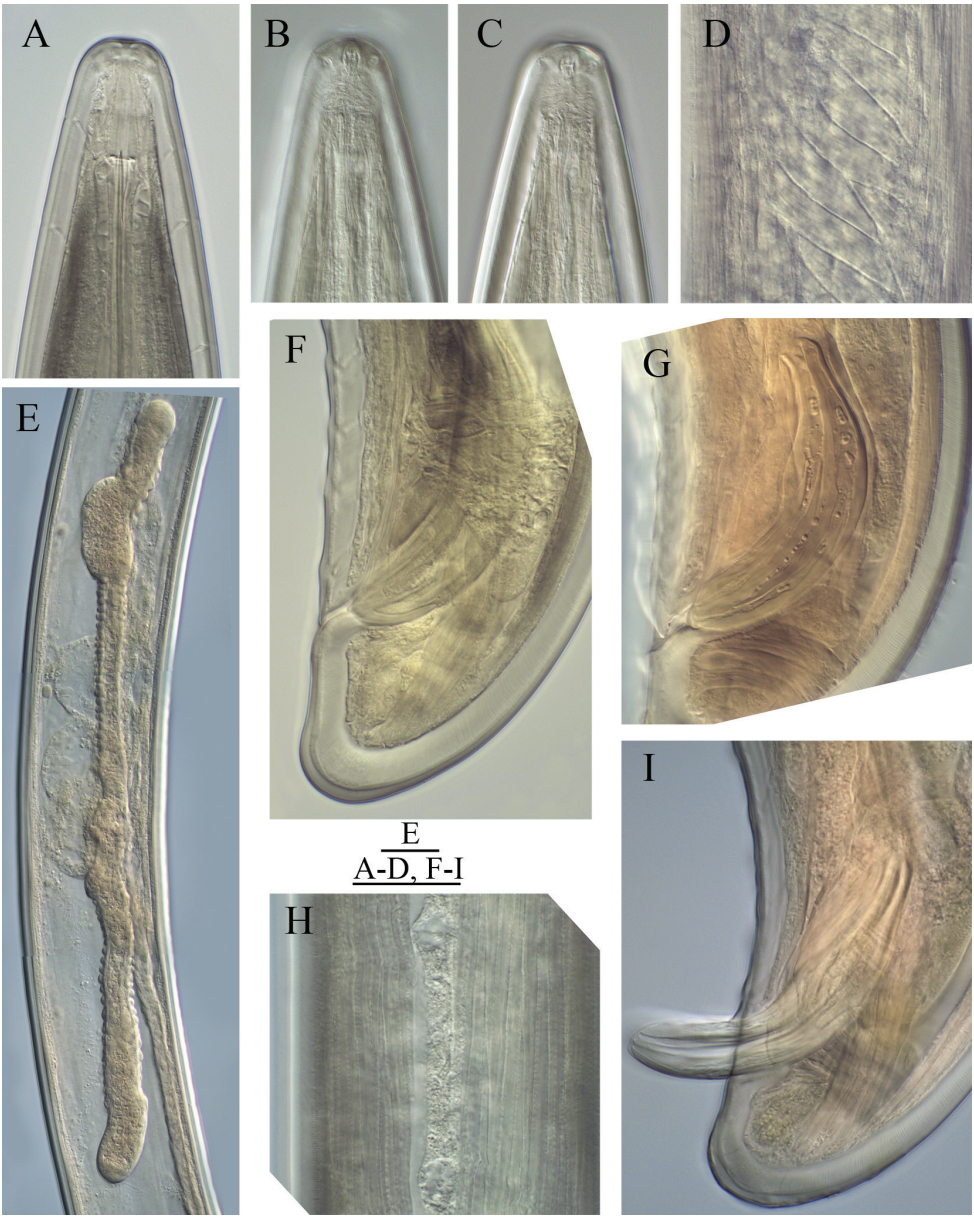

Figure 5. *Longidorus cholevae* sp. n. *Male:* **A** Lip region **B, C** Amphidial fovea, **B** upper view **C**, lower view **D** Part of testis with radial muscles **E** Genital system of a young male **F** Tail, **G** Spicule **H** Lateral field **I** Protracted spicule. Scale bars: **A–D, F–I** 30 μm; **E** 40 μm.

37.5–48 μm in *L. helveticus*; 42–47 μm in *L. carniolensis*; 38 μm in *L. macroteromucronatus* and ave. 38.7 (35–41) μm in *L. pius*). Among the above group the new species appears most similar to *L. poessneckensis* from which it differs by adult specimens

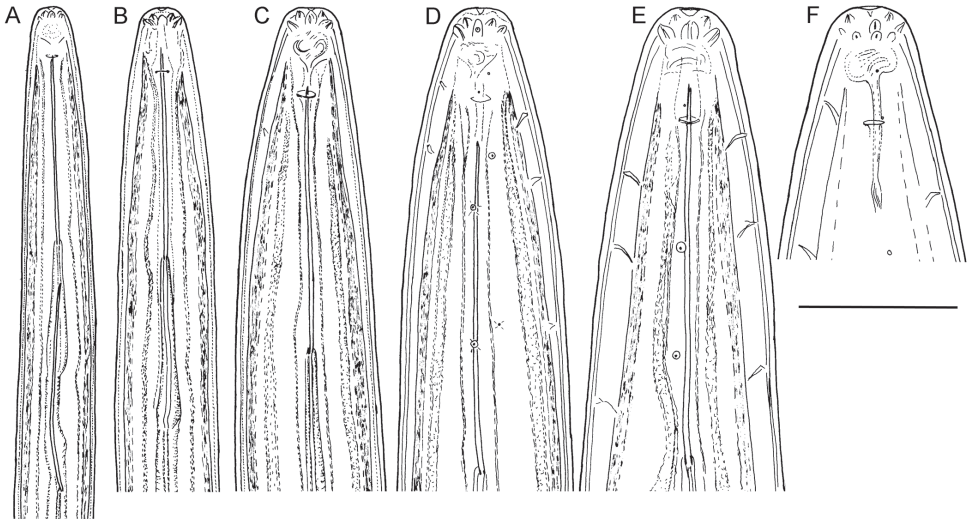

Figure 6. *Longidorus cholevae* sp. n. Anterior region of: **A–D** First-fourth juvenile stage **F** Female **G** Male. Scale-bar: 50 μm.

having different shape of amphidial pouches (almost as long as wide *vs* visbly longer than wide), males abundant *vs* males rare and different tail shape in first stage juveniles (elongate conoid with narowly tapering terminus *vs* elongate conoid with blunly rounded terminus) (Sturhan and Loof 2001, Kumari et al. 2009, Lišková and Kumari 2010, Kornobis and Peneva 2011). Further, it can be differentiated from:

L. caespiticola by females having wider (21.5–24 *vs* 16–18 μm) and differently shaped lip region (rounded *vs* smoothly rounded, almost conical), shorter tail (28.5–38 *vs* 39–47 μm) and longer spicules (96–120 *vs* 88.5–93 μm), and tail in first stage juveniles (elongate conoid *vs* bluntly conoid) (Boag and Brown 1975);

L. macrosoma by female specimens having a somewhat shorter body (L= ave. 6.8 mm (6.1–8.1) *vs* ave. 9.1 mm (6.8–12), diferently shaped lip region (rounded *vs* slightly concave) and differently shaped tail of the first stage juvenile (elongated conoid *vs* digitate) (Brown and Boag 1975);

L. helveticus by females having different shape of amphidial fovea (almost rounded *vs* elongated), shorter odontostyle (ave. 120.1 (106–129) μm *vs* ave. 135. 4 (127–145.5) in the type population and reported range for other populations 127–142 μm, differently shaped tail in first stage juvenile (elongated conoid *vs* mucronated) and shorter hyaline portion of tail (J=10–14 *vs* J=17.5–33 μm) (Lamberti et al. 2001, Barsi and De Luca 2005, Širca and Urek 2009, Kumari and Subbotin 2012);

L. carniolensis – by having a shorter odontostyle (106–129 *vs* 136–157 μm); males with shorter spicules (96–120 *vs* 122–145 μm); different tail shape in first stage juvenile (elongate conoid *vs* rounded, conoidal, c'=1.8–2.2 vs c'=1.2–1.5) (Širca et al. 2011);

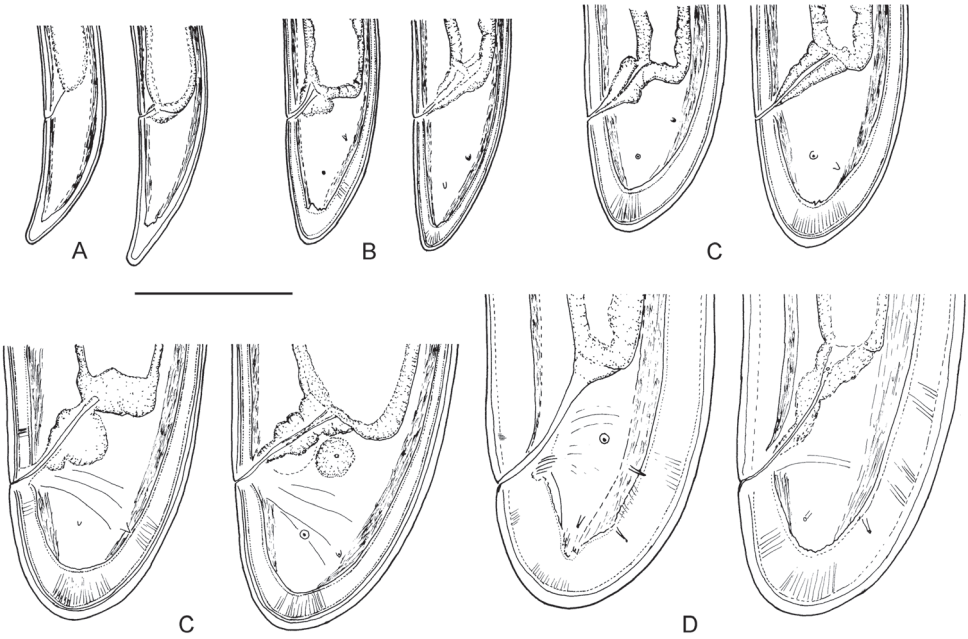

Figure 7. *Longidorus cholevae* sp. n. Variations in tail shape: **A–D** Tail of first-fourth juvenile stage **E** Female Scale-bar: 50 μm.

L. macroteromucronatus – by females having wider lip region (21.5–24 *vs* 17.5 μm (calculated from the drawing by Altherr (1974), shorter odontostyle (106–129 *vs* 133 μm) and higher **c** values (**c**=171.2–220.4 *vs* **c**=160);

L. pius - by different **d** and **d'** values (following Brown et al. 1994) (**d**= 1.2–1.4 *vs* **d**=1.7–1.9; **d'**= 1.4–1.6 *vs* **d'**=1.9–2.1), shorter odontostyle (ave. 120.1 (106–129 *vs* ave. 136.5 and 137.5 (128–147.5) μm), shorter tail (28.5–38 *vs* 37–46.5 μm), higher **c** value (**c**=171.2–220.4 *vs* **c**=114.6–166.5) in females; males abundant *vs* males rare, and different tail shape in first stage juveniles (elongate conoid *vs* subdigitate, J=10–14 *vs* J=15–20 μm) (Barsi and Lamberti 2001, Barsi and De Luca 2008). Although in the original description the code for amphidial fovea shape is D1, but in the photos it appears more like that in the new species;

L. pseudoelongatus – by having a longer body (L=6.1–8.1 *vs* L=5.1–5.6 mm), differently shaped (continious *vs* separated by constriction) and wider lip region (21.5–24 *vs* 12 μm), higher **c** (**c**=171.2–220.4 *vs* **c**=100–150) and lower **c'** values (**c'**=0.5–0.7 *vs* **c'**=0.93) (Altherr 1976).

Further, *L. cholevae* sp. n. is similar in body and odontostyle lengths (codes F34 and A45), and shape of anterior region and tail (codes D1 and H1) with a group of several other species from which it differs in amphidial fovea shape (see Appendix 2: a partial polytomous key): *L. kheirii*, *L. raskii* Lamberi & Agostinelli, 1993, *L. arthensis*

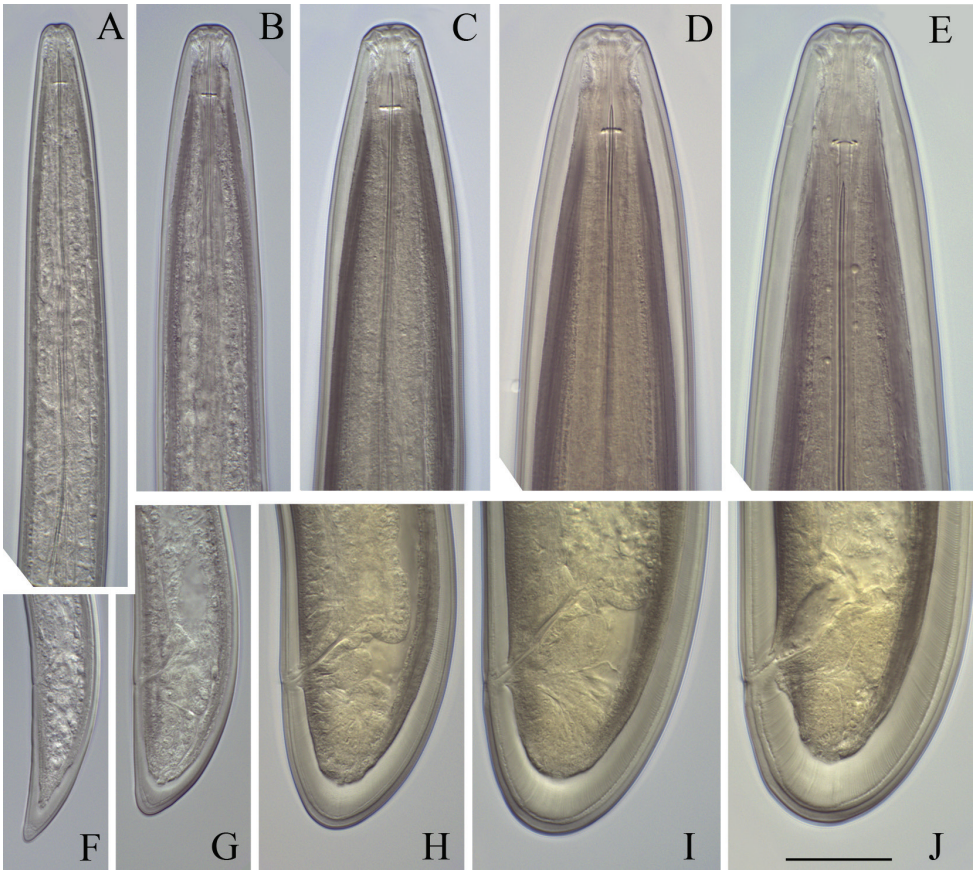

Figure 8. *Longidorus cholevae* sp. n. *Juveniles:* **A–D** Head ends of first- to fourth-stages **F–I** Tail end of first- to fourth-stages *Female:* **E** Anterior end **J** Tail. Scale-bar: 30 µm.

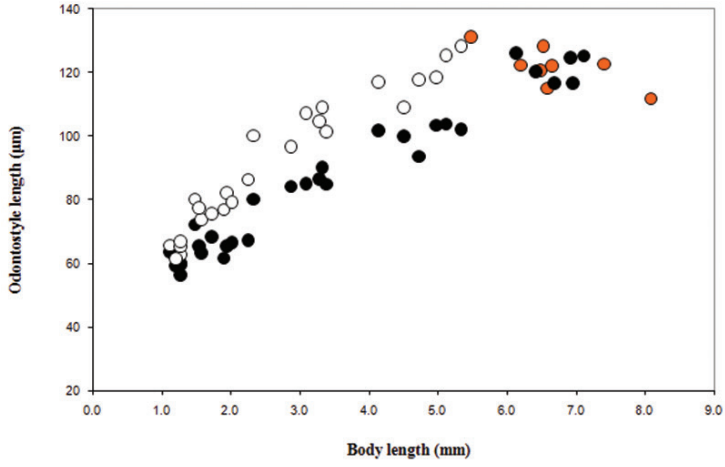

Figure 9. *Longidorus cholevae* sp. n. Scatter plot of the functional (●, juveniles and adults, females in orange) and replacement (○, juveniles) odontostyle in relation to body length of the juvenile developmental stages and adults.

Brown, Grunder, Hooper, Klingler & Kunz, 1994, *L. fasciatus* Roca & Lamberti, 1981, *L. uroshis* Krnjaić, Lamberti, Krnjaić, Agostinelli & Radicci, 2000, *L. silvae* Roca, 1993, *L. iuglandis* Roca, Lamberti & Agostinelli, 1984, *L. saginus* Khan, Seshardi, Weischer & Mathen, 1971, *L. picenus* Roca, Lamberti & Agostinelli, 1984, *L. baeticus*). The new species can be distinguished from *L. raskii, L. arthensis, L. fasciatus L. uroshis, L. silvae, L. picenus* and *L. baeticus* by its wider lip region (21.5–24 µm *vs* 15–19 µm; 14–19 µm; 12–14 µm; 14–20.5; 14–17 µm; 14–16 µm; 19–22 µm; 12–14.5 µm); from *L. raskii, L. uroshis* and *L. saginus* by having different odontostyle length (106–129 µm *vs* 76–103 µm; 120–152 µm and 135–155 µm); from *L. kheirii*, *L. raskii, L. arthensis* and *L. uroshis* by the shorter tail (28.5–38 *vs* 47–72 µm; 36–50 µm; 36–46.5 µm and 38–57 µm); from *L. kheirii*, *L. silvae* and *L. picenus* by having more anteriorly situated guide ring (30–37 *vs* 36.5–45 µm, 36–48 µm and 37–42 µm). Additionally, it can be differentiated from:

L. kheirii by females having differently shaped lip region (rounded *vs* slightly concave), higher **c** value (171.2–220.4 *vs* 119–167.8), smaller pharyngeal bulb (114.5–146.5 × 30–38 *vs* 149.5–193.5 × 39.5–48 µm), males abundant, functional *vs* rare and not functional, differently shaped tail of the first stage juvenile as well as different morphometrics concerning the main characters such as body and tail length, functional and replacement odontostyle length (Table 1; Table 2 in Pedram et al. 2008).

L. raskii by females having different tail shape in first stage juveniles (elongate conoid *vs* bluntly conoid); (Lamberti et al. 2001, Krnjaić et al. 2002, Barsi and De Luca 2005);

L. arthensis by females having lower **c'** value (**c'**=0.5–0.7 *vs* **c'**=0.8–1.1 and 0.9–1.1); males with longer spicules (96–120 vs 60–66 µm); different tail shape in first stage juveniles (elongate conoid *vs* digitate) (Brown et al. 1994, Lamberti et al. 2001);

L. fasciatus by females having a more plump body (**a**=61.1–83.3 *vs* **a**=121–143) (Roca and Lamberti 1981);

L. uroshis by males with longer spicules (96–120 *vs* 59–72 and 64–78 µm) and different tail shape in first stage juveniles (elongate conoid *vs* digitated) (Krnjaić et al. 2000, Krnjaić et al. 2002, Sturhan and Lišková 2002);

L. silvae by female specimens having differently shaped lip region (rounded *vs* subacute and flattened anteriorly) and tail of the first and second stage juvenile (elongated conoid *vs* mucronated; conoid *vs* bluntly rounded, respectively), and males abundant *vs* males rare (Roca 1993, Barsi and Lamberti 2004, Barsi et al. 2007).

L. iuglandis by having longer uteri (357.5–662.5 *vs* 140–160 µm) and differently shaped tail in the first stage juvenile (elongate conoid *vs* bluntly rounded) (Roca et al. 1984);

L. saginus by having a longer body (L=6.1–8.1 *vs* 4.8–6.4 mm); lower **c'** value (**c''**=0.5–07 *vs* **c'**=0.8); more posteriorly situated vulva (**V**=46.7–53.4 *vs* **V**=40–45) (Khan et al. 1971);

L. picenus by having, differently shaped tail in the first stage juvenile (elongate conoid *vs* mucronated) (Roca et al. 1985);

L. baeticus by males having longer spicules (96–120 *vs* 80–95 µm) and differently shaped tail in the first stage juvenile (elongate conoid *vs* bluntly rounded to cylindrical).

Etymology. The species is named after Dr Boryana Choleva, Faculty of Biology, University of Sofia, retired, for her substantial contribution to the knowledge of the fam. Longidoridae in Bulgaria.

Phylogenetic relationships of *Longidorus cholevae* with other *Longidorus* species

The amplification of D2-D3 expansion domains of the 28S rDNA and the ITS containing region yielded single fragments of 800 bp and 1384 bp, respectively, based on sequencing. The ITS1 and ITS2 sizes were 579 bp and 338 bp, respectively that resulted in the shortest ITS recorded for *Longidorus* so far. Intra-individual and intra-population sequence variability in ITS and no variability in D2D3 domains have been observed.

A BLAST search for D2-D3 region showed a 80-93% degree of similarity among *Longidorus* spp. suggesting that *L. cholevae* can be easily identified from other species by using this ribosomal region. The closest species were *L. poessneckensis* (93% similarity), *L. caespiticola*, *L. macrosoma* and *L. helveticus* (92% similarity). Pairwise BLAST comparisons of the ITS sequence of *L. cholevae* with those of *Longidorus* spp. from the database displayed high nucleotide dissimilarity and considerable variation in length.

Our preliminary phylogenetic analyses based on all the D2-D3 *Longidorus* sequences deposited in NCBI revealed that the new species clusters into a well-supported group of *Longidorus* species having a European distribution: *L. caespiticola, L. macrososma, L. poessneckensis, L. helveticus* and *L. carniolensis* (trees not presented). The monophyly of this group has been highly supported also in other studies, including SSU phylogenetic analyses (Robbins et al. 2009, Gutiérrez-Gutiérrez et al. 2013). All these are large species, very similar in their morphology having long odontostyles, elongated or short not bilobed pouch-like amphidial fovea, continuous head region, short bluntly conoid to almost hemisphaercial tail, mainly amphimictic (only with *L. macrosoma* and *L. poessneckensis* males are rare). *Longidorus caespiticola* and *L. macrososma* occur mainly in western Europe including the British Isles, *L. poessneckensis* was reported from central (Germany, Slovakia and Czech Republik) and northern Europe (Poland); the first two species were found in association with a wide range of crops and forest trees (Brown and Boag 1975, Boag and Brown 1975); *L. poessneckensis* with preference to flood plains and hill deciduos forest habitat (Lišková and Kumari 2010) and *L. helveticus* associated with deciduous forest and orchard threes in central Europe (Lamberti et al. 2001, Širca and Urek 2009, Kumari and Subbotin 2012). *Longidorus carniolensis* is known only from Slovenia (grapevine) and *L. cholevae* sp. n. - only from Bulgaria (riparian vegetation). Probably, *L. pius*, known so far only from Macedonia and having similar morphology, is part of this group, however, no sequences of D2-D3 region are available.

Further, for phylogenetic analysis *Longidorus* species from GenBank with the highest match of BLAST search were aligned along with *L. cholevae* D2-D3 and partial 18S-ITS1 sequences and these alignments included sequences from various populations (Table1).

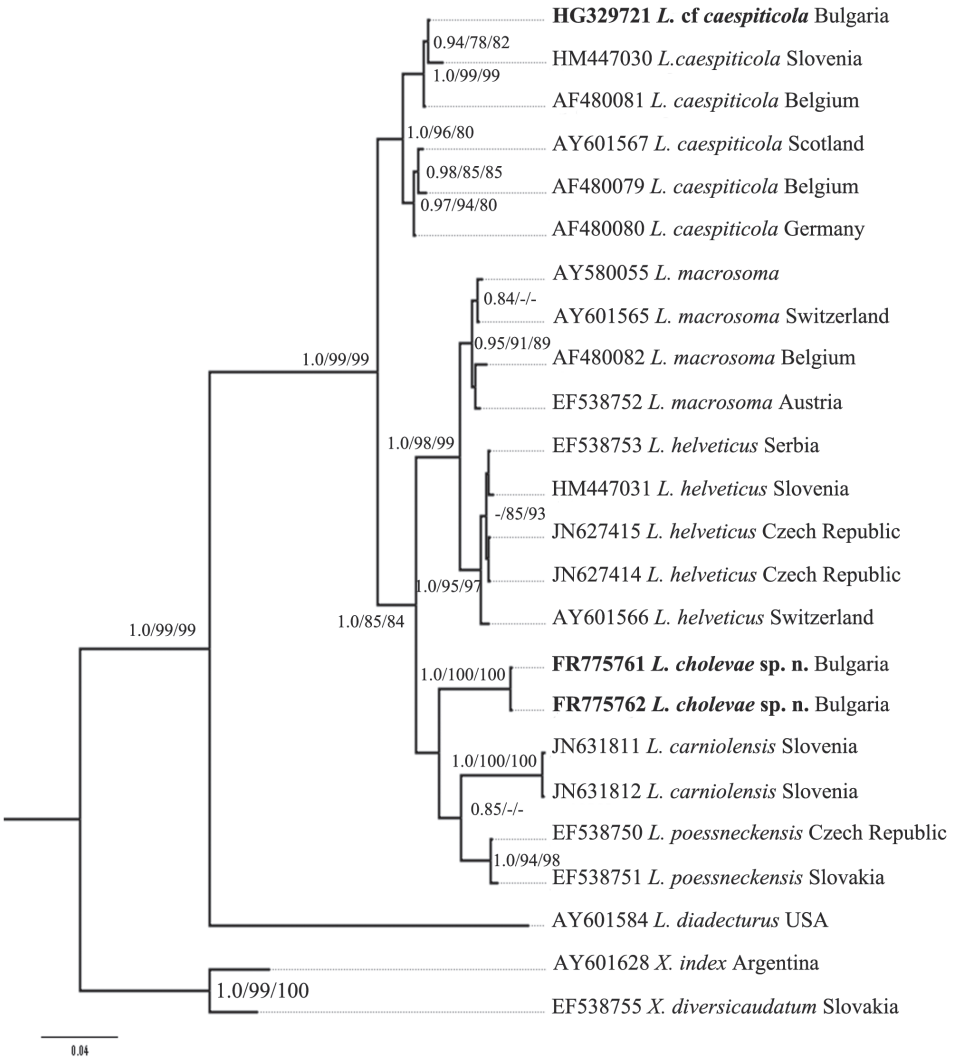

Figure 10. Phylogenetic relationships of *Longidorus cholevae* sp. n. and its closest species for the D2-D3 rDNA. Bayesian Inference strict consensus tree acquired under GTR+G model. Numbers at the nodes indicating posterior probabilities higher that 0.8 and bootstrap values more that 70% for ML and NJ are presented.

The trees obtained by NJ, ML and BI methods showed similar topology and differed in the position of poorly supported clades, and thus only the BI trees with posterior probabilities higher than 0.8 and bootstrap values above 70% (NJ and ML) are presented (Figs 10–11).

The phylogenetic tree of the D2-D3 region (Fig. 10) showed two well-supported clades: Clade I consists of three subclades: two highly supported subclades containing various populations of I1) *L. helveticus* and I2) *L. macrosoma*, and one subclade having lower values for ML bootstrap support (52%) and BI posterior probabilities (0.72) I3) that includes the new species *L. cholevae*, two populations of *L. carniolensis* from

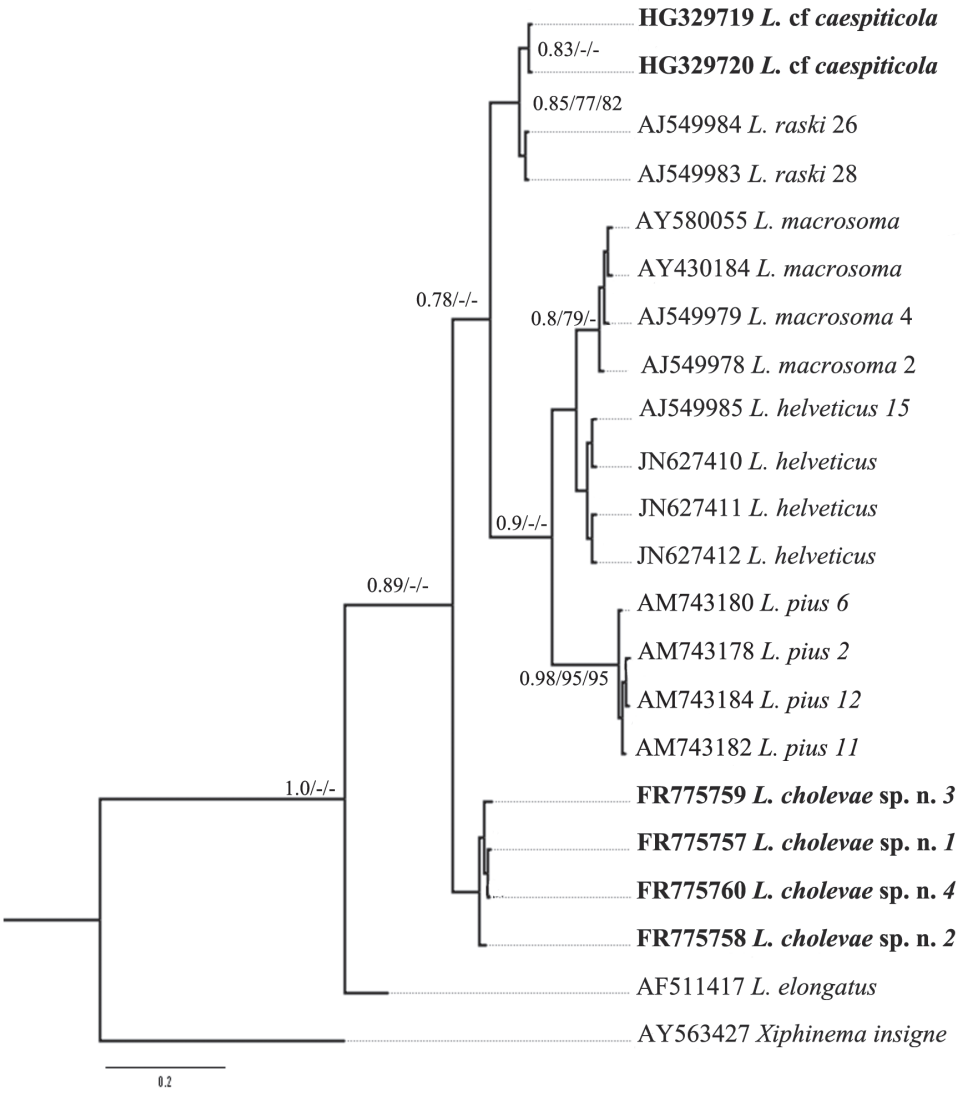

Figure 11. Phylogenetic relationships of *Longidorus cholevae* sp. n. and its closest species for the partial 18S-ITS1 rDNA regions. Bayesian Inference strict consensus tree acquired under K2+G model. Numbers at the nodes indicating posterior probabilities higher that 0.8 and bootstrap values more that 70% for ML and NJ are presented.

Slovenia and two populations of *L. poessneckensis* from the Czech Republic and Slovakia. The second clade (II) consists of two well-supported subclades: II1) consisted of *L. caespiticola* from Slovenia and Belgium and one *L.* cf. *caespiticola* from Bulgaria and subclade II2) consisted of three populations of *L. caespiticola* from Scotland, Belgium and Germany. It is possible that these populations represent two different species that requires further investigation.

The phylogenetic reconstructions of the partial 18S-ITS1 region revealed more unstable groups due to the shorter sequence length and higher sequence variability. Three of the *Longidorus* spp. belonging to the above mentioned group (*L.* cf. *caespiticola*, *L. helveticus* and *L. macrosoma*) and two additional species (*L. pius* and *L. raskii*) originating from Macedonia and Switzerland have been separated from other ITS1 *Longidorus* sequences (the tree not presented) and further analysed (Fig. 11). Three clades were distinguished, two well supported clades consisting of: 1) *Longidorus macrosoma*, *L. helveticus* and *L. pius* and 2) *Longidorus* cf. *caespiticola* and *L. raskii*, and one not well resolved 3) containing only *L. cholevae* sp. n. The species forming these clades have similar tail shape in first stage juveniles: digitate in clade 1, bluntly conoidal in clade 2, elongate conoidal in clade 3.

Acknowledgements

The study was supported by the National Science Fund grants: No B-1405/04, CEBDER (DO 02-15) and BioCORE (INI 03/01.08.2005) projects and the project ANIDIV, funded by the Bulgarian Academy of Sciences. Mutual visits of authors have been supported by a mobility program between the Bulgarian Academy of Sciences and the Consiglio Nazionale delle Ricerche (Bilateral Collaboration Project between BAS and CNR).

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Appendix 1

List of the species of the genus *Longidorus* (doi: 10.3897/zookeys.330.5750.app1) File format: Microsoft Word Document (doc).

Explanation note: List of the species of the genus *Longidorus* Micoletzky, 1922.

Citation: Peneva VK, Lazarova SS, De Luca F, Brown DJF (2013) Description of *Longidorus cholevae* sp. n. (Nematoda, Dorylaimida) from a riparian habitat in the Rila Mountains, Bulgaria. ZooKeys 330: 1–26. doi: 10.3897/zookeys.330.5750 List of the species of the genus *Longidorus*. doi: 10.3897/zookeys.330.5750.app1

Appendix 2

A partial polytomous key to the species of *Longidorus* (doi: 10.3897/zookeys.330.5750.app2) File format: Microsoft Word Document (doc).

Explanation note: A partial polytomous key to the species of *Longidorus* with long odontostyle (A45) and short tail (H1) based on the key by Chen et al. (1997) incorporating species described after 1997 and those transferred from other genera, see Appendix 1.

Citation: Peneva VK, Lazarova SS, De Luca F, Brown DJF (2013) Description of *Longidorus cholevae* sp. n. (Nematoda, Dorylaimida) from a riparian habitat in the Rila Mountains, Bulgaria. ZooKeys 330: 1–26. doi: 10.3897/zookeys.330.5750 A partial polytomous key to the species of *Longidorus*. doi: 10.3897/zookeys.330.5750.app2

ZooKeys 330: 27–52 (2013)
doi: 10.3897/zookeys.330.5852
www.zookeys.org

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Systematics of a widely distributed western North American springsnail, *Pyrgulopsis micrococcus* (Caenogastropoda, Hydrobiidae), with descriptions of three new congeners

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Academic editor: *Eike Neubert* | Received 24 June 2013 | Accepted 26 August 2013 | Published 10 September 2013

http://www.zoobank.org/64BCF75A-87C9-4302-B4B4-CEC439450805

Citation: Hershler R, Liu H-P, Bradford C (2013) Systematics of a widely distributed western North American springsnail, *Pyrgulopsis micrococcus* (Caenogastropoda, Hydrobiidae), with descriptions of three new congeners. ZooKeys 330: 27–52. doi: 10.3897/zookeys.330.5852

Abstract

We describe three new species of springsnails (genus *Pyrgulopsis*) from the Amargosa River basin, California and Nevada (*P. licina* **sp. n.**, *P. perforata* **sp. n.**, *P. sanchezi* **sp. n.**), each of which was previously considered to be part of *P. micrococcus*. We also restrict *P. micrococcus* to its type locality area (Oasis Valley) and redefine a regional congener, *P. turbatrix*, to include populations from the central Death Valley region and San Bernardino Mountains that had been previously identified as *P. micrococcus*. The five species treated herein form genetically distinct lineages that differ from each other by 4.2–12.6% for mtCOI and 5.2–13.6% for mtNDI (based on previously published and newly obtained data), and are diagnosable by shell and/or penial characters. The new molecular data presented herein confirm sympatry of *P. licina* and *P. sanchezi* in Ash Meadows (consistent with morphological evidence) and delineate an additional lineage of *P. micrococcus* (in the broad sense) that we do not treat taxonomically owing to the paucity of morphological material. Conservation measures are needed to ensure the long term persistence of populations of *P. micrococcus* and a genetically differentiated lineage of *P. sanchezi* which live in disturbed habitats on private lands.

Keywords

Pyrgulopsis, Hydrobiidae, Gastropoda, United States, California, Nevada, freshwater, taxonomy, conservation

Introduction

The western North American hydrobiid gastropod genus *Pyrgulopsis* (commonly known as springsnails) is composed of 134 currently recognized species (Hershler and Liu 2012) which typically live in springs and have very narrow geographic ranges. This large radiation is characterized by a high degree of morphological conservatism and homoplasy, which has posed difficulties in delineating species limits and phylogenetic relationships (Hershler 1994, Liu and Hershler 2005). Although the recent use of molecular tools has facilitated considerable progress in establishing a species level taxonomy that reflects the phylogenetic history of *Pyrgulopsis* (e.g., Hershler et al. 2003, Hershler and Liu 2004, Hershler and Liu 2009), a number of issues have yet to be addressed, including the unsettled status of several widely ranging congeners which have been shown to be composites of genetically divergent lineages (Hurt 2004, Liu et al. 2003, Liu and Hershler 2007). There is an urgent need to resolve these taxonomic problems to help identify conservation priorities for *Pyrgulopsis*, which is a current focus of attention of land managers owing to the increasing threats to its groundwater-dependent habitats (e.g., USFWS 2012a–c).

Pyrgulopsis micrococcus (Pilsbry in Stearns, 1893) was originally described based on shells from two localities in the Amargosa River basin and early treated as endemic to the upper portion of this watershed (Gregg and Taylor 1965, Taylor 1985: 317). This species was subsequently revised to include additional populations scattered within large portions of the Mojave Desert (southeastern California and southwestern Nevada) that resembled specimens from the type locality area in having a globose to ovate-conic shell and distally lobate penis with a terminal gland (sometimes reduced or absent) on the ventral surface (Hershler and Sada 1987, Hershler 1989, Hershler and Pratt 1990). A recent phylogenetic analysis resolved mtDNA sequences from 29 populations of *P. micrococcus* into five deeply divergent, allopatric clades–one of which also included morphologically similar and geographically proximate *P. turbatrix* Hershler, 1998–which were postulated to be distinct species (Liu et al. 2003, also see Hershler and Liu 2008). In this paper we detail previously unrecognized shell and penial differences supporting recognition of three of these lineages as new species which we describe herein while also clarifying the limits of *P. micrococcus* and *P. turbatrix*. We also present additional molecular data that confirm sympatry of two of these novelties at various sites in Ash Meadows (consistent with our morphological evidence) and delineate a new lineage of *P. micrococcus* (in the broad sense) in northern Death Valley that is not taxonomically treated owing to inadequate material.

Methods

The previous phylogeographic investigation (Liu et al. 2003) was based on sampling across the entire broad range of *P. micrococcus*, including each of the drainage basins inhabited by this species. For the current study additional molecular sampling was done to confirm the apparent sympatry of two *P. micrococcus* lineages at various sites in Ash

Meadows that was discovered during the course of this taxonomic study, and to evaluate the relationships of a distinctive morphotype (of *P. micrococcus*) in Grapevine Springs (northern Death Valley) that was not included in our previous analysis. Several previously analyzed populations–Grapevine Springs (M2), Purgatory Spring (M8), Tecopa Spring (M25), Shoshone Spring (M26)–were also additionally sampled to increase sample size and further evaluate their genetic distinctiveness. Newly collected material was preserved in 90% ethanol in the field. Genomic DNA was extracted from entire snails (2–10 specimens per sample) using a CTAB protocol (Bucklin 1992); each specimen was analyzed for mtDNA individually. LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify a 710 base pair (bp) fragment of COI, and ND43F and RND592F (Liu et al. 2003) were used to amplify a 550 bp fragment of NADH dehydrogenase subunit I (NDI). Amplification conditions and sequencing of amplified polymerase chain reaction product followed Liu et al. (2003). Sequences were determined for both strands and then edited and aligned using SEQUENCHERä version 5.0.1. The 51 newly sequenced specimens were analyzed together with our previously published *P. micrococcus* dataset (Liu et al. 2003, Hershler and Liu 2008). The new haplotypes from each sampling locality were deposited in GenBank (accession numbers KF559184-KF559202). Sample information and GenBank accession numbers are given in Appendix I. One example of each haplotype detected in a given sample was used in our analyses.

The partition homogeneity/incongruence length difference test (Farris et al. 1994) was used to determine whether the COI and NDI datasets were consistent and could be combined for the phylogenetic analysis. The test, which was conducted using parsimony-informative sites only and 1,000 replicates, indicated no significant incongruence (P=0.36) and consequently we combined the two datasets in our phylogenetic analysis. MRMODELTEST 2.3 (Nylander 2004) was used to obtain an appropriate substitution model (using the Akaike Information Criterion) and parameter values for this analysis. Phylogenetic relationships were inferred by Bayesian analysis using MRBAYES 3.1.2 (Huelsenbeck and Ronquist 2001). Metropolis-coupled Markov chain Monte Carlo simulations were run with four chains (using the model selected through MRMODELTEST) for 5,000,000 generations, and Markov chains were sampled at intervals of 10 generations to obtain 500,000 sample points. We used the default settings for the priors on topologies and the GTR + I + G model parameters selected by MRMODELTEST as the best fit model. At the end of the analysis, the average standard deviation of split frequencies was less than 0.01 (0.0063) and the Potential Scale Reduction Factor (PSRF) was 1, indicating that the runs had reached convergence. The sampled trees with branch lengths were used to generate a 50% majority rule consensus tree with the first 25% of the samples removed to ensure that the chain sampled a stationary portion. Genetic distances (maximum composite likelihood) within and between species/lineages were calculated using MEGA5 (Tamura et al. 2011), with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of missing data.

Types and other voucher material were deposited in the National Museum of Natural History (USNM) collection. Relevant material from the Academy of Natural Sciences of Philadelphia (ANSP), Bell Museum of Natural History (BellMNH) and

the Santa Barbara Museum of Natural History (SBMNH) was also examined during the course of this study. Series of large adults (n=10) were used for shell measurements. Whorl counts refer to the entire shell. Sexual dimorphism in shells, which is occasionally observed in *Pyrgulopsis* (Taylor 1987), could not be quantified owing to small sample sizes. The total number of shell whorls was counted (WH) for each specimen; and the height and width of the entire shell (SH, SW), body whorl (HBW, WBW), and aperture (AH, AW) were measured from camera lucida outline drawings using a digitizing pad linked to a personal computer (see Hershler 1989). In addition, three ratios were generated from the raw data (SW/SH, HBW/SH, AH/SH). Descriptive statistics were generated using SYSTAT FOR WINDOWS 11.00.01 (SSI 2004). Penial variation was described from series of adult specimens (typically n=30) that were relaxed with menthol crystals and fixed in dilute formalin prior to preservation in 70% ethanol. Descriptive penial terminology is from Taylor (1987) and Hershler (1994, 1998). Variation in the number of cusps on the radular teeth (n=5) was assessed using the method of Hershler et al. (2007).

We used a conservative, evolutionary lineage concept in describing new species only for those snails that are morphologically diagnosable as well as phylogenetically independent and substantially divergent genetically (Hershler et al. 2007). Inasmuch as the principal goal of our paper is to delimit species, we provide only brief taxonomic descriptions which focus on those aspects of morphology that have proven most useful in previous such studies of *Pyrgulopsis* (Taylor 1987, Hershler 1994, Hershler 1998).

Results

The alignment of COI and NDI sequences yielded 1188 bp. The five previously reported clades (A–E) were similarly recovered in the Bayesian analysis of this combined dataset (Fig. 1). The *P. micrococcus* morphotype in Grapevine Springs which was not included in our prior analysis formed an additional lineage (F) together with specimens from a spring in the Southern California coastal drainage. This clade is not formally treated herein owing to the paucity of morphological material. The additional molecular sampling conducted for this study also confirmed sympatry of morphologically distinctive clades C and E at three localities in Ash Meadows (M51–52, M53–54, M57–58) (Fig. 1), providing additional support for recognizing these as separate species.

Clades A–F differed from each other by 4.2–12.6% for COI and 5.2–13.6% for NDI; variation within clades ranged from 0–2.5% for COI and 0–3.5% for NDI (Appendix II). The geographic distributions of these genetic lineages are shown in Fig. 2. Based on the genetic evidence of distinctiveness and diagnosable shell and/or penial characters (detailed below) we recognize three of these lineages as new species which are described below (clade B as *P. perforata*, clade C as *P. licina*, clade E as *P. sanchezi*), restrict *P. micrococcus* to its type locality area (Oasis Valley, clade A), and revise *P. turbatrix* to include populations from the central Death Valley region and San Bernardino Mountains that had been previously identified as *P. micrococcus* (clade D).

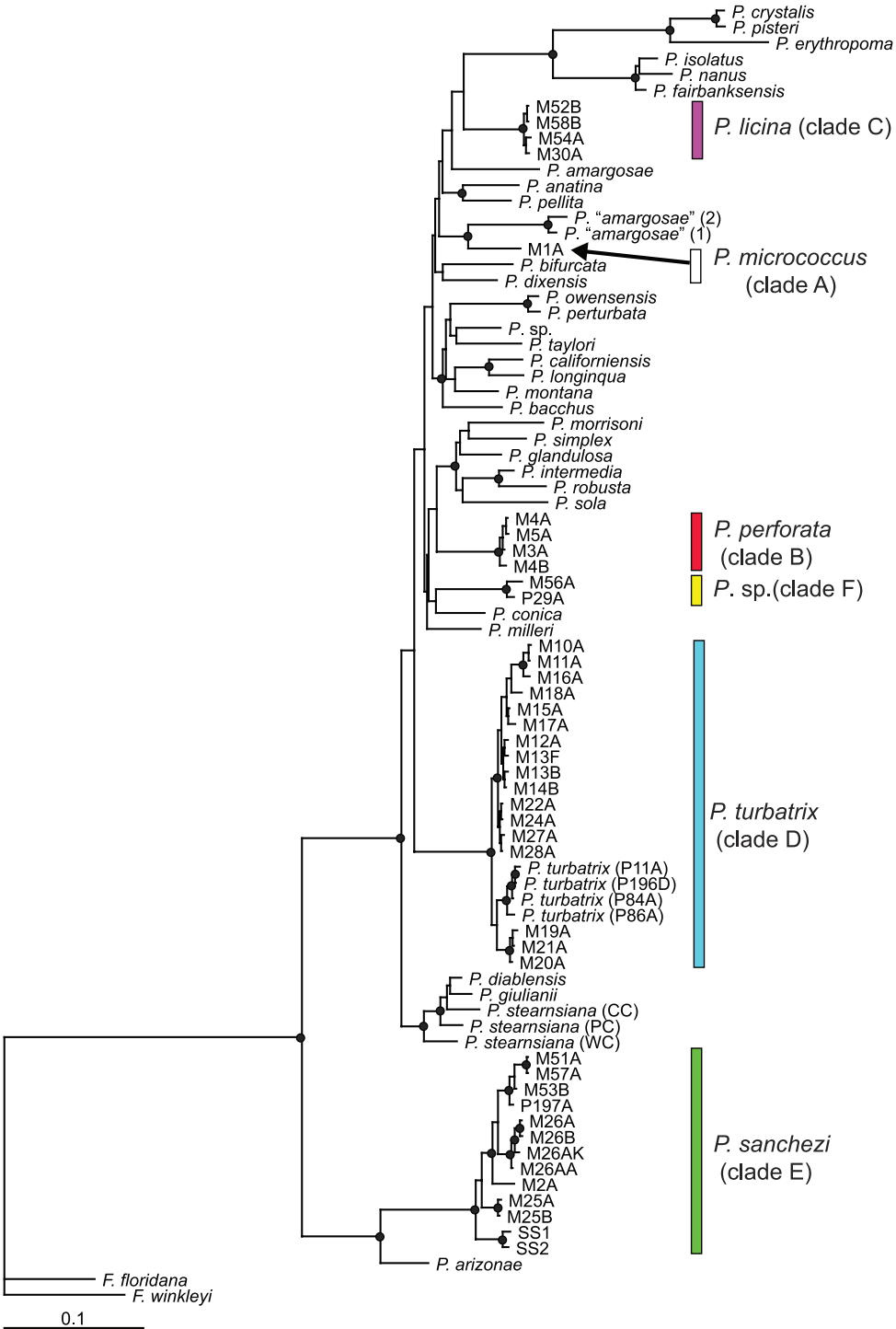

Figure 1. Bayesian tree based on the combined (COI, NDI) dataset. Nodes having posterior probabilities >95% are identified by filled circles. Specimen codes are from Appendix I.

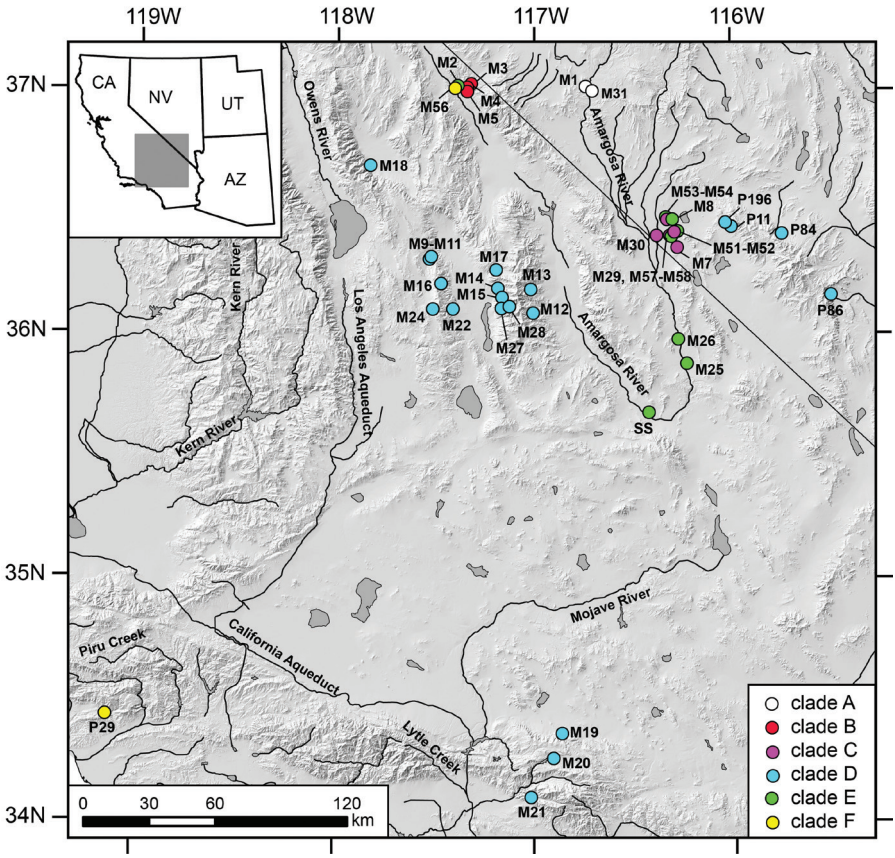

Figure 2. Map showing the distribution of mtDNA clades **A–F** with color codes matching those in Fig. 1.

Systematic descriptions

Family Hydrobiidae
Subfamily Nymphophilinae
Genus Pyrgulopsis Call & Pilsbry, 1886

***Pyrgulopsis licina* Hershler, Liu & Bradford, sp. n.**
http://zoobank.org/5F006641-38E0-4AA0-B3D2-AE78145B51CC
http://species-id.net/wiki/Pyrgulopsis_licina
Figs 3, 4A–B

Pyrgulopsis micrococcus.–Hershler and Sada 1987 (in part).
[*Pyrgulopsis micrococcus*] clade C.–Liu et al. (2003).

Types. Holotype, USNM 850347 (a dry shell), spring south of Clay Pits, Ash Meadows, Nye County, Nevada, 36.40719°N, 116.37856°W, 11 November 1985, R. Hershler and D. W. Sada. Paratypes, USNM 1204732 (from same lot).

Referred material. NEVADA. *Nye County*: USNM 859186, USNM 903997, spring south of Clay Pits, USNM 850345, USNM 850346, USNM 850347, USNM 850348, USNM 859185, spring at Clay Pits, Ash Meadows (36.41608°N, 116.37802°W), USNM 850343, USNM 850344, USNM 859184, spring north of Clay Pits, Ash Meadows (36.41613°N, 116.37808°W), USNM 850334, Rogers Spring, Ash Meadows (36.47931°N, 116.32622°W), USNM 850336, USNM 1122742, USNM 1122754, USNM 1197782, USNM 1204745, springs south of Rogers Spring, Ash Meadows (36.47467°N, 116.32747°W), USNM 850349, USNM 1197775, spring east of Crystal Reservoir, Ash Meadows (36.40790°N, 116.31297°W), USNM 850350, spring east of Crystal Reservoir, Ash Meadows (36.40742°N, 116.31197°W), USNM 903982, spring east of Crystal Reservoir, Ash Meadows (36.40836°N, 116.31042°W), USNM 1197780, spring ca. 100 m north of Collins Ranch, Ash Meadows (36.42038°N, 116.29921°W), USNM 850352, USNM 850351, USNM 859188, USNM 859189, USNM 1122848, Frenchy Springs, Ash Meadows (36.36364°N, 116.27432°W), USNM 850353, USNM 859190, USNM 894336, USNM 1122849, Last Chance Spring, Ash Meadows (36.35700°N, 116.27400°W).

Diagnosis. A small congener (maximum shell height, 2.4 mm) having a narrow-conic shell. Distinguished from similar regional species by its strongly curved penial filament and absence of glands on the penis. Further differentiated from frequently sympatric *P. sanchezi* (described below) by its highly convex, deeply incised teleoconch whorls and ovate shell aperture.

Description. Shell (Fig. 3A–C) narrow-conic, whorls 3.75–4.50. Teleoconch whorls highly convex, sutures deeply impressed. Aperture ovate, parietal lip complete, narrowly adnate or slightly disjunct, umbilicus narrow. Outer lip thin, orthocline or prosocline. Sculpture of faint, irregular spiral striae.

Operculum (Fig. 3D–E) as for genus; edges of last 0.5 whorl frilled on outer side; muscle attachment margins variably thickened on inner side. Radula (Fig. 3F–H) as for genus; dorsal edge of central teeth concave, lateral cusps four–six, basal cusp one. Lateral teeth having three–four cusps on both inner and outer sides. Inner marginal teeth with 20–25 cusps, outer marginal teeth with 24–31 cusps. Radula data are from USNM 850348.

Penis (Fig. 4A–B) medium-sized; filament medium length, narrow, weakly tapering, strongly curved (to outer side); lobe small, rectangular, horizontal or oblique; glands almost always absent (87/90 specimens), two specimens had a small, dot-like

Table 1. Shell parameters for *P. licina*. Measurements are in mm.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
Holotype, USNM 850347										
	4.25	1.94	1.33	1.42	1.09	0.78	0.76	0.69	0.73	0.44
USNM 1204732 (n=10)										
Mean	4.00	2.00	1.33	1.45	1.15	0.83	0.76	0.67	0.73	0.42
S.D.	0.12	0.12	0.06	0.08	0.06	0.05	0.03	0.03	0.02	0.02
Range	3.75–4.25	1.88–2.22	1.28–1.44	1.34–1.60	1.09–1.27	0.75–0.92	0.72–0.83	0.63–0.72	0.70–0.76	0.38–0.45

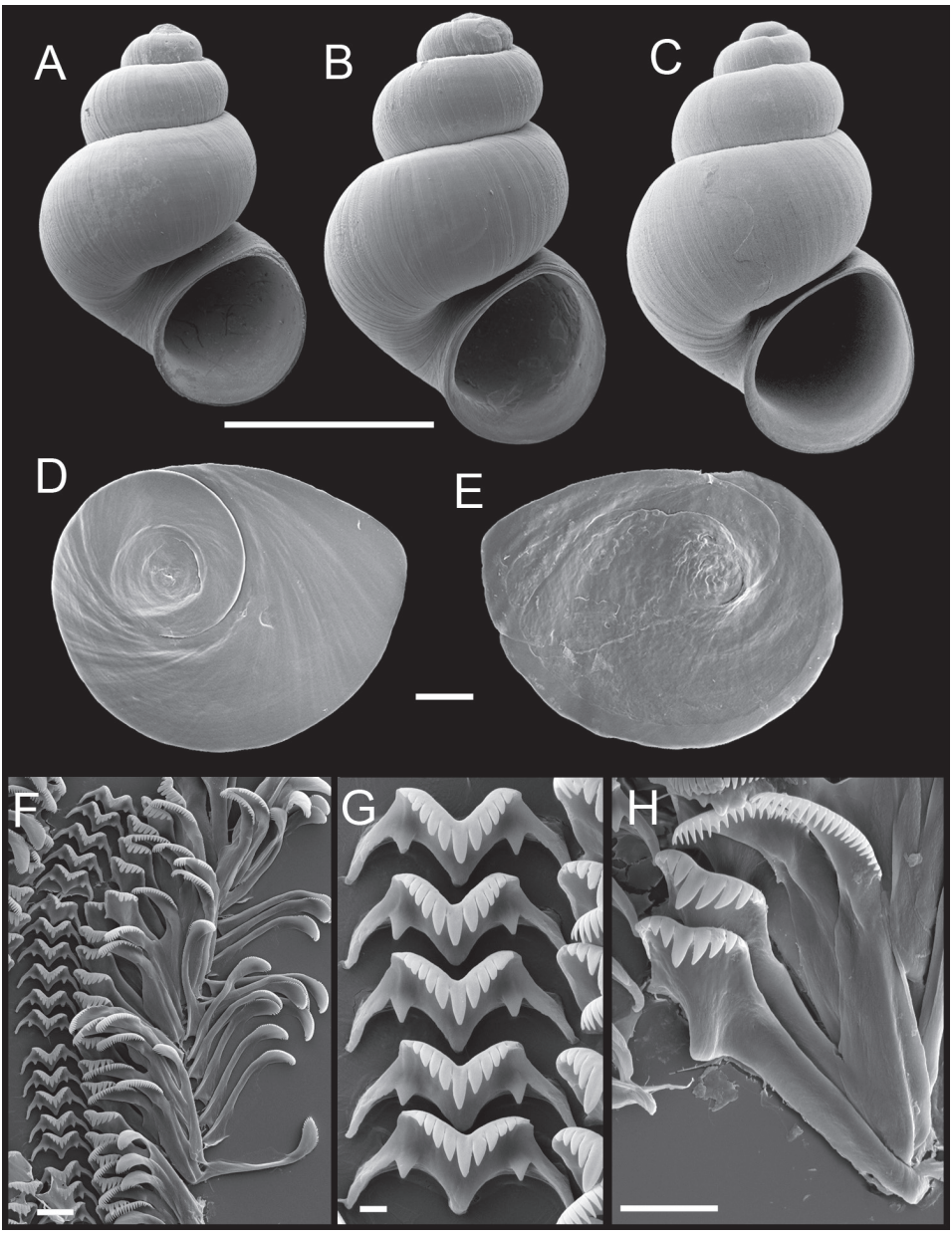

Figure 3. Shells, opercula and radula, *P. licina* sp. n. **A** Holotype, USNM 850347 **B, C** Shells, USNM 1204732, USNM 1188732 **D, E** Opercula (outer, inner sides), USNM 850348 **F** Portion of radular ribbon, USNM 850348 **G** Central teeth, USNM 850348 **H** Lateral and inner marginal teeth, USNM 850348. Scale bars **A–C** 1.0 mm; **D, E** 100 μm; **F, H** 10 μm; **G** 2 μm.

gland along the distal edge of the lobe and one specimen had a glandular smear near the distal edge of the ventral surface of the lobe. Penial data are from USNM 850334, USNM 850348, USNM 850351.

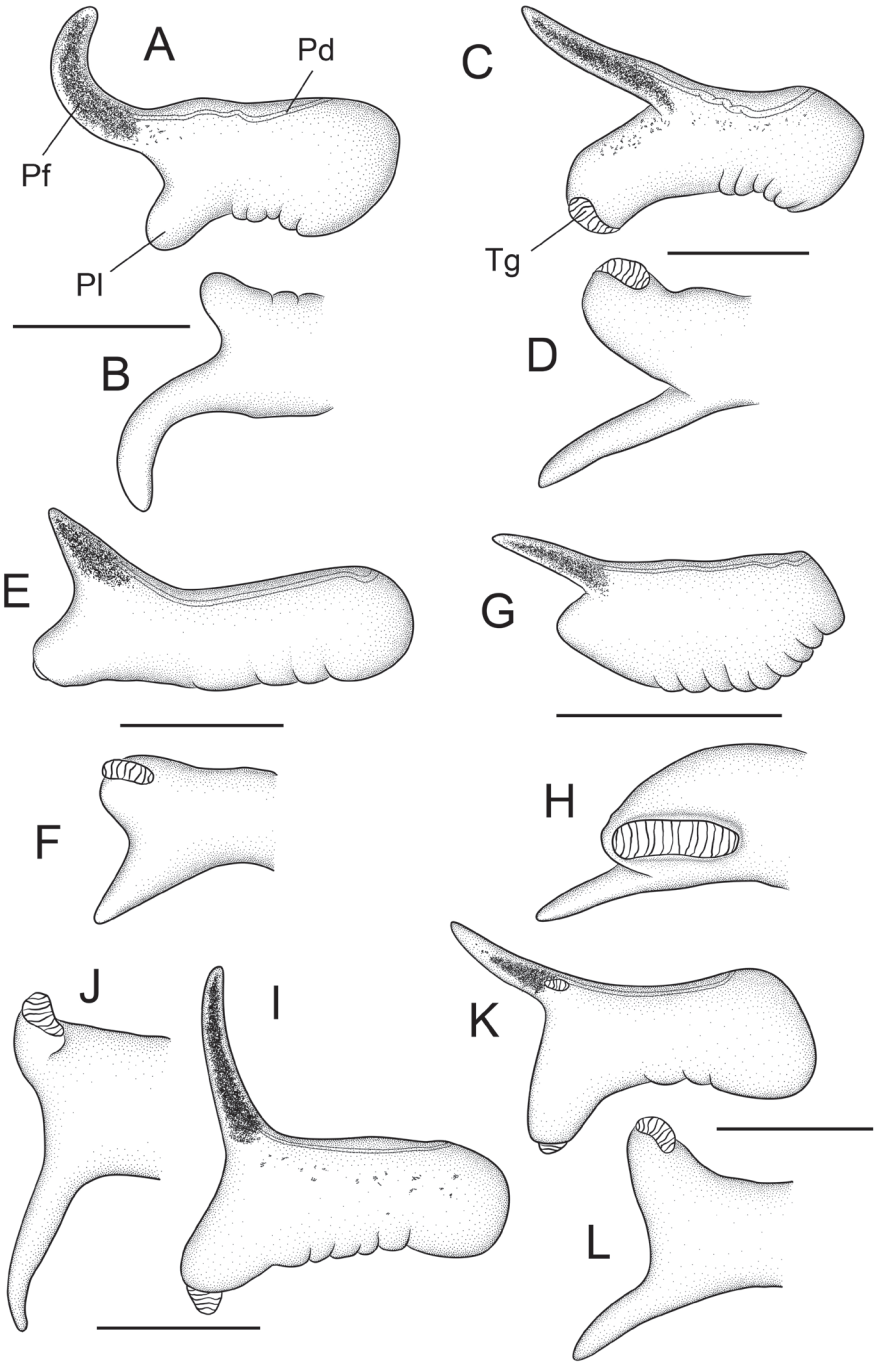

Figure 4. Penes (dorsal, ventral surfaces). **A, B** *P. licina* sp. n., USNM 850346 **C, D** *P. perforata* sp. n., BellMNH 20891 **E, F** *P. sanchezi* sp. n., USNM 883361 **G, H** *P. micrococcus*, BellMNH 20663 **I, J** *P. turbatrix*, USNM 860699 **K, L** *P. turbatrix*, USNM 883373. Scale bars **A–C** 250 μm; **D–L** 500 μm. **Pd** penial duct **Pf** penial filament **Pl** penial lobe **Tg** terminal gland.

Etymology. The epithet is an adjective derived from the New Latin *licinus*, meaning bent or turned upward, and refers to the distinctive shape of the penial filament in this species.

Distribution. Ash Meadows, Amargosa River basin (M7, M29, M30, M52, M54, M58, Fig. 2). The type locality is a broad spring brook that courses through a pit-like depression (Fig. 5A).

Remarks. The relationships of *P. licina* were not well resolved in the molecular phylogenetic analysis (Fig. 1). Haplotype variation within this clade was relatively small (Appendix II).

***Pyrgulopsis perforata* Hershler, Liu & Bradford, sp. n.**
http://zoobank.org/B246F89B-53AD-4476-8F47-DE21324ED20F
http://species-id.net/wiki/Pyrgulopsis_perforata
Figs 4C–D, 6

Pyrgulopsis micrococcus.–Hershler 1989 (in part).
[*Pyrgulopsis micrococcus*] clade B.–Liu et al. (2003).

Types. United States: Holotype, USNM 853507 (a dry shell), easternmost spring from Scotty's Castle along California Highway 72, Grapevine Canyon, Death Valley, Inyo County, California, 37.03233°N, 117.32333°W, 26 February 1985, R. Hershler. Paratypes, USNM 1204734 (from same lot).

Referred material. CALIFORNIA. *Inyo County*: BellMNH 20891, USNM 857965, USNM 883371, USNM 883374, USNM 883375, USNM 883376, USNM 883377, USNM 894332, easternmost spring from Scotty's Castle along CA Hwy 72, Grapevine Canyon, Death Valley, USNM 883369, spring east of Scotty's Castle along CA Hwy 27, Grapevine Canyon, Death Valley (37.03259°N, 117.33118°W), USNM 883368, USNM 883379, spring just east of Scotty's Castle, Grapevine Canyon, Death Valley (37.03205°N, 117.33715°W), BellMNH 20999, USNM 894333, spring ca. 0.8 km west of Scotty's Castle along CA Hwy 72, Grapevine Canyon, Death Valley (37.01400°N, 117.34867°W), USNM 894334, Surprise Springs, Death Valley (36.99933°N, 117.34400°W).

Diagnosis. A small to medium-sized congener (maximum shell height, 2.6 mm) having a broadly to ovate conic shell. Differentiated from similar regional species except *P. micrococcus* by its low-spired, broadly umbilicate shell. Differs from *P. micrococcus* in having a larger distal lobe and smaller gland on the penis.

Description. Shell (Fig. 6A–B) broadly to ovate conic, whorls 3.00–4.25. Teleoconch whorls medium convex, shouldered. Aperture ovate, parietal lip complete, narrowly adnate or slightly disjunct, last 0.25–0.5 whorl rarely loosened behind aperture, umbilicus broad (Fig. 6C). Outer lip thin, orthocline or prosocline.

Operculum (Fig. 6D–E) as for genus; outer side smooth; inner side smooth or weakly thickened along portions of the muscle attachment margin. Radula (Fig. 6F–H) as for genus; dorsal edge concave, lateral cusps four–eight, basal cusp one. Lateral

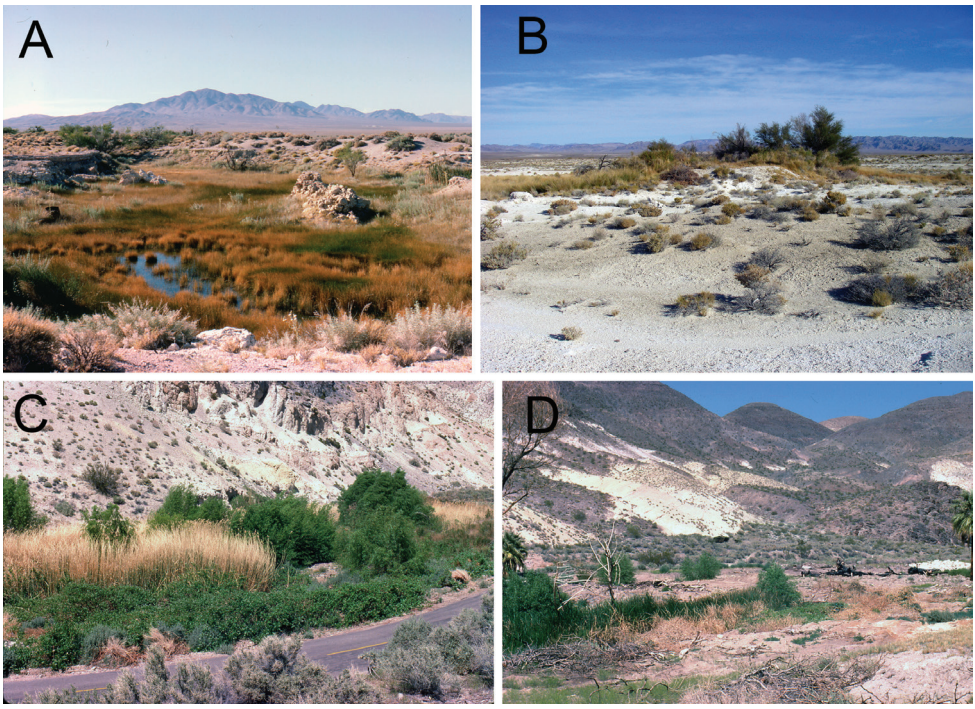

Figure 5. Photographs of habitats. **A** Spring south of Clay Pits, Ash Meadows, Nye County, Nevada, type locality of *P. licina* sp. n. (photograph taken on 7/VII/1986) **B** Purgatory Spring, Ash Meadows, Nye County, Nevada, type locality of *P. sanchezi* (15/XI/2011) **C, D** Uppermost spring east of Scotty's Castle, Death Valley, Inyo County, California, type locality of *P. perforata* sp. n. (18/IV/1980).

Table 2. Shell parameters for *P. perforata*. Measurements are in mm.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
Holotype, USNM 853507										
	3.75	1.82	1.51	1.46	1.30	0.87	0.79	0.83	0.80	0.48
USNM 1204734 (n=10)										
Mean	3.63	1.83	1.52	1.50	1.29	0.91	0.82	0.84	0.82	0.50
S.D.	0.21	0.21	0.08	0.13	0.09	0.07	0.05	0.08	0.03	0.04
Range	3.25–4.00	1.51–2.09	1.41–1.67	1.31–1.68	1.15–1.41	0.83–1.03	0.75–0.90	0.74–0.98	0.77–0.87	0.45–0.55

teeth having two–four cusps on inner sides and three–six cusps on outer sides. Inner marginal teeth with 14–24 cusps, outer marginal teeth with 18–31 cusps. Radula data are from USNM 857965.

Penis (Fig. 4C–D) medium-sized; filament medium length, narrow, tapering, oblique; lobe medium-sized, rectangular, horizontal or slightly oblique; small (terminal) gland present on ventral edge of lobe (60/60 specimens), one specimen had an additional dot-like gland on the ventral surface of the lobe and one specimen had a similar glandular unit on the dorsal surface of the lobe. Penial data are from BellMNH 20891, USNM 883371.

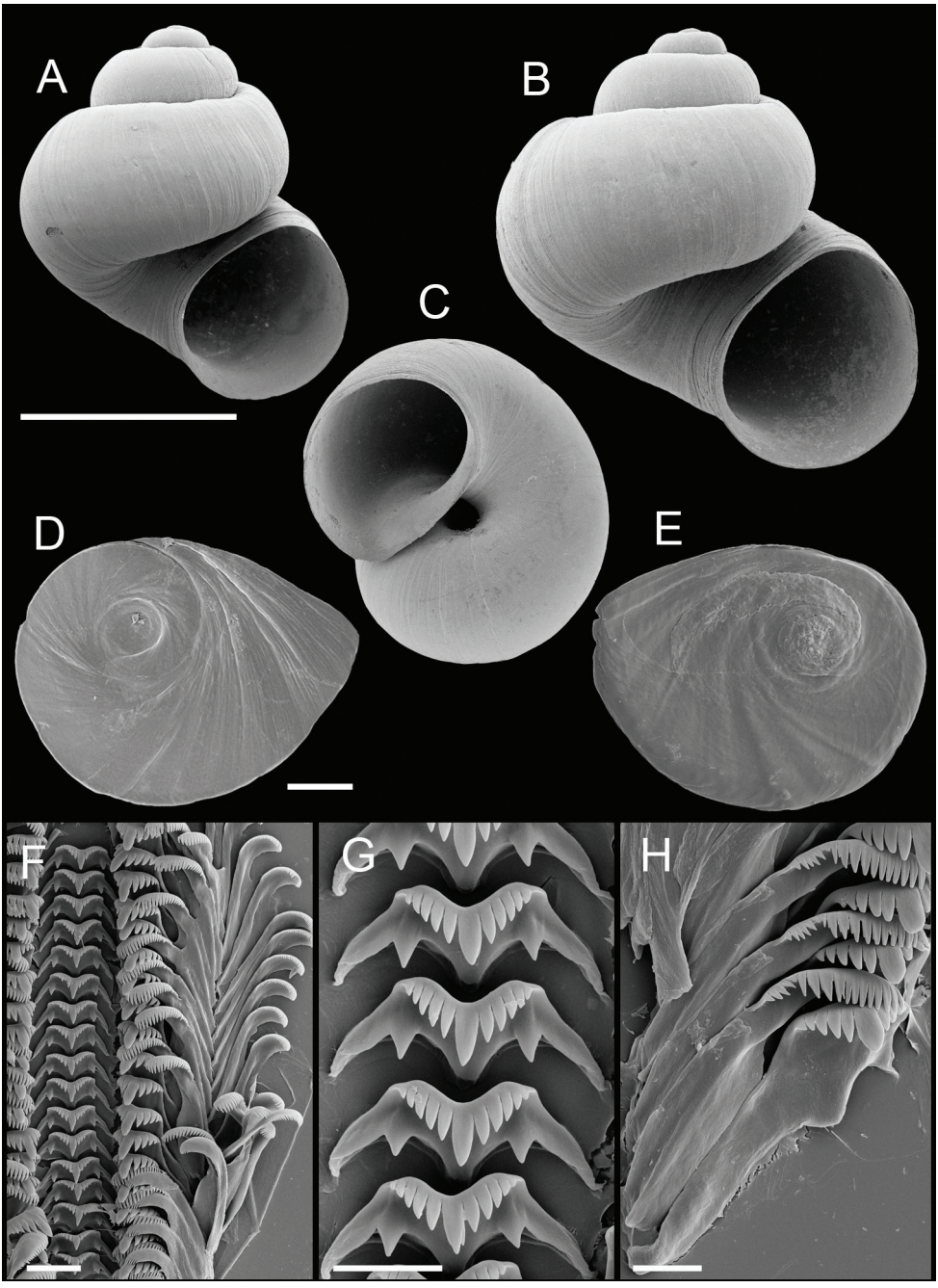

Figure 6. Shells, opercula and radula, *P. perforata* sp. n. **A** Holotype, USNM 853507 **B, C** Shells, USNM 1204734 **D, E** Opercula (outer, inner sides), USNM 857965 **F** Portion of radular ribbon, USNM 857965 **G** Central teeth, USNM 857965 **H** Lateral and inner marginal teeth, USNM 857965. Scale bars **A–C** 1.0 mm; **D, E** 200 μm; **F** 20 μm; **G, H** 10 μm.

Distribution. Lower portion of Grapevine Canyon, and Grapevine Mountains, lower Amargosa River basin (M3, M4, M5, Fig. 2). The type locality (Fig. 5C–D) is the uppermost of a small series of springs to the east of Scotty's Castle.

Etymology. An adjective derived from the New Latin, *perforare*, meaning to pierce, and referring to the broad umbilicate shells of this species.

Remarks. The relationships of *P. perforata* were not well resolved in the molecular phylogenetic analysis (Fig. 1). Haplotype variation within this clade was relatively small (Appendix II).

***Pyrgulopsis sanchezi* Hershler, Liu & Bradford, sp. n.**
http://zoobank.org/DA1A41D8-E257-466B-B28E-E500E89D16A9
http://species-id.net/wiki/Pyrgulopsis_sanchezi
Figs 4E–F, 7

Pyrgulopsis micrococcus.–Hershler and Sada 1987 (in part).
Pyrgulopsis micrococcus.–Hershler 1989 (in part).
[*Pyrgulopsis micrococcus*] clade E.–Liu et al. (2003).

Types. United States: Holotype, USNM 850333 (a dry shell), Purgatory Spring, Ash Meadows, Nye County, Nevada, 36.47200°N, 116.31617°W, 26 February 1985, R. Hershler and D.W. Sada. Paratypes, USNM 1204735 (from same lot).

Referred material. CALIFORNIA. *Inyo County*: USNM 853505, USNM 853506, USNM 854609, USNM 854610, Grapevine Springs, spring brook on travertine bench above Scotty's Ranch, Death Valley (37.019210°N, 117.38649°W), USNM 857964, USNM 883372, USNM 1152507, Grapevine Springs, spring outflow at Scotty's Ranch, Death Valley (37.01830°N, 117.38770°W), USNM 1197772, Grapevine Springs, spring outflow below Scotty's Ranch, Death Valley (37.01760°N, 117.39420°W), USNM 853503, Grapevine Springs, northern-most spring complex, outflow below base of hill, Death Valley (37.01970°N, 117.39288°W), USNM 894331, Grapevine Springs, third stream north of ranch, Death Valley (37.01867°N, 117.38900°W), BellMNH 21116, USNM 853501, USNM 857962, USNM 894335, USNM 1152506, Shoshone Spring, (35.98022°N, 116.27308°W), USNM 853502, USNM 857963, USNM 873153, USNM 883366, USNM 894354, Tecopa Hot Springs, northern-most spring, (35.88011°N, 116.22992°W), USNM 874035, Spring brook north of Tecopa, (35.85346°N, 116.22361°W). *San Bernardino County*: USNM 123904, USNM 883365, USNM 899902, USNM 1008345, USNM 1008725, USNM 1011485, USNM 1152503, Saratoga Springs, Death Valley (35.68099°N, 116.42245°W). NEVADA. *Nye County*: USNM 850339, USNM 859183, USNM 1122825, Shaft Spring, Ash Meadows (36.45109°N, 116.31552°W), USNM 850340, USNM 1122826, Chalk Spring, Ash Meadows (36.44913°N, 116.31497°W), BellMNH 20664, School Spring, Ash Meadows (36.42741°N, 116.30397°W), USNM

1204746, Rogers Spring, Ash Meadows (36.47931°N, 116.32632°W), USNM 850335, USNM 859180, USNM 859181, USNM 204755, USNM 1122554, springs south of Rogers Spring, Ash Meadows (36.47467°N, 116.32747°W), USNM 850337, USNM 850338, Five Springs, Ash Meadows (36.46476°N, 116.32023°W), USNM 859182, USNM 1122821, spring south of Five Springs, Ash Meadows (36.45109°N, 116.31552°W), BellMNH 20666, BellMNH 20743, BellMNH 21149, USNM 850341, USNM 850342, USNM 859195, USNM 1204752, spring ca. 100 m north of Collins Ranch, Ash Meadows (36.42038°N, 116.29921°W), BellMNH 20741, USNM 859179, USNM 883361, USNM 894337, USNM 1074313, USNM 1122759, USNM 1152498, Purgatory Spring, Ash Meadows, USNM 1204738, USNM 1197773, spring east of Crystal Reservoir, Ash Meadows (36.40790°N, 116.31297°W), USNM 859187, USNM 1204744, spring east of Crystal Reservoir, Ash Meadows (36.40742°N, 116.31197°W).

Diagnosis. A small to medium-sized congener (maximum shell height, 2.9 mm) having an ovate to narrow conic shell. Differentiated from similar regional species by its short, strongly tapering penial filament.

Description. Shell (Fig. 7A–D) ovate to narrow conic, whorls 3.5–4.75. Teleoconch whorls medium convex, sometimes strongly shouldered, last 0.25–0.50 whorl sometimes slightly loosened. Aperture ovate, sometime strongly angled adapically, parietal lip complete, narrowly adnate or slightly disjunct, umbilicus usually narrow. Apertural lip sometimes rather thickened and/or slightly reflected, outer lip orthocline or prosocline.

Operculum (Fig. 7E–F) as for genus; outer side smooth or with last 0.5 whorl weakly frilled; inner side smooth or slightly thickened along a small portion of the muscle attachment margin. Radula (Fig. 7G–I) as for genus; dorsal edge concave, lateral cusps three–six, basal cusp one. Lateral teeth having one–four cusps on inner sides and two–six cusps on outer sides. Inner marginal teeth with 10–26 cusps, outer marginal teeth with 12–33 cusps. Radula data are from BellMNH 21116, USNM 857963, USNM 883361, USNM 883365, USNM 883372.

Penis (Fig. 4E–F) medium-sized; filament short, broad, strongly tapering, oblique; lobe short, rectangular, horizontal or slightly oblique; small (terminal) ovate gland almost always present on ventral surface of lobe (92/93 specimens), gland usually positioned horizontally, rarely borne on a raised swelling (one specimen), one specimen had a second, dot-like gland on the ventral surface of the lobe. Penial data are from BellMNH 2116, USNM 857963, USNM 857964, USNM 883361, USNM 883666.

Table 3. Shell parameters for *P. sanchezi*. Measurements are in mm.

	WH	SH	SW	HBW	WBW	AH	AW	SW/SH	HBW/SH	AH/SH
Holotype, USNM 850333										
	4.25	2.50	1.73	1.95	1.40	1.10	1.07	0.69	0.78	0.44
USNM 1204735 (n=10)										
Mean	4.33	2.43	1.66	1.82	1.33	1.06	0.99	0.68	0.75	0.43
S.D.	0.21	0.14	0.10	0.10	0.06	0.06	0.07	0.04	0.02	0.02
Range	4.00–4.75	2.22–2.70	1.46–1.81	1.68–1.94	1.22–1.44	0.96–1.16	0.90–1.08	0.62–0.73	0.70–0.78	0.40–0.46

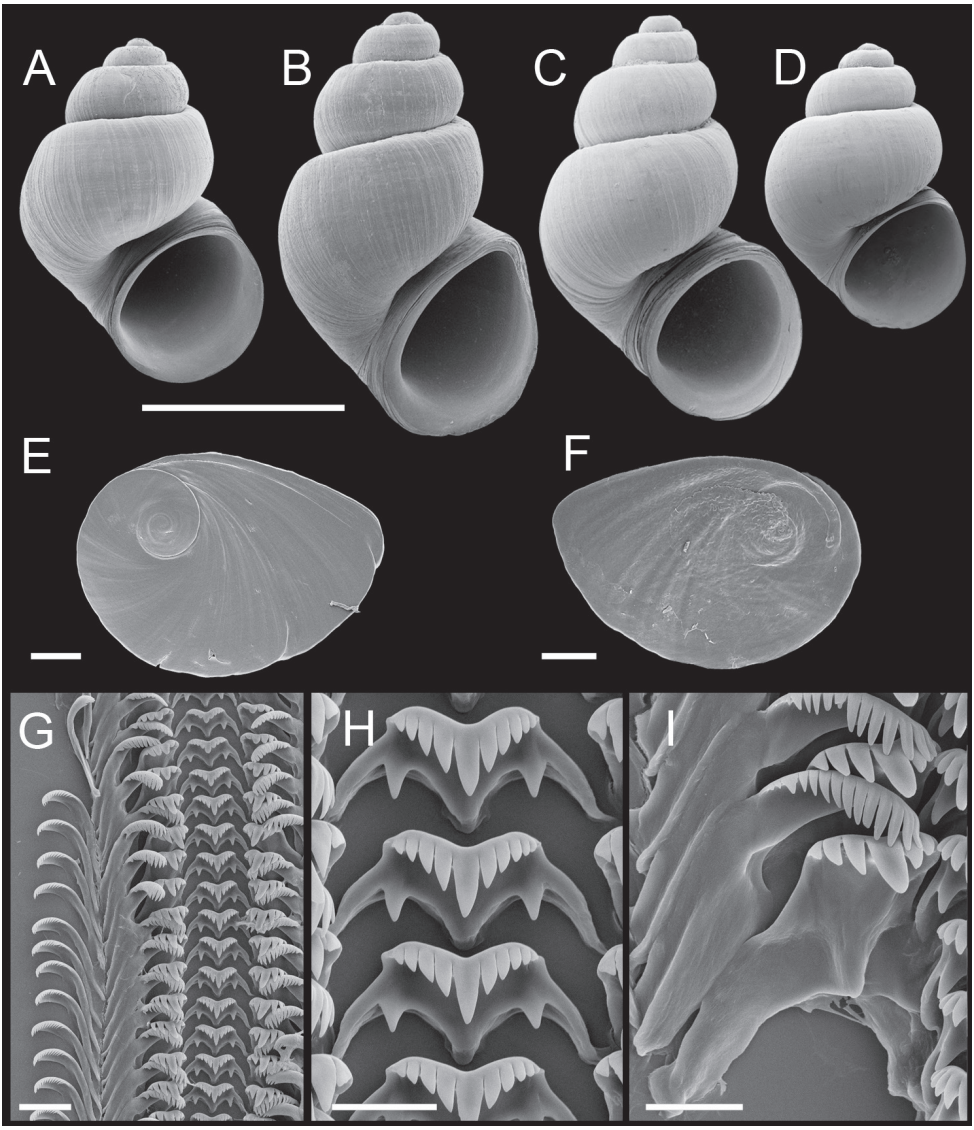

Figure 7. Shells, opercula and radula, *P. sanchezi* sp. n. **A** Holotype, USNM 850333 **B–D** Shells, USNM 853505, USNM 1204755, USNM 853501 **E, F** Opercula (outer, inner sides), USNM 883361 **G** Portion of radular ribbon, USNM 883361 **H** Central teeth, USNM 883361 **I** Lateral and inner marginal teeth, USNM 883361. Scale bars **A–D** 1.0 mm; **E, F** 100 μm; **G** 20 μm; **H, I** 10 μm.

Distribution. Distributed in five separate groundwater discharge areas of the Amargosa River basin: Grapevine Springs (M2), Ash Meadows (M8, M51, M53, M57), Tecopa (M25), Shoshone (M26), Saratoga Spring (SS) (Fig. 2). The type locality (Fig. 5B) is a flowing well that was drilled into a small spring mound (Dudley and Larson 1976).

Etymology. This species is named for Peter G. Sanchez, who spearheaded early efforts to protect and conserve regional springsnails and their associated aquatic

habitats while serving as a Resource Management Specialist in the Death Valley National Monument (now National Park) and Chair of the Desert Fishes Council (1978–1980).

Remarks. *Pyrgulopsis sanchezi* was resolved as sister to *P. arizonae* (Gila River basin, Arizona) in the Bayesian analysis (Fig. 1). The five geographically separated groups of *P. sanchezi* populations are genetically differentiated—e.g., mean genetic distance is 1.5+/-0.3% (ranging from 1.3–2.3%) for COI and 2.1+/-0.6% (ranging from 1.8–3.2%) for NDI, however we have not found consistent morphological differences to support their recognition as distinct species.

Pyrgulopsis micrococcus (Pilsbry, 1893)

http://species-id.net/wiki/Pyrgulopsis_micrococcus
Figs 4G–H, 8

Amnicola micrococcus Pilsbry in Stearns 1893: 277, fig. 1 (small spring in Oasis Valley Nevada; also from Death Valley). Baker 1964: 174 (lectotype designation).
Fontelicella (*Microamnicola*) *micrococcus*.–Gregg and Taylor 1965: 109 (comb. n.).
Pyrgulopsis micrococcus.–Hershler and Thompson 1987: 29 (new combination). Hershler and Sada 1987: 788–791 (in part). Hershler 1989: 182–187 (in part). Hershler 1998: 15.
[*Pyrgulopsis micrococcus*] clade A.–Liu et al. (2003).

Types. Lectotype, ANSP 67279; paralectotypes, ANSP 368399, USNM 123622 (from same lot).

Other material examined. NEVADA. *Nye County*: USNM 847246, springs at Springdale (37.03049°N, 116.75117°W), BellMNH 20674, spring east of Springdale (37.03858°N, 116.71730°W), BellMNH 20671, BellMNH 20672, BellMNH 20673, BellMNH 20739, USNM 850297, USNM 857961, USNM 874778, USNM 894330, USNM 905091, USNM 1002348, USNM 1004184, USNM 1004185, USNM 1068649, USNM 1068650, USNM 1068794, spring at Fleur de Lis Ranch, ca. 0.8 km south of Springdale (37.01700°N, 116.73300°W), BellMNH 20670, USNM 874771, USNM 1002349, USNM 1004182, USNM 1004183, USNM 1146338, Goss Springs (36.99906°N, 116.70725°W), BellMNH 20669, BellMNH 20774, Ute Springs (36.95729°N, 116.71648°W), BellMNH 20667, BellMNH 20740, USNM 874758, Revert Springs (36.91795°N, 116.74397°W).

Revised diagnosis. A medium-sized congener (maximum shell height, 4.4 mm) having a broadly to elongate conic shell. Differentiated from similar regional species by the large size of the gland on the ventral surface of the penis.

Description. Shell (Fig. 8A–D) broadly to narrow-conic, whorls 3.50–5.0. Teleoconch whorls weakly to strongly convex, sutures impressed. Aperture ovate, parietal lip complete, usually disjunct, last 0.25–0.5 whorl often loosened behind aperture, umbilicus small. Outer lip usually thin, orthocline. Sculpture of faint, irregular spiral striae.

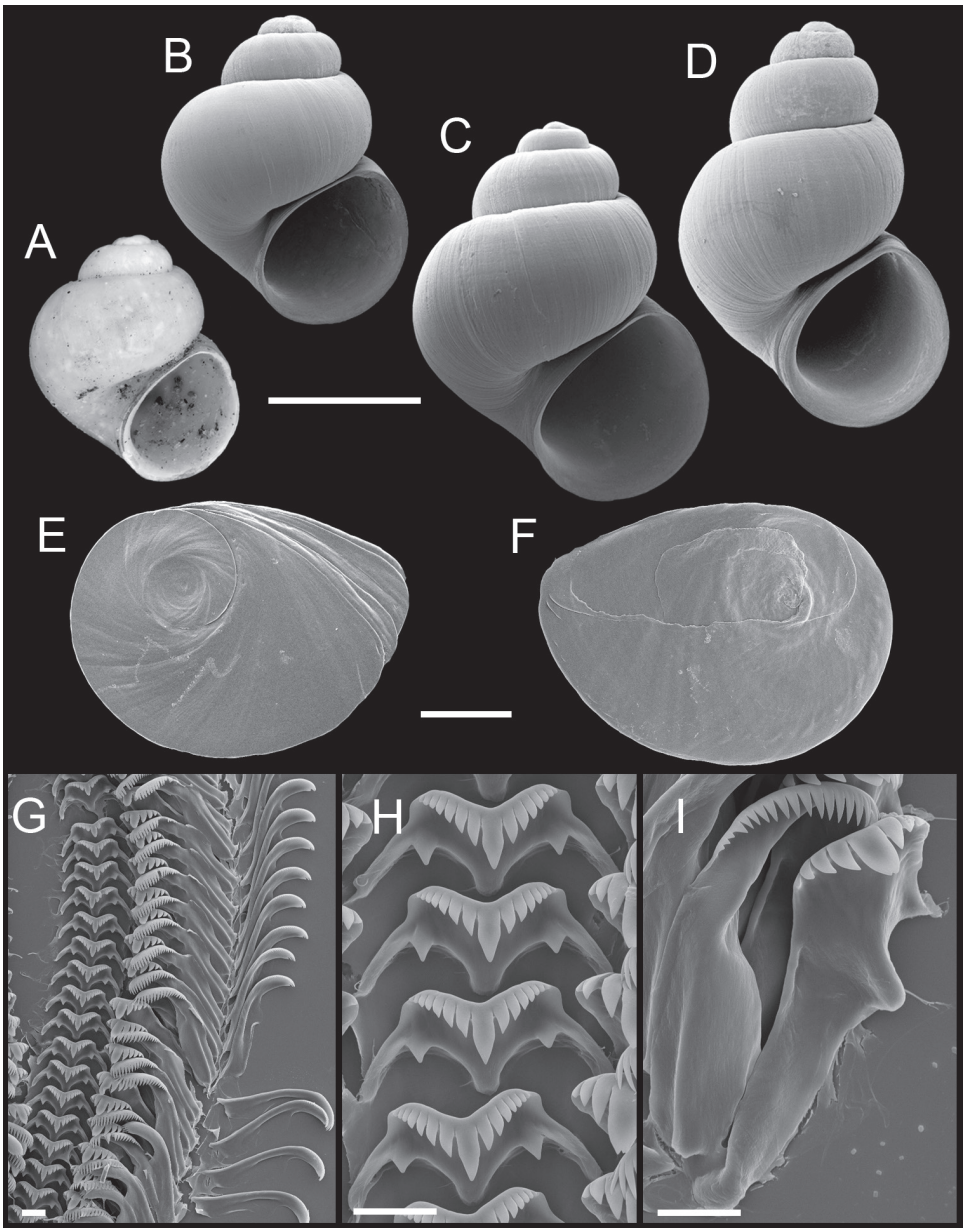

Figure 8. Shells, opercula and radula, *P. micrococcus*. **A** Lectotype, ANSP 67279a **B–D** Shells, USNM 1004185, USNM 905091, USNM 1004183 **E, F** Opercula (outer, inner sides), USNM 847246 **G** Portion of radular ribbon, USNM 847246 **H** Central teeth, USNM 847246 **I** Lateral and inner marginal teeth, USNM 847246. Scale bars **A–D** 1.0 mm; **E, F** 250 μm; **G–I** 10 μm.

Operculum (Fig. 8E–F) as for genus; edges of last 0.5 whorl frilled on outer side; muscle attachment margins thickened on inner side. Radula (Fig. 8G–I) as for genus; dorsal edge of central radular teeth concave, lateral cusps five–eight, basal cusp

one. Lateral teeth having three–four cusps on inner sides and four–five cusps on outer sides. Inner marginal teeth with 18–23 cusps, outer marginal teeth with 21–29 cusps. Radula data are from USNM 847246.

Penis (Fig. 4G–H) medium-sized; filament short, narrow, tapering, slightly oblique; lobe small, tapering, horizontal; a large (terminal) gland (borne on a raised swelling) present on ventral surface of penis, extending from near mid-length almost to tip of lobe (90/90 specimens), one–two additional small glands sometimes present on ventral surface of lobe (8 specimens), one specimen had a glandular dot on the dorsal surface near the base of the filament. Penial data are from BellMNH 20663, BellMNH 20669, BellMNH 20744.

Distribution. Several groups of springs in Oasis Valley, upper Amargosa River basin (M1, M31, Fig. 2).

Remarks. Pilsbry (in Stearns 1893; also see Stearns 1901) listed a single "type" lot for *P. micrococcus*, [USNM] 123622, which is composed of six dry shells. Baker (1964) subsequently designated ANSP 67279a as the "type" without explaining his rationale for this action. ANSP 67279a (Fig. 8A) closely conforms to Pilsbry's description and figure and is also very similar to the USNM type material (Hershler and Sada 1987, fig. 8a). The labels associated with ANSP 67279a indicate that it was part of the original collection of *P. micrococcus* (made by C. Hart Merriam) and this lot was almost certainly known to Pilsbry, who was the curator of mollusks at the Academy of Natural Sciences during the time period when his description was prepared and published. Based on this evidence we conclude that ANSP 67279a is part of the type series and thus Baker's subsequent lectotype designation is valid.

Pyrgulopsis micrococcus was resolved in the Bayesian tree as sister to an undescribed species from the Amargosa Canyon, south of Tecopa (Fig. 1). Specimens assigned to *P. micrococcus* vary somewhat in size and shell shape, but are closely similar both genetically (Liu et al., 2003) and in penial morphology.

Pyrgulopsis turbatrix Hershler, 1998

http://species-id.net/wiki/Pyrgulopsis_turbatrix
Figs 4I–L, 9

Pyrgulopsis turbatrix Hershler, 1998: 50, figs. 6K, 18G–J, 30D–F (Horseshutem Springs, Pahrump Valley, Nye County, Nevada).
Pyrgulopsis micrococcus.–Hershler 1989 (in part).
Pyrgulopsis micrococcus.–Hershler and Pratt 1990 (in part).
[*Pyrgulopsis micrococcus*] clade D.–Liu et al. (2003).

Types. Holotype, USNM 883978; paratypes, USNM 860699 (from same lot).

Other material examined. CALIFORNIA. *Inyo County*: USNM 853508, USNM 883373, Hanaupah Spring, Hanaupah Canyon, Death Valley (36.18684°N, 117.02537°W), USNM 853512, spring above Darwin Falls, Panamint Valley

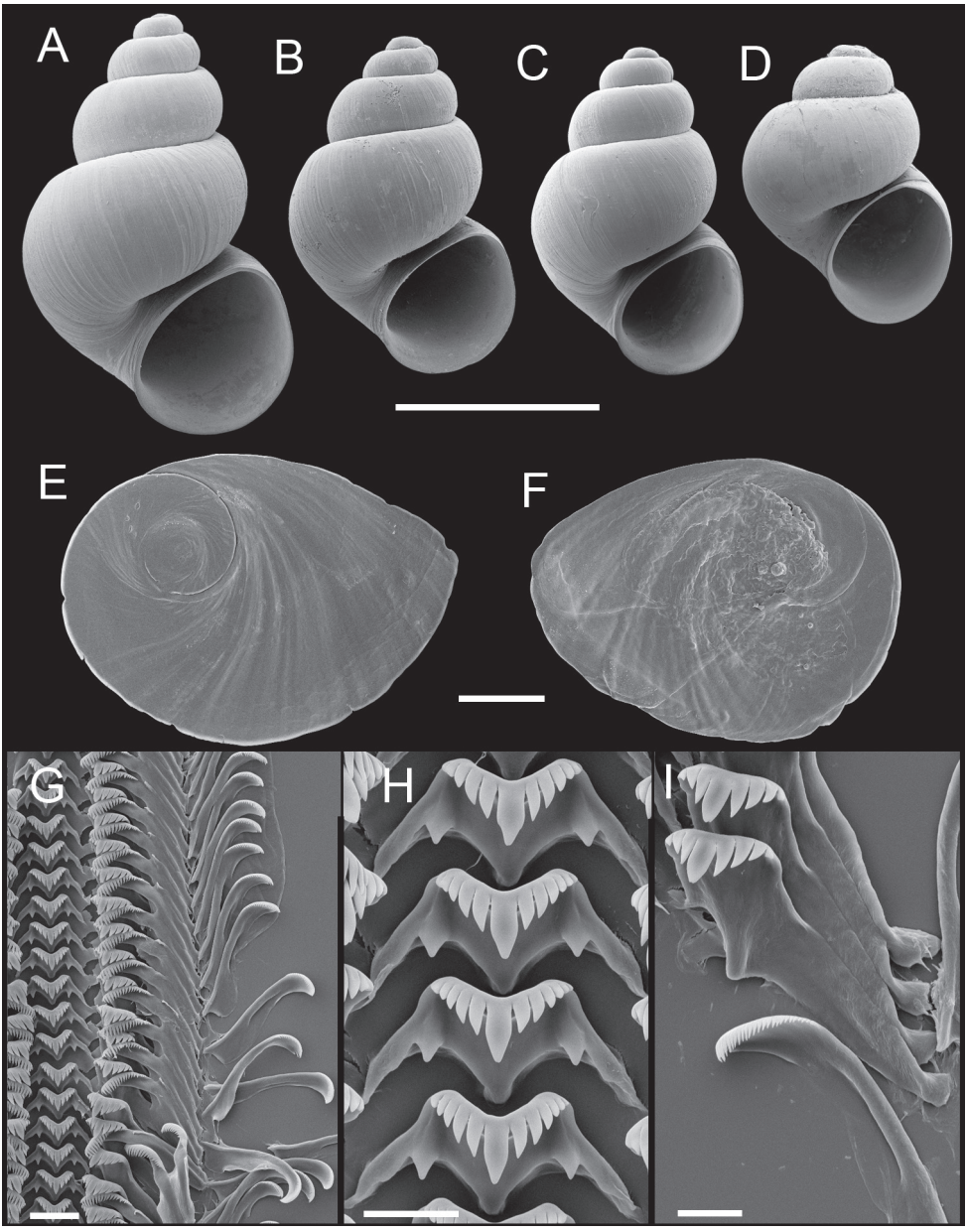

Figure 9. Shells, opercula and radula, *P. turbatrix*. **A** Paratype, USNM 860699. **B–D** Shells, SBMNH uncat., USNM 853508, USNM 853510 **E, F** Opercula (outer, inner sides), USNM 860699 **G** Portion of radular ribbon, USNM 860699 **H** Central teeth, USNM 860699 **I** Lateral and outer marginal teeth, USNM 860699. Scale bars **A–D** 1.0 mm; **E, F** 250 μm; **G** 20 μm; **H, I** 10 μm.

(36.31783°N, 115.52700°W), USNM 857969, stream below Darwin Falls, Panamint Valley (36.32033°N, 117.51917°W), USNM 857968, Saline Marsh, Saline Valley (36.69350°N, 117.83033°W). *San Bernardino County*: SBMNH uncat., roadside

spring between north shore highway and Big Bear Lake at point 1.2 km east of road which crosses lake, Southern California coastal drainage (34.26424°N, 116.87497°W), USNM 860450, spring southwest of Big Bear Ranger Station, southern California coastal drainage (34.26281°N, 116.90185°W). NEVADA. *Clark County*: USNM 883551, Willow Spring, Indian Springs Valley (36.41700°N, 115.76419°W), USNM 883981, La Madre Spring, Las Vegas Valley (36.18381°N, 115.50638°W), USNM 1002785, Harris Spring, Las Vegas Valley (36.24071°N, 115.54351°W). *Nye County*: USNM 854967, Wood Canyon Spring, Pahrump Valley (36.39924°N, 115.93258°W).

Revised diagnosis. A medium-sized congener (maximum shell height, 3.7 mm) having an ovate to narrow-conic shell. Differentiated from similar regional species by the combination of its relatively large, narrow shell; elongate penial filament; and small size of the terminal gland on penis.

Description. Shell (Fig. 9A–D) ovate to narrow conic, rarely broadly conic, whorls 4.25–5.25. Teleoconch whorls strongly convex, shouldered. Aperture ovate, parietal lip complete, usually slightly disjunct, last 0.25 whorl sometimes loosened behind body whorl, umbilicus narrow. Outer lip thin, prosocline.

Operculum (Fig. 9E–F) as for genus; edges of last 0.5 whorl frilled on outer side; muscle attachment margins slightly thickened on inner side. Radula (Fig. 9G–I) as for genus; dorsal edge of central teeth moderately to highly concave, lateral cusps three–seven, basal cusps one–two. Lateral teeth having two–six cusps on inner sides and three–six cusps on outer sides. Inner marginal teeth with 14–31 cusps, outer marginal teeth with 15–33 cusps. Radula data are from USNM 857968, USNM 860450, USNM 860699, USNM 883373.

Penis (Fig. 4I–L) medium-sized; filament long, narrow, tapering, oblique; lobe medium-sized, tapering, slightly oblique; ventral surface of lobe having a small (terminal) gland (199/200 specimens) and rarely one or two additional glandular dots (3 specimens), dorsal surface sometimes having a small (penial) gland at base of filament (24/200 specimens) and rarely having an additional glandular dot (one specimen). Penial data are from USNM 854967, USNM 857969, USNM 860450, USNM 860699, USNM 883373, USNM 883981, USNM 1002785.

Distribution. Spring Mountains region (Frenchman Flat; Indian Springs, Las Vegas, Pahrump Valleys [*P. turbatrix*]), San Bernardino Mountains (Mojave, Southern California Coastal drainages [M19, M20, M21]), central Death Valley region (Amargosa River drainage, Panamint and Saline Valleys [M9-M22, M24, M27, M28]). The populations from the latter two areas were previously assigned to *P. micrococcus*.

Remarks. The penial gland was not observed in >25% of the males in any of the seven samples that we studied and consequently has been removed from the diagnosis. The three geographically separated subunits of *P. turbatrix* are somewhat diverged genetically—e.g., mean sequence divergence is 0.9+/-0.2% (ranging from 1.1–1.5%) for COI and 0.9+/-0.2% (ranging from 1.1–1.3%) for NDI, but we have not found any consistent morphological differences among them.

Discussion

The three novelties described herein increase the number of *Pyrgulopsis* species in the Death Valley region to 17 (Hershler and Sada 2002) and add to the large body of evidence supporting recognition of this desert area as one of the most significant hotspots of rarity and richness in the United States (Chaplin et al. 2000). Our revision of *P. micrococcus* obviously is not yet complete as we have not treated clade F, which differs from the other lineages of *P. micrococcus* (in the broad sense) by 4.3–12.6% for COI and 7.4–13.3% for NDI (Appendix II). The two populations in clade F differ by only 0.8+/-0.3% for COI and 1.2+/-0.5% for NDI, suggesting that they may be conspecific. Additional studies are needed to clarify the taxonomy of this clade and to evaluate the biogeographic cause of its broadly disjunct distribution.

All of the new species described herein are endemic to the Amargosa River basin. *Pyrgulopsis perforata* and *P. licina* are distributed entirely within the confines of Death Valley National Park and Ash Meadows National Wildlife Refuge, respectively, and consequently are being afforded some measure of protection. The *P. licina* populations are also being monitored by The Nature Conservancy as part of their Nevada Springs Conservation Plan (Abele 2011). Three of the five genetically differentiated lineages of *P. sanchezi* are distributed in the land management areas mentioned above. The Tecopa lineage (M25) is distributed on private and public water resources lands and is being monitored as part of the Bureau of Land Management's Amargosa River Area of Environmental Concern Implementation Plan (BLM 2006). The Shoshone lineage (M26) lives in a spring on private land that has a long history of disturbance which includes diversion of most of its flow and extensive modification of its outflow channel (USFWS 1984, Castleberry et al. 1990). This population appears to be restricted at present to a small area of leakage from a spring box (RH, HPL, CB, personal observation 15.XI.2011) and some measures will need to be taken to ensure its long term persistence. Our finding that *P. micrococcus* is restricted to its type locality area (Oasis Valley) suggests a need for additional conservation-related activities. The known populations of this species are in disturbed springs (per Maciolek 1983) on private land and some protection is needed to ensure their long term persistence. Surveys are also needed to evaluate the current status of several populations of this species that have not been sampled for several decades (e.g., that in Ute Springs) and to determine whether there may be previously unrecorded populations in Oasis Valley, which contains a large area of groundwater discharge (Reiner et al. 2002). *Pyrgulopsis micrococcus* (as currently constituted) was a candidate for addition to the Federal List of Endangered and Threatened Wildlife and Plants (USFWS 1976) prior to its removal from this list in 1994 (USFWS 1994).

Acknowledgements

We thank Paul Callomon and Amanda Lawless (ANSP), Andrew Simons and Jonathan Slaght (BellMNH), and Eric Hochberg and Paul Scott (SBMNH) for loans of specimens under their care. We thank Kevin P. Wilson (Death Valley National Park) and Sharon McKelvey (Ash Meadows National Wildlife Refuge) for provision of collection permits and logistical assistance in the field. We also thank Don Sada and Rob Robinson for collecting specimens that were used in this study. Yolanda Villacampa measured shells and prepared scanning electron micrographs, and Karolyn Darrow photographed the lectotype of *P. micrococcus* and inked the anatomical drawings.

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Appendix I

Specimen codes, locality details and GenBank accession numbers for COI and NDI sequences. (doi: 10.3897/zookeys.330.5852.app1) File format: Microsoft Word file (doc).

Citation: Hershler R, Liu H-P, Bradford C (2013) Systematics of a widely distributed western North American springsnail, *Pyrgulopsis micrococcus* (Caenogastropoda, Hydrobiidae), with descriptions of three new congeners. ZooKeys 330: 55–80. doi: 10.3897/zookeys.330.5852 Specimen codes, locality details and GenBank accession numbers for COI and NDI sequences. doi: 10.3897/zookeys.330.5852.app1

Appendix II

Genetic distances (maximum composite likelihood) of clades A–F ("*Pyrgulopsis micrococcus* complex"). (doi: 10.3897/zookeys.330.5852.app2) File format: Microsoft Word file (doc).

Citation: Hershler R, Liu H-P, Bradford C (2013) Systematics of a widely distributed western North American springsnail, *Pyrgulopsis micrococcus* (Caenogastropoda, Hydrobiidae), with descriptions of three new congeners. ZooKeys 330: 55–80. doi: 10.3897/zookeys.330.5852 Genetic distances (maximum composite likelihood) of clades A–F ("*Pyrgulopsis micrococcus* complex"). doi: 10.3897/zookeys.330.5852.app2

ZooKeys 330: 53–74 (2013)
doi: 10.3897/zookeys.330.5939
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Thrips (Insecta, Thysanoptera) of Iran: a revised and updated checklist

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Academic editor: *L. Mound* | Received 7 July 2013 | Accepted 15 August 2013 | Published 11 September 2013

Citation: Minaei K (2013) Thrips (Insecta, Thysanoptera) of Iran: a revised and updated checklist. ZooKeys 330: 53–74. doi: 10.3897/zookeys.330.5939

Abstract

In Iran, as a result of recent changes in nomenclature 201 species and one species group of the insect Order Thysanoptera, are here listed in 70 genera and five families. In considering species listed previously from this country, the presence of 7 species is considered not confirmed, and 12 species are excluded from the Iranian list. Problems in the study of Iranian Thysanoptera are discussed briefly.

Keywords

Iran, list, species, Thysanoptera

Introduction

Iran forms a large part of the Iranian plateau, and covers an area of 1,623,779 km². It is bordered in the north by the Caucasus Mountains, Middle Asian natural regions and the Caspian Sea (-27 m below sea level); in the west by the Anatolian and Mesopotamian regions; in the east by the eastern part of the Iranian plateau (Afghanistan and adjacent west Pakistan) and the Baluch-Sindian region; and finally in the south by the Persian Gulf and Oman Sea, which are connected by the latter to the Indian Ocean (Zehzad et al. 2002).

In Iran, the first record of thrips species was of three species, *Frankliniella intonsa* (Trybom), *Thrips flavus* Schrank and *T. tabaci* Lindeman, as pests of summer crops (Afshar 1938), and after that there were several scattered studies of this group in vari-

ous parts of this country. Recently the extensive Iranian literature on these insects was summarised by Bhatti et al. (2009a), who listed 177 species in 62 genera. However that checklist needs further consideration for four reasons:

1. The checklist by Bhatti et al. (2009a) covers the literature until 2007 and since then several important works on Thysanoptera of Iran have been published, including a further 13 genera and 38 species recorded or described. Moreover, a few recent name changes have become available.
2. There are some misinterpretations of "Iranian Persian literature" in Bhatti et al. (2009a). Thus a few species have appeared in Iranian literature as potential pests or as exotic pests without any supporting data or records from Iran.
3. Bhatti et al. (2009a) did not employ the standard suprageneric classification of Thysanoptera, so the utility of the checklist for students is limited.
4. The restricted distribution of the journal in which the book (Bhatti et al. 2009a) was published limits its utility to entomologists in Iran as well as the world.

Thrips studies in Iran: problems

Relevant information about thrips species recorded from Iran is severely lacking. For example, until the end of 2007, 187 primary references had been published on Iranian Thysanoptera, but, of these, 123 (65%) appeared only as "abstracts". Almost all of these consisted solely of species lists, without any further information being provided as to the number of collected specimens, their sex, or the habitats in which the species were collected. In one of these abstracts (Mortazaviha 1995) even the specific locality where the species were collected is not given, and for 15 thrips species collection details are restricted to the country "Iran". A further problem is the difficulty in tracing collections in which relevant voucher specimens were placed; and for many species there appear to be no extant voucher specimens. For example, *Haplothrips minutus* was recorded by Kheyrandish Koshkoei et al. (2000), but when asked for a loan of material Kheyrandish Koshkoei (personal communication, 2006) responded that he did not have access to any specimens of that species. Similarly, two papers (Mehrnejad and Panahi 2006; Kazemi and Mehrnejad 2011) concerning the biology and pest status of *Liothrips austriacus* (Karny) have been published from work carried out at the "Pistachio Research Institute" in Rafsenjan, Kerman Province, but no specimens of that species are available from that Institute at present (F. Kazemi, personal communication, 2013). Furthermore, in recent years Majid Mirab-balou has described or recorded several thrips species from Iran, but the specimens (including type specimens) have been deposited in China (Mirab-balou and Chen 2012a, b).

The third problem, related to the above, is imprecise reporting by Iranian authors. Several species have been reported from Iran despite the original specific identifications on which these reports are based remaining tentative. For example, the Iranian

records for three *Haplothrips* species reported by Bagheri and Alavi (2007) are based on specimens identified by zur Strassen as "perhaps" those species (Minaei and Mound 2008). Similarly, three *Aeolothrips* species recorded by Fallahzadeh et al. (2011) were only tentatively identified to species by Bhatti (Minaei 2013a).

A revised checklist of Thysanoptera from Iran

The following checklist is organized following the standard taxonomic hierarchy, and is based on published literatures including Bhatti et al. (2009a). For each suprageneric category a brief description is provided based largely on the Iranian fauna. Higher level taxonomy in the checklist follows Mound (2011a). Nomenclature follows that used in a web-based world checklist (ThripsWiki 2013), which should also be referred to for full synonymies for the names listed here. The checklist includes references for all additions and changes in taxonomic status or changes in synonymy made since the publication of the previous checklist by Bhatti et al. (2009a), and the symbol + is used to indicate these changes.

Suborder Terebrantia

The Terebrantia comprises eight families (Mound 2011a) of which four (Aeolothripidae, Melanthripidae, Stenurothripidae, Thripidae) are represented in Iran. Terebrantia species are largely phytophagous, feeding in flowers and on leaves.

Family Aeolothripidae

The family includes 194 extant species in 23 genera (ThripsWiki 2013), mostly from the temperate areas of the northern and southern hemispheres. Adults and larvae of many species in this family appear to be facultative predators of other small arthropods, in that they feed on both floral tissues as well as on thrips and mites that live in flowers. However, some species are almost certainly solely phytophagous, a few being univoltine in flowers of particular plant species (Tyagi et al. 2008), whereas in the warmer parts of the world, a considerable number of species are obligate predators (Hoddle 2003).

In this family, the most species-rich genus, *Aeolothrips* was interpreted by Bhatti (1988) in a different way to other specialists, with *Aeolothrips* restricted to *albicinctus* Haliday. Bhatti's interpretation put all other species from the original genus into four further genera (*Arabthrips* Bhatti, *Coleothrips* Haliday, *Fabothrips* Bhatti, *Podaeolella* Priesner). In this paper, that interpretation is not accepted. Four genera including 23 species are recognized in this family in Iran.

***Aeolothrips* Haliday, 1836**	
+ *afghanus* Jenser, 1984	male described by Minaei et al. (2013)
albicinctus Haliday, 1836	
collaris Priesner, 1919	
+ *cursor* Priesner, 1939	added by Mirab-balou and Chen (2012c)
deserticola Priesner, 1929	
+ *eremicola* Priesner, 1938	added by Zolfaghari et al. (2012); male described by Alavi et al. (2013)
fasciatus (Linnaeus, 1758)	
gloriosus Bagnall, 1914	
heinzi zur Strassen, 1990	
intermedius Bagnall, 1934	
+ *modestus* zur Strassen, 1966	added by Minaei (2013a)
mongolicus Pelikan, 1985	
+ *montivagus* Priesner, 1948	added by Mirab-balou and Chen (2012c)
tenuicornis Bagnall, 1926	
versicolor Uzel, 1895	
+ *wittmeri* Priesner, 1935	added by Alavi et al. (2012)
+ *zurstrasseni* Minaei	described by Minaei (2013a)
***Indothrips* Bhatti, 1967**	
bhushani Bhatti, 1967	
***Orothrips* Moulton, 1907**	
priesneri (Titschack, 1958)	
***Rhipidothrips* Uzel, 1895**	
brunneus Williams, 1913	
flavus Tunç, 1991	
gratiosus Uzel, 1895	
unicolor zur Strassen, 1965	

Family Melanthripidae

Melanthripids were considered to be members of the Aeolothripidae until recently. The family now includes 66 extant species in four genera: *Ankothrips* (13 species), *Cranothrips* (11 species), *Dorythrips* (6 species) and *Melanthrips* (35 species). All species in the family are flower-feeding but each genus exhibits a remarkable discontinuity in geographical distribution: *Cranothrips* and *Dorythrips* are known only from the Southern Hemisphere, whereas *Ankothrips* and *Melanthrips* are mainly from the Northern Hemisphere but each with one or two species from South Africa (Pereyra and Mound 2009, Hoddle et al. 2013). In Iran, seven species in two genera have been recorded.

***Ankothrips* Crawford, 1909**

+ *zayandicus* Minaei, Haftbaradarn & Mound, 2012 described by Minaei et al. (2012)

***Melanthrips* Haliday, 1836**

fuscus (Sulzer, 1776)
+ *hei* Mirab-balou & Chen, 2012 described by Mirab-balou and Chen (2012a)
knechteli Priesner, 1936
pallidior Priesner, 1919
rivnayi Priesner, 1936
separandus Priesner, 1936

Family Stenurothripidae

The extant species in this group were placed in the family Adiheterothripidae (Bhatti 1986), but this is now considered a synonym of Stenurothripidae (Bhatti 2006). The three extant genera of this family occur in California and in the Mediterranean region through to India (Mound and Marullo 1999). The species in this family apparently all breed in flowers, and they probably have a high degree of host specificity. All four species of *Holarthrothrips* breed in the male flowers of date palms and its relatives (Mound et al. 2013b). Only one species is recorded in Iran.

***Holarthrothrips* Bagnall, 1927**

josephi Bhatti, 1986

Family Thripidae

Thripids include 2020 species in 284 genera worldwide (ThripsWiki 2013). Most of them are phytophagous on higher plants, with a few species on ferns (Mound 2002), and a few are obligate predators (Mound 2011b). However, some polyphagous pest thrips such as *Frankliniella occidentalis* and *Thrips tabaci* can behave as facultative predators (Wilson et al. 1996). One genus in Brazil comprises species that are ectoparasitic on Hemiptera (Cavalleri et al. 2010). Four subfamilies within the Thripidae are currently recognized worldwide, and each of these is represented in Iran.

Thripidae—Dendrothripinae

More than 90 species, in 11 genera, are recognized worldwide in this subfamily (ThripsWiki 2013). All of the species live on leaves. Five species in two genera have been recorded in Iran.

***Dendrothrips* Uzel, 1895**
degeeri Uzel, 1895
karnyi Priesner, 1921
phyllireae (Bagnall, 1927)
saltator Uzel, 1895

***Pseudodendrothrips* Schmutz, 1913**
mori (Niwa, 1908)

Thripidae—Panchaetothripinae

Wilson (1975) provided an account of the members of this subfamily that is now considered to include 136 species in 38 genera. The species in this subfamily are all leaf feeding usually associated with older, senescing leaves (Mound et al. 2013b). Seven species in six genera have been found in Iran so far.

***Caliothrips* Daniel, 1904**
impurus (Priesner, 1928)
+ *quadrifasciatus* — the species is recorded as *Caliothrips graminicola* Bagnall & Cameron, 1932 in Iranian literature

***Heliothrips* Haliday, 1836**
haemorrhoidalis (Bouche, 1833)

***Parthenothrips* Uzel, 1895**
dracaenae (Heeger, 1854)

***Retithrips* Marchal, 1910**
syriacus (Mayet, 1890)

***Rhipiphorothrips* Morgan, 1913**
cruentatus Hood, 1919

***Selenothrips* Karny, 1911**
+ *rubrocinctus* (Giard, 1901) — added by Mirab-balou and Chen (2012d)

Thripidae—Sericothripinae

This group is treated as a subfamily of Thripidae to include three genera: *Hydatothrips* Karny, *Neohydatothrips* John, S*ericothrips* Haliday (ThripsWiki 2013). This subfamily

includes 148 species worldwide, and these are usually found in association with flowers but with some species breeding on leaves (Mound and Tree 2009). Bhatti (2006) proposed *Papiliothrips* as a new genus and transferred *Neohydatothrips gracilicornis* and two species of *Sericothrips* to the genus, but this is not accepted here. Two species in one genus are reported in Iran so far.

***Neohydatothrips* John, 1929**
gracilicornis (Williams, 1916)
tadzhicus (Pelikan, 1964)

Thripidae—Thripinae

This is the largest group of Thripidae with 1644 species in 232 genera (ThripsWiki 2013). The species exhibit a wide range of biologies, and most of the species of thrips regarded as pests are included in this subfamily (Mound 1997). Bhatti et al. (2009a) synonymized *Chirothrips aculeatus* Bagnall with *Chirothrips pedestris* (Karny). However, this was not accepted by other researchers (Minaei and Mound 2010a). Moreover, in contrast to zur Strassen (2003) and Bhatti et al. (2009a), three *Chirothrips* species recorded in Iran (*africanus* Priesner, *manicatus* (Haliday), *pallidicornis* Priesner), together with *ammophilae* Bagnall, were placed in a *manicatus* species group by Minaei and Mound (2010a) due to the difficulty in separating them from each other by morphological characters, and this approach is accepted here. Two species listed under the genus *Thrips*, *T. iranicus* Yakhontov and *T. pistaciae* Yakhontov, are not recognizable at present due to the poor descriptions (see Bhatti et al. 2009a). In addition, *T. fraudulentus* (Priesner) is very similar to *T. atratus* Haliday. Laurence Mound (personal communication 2010) examined the holotype of *fraudulentus* in the Forschungsinstitut Senckenberg, Frankfurt, and although there are differences in the lengths of the antennae the recognition of *fraudulentus* as a distinct species remains doubtful. In Iran, 110 species and one species-group in 35 genera in this subfamily are recognized.

***Agalmothrips* Priesner, 1965**
parviceps (Priesner, 1965)

***Anaphothrips* Uzel, 1895**
+ *obscurus* (Müller, 1776) male described by Mirab-balou and Chen (2010)
sudanensis Trybom, 1911

***Aptinothrips* Haliday, 1836**
elegans Priesner, 1924
rufus (Haliday, 1836)
stylifer Trybom, 1894

***Arorathrips* Bhatti, 1990**
+ *mexicanus* (Crawford DL, 1909) added by Minaei and Alichi (in press)

Bregmatothrips Hood, 1912
bournieri Pelikan, 1988

***Chirothrips* Haliday, 1836**
aculeatus (Bagnall, 1927)
+ *atricorpus* (Girault, 1927) stat. rev. by Minaei and Mound (2010a)
kurdistanus zur Strassen, 1967
+ *manicatus* species-group defined by Minaei and Mound (2010a)
+ *maximi* Ananthakrishnan, 1957 added by Mirab-balou et al. (2013)
+ *meridionalis* Bagnall, 1927 stat. rev. by Minaei and Mound (2010a)
molestus Priesner, 1926

***Collembolothrips* Priesner, 1935**
mediterraneus Priesner, 1935

***Drepanothrips* Uzel, 1895**
reuteri Uzel, 1895

***Eremiothrips* Priesner, 1950**
antilope (Priesner, 1923)
arya (zur Strassen, 1975)
+ *bhattii* Minaei, 2012 described by Minaei (2012)
dubius (Priesner, 1933)
efflatouni (Priesner, 1965)
farsi Bhatti & Telmadarraiy, 2003
shirabudinensis (Yakhontov, 1929)
+ *similis* Bhatti, 1988 added by Ramezani et al. (2009)
taghizadehi (zur Strassen, 1975)
tamaricis (zur Strassen, 1975)
varius (Bhatti, 1967)
+ *zurstrasseni* Bhatti, Bagheri & Ramezani, 2009 described by Bhatti et al. (2009b)

***Euphysothrips* Bagnall, 1926**
minozzii Bagnall, 1926

***Exothrips* Priesner, 1939**
redox Bhatti, 1975

***Ficothrips* Minaei, 2012**
+ *moundi* Minaei, 2012 described by Minaei (2012a)

***Frankliniella* Karny, 1910**
intonsa (Trybom, 1895)
occidentalis (Pergande, 1895)
pallida (Uzel, 1895)
schultzei (Trybom, 1910)
tenuicornis (Uzel, 1895)

***Kakothrips* Williams, 1914**
+ *dentatus* Knechtel, 1939 added by Mirab-balou and Chen (2011a)
pisivorus (Westwood, 1880)
priesneri Pelikan, 1965

***Limothrips* Haliday, 1836**
angulicornis Jablonowski, 1894
+ *cerealium* (Haliday, 1836) added by Mirab-balou et al. (2013)
denticornis (Haliday, 1836)
schmutzi Priesner, 1919
transcaucasicus Savenko, 1944

***Megalurothrips* Bagnall, 1915**
+ *distalis* (Karny, 1913) added by Mirab-balou and Chen (2011b)

***Microcephalothrips* Bagnall, 1926**
abdominalis (Crawford DL, 1910)

***Mycterothrips* Trybom, 1910**
consociatus (Targioni-Tozzetti, 1887)
+ *hamedaniensis* Mirab-balou, Shi & Chen, 2011 described by Mirab-balou et al. (2011)
latus (Bagnall, 1912)
salicis (Reuter, 1879)
tschirkunae (Yakhontov, 1961)
+ *weii* Mirab-balou, Shi & Chen, 2011 described by Mirab-balou et al. (2011)

***Odontothrips* Amyot & Serville, 1843**
confusus Priesner, 1926
loti (Haliday, 1852) added by Mirab-balou and Chen (2011b)
meliloti Priesner, 1951
phlomidinus Priesner, 1954

***Oxythrips* Uzel, 1895**
+ *claripennis* Priesner, 1940 added by Mirab-balou and Chen (2013)
halidayi Bagnall, 1924

retamae (Priesner, 1934)
ulmifoliorum (Haliday, 1836)
wiltshirei Priesner, 1954

***Parascolothrips* Mound, 1967**
priesneri Mound, 1967

***Pezothrips* Karny, 1907**
bactrianus (Pelikan, 1968)

***Psilothrips* Hood, 1927**
bimaculatus (Priesner, 1932)

***Rubiothrips* Schliephake, 1975**
+ *parisae* Mirab-balou & Chen, 2013 described by Mirab-balou and Chen (2013)
+ *tongi* Mirab-balou & Chen, 2013 described by Mirab-balou and Chen (2013)
+ *vitalbae* (Bagnall, 1926) added by Mirab-balou and Chen (2013)
vitis (Priesner, 1933)

***Scirtothrips* Shull, 1909**
mangiferae Priesner, 1932

***Scolothrips* Hinds, 1902**
latipennis Priesner, 1950
longicornis Priesner, 1926
rhagebianus Priesner, 1950

***Sitothrips* Priesner, 1931**
arabicus Priesner, 1931

***Sphaeropothrips* Priesner, 1928**
vittipennis (Bagnall, 1927)

***Stenchaetothrips* Bagnall, 1926**
+ *biformis* (Bagnall, 1913) added by Mirab-balou and Chen (2011a)

***Stenothrips* Uzel, 1895**
graminum Uzel, 1895

***Taeniothrips* Amyot & Serville, 1843**
inconsequens (Uzel, 1895)

***Tamaricothrips* Priesner, 1964**
tamaricis (Bagnall, 1926)

***Tenothrips* Bhatti, 1967**
discolor (Karny, 1907)
frici (Uzel, 1895)
latoides (Pelikan, 1968)
reichardti (Priesner, 1926)

***Thermothrips* Pelikan, 1949**
+ *mohelensis* (Pelikan, 1949) added by Mirab-balou and Chen (2013)

***Thrips* Linnaeus, 1758**
alavii Mirab-balou, Tong & Chen, 2012 described by Mirab-balou et al. (2012b)
albopilosus Uzel, 1895
+ *alliorum* (Priesner, 1895) added by Mirab-balou et al. (2012b)
angusticeps Uzel, 1895
atratus Haliday, 1836
+ *australis* Bagnall, 1915 added by Minaei (2012b)
dubius Priesner, 1927
euphorbiae Knechtel, 1923
flavus Schrank, 1776
fraudulentus (Priesner, 1954)
fuscipennis Haliday, 1836
hawaiiensis (Morgan, 1913)
iranicus Yakhontov, 1951
major Uzel, 1895
mareoticus (Priesner, 1932)
meridionalis (Priesner, 1926)
minutissimus Linnaeus, 1758
nigropilosus Uzel, 1895
pelikani Schliephake, 1964
physapus Linnaeus, 1758
pillichi Priesner, 1924
pistaciae Yakhontov, 1951
simplex (Morison, 1930)
tabaci Lindeman, 1889
trehernei Priesner, 1927
verbasci (Priesner, 1920)
vuilleti (Bagnall, 1933)
vulgatissimus Haliday, 1836

Suborder Tubulifera- Family Phlaeothripidae

Only a single family is recognized in this suborder, the Phlaeothripidae, with two subfamilies, Idolothripinae and Phlaeothripinae. Species of Phlaeothripidae are diverse in their biologies. Idolothripinae are all considered to feed on fungal spores (Mound and Palmer 1983). In the Phlaeothripinae, three "lineages" (*Haplothrips*, *Liothrips* and *Phlaeothrips*) have been recognized (Mound and Marullo 1996). The *Haplothrips* lineage is now well defined as the tribe Haplothripini (Mound and Minaei 2007, Minaei and Mound 2008). Members of this tribe are usually phytophagous, but some *Haplothrips* species are predators on other small arthropods, and one unusual Haplothripine species has been demonstrated to be a predator of the eggs of social wasps (Cavalleri et al. 2013). Members of the "*Phlaeothrips* lineage" are fungus feeders on fungal hyphae (Mound et al. 2013a). Species in the "*Liothrips* lineage" are leaf-feeding on the leaves of shrubs and trees, and many of these are involved in the induction of galls on leaves (Ananthakrishnan and Raman 1989). Four species in four genera of Idolothripinae, and 41 species in 15 genera of Phlaeothripinae, are recognized in Iran.

Subfamily Idolothripinae

***Allothrips* Hood, 1908**
+ *pillichelus bournieri* Mound, 1972 added by Minaei (2011)

***Compsothrips* Reuter, 1901**
albosignatus (Reuter, 1884)

Megathrips Targioni-Tozzetti, 1881
flavipes (Reuter, 1901)

Pseudocryptothrips Priesner, 1919
meridionalis Priesner, 1919

Subfamily Phlaeothripinae
Tribe Haplothripini

***Bagnalliella* Karny, 1920**
+ *yuccae* (Hinds, 1902) added by Mirab-balou et al. (2012a)

***Dolicholepta* Priesner, 1932**
micrura (Bagnall, 1914)

***Haplothrips* Amyot & Serville, 1843**
aculeatus (Fabricius, 1803)
andresi Priesner, 1931
clarisetis Priesner, 1931
distinguendus (Uzel, 1895)
eragrostidis Priesner, 1931
flavicinctus (Karny, 1910)
flavitibia Williams, 1916
ganglbaueri Schmutz, 1913
globiceps (Bagnall, 1934)
+ *herajius* Minaei & Aleosfoor, 2013 described by Minaei and Aleosfoor (2013)
kermanensis zur Strassen, 1975
kurdjumovi Karny, 1913
leucanthemi (Schrank, 1781)
maroccanus Priesner, 1950
phyllophilus Priesner, 1914
reuteri (Karny, 1907)
subtilissimus (Haliday, 1852)
tamaricinus Priesner, 1939
tritici (Kurdjumov, 1912)
vuilleti Priesner, 1920

***Neoheegeria* Schmutz, 1909**
dalmatica Schmutz, 1909
gigantea (Priesner, 1934) added by Minaei and Behmanesh (2012)
persica Priesner, 1954

***Plicothrips* Bhatti, 1979**
apicalis (Bagnall, 1915)

***Liothrips* lineage**

***Ataliothrips* Bhatti, 1995**
reuteri (Bagnall, 1913)

***Cephalothrips* Uzel, 1895**
coxalis Bagnall, 1926
monilicornis (Reuter, 1885)

***Liothrips* Uzel, 1895**
austriacus (Karny, 1909)
jakhontovi Kreutzberg, 1955
pragensis Uzel, 1895
setinodis (Reuter, 1880)

***Phlaeothrips* lineage**

***Aleurodothrips* Franklin, 1909**

+ *fasciapennis* Franklin, 1909	added by Mirab-balou and Chen (2012b)

***Hindsiothrips* Stannard, 1958**

+ *sisakhti* Minaei, 2013	described by Minaei (2013b)

***Hoplandrothrips* Hood, 1912**

bidens (Bagnall, 1910)
hungaricus Priesner, 1961

***Hoplothrips* Amyot & Serville, 1843**

+ An unknown species	added by Jalali Sandi et al. (2011)

***Idiothrips* Faure, 1933**

+ *bellus* Faure, 1933	*Idiothrips ficus* Bhatti, 1967 is synonymized with *bellus* by Minaei (2013b)

***Phlaeothrips* Haliday, 1836**

coriaceus Haliday, 1836

***Stictothrips* Hood, 1924**

faurei Hood, 1924

Unconfirmed Thysanoptera species

Aeolothripidae

Aeolothrips

Cheraghian and Barimani Varandi (2000) reported *A. insularis* Priesner from Iran based on a specimen identified by zur Strassen as "near *insularis*" (e-mail from zur Strassen to Bhatti, see Bhatti et al. 2009a). Therefore, the record of *Aeolothrips insularis* in Iran is doubtful (see also Bhatti et al. 2009a). Moreover, the records of two other species in the genus, *balati* Pelikan and *citricinctus* Bagnall from Iran are also not confirmed (see Minaei 2013a).

Phlaeothripidae

Haplothrips

The report of *H. minutus* (Uzel) from Iran is based on specimens identified by zur Strassen with a query (?). Similarly, the reports of three other species of *Haplothrips*, (*caespitis* Priesner, *longipes* Bagnall, and *rabinovitchi* Priesner) from Iran have also not been confirmed (Minaei and Mound 2008).

Species removed from the Iranian Thysanoptera list

Thripidae

***Caliothrips striatopterus* (Kobus, 1892):** this species was recorded by Manzari (2004) in an informal newsletter as a cursory report, and so is excluded from the Iranian list (see also Bhatti et al. 2009a, Minaei and Aleosfoor 2013).

***Chaetanaphothrips* Haliday, 1836**

The only mention of this genus in an Iranian context appeared in a text book (Esmaili 1983) with these thrips noted as potential pests in the north of Iran, but no species was recorded. The species in the genus are widespread in tropical and subtropical countries, and also in greenhouses in temperate areas including Europe (zur Strassen 2003); it is possible the genus may be found in Iran as well. However, at present there is no evidence to indicate the occurrence of any species of the genus in Iran.

Frankliniella

***cephalica* (Crawford DL, 1910):** the species appeared in the Iranian literature as a potential pest in the north of Iran but with no recorded details of occurrence (Esmaili 1983). *F. cephalica* has been recorded between Bermuda and Trinidad, and in Mexico and Colombia as well as Japan and Taiwan (Hoddle et al. 2013).

***sulphurea* Schmutz, 1913:** the species is considered as a good species by Bhatti et al. (2009a), but is usually considered a synonym of *schultzei*. The body colour in *schultzei* is variable and the species has 17 synonyms from various tropical countries around the world (Cavalleri and Mound 2012).

***tritici* (Fitch, 1855):** the species appeared in the Iranian literature as an external plant quarantine element (but not recorded) (Salavatian 1996). *F. tritici* is widespread in North America (Hoddle et al. 2013).

***Scirtothrips citri* (Moulton, 1909)**: the Californian citrus thrips was mentioned in the text book by Esmaili (1983) in which the author described damage to the flowers, leaves and fruits of citrus plants. However, no evidence was provided concerning the presence of this species in Iran. In addition to California, the species has been found in Arizona and Mexico (Hoddle et al. 2013).

***Scolothrips sexmaculatus* (Pergande, 1890):** the species was first reported from Iran by Shishehbor (1991) based on specimens that were not authentically determined. The name was used subsequently by a few other Iranian authors. The species identified has been recorded with certainly only from North America (including California) (Hoddle et al. 2013). According to Mound (2011b), Old World records probably all refer to *S. rhagebianus*, which has been recorded in Iran (see also Bhatti et al. 2009a).

***Thrips coloratus* Schmutz, 1913**: the species was recorded by Manzari (2004) in an informal newsletter as a cursory report and although potentially it might occur in Iran (zur Strassen 2003), it is excluded here.

Phlaeothripdae

Haplothrips

***bagnalli* (Trybom, 1910)**
nr. *bagrolis* Bhatti, 1973
***cerealis* Priesner**

The first two species listed above have already been excluded from the Iranian list by Minaei and Aleosfoor (2013), whilst the third is a misidentification of *Haplothrips tritici* (Kurdjumov) (Minaei and Mound 2008). There is no evidence of the presence of *cerealis* in Iran (Minaei and Mound 2010b).

***rasouliani* Mirab-balou & Chen:** this name recently appeared in a paper (Mirab-balou et al. 2012c) but it is not available under the terms of the International Commission on Zoological Nomenclature International Commission on Zoological Nomenclature according to article 8.1, and so it is excluded here.

Discussion

Knowledge of the natural biological systems of Iran is variable. Despite excellent floristic studies, such as Flora Iranica that now provides an identification system to more than 10,000 plant species (Rechinger 1989), comprehensive studies on the insect fauna of this country are lacking. Iran, in particular, is a bridge between the faunas of the European and Oriental Realms, and this produces considerable difficulties in studying any single group. In addition, the number of species recorded from any given

area in this country almost totally depends on where particular specialists have lived or spent their careers. Consequently, field sampling of the thrips fauna of Iran has been uneven across the various Provinces, and so the results do not necessarily represent the biological diversity of any given area. Although there are a few thrips (especially those that are well known as crop pests) that are found almost all over Iran, several Provinces have yet to be surveyed for their thrips fauna. Even in those Provinces that have been apparently well-surveyed, there are still thrips species remaining to be discovered. For instance, Fars Province has been surveyed for at least 14 years continuously yet there are still several examples of recently collected material from the Province in the Collection of Department of Plant Protection, Shiraz University, that represent unrecorded, or even undescribed species. Despite this, faunistic knowledge of these tiny insects in Iran is better than in neighbouring countries, presumably due to political unrest in most of the neighbouring countries.

Although the fauna of Iran shares many species with the European Mediterranean region, other areas have a considerable effect on the Iranian fauna. For example, among the 125 species from the family Thripidae recorded here, 91 are also present in the European Mediterranean area (zur Strassen 2003). Of the remaining species, 11 have been described from Iran and most of the other 23 species are from the Oriental.

Acknowledgement

I am grateful to Dom Collins (The Food and Environment Research Agency, Sand Hutton, York, United Kingdom) for reviewing an earlier draft of this manuscript as well as editorial help. The manuscript was improved through the advice and critics kindly provided by Laurence Mound (CSIRO Ecosystem Sciences, Canberra, Australia) and two anonymous referees.

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ZooKeys 330: 75–103 (2013)
doi: 10.3897/zookeys.330.5795
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Three new species of *Oreophryne* (Anura, Microhylidae) from Papua New Guinea

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Academic editor: *F. Andreone* | Received 15 June 2013 | Accepted 20 August 2013 | Published 13 September 2013

http://zoobank.org/B8F0757A-63B3-4FE7-B1A3-17DF76A2F951

Citation: Kraus F (2013) Three new species of *Oreophryne* (Anura, Microhylidae) from Papua New Guinea. ZooKeys 330: 75–103. doi: 10.3897/zookeys.330.5795

Abstract

I describe three new species of the diverse microhylid frog genus *Oreophryne* from Papua New Guinea. Two of these occur in two isolated mountain ranges along the northern coast of Papua New Guinea; the third is from Rossel Island in the very southeasternmost part of the country. All three are the first *Oreophryne* known from these areas to have a cartilaginous connection between the procoracoid and scapula, a feature usually seen in species far to the west or from the central cordillera of New Guinea. Each of the new species also differs from the many other Papuan *Oreophryne* in a variety of other morphological, color-pattern, and call features. Advertisement-call data for *Oreophryne* species from the north-coast region suggest that they represent only two of the several call types seen in regions further south, consistent with the relatively recent derivation of these northern regions as accreted island-arc systems. The distinctively different, whinnying, call type of the new species from Rossel Island occurs among other *Oreophryne* from southeastern Papua New Guinea but has been unreported elsewhere, raising the possibility that it may characterize a clade endemic to that region.

Keywords

Adelbert Mts., advertisement calls, Frog, North-coast ranges, Rossel Island, Torricelli Mts

Introduction

Asterophryinae is a subfamily of Microhylidae consisting of 21 genera and hundreds of species that is largely restricted to New Guinea and its satellite islands. Of the constituent genera, *Oreophryne* is presently one of the largest within the Papuan Region, which consists of New Guinea and immediately adjacent islands, the Bismarck and Admiralty Archipelagos, and the Solomon Islands. Currently, the genus has 40 named species in this area (Frost 2013), second in local asterophryine species diversity only to *Cophixalus*, which currently has 42 species (Kraus 2012). Both genera also range outside this area: *Oreophryne* has ten additional species distributed across the Moluccas, Lesser Sundas, Sulawesi, and southern Philippines (Parker 1934, Frost 2013); *Cophixalus* has 18 additional species in northeastern Australia and one more on Halmahera (Parker 1934, Hoskin 2008, 2012, Hoskin and Aland 2011).

Species of *Oreophryne* are usually arboreal dwellers of rainforest habitats, but a few species are terrestrial inhabitants of alpine grasslands (Zweifel et al. 2005, Günther and Richards 2011). The genus is approximately evenly divided into two groups that differ in whether the connection between the procoracoid and scapula consists of a cartilaginous rod or a ligament (Parker 1934). The former group is largely restricted to the central cordillera of New Guinea or areas to the west; *O. kampeni* is currently the only recognized representative of this group in Papua New Guinea outside of that region, being known only from its type locality near Port Moresby. In contrast, the group having a ligamentous connection is distributed across the entire Papuan range of *Oreophryne* but appears to be absent from the islands west of New Guinea (Parker 1934, Brongersma 1948, Brown and Alcala 1967). There is currently no evidence to suggest if either of these groups is monophyletic. Additional phenotypic features of widespread systematic use for distinguishing among *Oreophryne* species include the presence and degree of toe webbing, relative length of the third vs. fifth toes, size of digital discs, aspects of color pattern, and (more recently) structure of advertisement calls (e.g., Parker 1934, Günther and Richards 2011, Zweifel et al. 2003, 2005).

Several of the early described species of *Oreophryne* have remained uncollected and little studied since their original descriptions, and this long hampered taxonomic study of the genus and diagnosis of new species. Consequently, the genus has received limited taxonomic attention until recently. Nonetheless, of the 40 species of Papuan *Oreophryne*, 24 have been described since 2000, and numerous additional species remain collected but undescribed. Most of these newly described species are from western New Guinea or from the central cordillera (e.g., Günther et al. 2001, Günther 2003a, b), but Zweifel et al. (2003) treated *Oreophryne* from the northern coast region of New Guinea. Herein, I describe a further three distinctive species from Papua New Guinea that belong to the group having a cartilaginous connection between the procoracoid and scapula. Two of these are from some of those same north-coast mountain ranges treated by Zweifel et al. (2003), and the third is from Rossel Island in southeasternmost Papua New Guinea. These bring to four the number of *Oreophryne* from outside

the central cordillera of Papua New Guinea that have a cartilaginous connection between the procoracoid and scapula. Other undescribed *Oreophryne* I have from this region have ligamentous connections between the scapulae and procoracoids and will be treated in a later paper.

Materials and methods

I collected specimens under all applicable institutional animal-care guidelines and provincial and national permits, euthanized them, fixed them in 10% buffered formalin, and then transferred them to 70% ethanol for storage. Livers were removed from representative specimens of each species and stored in 70% ethanol. I made all measurements with digital calipers (SV) or an optical micrometer to the nearest 0.1 mm, with the exception that disc widths were measured to the nearest 0.01 mm. Measurements, terminology, and abbreviations follow Zweifel (1985) and Kraus and Allison (2006): body length from snout to vent (SV); tibia length from heel to outer surface of flexed knee (TL); horizontal diameter of eye (EY); distance from anterior corner of eye to center of naris (EN); internarial distance, between centers of external nares (IN); distance from anterior corner of eye to tip of snout (SN); head width at widest point, typically at the level of the tympana (HW); head length, from tip of snout to posterior margin of tympanum (HL); horizontal tympanum diameter (TY); width of third finger disc (3rdF); and width of the fourth toe disc (4thT). I measured mass to the nearest 0.05 g on freshly euthanized animals using a 10-g Pesola spring scale. I determined sex of animals by examination of vocal slits or by dissection; I determined the cartilaginous nature of the connection between procoracoid and scapula by dissection of alcoholic specimens with a binocular dissecting microscope. Webbing formulae follow Savage and Heyer (1967) as modifed by Myers and Duellman (1982).

I recorded calls in the field using a Sennheiser ME66 microphone with a K6 powering module and either a Sony Professional Walkman WM-D6C cassette recorder, a Sony MDSJE480 minidisc recorder, or a Marantz PMD660 digital audio recorder. I analyzed call structure using the computer program Avisoft-SASLab Pro(v4.34), available from Avisoft Bioacoustics (http://www.avisoft.com/).

I confirmed generic assignment of the frogs using the presence of eleutherognathine maxillae, procoracoids, and clavicles that do not extend to the scapulae (Parker 1934). For discrimination from congeners I relied on direct comparison to museum material (Appendix) as well as to information from Günther (2003a, b), Günther and Richards (2011), Günther et al. (2001, 2009, 2012), Parker (1934), Richards and Iskandar (2000), van Kampen (1913), Zweifel (1956, 2003), and Zweifel et al. (2003, 2005).

Type specimens of new species are deposited in the Bernice P. Bishop Museum, Honolulu (BPBM) and Papua New Guinea National Museum and Art Gallery, Port Moresby (PNGNM). Additional museum abbreviations (Appendix) follow Leviton et al. (1985). Specimens have latitude and longitude coordinates using the Australian Geodetic Datum, 1966 (AGD 66).

Taxonomy

***Oreophryne cameroni* sp. n.**
http://zoobank.org/2F75551A-847F-41C0-A6A1-7ACBA408B37E
http://species-id.net/wiki/Oreophryne_cameroni
Figs 1, 2A, B

Holotype. BPBM 34677 (field tag FK 13704), adult male, collected by F. Kraus at Keki Lodge, Adelbert Mts., 4.7048°S, 145.4042°E, 850 m, Madang Province, Papua New Guinea, 1 October 2009.

Paratypes (n = 3). BPBM 34678, same data as holotype, except collected 4 October 2009; BPBM 22689, Siruohu, ~3 km SSE Mt. Sapau summit, Torricelli Mts., 3.3908°S, 142.5297°E, 550–700 m, West Sepik Province, Papua New Guinea; AMNH 78139, Mt. Nibo, 19 km NE Lumi, 700–1550 m, West Sepik Province, Papua New Guinea.

Diagnosis. *Oreophryne cameroni* can be distinguished from all congeners by its unique combination of small size (adult male SV = 19.5–20.4 mm); cartilaginous connection between the scapula and procoracoid; basal toe webbing; fifth toe longer than third; leg moderately long (TL/SV = 0.49–0.51); snout slightly shorter than broad (EN/IN = 0.94–0.95, IN/SV = 0.097–0.103); head relatively broad (HW/SV = 0.37–0.42); finger discs relatively narrow (3rdF/SV = 0.048–0.068); dorsum brown with scattered pustules, white flecks, and darker lateral blotches; venter heavily stippled with brown; dark-brown subocular blotch; dark-brown iris; and call a series of short peeps delivered at a rate of 2.8–2.9 notes/s with a dominant frequency of around 2900 Hz.

Comparisons with other species. The new species differs from all other Papuan *Oreophryne* except *O. idenburghensis*, *O. oviprotector*, and *O. waira* in its unique combination of having a cartilaginous connection between the scapula and procoracoid and basal webbing between the toes. It is easily distinguished from *O. idenburghensis* by its much smaller size (SV = 19.5–20.4 mm vs. 43–47 mm in *O. idenburghensis*), longer leg (TL/SV = 0.49–0.51 vs. 0.42–0.44 in *O. idenburghensis*), broader head (HW/SV = 0.37–0.42 vs. 0.34–0.35 in *O. idenburghensis*), broader snout (EN/IN = 0.94–0.95 vs. 0.87–0.91 in *O. idenburghensis*, IN/SV = 0.097–0.103 vs. 0.076–0.081 in *O. idenburghensis*), and narrower finger discs (3rdF/SV = 0.048–0.068 vs. 0.077–0.091 in *O. idenburghensis*). It differs from *O. oviprotector* in its brown dorsal color with scattered white flecks (lime green, without flecks in *O. oviprotector*), dark-gray ventral coloration (pale translucent gray in *O. oviprotector*), dark-brown subocular blotch (absent in *O. oviprotector*), dark-brown iris (coppery brown around pupil and yellowish distally from pupil in *O. oviprotector*), absence of yellow inguinal and axillary blotches (present in *O. oviprotector*), absence of conspicuous green bar between eyes (present in *O. oviprotector*), absence of a white ring around eye (present in *O. oviprotector*), and call a series of peeps delivered at a rate of 2.8–2.9 notes/s (call a rattle delivered at a rate of 26–28 notes/s in *O. oviprotector*) and with each note of 71–134 ms duration (each note of approximately 12 ms duration in *O. oviprotector*). From *O. waira*, the new species differs

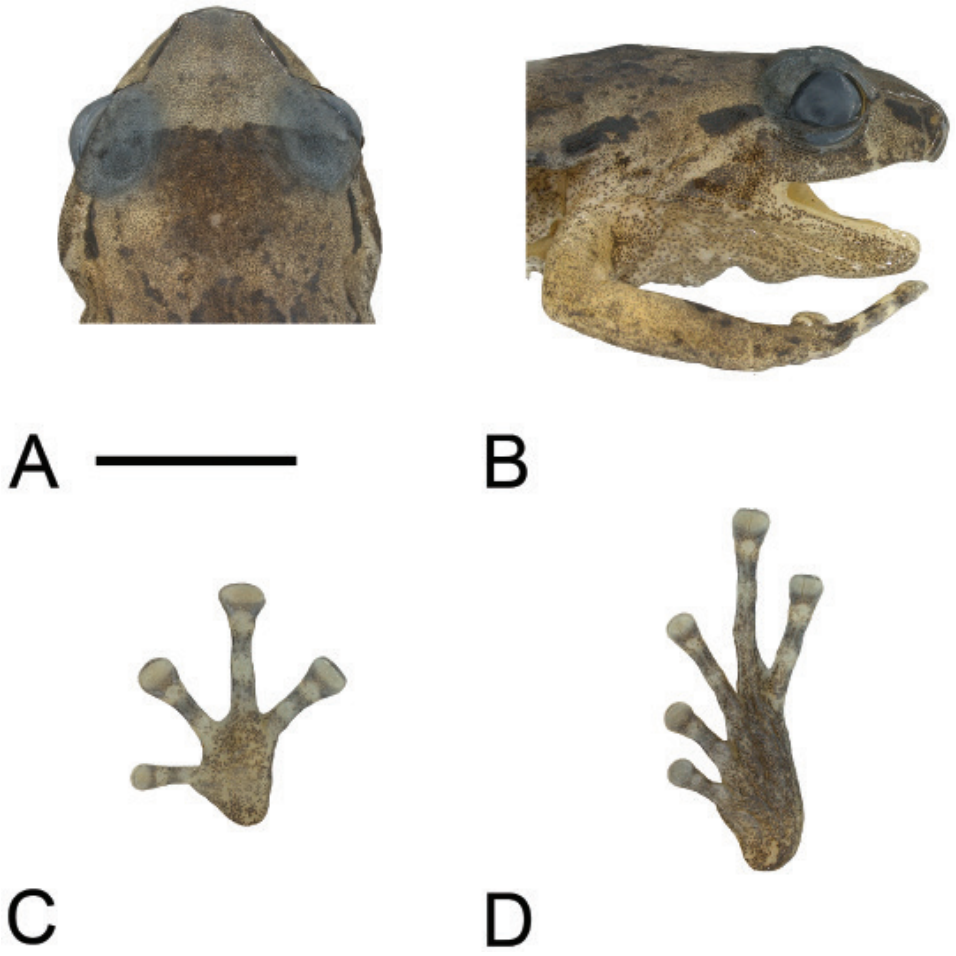

Figure 1. A Top of head **B** side of head **C** palmar view of left hand, and **D** plantar view of left foot of holotype of *Oreophryne cameroni* sp. n. (BPBM 34677). Scale bar = 5 mm.

in having the fifth toe longer than the third (subequal in *O. waira*), longer leg (TL/SV = 0.49–0.51 vs. 0.43–0.46 in *O. waira*), and call a series of peeps of around 2900 Hz and delivered at a rate of 2.8–2.9 notes/s (call a rattle of around 3600 Hz and delivered at a rate of 15–19 notes/s in *O. waira*).

Description of holotype. An adult male with an incision on right side and left pectoral region dissected. Procoracoid is connected to the scapula by a cartilaginous rod. Head wide (HW/SV = 0.42), with steep, almost vertical loreal region; upper lip inflated. Canthus rostralis rounded, straight when viewed from above (Fig. 1A). Nostrils directed laterally, much closer to tip of snout than to eyes. Internarial distance slightly wider than distance from naris to eye (EN/IN = 0.95, IN/SV = 0.101, EN/SV = 0.096). Snout slightly rounded when viewed from the side (Fig. 1B), shallowly angulate when viewed from above (Fig. 1A). Eyes moderately large (EY/SV = 0.14); eyelid

approximately two-thirds width of interorbital distance. Tympanum small (TY/SV = 0.045), but with a distinct annulus. Skin smooth above and below, except abdomen granular. Supratympanic fold absent. Fingers unwebbed, bearing discs with terminal grooves; relative lengths 3>4>2>1 (Fig. 1C). Finger discs approximately twice width of penultimate phalanges (3rdF/SV = 0.066). Subarticular tubercles low but distinct; inner metacarpal tubercle large, low, oval; outer not apparent. Toes with basal webbing between T2–T5, but absent between T1 and T2; bearing discs with terminal grooves; relative lengths 4>5>3>2>1 (Fig. 1D). Toe discs smaller than those of fingers (4thT/SV = 0.060, 3rdF/4thT = 1.10), somewhat less than twice width of penultimate phalanges. Subarticular tubercles low but distinct; inner metatarsal tubercle a narrow oval; outer not apparent. Hind legs moderately long (TL/SV = 0.51).

In preservative, dorsum dark tan, with a dark-brown smudge between the shoulders, flecked with dark brown dorsally and with a series of darker and larger dashes dorsolaterally extending from behind eye to mid-body. Ground color paler tan on snout and sides; pale region on snout sharply demarcated from darker body color along a front extending between eyes (Fig. 1A). Face pale tan, darker below eye, and with elongate dark-brown blotch on canthus (Fig. 1B). Dorsal surfaces of limbs medium brown; rear of thighs with small pale-straw patch of ground color proximally, distal three-fourths uniform medium brown; front of thighs uniformly medium brown. Ventral surfaces pale straw heavily and uniformly stippled with brown punctations throughout; plantar surfaces more densely stippled with brown. A vaguely defined dark-brown blotch present on each wrist, and a dark-brown ring or blotch present on each finger and toe, each followed distally by a pale-straw blotch at the junction of the last two phalanges. Iris dark brown.

Measurements of holotype (in mm).—SV = 19.8, TL = 10.1, HW = 8.4, HL = 7.0, IN = 2.0, EN = 1.9, SN = 3.2, EY = 2.8, TY = 0.9, 3rdF = 1.30, 4thT = 1.18, mass = 0.7 g.

Variation. Mensural variation is limited (Table 1), as is to be expected from such a small sample. In life, animals have obvious scattered tubercles (Fig. 2A, B), but these become obscure in preservative. Color pattern shows somewhat more variation. The subadult male from the Torricelli Mts. (BPBM 22689) is similar to the holotype in color pattern except that the dorsum is somewhat darker brown, the brown blotch below the eye is more sharply delimited, and the venter is darker brown overall, with the brown punctations more aggregated and less uniformly dispersed than in the holotype. The second specimen from the Adelbert Mts. (BPBM 34678) is also darker dorsally and laterally than the holotype, and it has an irregular pale-straw stripe mid-dorsally that is broadened into one mid-dorsal pale-straw blotch midway along the back and another one in the sacral region. This pale stripe itself is intermittently bisected by a dark-brown vertebral line. In life, this broadening into blotches along the spine was not evident, and the bisecting brown line was continuous (Fig. 2A). The top of the snout in this specimen is also darker than that in the holotype, but the area between the eyes is pale. The venter is as seen in the holotype. The final specimen (AMNH 78139) also has a broad vertebral stripe on a dark-brown ground color; its venter too is like that seen in the holotype.

Figure 2. Portraits in life of **A** paratype of *Oreophryne cameroni* sp. n. (BPBM 34678) from Keki Lodge, Adelbert Mts., 850 m elevation **B** paratype of *Oreophryne cameroni* sp. n. (BPBM 22689) from Torricelli Mts., 550 m elevation **C** paratype of *Oreophryne parkopanorum* sp. n. (BPBM 22788) from near summit of Mt. Sapau, Torricelli Mts., 1100–1300 m elevation; and **D** holotype of *Oreophryne parkopanorum* sp. n. (BPBM 22789) from near summit of Mt. Sapau, Torricelli Mts., 1100–1300 m elevation **E** holotype of *Oreophryne gagneorum* sp. n. (BPBM 20542) from Rossel Island, 720 m elevation; and (F) paratype of *Oreophryne gagneorum* sp. n. (BPBM 20544) from Rossel Island, 720 m elevation.

Color in life. In life, BPBM 34678 was mottled dark brown on a burnt-orange ground dorsally, with the front and rear of thighs the same. An orange-tan vertebral stripe was bisected by a narrow brown vertebral line, the heels were paler than the remainder of the legs, a pale dash extended posteroventrally from the corner of the eye, and white flecks were scattered thoughout the lateral surfaces (Fig. 2A). The venter was pale yellow, heavily stippled with brown, and under the legs was the same. Iris was

Table 1. Mensural data for the type series of *Oreophryne cameroni* sp. n. All measurements except mass are in mm.

Character	BPBM 34677	BPBM 34678	BPBM 22689	AMNH 78139
Sex	M	M	subadult M	M
mass (g)	0.7	–	0.3	–
SV	19.8	20.4	15.6	19.5
TL	10.1	9.9	7.9	9.6
EN	1.9	2.0	1.5	1.8
IN	2.0	2.1	1.6	1.9
SN	3.2	3.1	2.4	2.6
TY	0.9	0.8	0.7	0.9
EY	2.8	2.7	2.1	2.7
HW	8.4	8.0	5.8	7.2
HL	7.0	7.0	5.3	6.4
3rd F	1.30	1.38	0.80	0.94
4th T	1.18	1.15	0.73	0.83
TL/SV	0.51	0.49	0.51	0.49
EN/SV	0.096	0.098	0.096	0.092
IN/SV	0.101	0.103	0.103	0.097
SN/SV	0.16	0.15	0.15	0.13
TY/SV	0.045	0.039	0.045	0.046
EY/SV	0.14	0.13	0.13	0.14
HW/SV	0.42	0.39	0.37	0.37
HL/SV	0.35	0.34	0.34	0.33
3rdF/SV	0.066	0.068	0.051	0.048
4thT/SV	0.060	0.056	0.047	0.043
EN/IN	0.95	0.95	0.94	0.95
3rd F/4th T	1.10	1.20	1.10	1.13
HL/HW	0.83	0.88	0.91	0.89

brown with a greenish cast on the upper half. The subadult animal from the Torricelli Mts. (BPBM 22689) had a paler ground color and greater contrast with the dark brown blotches (Fig. 2B). Field notes for that animal state: "Dorsum mottled tan and brown with black spots on face and sides. Venter light gray heavily flecked with black and silver-gray. Iris brown with upper edge tan."

Call. I could identify perches of only two calling animals; both called from hidden locations approximately 2.5–3.5 m above ground. I was able to record two calls from the holotype. The call is a short series of relatively rapid peeps.

Recorded calls comprised series of 8 and 27 peeps emitted at a rate of 2.64–2.75 notes/s; calls ranged from 2.74–9.55 s in duration (Table 2). Each note was brief, with a mean duration of 0.088 s (range 0.071–0.134 s). The interval between notes was approximately three times longer, averaging 0.280 s and ranging from 0.241–0.389 s. The first 1–3 notes in a series were longer than the remainder; as were the first inter-note intervals. In the second call, the terminal three inter-note intervals were also longer

Table 2. Data for two calls from the holotype of *Oreophryne cameroni* sp. n., BPBM 34677, recorded 1 October 2009, air temperature 23.0 °C. Numbers for call parameters are mean ± SD (range).

Call series	Number of notes	Call duration (s)	Note duration (s)	Inter-note duration (s)	Repetition rate (notes/s)	Dominant frequency (kHz)
a	8	2.74	0.101 ± 0.0051 (0.087–0.134)	0.278 ± 0.0091 (0.249–0.316)	2.64	2.906 ± 0.0065 (2.871–2.940)
b	27	9.55	0.084 ± 0.0025 (0.071–0.128)	0.281 ± 0.0071 (0.241–0.389)	2.75	2.900 ± 0.0032 (2.872–2.940)

than the preceding intervals. Hence, calls begin somewhat slowly, rapidly reach a regular pace, and may also slow down as they approach termination. There was approximately twice as much variation in inter-note duration than in note duration (Table 2). Notes may have a rounded amplitude envelope or may begin at maximum volume and decrease more or less monotonically, creating either a rounded or an approximately triangular amplitude envelope (Fig. 3A). Notes lack harmonic structure, pulsing, and frequency modulation (Fig. 3C). The dominant frequency of calls varied within a very narrow window (Fig. 3B), averaging 2901 Hz and ranging from 2871–2940 Hz.

Etymology. The name is an honorific for my friend H. Don Cameron, professor emeritus of classical studies at University of Michigan and provider of much etymological and grammatical advice on Greek and Latin over the years.

Range. Known from the Adelbert Mts., Madang Province, and the Torricelli Mts., West Sepik Province, Papua New Guinea at elevations of 550–850 m (Fig. 4, circles). The species will certainly be found at appropriate elevations in the intervening Prince Alexander Mts. and may, as well, occur in mountain ranges to the west.

Ecological notes. I collected the subadult male in primary lowland rainforest on a steep slope at 550 m elevation in the Torricelli Mts. Canopy was at approximately 30–35 m; understory was rather open and uncrowded; soil was greasy mud. I collected the two calling animals at 850 m in the Adelbert Mts. perched at night in trees approximately 2.5–3.5 m above the ground. These animals were the nearest to the ground that I heard; all others were calling from higher up in the trees. Forest at this site was a clearing edge in remnant primary rainforest on a ridgetop with gentle slopes and rather open understory; soil was thick, sticky clay.

Mature males were 19.5–20.4 mm SV, but one male was still immature at 15.6 mm SV.

Frogs syntopic with this species include *Albericus gudrunae*, *Austrochaperina basipalmata*, *A. blumi*, an undescribed *Austrochaperina*, *Callulops microtis*, *C. personatus*, *Choerophryne proboscidea*, *C. rostellifer*, *Cophixalus balbus*, *C. cheesmanae*, *C. pipilans*, *Copiula fistulans*, *C. tyleri*, *Hylarana arfaki*, *H. garritor*, *H. jimiensis*, *H. papua*, *H. volkerjane*, *Hylophorbus macrops*, *H. proekes*, two undescribed *Hylophorbus*, *Liophryne schlaginhaufeni*, *Litoria arfakiana*, *L. genimaculata*, *L. wollastoni*, *Oreophryne biroi*, *Mantophryne lateralis*, *Nyctimystes fluviatilis*, *N. pulcher*, *Platymantis papuensis*, *Sphenophryne cornuta*, *Xenorhina obesa*, *X. oxycephala*, and *X. tumulus*.

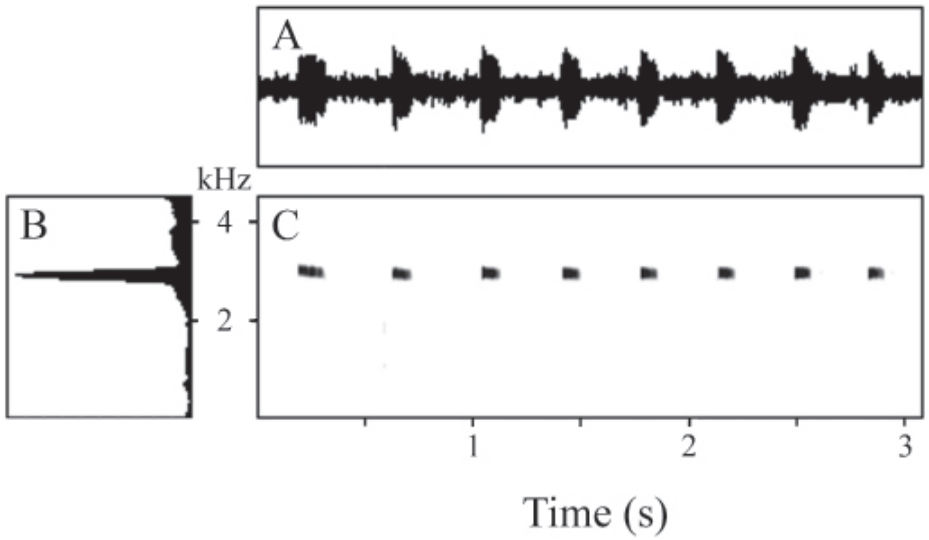

Figure 3. A Waveform **B** power spectrum, and **C** spectrogram of 8-note call of the holotype of *Oreophryne cameroni* sp. n. (BPBM 34677) recorded at Keki Lodge, Adelbert Mts., 1 October 2009, air temperature 23.0 °C.

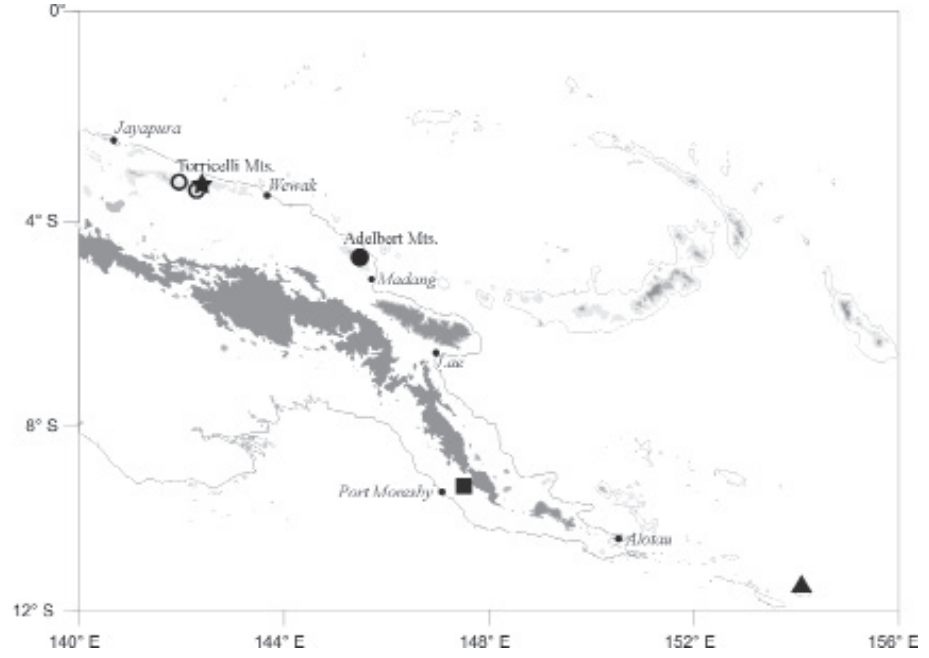

Figure 4. Map of eastern New Guinea showing type locality for *Oreophryne cameroni* sp. n. (filled circle), additional localities for *Oreophryne cameroni* sp. n. (open circles), type locality for *Oreophryne parkopanorum* sp. n. (star), and type locality for *Oreophryne gagneorum* sp. n. (triangle). The square shows the type locality for *Oreophryne kampen*i, the only previous member of the genus with a cartilaginous connection between the scapula and procoracoid known to occur in Papua New Guinea outside the Central Highlands.

Remarks. In their revision of *Oreophryne* species from the northern coast of New Guinea, Zweifel et al. (2003) pointed out that AMNH 78139 was problematic in its identification, not clearly fitting with the other species discussed. In assigning the specimen to this new species, that problem is resolved.

***Oreophryne parkopanorum* sp. n.**
http://zoobank.org/552EF19B-8CA3-4DD0-9271-DEB337E78FA9
http://species-id.net/wiki/Oreophryne_parkopanorum
Figs 2C, D, 5

Holotype. BPBM 22789 (field tag FK 11847), adult female, collected by F. Kraus 1.2 km S Mt. Sapau summit, Torricelli Mts., 3.3773°S, 142.5180°E, 1120–1320 m, West Sepik Province, Papua New Guinea, 27 May 2005.

Paratypes (n = 4). BPBM 22787–88, PNGNM 24152, same data as holotype; BPBM 22790, 1.6 km SSW Mt. Sapau summit, Torricelli Mts., 3.3807° S, 142.5155° E, 1050 m, West Sepik Province, Papua New Guinea.

Diagnosis. *Oreophryne parkopanorum* can be distinguished from all congeners by its unique combination of small size (adult male SV = 17.5–17.7 mm, adult female SV = 20.1 mm); cartilaginous connection between the scapula and procoracoid; unwebbed toes; third toe longer than fifth; leg moderately long (TL/SV = 0.45–0.51); head short (HL/SV = 0.35–0.36, HL/HW = 0.89–0.91), snout long and broad (EN/IN = 0.76–0.89, EN/SV = 0.085–0.097, IN/SV = 0.102–0.120); eye large (EY/SV = 0.14–0.15); finger and toe discs broad (3rdF/SV = 0.048–0.068, 4thT/SV = 0.044–0.053, 3rdF/4thT = 1.08–1.30); longitudinal rows of ridges or pustules on dorsum; dorsum paler mid-dorsally than dorsolaterally; and coppery-brown iris.

Comparisons with other species. The new species differs from all other Papuan *Oreophryne* except *O. alticola* and *O. habbemensis* in its unique combination of having a cartilaginous connection between the scapula and procoracoid, absence of toe webbing, and third toe longer than the fifth. *Oreophryne parkopanorum* differs from these species in its longer leg (TL/SV = 0.45–0.51 vs. 0.33–0.38 in *O. alticola* and *O. habbemensis*), longer and wider snout (EN/SV = 0.085–0.097 vs. 0.064–0.065 in *O. alticola*, 0.073–0.081 in *O. habbemensis*; IN/SV = 0.102–0.120 vs. 0.079–0.088 in *O. alticola*, 0.082–0.089 in *O. habbemensis*), larger eye (EY/SV = 0.14–0.15 vs. 0.10–0.13 in *O. alticola*, 0.12–0.13 in *O. habbemensis*), broader finger discs (3rdF/SV = 0.048–0.068 vs. 0.031–0.040 in *O. alticola*, 0.034–0.044 in *O. habbemensis*), and broader toe discs (4thT/SV = 0.044–0.053 vs. 0.026–0.033 in *O. alticola*, 0.034–0.038 in *O. habbemensis*).

Several other species also share with *O. parkopanorum* the combination of a cartilaginous connection between the scapula and procoracoid and absence of toe webbing. Of these, *Oreophryne anamiatoi*, *O. asplenicola*, *O. flava*, *O. graminus*, *O. notata*, *O. pseudasplenicola*, and *O. streiffeleri* are easily distinguished from *O. parkopanorum* in having the fifth toe obviously longer than the third. *Oreophryne brevicrus*, *O. clamata*, *O. geminus*, and *O. terrestris* are somewhat less distinct in this respect in having the

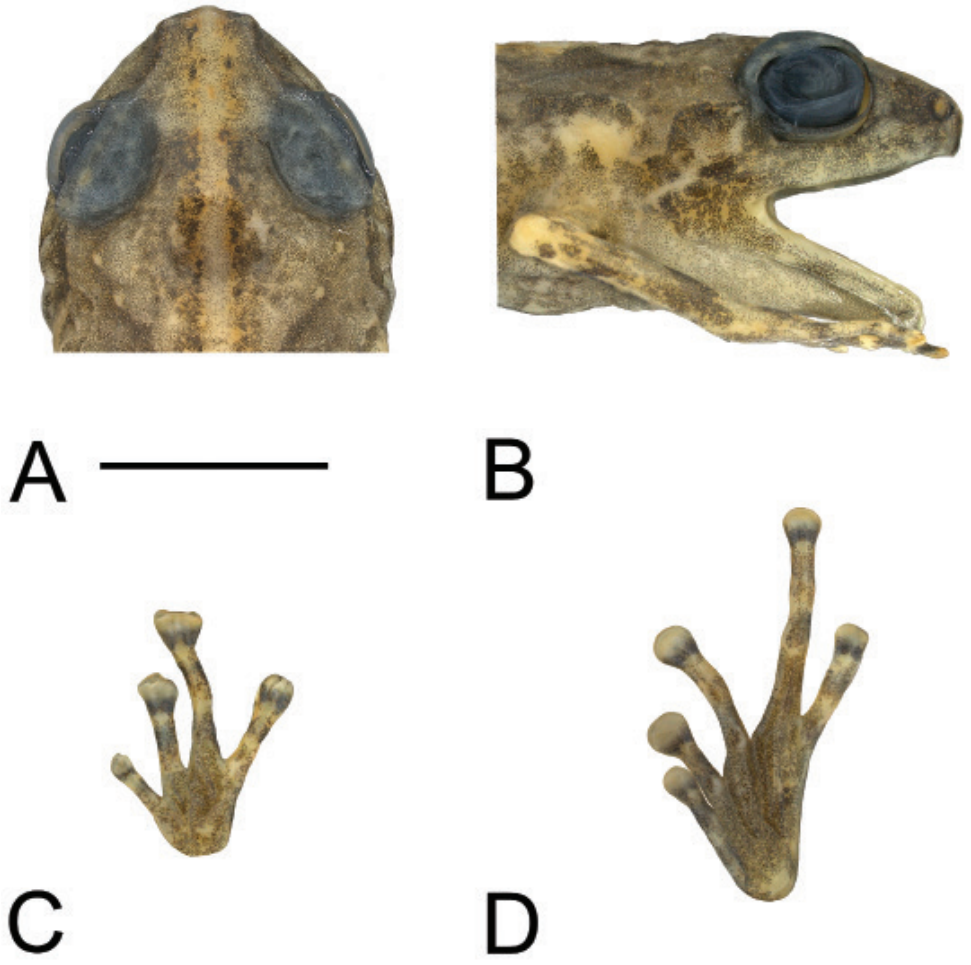

Figure 5. A Top of head **B** side of head **C** palmar view of left hand, and **D** plantar view of left foot of holotype of *Oreophryne parkopanorum* sp. n. (BPBM 22789). Scale bar = 5 mm.

third toe subequal to the fifth, instead of distinctly longer. Besides relative toe length, *Oreophryne parkopanorum* further differs from *O. clamata* in its broader snout (IN/SV = 0.102–0.120 vs. 0.091–0.103 in *O. clamata*), lesser relative size of finger discs to toe discs (3rdF/4thT = 1.08–1.30 vs. 1.50–1.63 in *O. clamata*), longer head (HL/SV = 0.35–0.36 vs. 0.28–0.30, HL/HW = 0.89–0.91 vs. 0.70–0.82 in *O. clamata*), and absence of a dark subocular blotch and black spots around arm insertion (both present in *O. clamata*).

O. brevicrus, *O. geminus*, and *O. terrestris* are all alpine species instead of mid-elevation forest dwellers. Beyond relative toe length, *O. parkopanorum* also differs from *O. brevicrus* in its longer leg (TL/SV = 0.45–0.51 vs. 0.36–0.42 in *O. brevicrus*), mid-dorsum paler than dorsolateral regions (mid-dorsum darker than dorsolateral regions in *O. brevicrus*), and venter with scattered large brown flecks (venter evenly stippled with brown in *O. brevicrus*); and it differs from *O. geminus* and *O. terrestris* in its longer leg

(TL/SV = 0.45–0.51 vs. 0.32–0.39 in *O. geminus*, 0.34–0.44 in *O. terrestris*), broader finger discs (3rdF/SV = 0.048–0.068 vs. 0.030–0.041 in *O. geminus*, 0.031–0.042 in *O. terrestris*), and broader toe discs (4thT/SV = 0.044–0.053 vs. 0.025–0.039 in *O. geminus*, 0.024–0.042 in *O. terrestris*).

Description of holotype. An adult female with lateral incision on right side and left pectoral region dissected. Head wide (HW/SV = 0.40), with steeply oblique, slightly concave loreal region; upper lip somewhat inflated. Canthus rostralis rounded, slightly concave when viewed from above (Fig. 5A). Nostrils directed laterally, closer to tip of snout than to eyes. Internarial distance wider than distance from naris to eye (EN/IN = 0.77, IN/SV = 0.109, EN/SV = 0.085). Snout truncate when viewed from the side (Fig. 5B), shallowly angulate when viewed from above (Fig. 5A). Eyes moderately large (EY/SV = 0.14); eyelid approximately two-thirds width of interorbital distance. Tympanum small (TY/SV = 0.050), with distinct annulus, partly covered by surrounding flesh dorsally, projecting ventrally. Dorsum with many raised ridges and series of tubercles, one paired series of tubercles forming an hourglass pattern from behind eyes to posterior of body, each line of warts constricting medially at shoulder and then diverging slightly laterally past this; sides tuberculate; ventral surfaces smooth anteriorly, granular on abdomen. Supratympanic fold absent; few tubercles posterior to tympanum. Fingers unwebbed, bearing discs with terminal grooves; relative lengths 3>4>2>1 (Fig. 5C). Finger discs slightly less than twice width of penultimate phalanges (3rdF/SV = 0.057), except for F1, which is only slightly wider than penultimate phalanx. Subarticular tubercles not obvious; inner metacarpal tubercle oval but low; outer not apparent. Toes unwebbed, bearing discs with terminal grooves; relative lengths 4>3>5>2>1 (Fig. 5D). Toe discs smaller than those of fingers (4thT/SV = 0.044, 3rdF/4thT = 1.32), somewhat less than 1.5 times width of penultimate phalanges on T4 and T5 but wider on T2 and T3. Subarticular tubercles very low or absent; inner metatarsal tubercle large, oval; outer absent. Hind legs moderately long (TL/SV = 0.50).

In preservative, dorsum with pale straw-yellow ground, heavily dusted with brown punctations, with areas having darker dusting and areas lacking dusting arrayed in rows. Pale straw-yellow vertebral stripe; pale straw-yellow blotch above each forearm insertion; pale straw-yellow triangle on top of snout (Fig. 5A). Series of darker-brown flecks dorsolaterally; dark-brown flecks widely scattered laterally; two dark-brown dashes behind eye, one largely superior to the tympanum, the other inferior to it and ending in a brown patch at rictus (Fig. 5B). Face irregularly dusted/mottled with brown, but not as dark as markings on body. Legs, including front and rear of thighs, pale straw yellow with scattered pale-brown flecks. Short, pale straw-yellow stripe on back surface of distal portion of shank and on heel. Irregular brown blotch dorsally on each wrist. Chin and throat evenly dusted with brown punctations except mid-ventrally on chin, where its absence forms a pale line, adjacent to which the brown dusting is more heavily concentrated; abdomen also heavily dusted with brown, but with more irregular distribution than on chin and throat. Palmar and plantar surfaces pale straw yellow evenly dusted with brown punctations. Iris dark brown.

Table 3. Mensural data for the type series of *Oreoprhyne parkopanorum* sp. n. All measurements except mass are in mm.

Character	BPBM 22787	BPBM 22788	BPBM 22789	BPBM 22790	PNGNM 24152
Sex	subadult M	M	F	M	M
mass (g)	0.35	0.60	0.80	0.55	–
SV	15.8	17.5	20.1	17.7	17.5
TL	8.3	9.0	10.0	8.0	8.7
EN	1.4	1.7	1.7	1.6	1.6
IN	1.8	1.9	2.2	1.8	2.1
SN	2.5	2.7	2.9	2.5	2.4
TY	0.7	0.8	1.0	0.8	0.8
EY	2.2	2.5	2.9	2.7	2.5
HW	6.3	7.1	8.0	6.9	7.0
HL	5.6	6.3	7.3	6.2	6.3
3rd F	0.78	0.90	1.14	0.85	1.14
4th T	0.70	0.79	0.88	0.79	0.93
TL/SV	0.53	0.51	0.50	0.45	0.50
EN/SV	0.089	0.097	0.085	0.090	0.091
IN/SV	0.114	0.109	0.109	0.102	0.120
SN/SV	0.16	0.15	0.14	0.14	0.14
TY/SV	0.044	0.046	0.050	0.045	0.046
EY/SV	0.14	0.14	0.14	0.15	0.14
HW/SV	0.40	0.41	0.40	0.39	0.40
HL/SV	0.35	0.36	0.36	0.35	0.36
3rdF/SV	0.049	0.051	0.057	0.048	0.065
4thT/SV	0.044	0.045	0.044	0.045	0.053
EN/IN	0.78	0.89	0.77	0.89	0.76
3rd F/4th T	1.11	1.14	1.30	1.08	1.23
HL/HW	0.89	0.89	0.91	0.90	0.90

Measurements of holotype (in mm).–SV = 20.1, TL = 10.0, HW = 8.0, HL = 7.3, IN = 2.2, EN = 1.7, SN = 2.9, EY = 2.9, TY = 1.0, 3rdF = 1.14, 4thT = 0.88, mass = 0.8 g.

Variation. The female is larger than the males and has a slightly larger tympanum and greater disparity in disc widths between the fingers and toes (Table 3). It remains to be determined from a larger sample size whether these represent instances of sexual dimorphism. Otherwise, there is little mensural variation of interest in the small sample.

Snout profile varies from truncate to shallowly angulate when viewed from the side, shallowly angulate to acutely rounded when viewed from above. The female holotype is more heavily tuberculate than the male paratypes, which typically have the hourglass-shaped rows of tubercles well-defined dorsolaterally and also have scattered tubercles on the lateral surfaces, as well as smaller pustules apparent elsewhere, especially posterior to the tympanum.

The holotype is the only specimen with a broad vertebral stripe and heel stripe (Fig. 2D), but two males (BPBM 22790 and PNGNM 24152) have narrower, inter-

mittent vertebral lines. All specimens have the mid-dorsal region paler than the sides, giving the impression of a paler hourglass-shaped region mid-dorsally. Most specimens are moderately heavily dusted with brown dorsally, as seen in the holotype, but the subadult male (BPBM 22787) is paler overall, with brown dusting less dense dorsally. This specimen also has two rows of dark-brown dashes laterally, extending from near forearm insertion to posterior third of body, the upper row at the level of the dark-brown supratympanic dash, the lower at the level of forearm insertion. BPBM 22790 also has these two rows of dark-brown lateral dashes well defined, but the other three specimens have brown flecking and spotting more irregularly distributed across the lateral surfaces. The snouts of all specimens are paler than the remainder of the head, but brown flecking occurs in this field in some specimens, thereby making the feature less obvious. The males all have an even dusting of brown punctations ventrally, as seen in the holotype, but also have large, darker-brown spots scattered across the ventrum, giving the impression of a pale venter with scattered large brown flecks; these spots are weaker in BPBM 22788 than in the other specimens. In the subadult male, these larger brown spots are arrayed more or less into two rows extending from the chin to the abdomen. None of the males has the pale, brown-bordered, mid-ventral line seen on the chin of the holotype.

Color in life. Field notes for BPBM 22787 note: "Dorsum light yellow brown with narrow dark-brown lines. Fore and aft of thigh and rear of shank orange-red. Venter pale straw with two rows of dark-gray flecks on chin and throat. Iris light brown." The holotype, BPBM 22789 (Fig. 2D) was similar but had a yellow stripe from chin to abdomen, another across the pectoral region, and an orange mid-dorsal stripe. Brown dorsolateral and postocular markings are more evident in some animals (Fig. 2C) than others (Fig. 2D). Animals are more orangish during the night and yellower during the day. The orange-red on the hidden surfaces of the thighs fades to pale straw in preservative.

Call. The call is uncertain. I heard two undetermined frog calls at the type locality that are consistent with *Oreophryne* species from the north-coast ranges. One of these was a rattle call, the other was a series of high-pitched peeps. But I could associate neither call with a particular frog, so the identities of both are undetermined. One of them almost certainly represents *O. parkopanorum*, but I cannot say which.

Etymology. The species name is a genetive plural honorific for the people of Parkop Village, whose unflagging help and friendliness made my expedition to the Torricelli Mts. successful and most pleasant.

Range. Known only from the upper elevations of Mt. Sapau, Torricelli Mts., West Sepik Province, Papua New Guinea at an elevation of 1050–1320 m (Fig. 4, star). It probably occurs in similar habitat elsewhere in the Torricelli Mts. and may occur in the upper elevations of other nearby north-coast ranges.

Ecological notes. This species inhabits primary mossy cloud forest at 1200–1300 m. We found our specimens active at night on moss-covered tree trunks from 6 cm to 2 m above ground. Forest in this area has a canopy of approximately 20 m height, many epiphytes, and a thick layer of leaf litter and duff.

Mature males were 17.5–17.7 mm in SV, but one male was still immature at 15.8 mm SV.

Syntopic frogs include *Albericus brunhildae*, *Austrochaperina septentrionalis*, *Choerophryne longirostris*, *C. rostellifer*, *Copiula tyleri*, *Hylarana jimiensis*, *H. volkerjane*, *Hylophorbus* sp., *Liophryne schlaginhaufeni*, *Litoria modica*, *L. wollastoni*, *Nyctimystes pulcher*, and *Xenorhina arboricola*.

***Oreophryne gagneorum* sp. n.**
http://zoobank.org/496AF6FA-988F-4805-B8A6-A0515CC8981E
http://species-id.net/wiki/Oreophryne_gagneorum
Figs 2E, F, 6

Holotype. BPBM 20542 (field tag FK 10121), adult female, collected by F. Kraus and local villagers on S slope of Mt. Rossel, 11.3555°S, 154.2246°E, 720 m, Rossel Island, Milne Bay Province, Papua New Guinea, 5 May 2004.

Paratypes (n = 52). Same data as holotype (BPBM 20538–41, 20543–57, PNGNM 24153–55); same data as holotype, except collected 3 May (BPBM 20531–37), 6 May (BPBM 20558–60, PNGNM 24156–60), 7 May (BPBM 20561–69), 8 May (BPBM 20570), 9 May (BPBM 20571), and 10 May (BPBM 20572) 2004; halfway between 11.3354°S, 154.2223°E and 11.3354°S, 154.2247°E, 275–280 m (BPBM 43075, PNGNM 24161).

Diagnosis. *Oreophryne gagneorum* can be distinguished from all congeners by its unique combination of small size (adult male SV = 16.3–20.0 mm, adult female SV = 19.0–23.5 mm); cartilaginous connection between the scapula and procoracoid; well-webbed toes; third and fifth toes subequal in length; steeply oblique lores; leg moderately long (TL/SV = 0.46–0.59); snout typically longer than broad (EN/IN = 1.00–1.33, EN/SV = 0.093–0.121, IN/SV = 0.080–0.109); head relatively broad (HW/SV = 0.36–0.43, HL/HW = 0.82–0.92); tympanum small (TY/SV = 0.034–0.051); finger and toe discs relatively broad (3rdF/SV = 0.063–0.086, 4thT/SV = 0.048–0.066, 3rdF/4thT = 1.18–1.47); shanks either unicolor or flecked/mottled with dark brown; pale-tan iris suffused or veined with black; and call a rapid series of short notes (23–190 ms) delivered at a rate of 9.57–11.32 notes/s with a dominant frequency of 3070–3510 Hz.

Comparisons with other species. The new species differs from all other Papuan *Oreophryne* except *O. crucifer* and *O. kampeni* in its unique combination of having a cartilaginous connection between the scapula and procoracoid and having well-webbed toes. *Oreophryne gagneorum* differs from *O. crucifer* in its longer leg (TL/SV = 0.46–0.59 vs. 0.45 in *O. crucifer*), longer snout (EN/SV = 0.093–0.121 vs. 0.092 in *O. crucifer*), shorter head (HL/HW = 0.82–0.92 vs. 0.78 in *O. crucifer*), smaller tympanum (TY/SV = 0.034–0.051 vs. 0.054 in *O. crucifer*), relatively wider finger discs (3rdF/4thT = 1.18–1.47 vs. 1.13 in *O. crucifer*), fourth finger longer than the second (second longer than fourth in *O. crucifer*), and absence of a golden-yellow bar between the eyes. It differs

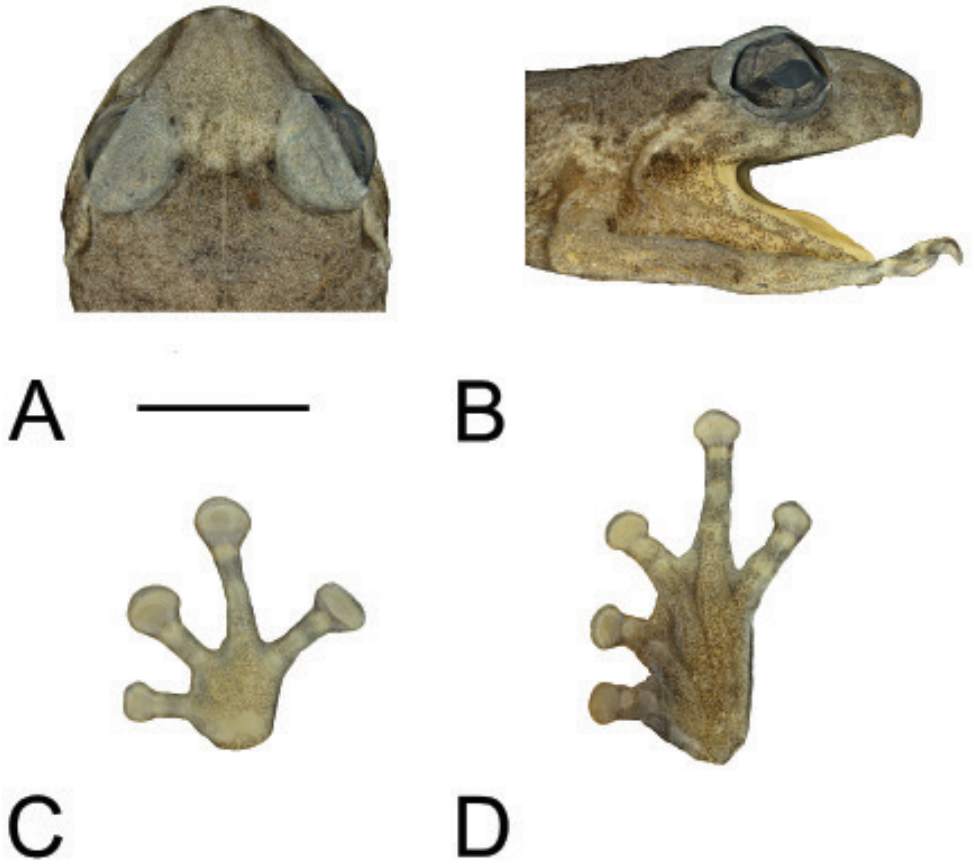

Figure 6. A Top of head **B** side of head **C** palmar view of left hand, and **D** plantar view of left foot of holotype of *Oreophryne gagneorum* sp. n. (BPBM 20542). Scale bar = 5 mm.

from *O. kampeni* in having the third and fifth toes subequal in length (third distinctly longer in *O. kampeni*), more oblique loreal region (lores almost vertical in *O. kampeni*), usually longer leg (TL/SV = 0.46–0.59 vs. 0.44–0.47 in *O. kampeni*) and longer snout (EN/IN = 1.00–1.33 vs. 0.094–1.05 in *O. kampeni*), larger toe discs (4thT/SV = 0.048–0.066 vs. 0.042–0.048 in *O. kampeni*), and the shanks without brown spots (shanks conspicuously patterned with round dark-brown spots in *O. kampeni*).

Oreophryne cameroni, *O. idenburghensis*, *O. oviprotector*, and *O. waira* also have a cartilaginous connection between the scapula and procoracoid and webbing between the toes, but they differ from *O. gagneorum* in having basal instead of extensive toe webbing. Furthermore, *O. cameroni* has the fifth toe distinctly longer than the third, a shorter snout (EN/IN = 0.94–0.95 vs. 1.00–1.3 in *O. gagneorum*), dark-brown iris, and call consisting of more slowly delivered notes (2.64–2.75 notes/s vs. 9.57–11.32 notes/s in *O. gagneorum*) of lower dominant frequeny (2870–2940 Hz vs. 3070–3510 Hz in *O. gagneorum*). *O. idenburghensis* is a much larger species (SV = 43–47 mm vs. 16.3–23.5 mm in *O. gagneorum*), with the fifth toe distinctly longer than the third,

and with a broader snout (EN/IN = 0.087–0.091 vs. 1.00–1.33 in *O. gagneorum*); *O. oviprotector* has the fifth toe distinctly longer than the third, is lime green dorsally with a green bar between the eyes and a white ring around the orbit, and has a rattle call; and *O. waira* is a slightly smaller species (SV = 17.8–21.0 mm vs. 16.3–23.5 mm in *O. gagneorum*) with a rattle call (vs. a high-pitched whinny in *O. gagneorum*) and a shorter snout (EN/IN = 0.094–0.105 vs. 1.00–1.33 in *O. gagneorum*). All other Papuan *Oreophryne* have either a ligamentous connection between the scapula and procoracoid, lack toe webbing entirely, or both.

Description of holotype. An adult female with an incision on right side and left pectoral region dissected. Procoracoid connected to the scapula by a narrow cartilaginous rod. Head wide (HW/SV = 0.40), with steeply oblique loreal region; upper lip inflated. Canthus rostralis rounded, straight when viewed from above (Fig. 6A). Nostrils directed laterally, much closer to tip of snout than to eyes. Internarial distance slightly wider than distance from naris to eye (EN/IN = 1.25, IN/SV = 0.085, EN/SV = 0.106). Snout truncate when viewed from the side (Fig. 6B), shallowly angulate when viewed from above (Fig. 6A). Eyes moderately large (EY/SV = 0.14); eyelid approximately two-thirds width of interorbital distance. Tympanum small (TY/SV = 0.034), but with a distinct annulus, partly covered by skin posterodorsally. Weak supratympanic fold present; another weak fold extends posteroventrally from rear margin of tympanum. Skin smooth above and below, except abdomen granular. Fingers unwebbed, bearing discs with terminal grooves; relative lengths 3>4>2>1 (Fig. 6C). Finger discs approximately twice width of penultimate phalanges except for F1, which is approximately 1.5 times as wide as penultimate phalanx (3rdF/SV = 0.074). Subarticular tubercles low but distinct; inner metacarpal tubercle a large, low oval; outer a low circle. Toes well webbed, formula **I** 2–2 **II** 2.7–3.6 **III** 3–4.5 **IV** 4.7–3.6 **V**; bearing discs with terminal grooves; relative lengths 4>5=3>2>1 (Fig. 6D). Toe discs smaller than those of fingers (4thT/SV = 0.060, 3rdF/4thT = 1.24), approximately 1.5 times width of penultimate phalanges. Subarticular tubercles low but distinct; inner metatarsal tubercle a narrow oval; outer not apparent. Hind legs rather long (TL/SV = 0.49).

In preservative, dorsum pale tan minutely speckled with brown, with a narrow, partially obscured tan vertebral line; limbs, including rear of thighs, same color as dorsum. Pale patch of lighter tan extends between eyes, narrowly margined posteriorly by dark brown. Short pale-cream dash extends from behind eye, through tympanum, to end near forearm insertion; this is bordered above and below by small, diffuse fields of brown. Lateral and ventrolateral surfaces suffused with pale cream. Venter pale straw yellow minutely stippled with brown, this more concentrated on chin and throat, somewhat sparser posteriorly; under limbs, hands, and feet stippled likewise. Sparse brown canthal stripe and subocular blotch present. Iris pale tan veined with black, which is especially concentrated in a horizontal plane before and behind the pupil.

Measurements of holotype (in mm).—SV = 23.5, TL = 11.6, HW = 9.5, HL = 8.3, IN = 2.0, EN = 2.5, SN = 3.5, EY = 3.2, TY = 0.8, 3rdF = 1.73, 4thT = 1.40, mass = 1.20 g.

Variation. The only apparent sexual dimorphism in this species is in size; females are larger than males in both mass and SV (Table 4). Otherwise, standard deviation of

Table 4. Mensural variation among adults of *Oreophryne gagneorum* sp. n. Measurements are in mm, except for mass (g).

Character	Males (n = 35)			Females (n = 14)		
	mean	SD	range	mean	SD	range
mass	0.61	0.0168	0.40–0.85	0.86	0.0412	0.65–1.20
SV	18.5	0.1790	16.3–20.0	20.5	0.3410	19.0–23.5
TL	9.5	0.0754	8.5–10.5	10.6	0.1626	9.9–11.7
EN	2.0	0.0189	1.8–2.2	2.2	0.0359	2.0–2.5
IN	1.8	0.0185	1.5–1.9	1.9	0.0277	1.8–2.1
SN	2.9	0.0234	2.5–3.2	3.2	0.0633	2.8–3.5
TY	0.8	0.0134	0.6–0.9	0.9	0.0334	0.7–1.1
EY	2.6	0.0346	2.2–3.0	2.8	0.0518	2.5–3.2
HW	7.4	0.0825	6.5–8.2	8.2	0.1482	7.6–9.5
HL	6.5	0.0577	5.8–7.1	7.3	0.1475	6.6–8.3
3rd F	1.34	0.0187	1.13–1.56	1.52	0.0479	1.26–1.84
4th T	1.03	0.0146	0.86–1.23	1.15	0.0364	0.94–1.41
TL/SV	0.51	0.0049	0.46–0.59	0.52	0.0044	0.49–0.54
EN/SV	0.106	0.0008	0.093–0.117	0.105	0.0009	0.097–0.111
IN/SV	0.095	0.0009	0.80–0.104	0.093	0.0010	0.085–0.098
SN/SV	0.16	0.0013	0.14–0.17	0.15	0.0019	0.14–0.17
TY/SV	0.042	0.0007	0.035–0.051	0.043	0.0015	0.034–0.051
EY/SV	0.14	0.0013	0.12–0.16	0.14	0.0016	0.13–0.15
HW/SV	0.40	0.0026	0.36–0.43	0.40	0.0022	0.38–0.41
HL/SV	0.35	0.0018	0.32–0.37	0.36	0.0023	0.35–0.37
3rdF/SV	0.072	0.0007	0.065–0.082	0.074	0.0016	0.063–0.086
4thT/SV	0.055	0.0007	0.049–0.065	0.056	0.0011	0.048–0.066
EN/IN	1.12	0.0129	1.00–1.33	1.13	0.0162	1.00–1.25
3rd F/4th T	1.31	0.0122	1.18–1.47	1.33	0.0200	1.20–1.43
HL/HW	0.87	0.0044	0.82–0.92	0.89	0.0056	0.85–0.91

variables largely accords with the size of the mensural character, and variation across most variables is tight (Table 4). The snout shape varies from truncate to slightly rounded in lateral view and from shallowly angulate to slightly rounded in dorsal view. The tympanum is usually partially embedded in the surrounding skin, giving the impression that it sits in a depression. Webbing between the toes is always well developed, as in the holotype, and never merely basal. In life, animals have obvious scattered tubercles (Fig. 2E, F), but these become obscure in preservative.

This species presents a diverse array of color patterns in brown and gray. The dorsum varies from pale tan to dark brown, and may be uniform in pattern but more often with ill-defined dark smudges or suffusions of dark color that frequently form a vague, paler hourglass pattern mid-dorsally and/or a poorly defined, dark scapular W. Occasionally, there will be a large orange-tan blotch mid-dorsally, usually on the posterior half of the dorsum; there are also orange-tan blotches on the heels of two specimens. The pale cream or tan postocular dash is always present and extends through the tym-

panum; this is invariably bordered above by a dark-brown dash followed by a brief hiatus and another short brown dash over the forearm insertion. There is typically a diffuse dark-brown field below the cream postocular stripe; occasionally this is better developed into another brown dash or blotch. The dark-brown canthal stripe and subocular blotch may be present, absent, or only vaguely suggested. Top of the snout is often, but not always, paler than the remainder of the dorsum; there is often either a dark-brown or pale-tan bar extending between the eyes, but these too are variably present. A narrow, pale-tan vertebral line is present in 25 of the specimens; this is often broken or developed only anteriorly. Lumbar ocelli are almost always absent and are poorly developed in the few specimens in which they occur. Ventral ground color is typically pale straw yellow with the overlying dark pigment varying from minute and evenly distributed stippling to dense evenly distributed stippling to dense, aggregated dark stippling. Consequently, the impression of ventral coloration to the naked eye varies from evenly pale brown to evenly dark brown to pale brown with dark-brown flecks. Four specimens have poorly defined, pale-straw lines mid-ventrally on the chin and throat, and another four have pale-gray flecks scattered across the belly. Iris color is always pale tan either suffused or veined with black, this is usually concentrated in a horizontal plane before and behind the pupil.

Color in life. Field notes for BPBM 20531 in life recorded the color as: "Dorsum brown with darker brown mottling and tan stripe laterally, below which is dark brown. Iris tan. Tan postocular stripe. Venter pale gray stippled with dark gray. Rear of thighs dark brown." BPBM 20532 was dark tan dorsally with a few dark-brown spots; the rear of thighs were the same as the dorsum. BPBM 20533 was also brown dorsally with vague brown markings, a pale tan postocular stripe, and a small amount of yellow in the groin. The rear of the thighs were brown with a few light-gray stipples. Chin to chest was dark gray with light-gray flecks, and the abdomen and undersides of the legs were light gray heavily flecked with dark gray. BPBM 20534 had a tan vertebral stripe and a dusky red patch in groin and front and rear of thighs. BPBM 20535 was chocolate brown dorsally with a cream postocular stripe, yellow in the inguinal region, and dusky brick red in groin and hidden surfaces of thighs. The holotype was pale tan-gray in life with a few, scattered red-brown spots (Fig. 2E); BPBM 20544 was dark brown with an orangish hourglass-shaped figure mid-dorsally, white-tipped tubercles, tan inter-ocular bar, and cream on the sides (Fig.2F). Both of these animals exhibited silver irises with a reddish-brown horizontal bar through the pupil.

Call. This species was the predominant frog calling around the summit of Mt. Rossel. I recorded 14 calls from six animals, and calls segregated into two types: a long and a short call (Table 5). The former was the most commonly produced call, with the shorter call being produced more frequently when conditions were drier. Note-delivery rate of both call types is so rapid that to the human ear calls sounds like a high-pitched whinny.

The more commonly delivered, long calls (n = 8) ranged from 1.98–2.92 s in duration and consisted of a series of 21–31 notes emitted at a rate of 10.30–11.02 notes/s (Table 5). The first note of each call was much longer than the remainder

Table 5. Call data for six specimens of *Oreophryne gagneorum* sp. n. from Rossel Island, Milne Bay Province, PNG. Numbers for call parameters are mean ± SD (range).

Specimen	Call type	Temperature (°C)	Number of calls	Call duration (s)	Notes/ call	Note duration (s)	Inter-note duration (s)	Repetition rate (notes/s)	Dominant frequency (kHz)
BPBM 20558	long	22.8	3	2.20 ± 0.1106 (1.98–2.33)	21–24	0.045 ± 0.0028 (0.033–0.157)	0.052 ± 0.0012 (0.032–0.082)	10.46 ± 0.0887 (10.30–10.61)	3.38 ± 0.0062 (3.26–3.51)
BPBM 20559	long	22.8	2	2.20 ± 0.0650 (2.13–2.26)	23–24	0.036 ± 0.0043 (0.028–0.190)	0.060 ± 0.0010 (0.051–0.078)	10.71 ± 0.0893 (10.62–10.80)	3.32 ± 0.0059 (3.19–3.37)
BPBM 20560	long	22.8	1	2.92	31	0.037 ± 0.0045 (0.023–0.170)	0.060 ± 0.0018 (0.036–0.079)	10.62	3.38 ± 0.0100 (3.23–3.47)
BPBM 20571	long	22.2	2	2.45 ± 0.0900 (2.36–2.54)	26–28	0.050 ± 0.0036 (0.041–0.182)	0.041 ± 0.0014 (0.029–0.067)	11.02 ± 0.0033 (11.02–11.02)	3.27 ± 0.0054 (3.21–3.31)
BPBM 20538	short	22.6	4	0.93 ± 0.0263 (0.85–0.96)	9–10	0.059 ± 0.0023 (0.047–0.105)	0.038 ± 0.0005 (0.032–0.044)	10.25 ± 0.2285 (9.57–10.59)	3.12 ± 0.0045 (3.07–3.18)
BPBM 20572	short	22.1	2	1.03 ± 0.0350 (0.99–1.06)	11–12	0.058 ± 0.0033 (0.046–0.112)	0.036 ± 0.0011 (0.026–0.043)	11.22 ± 0.1048 (11.11–11.32)	3.46 ± 0.0104 (3.30–3.50)

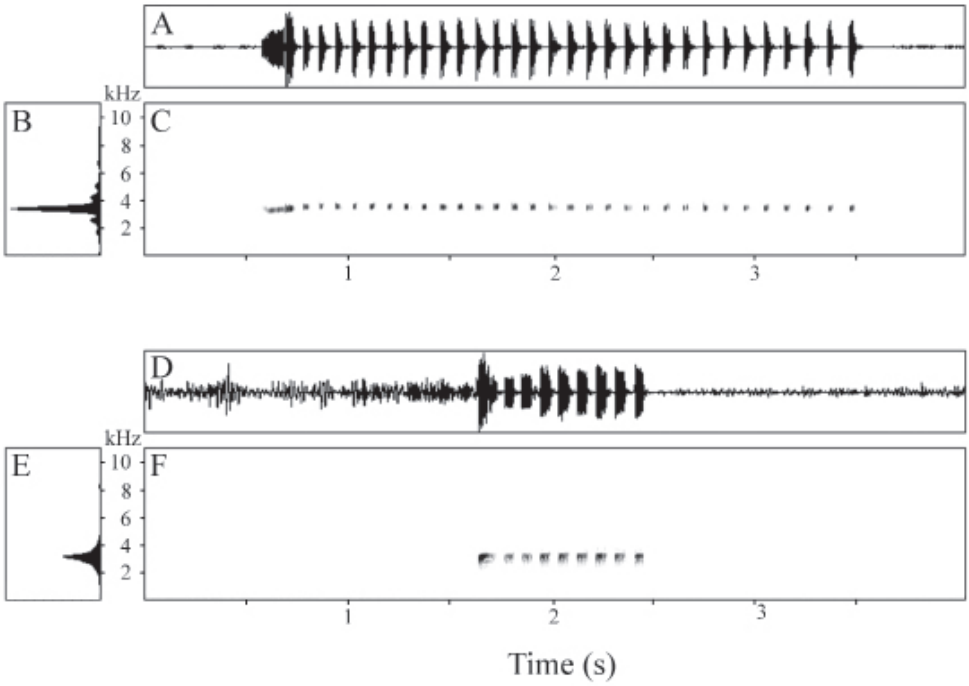

Figure 7. A Waveform **B** power spectrum, and **C** spectrogram of 31-note long call of paratype of *Oreophryne gagneorum* sp. n. (BPBM 20560) recorded on Mt. Rossel, Rossel Island, 6 May 2004, air temperature 22.8 °C, and **D** waveform **E** power spectrum, and **F** spectrogram of 9-note short call of paratype of *Oreophryne gagneorum* sp. n. (BPBM 20538), recorded on Mt. Rossel, Rossel Island, 5 May 2004, air temperature 22.6 °C.

(Fig. 7A), being 143–190 ms in length (mean 167 ms); subsequent notes were much briefer, with a mean of means of 37 ms (range 23–56 ms). The interval between notes was somewhat longer than the notes themselves, with a mean of means of 53 ms and range of 29–82 ms. The first note had a rounded amplitude envelope initially, followed by a short, sharp drop in volume, quickly succeeded by a large terminal spike (Fig. 7A); subsequent notes attained maximum volume rapidly and then decreased at an increasing rate, resulting usually in a concavely triangular amplitude envelope (Fig. 7A). Notes lacked harmonics, pulsing, and frequency modulation (Fig. 7C). The dominant frequency of calls varied within a very narrow window (Fig. 7B), with a mean of means of 3337 Hz and range of 3212–3514 Hz.

The less-frequently delivered short calls (n = 6) contained only 9–12 notes but were emitted at a rate similar to that found in the longer calls (9.57–11.32 notes/s); calls ranged from 0.85–1.06 s in duration (Table 5). Notes of these calls were not so internally divergent in length as those in the long calls. For each call, the first note was only approximately twice the length of the remainder (Fig. 7D), being 92–112 ms in length (mean 102 ms), compared to a mean of means of 54 ms (range 46–62

ms) for subsequent notes (Table 5). The interval between notes was shorter than in the long calls, with a mean of means of 37 ms and range of 26–44 ms. Hence, the length difference between notes and inter-note intervals was not as great as seen in the long calls. The first note attained maximum volume rapidly, decreased rapidly to a lower amplitude, and then maintained that until the end of the note (Fig. 7D); subsequent notes also increased to maximum amplitude quickly, maintained that volume rather evenly, and then decreased quickly to termination, producing an approximately square-shaped amplitude envelope (Fig. 7D). Notes lacked harmonics, pulsing, and frequency modulation (Fig. 7F). The dominant frequency of calls varied within a very narrow window (Fig. 7E), with a mean of means of 3289 Hz and range of 3068–3497 Hz.

Etymology. The name is an honorific for Betsy and Wayne Gagné, dedicated and inspiring conservationists of Pacific island biotas and among the few western researchers to visit Mt. Rossel, being members of the 1979 Lae Forestry Institute botanical expedition to that mountain.

Range. Endemic to Rossel Island, Milne Bay Province, PNG. It was very common along the upper elevations of Mt. Rossel at 720–750 m elevation, but I found it to occur as low as 280 m elevation.

Ecological notes. The type locality consists of dense cloud forest on a steep ridge on the south slope of Mt. Rossel. Forest here is approximately 5–10 m high, and large gingers and tree ferns are common. Even when rainfall is absent moisture at this site is largely constant due to fog drip from clouds blowing over the ridge. Soil consists of mud on the slopes but with pockets of humus, especially along the ridge. The region is subject to major landslides, with a large landslide extending from just below the type locality to the bottom of an adjacent valley at approximately 250 m elevation. Animals were abundant at the type locality. They also occured less commonly in tall, lowland secondary forest growing at 280 m on clay mud and scree slides. In this area the undergrowth was not dense, and palms, pandanus, and ferns were common.

Frogs called from late afternoon through early morning at the type locality; calling perches were typically stems or leaves from 1–4 m above ground. Frogs typically emitted the longer advertisement calls when conditions were wet. In those circumstances they were not shy and were easily captured. Under drier conditions, the frogs gave slower calls at more erratic intervals, and they often called from hidden perches. Calls in the population would often move in a wave of chorusing activity across the mountain.

The smallest mature male was 16.3 mm SV, and it was recorded calling, but another male at 16.5 mm SV was not yet mature. The smallest mature female had a SV of 19.0 mm; two immature females were 17.0 and 17.4 mm long. Hence, males mature at a smaller size than do females.

The frog community on Rossel Island is rather depauperate; syntopic frogs include only *Austrochaperina yelaensis*, *Barygenys exsul*, *Cophixalus cupricarenus*, *C. kethuk*, an undescribed *Copiula*, *Litoria eschata*, *L. louisiadensis*, *Mantophryne louisiadensis*, *Nyctimystes perimetri*, and an undescribed *Oreophryne*.

Discussion

The outlying mountain ranges that occur along the northern coast of New Guinea are derived from a series of offshore island arcs that have been sequentially accreted onto the New Guinea mainland in a west-to-east progression over the past 20 million years (Davies et al. 1997; Davies 2012). This region in composite may be referred to as the Northern Island-Arc Terranes (Pigram and Symonds 1991) and is separate in origin from the adjacent Vogelkop Composite Terrane (the "bird's-head" region of New Guinea) to the west, which was sutured to New Guinea approximately 12 MYA (Pigram and Symonds 1991; Polhemus and Polhemus 1998). Although currently remaining as offshore islands, Yapen and Biak islands in the west and New Britain in the east are parts of the same Northern Island-Arc Terranes system (cf., map in Polhemus and Polhemus 1998); they just haven't accreted to the mainland yet.

Seven species of *Oreophryne* are now known from the mainland north-coast ranges of the Northern Island-Arc Terranes: *O. biroi*, *O. cameroni*, *O. geislerorum*, *O. hypsiops*, *O. parkeri*, *O. parkopanorum*, and *O. wolterstorffi* (Zweifel et al. 2003; this study). On geologically allied offshore terranes, *O. brachypus* is restricted to New Britain (Zweifel et al. 2003), *O. kapisa* to Biak Island (Günther 2003b), and *O. asplenicola*, *O. pseudasplenicola*, and *O. waira* to Yapen Island (Günther 2003b). In the eastern portion of the Vogelkop Composite Terrane – immediately adjacent to the Northern Island-Arc Terranes but geologically independent of them – *O. atrigularis*, *O. clamata*, *O. sibilans*, and *O. unicolor* are known from the Wandammen Peninsula (Günther et al. 2001; Günther 2003a). As yet, none of the species described from the Northern Island-Arc Terranes system has been reported in the Vogelkop Composite Terrane, or vice versa.

As a biogeographically related community, the species of the Northern Island-Arc Terranes show interesting patterns of phenotypic variation in a few characters that may be useful in indicating phylogenetic relationships among them. Of the mainland species, *O. cameroni* and *O. parkopanorum* are the only north-coast species to have a cartilaginous connection between the procoracoid and scapula, a situation shared only with the three species endemic to Yapen Island in the west. However, one would expect additional species with this feature to surface once the large expanses of intervening terrain in Indonesian New Guinea are better surveyed. Most other Papuan *Oreophryne* with a cartilaginous connection occupy portions of the central cordillera, although *O. clamata* is known from the eastern portion of the Vogelkop Composite Terrane, *O. kampeni* is known only from the type locality near Port Moresby and *O. gagneorum* is restricted to Rossel Island. These latter two locations are part of the East Papuan Composite Terrane that comprises southeastern New Guinea and adjacent islands. The remaining species of the Northern Island-Arc Terranes and of the East Papuan Composite Terrane, whether occurring insularly or on the mainland, all have a ligamentous connection between these pectoral elements. As a hypothesis for future testing, it will be interesting to determine whether these *Oreophryne* from Yapen Island are in fact closely related to the two species described herein or whether they have independently acquired this pectoral feature. The preliminary phylogenetic tree for

western *Oreophryne* obtained by Köhler and Günther (2008) suggests that the latter may be the case.

Similarly interesting is that all *Oreophryne* from the Northern Island-Arc Terranes for which data are available exhibit one of only two call types: either a series of unpulsed peeps or a pulsed rattle. The calls of *O. asplenicola*, *O. cameroni*, *O. hypsiops*, *O. parkeri*, and *O. pseudasplenicola* are a series of peeps; those of *O. biroi*, *O. brachypus*, *O. geislerorum*, *O. kapisa*, and *O. waira* are rattles. The calls of *O. parkopanorum* and *O. wolterstorffi* remain unknown. This pattern also holds true for the *Oreophryne* of the Vogelkop Composite Terrane: the calls of *O. sibilans* and *O. unicolor* are peeps, those of *O. atrigularis*, and *O. clamata* are rattles. Both call types also occur among *Oreophryne* species in the central cordillera, but call types there are more diverse and include calls not easily placed in either of the preceding two categories (Zweifel et al. 2005; Kraus and Allison 2009a). More interesting is that most *Oreophryne* species from the East Papuan Composite Terrane have calls that represent two additional call types: either the high-pitched, rapid whinny found in *O. gagneorum* and a number of other, current undescribed, species, or a short honk (F. Kraus, unpubl. data), although additional call types that do not fit into these primary groups also occur (Menzies 2006; Kraus and Allison 2009b; F. Kraus, unpubl. data). In no case have I encountered *Oreophryne* species in this region having peep calls, but *O. geislerorum* and at least one undescribed species from this region have rattle calls. It is perhaps informative of phylogenetic history that some of these diverse call types (e.g., peep call, whinny call, honk call) should be exclusive to areas of New Guinea having very different geological histories. It remains to be determined whether call types will provide a better indicator of phylogenetic propinquity than several of the morphological features used for species discrimination. Because most of the species from southeastern New Guinea remain undescribed, I will explore this issue in greater detail upon their description.

In contrast to these characters, variation in toe webbing and relative length of third and fifth toes does not appear to divide into geographically discrete patterns. Species without toe webbing are largely, but not entirely, confined to the central cordillera and islands to the west, whereas those with either basal webbing or well-webbed toes are found throughout the region. And all variants in relative toe length are found throughout the region. Given that most *Oreophryne* species seem to have narrowly circumscribed geographic ranges suggestive of limited dispersal ability, the distribution patterns of these phenotypic features are consistent with independent origins of each character state.

Although the description of the new species treated herein now brings to seven the number of *Oreophryne* species reported from the north-coast region of New Guinea, the presence from these areas of additional specimens of uncertain identity (Zweifel et al. 2003) suggests that additional species likely await description. Furthermore, the large expanse of unexplored north-coast mountains in adjacent Papua Province of Indonesia will certainly disclose new species once they become more thoroughly investigated. Similarly, the description of *O. gagneorum* brings to eight the number of *Oreophryne* species described from the East Papuan Composite Terrane system (Parker 1934; Men-

zies 2006; Kraus and Allison 2009b). However, I have at least a dozen more new *Oreophryne* species remaining to be described from this region, and large portions of this terrane system remain unsurveyed. Hence, interpreting patterns of phenotypic variation in that region is premature at this time. But it is clear that the number of Papuan species contained within *Oreophryne* remains only poorly approximated.

Acknowledgements

I thank M. Wilkinson for kindly hosting my visit to BMNH; D. Frost and D. Kizirian (AMNH), M. Hagemann (BPBM), G. Doria (MSNG), and M. Praagman and R. Vonk (ZMA) for loans of specimens; D. Kizirian for providing photos of type specimens of *Oreophryne brevicrus*; S. Myers for producing Figs 1, 5, and 6; H.D. Cameron for providing advice on Latin grammar; C. Bernard, C. Graham, I. Haguna, R. Henry, F. Hurahura, S. John, C. Kembwa, F. Malesa, D. Mitchell, M. Okira, S. Simalken, T. Simalken, J. Slapcinsky, I. Yikida, Conservation International, and all the people of Parkop for providing field or logistical assistance during my expeditions. I thank the PNG National Museum and Art Gallery for providing in-country collaborative assistance and the Department of Environment and Conservation; National Research Institute; and the Madang, Milne Bay, and West Sepik provincial governments for permission to conduct this research. This research was supported by National Science Foundation grants DEB-0103794 and DEB-0743890 and a grant from Conservation International.

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Appendix

Specimens examined of Papuan *Oreophryne* with cartilaginous connection between procoracoid and scapula

Oreophryne anamiatoi (n = 20): Papua New Guinea: Southern Highlands Province: E slope Mt. Itukua, 5.6695°S, 142.6233°E, 2177 m (BPBM 33768, holotype; 33763–67, 33769–72, PNGNM 24097–99, paratypes), E slope Mt. Paramo, 5.6451°S, 142.6362°E, 1874 m (BPBM 33773–79, PNGNM 24100, paratypes).

Oreophryne crucifer (n = 1): Indonesia: Papua Province: Went Mts. (ZMA 5819, syntype).

Oreophryne flava (n = 1): Indonesia: Papua Province: Lorentz River, Kloofbivak (ZMA 5823, holotype).

Oreophryne idenburghensis (n = 2): Indonesia: Papua Province: 18 km SW Bernhard Camp, Idenburg River, 2150 m (AMNH 49663, holotype, AMNH 49666, paratype).

Oreophryne kampeni (n = 12): Papua New Guinea: Central Province: Moroka (BMNH 1947.2.12.14, holotype, BMNH 1947.2.12.43–44, paratypes, MSNG 29127, nine paratypes under the same number).

Oreophryne notata (n = 35): Papua New Guinea: Southern Highlands Province: E slope Mt. Itukua, Muller Range, 2170 m (BPBM 33672–706).