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Biogeography and conservation assessment of *Bactrurus* groundwater amphipods (Crangonyctidae) in the central and eastern United States

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Abstract

The subterranean amphipod genus *Bactrurus* (Amphipoda: Crangonyctidae) is comprised of eight species that occur in groundwater habitats in karst and glacial deposits of the central and eastern United States. We reexamine the distribution, biogeography, and conservation status of *Bactrurus* in light of new species distribution records and divergence time estimates in the genus from a recent molecular study. In particular, we discuss hypotheses regarding the distribution and dispersal of *B. mucronatus* and *B. brachycaudus* into previously glaciated regions of the Central Lowlands. We also conducted the first IUCN Red List conservation assessments and reassessed global NatureServe conservation ranks for each species. We identified 17 threats associated with increased extinction risk that vary in source, scope, and severity among species, with groundwater pollution being the most significant threat to all species. Our conservation assessments indicate that five of the eight species are at an elevated risk of extinction under IUCN Red List or NatureServe criteria, with one species (*B. cellulanus*) already extinct. However, none of the eight species are considered threatened or endangered by any state or federal agency. Significant knowledge gaps regarding the life history, ecology, and demography of each species exist. Given results of our conservation assessments and available information on threats to populations, we offer recommendations for conservation, management, and future research for each species.

Keywords

IUCN, Red List, NatureServe, glaciation, groundwater pollution, climate change, Teays River, Pleistocene

Introduction

The type species of the subterranean amphipod genus *Bactrurus* was originally described (as *Crangonyx mucronatus*) from specimens collected from a well in Normal, McClean County, Illinois, USA by Stephan A. Forbes (Forbes 1876). The description was published shortly before his appointment as the new director of the State Laboratory of Natural History, which would later become the Illinois Natural History Survey, with Forbes serving as its first director (Hays 1980; Mills 1958). Nearly 140 years later, we still have much to learn about *Bactrurus*, even beneath the very institution where study of this genus began. Over a century passed before a proper revision of this genus was completed (Koenemann and Holsinger 2001).

At present, *Bactrurus* comprises eight described species (Table 1) found in the eastern and central United States, in groundwater habitats including caves, springs, wells, and interstitial spaces in glacial sediments. Seven of the eight species are associated with groundwater habitats in karst terranes. Three species found in the Interior Low Plateau and Appalachian Valley are thought to be isolated, endemic relict species (the *B. wilsoni* group), while the other four species associated with karst terranes occur primarily in the Ozark Plateaus. In contrast, *Bactrurus mucronatus* (Forbes, 1876), which is the most widely distributed member of the genus, occurs almost exclusively in non-karst habitats in previously glaciated regions of the Central Lowlands. However, *Bactrurus brachycaudus* Hubricht & Mackin, 1940, a species largely associated with karst on either side of the Mississippi River Valley in Illinois and Missouri, also has been collected from previously glaciated regions of central Illinois where Koenemann and Holsinger (2001) reported specimens from drain tile outlets in glacial drift (Montgomery and Sangamon counties, Illinois). Unlike *B. brachycaudus* from most karst populations, specimens from central Illinois exhibit sexual dimorphism in the length of the telson, where males have about 20% longer telsons than females. Rather than a new species, Koenemann and Holsinger (2001) treated the Illinois populations as geographic variants of *B. brachycaudus*. Molecular analyses of the small-subunit rDNA gene also supported conspecific treatment (Englisch and Koenemann 2001).

In the course of ongoing studies of groundwater amphipods in North America, we collected specimens of *Bactrurus* from central Illinois, unexpectedly discovering specimens of *B. brachycaudus* significantly northeast of its known distribution in central Illinois. Here, we reexamine the distribution and biogeography of *Bactrurus* in light of the discovery of this new population, the recent description of a new species from the Ozark Plateaus of Arkansas (Holsinger et al. 2006), and the results of a recent divergence time analysis of amphipods that included three species of *Bactrurus* (Corrigan et al. 2014). In particular, we explore hypotheses regarding the distribution and dispersal of *B. mucronatus* and *B. brachycaudus* into previously glaciated regions of the Central Lowlands. Finally, we reviewed threats and conducted the first IUCN Red List conservation assessments for each of the eight species of *Bactrurus* and reassess the NatureServe global and state conservation ranks. No *Bactrurus* species have been subject to an IUCN Red List conservation status assessment, and NatureServe global conservation status for *Bactrurus* species have not been reviewed in 9–14 years (since 2002–2007).

Table 1. The described species of the genus *Bactrurus* (Amphipoda: Crangonyctidae), number of occurrences, type localities, general distribution, and conservation status.

Species	Common Name	Type Locality	States (Counties)	Occurrences	EOO (km^2)	AOO (km^2)	Occurrences on Protected Land	Overall Threat Impact	Previously Assessed	Previous NatureServe Rank	Calculated NatureServe Rank	IUCN Red List Rank	IUCN Red List Criteria	State T & E Status
Bactrurus angulus Koenemann & Holsinger, 2001	Cumberland Gap Cave Amphipod	TN: Claiborne Co.: Sour Kraut Cave (CB46)	TN (1), VA (1)	3	29	12	1	High	2002	G1	G1	CR	B1ab (i,ii, iii,iv)	SGCN[‡] (VA), Rare Animal List (TN)
Bactrurus brachycaudus Hubricht & Mackin, 1940	Short-Tailed Groundwater Amphipod	MO: St. Louis Co.: Spring on Keifer Creek	IL (12), MO (19)	114	90,012	408	41	Medium	2002	G4	G4	LC		absent[¶] (IL), Species of Concern (MO)
Bactrurus cellulanus Koenemann & Holsinger, 2001	Indiana Groundwater Amphipod[§]	IN: Monroe Co.: seep in basement of Jordan Hall, Indiana University, Bloomington	IN (1)	1	na	4	0	Very High	2003	GX	GX	EX		absent[¶] (IN)
Bactrurus hubrichti Shoemaker, 1945	Kansas Well Amphipod	KS: Shawnee Co.: well at Topeka	KS (8), MO (1), OK (2)	15	90,935	52	3	Medium	2002	G4	G3	LC		absent[¶] (KS), Species of Concern (MO), ODWC-II[#] (OK)
Bactrurus mucronatus (Forbes, 1876)	Glacial Till Groundwater Amphipod[§]	IL: McClean Co.: well at Normal	IA (3), IL (36), IN (26), MI (2), OH (11)	153	259,076	596	8	Medium	2002	G5	G5	LC		absent[¶] (IA, IL, IN, MI, OH)
Bactrurus pseudomucronatus Koenemann & Holsinger, 2001	Ozark Groundwater Amphipod[§]	AR: Randolph Co.: Mansell Cave	AR (2), MO (4)	20	7,230	80	14	Medium	2004	G2G3	G3	NT		SGCN[‡] (AR), Species of Concern (MO)

Species	**Common Name**	**Type Locality**	**States (Counties)**	**Occurrences**	**EOO (km^2)**	**AOO (km^2)**	**Occurrences on Protected Land**	**Overall Threat Impact**	**Previously Assessed**	**Previous NatureServe Rank**	**Calculated NatureServe Rank**	**IUCN Red List Rank**	**IUCN Red List Criteria**	**State T & E Status**
Bactrurus speleopolis Holsinger, Sawicki & Graening, 2006	Cave City Groundwater Amphipod	AR: Sharp Co.: Cave City Cave	AR (2)	2	na	8	0	High	2007	G1	G1	VU	D2	absent¶ (AR)
Bactrurus wilsoni Koenemann & Holsinger, 2001	Alabama Groundwater Amphipod§	AL: Blount Co.: well in kitchen of residence	AL (1)	1	na	4	0	High	2002	G1G2	G1	VU	D2	absent¶ (AL)

‡ SGCN = Species of Greatest Conservation Need.

§ New common name – where prior common names have not been used or accepted, we propose common names to facilitate better communication for conservation and management awareness.

¶ absent = absent from state list.

ODWC-II = Oklahoma Department of Wildlife Conservation tier status II.

na = not enough occurrences to calculate a minimum convex hull.

Methods

Field surveys

Amphipods were collected from field drain tile outlets and caves by hand and using dip nets. Specimens were transported back to the laboratory alive for photography and subsequent preservation in 80 and 100% ethanol for morphological and future molecular analyses, respectively. Morphological identifications utilized the key and descriptions in Koenemann and Holsinger (2001), original descriptions of species (Forbes 1876; Hubricht and Mackin 1940), and comparisons with museum material.

Compiling distributional records

Distributional data for all *Bactrurus* species were compiled from literature sources and biological databases, including Hubricht and Mackin (1940), Hubricht (1943), Barnett (1970), Webb et al. (1998), Koenemann and Holsinger (2001), Reid et al. (2002), Sutton (2003), Holsinger et al. (2006), and Lewis (2015). Additional records from Indiana, collected by Julian Lewis, were obtained from the US National Museum (USNM) and from Lewis (unpublished data, 23 October 2015). Additional records from Illinois were obtained from the Illinois Natural History Survey Crustacean Collection (INHS-CC). Specimen identifications for INHS-CC material were confirmed by microscopic examination (SJT). Distributional data were georeferenced and mapped in ArcMap 10.1 (ESRI 2012) onto the United States karst map (Weary and Doctor 2014). Pleistocene glacial episodes in the central U.S. (Fullerton et al. 2003) also were overlain. Pre-Pleistocene river drainage patterns were adapted from various sources (Teller and Goldthwait 1991; Anderson 1988; Mayden 1988; Cupples and Van Arsdale 2014; Dutch 2015).

Conservation assessments

We conducted conservation assessments under both the IUCN Red List of Threatened Species (http://www.iucnredlist.org/) criteria and NatureServe conservation rank protocols (http://www.natureserve.org/). In addition to presenting IUCN Red List and NatureServe conservation status ranks, we present conservation status based on state agency listings.

IUCN Red List. Seven IUCN Red List categories are recognized on a continuum of increasing extinction risk (IUCN 2001): Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW), and Extinct (EX). Two additional categories are also recognized: Data Deficient (DD) in which a species has been evaluated but insufficient data are available to make a determination on conservation rank, and Not Evaluated (NE) in which a species has yet to be evaluated. The categories Critically Endangered, Endangered, and Vulnerable are considered 'Threatened' categories. A species may be classified under one of the 'Threatened' categories if it meets specific conditions under any one of five criteria (IUCN 2001): (A) past, present, or projected reduction in population size over three generations; (B) small geographic range in combination with fragmentation, population decline or fluctuations; (C) small population size in combination with decline or fluctuations; (D) very small population or very restricted distribution; or (E) a quantitative analysis of extinction risk. Criteria for threat classification under categories A, C, and E require evidence of declining trends in population size. IUCN Red List assessments followed definitions and guidelines outlined in IUCN (2010). We assessed the status of each species using IUCN Red List categories and criteria (IUCN 2001) using RAMAS Red List 3.0 (Akcakaya et al. 2007) to calculate risk categories.

NatureServe. NatureServe conservation status ranks are based on a one to five scale, from most to least at risk of extinction: G1 (Critically Imperiled), G2 (Imperiled), G3 (Vulnerable), G4 (Apparently Secure), and G5 (Secure). Two additional ranks associated with extinction exist: GH (Possibly Extinct) and GX (Presumed Extinct). Status ranks are assessed at three geographic scales: global (G), national (N), and state (S). We present ranks at the global and state scales. NatureServe ranks are based on ten primary factors grouped into three main categories: rarity, trends, and threats (Master et al. 2009). Rarity factors include range extent of occurrence (EOO), area of occupancy (AOO), number of occurrences, number of occurrences with good viability or ecological integrity, population size, and environmental specificity. Trend factors include both short-term and long-term trends in population size, EOO, AOO, number of occurrences, and viability or ecological integrity of occurrences. Threat factors include threat impact and intrinsic vulnerability to threats. Number of protected or managed occurrences and other factors can also be included to assess conservation status.

NatureServe global conservation status assessments for each lineage were calculated using default points and weights with the NatureServe Rank Calculator v3.186 (Faber-Langendoen et al. 2009; available at www.natureserve.org/conservation-tools/conservation-rank-calculator). We assigned a value of "Very Narrow" for environmental specificity for *B. angulus*, *B. cellulanus*, *B. pseudomucronatus*, *B. speleopolis*, and *B. wilsoni*, as these species are primarily known from only a single habitat type. We assigned a value of "Narrow" for environmental specificity for *B. brachycaudus*, *B. hubrichti*, and *B. mucronatus*. These species are known from subterranean habitats but also springs and seeps.

Geographic range size. IUCN Red List and NatureServe conservation assessments use two different measures of geographic range size: EOO (also referred to as range extent) and AOO. We calculated EOO and AOO using the web-based program GeoCAT (Bachman et al. 2011; available at geocat.kew.org). EOO was calculated as a minimum convex hull. A grid size of 2 km (4 km^2) was used to estimate AOO (Faber-Langendoen et al. 2009; IUCN 2010).

Abundance. Accurate estimates of population size and abundance and trends through time are rare for invertebrates in conservation assessments, as such data are particularly difficult to obtain for most species (Cardoso et al. 2011, 2012; Fox et al. 2011; Adriaens et al. 2015). Consequently, many IUCN assessments of invertebrates have used criteria B and D (Cardoso et al. 2012). Estimates of the reduction in AOO have been used as a surrogate for suspected reductions in population abundance under criterion A in some assessments (e.g., Adriaens et al. 2015), based on the assumption that abundance is correlated with the range of a species (Gaston 1994; Cardoso et al. 2011). However, this approach also has shortcomings (discussed in Cardoso et al. 2011). Abundance data are particularly difficult to obtain for subterranean taxa, because of the inaccessibility of and challenges associated with sampling subterranean habitats. Unsurprisingly, abundance data are limited for most populations of *Bactrurus*. However, historical and contemporary abundance data do exist for select populations, particularly those of range restricted species (i.e., *B. angulus*, *B. cellulanus*, and *B. wilsoni*). These data were included when applicable in NatureServe and IUCN Red List assessments.

Trends. The change in EOO, AOO, number of occurrences, and quality of habitat were determined over short- and long-term timescales when such data were available. Trends in abundance over time were largely not incorporated in assessments for reasons mentioned above. Long-term trends were considered from the year of first discovery of a species to the present day, while short-term trends were considered over the last 10 years.

Threats. We followed the threat assessment protocol outlined in Master et al. (2009) and the IUCN-Conservation Measures Partnership Classification of Threats (Salafsky et al. 2008) to evaluate the scope, severity and timing of observed, inferred, and suspected threats to each species. To assist in the identification of current and potential threats, we examined land cover and human population density surrounding occurrences for each species. Land cover data from the 2011 release of the National Land Cover Database (NLCD; Homer et al. 2015) were analyzed around a 2.5 km buffer (19.6 km^2 area) around each occurrence in ArcGIS v10.3. Because many of these cover types occur only in Alaska or coastal areas, we analyzed 15 major cover types that were collapsed into six broad categories: (1) developed land – open space, low-intensity, medium-intensity, and high-intensity urbanization; (2) forest – deciduous forest, evergreen forest, and mixed forest; (3) grassland/shrub – grassland with >80% total herbaceous vegetation and shrub/scrub; (4) pasture/hay planted for livestock grassing or the production of seed or hay crops; (5) cultivated crops, orchards, and vineyards; and (6) water, including open water and wetlands. We also examined human population density surrounding occurrences as a proxy for urbanization. Human population data were obtained for the 2010 U.S. Census from the U.S. Census Bureau (TIGER/Line® shapefiles available at https://www.census.gov/geo/maps-data/data/tiger-data.html). We used the Intersect geoprocessing tool with the buffer shapefile generated previously and the polygon population and housing unit shapefile as input to create a shapefile used to calculate total population and population density within the buffer area around each occurrence. The Dissolve data management tool was used to summarize total population.

Protected and managed occurrences. Although the number of protected or managed occurrences is no longer considered in NatureServe conservation assessments, this information is of value for developing and prioritizing management decisions. We determined whether occurrences for each species occurred on state or federal protected areas (e.g., state parks, natural areas, national parks, state and national forests, etc.). Protected areas were obtained from the USGS Protected Areas Database (PAD-US) version 1.3 (shapefiles available at http://gapanalysis.usgs.gov/padus/).

Uncertainty. Uncertainty in values of assessment criteria is an important consideration when assessing conservation status, as uncertainty can strongly influence the assessment of extinction risk (Akcakaya et al. 2000; IUCN 2001; Gillespie et al. 2011). NatureServe accounts for uncertainty by allowing a range of ranks to show the degree of uncertainty in a conservation status when available information does not permit a single status rank (Master et al. 2009). The IUCN Red List assessment also deals with uncertainty by allowing a plausible range of values to be used to evaluate criteria (IUCN 2001, 2010; Mace et al. 2008). For both assessments, we adopted a moderate dispute tolerance considering the most likely plausible range of values for a criterion and excluding extreme or very unlikely values (Faber-Langendoen et al. 2009; IUCN 2010). RAMAS Red List allows the specification of user's attitude to risk and uncertainty by setting values for risk tolerance and dispute tolerance (Akcakaya et al. 2000). Risk tolerance ranges from 0 (extremely precautionary) to 1 (extremely evidentiary), while dispute tolerance ranges from 0 (inclusion of all estimates) to 1 (inclusion of only the consensus estimates). For all assessments, we set risk tolerance to 0.5 (risk neutral) and dispute tolerance to 0.5.

Results

New distribution records

In 2011, David J. Soucek (Illinois Natural History Survey, University of Illinois), collected specimens identified as *B. brachycaudus* from the outlet of a field tile draining into the East Fork Embarras River in Champaign County, Illinois, USA (39.94505°N, 88.12310°W; 23 March 2011; Figs 1, 2, arrow A), and additional material of the same species was later collected from the same field tile outlet in 2015 (MLN & SJT; 28 May 2015). Also on 28 May 2015, we made a collection of *B. mucronatus* in the same county from a historical locality (large concrete drain pipe flowing into drainage ditch, 5.1 km N of Mayview in the Vermilion River Basin; 40.15754°N, 88.10506°W) as was collected by Koenemann and Englisch on 13 May 1999 and identified as *B. mucronatus* by Koenemann and Holsinger (2001). The East Fork Embarras River record (Figs 1, 2, arrow A) represents a 129 km northeastern range extension for *B. brachycaudus*. The distribution of *B. mucronatus* is extended to the southwest in Indiana on the basis of USNM records, and northwards in Illinois to Cook and Lee counties based on INHS-CC records. Finally,

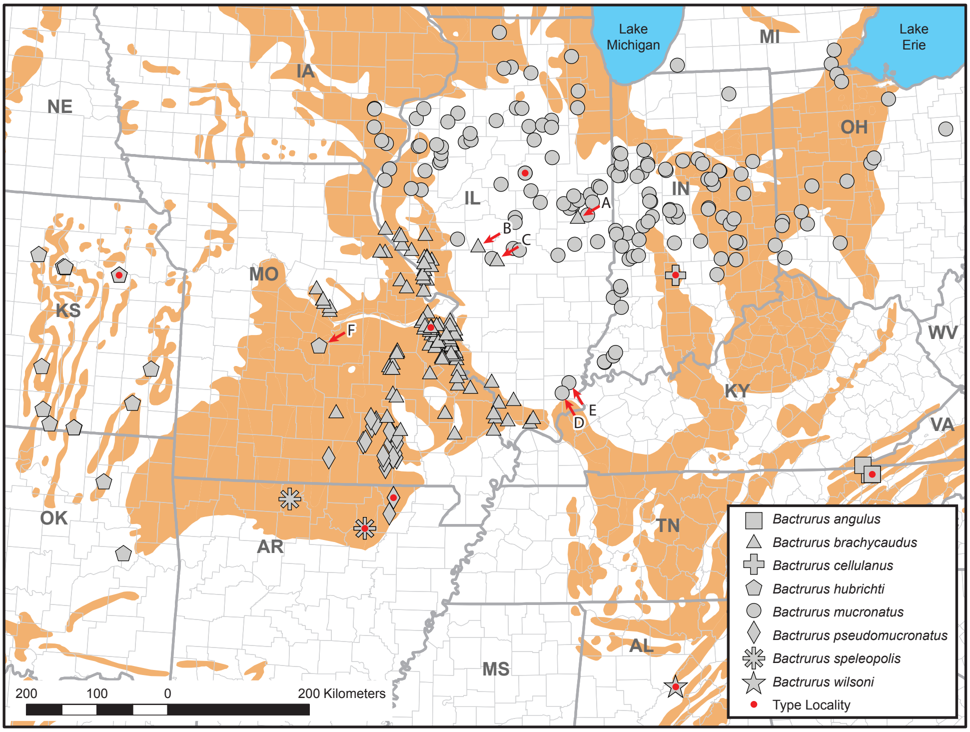

Figure 1. Distribution of species of the genus *Bactrurus* (Amphipoda, Crangonyctidae) in relation to karst. Karst areas are based on Weary and Doctor (2014). Letters **A–F** (red arrows) are localities discussed in the body of the text.

we (MLN, SJT, D. Soares, and T. Haspel-Soares) collected a single adult *B. hubrichti* on 13 July 2015 from Klug's Cave, Miller Co., Missouri.

Distribution and biogeography

Almost all *Bactrurus* species are associated with karst terranes (Fig. 1), with the majority of species restricted to a single karst region. Three species, *B. angulus*, *B. cellulanus*, and *B. wilsoni*, have extremely restricted distributions and are endemic to isolated karst areas in the Interior Low Plateau and Appalachian Ridge and Valley. *Bactrurus speleopolis* also has a limited distribution but is parapatric to *B. pseudomucronatus* in the Ozark Highlands. Among the broadly distributed species, *B. hubrichti* is associated with the Flint Hills karst in Kansas and Oklahoma (with one notable exception in Missouri: Figs 1, 2, arrow F). *Bactrurus brachycaudus* is associated with springs and caves along either side of the Mississippi River. However, three localities in central Illinois (Figs 1, 2, arrows A, B, and C) are disjunct from the main distribution in areas strongly impacted by Pleistocene glacial drift, including the new locality reported above.

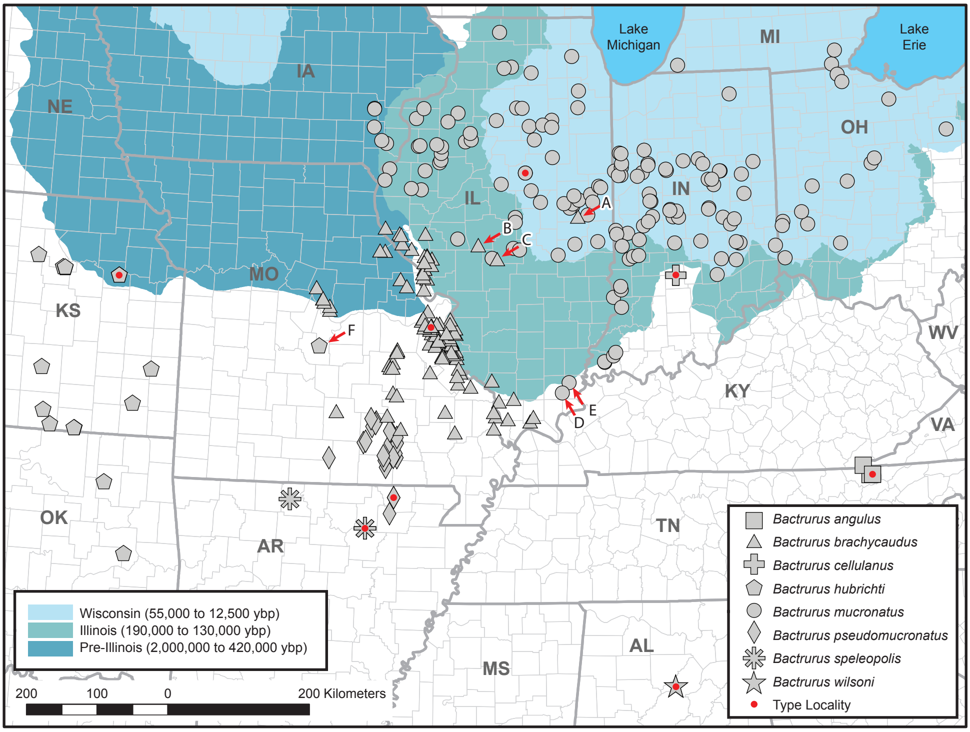

Figure 2. Distribution of species of the genus *Bactrurus* (Amphipoda, Crangonyctidae), overlain on a map of the maximum extent of Pleistocene glacial episodes derived from Fullerton et al. (2003). Letters **A–F** (red arrows) are localities discussed in the body of the text.

Occurrences, EOO, and AOO

We compiled 309 occurrence records from several literature sources, state and personal biological databases, and museum collections for the eight described *Bactrurus* species. The vast majority (86.4%) of these records are associated with two species (Table 1): *B. brachycaudus* and *B. mucronatus*. Two species, *B. cellulanus* and *B. wilsoni*, are known from only a single locality, and two others, *B. angulus* and *B. speleopolis*, are known from three and two localities, respectively. EOO was quite variable among species (Table 1), ranging from 29 km^2 to 259,076 km^2 with a mean of 89,456 ± 104,321 km^2. EOO could not be calculated for three species known from two or fewer occurrences: *B. cellulanus*, *B. speleopolis*, and *B. wilsoni*. AOO averaged 146 ± 227 km^2, with a minimum of 4 km^2 and a maximum of 596 km^2.

Abundance

Less than 15 individuals in total have been observed or collected for three species. A total of four specimens of *B. cellulanus* have been collected on three occasions in

1962–1963 from a groundwater seep-stream in the subbasement of Jordan Hall on the campus of Indiana University in Bloomington, Indiana (Koenemann and Holsinger 2001). *Bactrurus wilsoni* is known from only one site and six specimens: four specimens were collected from a residential well in Blount Co., Alabama, on three occasions in 1982–1983. However, a single specimen was collected on two separate occasions in June 1999 (Koenemann and Holsinger 2001). The type locality of *B. wilsoni* has not been visited since 1999 to our knowledge. Seven specimens of *B. angulus* were collected from three localities in Claiborne Co., Tennessee, and Lee Co., Virginia, in the 1970s. Repeat visits to these caves in 1996–1997 did not yield any additional observations or specimens (Koenemann and Holsinger 2001). However, six individuals were observed at one locality in Tennessee in June 2015, confirming the continued presence of this species (MLN, unpublished data). Up to 25 individuals have been observed at the type locality of *B. speleopolis* in Sharp Co., Arkansas during surveys in 2001–2004 (Graening et al. 2005; Holsinger et al. 2006).

The other four *Bactrurus* species are comparatively more abundant. Although fewer than 10 individuals have been reported during surveys for the majority of occurrences, all four species can be locally abundant. For example, over 30 individuals have been observed during single surveys at one locality of *B. hubrichti*, two localities of *B. pseudomucronatus*, five localities of *B. brachycaudus*, and 13 localities of *B. mucronatus*. Over 1,000 individuals of *B. hubrichti* were reported over a 20-month period in 1990–1992 during a study of a spring at the Konza Prairie Research Natural Area in Riley Co., Kansas (Edler and Dodds 1996).

Trends

EOO and AOO for each *Bactrurus* species, with the exception of *B. cellulanus* and *B. angulus*, likely have not decreased significantly in the last 10 or even 50 years. *Bactrurus cellulanus* has not been seen since 1963, as termiticide applications to the grounds around the type locality extirpated all aquatic subterranean life, including *B. cellulanus* (Lewis 2012, 2015). *Bactrurus angulus* has not been observed from two of the three known occurrences since the 1970s: the type locality in Tennessee since 1977 and from the locality in Virginia since 1979. EOO and AOO have increased for *B. brachycaudus* and *B. mucronatus* in recent years due to increased study and inventory efforts in Illinois and Indiana.

Threats

Dominant land use surrounding occurrences of each species varied widely among species (Fig. 3). Developed (urban) areas was the prevalent land use surrounding occurrences of *B. cellulanus* (86.8% of the area), which occurred in Bloomington, Indiana, but the percentage of developed land was not greater than 15% for any other species.

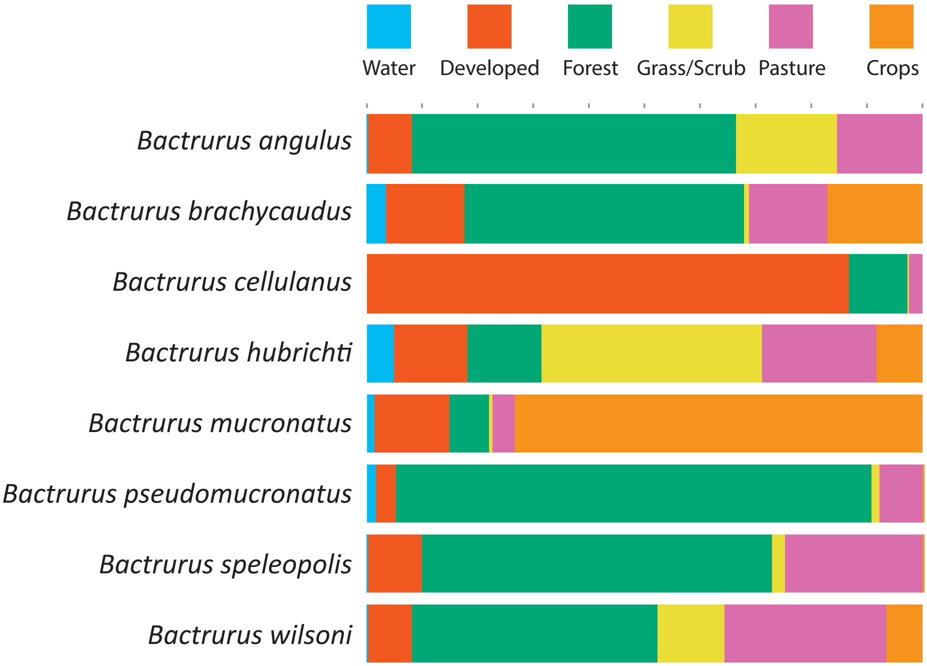

Figure 3. Land use within the ranges of the eight species of the genus *Bactrurus*. Based on the National Land Cover Database (Homer et al. 2015), collapsed into six categories (see Methods).

Cropland was the most prevalent land use surrounding occurrences of *B. mucronatus* (73.2%) but was only greater than 10% in one other species (*B. brachycaudus*). Pasture was common (>20%) surrounding occurrences of three species: *B. hubrichti*, *B. speleopolis*, and *B. wilsoni*. Forest was the prevalent land use surrounding occurrences of five species: *B. pseudomucronatus* (85.6%), *B. speleopolis* (62.7%), *B. angulus* (58.3%), *B. brachycaudus* (50.2%), and *B. wilsoni* (44.0%). Grassland-scrub was the prevalent land use surrounding occurrences of *B. hubrichti* (39.8%).

We identified 17 threats that either have been documented or may affect populations of *Bactrurus* at present or in the near future (Table 2). These threats vary in impact among *Bactrurus* species and likely among regions within the widely distributed species, such as *B. mucronatus*. Groundwater pollution is the primary threat that has the greatest probability of impacting local populations of *Bactrurus* species. However, the potential sources of groundwater pollution vary among species depending on land use within the recharge areas of documented occurrences. For example, contamination associated with agriculture, such as pesticides, herbicides, and livestock waste, are more likely to impact populations of *B. mucronatus*, where cropland is the prevailing land use surrounding occurrences. Application of insecticides, herbicides, and fungicides onto lawns, gardens, and landscaping is a threat to populations in urban residential areas. Repeated termiticide application is believed to be the primary cause of extinction of *B. cellulanus* (Lewis 2012, 2015). *Bactrurus* populations in urban and rural residen-

Table 2. List of possible threats facing each species of *Bactrurus* following the classification proposed by Salafsky et al. (2008). Threat impacts are negligible (N), low (L), medium (M), high (H), and very high (VH).

Threat	*B. angulus*	*B. brachycaudus*	*B. cellulanus*	*B. hubrichti*	*B. mucronatus*	*B. pseudomucronatus*	*B. speleopolis*	*B. wilsoni*
1. Residential & commercial development	L	M	H	L	L	L	L	L
1.1. Housing & urban areas	L	L	H	L	L	L	L	L
1.2. Commercial & industrial areas	N	L	M	L	L	N	L	N
2. Agriculture & aquaculture	L	M	L	M	M	L	L	L
2.1. Annual & perennial non-timber crops	N	L	N	L	H	N	N	L
2.3. Livestock farming & ranching	L	L	L	L	L	L	L	L
3. Energy production & mining	N	L	N	N	N	N	N	N
3.1. Oil & gas drilling	N	L	N	N	N	N	N	N
3.2. Mining & quarrying	N	L	N	N	L	N	N	N
4. Transportation & service corridors	L	L	H	L	L	L	L	L
4.1. Roads & railroads	L	L	H	L	L	L	L	L
5. Biological resource use	L	L	L	L	L	L	L	L
5.1. Hunting & collecting animals	L	L	L	L	L	L	L	L
6. Human intrusions & disturbance	L	L	N	L	L	L	L	N
6.1. Recreational activities	L	L	N	L	L	L	L	N
7. Natural system modifications	L	L	N	L	L	L	L	L
7.1. Dams & water management/use	L	L	N	L	L	L	L	L
9. Pollution	L	L	VH	L	M	L	L	L
9.1. Domestic & urban waste water	L	L	VH	L	L	L	L	L
9.2. Industrial & military effluents	N	L	M	L	N	N	N	N
9.3. Agricultural & forestry effluents	L	L	N	L	M	L	L	L
9.4. Garbage & solid waste	N	L	N	N	N	N	L	N
11. Climate change & severe weather	M	L	M	L	L	M	M	M
11.1. Habitat shifting & alteration	M	L	M	L	L	M	M	M
11.2. Droughts	L	L	L	L	L	L	L	L
11.4. Storms & flooding	N	N	N	N	N	N	N	N

tial areas are also susceptible to exposure to leachate from septic tanks and sewers. A growing threat is the increase in conversion of land into impervious surfaces, such as roads, parking lots, and sidewalks, associated with urbanization, particularly for populations of *Bactrurus* near growing metropolitan areas.

Any change in land use from natural to disturbed states has the potential to impact populations of *Bactrurus* through habitat degradation associated with changes in sediment and nutrient loads, hydrological regimes, and thermal profiles. Other potential threats include mining operations, climate change, over-collection associated the scientific studies, and disturbance of cave populations associated with human visitation for research, recreational, or commercial purposes. At the present time, no evidence exists for a documented decline as a result of these additional threats. The known exception is one population of *B. brachycaudus* was extirpated when a cave system was destroyed by mining in Ste. Genevieve Co., Missouri.

Occurrences on protected areas

The majority of *Bactrurus* occurrences are located on private land (78.3%). However, the percentage of protected occurrences (those that occur on state or federal protected land) is highly variable among species. No occurrences of *B. cellulanus*, *B. speleopolis*, and *B. wilsoni* occur on protected lands. In contrast, 70.0% of occurrences of *B. pseudomucronatus* occur on protected land, with most occurring on land units of Mark Twain National Forest or conservation areas owned by the Missouri Department of Conservation. Just 5.2% occurrences of *B. mucronatus* occur on protected land, despite 153 occurrences in total. Between 20.0 and 36.0% of occurrences occur on protected land for the remaining three species (*B. angulus*, *B. brachycaudus*, and *B. hubrichti*).

Conservation status

IUCN Red List. None of the eight species of *Bactrurus* had been assessed previously under IUCN Red List criteria (IUCN 2015). Applying IUCN criteria and their associated subcriteria to *Bactrurus* species resulted in three species being classified as threatened (Table 1): *B. angulus* as Critically Endangered under criterion B1ab(i.ii,iii,iv), *B. speleopolis* as Vulnerable under criterion D2, and *B. wilsoni* as Vulnerable under criterion D2. *Bactrurus cellulanus* was classified as “Extinct.” *Bactrurus pseudomucronatus* was classified as “Near Threatened,” as there is no current evidence for a decline in EOO, AOO, or quality of habitat. However, only 20 occurrences are known, and any significant threat could result in the species soon qualifying for “Vulnerable” under criterion B1 or B2. The remaining three species (*B. brachycaudus*, *B. hubrichti*, and *B. mucronatus*) were classified as “Least Concern.”

NatureServe. *Bactrurus cellulanus* is “Presumed Extinct” (GX), as this species has not been observed since the 1960s, and additional populations have not been discovered despite intensive surveys of cave and spring communities in karst of southern Indiana. Five of the eight species are considered threatened under NatureServe criteria (Table 1) with a ranking of “Vulnerable” or higher (G1–G3). *Bactrurus angulus*, *B. wilsoni*, and *B. speleopolis* are at the highest risk of extinction, with a ranking of “Critically Imperiled” (G1). These three species are known from three or fewer occurrences and have extremely restricted distributions. *Bactrurus pseudomucronatus* and *B. hubrichti* were assessed as “Vulnerable” (G3). Only *B. brachycaudus* (G4) and *B. mucronatus* (G5) are not considered at risk of extinction.

Discussion

Biogeography

Of the eight described *Bactrurus*, only *B. brachycaudus* and *B. mucronatus* are not entirely associated with karst, although the majority of *B. brachycaudus* localities are known from karst terranes. Only *B. mucronatus* fails by any measure to demonstrate an association with karst terrane, with specimens found broadly across the Midwestern United States from southeastern Iowa to central Ohio and southern Michigan (Fig. 1). Koenemann and Holsinger (2001) discussed the biogeographic distribution of *Bactrurus*, noting a concentration of diversity in the Central Lowland and Ozark Plateaus. Only *B. mucronatus* occurs almost exclusively to the north of the maximum extent of glacial ice during the Pleistocene, which is in contrast to spatial patterns of subterranean biodiversity in temperate North America where the vast majority of subterranean biodiversity occurs exclusively south of this line (Culver et al. 2003).

It is often assumed that subterranean habitats were either destroyed or too inhospitable for most subterranean fauna to survive beneath glacial ice sheets during the Pleistocene (Vandel 1965; Peck and Christiansen 1990; Culver and Pipan 2009). Both geological and climatic processes during this epoch have influenced the distributions of temperate subterranean fauna in North America (Barr 1968; Holsinger 2000; Culver and Pipan 2009; Niemiller et al. 2013b). Two primary hypotheses have been proposed to explain the distribution of *B. mucronatus* and other subterranean species that occur north of the line of maximal extent of Pleistocene glacial ice. One possibility is that groundwater amphipods, including *Bactrurus*, were able to survive in groundwater refugia beneath the ice (Holsinger 1978, 1981, 1986). Alternatively, amphipods dispersed northward colonizing groundwater in the glacial deposits left behind by the receding glaciers (Holsinger 1978; Lewis and Bowman 1981). Because the closest relative of *B. mucronatus* (*B. pseudomucronatus*) occurs further south in the unglaciated Ozark Plateaus, Holsinger (1986) considered the present-day distribution of *B. mucronatus* more likely reflected the latter scenario. *Bactrurus mucronatus* is the only species in the genus not generally restricted to karst, and it is instead adapted to life in saturated soils and underlying layers (Koenemann and Holsinger 2001). Because it is not restricted to karst, *B. mucronatus* was uniquely (unlike most of its congeners) suited to recolonize interstices of loosely consolidated, saturated drift and underlying strata as glaciers retreated, moving into areas formerly covered by the Wisconsin glacial ice sheet only after its retreat. Under this scenario, *B. mucronatus* populations in western and southern Illinois would represent much older populations, established at least 130,000 to 55,000 years ago (i.e., prior to the Illinoisian and Wisconsinan glacial episodes) and possibly much older (e.g., Miocene, 23.03 to 5.33 mya), while those in central Illinois and areas north and east would represent much younger populations derived from these older, southwestern populations. Under this scenario, the youngest populations of *B. mucronatus* would occur in northern Indiana, northern Ohio, and southern Michigan.

However, Koenemann and Holsinger (2001) suggested post-glacial range expansion may be less likely given the relatively short post-glacial time available for dispersal from the Ozark Plateaus or southwestern Illinois (19,000–10,000 ybp) and the presumed poor dispersal ability of *B. mucronatus*. Koenemann and Holsinger (2001) further argued against a post-glacial range expansion hypothesis by noting that the range of *B. brachycaudus* bisects the collective distributions of *B. mucronatus* and *B. pseudomucronatus*. *Bactrurus mucronatus* and *B. pseudomucronatus* are sister species (Koenemann and Holsinger 2001; Corrigan et al. 2014), with the latter occurring in karst terranes of the Ozark Plateaus. Koenemann and Holsinger (2001) hypothesized that *B. mucronatus* originated south of the maximum extent of Pleistocene glaciation, perhaps in southern Illinois and Missouri. As the glaciers receded, divergence of *B. mucronatus* and *B. pseudomucronatus* occurred. In this scenario, relict populations of *B. mucronatus* would be expected to occur between the gap between the distributions of each species that is now occupied by *B. brachycaudus*. Koenemann and Holsinger (2001) hypothesized further that a geographic distributional pattern associated with major post-glacial drainage basins would be expected in *B. mucronatus* under a post-glacial expansion hypothesis; however, such a pattern is notably absent. Koenemann and Holsinger (2001) also suggested that the occurrence of *B. brachycaudus* at several localities north of the maximum extent of glaciation "appear to be exceptional occurrences," indicating possible barriers to dispersal in glacial sediments in western Illinois. However, our new record 129 km northeast suggests that barriers to dispersal may not be as strong as suggested by Koenemann and Holsinger (2001).

Instead, Koenemann and Holsinger (2001) favored a subglacial refugia hypothesis whereby *B. mucronatus* survived *in situ* under episodic glaciation of the Wisconsin (55,000 to 12,500 ybp) and Illinois (190,000 to 130,000 ybp), moving vertically rather than horizontally into sand and gravel layers deposited by glaciers. While Koenemann and Holsinger (2001) stated that permafrost did not penetrate the ground deeper than 5 meters, allowing persistence of *B. mucronatus* in aqueous media below this level, it is possible that the Pleistocene glaciers were largely free of an underlying permafrost layer because the weight of the glaciers lowers the melting point of water, and temperate glaciers typically have subglacial flowing waters (Sharp 1988). Moreover, there is growing evidence that groundwater amphipods have persisted beneath glacial ice. At least six species of amphipods are thought to have persisted in subglacial refugia. *Stygobromus canadensis* occurs in a section of Castlegaurd Cave overlain by Mount Castleguard glaciers and the Columbian Icefields in Alberta, Canada (Holsinger 1980, 1981; Holsinger et al. 1983). Three other stygobromids occur in previously glaciated regions and are hypothesized to have persisted in subglacial refugia during the Pleistocene: *S. iowae* (Peck and Christiansen 1990), *S. putealis* (Peck and Christiansen 1990), and *S. tenuis tenuis* (Smith 1985). A species and family of Crangonyctoidea (family Crymostygidae), *Crymostygius thingvallensis* Kristjánsson & Svavarsson, 2004 from Iceland, and another crangonyctid, *Crangonyx islandicus* Svavarsson & Kristjánsson, 2006, also from Iceland, are thought to have persisted in subglacial refugia since the Pliocene when glaciers covered Iceland, from 2.6 mya until about 10,000–12,000 ybp (Kristjánsson and Svavarsson 2004, 2007; Svavarsson and Kristjánsson 2006; Kornobis et al. 2010, 2011). Groundwater amphipods in the genus *Niphargus* (family Niphargidae) likely persisted beneath glaciers during the Pleistocene in northern Europe (McInerney et al. 2014). Finally, experiments by Espinasa et al. (2015) demonstrate that the crangonyctid *Stygobromus allegheniensis* Holsinger, 1967 in the eastern United States can survive being frozen in ice, at least for a short duration.

A recent molecular study by Corrigan et al. (2014) sheds further light on the biogeography of *Bactrurus*. This study reconstructed the time-calibrated phylogeny of deep sea amphipods in North America using five loci but also included three species of *Bactrurus* as outgroup taxa: *B. brachycaudus*, *B. mucronatus*, and *B. pseudomucronatus* (Fig. 4). Divergence between *B. mucronatus* and *B. pseudomucronatus* was dated at 12–27 mya, while divergence between *B. brachycaudus* and *B. mucronatus* + *B. pseudomucronatus* was dated at 30–58 mya (Corrigan et al. 2014), well before glacial episodes of the Pleistocene. These three lineages were likely well established before the Pleistocene, and, consequently, divergence between *B. mucronatus* and *B. pseudomucronatus* was not driven or associated with Pleistocene glaciation, as suggested by Koenenmann and Holsinger (2001).

We find further support for the subglacial refugia hypothesis by examining the present-day distribution of *B. mucronatus* overlaid onto the pre-Pleistocene drainage pattern of the ancient Teays River Valley (Fig. 5). The distributions of all of the species within *Bactrurus*, with the possible exception of *B. brachycaudus*, appear well correlated with pre-Pleistocene river drainage patterns rather than contemporary drainages (Fig. 5). The timing of speciation within *Bactrurus* (Corrigan et al. 2014) also suggests that these species or their ancestors had well-established lineages during the Pliocene, when the Teays River flowed across what is now the Midwestern United States.

Two populations in Saline and Gallatin counties in southern Illinois occur south of the glacial limit (Figs 1 and 2, arrows D and E; Fig. 5). The presence of these populations, as well as the fact that non-karst populations are directly associated with glacial deposits, has been taken as evidence against *B. mucronatus* persisting in subglacial refugia during the Pleistocene (Holsinger 1981, 1986; Koenemann and Holsinger 2001). It is possible, however, that these populations reflect more recent dispersal along the Wabash River into karst following glacial advances during the Pleistocene. This view is further supported by collections in Indiana along the lower Wabash River basin that were unavailable to Koenemann and Holsinger (2001). Molecular studies presently underway should help elucidate the biogeography history of this species.

Bactrurus brachycaudus appears able to occupy habitats similar to those where *B. mucronatus* is found, at least in central Illinois. However, nearly all populations of this species outside of central Illinois are associated with caves or karst springs (see Koenemann and Holsinger 2001). One possible explanation is that the central Illinois populations formerly resided in solutionally enlarged conduits within carbonate rocks that were formerly exposed at the surface, or at least at the water table, during the Pliocene. Devonian limestones are present very near the surface in southern Champaign Co., Illinois (SSW of our new northeastern range extension for *B. brachycaudus*) and just to

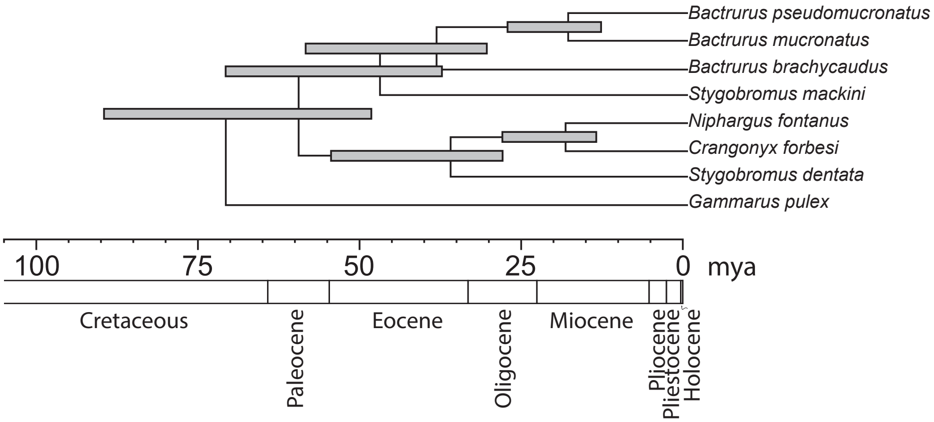

Figure 4. Groundwater amphipod relationships modified after the maximum clade credibility diagram of Corrigan et al. (2014) with 95% highest posterior density intervals shown as gray bars, and with additional details of geological timeline following Cohen et al. (2013).

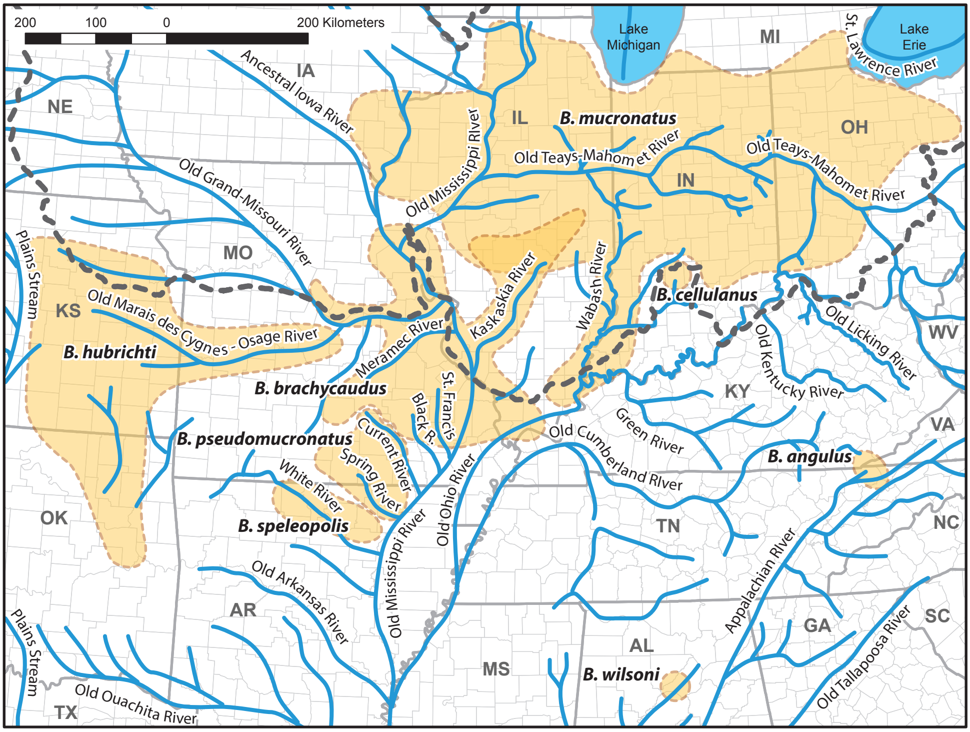

Figure 5. Species ranges for the genus *Bactrurus* (Amphipoda, Crangonyctidae) in relation to pre-Pleistocene rivers of the mid-western United States. Shaded areas with thin dashed lines represent species ranges. For *Bactrurus brachycaudus*, *Bactrurus hubrichti* and *Bactrurus mucronatus*, the range boundary has been interpreted in light of these rivers. Heavy dashed line is maximum extent of Pleistocene glaciation.

the south in Douglas Co., Illinois. Similarly, glacial deposits in Montgomery Co., Illinois, where one of the other two vexing localities of this species is located (Figs 1 and 2, arrow C), is underlain by carbonates of the Bond Formation (Willman et al. 1975, p. 196), which would have been exposed along river valleys during the Pliocene, when the Teays River was flowing westward through what is now central Illinois. It seems plausible, then, that this species, in addition to *B. mucronatus*, may have persisted throughout the Pleistocene in subglacial refugia.

The single Missouri population of *B. hubrichti* (Figs 1 and 2, arrow F) is interesting because it lies deep within the relatively continuous karst of the Missouri and Arkansas Ozark Plateaus developed in Ordovician dolomite. We confirmed the continued presence of this species from Miller Co., Missouri, where it had not been observed in nearly 75 years — since L. Hubricht collected seven specimens in August 1940 (Koenemann and Holsinger 2001). Remaining populations of *B. hubrichti* are associated with caves, springs, seeps, and wells in small disjunct carbonate outcrops, primarily in younger rocks of the Lower Permian Barnestone Formation of northeastern Oklahoma and eastern Kansas (Fig. 1). However, pre-Pleistocene river drainage patterns also may best explain the distribution of *B. hubrichti*, where the present-day distribution corresponds well with the old Marais des Cygnes and contemporary Osage rivers (Fig. 5). Other Ozark *Bactrurus* species have distribution patterns that match both current and Pliocene river drainage basins: *B. speleopolis* is found in the White River and Curia Creek watersheds in Arkansas, *B. pseudomucronatus* is found in the Spring, Eleven Point, and Current river watersheds (Fig. 5). These two species have non-overlapping ranges and also do not co-occur with the more widespread *B. brachycaudus*. *Bactrurus brachycaudus* presents a somewhat more confusing picture, but is associated with the Black River, St. Francis River, Meramec River and middle Mississippi River watersheds, perhaps extending up the Kaskaskia River watershed into central Illinois (Fig. 5).

Conservation assessments

Our conservation assessments indicate that five of the eight species of *Bactrurus* are at an elevated risk of extinction under IUCN Red List or NatureServe criteria (Table 1), with one species (*B. cellulanus*) already extinct. Three species (*B. angulus*, *B. speleopolis*, and *B. wilsoni*) are ranked as “Critically Imperiled” (G1) under NatureServe criteria primarily because of very restricted distributions and very few number of occurrences. IUCN Red List assessments of these three species also reveal that each species is threatened, however, *B. speleopolis* and *B. wilsoni* were ranked as “Vulnerable” under criterion D2 rather than at higher risk category. This is because although both species have very restricted distributions and are known from very few occurrences, there is no current evidence for continuing, observed, inferred, or projected decline in EOO, AOO, quality of habitat, or number of occurrences or subpopulations. Lack of evidence for declines in these factors also explains differences in threat categories between Nature-

Serve and IUCN Red List assessments for *B. pseudomucronatus* and *B. hubrichti*. Both species were assessed as "Vulnerable" (G3) under NatureServe criteria but were assessed at lower risk categories under IUCN Red List criteria. *Bactrurus brachycaudus* and *B. mucronatus* are two species at lowest risk of extinction according to both NatureServe and IUCN Red List assessments. Although populations of these species are not free from threats, both species are known from >100 occurrences, and there is no evidence that EOO or AOO has significantly declined.

Our NatureServe conservation ranks were generally very similar to previous assessments (Table 1; NatureServe 2015). *Bactrurus wilsoni* was previously assessed as "Imperiled-Critically Imperiled" (G1G2), but we downgraded the status rank to "Critically Imperiled" (G1) because inventory efforts of caves and springs in northern and central Alabama since 2002 have not resulted in the documentation of additional populations. *Bactrurus pseudomucronatus* was previously assessed as "Imperiled-Vulnerable" (G2G3). We upgraded the status rank to "Vulnerable" (G3) because of lower overall threat impacts, as most localities of this species occur on protected lands in rural areas that are predominantly forested. We downgraded the status rank of *B. hubrichti* from "Apparently Secure" (G4) to "Vulnerable" (G3). Although *B. hubrichti* has a large EOO, its distribution is likely fragmented, given the discontinuous extent of karst this species inhabits (Fig. 1). In addition, this species is still known from just 15 occurrences, as additional populations have not been reported in several years. Calculated status ranks were the same as previous assessments for all other species.

Threats

The most significant threat to all *Bactrurus* species is groundwater pollution, but the sources, scope, and potential severity of groundwater pollution varies among species and also among populations within a species. Impacts from groundwater pollution can be chronic, occurring over years to decades, or acute, on the order of hours or days. Common sources of groundwater pollution include septic system leachate, sewage, urban and storm water runoff, livestock waste, and pesticides and other chemicals used in agriculture and residential areas (lawns and landscaping). Termiticide treatment on the grounds around Jordan Hall on the campus of Indiana University in Bloomington, Indiana, in the 1960s resulted in the extirpation of all groundwater life at the type locality of *B. cellulanus* (Koenemann and Holsinger 2001; Lewis 2012, 2015). Populations associated with karst are particularly vulnerable to groundwater pollution, as karst aquifers often have low potential for auto-depuration and have a high probability of retention of contaminants (White 1988; Kačaroğlu 1999; Ford and Williams 2007).

Conversion of land for development, agriculture, and logging can lead to increased sedimentation and changes in local hydrology, which may degrade the quality of habitat or reduce the amount of habitat available. Additionally, groundwater extraction for various human needs can result in reduction or complete loss of amphipod habitat. An increasing threat to populations of *Bactrurus* in urban areas is the conversion of

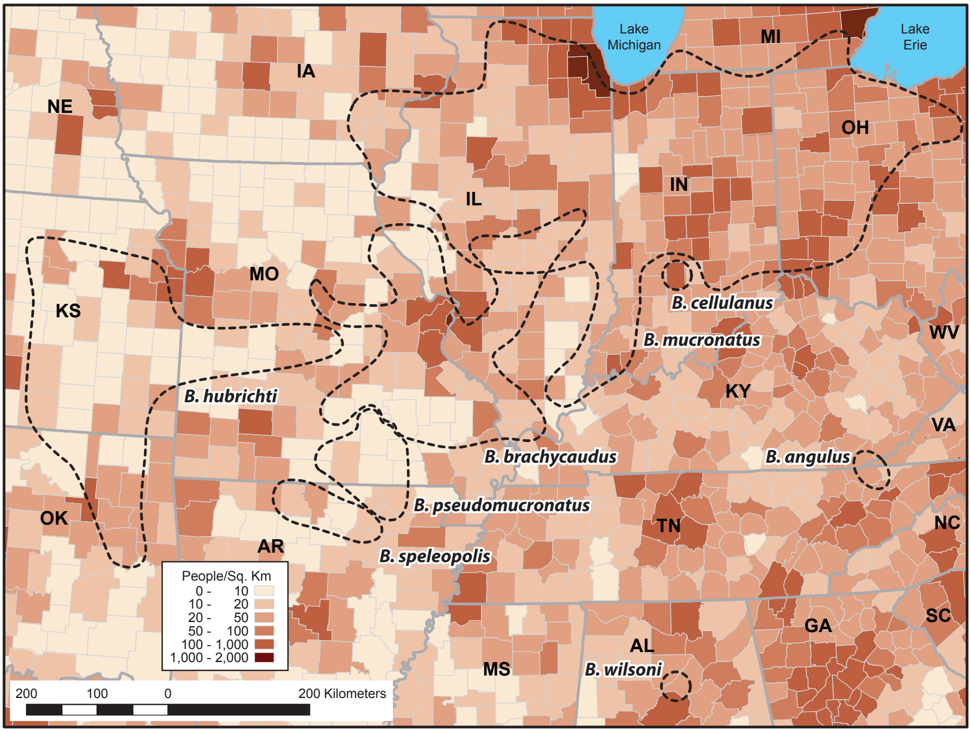

Figure 6. Human population density (2010 U.S. Census from the U.S. Census Bureau) by county with ranges for the eight *Bactrurus* species. For *Bactrurus brachycaudus*, *B. hubrichti*, and *B. mucronatus*, the range boundary has been interpreted as in Fig. 5.

land into impervious surfaces, such as roads, parking lots, and sidewalks. Impervious surfaces increase the speed and amount of storm water runoff leading to degradation of aquatic habitat through more rapid transport of contaminants and increased sediment load (Booth and Jackson 1997). Some populations of *B. brachycaudus* in the St. Louis, Missouri, metropolitan area and *B. mucronatus* in the Indianapolis, Indiana, metropolitan area occur where a significant (>30%) of land cover surrounding these populations has been converted to impervious surfaces, and these areas typically have high human population densities (Fig. 6).

Mining, natural gas, and oil drilling have impacted some populations of *Bactrurus*. At least one population of *B. brachycaudus* was extirpated due to complete removal of a cave system by limestone mining operations in Ste. Genevieve Co., Missouri. A population of *B. mucronatus* is known from a reclaimed limestone mine in La Salle Co., Illinois, but this population wasn't discovered until after mining operations ceased. Coal strip-mining potentially impacts populations of *B. mucronatus* in southwestern Indiana (Lewis 2015).

Over-collection for scientific purposes can reduce or possibly extirpate local populations. However, there is no evidence to suggest that any populations have been extirpated directly or indirectly due to scientific collection. Habitat disturbance caused by recreational cavers may pose a threat to some cave populations. *Bactrurus* are typically found underneath rocks or within interstices in gravel and cobble in pools and shallow streams in caves. Consequently, there is a risk of mortality caused by trampling with increased cave visitation, but data are lacking regarding whether increased recreational caving significantly impacts *Bactrurus* populations. Moreover, it is unclear whether most cave occurrences represent source or sink subpopulations. Source subpopulations of *Bactrurus* species primarily associated with karst may occur in epikarst, the zone below the soil layer but perched above caves where water percolates from the surface into caves through pores, joints, and fissures. Epikarst has been poorly sampled and studied, and underappreciated as important habitat for subterranean species until recently (Culver and Pipan 2014). At least 38 species of the crangonyctid genus *Stygobromus* are associated with epikarst in the United States (Culver et al. 2010; Culver and Pipan 2014).

Climate change is predicted to have significant impacts on the levels, quality, and sustainability of groundwater (Taylor et al. 2012; Treidel et al. 2012; Klove et al. 2014). Climate change is expected to impose changes in several important environmental variables that directly influence groundwater organisms and ecosystem processes (reviewed in Klove et al. 2014), including changes in water temperature, dissolved oxygen, recharge rates, altered hydrological regime, and groundwater levels, as well as groundwater quality (Earman and Dettinger 2011; Treidel et al. 2012). Many subterranean species may be particularly vulnerable to impacts of climate change, because of their unique habitat requirements, endemicity, adaptations, and often limited dispersal abilities. However, gaps in our understanding of distribution of groundwater organisms and groundwater ecosystem dynamics and services impair our ability to predict and manage species' responses to climate change.

Recommendations

Given results of our conservation assessments and available information on threats to populations, we offer several recommendations for study and management. First, studies are greatly needed to better ascertain the physical, chemical, and biological habitat variables that influence the survival of each species. These data are needed to better inform models of species distributions and responses to land use and climate change. Life history and demographic information are lacking for all *Bactrurus* species. Research is greatly needed to determine population sizes, generation time, reproductive cycles, life span, fecundity, sex ratios, and survivorship. Such data are needed to quantitatively predict the future status of populations and species (e.g., population viability analysis). In addition, little information exists on diet, diseases, parasites, and other basic life history traits. Such information would be useful in the management of individual species and groundwater ecosystems. Future efforts should focus on locating additional populations of *B. angulus*, *B. speleopolis*, and *B. wilsoni*. Inventory efforts may be aided by the development of species distribution models to predict where each species may potentially occur.

Groundwater recharge zones and flow patterns should be delineated for populations of species of greatest conservation concern (i.e., *B. angulus*, *B. speleopolis*, and *B. wilsoni*). Vulnerability mapping can then be conducted to estimate the risk and impacts of groundwater pollution to aid in land management decisions and protection of sensitive groundwater species. Studies are needed to determine the sources, nature, and extent of local threats to significant populations. Water quality should be regularly assessed at select sites to monitor for possible changes that might negatively impact populations. Recent molecular studies have discovered high levels of cryptic diversity within widely distributed stygobiotic morphospecies (Finston et al. 2007; Zaksek et al. 2009; Niemiller et al. 2012; Eme et al. 2013), which has important conservation and management implications (Niemiller et al. 2013a). Phylogeographic and species delimitation studies should be a priority to ascertain if any of the three widely distributed species (*B. brachycaudus*, *B. hubrichti*, and *B. muconatus*) are actually comprised of multiple morphologically cryptic, genetically distinct lineages. Finally, it is somewhat surprising that *B. angulus* (in Tennessee), *B. speleopolis*, and *B. wilsoni* are not included on state endangered and threatened species lists, as all three species were assessed as threatened under both NatureServe and IUCN Red List criteria. We recommend that the conservation status of these species be reevaluated in their respective states in light of our conservation assessments.

Conclusions

Subterranean amphipods in the genus *Bactrurus* are excellent candidates for addressing important questions in subterranean biogeography, as species in this genus occur in karst and non-karst habitats in both previously glaciated and non-glaciated regions in the central and eastern United States. Three species have extremely restricted distributions and are endemic to isolated karst areas. In contrast, three other species have exceptionally large distributions for subterranean fauna, which raises an important question of whether these species are presumably good dispersers or consist of assemblages of cryptic species with much smaller distributions. We suggest that the current distribution of *Bactrurus* has been more influenced by drainage patterns and other events dating before the Pleistocene than to post-glacial dispersal. Five of eight *Bactrurus* species were found to be at an elevated risk of extinction under IUCN Red List or NatureServe criteria, with one species already extinct (*B. cellulanus*). We identified 17 threats that either currently or may affect populations of *Bactrurus* in the near future. Groundwater pollution represents the most insidious threat to all *Bactrurus* species, although the sources, scope, and potential severity vary among species. Climate change may impact *Bactrurus* and other groundwater species in the coming decades, particularly endemic taxa with small distributions, such as *B. angulus* and *B. wilsoni*. However, knowledge gaps in our understanding of distributions of groundwater taxa and ecosystem dynamics impair our ability to model and effectively manage species' responses to climate change and other threats. Research is also needed to better understand the life history, ecology, and demography of *Bactrurus*. Nonetheless, our conservation assessments strongly suggest that several species warrant consideration for state or federal listing as protected species and highlight the need for new conservation assessments (in the case of IUCN Red List) or reassessments (in the case of NatureServe) for groundwater and other subterranean species.

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The first survey on harvestmen in Brazilian artificial cavities, with notes on distribution and natural history

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Abstract

Several species of harvestmen occur in natural subterranean cavities using these habitats as shelters and sites of oviposition. Many species have evolved in these environments, thus becoming cave-dwellers. In a few cases harvestmen have been reported in artificial cavities (mines), but without details about their distribution or natural history. Based on faunal inventories carried out in 111 artificial cavities in the state of Minas Gerais, Brazil, this work aimed to register species of harvestmen that are associated with these artificial cavities. Seventeen species were found in 12 municipalities, in addition to new occurrences for 14 of these species and some new behavioral notes. Two undescribed species were also recorded. This study highlights that artificial cavities may be used as shelters by harvestmen, mainly in human-modified landscapes.

Keywords

Opiliones, artificial subterranean cavities, mines, new records, aggregation, Neotropical, Brazil

Introduction

Subterranean cavities can be formed by the action of environmental agents (e.g. water action and eruptions) (Allred and Allred 1997, Culver and Pipan 2009) or anthropogenic activities (Peck 1988, Bernardi et al. 2010, 2011, Isaia et al. 2011). Some natural cavities (paleo-burrows) were also made by the activity of large mammals extinct during the Pleistocene (Buchmann et al. 2009).

Natural subterranean cavities (karstic caves) are originated mainly by the dissolution of rock through water action, thus forming galleries (Ford and Williams 2007). In contrast, artificial subterranean cavities are generated by human action with the intention of extracting minerals or metals of economic value, for the construction of structures such as access tunnels or even for military purposes (Peck 1988, Bernardi et al. 2010, 2011, Isaia et al. 2011).

Although natural and artificial cavities present different genesis, these habitats may have similar environmental characteristics, which are primarily determined by the permanent absence of light, limited food, constantly high relative humidity and thermic stability (Ferreira 2004, Culver and Pipan 2009, Bernardi et al. 2010, 2011, Isaia et al. 2011). These environmental similarities generally enable a similar faunistic composition in these two systems (natural and artificial cavities) (Peck 1988, Ferreira 2004, Bernardi et al. 2010, 2011) in the same region. In this context, several taxa are common to both habitats, such as amphibians, arachnids, bats, crickets, moths and a large number of other invertebrates (Peck 1988, Gnaspini and Trajano 1994, Ferreira 2004, Bernardi et al. 2010, 2011, Isaia et al. 2011, Rosa and Penado 2013, Kurta and Smith 2014, Ueti et al. 2015). Among these groups, the members of the order Opiliones are also a common component inside natural and artificial cavities.

The order Opiliones Sundevall, 1833 is divided into four extant suborders, comprising 49 families and 6519 species worldwide (Kury 2011), representing the third most diverse group in the class Arachnida. In Brazil, there are 855 described species (Kury 2003). Epigean species of this order can be found in the soil, moss, leaf litter, under rocks, tree barks, stone walls, under the vegetation and in caves (Machado et al. 2007). The vast majority of species is nocturnal and need high levels of humidity to survive (Machado et al. 2007). This last condition is generally found both in natural (Culver and Pipan 2009) and artificial cavities (Peck 1988, Ferreira 2004, Bernardi et al. 2010, 2011, Isaia et al. 2011). Many harvestmen species use caves as shelters, where they may lay eggs and raise offsprings, and are often found in large aggregations on the cave walls. These species are classified as trogloxenes (e.g. *Mitogoniella indistincta* Mello-Leitão, 1936, *Serracutisoma spelaeum* (Mello-Leitão, 1933)). There are also troglophile species that may remain inside the cavities to complete their life cycle (e.g. "*Daguerreia inermis*" Soares & Soares, 1947, *Pararezendesius luridus* H. Soares, 1972). The two previously conditions may be facultative, while the troglobitic species are exclusively found in caves and generally present morphological modifications, such as eye reduction, depigmentation and elongation of legs, which are considered adaptations to subterranean conditions (e.g. *Spinopilar moria* Kury & González, 2008, *Giupponia chagasi* González & Kury, 2002).

It has been shown by several studies that caves are natural shelters for many epigean harvestmen species (Ferreira et al. 2005, DaSilva and Gnaspini 2010, Chelini et al. 2011). However, few studies registered the presence of these arthropods in artificial cavities in Brazil (Gnaspini and Trajano 1994, Bernardi et al. 2010, 2011, Ázara et al. 2013) and worldwide (Holmberg et al. 1984, Angerilli and Holmberg 1986, Moseley and Hebda 2001, Isaia et al. 2011). This is probably due to the small number of faunal surveys conducted in these environments when compared to natural caves (Trajano and Bichuette 2010, Reboleira et al. 2011, Silva et al. 2011a, Silva et al. 2011b, Iniesta et al. 2012, Pellegrini and Ferreira 2012, Simões et al. 2014, Silva et al. 2014, Silva and Ferreira 2015).

Considering the small number of faunal surveys performed in artificial subterranean cavities, the present study aimed to: (i) register harvestmen species associated with artificial cavities in Minas Gerais state, Brazil and (ii) present data regarding the species distribution, new occurrences and presence of aggregations in these environments.

Methods

A total of 111 subterranean artificial cavities were sampled in 13 municipalities in the state of Minas Gerais, Southeast Brazil. Each municipality is shown in Figure 1 indicated by a letter in brackets: Alagoa (a, 2 cavities), Ataléia (b, 1 cavity), Caeté (c, 4 cavities), Caraí (d, 17 cavities), Mariana (e, 11 cavities), Mateus Leme (f, 4 cavities), Medina (g, 13 cavities), Nova Lima (h, 1 cavity), Novo Oriente de Minas (i, 7 cavities), Ouro Preto (j, 4 cavities), Padre Paraíso (k, 30 cavities), São José da Safira (l, 16 cavities) and Vazante (m, 1 cavity) (Figure 1). Pictures from some of the surveyed cavities are displayed in Figure 2. This study was based on data obtained through direct observations from the fieldwork and on a literature review to search for additional information about the records of harvestmen species.

Among the sampled municipalities, Alagoa, Ataléia, Caeté, Caraí, Mariana, Mateus Leme, Medina, Nova Lima, Novo Oriente de Minas, Ouro Preto, Padre Paraíso and São José da Safira are in the Atlantic Forest biome; Caeté and Nova Lima are situated in the transition between Cerrado and Atlantic Forest, while Vazante is located in the Cerrado (IBGE 2004) (Figure 1). However, most of the cavities were located in modified landscapes of these biomes, which were mainly surrounded by pastures. All collections were performed between April 2008 and November 2009.

The original names of the artificial cavities, most of them given by local residents, were maintained since there is no agency in Brazil responsible for the registration of these habitats. Besides, many names refer to the location of the cavities, what might be a helpful tool to find the locality. Since all the artificial cavities of this study are mines we will use further the term mines.

The specimens found in the mines were sampled with the aid of tweezers, after a detailed and extensive search. In order to assess the position of the species in the cavities, each observed individual was plotted in a schematic map of the mine according to the methodology proposed by Ferreira (2004). After the survey, information about the abundance

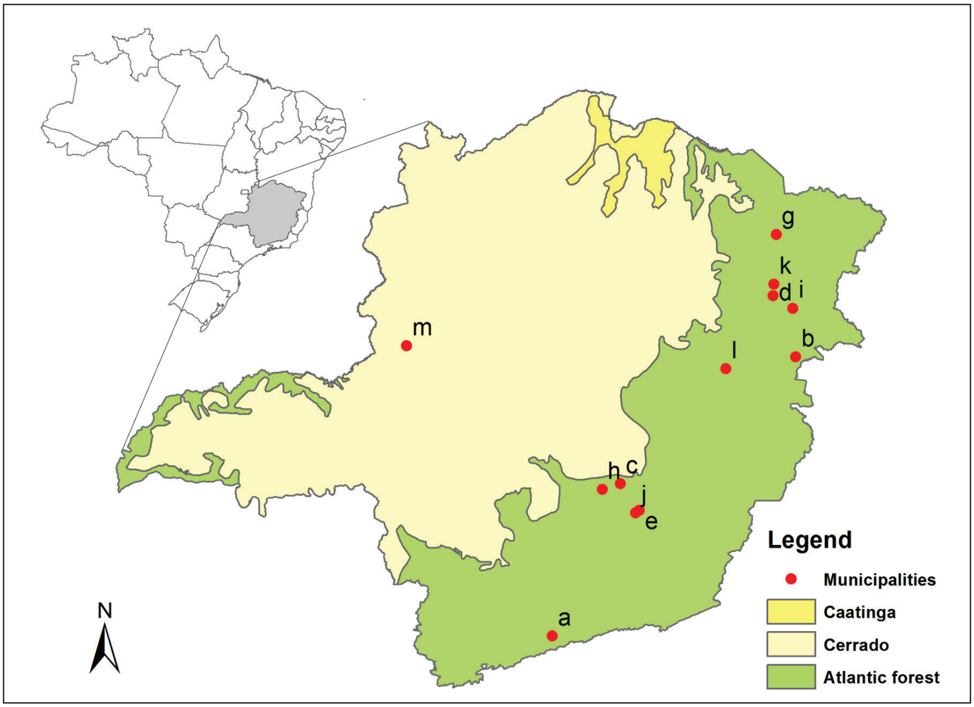

Figure 1. Map of the state of Minas Gerais, Brazil, showing the biomes and municipalities in which the artificial cavities are situated: **a** Alagoa **b** Ataléia **c** Caeté **d** Caraí **e** Mariana **f** Mateus Leme **g** Medina **h** Nova Lima **i** Novo Oriente de Minas **j** Ouro Preto **k** Padre Paraíso **l** São José da Safira **m** Vazante.

and distribution of each species were obtained. In addition, the presence of loose aggregations was recorded, which were represented by groups of three or more individuals with their bodies between 0 to 5 cm apart from each other with their legs overlapping, such as proposed by Machado et al. (2000) and revised by Machado and Macías-Ordóñez (2007).

Individuals were identified whenever possible. In cases of nymphs, females and undescribed species, the individuals were just separated into morphospecies. However these morphospecies were theoretically considered as species. When there was more than one morphospecies in the same genus, they were separated using correlative numbers.

To determine the extension of the aphotic zone, a luximeter was positioned 1.2 m above the ground with its reception cell directed to the cavity entrance. The device was then conducted from the entrance to the interior of the subterranean system. The location where the luximeter presented luminosity equal to zero was considered the transition between the aphotic and the photic zone.

Specimens were deposited in the Collection of Subterranean Invertebrates of Lavras (ISLA) at the Federal University of Lavras, Lavras, Minas Gerais, and in the National Museum of the Federal University of Rio de Janeiro (MNRJ), Rio de Janeiro, both in Brazil.

The occurrences of harvestmen species are indicated in alphabetical order in the results with the abundance of each species in each locality indicated in parentheses.

Figure 2. Artificial cavities in: **A**, **B**, **C** Alagoa **D** and **E** Padre Paraíso **F** Caraí **G** Mariana **H** São José da Safira **I** Caeté **J** Mariana.

Results

From all the 111 sampled mines 52 contained harvestmen distributed in 12 municipalities (Figure 1). Overall 6,066 individuals were observed, which belong to 17 species, 10 genera, 7 subfamilies and 4 families. We found different distribution and abundance patterns: from species with just one individual (e.g. *Paratricommatus* sp. 1, *Goniosoma carum*, *Pseudopucrolia mutica* and *Eusarcus* sp. 2) to broadly distributed and abundant species (e.g. *Mitogoniella indistincta*) presenting more than 500 individuals in 4 municipalities and 21 mines (Table 1).

The following species and genera were new occurrences for the state of Minas Gerais (letters in brackets correspond to the municipality): *Abaetetuba bahiensis* (g), *Gryne perlata* (d, g, k), *Paratricommatus* sp. 1 (c), *Spinopilar* sp. 1 (f), *Goniosoma carum* (j), *Goniosoma vatrax* (h), *Liogonyleptoides inermis* (i), *Pseudopucrolia mutica* (d), *Longiperna trembao* (c, e), *Mitobatula* sp. 1 (a), *Eusarcus aduncus* (c, l), *Eusarcus cavernicola* (m), *Eusarcus* sp. 1 (e, l), *Eusarcus* sp. 2 (f) and *Planiphalangodus* sp. 1 (a). Additionally, this study improved the information about the distribution of some species, such as *Goniosoma vatrax* with about 40 km, and *Gryne perlata* with 1000 km. It was also registered for the first time an aggregation behavior for *Abaetetuba bahiensis* and *Gryne perlata*, besides the record of two undescribed species.

Table 1. Distribution of harvestmen species in the municipalities and mines with information about their total abundance and presence (P)/absence (A) in aphotic and/or photic zones. *The abbreviated name of the species will be used to refer to each species in Table 2. The symbol "-" means the absence of data. N = abundance.

Abreviated name of the species	Species	N	Family	Municipality	Mine	Aphotic zone	Photic zone
Ab. b.	*Abaetetuba bahiensis* (Mello-Leitão, 1931)	66	Sclerosomatidae	g	4	A	P
Gr. p.	*Gryne perlata* Mello-Leitão, 1936	5298	Cosmetidae	d, g, k	11	A	P
Pa. 1	*Paratricommatus* sp. 1	1	Cryptogeobiidae	c	1	-	-
Sp. 1	*Spinopilar* sp. 1	3	Cryptogeobiidae	f	1	-	-
Go. c.	*Goniosoma carum* (Mello-Leitão, 1936)	1	Gonyleptidae	j	1	-	-
Go. v.	*Goniosoma vatrax* Koch, 1848	14	Gonyleptidae	e, h	5	P	P
Mi. i.	*Mitogoniella indistincta* Mello-Leitão, 1936	555	Gonyleptidae	c, e, j, l	21	P	P
Mi. m.	*Mitogoniella mucuri* Ázara et al., 2013	12	Gonyleptidae	a, d	2	P	P
Li. i.	*Liogonyleptoides inermis* (Mello-Leitão, 1922)	4	Gonyleptidae	i	1	A	P
Ps. m.	*Pseudopucrolia mutica* (Perty, 1833)	1	Gonyleptidae	d	1	A	P
Lo. t.	*Longiperna trembao* Pinto-da-Rocha & Bragagnolo, 2010	26	Gonyleptidae	c, e, j	5	P	P
Mi. 1	*Mitobatula* sp. 1	22	Gonyleptidae	a	2	P	P
Eu. a.	*Eusarcus aduncus* (Mello-Leitão, 1942)	24	Gonyleptidae	c, l	7	P	P
Eu. c.	*Eusarcus cavernicola* Hara & Pinto-da-Rocha, 2010	23	Gonyleptidae	m	1	P	A
Eu. 1	*Eusarcus* sp. 1	14	Gonyleptidae	e, j, l	6	P	P
Eu. 2	*Eusarcus* sp. 2	1	Gonyleptidae	f	1	-	-
Pl. 1	*Planiphalangodus* sp. 1	1	Gonyleptidae	a	1	P	A

Table 2. List of mines containing harvestmen, with the geographic coordinates (in decimal degrees, DATUM WGS 84), age (in years), use (not in use = D., daily tourism = D.T., mineral extraction = M.E., scientific experiment = S.E., sparse tourism = S.T., sparse visitation = S.V., urban zone = U.Z.), type of the surrounding vegetation/rock (Atlantic Forest = A.F., *Eucalyptus* forest = E., pasture = P., ferriferous formations = R.F.) on a 30 m (S. 30 m) and 250 m radius (S. 250 m), extension and identified harvestmen species (see Table 1 for species abbreviations).

Municipality	Mine	Latitude	Longitude	Age	Usage	S. 30 m	S. 250 m	Extension	Species
Alagoa	Mina da Companhia	-22.17812	-44.70546	> 100	S.T.	A.F.	P. - A.F.	46	*Mi.* 1, *Pl.* 1

Municipality	Mine	Latitude	Longitude	Age	Usage	S. 30 m	S. 250 m	Extension	Species
Alagoa	Túnel do Garrafão	-22.18221	-44.71956	> 100	S.T.	A.F.	P. - A.F.	73	*Mi. m.*, *Mi.* 1
Caeté	Mina do Morro Vermelho I	-19.96525	-43.71271	< 30	S.V.	E.	P. - E.	76	*Mi. i.*
Caeté	Mina do Morro Vermelho II	-19.96520	-43.71364	< 30	S.V.	E.	P. - E.	12	*Pa. 1*, *Eu. a.*
Caeté	Mina do Morro Vermelho III	-19.96538	-43.68757	< 30	S.V.	E.	P. - E.	60	*Mi. i.*, *Mi.* 1
Caeté	Mina dos Matarelli	-19.94719	-43.74017	< 50	D.	E.	P. - E - A.F.	62	*Lo. t.*
Caraí	Túnel da Br116	-17.22967	-41.49569	< 5	D.	P.	P.	8	*Ps. m.*
Caraí	Túnel do Noel I	-17.11623	-41.51527	< 5	M.E.	P.	P.	69	*Gr. p.*
Caraí	Túnel do Noel II	-17.11727	-41.51627	< 5	M.E.	P.	P.	38	*Gr. p.*
Caraí	Túnel da Fazenda Cilindro I	-17.11920	-41.32715	< 20	D.	P.	P.	10.2	*Mi. m.*
Mariana	Mina dos Canelas Casa	-20.35245	-43.43990	> 100	S.V.	R.F.	P.	49	*Go. v.*, *Eu.* 1
Mariana	Minas dos Canelas VI	-20.35315	-43.44090	> 100	S.V.	R.F.	R.F.	68	*Go. v.*
Mariana	Mina dos Canelas VII	-20.54384	-43.81015	> 100	S.V.	R.F.	R.F.	229	*Go. v.*, *Lo. t.*, *Eu.* 1
Mariana	Mina dos Canelas XI	-20.35256	-43.43936	> 100	S.V.	R.F.	R.F.	68	*Go. v.*, *Mi. i.*
Mariana	Mina dos Canelas XII	-20.35377	-43.43932	> 100	S.V.	R.F.	R.F.	98	*Go. v.*, *Mi. i.*
Mariana	Mina do Meio do Mato	-20.34518	-43.44805	> 100	S.V.	R.F.	R.F.	28	*Mi. i.*
Mariana	Mina da Cachoeira	-20.34507	-43.44678	> 100	S.V.	R.F.	R.F.	26	*Mi. i.*, *Eu.* 1
Mateus Leme	Mina do Aqueduto I	-19.95938	-44.42231	> 100	S.V.	R.F.	R.F.	11	*Eu.* 2
Mateus Leme	Mina do Aqueduto II	19.959223	-44.42196	> 100	S.V.	R.F.	R.F.	8	*Sp.* 1
Medina	Mina da Fazenda do Sol V	-16.34362	-41.45138	< 30	D.	P.	P.	27	*Ab. b.*
Medina	Mina da Fazenda Serra Azul I	-16.20885	-41.47934	< 30	D.	P.	P.	14	*Gr. p.*
Medina	Mina da Fazenda Serra Azul II	-16.21157	-41.47938	< 30	D.	P.	P.	74	*Gr. p.*
Medina	Mina do Bloco I	-16.21425	-41.46832	< 30	D.	P.	P.	126	*Ab. b.*, *Gr. p.*
Medina	Mina do Bloco II	-16.21610	-41.46872	< 30	D.	P.	P.	56	*Ab. b.*, 2
Medina	Mina do Bloco III	-16.21460	-41.46815	< 30	D.	P.	P.	31	*Ab. b.*, 2
Nova Lima	Mina de Capão Xavier	-20.0469	-43.9798	<5	S.C/ S.V.	R.F.	R.F>	15	*Go. v.*

Municipality	**Mine**	**Latitude**	**Longitude**	**Age**	**Usage**	**S. 30 m**	**S. 250 m**	**Extension**	**Species**
Novo Oriente de Minas	Mina do João Bufinha	-17.4137	-41.2097	-	S.V.	P.	P.	-	*Li. i.*
Ouro Preto	Mina da Volta do Córrego	-20.37922	-43.51066	> 100	S.V.	A.F.	A.F. - P.	22	*Go. c.*
Ouro Preto	Mina de Vila Rica	-20.38757	-43.49346	> 100	D.T.	U.Z.	U.Z.	96	*Lo. t.*
Ouro Preto	Mina do Chico Rei	-20.38608	-43.49930	> 100	D.T.	U.Z.	U.Z.	157	*Mi. i.*, *Lo. t.*, *Eu.* 1
Ouro Preto	Mina Velha	-20.38838	-43.49163	> 100	D.T.	U.Z.	U.Z.	174	*Mi. i.*
Padre Paraiso	Túnel dos Meninos I	-17.06916	-41.46849	< 20	D.	P.	P.	42	*Gr. p.*
Padre Paraiso	Túnel dos Meninos II	-17.06856	-41.46903	< 20	M.E.	P.	P.	29.8	*Gr. p.*
Padre Paraíso	Túnel do Hotel Entre Vales I	-17.06380	-41.48198	< 20	D.	P.	P. - A.F.	12.5	*Gr. p.*
Padre Paraíso	Túnel do Hotel Entre Vales II	-17.06380	-41.48198	< 20	D.	P.	P. - A.F.	39	*Gr. p.*
São José da Safira	Mina do Chiá Donizete I	-18.29455	-42.18781	< 50	D.	A.F.	A.F.	72	*Mi. i.*, *Eu. a.*
São José da Safira	Mina do Chiá Donizete II	-18.29064	-42.18758	< 50	M.E.	A.F.	A.F.	68	*Mi. i.*, *Eu. a.*
São José da Safira	Mina do Chiá Donizete III	-18.29163	-42.18846	< 50	D.	A.F.	A.F.	15.5	*Mi. i.*, *Eu. a.*
São José da Safira	Mina do Milto Godinho I	-18.29614	-42.18276	< 50	M.E.	P.	P.	33	*Eu. a.*
São José da Safira	Mina do Milto Godinho II	-18.29631	-42.18286	< 50	D.	P.	P.	12	*Mi. i.*
São José da Safira	Mina do Milto Godinho III	-18.29639	-42.18329	< 50	D.	P.	P.	52	*Mi. i.*, *Eu.* 1
São José da Safira	Mina do Milto Godinho IV	-18.29598	-42.18292	< 50	M.E.	P.	P.	72	*Mi. i.*, *Eu. a.*
São José da Safira	Mina do Milto Godinho VII	-18.29697	-42.18121	< 50	D.	P.	P.	37	*Mi. i.*
São José da Safira	Mina do Milto Godinho VIII	-18.29447	-42.18075	< 50	D.	P.	P.	84	*Mi. i.*, *Eu. a.*
São José da Safira	Túnel do Milto Godinho IX	-18.29395	-42.18040	< 50	D.	A.F.	P. - A.F.	12	*Mi. i.*
São José da Safira	Túnel do Milto Godinho X	-18.29268	-42.18421	< 50	D.	A.F.	P. - A.F.	67.6	*Mi. i.*
São José da Safira	Túnel do Milto Godinho XI	-18.29268	-42.18421	< 50	D.	A.F.	P. - A.F.	31	*Mi. i.*
São José da Safira	Túnel do Milto Godinho XII	-18.29292	-42.18485	< 50	D.	A.F.	P. - A.F.	48.5	*Mi. i.*, *Eu.* 1
São José da Safira	Túnel do Milto Godinho XIII	-18.29250	-42.18418	< 50	D.	A.F.	P. - A.F.	100.3	*Mi. i.*
Vazante	Mina da Vmetais	-17.95928	-46.82371	< 30	S.V.	P.	P.	600	*Eu. c.*

The mines with the highest number of species were Mina dos Canelas VII in Mariana, and Mina Turística do Chico Rei in Ouro Preto, each with 3 species (Table 2). All other cavities had only 1 or 2 species.

Species from mines with behavioral observations

Suborder Eupnoi Hansen & Sørensen, 1904
Family Sclerosomatidae Simon, 1879
Gagrellinae Thorell 1889

Abaetetuba bahiensis (Mello-Leitão, 1931)

Previously known distribution. Bahia: Ilhéus (Mello-Leitão 1931), Itamajarú, Juçari; Espírito Santo: Colatina (Tourinho-Davis 2004).

Examined material. *Medina*: 1 female (ISLA 16035) from Mina da Fazenda do Sol V (50), 08/IV/2009; 1 male (ISLA 16048) from Mina do Bloco I (8), 14/VII/2009; 1 male (MNRJ 2336) from Mina do Bloco II (5), 18/VII/2009; 1 male (ISLA 16030) from Mina do Bloco III (3), 14/VII/2009, new record for locality.

This species was observed forming aggregations of 8, 19 and 26 individuals at a maximum distance of 5 meters from the entrance representing the first record of an aggregation behavior for this species. In other cavities, sparsely distributed individuals were observed, but always located in the photic zone no more than 10 meters from the entrance. These records represent a new occurrence of this species for this municipality, thus expanding its distribution with 250 km from the southwest of Juçari, Bahia state.

Suborder Laniatores Thorell, 1876
Cosmetidae Koch, 1839
Discosomaticinae Pickard-Cambridge, 1905

Gryne perlata Mello-Leitão, 1936

Figure 3A–B

Previously known distribution. Pernambuco: municipality not specified (Mello-Leitão 1936).

Examined material. *Caraí*: 1 male (ISLA 16033) from Túnel do Noel I (1), 13/VII/2008; 1 female (MNRJ 2270) from Túnel do Noel II (3), 13/VII/2008; *Medina*: 2 males and 1 female (ISLA 16025) from Mina da Fazenda Serra Azul I (2400), 16/VII/2009; 1 male (ISLA 16036) from Mina da Fazenda Serra Azul II (2420), 16/VII/2009; 1 male (ISLA 16041) from Mina do Bloco I (28), Mina do Bloco II (218), 14/VII/2009, 1 male (ISLA 16029) from Mina do Bloco III (4), 14/VII/2009. *Padre Paraíso*: 1 male (ISLA 16037) from Túnel dos Meninos I (148), 12/VII/2008; 2 males

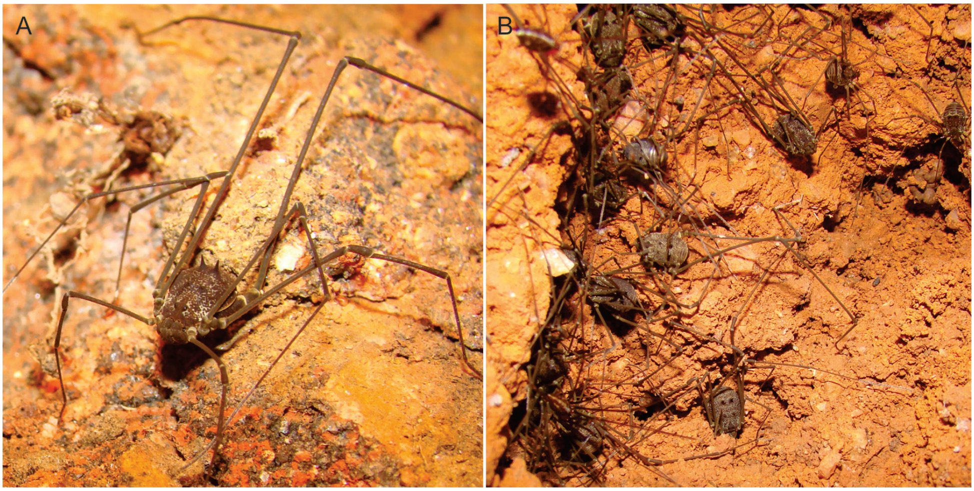

Figure 3. *Gryne perlata* **A** Individual inside the mine Túnel dos Meninos II, Padre Paraíso **B** aggregation of individuals in the same cavity.

and 1 female (ISLA 16028) from Túnel dos Meninos II (49), 12/VII/2008; 2 males and 1 female (ISLA 16031) from Túnel do Hotel Entre Vales I (9), 22/VII/2008; 1 male (ISLA 16034) from Túnel do Hotel Entre Vales II (18), 22/VII/2008, new record for state and localities.

This species was observed forming aggregations of 28, 45, 49, 103, 200, 400 and 2200 individuals at the entrance of seven cavities at a maximum distance of 5 m from the entrance. This was the first record of an aggregation behavior for this species. In other cavities, sparsely distributed individuals were also recorded, but always located in the photic zone at a maximum distance of 10 m from the entrance. During one of the fieldtrips, three aggregations with about 1000 individuals were observed. These aggregations were found in the epigean environment, next to the mine Túnel do Hotel Entre Vales I in holes and cracks in a ravine of exposed soil. Such shelters had a longitudinal form of ~ 80 cm in depth, 20 cm width and 1.5 cm height. The three municipalities represent new occurrences for this species, expanding its distribution ~ 1000 km from the south of Pernambuco state.

Family Cryptogeobiidae Kury, 2014

Paratricommatus sp. 1

Previously known distribution of the genus. Alto Paraná (Paraguai), Espírito Santo, Rio de Janeiro and São Paulo (Mello-Leitão 1940, Piza Jr 1943, H. Soares 1945, 1966, Soares and Soares 1946, 1954, 1985, H. Soares 1966, Kury 2014).

Examined material. *Caeté*: 1 female (MNRJ 2266) from Mina do Morro Vermelho II (1), 19/VI/2009, new record for state and locality.

Only one female was found. This is a new occurrence in the municipality, expanding the genus distribution with about 300 km from the southwest of Colatina, Espírito Santo state.

Spinopilar sp. 1

Previously known distribution of the genus. Alto Paraná (Paraguai), Argentina (province not specified), Espírito Santo, Minas Gerais, Rio de Janeiro, Santa Catarina (Sørensen 1932, Mello-Leitão 1940, Soares and Soares 1946, Roewer 1949, Soares 1972, Soares et al. 1985, Kury 1992, Acosta and Maury 1998, Kury and Pérez-González 2008).

Examined material. *Mateus Leme*: 1 female (MNRJ 2263) from Mina do Aqueduto II (3), 05/IV/2008, new record for locality.

Only one female was found and Mateus Leme corresponds to a new occurrence for the genus.

Family Gonyleptidae Sundevall, 1833
Goniosomatinae Mello-Leitão, 1935

Goniosoma carum (Mello-Leitão, 1936)

Previous known distribution. Minas Gerais: Viçosa (Mello-Leitão 1936).

Examined material. *Ouro Preto*: 1 male (ISLA 1468) from Mina da Volta do Córrego (1), 13/VI/2009, new record for locality.

Ouro Preto corresponds to a new occurrence for this species, expanding its distributions with about 100 km from the northwest of Viçosa, Minas Gerais state.

Goniosoma vatrax Koch, 1848
Figure 4D

Previously known distribution. Minas Gerais: Catas Altas (DaSilva and Gnaspini 2010), Ouro Preto (Mello-Leitão 1932) and Santa Bárbara (Mello-Leitão 1936).

Examined material. *Mariana*: 1 male (ISLA 16019) from Mina dos Canelas Casa (4), 01/V/2009; 1 male (MNRJ 2339) from Minas dos Canelas VI (3), 02/V/2009; 1 female (ISLA 16020) from Mina dos Canelas VII (3), 11/VI/2009; 1 male (ISLA 9573) from Mina dos Canelas XI (2), 11/VI/2009; 1 male (ISLA 16047) from Mina dos Canelas XII (2), 11/VI/2009 (Bernardi et al. 2011); *Nova Lima*: 1 male (ISLA 160) from Mina de Capão Xavier (15), 10/V/2009, new record for locality.

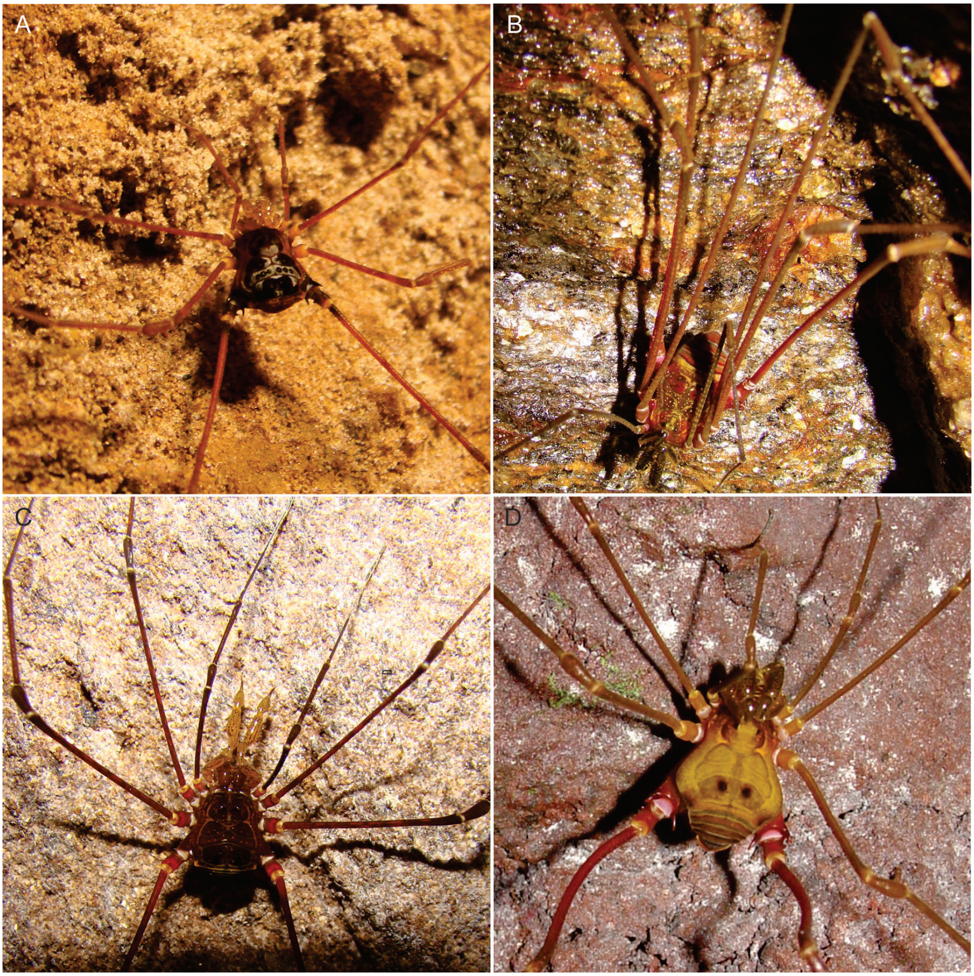

Figure 4. Individuals inside the artificial cavities **A** *Longiperna trembao* (Caeté: Mina dos Matarelli) **B** *Mitogoniella mucuri* (Alagoa: Túnel do Garrafão) **C** *Mitobatula* sp. 1 (Alagoa: Mina da Companhia) **D** *Goniosoma vatrax* (Nova Lima: Mina de Capão Xavier).

Sparse individuals were registered in the photic and aphotic zones of the mines at a maximum distance of 45 meters from the entrance. Nova Lima corresponds to a new occurrence for this species, expanding its distribution with about 40 km from the east of Santa Bárbara, Minas Gerais. However, the Mina de Capão Xavier was destroyed by the expansion of mining activities, and its fauna was translocated to another artificial mine constructed in the same region of the original one (Ferreira, R.L. pers. observation).

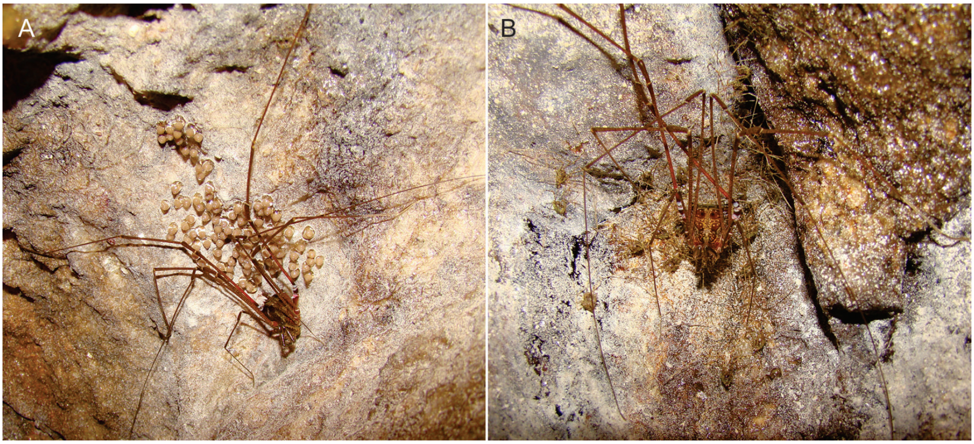

Figure 5. *Mitogoniella mucuri* inside the Túnel do Garrafão (Alagoa) **A** Female guarding eggs on the wall of the mine **B** Female guarding immatures on the wall of the mine.

Mitogoniella indistincta Mello-Leitão, 1936

Figure 5A–B

Previously known distribution. Minas Gerais: Alto Caparaó, Baependi, Barão de Cocáis (Ázara et al. 2013), Brumadinho (DaSilva and Gnaspini 2010), Caeté (Ázara et al. 2013), Catas Altas (DaSilva and Gnaspini 2010), Itabirito, Itambé do Mato Dentro (Ázara et al. 2013), Jaboticatubas (DaSilva and Gnaspini 2010), Lima Duarte, Mariana, Minduri, Ouro Branco, Ouro Preto, Prados (Ázara et al. 2013), Santa Bárbara (Mello-Leitão 1936), Santana do Riacho (DaSilva and Gnaspini 2010).

Examined material. *Caeté*: 1 female (ISLA 16038) from Mina do Morro Vermelho I (6), 17/VI/2009; 1 male (MNRJ 2337) from Mina do Morro Vermelho III (6), 17/VI/2009. *Mariana*: 1 male (ISLA 16057) from Mina dos Canelas XI (12), 11/VI/2009; 1 male (ISLA 1491) from Mina dos Canelas XII (6), 11/VI/2009; 1 male (ISLA 16022) from Mina do Meio do Mato (5), 12/VI/2009; 1 male (ISLA 16023) from Mina da Cachoeira (3), 12/VI/2009 (Ázara et al. 2013). *Ouro Preto*: 1 male (ISLA 16049) from Mina do Chico Rei (3), 29/XI/2009; 1 male (ISLA 16053) from Mina Velha (6), 29/XI/2009 (Bernardi et al. 2010). *São José da Safira*: 1 male (ISLA 16027) from Mina do Chiá Donizete I (78), 13/VII/2009; 1 male (ISLA 9575) from Mina do Chiá Donizete II (39), 13/VII/2009; 1 male (ISLA 9577) from Mina do Chiá Donizete III (5), 13/VII/2009; 1 male (ISLA 16050) from Mina do Milto Godinho II (5), 11/VII/2009; 1 male (ISLA 16051) from Mina do Milto Godinho III (34), 11/VII/2009; 1 male (ISLA 16052) from Mina do Milto Godinho IV (17), 11/VII/2009; 1 male (ISLA 9576) from Mina do Milto Godinho VII (84), 12/VII/2009; 1 male (MNRJ 2340) from Mina do Milto Godinho VIII (47), 12/VII/2009; 1 male (ISLA 16054) from Túnel do Milto Godinho IX (1), 12/VII/2009; 1 male (ISLA 16055) from Túnel do Milto Godinho X (38), 12/VII/2009; 1 male (ISLA 3690) from Túnel do Milto Godinho XI (48), 21/VII/2009; 1 male (ISLA 9572) from Túnel do Milto

Godinho XII (51), 12/VII/2009; 1 male (ISLA 16056) from Túnel do Milto Godinho XIII (71), 12/VII/2009 (Ázara et al. 2013).

In seven cavities, this species was observed forming aggregations of 10, 15, 18, 22, 41 and 43 individuals up to 5 meters from the entrance. In other cavities, sparsely distributed individuals were also recorded both in the photic and aphotic zones at a maximum distance of 60 m from the entrance. In some cases, individuals were found guarding their eggs and nymphs. This species also occurs in natural cavities in Minas Gerais State (Machado 2002, DaSilva and Gnaspini 2010, Ázara et al. 2013)

Mitogoniella mucuri Ázara et al., 2013

Figure 5A–B

Previously known distribution. Minas Gerais: Alagoa, Caraí, Novo Oriente de Minas, Padre Paraíso (Ázara et al. 2013).

Examined material. *Alagoa*: 1 male (ISLA 3983) from Túnel do Garrafão (10), 31/VIII/2008 (Ázara et al. 2013). *Caraí*: 1 male (ISLA 3970) from Túnel da Fazenda Cilindro I (2), 21/VII/2008 (Ázara et al. 2013).

Individuals of this species were recorded sparsely distributed inside the mines both in the photic and aphotic zones at a maximum distance of 36 m from the entrance. In some mines, specimens were found guarding their eggs and nymphs. This species also occurs in natural cavities in Minas Gerais state (Ázara et al. 2013).

Gonyleptinae Sundevall, 1833

Liogonyleptoides inermis (Mello-Leitão, 1922)

Previously known distribution. Minas Gerais: Tapira (H. Soares 1977); Rio de Janeiro: Petrópolis (Mello-Leitao 1923); Santa Catarina: Mafra (Mello-Leitão 1937); São Paulo: Santo André (Mello-Leitao 1922), Amparo, Anápolis (B. Soares 1945b), Barretos (Mello-Leitão 1937), Cascalho, Corumbataí (B. Soares 1945c), Espírito Santo do Pinhal (H. 1966), Funil (B. Soares 1946), Guaianaz (B. Soares 1945b), Laranja Azeda (Soares and Soares 1945), Lusitânia (B. Soares 1945c), Mogi Guaçu (H. Soares 1966), Piracicaba (B. Soares 1946), Rio Claro (B. Soares 1945a), Santa Adélia (H. Soares 1966), São Paulo (B. Soares 1945c), Silvânia (B. Soares 1946), Vista Alegre do Alto (H. Soares 1977).

Examined material. *Novo Oriente de Minas*: 1 male (MNRJ 2338) from Mina do João Bufinha (4), 05/I/2009, new record for locality.

Individuals were sparsely distributed inside the mines, both in the photic and aphotic zones at a maximum distance of 10 m from the entrance. Novo Oriente de Minas corresponds to a new occurrence for this species, expanding its distribution with about 700 km from the northeast of Petrópolis, Rio de Janeiro state.

Heteropachylinae Kury, 1994

Pseudopucrolia mutica (Perty, 1833)

Previously known distribution. Bahia: Salvador (Roewer 1912), municipality not specified (Mello-Leitao 1926); Pernambuco: Arquipélago de Fernando de Noronha (Roewer 1930), Tapera (Mello-Leitão 1927); Sergipe: Aracaju, Maroim, Santo Amaro das Brotas (Soares and Bauab-Vianna 1972); São Paulo: municipality not specified (Roewer 1913).

Examined material. *Caraí*: 1 male (MNRJ 2271) from Túnel da Br116 (1), 20/VII/2008, new record for state and locality.

Only one individual was recorded in the photic zone of the cavity, at less than 10 m from the entrance. The record at Caraí expands the distribution of this species with about 750 km from the southwest of Salvador, Bahia.

Mitobatinae Simon, 1879

Longiperna trembao Pinto-da-Rocha & Bragagnolo, 2010

Figure 4A

Previously known distribution. Minas Gerais: Belo Horizonte, Ouro Preto (Pinto-da-Rocha and Bragagnolo 2010).

Examined material. *Caeté*: 1 male (ISLA 16059) from Mina do Morro Vermelho III (1), 17/VI/2009; 1 male (MNRJ 2269) from Mina dos Matarelli (11), 18/VII/2009. *Mariana*: 1 male (ISLA 16060) from Mina dos Canelas VII (3), 11/VI/2009. *Ouro Preto*: 1 male (ISLA 16058) from Mina de Vila Rica (2), 28/XI/2009; 1 male (MNRJ 2268) from Mina do Chico Rei (9), 27/XI/2009, new records for localities.

Individuals of this species were sparsely distributed in the photic and aphotic zones of the mine at a maximum distance of 30 m from the entrance. The records at Caeté and Mariana expand its distribution with about 60 km from the northwest of Ouro Preto, Minas Gerais state.

Mitobatula sp. 1

Figure 4C

Previously known distribution of *Mitobatula castanea* Roewer, 1931 (the genus is monospecific). Santa Catarina: Serra Azul (Roewer 1931); Rio de Janeiro: Teresópolis (Kury 2003).

Examined material. *Alagoa*: 1 male (MNRJ 2374) from Mina da Companhia (7), 01/XI/2008; 1 male (MNRJ 2342) from Túnel do Garrafão (15), 31/X/2008, new record for state and locality.

Individuals of this morphospecies were sparsely distributed in the photic and aphotic zones at a maximum distance of 12 m from the entrance. The record at Alagoa expands the distribution of the genus with about 150 km from the west of Teresópolis, Rio de Janeiro state.

Pachylinae Sørensen, 1884

Eusarcus aduncus (Mello-Leitão, 1942)

Previously known distribution. Bahia: Carinhanha, Itagibá, Una (Hara and Pinto-da-Rocha 2010); Distrito Federal: Brasília, Brazlândia (Hara and Pinto-da-Rocha 2010); Espírito Santo: Barra do São Francisco (Hara and Pinto-da-Rocha 2010), Colatina (Mello-Leitão 1942), Linhares (Hara and Pinto-da-Rocha 2010); Goiás: Corumbá (Hara and Pinto-da-Rocha 2010), Formosa (Gnaspini and Trajano 1994), Itaberaí, Mabaí (Hara and Pinto-da-Rocha 2010), São Domingos (Trajano and Gnaspini 1991); Minas Gerais: Itacarambi (Chaimowicz 1986).

Examined material. *Caeté*: 1 male (MNRJ 2265) from Mina do Morro Vermelho II (2), 19/VI/2009. *São José da Safira*: 2 males (ISLA 16026) from Mina do Chiá Donizete I (10), 13/VII/2009; 1 female (ISLA 16042) from Mina do Chiá Donizete II (1), 11/VII/2009; 1 female (ISLA 16043) from Mina do Chiá Donizete III (1), 11/VII/2009; 1 male (MNRJ 2267) from Mina do Milto Godinho I (2), 11/VII/2009; 1 female (ISLA 16044) from Mina do Milto Godinho IV (4), 11/VII/2009; 1 male (MNRJ 2377) from Mina do Milto Godinho VIII (4), 11/VII/2009, new record for localities.

Individuals of this species were found sparsely distributed in the photic and aphotic zones at a maximum distance of 60 m from the entrance. The records at Caeté and São José da Safira expand its distribution with about 100 km from the south of Carinhanha, Bahia. This species also occurs in Minas Gerais and São Paulo state in natural cavities (Chaimowicz 1986, Trajano and Gnaspini 1991, Gnaspini et al. 1994, Hara and Pinto-da-Rocha 2010).

Eusarcus cavernicola Hara & Pinto-da-Rocha, 2010

Previously known distribution. Bahia: Santana; Goiás: Anápolis, São Domingos; Minas Gerais: Itacarambi, Montes Claros, Unaí (Hara and Pinto-da-Rocha 2010).

Material examined. *Vazante*: 1 male (MNRJ 2344) from Mina da Vmetais, 08/IV/2009, new record for locality.

Individuals of this species were observed sparsely distributed inside the mine. They were located in the photic and aphotic zones at a maximum distance of 500 m from the entrance. The record at Vazante expands its distribution with about 200 km from the south of Unaí, Minas Gerais. This species also occurs in natural cavities in Bahia, Goiás and Minas Gerais state (Hara and Pinto-da-Rocha 2010).

Eusarcus sp. 1

Previously known distribution of the genus. From the northeast to the southeast of Brazil, northeast of Argentina, east of Paraguai and Uruguai (Hara and Pinto-da-Rocha 2010).

Examined material. *Mariana*: 1 male (ISLA 16061) from Mina dos Canelas I (1), 11/VI/2009; 1 male (ISLA 16021) from Mina dos Canelas VII (2), 11/VI/2009; 1 male (ISLA 16024) from Mina da Cachoeira (4), 12/VI/2009 (Bernardi et al. 2011). Ouro Preto: 1 female (ISLA 16045) from Mina do Chico Rei (3), 02/V/2009 (Bernardi et al. 2010). *São José da Safira*: 1 male (MNRJ 2376) from Mina do Milto Godinho III (2), 13/IV/2009; 1 male (ISLA 16018) from Túnel do Milto Godinho XII (2), 12/IV/2009, new record for locality.

Individuals were observed sparsely distributed inside the cavities both in the photic and aphotic zones, at a maximum distance of 22 m from the entrance. Individuals were also observed next to the touristic path. The records at Mariana and São José da Safira correspond to new occurrences for the genus.

Eusarcus sp. 2

Previoulys known distribution of the genus. From the northeast to the southeast of Brazil, northeast of Argentina, east of Paraguai and Uruguai. (Hara and Pinto-da-Rocha 2010).

Examined material. *Mateus Leme*: 1 male (ISLA 16062) from Mina do Aqueduto I (1), 05/IV/2008, new record for locality.

Only one female was found and the record of Mateus Leme corresponds to a new occurrence for this genus.

Planiphalangodus sp. 1

Previously known distribution of *Planiphalangodus robustus* Roewer, 1929 (the genus is monospecific). Santa Catarina: municipality not specified (Roewer 1929); Argentina, Missiones: *Iguazú* (Ringuelet 1959).

Examined material. *Alagoa*: 1 male (MNRJ 2264) from Mina da Companhia (1), 01/XI/2008, new record for state and locality.

Only one male was found and the record of Alagoa corresponds to a new occurrence for this genus.

Discussion

This study presented the occurrence of two undescribed species (*Eusarcus* sp. 1 and *Mitobatula* sp. 1) and new records for states and localities in the case of nine species and four genera of harvestmen. The results obtained demonstrate the importance of observations in unusual environments, like mines for the potential of finding new species, provide more information about the occurrence of species and register new behavioral patterns, thus improving the knowledge about the group.

Although there are studies in Brazil and worldwide about the fauna associated to mines (Peck 1988, Gnaspini and Trajano 1994, Ferreira 2004, Bernardi et al. 2010, 2011, Isaia et al. 2011), most of them are focused on community ecology. Until present only few groups received special attention in studies of mines: anurans (Rosa and Penado 2013), bats (Kurta and Smith 2014) and salamanders (Manenti et al. 2009, Manenti and Ficetola 2013, Balogová and Uhrin 2014, Herrero and Hinckley 2014).

The only record of harvestmen in artificial subterranean environments outside the state of Minas Gerais was for the species *Pararezendesius luridus* H. Soares, 1972, which was found in mines of Iporanga and also in natural cavities of Apiaí, Iporanga and Ribeira municipalities, São Paulo state (Soares 1972, Trajano et al. 1991, Gnaspini et al. 1994, Pinto-da-Rocha 1995). Three species, *Eusarcus* sp. 1, *Goniosoma vatrax* and *Mitogoniella indistincta,* were recorded in touristic mines of Mariana and Ouro Preto, Minas Gerais state (Bernardi et al. 2010, 2011).

The age of the majority of the mines in our study varied from less than five years to more than 100 years (Table 2). Twenty of the 52 cavities containing harvestmen have only pastures surrounding them (30 and 250 m radius). The other cavities present different phytophysiognomies arround them, such as Atlantic Forest, Caatinga and Cerrado fragments, *Eucalyptus* plantations and pastures.

As shown by Bragagnolo et al. (2007), richness and abundance of harvestmen species in the Atlantic Forest are negatively affected by fragmentation, reduction of fragment size and forest quality (stratification and leaf density). The replacement of natural environments by areas with mineral exploitation can destroy the habitats. However, mines apparently might ensure the permanence for a number of species that otherwise might get locally extinct through the destruction of epigean habitats (Ferreira 2004). However, it is still unknown how populations inside mines surrounded by pastures and small fragments can maintain.

The results of this work show that harvestmen can use mines as shelters during the day and for reproduction. These mines are important because many of the studied regions are fragmented, with forest areas replaced by pastures and agricultural land. Future studies can demonstrate whether harvestmen feed in the pastures or use the organic material inside the mines.

The presence of *Eusarcus cavernicola* individuals, a species commonly found in caves (Hara and Pinto-da-Rocha 2010), inside of the mine Mina da Vmetais (500 m from the entrance) suggests that this habitat is a permanent shelter for the species, due the presence of the food resource in the cavity.

Further studies should be concentrated on understanding the dynamics of species inside mines and the taxonomic identification. Artificial cavities can contribute to the conservation of some harvestmen species in many areas where the original vegetation was removed and replaced by pastures, monocultures or constructions.

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Biodiversity and biogeography of groundwater invertebrates in Queensland, Australia

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Abstract

Groundwater systems, traditionally considered lifeless conduits of water (Hancock and Boulton 2008, Schulz et al. 2013), are now known to provide critical habitat for a diverse range of fauna collectively called stygofauna. Stygofauna communities can have significant conservation value as exemplified by relatively high levels of endemism and biodiversity. Despite this the biogeography and taxonomic diversity of stygofauna communities largely remains undocumented. This paper describes the development and interrogation of a state-wide database of 755 samples from 582 sites, and reviews the current knowledge of stygofauna biodiversity and biogeography across Queensland (north-eastern Australia).

Queensland is known to host 24 described families of stygofauna with stygofauna composition broadly consistent with other regions around the world. However Queensland assemblages tend to be unusually rich in both oligochaetes (16% cf. 2%) and syncarids (12% cf. 4%). Associations between stygofauna taxonomic richness and key environmental variables were consistent with many general assumptions of habitat suitability. However there were also notable exceptions, including stygofauna records from: groundwater 60 meters below ground level; groundwater with electrical conductivity above 50,000 µS/cm, and; both highly acidic (pH 3.5) and alkaline (pH 10.3) environments. These exceptions clearly demonstrate that strict adherence to general assumptions about habitat suitability when planning sampling activities may mask the true diversity of groundwater ecosystems.

Keywords

Stygofauna, biogeographic patterns, subterranean aquatic fauna

Introduction

Ecological and microbiological exploration of groundwater over the past two decades has identified a diverse range of organisms inhabiting groundwater systems, collectively called stygofauna (Danielopol et al. 2003, Boulton et al. 2008, Schulz et al. 2013). The term stygofauna is commonly thought to encompass: 1. stygophilic fauna that inhabit surface water, groundwater and epigean environments; 2. stygoxenic fauna that mostly inhabit epigean environments but occasionally or accidentally inhabit groundwater; and 3. stygobitic fauna that live exclusively in groundwater throughout their entire life cycle and are thought to be relics from groups of surface organisms that existed during a more mesic time period (Gibert et al. 1994, Sket 2008). Habitats created by groundwater systems are generally geographically restrictive (Eberhard et al. 2005, Majer 2009) and relatively stable across geological time (Humphreys 2006a), contributing to the typically narrow distributions (Asmyhr et al. 2014), high endemism (Cooper et al. 2002, Eberhard et al. 2005, Humphreys 2006b, Majer 2009, Asmyhr et al. 2014), and high diversity (Eberhard et al. 2005, Majer 2009) of stygofauna communities.

Tomlinson et al. (2007) identified that stygofauna are valued as a biodiversity resource, as indicators of groundwater ecosystem health, and potential providers of ecosystem goods and services. Such ecosystem goods and services may include nutrient cycling and storage (e.g. carbon, nitrogen, phosphorus) (Danielopol et al. 2003, Murray et al. 2006, Schulz et al. 2013, Asmyhr et al. 2014), organic matter cycling and redistribution (Danielopol et al. 2003), water treatment (e.g. filtering water to remove toxins) (Danielopol et al. 2003, Murray et al. 2006, Boulton et al. 2008, Majer 2009, Schulz et al. 2013, Asmyhr et al. 2014), water regulation (e.g. increasing the size of interstitial pore spaces to maintain hydraulic flow pathways and infiltration rates) (Hancock et al. 2005, Murray et al. 2006, Boulton et al. 2008, Majer 2009, Nwankwoala 2012, Schulz et al. 2013), and mineral weathering and formation (Danielopol et al. 2003).

The major pressures on groundwater systems in Australia, as elsewhere, are from anthropogenic activities that modify aspects of the groundwater regime, including flow, flux, pressure, level and quality (Danielopol et al. 2003, Eamus et al. 2006), and the transport of nutrients and organic matter (Menció et al. 2014). Activities such as agriculture, industrial production and domestic water supply result in a depletion in groundwater quantity and may introduce pollutants that impact groundwater quality (Danielopol et al. 2003), potentially altering ecosystem function (Danielopol et al. 2003) and driving changes in stygofauna distribution and composition (Menció et al. 2014). The pressures on groundwater ecosystems are cumulative (Danielopol et al. 2003) and their impacts may be observed earlier in more vulnerable groundwater ecosystems such as stygofauna communities of the hyporheic zone or in shallow, dynamic groundwater systems (Hancock 2002, Nwankwoala 2012).

Biological inventories have been used extensively to support management and conservation activities including development of conservation goals and identification of priority areas for conservation (Funk et al. 1999, Fleishman et al. 2000, Groves et al. 2002). Biological inventories containing species location data are a cost-effective option (Groves et al. 2002) to compile and manage historical records of the presence/absence of species at a particular location (Funk et al. 1999, Groves et al. 2002). Such baseline information commonly underpins assessments of biological diversity (Funk et al. 1999) including robust statistical analyses (Fleishman et al. 2000). While biological inventories include presence/absence data, further information on abundance and variance in abundance can support analysis of population viability (Fleishman et al. 2000). This paper analyses a recently developed subterranean aquatic fauna database to review the current knowledge of stygofauna distribution and diversity in Queensland.

Method

Description of Queensland study area and its groundwater systems

Queensland is a large state covering over 1.7 million square kilometres (Department of Environment and Heritage Protection 2015) and comprising 18 geographically distinct bioregions based on commonalities in climate, geology, landform, vegetation and species (Interim Biogeographical Regionalisation of Australia, IBRA) divided into 133 relatively homogenous geomorphological units called biogeographic subregions (Department of the Environment n.d.). Queensland encompasses six climatic zones with mean annual rainfall ranging from less than 200 millimetres (in south–western Queensland) to over 3,000 millimetres (in north–eastern Queensland, near Cairns) (Bureau of Meteorology n.d.).

Groundwater occurs throughout Queensland in Mesozoic sedimentary basins and overlying Cainozoic deposits. Broad types of geologies that are sources of groundwater include unconsolidated sedimentary material (e.g. Quaternary alluvial and colluvial deposits, Quaternary coastal or inland sand deposits), consolidated sedimentary rocks (e.g. sandstone), fractured rocks (e.g. Cainozoic igneous rocks), and cavernous rocks (e.g. limestone karst systems).

Stygofauna inventory database

A database of stygofauna inventory data for Queensland, the 'Queensland Subterranean Aquatic Fauna Database' (the database), was developed to compile available data in a standard format that facilitates value-adding activities such as comparative analysis and interpretation. From mid-2000 the Queensland Government has required stygofauna sampling to be undertaken as part of an environmental impact assessment for relevant mining, petroleum or gas developments under the Environmental Protection Act 1994 (Qld). Over twenty developments have undertaken stygofauna sampling as part of the environmental impact assessment process with results publicly reported in written reports. The database was designed as a low-cost, central repository of stygofauna data managed using MICROSOFT ACCESS® software and available to inform environmental planning and management.

The database was designed to meet initial data management requirements in terms of capturing available existing sampling data and providing flexibility to allow for the addition of new information in the future as monitoring and taxa identification techniques evolve. Currently the database has six major component tables and two supporting tables linked by two primary keys, a site identification number (e.g. bore hole registration number) and sample number (i.e. date of sampling event) (Figure 1). Provision was made in the database to explicitly identify any intellectual property restrictions and level of data access provided by data owners.

The wide variety of data contributors to the database required the establishment of the 'Guideline for the Environmental Assessment of Subterranean Aquatic Fauna' (Department of Science, Information Technology and Innovation 2015) (the guideline) that details minimum data requirements and provision of data in suitable formats (e.g. MICROSOFT EXCEL®) for inclusion in the database. This guideline is supported by an established preferred sampling method document. Any deviation from the preferred method (particularly for historical data) is noted in the database metadata. The establishment of the guideline addresses concerns that stygofauna sampling undertaken in

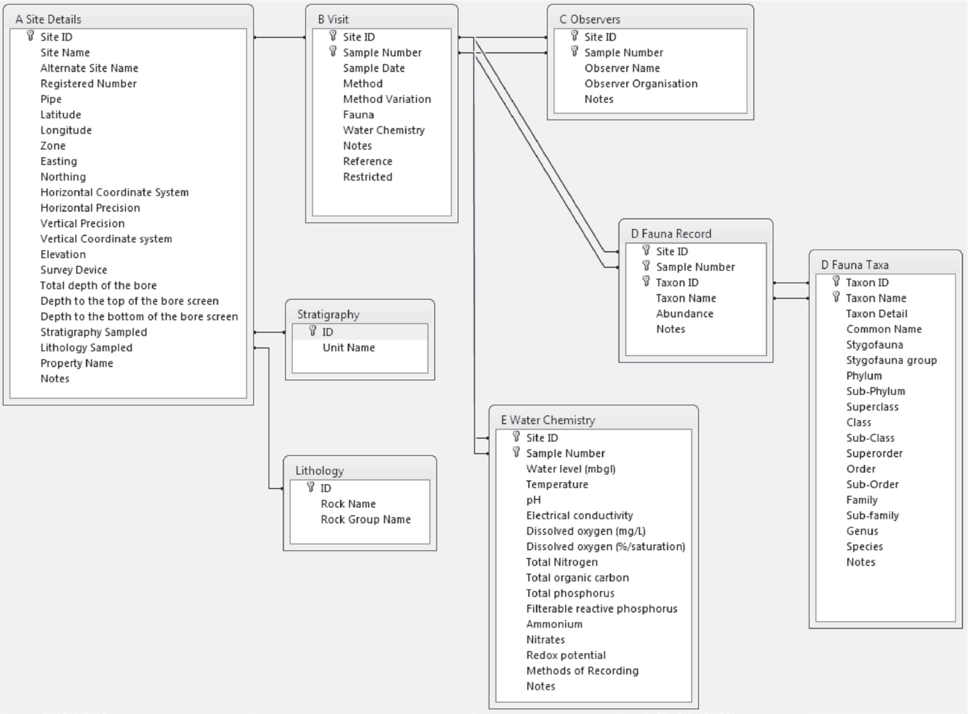


Figure 1. Diagram map of the structure of the 'Queensland Subterranean Aquatic Fauna Database'.

environmental impact assessment processes is usually data-poor or in formats unsuitable for other uses (Office of the Environmental Protection Authority 2012).

The Queensland Government is currently in the process of establishing regular dissemination of the releasable portions of the database to the general public through existing online delivery mechanisms including the Queensland Globe and the Queensland Spatial Catalogue, consistent with the government's policy for open data. The regular release of the database is intended to facilitate value-adding activities and the development of data derivatives by users.

Data analysis

As of 1 October 2015, the database contained information from a total of 755 samples across 582 sites in Queensland. This includes comprehensive coverage of Queensland Government data, largely collected to support water planning activities, as well as industry data made publicly available through environmental impact statements. At present there is only limited incorporation of data from other sources such as research institutions. The database contains information on all sampling events, regardless of whether fauna were present or absent, because information on where fauna have not been found may be as valuable as where they have been recorded for planning and conservation purposes (Gibert et al. 2009). RSTUDIO (v0.99.489, RStudio 2015) was used to analyse sampling methods, sampling effort, biodiversity, and correlation between stygofauna and environmental variables.

Results and discussion

Sampling methods and effort

Stygofauna sampling in Queensland has predominantly involved hauling a plankton net of variable mesh size through the water column of a bore hole either exclusively (77.4%) or in combination with other sampling methods such as pumping and scraping (11.3%). The prevalence of plankton net sampling is due to the ease of application and the minimal time required for sample collection. Plankton net sampling assumes that the water column in the bore hole is representative of the biota and physico-chemical properties of water within the broader groundwater system (Hahn and Matzke 2005). Research by Hahn and Matzke (2005) has illustrated that this assumption is appropriate when discussing water chemistry and stygofauna taxonomic composition but may not hold for assessment of the relative abundance of different stygofauna. Despite the relative ease of plankton net sampling, only 19.9% of sites have been sampled more than once and less than 6.5% have been sampled three or more times. Given that species accumulation curves do not plateau after one or two samples at a site, this lack of repeat sampling almost certainly underrepresents stygofauna diversity (Humphreys 2008).

Analysis of the diversity and biogeography of stygofauna is complicated by variations in the sampling methods. While 77.4% of samples were obtained using netting methods, in only 58.5% of these samples the netting method used was that specified in the 'Guideline for the Environmental Assessment of Subterranean Aquatic Fauna' (Department of Science, Information Technology, and Innovation 2015). The remaining 41.5% of these samples used netting methods with varying plankton net mesh size, decontamination procedures, and/or included bore hole purging prior to sampling. Since 2003 our understanding of groundwater ecology has rapidly expanded resulting in improvements to sampling methods and the establishment of standardised sampling protocols. This accounts for some of the variation in netting methods between different samples in the database.

Sampling coverage

Stygofauna sampling across Queensland is extremely sparse and geographically patchy. The spatial coverage of sampling has been clustered around locations of intensive groundwater resource development (e.g. the Murray–Darling Basin) and extractive industries (e.g. the Bowen Basin) (Figure 2). There are many groundwater systems outside these locations which remain largely unexplored for subterranean ecological communities (Schulz et al. 2013). Large areas of northern and western Queensland for example remain un-sampled entirely despite the presence of potentially suitable stygofauna habitat.

The skew in sampling coverage extends to the types of groundwater systems sampled (Figure 3). Lithology information associated with bore hole screening depths was available for 50.9% of samples (n=384) across 294 sites and exhibited a clear bias towards the sampling of alluvial and coal deposits. This preference reflects the focus towards general assumptions of good habitat suitability and major coal or water bearing formations. While some sampling has been undertaken across a range of lithologies, the clear geographic and lithological sampling skew towards permeable alluvial aquifers is consistent with other Australian experiences (Office of the Environmental Protection Authority 2012). More recently, research internationally has begun to increase sampling effort in other lithologies including limestone, glacial till, colluvium and clay (Dole-Olivier et al. 2009).

Stygofauna biogeography

Despite a clear sampling skew a wide range of lithologies has been found to support stygofauna communities, including unconsolidated sedimentary material (e.g. alluvium, sand), consolidated sedimentary rocks (e.g. sandstone), and fractured rocks (e.g. basalt, granite, volcanics), mirroring other Australian (Guzik et al. 2010) and European experiences (Stein et al. 2012). Overall stygofauna were discovered in 28.0% of

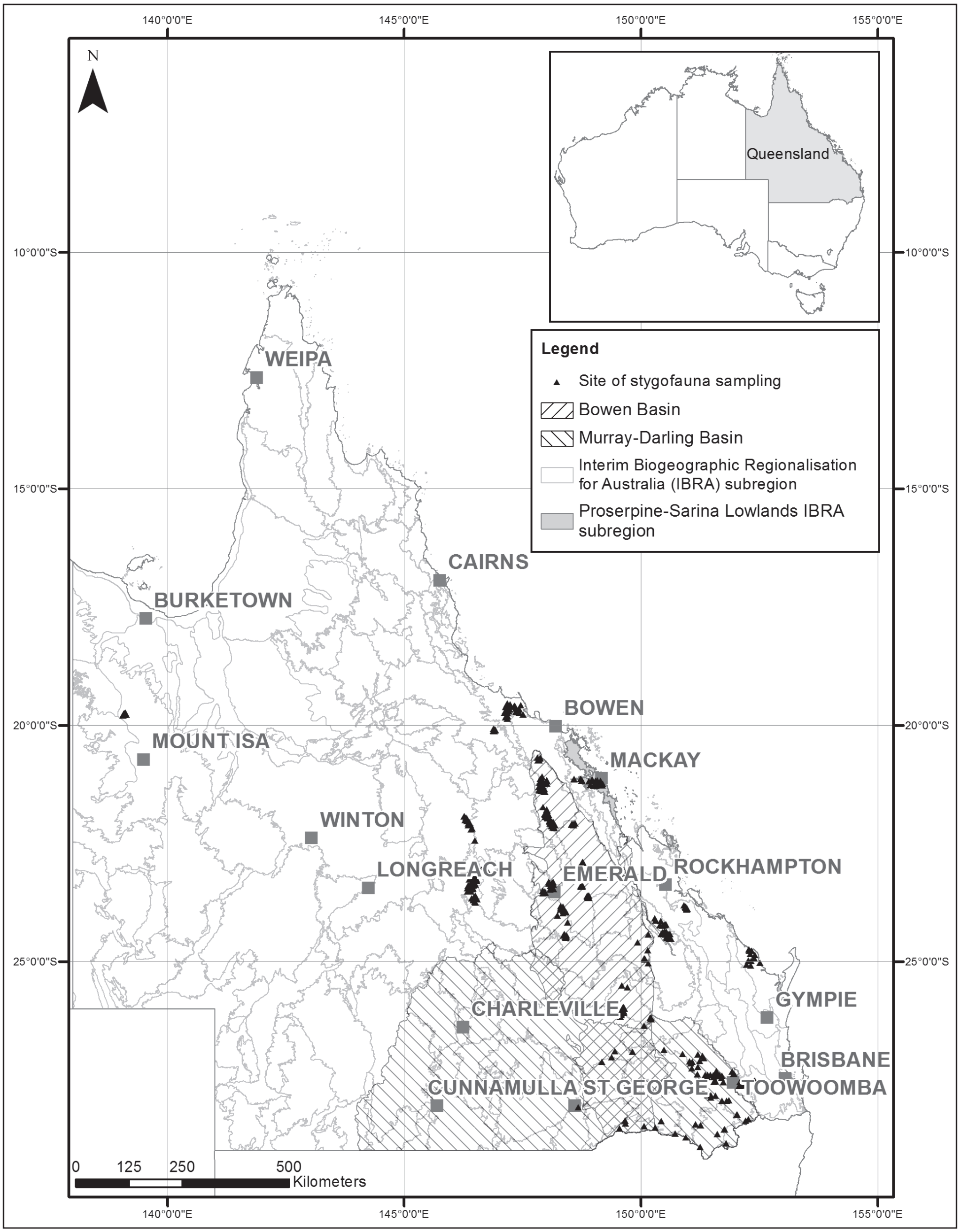


Figure 2. Outline map of Queensland, Australia highlighting the location of stygofauna sampling sites and other localities; In Figure 2 an outline map shows the location of all 582 stygofauna sampling sites and other key localities mentioned in the text (e.g. Bowen Basin, Murray–Darling Basin, Proserpine–Sarina Lowlands IBRA region).

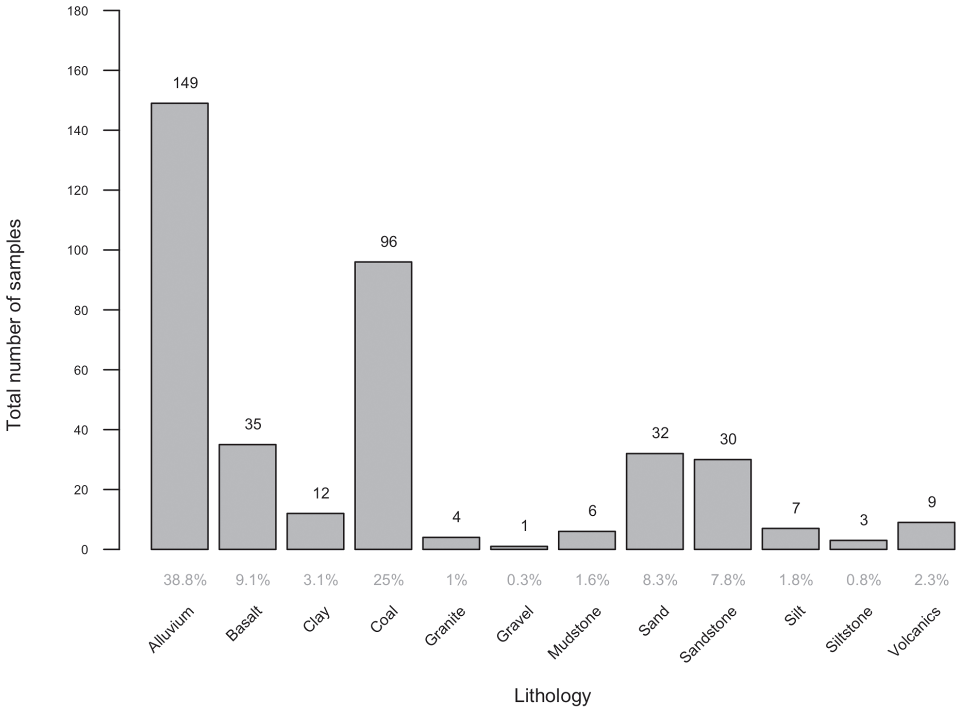


Figure 3. Distribution of stygofauna sampling effort by lithology in Queensland, Australia; In Figure 3 the total number of samples is indicated by numerical figures located above the columns and the percentage of samples is indicated by numerical figures along the x-axis.

samples, however variability in the proportion of samples found to contain at least one stygofauna individual among lithologies suggests differences in habitat suitability (Figure 4). The proportion of samples found to contain stygofauna varied from 11%–55% for those lithologies in the database with more than 20 samples. Sample sizes for some lithologies are extremely small, so any variation in the proportion of samples found to contain stygofauna may also appear due to chance.

Stygofauna biodiversity

Available data include records of 24 described families and 23 described genera of stygofauna in Queensland (Supplementary material 1, Figure 5). The most widely distributed groundwater taxon in Queensland is syncarids from the order Bathynellacea with individuals identified in approximately 60% of subregions sampled. Stygofauna from this order are comprised of two described families (Bathynellidae and Parabathynellidae) and two described genera (*Bathynella* and *Notobathynella*), however some samples did not identify fauna taxonomy below the Bathynellacea order. The di-

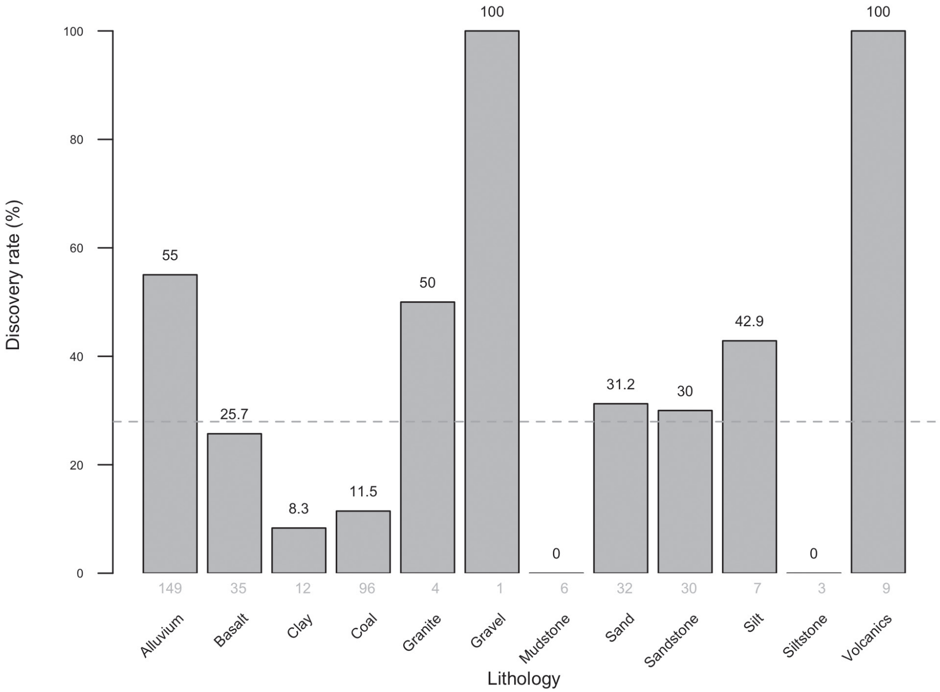


Figure 4. Stygofauna discovery rates by lithology in Queensland, Australia; In Figure 4 the discovery rate of stygofauna is indicated by numerical figures located above the columns, the total number of samples is indicated by numerical figures located along the x-axis, and the average stygofauna discovery rate (28%) is plotted as a grey, dashed line.

versity of described families varies by subregion (Figure 6) with the Proserpine–Sarina Lowlands subregion along the central east coast of Queensland exhibiting the highest diversity. The relatively high diversity recorded in this subregion may result from the high taxonomic effort employed to identify samples to at least family level (61.4% cf. 47.3%) but often to the genus or species level (42.9% cf. 21.3%). Other regions may have similar diversity levels to the Proserpine-Sarina Lowlands however, low sampling effort and/or limited taxonomic resolution may hide their diversity. In addition to high taxonomic resolution, 62.5% of all sites sampled in the Proserpine–Sarina Lowlands were alluvial groundwater systems, which are thought to have relatively high habitat suitability. Once again, low sampling effort and/or limited sampling in lithologies with relatively high habitat suitability may hide similar diversity levels in other regions.

A comparison of the habitat suitability of different lithologies is limited by the inconsistent sampling effort. However, variation in the diversity of described species suggests differences in habitat suitability exist between types of groundwater systems (Figure 7). Some described families of syncarids, copepods and oligochaetes are able to inhabit a wide range of lithologies (Figure 8). Consistent with their wide geographic distribution, syncarids from the two described families of the order Bathynellacea have

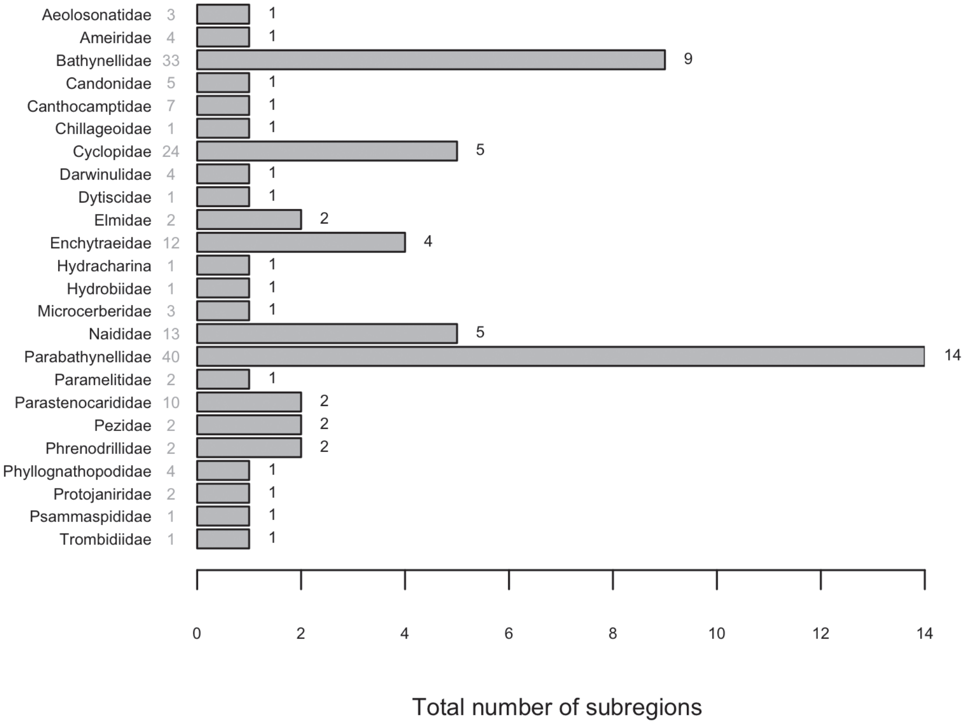


Figure 5. Biogeography of described families in Queensland, Australia; In Figure 5 the total number of subregions a described family has been recorded inhabiting is indicated by numerical figures located to the right of the bars and the total number of samples is indicated by numerical figures located along the y-axis.

been recorded across six different lithologies in Queensland including unconsolidated material (e.g. alluvium, gravel, sand), consolidated sedimentary rocks (e.g. sandstone) and fractured rocks (e.g. basalt). Similarly, copepods from the family Cyclopidae and oligochaetes from the family Naididae have also been recorded inhabiting a wide range of lithologies including unconsolidated material (e.g. alluvium, sand), consolidated sedimentary rock (e.g. coal), and fractured rock (e.g. basalt). While sampling data are scarce or absent for many lithologies, the results suggest that groundwater systems cannot be eliminated as potential habitat for stygofauna based solely on geology or lithology.

Systematic composition of stygofauna

The composition of Queensland stygofauna communities is comparable with knowledge of global stygofauna (Humphreys 2006b, Deharveng et al. 2009, Gibert et al. 2009). Individuals from 9 of the 17 major stygofauna taxonomic groups identified by Botosaneanu (1986) have been recorded in the groundwater ecosystems of Queensland

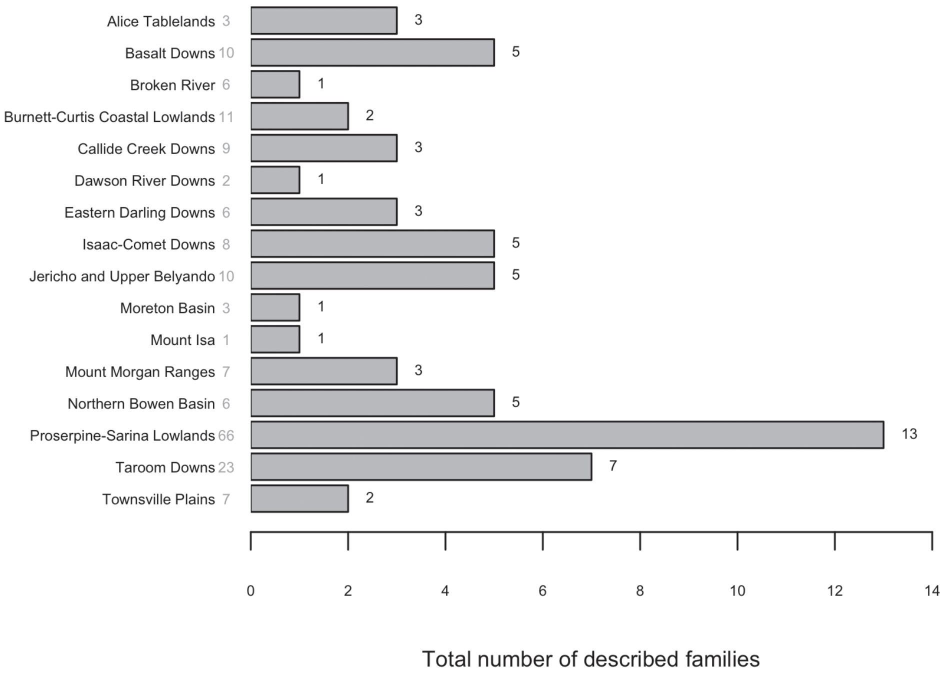


Figure 6. Diversity of described families in different IBRA subregions in Queensland, Australia; In Figure 6 the total number of described families is indicated by numerical figures located to the right of the bars and the total number of samples is indicated by numerical figures located along the y-axis.

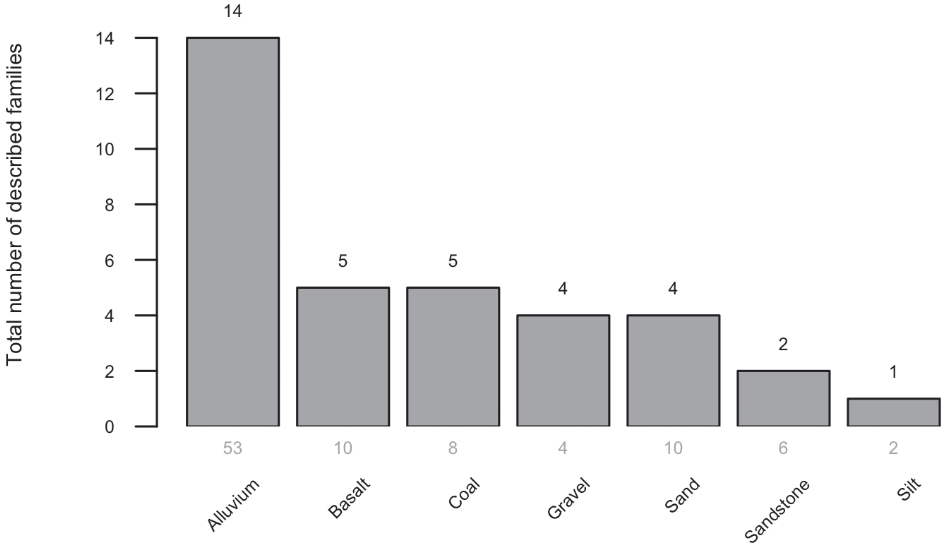


Figure 7. Diversity of described families across different lithologies in Queensland, Australia; In Figure 7 the total number of described families is indicated by numerical figures located above the columns and the total number of samples is indicated by numerical figures located along the x-axis.

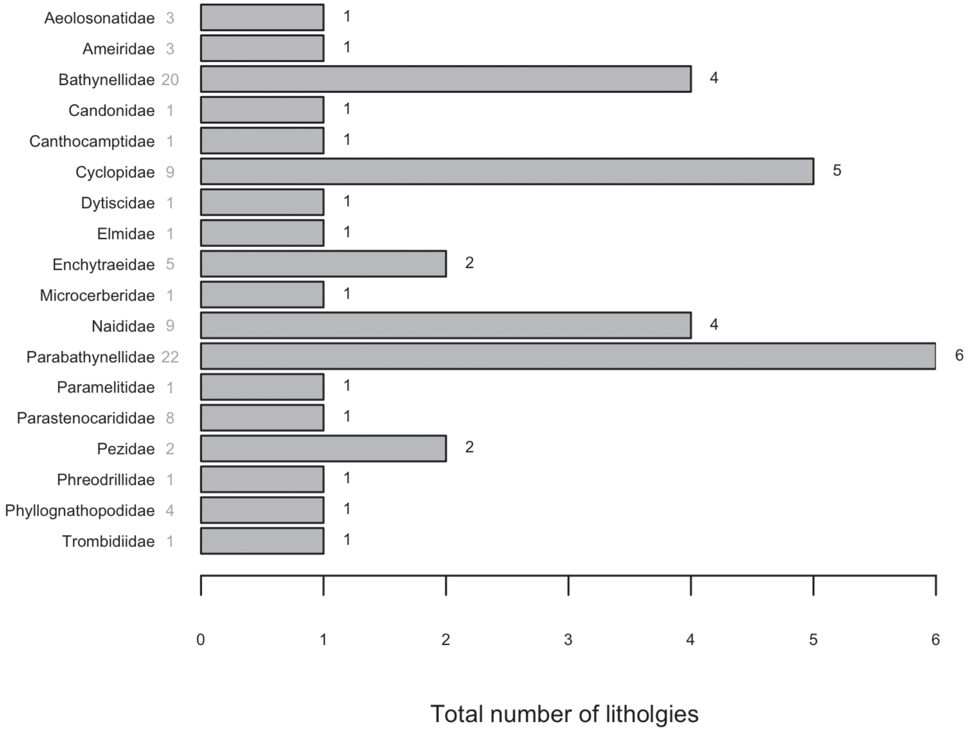


Figure 8. Distribution of described families across different lithologies in Queensland, Australia; In Figure 8 the total number of lithologies is indicated by numerical figures located to the right of the bars and the total number of samples is indicated by numerical figures located along the y-axis.

(Figure 9) with undescribed families identified across a further 3 taxonomic groups (Nematoda, Rotifera, and Turbellaria). Groundwater fauna from the 5 remaining taxonomic groups yet to be identified in Queensland groundwater ecosystems include Decapoda, Polychaeta, Remipedia, Spelaeogriphacea, and Thermosbaenacea. Knowledge of groundwater ecosystem composition in Queensland is in its infancy mirroring other Australian experiences and more broadly experiences in Africa, Asia and South America (Eberhard et al. 2005, Halse et al. 2014). As further survey and taxonomic research is completed it is likely that the range of taxonomic groups represented and their relative richness will change.

Many of the described families in Queensland are crustaceans (36%) including amphipods (e.g. Chillagoeidae, Paramelitidae), copepods (e.g. Cyclopidae) and ostracods (e.g. Candonidae, Darwinulidae) (Figure 9). Unlike the stygofauna from the Pilbara region (Western Australia, Australia) that are disproportionally rich in ostracods (27%) (Eberhard et al. 2005), Queensland has an ostracod richness much closer to the world average (8% cf. 3%, Eberhard et al. 2005) but is disproportionately rich in both oligochaetes (16% cf. 2%, Eberhard et al. 2005) and syncarids (12% cf. 4%, Eberhard et al. 2005). Queensland has a significantly smaller proportion of amphipods (8%)

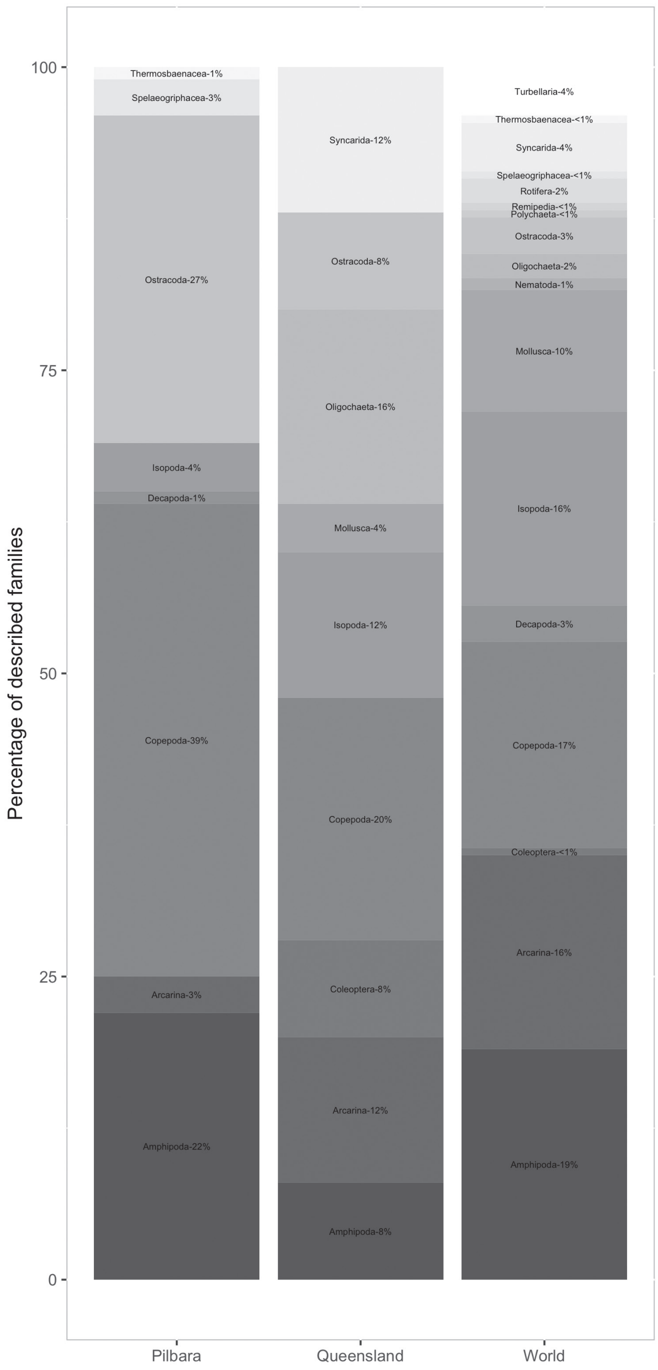


Figure 9. Comparison of systemic composition of described families from Australia and the World Average; In Figure 9 the systemic composition of described stygofauna families is compared between the Pilbara region (Western Australia, Australia) derived from Eberhard et al. (2005), Queensland (Australia), and the World Average derived from Eberhard et al. (2005).

compared to both the Pilbara region (22%, Eberhard et al. 2005) and the world average (19%, Eberhard et al. 2005). Despite some variation in the taxonomic richness of specific groups, the overall systematic composition of stygofauna in Queensland more closely resembles that of the world average than the composition found in the Pilbara region. This similarity may reflect the different scales of analysis being compared from regional (Pilbara) to state (Queensland) to global.

The lack of detailed taxonomic identification undertaken for many samples precludes a more detailed analysis of stygofauna diversity in Queensland. The diversity analysis described above used family level data, utilising just 47.3% of all available samples. The remaining samples were predominantly identified to the order level, however about 5.7% of all available samples underwent no taxonomic identification at all. Available data (Supplementary material 2) highlights that undescribed families and/or genera from a broad range of higher taxonomic ranks exist in almost all subregions sampled. This lack of detailed taxonomic resolution probably reflects the historic requirement provided in the terms of reference for environmental impact assessments to identify sampled groundwater fauna to the level of Order. In December 2015 a new version of the 'Guideline for the Environmental Assessment of Subterranean Aquatic Fauna' was released in Queensland, which specified minimum taxonomic resolution (i.e. genus, family, order) based on major stygofauna taxonomic groups while noting that "assessing risk to subterranean aquatic fauna ideally requires identification at the species level" (Department of Science, Information Technology and Innovation 2015). It is likely that stygofauna diversity in Queensland is largely undocumented and underrepresented in the current database given the limited taxonomic resolution (Tomlinson and Boulton 2008) and the tendency of stygofauna to exhibit morphological similarities (Gibert et al. 2009).

Correlation between stygofauna discovery and environmental data

The physico-chemical characteristics of groundwater systems can vary significantly on temporal and spatial scales, including variable depth to watertable, groundwater salinity, pH and the availability of organic carbon and oxygen (Humphreys 2006b). Widespread assumptions about the suitability of groundwater systems to support ecosystems based on physico-chemical characteristics may bias the diversity of groundwater habitats sampled to date (Tomlinson and Boulton 2010). Groundwater systems with a wide range of physico-chemical conditions have been recorded as supporting groundwater ecosystems in Queensland (Table 1). Stygofauna are not necessarily limited by common assumptions about the suitability of the physico-chemical properties of groundwater systems for supporting stygofauna (Schulz et al. 2013). Stygofauna were recorded living in physico-chemically diverse groundwater systems, including in systems with: groundwater ranging in depth from 0.1 and 63.2 metres below ground level; electrical conductivity ranging from 11.5 to 54,800 µS/cm; groundwater temperatures ranging from 17.0 to 30.7 degrees Celsius; and groundwater pH ranging

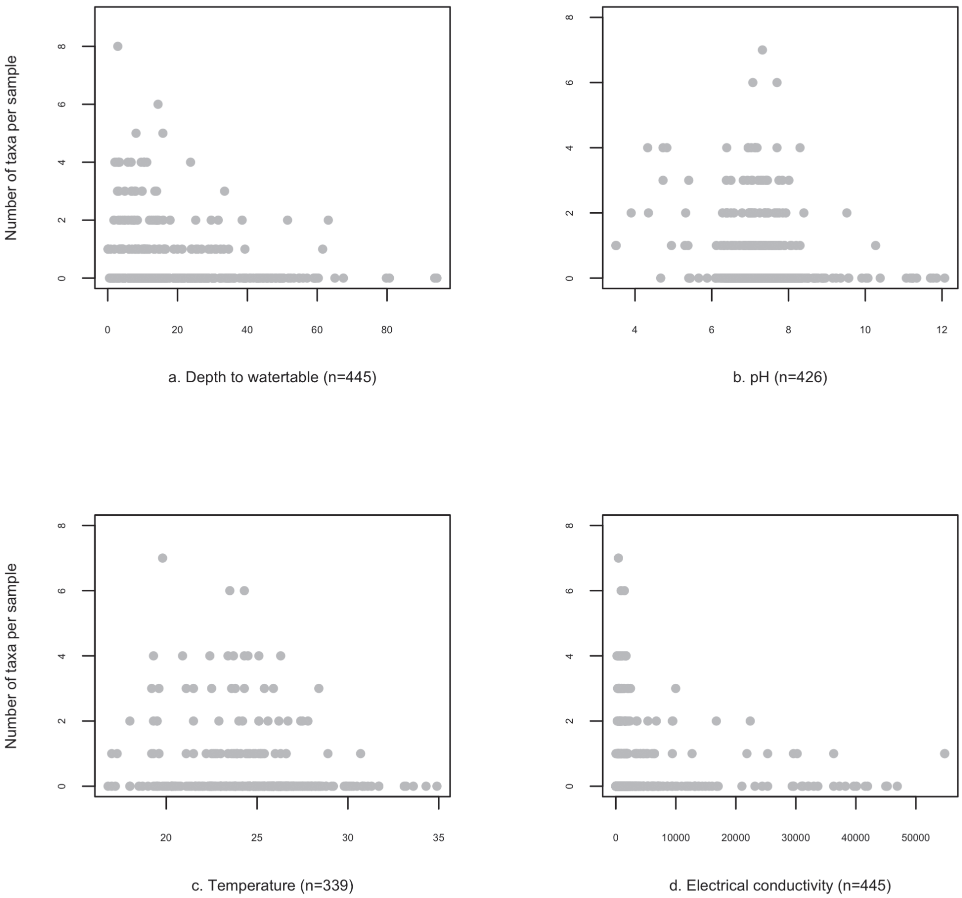
Table 1. Descriptive statistics of the physico-chemical properties of groundwater systems known to support stygofauna in Queensland, Australia. In Table 1 the descriptive statistics presented are based on available data in the Queensland Subterranean Aquatic Fauna database where: depth to groundwater is available for 113 samples in meters below ground level (mbgl); electrical conductivity is available for 137 samples in microSiemens per centimetre (µS/cm); pH is available for 130 samples; and temperature is available for 77 samples in degrees Celsius (°C).

Physico-chemical variable	Range	Mean	Median	Standard error	Source[1]
Depth to groundwater (mbgl)	0.1–63.2	13.7	10.5	1.1	1–9, 12–14, 16–19, 21–25
Electrical conductivity (µS/cm)	11.5–54,800.0	3,924.0	1,348.0	733.1	1–4, 7–15, 17–26
Groundwater pH	3.5–10.3	7.0	7.1	0.1	1–4, 7, 8, 10–15, 17–22, 24–26
Groundwater temperature (°C)	17.0–30.7	23.5	23.9	0.3	1–4, 7, 8, 10, 13–15, 17–19, 21, 22, 24–26

[1] Sources: 1 (ALS Laboratory Group 2010); 2 (ALS Laboratory Group 2011a); 3 (ALS Laboratory Group 2011b); 4 (Subterranean Ecology 2012a); 5 (Subterranean Ecology 2012b); 6 (Subterranean Ecology 2010a); 7 (ALS Laboratory Group 2013); 8 (AustralAsian Resource Consultants 2011); 9 (Sinclair Knight Merz 2008); 10 (C&R Consulting 2013); 11 (FRC Environmental 2013); 12 (Schulz et al. 2013); 13 (GHD 2012c); 14 (GHD 2012b); 15 (GHD 2013); 16 (Hancock 2004); 17 (Hancock n.d.); 18 (Little 2014); 19 (Byerwen Coal Proprietary Limited 2013); 20 (Department of Science, Information Technology and Innovation 2013); 21 (ALS Laboratory Group 2012c); 22 (ALS Laboratory Group 2012b); 23 (ALS Laboratory Group 2012a); 24 (AustralAsian Resource Consultants 2013); 25 (GHD 2012a); 26 (Subterranean Ecology 2010b).

from 3.5 to 10.3. Information on the wide variance in the physico-chemical properties of known groundwater habitats is valuable in developing our understanding of the characteristics of groundwater systems that support groundwater communities.

Stygofauna taxon richness shows a general negative trend with increasing depth to groundwater (Figure 10a) or electrical conductivity (Figure 10d). Taxon richness was highest in neutral to slightly alkaline pH groundwater systems (Figure 10b) and in water temperatures between approximately 18 and 27 degrees Celsius (Figure 10c). Humphreys (2008) considered that groundwater systems in igneous and metamorphic rocks may tend towards acidic environments that would be less suited to supporting stygofauna due to constraints imposed by the reducing environment. This is consistent with Queensland experience where taxon richness decreases sharply with increasing groundwater acidity and particularly alkalinity. The preferences inferred from stygofauna taxon richness may partially reflect the limited sampling undertaken across physico-chemically diverse groundwater systems, particularly for groundwater temperature and pH. It is also difficult to robustly analyse correlations as available data are predominantly from sites sampled only once. These point-in-time measurements may not reflect the prevailing physico-chemical habitat characteristics or microhabitat characteristics in which the stygofauna actually reside (Boulton 2009).


a. Depth to watertable (n=445)

b. pH (n=426)

c. Temperature (n=339)

d. Electrical conductivity (n=445)

Figure 10. Scatterplots showing the relationship between stygofauna taxon richness per sample and different physico-chemical variables; In Figure 10 the scatterplots presented are based on available data in the Queensland Subterranean Aquatic Fauna database where: depth to groundwater is available for 113 samples in meters below ground level (mbgl); electrical conductivity is available for 137 samples in microSiemens per centimetre (µS/cm); pH is available for 130 samples; and temperature is available for 77 samples in degrees Celsius (°C).

Global and local significance of Queensland fauna

Many of Queensland's stygofauna communities are unstudied or understudied hampering both global and local comparisons. Despite this, Europe, North America and other areas of Australia (e.g. Western Australia) provide the most appropriate baseline for comparison given the higher survey effort employed in these regions (Deharveng et al. 2009, Halse et al. 2014). While research has identified that eastern Queensland supports moderate richness stygofauna communities (Hancock and Boulton 2008, Cook et al. 2012, Halse et al. 2014), our analysis highlights that this estimate is too low due to the low sampling effort and limited sampling coverage that largely excludes arid regions and low taxonomic resolution.

Many stygofauna communities around the world are dominated by amphipods, copepods, and isopods (Deharveng et al. 2009, Halse et al. 2014). Queensland stygofauna communities comprise copepods and isopods in proportions comparable with world averages (20% cf. 17%, 12% cf. 16% respectively; Eberhard et al. 2005) and copepod proportions comparable to experiences in eastern Australia and the Pilbara (Eberhard et al. 2005, Halse et al. 2014). However, Queensland stygofauna communities differ due to the dominance of oligochaetes (16% cf. 2%; Eberhard et al. 2005), syncarids (12% cf. 4%; Eberhard et al. 2005) and beetles (8% cf. <1%; Eberhard et al. 2005).

Dissimilar to many other stygofauna communities around the world, stygofauna communities in Queensland have a low proportion of molluscs (4% cf. 10%; Eberhard et al. 2005). This compositional feature more closely reflects that of other Australian stygofauna communities (4% cf. 3%; Hancock and Boulton 2008) including the Pilbara (4% cf. 1%; Eberhard et al. 2005, Halse et al. 2014) than global experiences. As previously stated these comparisons are limited by low sampling effort in many regions, however, the composition of Queensland stygofauna communities is clearly differentiated from that of most of the world.

Conclusion

Biological inventories are a cost-effective option to capture and maintain baseline records to support management and conservation activities such as assessments of biological diversity and endemism. Interrogation of the database developed to collate available biological information on stygofauna enabled the authors to complete comparative analysis and interpretation at the state scale providing significant insights into the biogeography and diversity of stygofauna in Queensland.

Queensland is known to host at least 24 described families and 23 described genera of stygofauna across 9 of the 17 major stygofauna taxonomic groups. Undescribed families have also been recorded across a further 3 major stygofauna taxonomic groups. The composition of stygofauna in Queensland is broadly consistent with the world average with the notable exception of high richness of oligochaetes and syncarids. Despite indications that a significant diversity of stygofauna is likely to exist across Queensland groundwater systems, stygofauna biodiversity largely remains undocumented and underrepresented in the above analysis. This underrepresentation is likely due to limited sampling coverage, limited taxonomic resolution (Tomlinson and Boulton 2008), and the tendency of stygofauna to exhibit morphological similarities (Gibert et al. 2009).

Stygofauna were recorded inhabiting a wide range of lithologies, including: unconsolidated sedimentary materials; consolidated sedimentary rocks; and fractured rocks. While the proportion of samples found to contain stygofauna varied considerably by lithology indicating some differences in habitat suitability, it was evident that a groundwater system cannot be excluded from the possibility of supporting stygofauna based purely on geology or lithology. Similarly, variations in stygofauna taxonomic richness indicate some degree of habitat preference based on the physico-chemical properties of groundwater systems. However there were sufficient notable exceptions to demonstrate that stygofauna may be found across a more diverse physico-chemical range of groundwater systems than is commonly assumed. These results clearly demonstrate that general assumptions of habitat suitability should not be used to guide sampling activities.

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Supplementary material 1

Biogeography of described families and genera of groundwater invertebrates in Queensland, Australia.

Authors: Katharine Glanville, Cameron Schulz, Moya Tomlinson, Don Butler

Data type: occurence

Explanation note: This dataset contains a supplementary table of described families and genera of groundwater invertebrates by higher rank in each subregion of Queensland, Australia.

Supplementary material 2

Biogeography of undescribed families and/or genera of groundwater invertebrates in Queensland, Australia.

Authors: Katharine Glanville, Cameron Schulz, Moya Tomlinson, Don Butler

Data type: occurence

Explanation note: This dataset contains a supplementary table of undescribed families and/or genera of groundwater invertebrates by higher rank in each subregion of Queensland, Australia.

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New records and new species of springtails (Collembola: Entomobryidae, Paronellidae) from lava tubes of the Galápagos Islands (Ecuador)

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Abstract

The Collembola fauna of the Galápagos Islands is relatively unexplored with only thirty-five reported species. Entomobryoidea, the most diverse superfamily of Collembola, is underrepresented, with only five species reported from the Galápagos. Here we present the findings of the first survey of Collembola from Galápagos lava tube caves, providing a significant update to the total number of entomobryoid Collembola species reported from the Galápagos Islands. Collections made during a March 2014 expedition to study lava tubes of the islands yielded new records for seven species of Entomobryoidea, including four genera not previously reported from the Galápagos Islands: *Coecobrya*, *Entomobrya*, *Heteromurus*, and *Salina*. As a result, three new species (*Entomobrya darwini* Katz, Soto-Adames & Taylor, **sp. n.**, *Pseudosinella vulcana* Katz, Soto-Adames & Taylor, **sp. n.**, and *Pseudosinella stewartpecki* Katz, Soto-Adames & Taylor, **sp. n.**) are described and new diagnoses are provided for *Heteromurus* (*Heteromurtrella*) *nitens* Yosii, 1964, *Lepidocyrtus nigrosetosus* Folsom, 1927 and *Pseudosinella intermixta* (Folsom, 1924). *Lepidocyrtus leleupi* Jacquemart, 1976 is synonymized with *L. nigrosetosus*. An updated checklist of all species within the superfamily Entomobryoidea reported from the Galápagos Islands is provided.

Keywords

Cave, chaetotaxy, Entomobryomorpha, Entomobryoidea, species checklist, synonymy, taxonomy

Introduction

Like the Hawaiian and Canary islands, major islands of the Galápagos archipelago are comprised of shield volcanoes (Fig. 1A). The Galápagos volcanoes are on the Nazca oceanic plate over the Galápagos hotspot, far from plate boundaries (Holden and Dietz 1972, Hey 1977, Toulkeridis 2011). The main Galápagos Islands are located east of the N-S-trending East Pacific Rise and south of the E-W-trending Galápagos Spreading Center and some 1000 km west of the Ecuadorian mainland. Associated with the shield volcanoes are hundreds of relatively small, cinder cones, ash cones, and spatter cones. These islands range in age from 0.0032 to 4 million years, with still active volcanoes on the younger islands (White et al. 1993, Geist et al. 2014, Global Volcanism Program 2015), with even older islands now represented only by seamounts. These geodynamics and the ever-changing volcanic development of the Galápagos Islands has given rise to conditions under which the unique endemic life on these islands has evolved (Darwin 1859). Evidence now suggests that for at least some animal taxa, colonization of the Galápagos archipelago likely took place before many of the present islands had emerged from the ocean (Christie et al. 1992, Torres-Carvajal et al. 2014, Husemann et al. 2015).

The composition of the fauna of the Galápagos Islands is shaped by the volcanic history of the islands, as well as the remoteness of the archipelago from the mainland. This is even more true for the subterranean fauna, comprised of "wrecks of ancient life" (Darwin 1859), living in the relatively stable, cool, humid conditions (Ashmole et al. 1992) of lava tubes on the slopes of the various shield volcanoes that form these islands. These lava tube caves (e.g., Fig. 1B) form from heated basalt flowing down the slopes of volcanoes, forming preferential flow paths that eventually drain, creating the caves. Various smaller cracks and other subterranean voids occur both in the relatively smooth pāhoehoe lava flows and in the more rugged ʻaʻā lava (Harris and Rowland 2015), providing innumerable places in which invertebrates may live (Howarth 1991, Peck 2001, Stone et al. 2004, Howarth et al. 2007, Toulkeridis 2011).

Diversity and relationships among subterranean animals, shaped by vicariance and dispersal are a common theme for island lava tube faunas of Hawaii (Howarth 1991, Juan et al. 2010), the Canary Islands (Oromí et al. 1991, Juan et al. 2000, Naranjo Morales and Abreu 2015), Rapa Nui (Easter Island) (Wynne et al. 2014), and the Galápagos Islands (Peck 1990). However, the inaccessibility of much of the Galápagos archipelago, due to a lack of roads and extremely rugged terrain, have restricted biological inventories of subterranean ecosystems in comparison to the Hawaiian (more than 50 terrestrial troglobites, Howarth 1991) and Canary archipelagos (more than 160 species of invertebrates unique to the underground environment, Naranjo Morales and Abreu 2015). Most recent work on Galápagos has been carried out, or summarized, by Stewart B. Peck (Peck and Kukalova-Peck 1986, Peck and Shear 1987a, 1987b, Peck

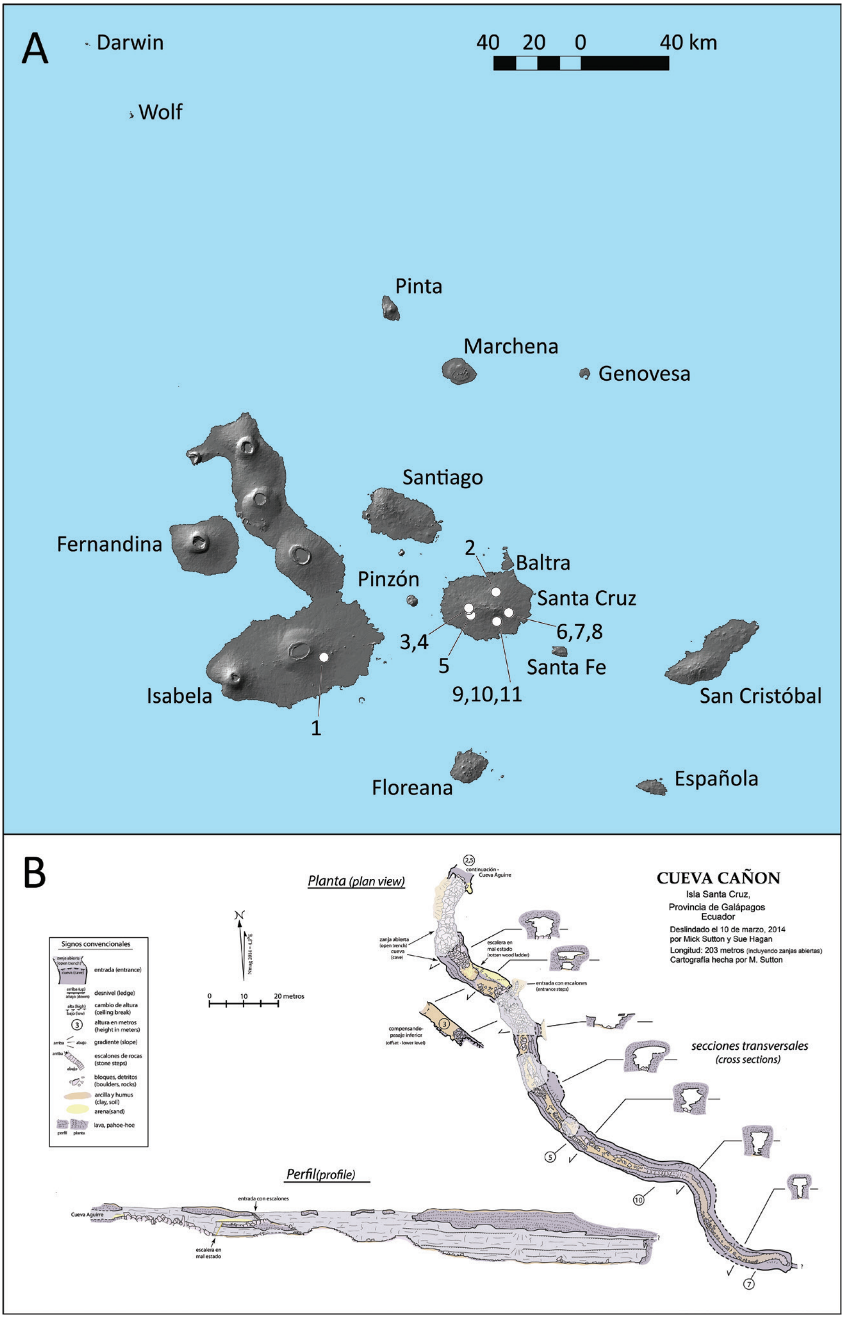

Figure 1. A Galápagos Islands, Ecuador, with major islands labeled and locations of caves sampled indicated by numbered circles: **1** La Cueve de Sucre **2** La Llegada **3** Cueva Chato 2 **4** Cueva Chato 1 **5** Cueva Primicias **6** Cueva Cañón, **7** Cueva Aguirre **8** Cueva Cascajo, **9** Cueva Soyla **10** Cueva JoAnn, and **11** Cueva Gallardo **B** Map of Cueva Cañón (Santa Cruz Island, Galápagos Islands, Ecuador), which is typical of the morphologies of the lava tube caves sampled. Maps of many of the other caves sampled during this study are available in Addison (2011) and Toulkeridis and Addison (2015).

1990, 2001, Peck and Finston 1993) who reports more than 55 eyeless and reduced eye arthropods, many of these from caves or cave entrances (Peck 1990, 2001).

Earlier analyses by Peck (1990) and Taylor et al. (2012) suggested that much remains to be learned regarding the subterranean fauna of the Galápagos Islands. These analyses helped build the justification for a multidisciplinary expedition, including the biological collections reported here, studying lava tube caves in the Galápagos Islands in March 2014. This expedition was conducted in association with the 16th International Symposium of Vulcanospeleology (Puerto Ayarro, Santa Cruz, Galápagos Islands, Ecuador) (Toulkeridis and Addison 2015), and built upon earlier field studies led by two of the authors (TT, AA). Here we make a contribution regarding the entomobryoid springtails (Collembola) of the Galápagos Islands as part of the ongoing studies of lava tube caves of the islands by our research team.

Thirty-five species of Collembola have been reported from the Galápagos Islands (Peck 2001); 18 in the order Poduromorpha (Stach 1932, Gama 1986, Najt et al. 1991) and 17 in the order Entomobryomorpha, 12 of which are in the family Isotomidae (Thibaud et al. 1994). The superfamily Entomobryoidea is underrepresented, with only 5 species formally reported from the Galápagos (Folsom 1924, Jacquemart 1976). The ostensible lack of diversity is most certainly due to the limited number of collections where specimens were subsequently identified and published. All 35 reported species were identified and described in six publications from material obtained from just four collections: the Williams Galápagos Expedition 1923–1925 (Folsom 1924); the Norwegian Zoological Expedition to the Galápagos Islands 1925, conducted by Alf Wollebæk (Stach 1932); the Belgian Zoological Mission to the Galápagos Islands and Ecuador led by N. and J. Leleup, 1964–1965 (Jacquemart 1976, Gama 1986, Najt et al. 1991, Thibaud et al. 1994); and collections made by Guy Coppois 1974–1976 (Najt et al. 1991, Thibaud et al. 1994).

Entomobryoidea is the most diverse superfamily of Collembola, representing more than one fourth of all described species worldwide (Bellinger et al. 2015). Species in the genera *Pseudosinella*, *Sinella*, *Coecobrya*, and *Trogolaphysa*, represent a large proportion of the springtail diversity found in New World caves, but the springtail fauna of Galápagos lava tubes has not been documented and these genera (except *Pseudosinella*) have not been reported from the islands. The purpose of this study is to describe and document all species in the superfamily Entomobryoidea collected during the recent 2014 bioinventories of Galápagos lava tubes. Here we provide descriptions for three new species endemic to the Galápagos, three new diagnoses, and new records for four genera and seven species. We also include an updated checklist of all species in the superfamily Entomobryoidea reported from the Galápagos Islands.

Methods

Geologic setting on Santa Cruz and Isabela islands. The Santa Cruz shield volcano is subdivided into two main units (Bow 1979), an older Platform Unit with an age of 1.3–1.1 Ma and a younger unit represented by lavas of the Shield Series with ages as young as 30–20 ka (Bow 1979, White et al. 1993, Geist et al. 2014). Sierra Negra on Isabela Island is a young, volcanically active shield volcano with various eruptive centers and lava fields that have been divided into five distinctive age groups, all being younger than 6000 years (Reynolds et al. 1995). Ten eruptions have occurred on Sierra Negra in the last 200 years, with the most recent eruption in October 2005 (Geist et al. 2008).

Specimen collection and preparation. Collections for this study were made at lava tube caves on Santa Cruz and Isabela islands, Galápagos (Fig. 1A) from 8 through 21 March 2014. Bioinventories were conducted in eleven lava tube caves (Table 1): ten on Santa Cruz Island and one on the Sierra Negra volcano on Isabela Island (Galápagos Islands, Ecuador). Caves were selected on the basis of opportunity, distribution across Santa Cruz Island, and coordination with other ongoing research (mapping, geology, soil science). For caves on Santa Cruz Island, handheld meters were used at most sites to collect temperature, humidity and light data in surface, entrance, twilight and dark zone habitats. Individual specimens were collected by hand from ceiling, walls, and floor in terrestrial or drip pool (Fig. 2A) microhabitats throughout the lava tubes, using a paintbrush moistened in alcohol, or with an aspirator. Where accumulated leaf litter deposits were encountered, litter samples were collected into plastic bags and then extracted for 2–4 days using cloth Berlese funnels heated with 25 watt light bulbs. All material was preserved in 70% ethanol.

Individuals sampled were sorted under a dissecting microscope to morphospecies and photographed to record color pattern and body shape prior to slide mounting. All slide-mounted specimens were cleared with Nesbitt's solution and mounted with Hoyer's medium (Mari Mutt 1979) in preparation for light microscopy. Sex was determined by the observation of genital plate morphology. Specimens where genital plate morphology was obscured are listed in material examined sections without a sex determination. Illustrations were hand-drawn under a camera lucida, scanned, with final drawings created using Adobe Illustrator.

Table 1. List of lava tube caves in the Galápagos Islands (Ecuador) that were sampled for invertebrates in March 2014. Elevations based on 3m DEM data. Lengths and cave maps are from cited sources. Cueva Aguirre is not the same as Cueva de Raul Aguirre of Hernández et al. (1992).

Cave	Island	Elevation (m)	Surveyed Length (m)	References
La Cueva de Sucre	Isabela	379	340	Addison (2011), Toulkeridis and Addison (2015)
Cueva Aguirre	Santa Cruz	304	574	Present study
Cueva Cañón	Santa Cruz	304	203	Present study (Figs 1B, 2B)
Cueva Cascajo	Santa Cruz	275	3010	Hernández et al. (1992), Gulden (2015)
Cueva Chato 1	Santa Cruz	344	515	Toulkeridis and Addison (2015), Gulden (2015)
Cueva Chato 2	Santa Cruz	373	457	Toulkeridis and Addison (2015), Gulden (2015)
Cueva Gallardo	Santa Cruz	213	2316	Hernández et al. (1992), Toulkeridis and Addison (2015), Gulden (2015)
Cueva JoAnn	Santa Cruz	208	80	Toulkeridis and Addison (2015)
Cueva Primicias	Santa Cruz	265	640	Toulkeridis and Addison (2015)
Cueva Soyla	Santa Cruz	212	1038	Toulkeridis and Addison (2015), Gulden (2015)
La Llegada	Santa Cruz	251	2066	Toulkeridis and Addison (2015), Gulden (2015)

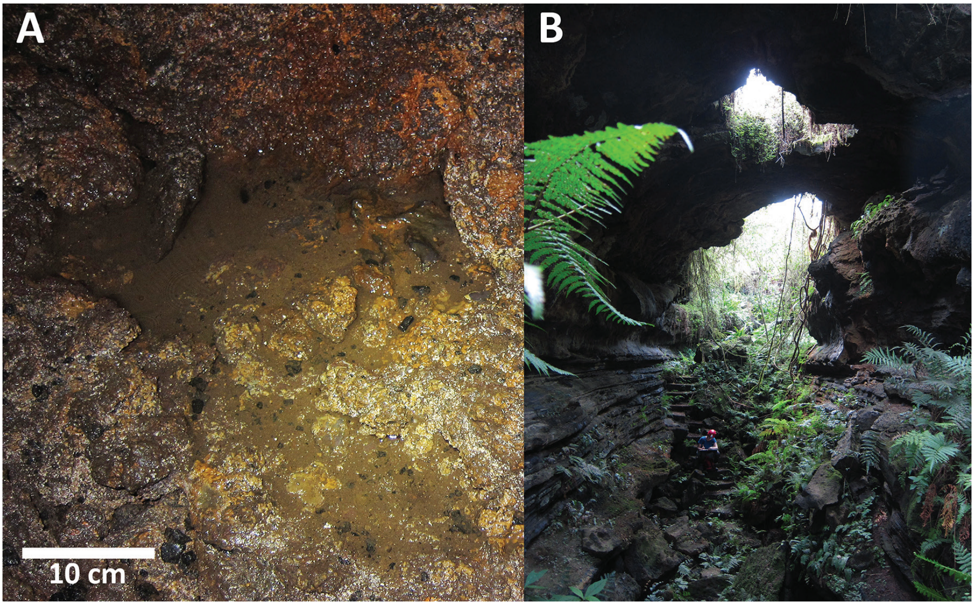

Figure 2. A Drip pool in dark zone of Cueva Chato 2 (Santa Cruz Island, Galápagos Islands, Ecuador), where *Pseudosinella vulcana* sp. n. was collected from surface film. Photo by SJT, 15 March 2014 **B** Entrance of Cueva Cañón (Santa Cruz Island, Galápagos Islands, Ecuador), where *Lepidocyrtus nigrosetosus* was collected. Photo by SJT, 10 March 2014.

Chaetotaxy nomenclature. Descriptions of dorsal body chaetotaxy follow the nomenclature established by Szeptycki (1979); the dorsal head chaetotaxy follows Jordana and Baquero (2005) and Soto-Adames (2008). Traditional dorsal chaetotaxy nomenclature for *Pseudosinella* established by Gisin (1967) is referred to in some descriptions for comparison and simplicity. See Soto-Adames (2010a) for comments and comparisons between nomenclature systems. All descriptions of labial chaetotaxy follow the nomenclature of Chen and Christiansen (1993).

Taxonomic classification. The suprageneric classification follows Soto-Adames et al. (2008).

Abbreviations and symbols. Abbreviations used in this paper are as follows: Ant. I-IV, antennal segments 1-4; Hd, head; Th. II, mesothorax; Th. III, metathorax; Abd. I-V, abdominal segments 1-5; Mc, macrosetae; mc, microsetae. See Figure 5F–J for symbol legend.

Deposition of types and material examined. Specimens examined are deposited in the following institutions: Illinois Natural History Survey Insect Collection, Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Urbana, Illinois, USA (INHS); Terrestrial Invertebrates Collection of the Charles Darwin Research Station, Puerto Ayora, Santa Cruz, Galápagos, Ecuador (CDRS); Royal Belgian Institute of Natural Sciences, Brussels, Belgium (RBINS).

Results

Invertebrate samples were collected from all eleven caves from all caves zones. Specimens of Entomobryoidea were found from eight caves (Santa Cruz Island: Cueva Aguirre, Cueva Cañón, Cueva Cascajo, Cueva Chato 1, Cueva Chato 2, Cueva Gallardo, La Llegada; Isabella Island: La Cueva de Sucre). The lava tube caves varied from dry and largely barren of organic deposits to moist and containing organic deposits, soils, and water pools. The sites examined during this study are on the slopes of two shield volcanoes – Santa Cruz Island (elevation: 964 m) and Sierra Negra on Isabela Island (elevation: 1124 m). These caves are all in lava flows of sufficient age to have become largely covered with vegetation and to have significant settling and collapse resulting in various surface connections and tube segmentation (e.g., Figs 1B, 2B). Measured light levels ranged from 91,900 lux on the surface to 0 lux in the dark zone, air temperatures ranged from 37.3 °C in direct sunlight and 32.8 °C in the shade on the surface to 21.9 °C in the dark zone, soil temperatures ranged from 31.7 °C on the surface to 20.8 °C in the dark zone, and relative humidity ranged from 37.3% on the surface to 93.7% in dark zone, deep-cave habitats (Fig. 3). Most springtails were taken in Berlese leaf

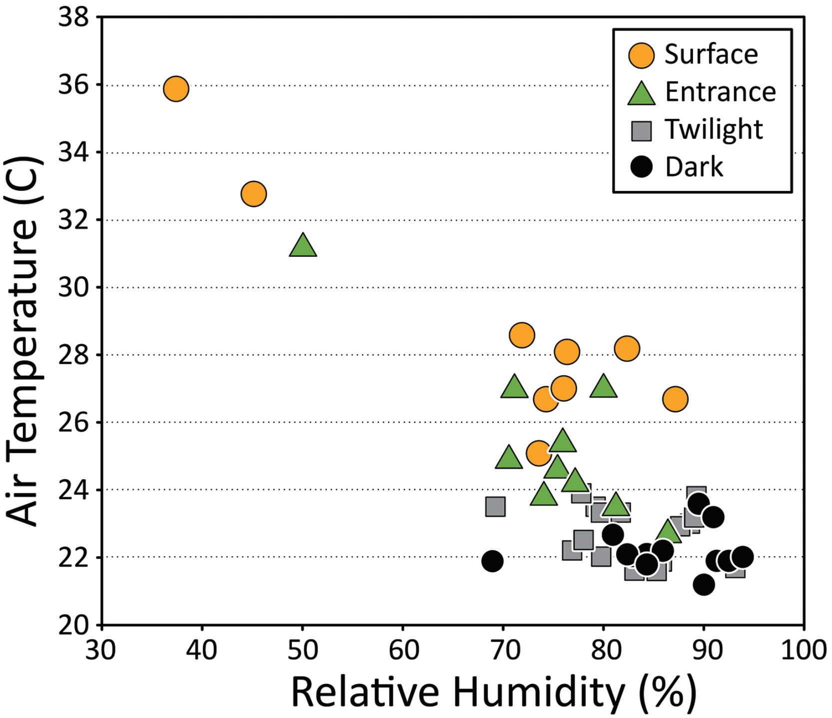

Figure 3. March 2015 relative humidity (%) and air temperature (°C) by cave zone for nine caves (Cueva Cañón, Cueva Cascajo, Cueva Chato 1, Cueva Chato 2, Cueva Gallardo, Cueva JoAnn, Cueva Primicias, Cueva Soyla, and La Llegada) on Santa Cruz Island, Galápagos Islands, Ecuador, where biological sampling took place during this study.

Figure 4. Entrance area of La Llegada (Santa Cruz Island, Galápagos Islands, Ecuador), where *Pseudosinella stewartpecki* sp. n. and *Cyphoderus* cf. *agnotus* were collected. Photo by SJT, 12 March 2014.

litter and moss samples in the entrance zone of caves (e.g., Figs 2B, 4), with specimens individually hand-collected in deep cave habitats, such as drip pools (Fig. 2A) or isolated woody debris.

Taxonomy

Family Entomobryidae

***Heteromurus* (*Heteromurtrella*) *nitens* Yosii, 1964**
Figs 5–6

Descriptive notes. *Color*. Background color white (Fig. 5A), with orange granules scattered across head and body.

Head. Apical pin seta on Ant. IV present (Fig. 5B). Dorsal chaetotaxy of head as in Figure 5C: row An with 7 or 8 Mc; Mc A0, A2, A3, A4 present, A5 present as mc; row M with Mc M1 and M2 displaced anteriorly, forming cluster with series A, mc M0, M3 and M4 present, 2 additional mc present, including M3p, posterior to

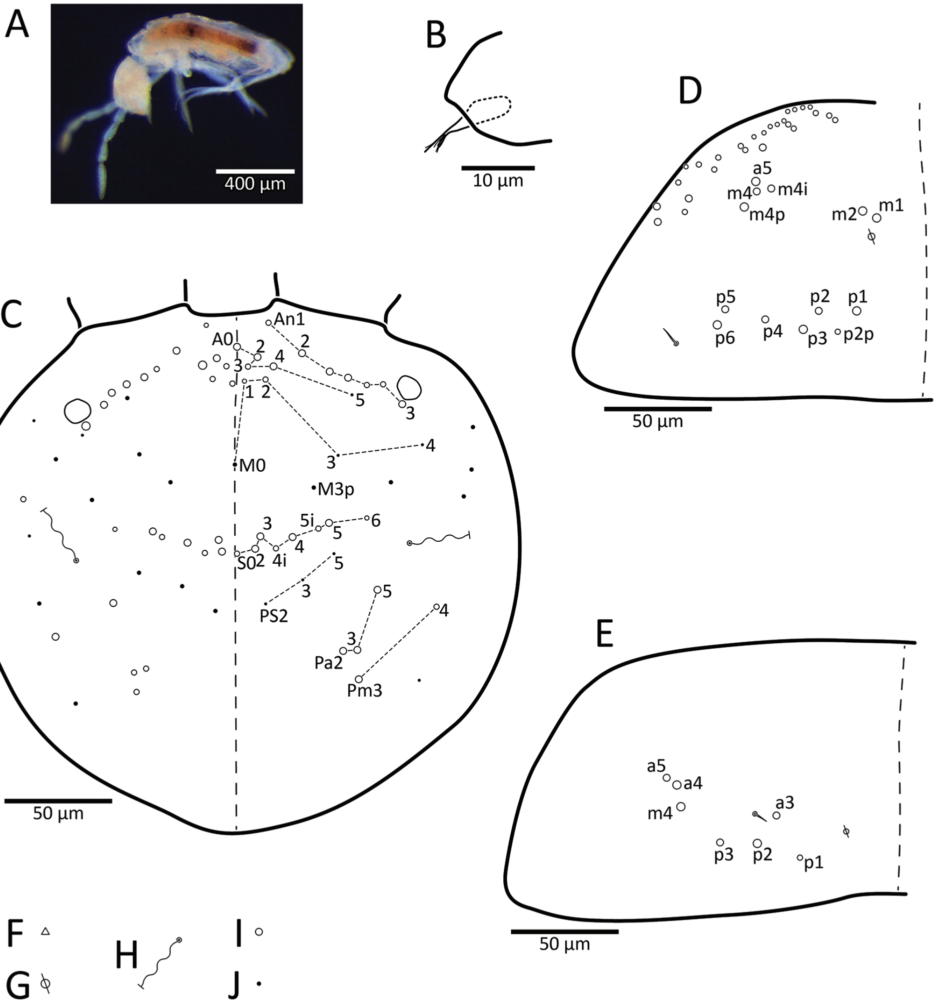

Figure 5. *Heteromurus* (*Heteromurtrella*) *nitens*. **A** habitus (INHS Acc. 567,401) **B** pin sensillum on Ant. IV **C–E** dorsal chaetotaxy: **C** head **D** mesothorax **E** metathorax **F–J** symbols used in illustrations: **F** fan-shaped ciliate microseta associated with bothriotricha **G** pseudopore **H** bothriotricha **I** macroseta **J** meso- or microseta.

M series; row S with 8 Mc, element S1 absent; row Ps with mc Ps2, Ps3, and Ps5; 5 posterior Mc present.

Dorsal body chaetotaxy. Dorsal chaetotaxy of Th. II as in Figure 5D: anterior and medial rows with Mc a5, M1, M2, M4, M4i, M4p; posterior row with 7 Mc. Dorsal chaetotaxy of Th. III as in Figure 5E: with Mc a3, a4, a5, m4, p1, p2 and p3. Dorsal chaetotaxy of Abd. I (Fig. 6A) with 3 Mc (m2, m3, m4). Dorsal chaetotaxy of Abd. II (Fig. 6B) with 2 Mc. Dorsal chaetotaxy of Abd. III (Fig. 6C) with Mc m3 and pm6 present. Dorsal chaetotaxy of Abd. IV (Fig. 6D) with 4 inner Mc; mc T3, ciliate.

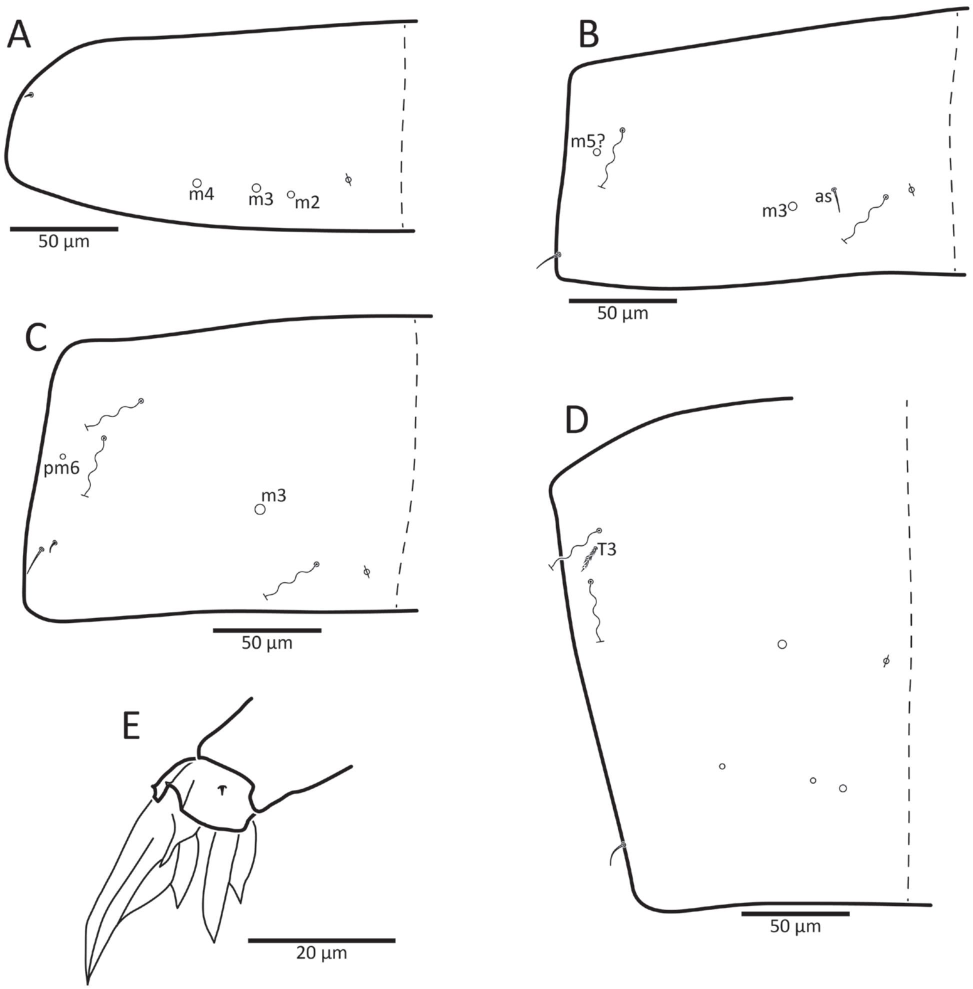

Figure 6. *Heteromurus* (*Heteromurtrella*) *nitens*. **A–D** dorsal chaetotaxy: **A** Abd. I **B** Abd. II **C** Abd. III **D** Abd. IV **E** hind claw complex.

Remarks. The chaetotaxy of the single individual collected (an adult female), is identical to that described for *H. nitens* from the Kingdom of Tonga by Yosii (1964). The individual from Galápagos differs from Yosii's (1964) description only in claw morphology, our specimen lacks the inner unpaired ungual tooth (Fig. 6E). This is the first record of the genus *Heteromurus* from the Galápagos Islands.

The description above is intended to supplement the original description by Yosii (1964) with detailed descriptions and illustrations of dorsal head and body chaetotaxy. Most scales and setae had fallen off our specimen during transport making it difficult to differentiate elements (i.e., scales, sensilla, microsetae). Therefore, the mc on Th. II-Abd. IV are not illustrated. Some sensilla may have also been omitted from the illustrations if not visible on the specimen.

This species' peculiar distribution (reported from Tonga and Galápagos) indicates its range extends across the Pacific Ocean and may occur on other Pacific Islands. The difference in number of inner ungual teeth and vast geographical distance separating the populations of Tonga and Galápagos hint at a species level differentiation. However, in view of the general morphological similarity and a lack of sufficient material, we have chosen not to erect a new name for this single individual.

Distribution. 'Eua Island, Kingdom of Tonga (Yosii 1964) and Isabela Island, Galápagos, Ecuador (new record).

Material examined. Ecuador, Galápagos, Isabela Island: 1♀ on slide, La Cueva de Sucre, 21.iii.2014 (G. Hoese), INHS Acc. 567,401.

***Lepidocyrtus nigrosetosus* Folsom, 1927**

Figs 7–12

= *Lepidocyrtus leleupi* Jacquemart, 1976: 145, **syn. n.**

Description. *Size*. Up to 2.6 mm

Color pattern. Background color white or light orange, with dark purple pigment limited to Ant. III-IV, latero-posterior margin of Th. II, lateral margin of Th. III and meso- and metathoracic coxae. Some individuals have and additional irregular purple spot on the base of the furcula, others have no pigment at all, except for the antennae. The black or dark brown look of living specimens is produced by the thick covering of black or dark brown scales (Fig. 7A).

Appendicular scales distribution. Dorsally on Ant. I, femur on middle and hind legs and ventral face of furcula. Scales absent from Ant. II-IV, fore legs, ventral tube and dorsal face of manubrium.

Head. Apical bulb on Ant. IV absent. Dorsal chaetotaxy of the head as in Figures 7B,C: Row An with 7-12 Mc; anterior Mc A0 and A2 present, relatively small but with differentiated sockets, and inserted among a group of enlarged fusiform elements field normally including only Mc A0 (Fig. 7B; fig. 2 in Jacquemart 1976), all other anterior Mc absent; posterior Mc absent, element Ps5 enlarged and fusiform; postocular bothiotrix displaced laterally and inserted behind eyes E and F. Prelabral and all labral setae smooth. Basal seta of outer maxillary palp smooth, subequal to terminal appendage; sublobal plate with 3 seta-like appendages and 1 minute, spine-like appendage on dorsal edge. Lateral appendage on labial papilla E curved anteriorly and short, not reaching tip of papilla. Labial palp with 5 smooth proximal setae. Labial triangle formula as m1M2rEL1L2, A1-5: m1 sometimes coarsely ciliate and always shorter than M2; r short, smooth and conic, sensilla-like (Fig. 8A). Anterior row of post-labial setae smooth or minutely denticulate; setae on posterior rows coarsely ciliate (Fig. 8B, C); cephalic groove with 5-6 +5-6 setae, anterior most seta smooth, setae becoming more coarsely ciliate from anterior to posterior rows; postlabial group C with 1–4 setae; modified post-labial setae 2-3, short, conic and smooth, similar to labial seta r, inserted among lateral columns.

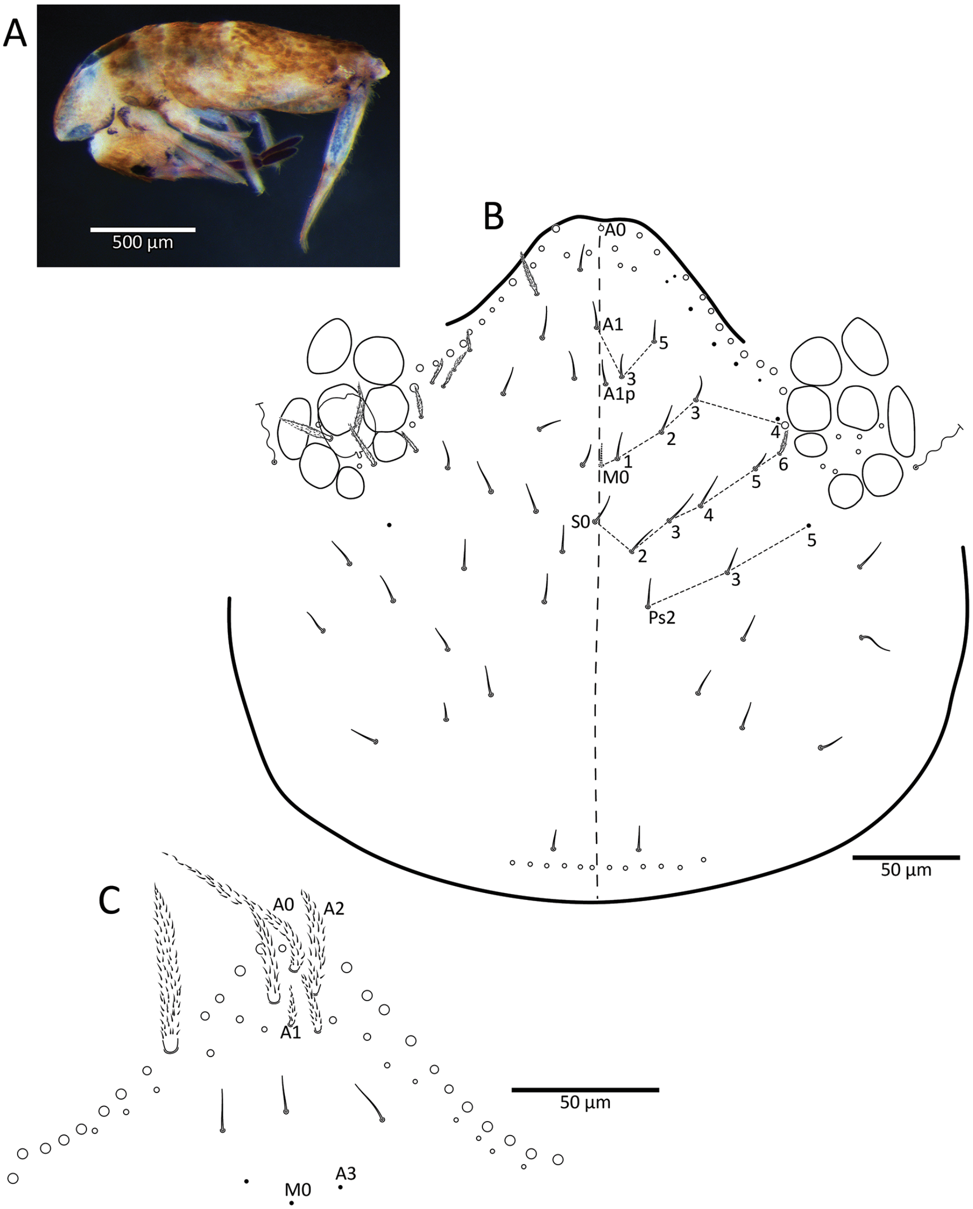

Figure 7. *Lepidocyrtus nigrosetosus*. **A** habitus (INHS Acc. 567,402) **B** dorsal chaetotaxy of head **C** detail of anterior chaetotaxy of head.

Dorsal body chaetotaxy. Dorsal macrosetae formula 00/0233+1+6. Dorsal S-seta 11/011n3; S-microseta 10/10100. Mesothoracic hood produced, anteriorly rounded, partially or completely shadowing head. Meso- and metathoracic chaetotaxy normal, with neither Th. II polychaetosis nor Th. III reductions. Chaetotaxy of Abd. I normal, with a6 present and 11 posterior setae. Abd. II (Fig. 9A, B) with all supplementary

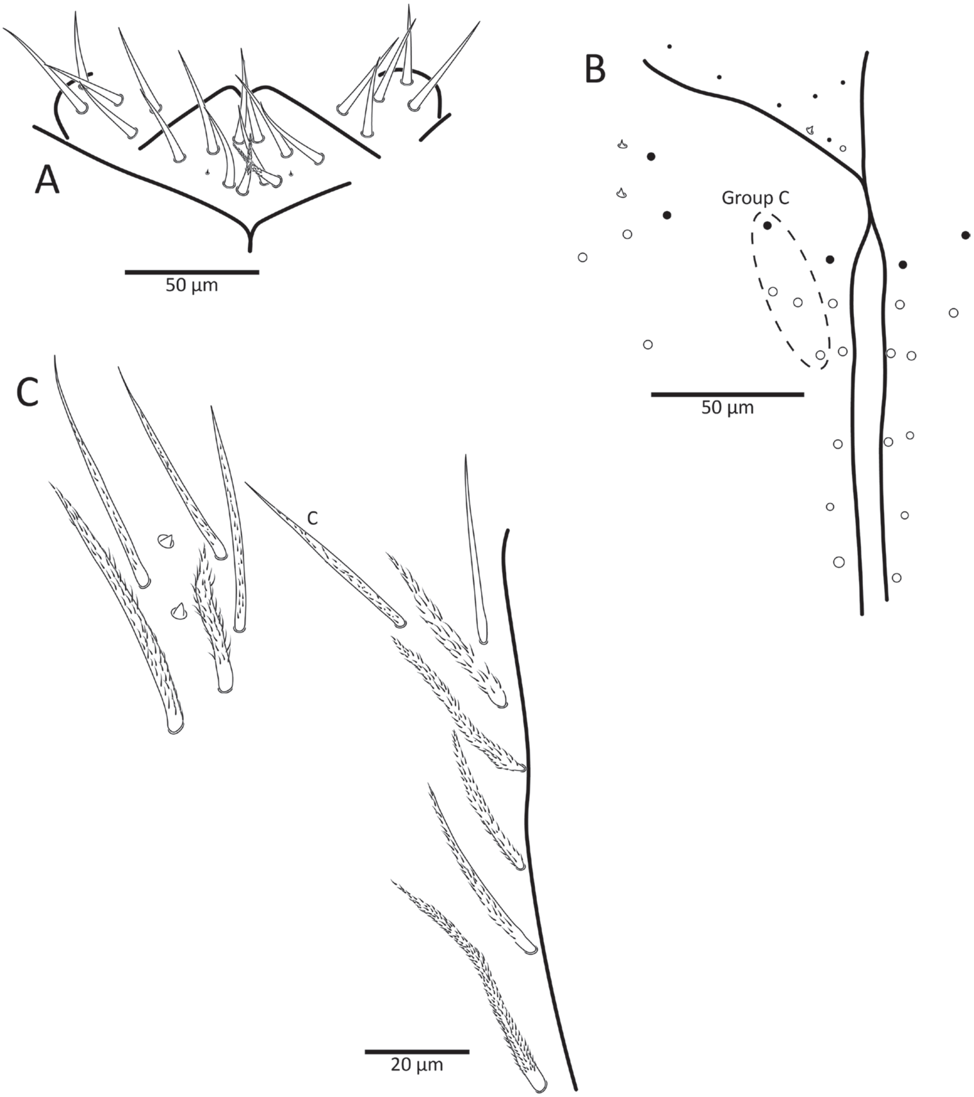

Figure 8. *Lepidocyrtus nigrosetosus*. **A** labial triangle **B** postlabial chaetotaxy **C** detail of postlabial chaetotaxy.

setae fan-shaped; a2 and a6 fusiform and finely ciliate in larger individuals, normal smooth setae in smaller individuals; a3 well anterior and not reaching sensillum as; as subequal to or shorter than a3; Mc m3 and m5 present; p4, m4, and p5 smooth; elements a2p, m3e, m4i and p5p absent. Abd. III (Fig. 9C) with all supplementary setae fan-shaped; a2, a6 and am6 fan-shaped; a3 well anterior and not reaching as; as shorter than a3 and m3; Mc pm6, p6 and p7 present; mc p3, m4, p5 and S-microseta d2 present; a7 smooth or very finely denticulate, displaced laterally, not reaching am6. Abd. IV (Fig. 10) with inner Mc B4, B5, B6 and C1; all supplementary setae of both-

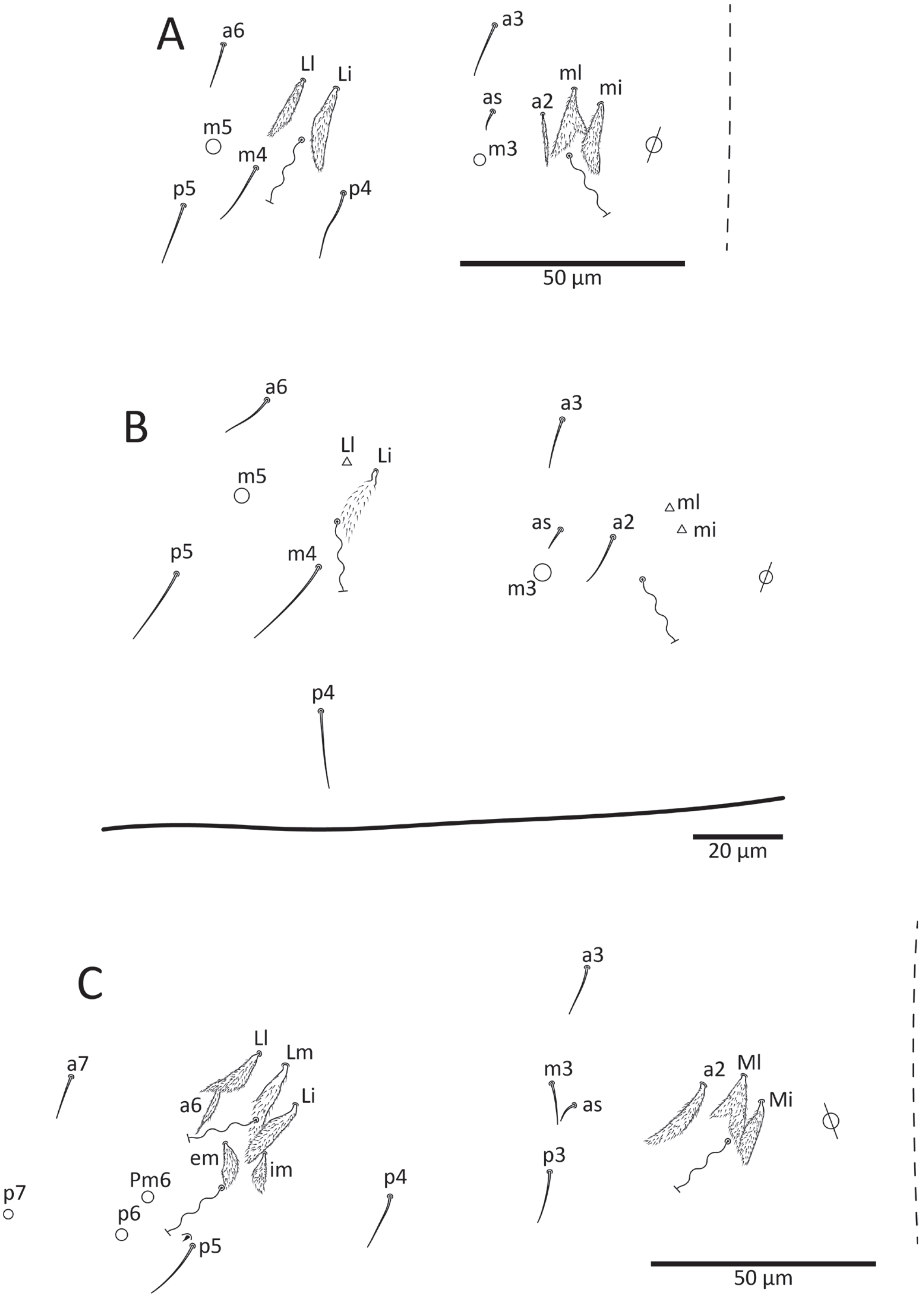

Figure 9. *Lepidocyrtus nigrosetosus*. **A–C** dorsal chaetotaxy: **A** Abd. II **B** Abd. II of *L. leleupi* paratype; **C** Abd. III.

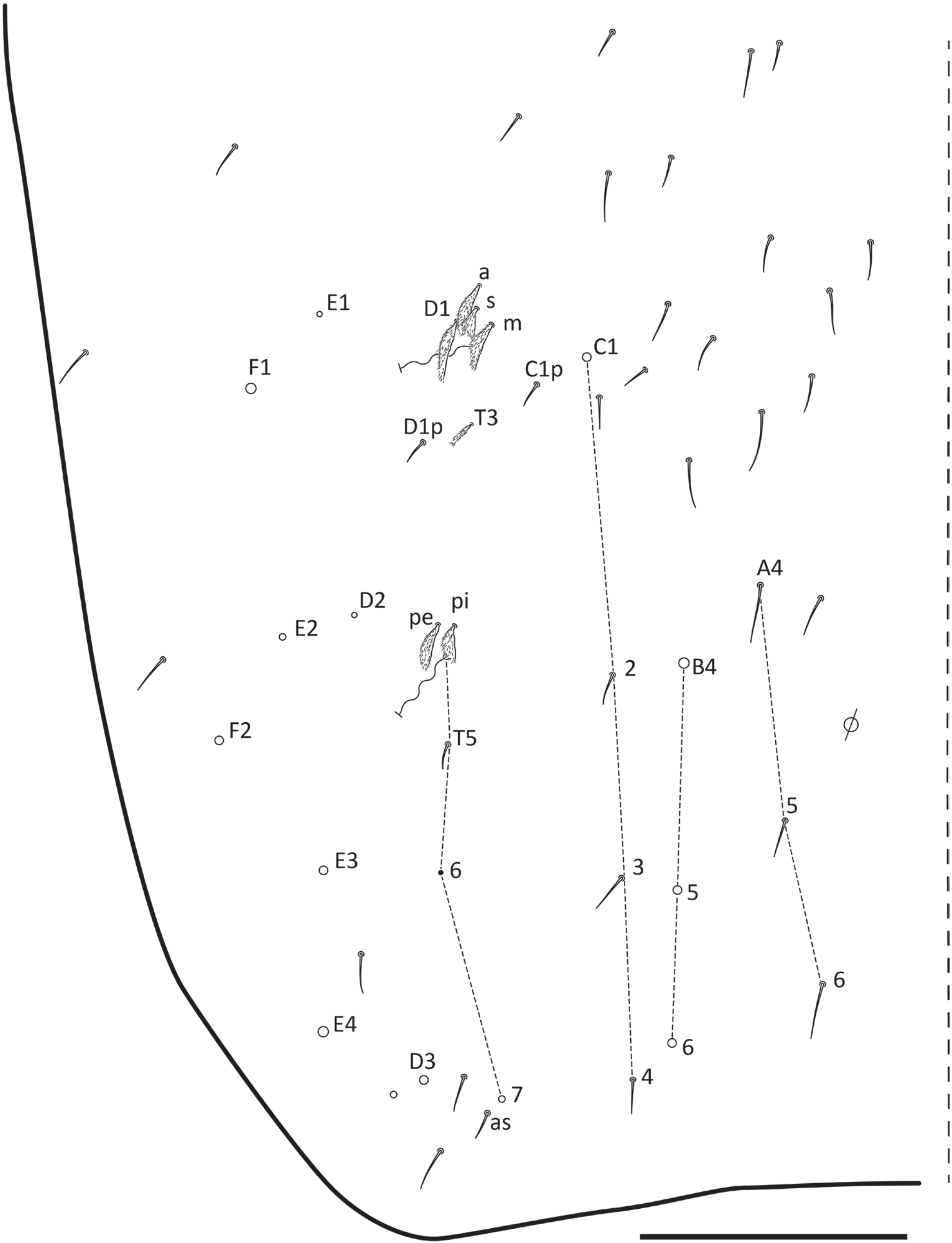

Figure 10. *Lepidocyrtus nigrosetosus*, dorsal chaetotaxy of Abd. IV.

riotrichal complex fan-shaped; mc s present (Fig. 11B); bothriotricha T2 and T4 well separated; mc T3 and D1p subequal, short, displaced anteriorly, closer to T2 than T4, and not reaching Pe or Pi; lateral Mc D3, E2, E3, F1, F2, F3 present (Fig. 11A); posterior setae 10–12.

Legs. Trochanteral organ with up to 41 setae. Tenent hair spatulate on all legs. Unguis with 3-4 inner teeth, distal unpaired tooth sometimes absent; all teeth small; proximal unpaired tooth well separated from basal paired teeth. Fore and middle unguiculi relatively short, strongly truncate, with well marked inner tooth and weakly serrate posterior edge; hind unguiculus usually lanceolate or weakly truncate, rarely strongly truncate, inner tooth absent or weakly delineated; hind unguiculus always clearly longer (surpassing inner proximal unpaired ungual tooth) than fore and middle unguiculi (barely reaching inner proximal unpaired tooth).

Ventral tube. All faces covered by many finely ciliate setae; posterior face with 1+1 smooth setae on distal margin in addition to ciliate setae (Fig. 11C)

Furcula. Manubrium and dens without smooth setae. Basal tubercle of dens apically rounded, somewhat asymmetrical. Mucro with apical tooth slightly longer than basal tooth. Mucronal spine with minute basal denticles.

Remarks. This species is characterized by the enlarged, rounded mesothoracic hood, absence of dorsal head Mc posterior to A2, smooth labial setae, absence of seta m3e on Abd. II, four inner Mc on Abd. IV, heteromorphic unguiculi (truncate on fore and middle legs, lanceolate or weakly truncate on hind leg), and a rounded but somewhat asymmetric tubercle on the dens.

As pointed out by Bernard et al. (2015) the large bodied members of the L. *nigrosetosus* species group (*L. nigrosetosus*, *L. immaculatus* Folsom, 1932, *L. leleupi* Jacquemart, 1976 and *L. geayides* Denis, 1931) are very similar and difficult to distinguish. Bernard et al. (2015) suggested that *L. leleupi*, originally described from the island of Santa Cruz in the Galápagos, was likely to be a junior synonym of *L. nigrosetosus*. Our collections of *Lepidocyrtus* from Santa Cruz fit the color pattern description of *L. leleupi* and at first the specimens were identified as that species, but evaluation of other morphological characters showed the specimens to fit the range of variation reported for *L. nigrosetosus* (Mari Mutt 1986). To confirm these observations we studied the type series of *L. leleupi* deposited in the Royal Belgian Institute of Natural Sciences in Brussels, Belgium.

The type series of *L. leleupi* comprises the holotype, 11 paratypes and 4 additional specimens mounted on slides. The slide labeled holotype holds two individuals. Jacquemart (1976) did not specify which of the two individuals was the holotype, hence here we designate an individual as the holotype (arrow in Figure 12). The holotype and other members of the type series *L. leleupi* lack mc m3e on Abd. II (Fig. 9B) and show the same range of morphological variation seen in specimens of *L. nigrosetosus* from Puerto Rico. For this reason we consider *L. leleupi* a junior synonym of *L. nigrosetosus*. This makes *L. nigrosetosus* the most geographically widespread member of the genus in the Neotropical region (Soto-Adames and Anderson in press).

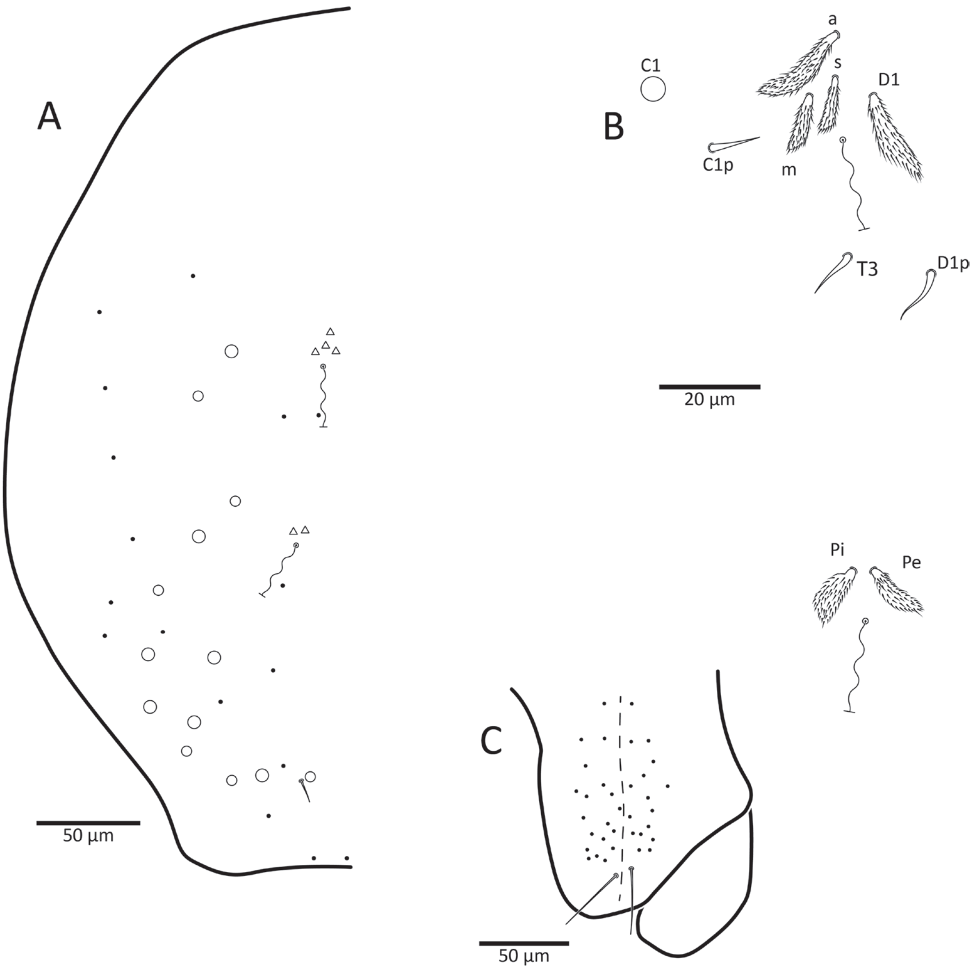

Figure 11. *Lepidocyrtus nigrosetosus*. **A** dorso-lateral chaetotaxy of Abd. IV **B** detail of bothriotricha complex chaetotaxy of Abd. IV **C** posterior face of collophore.

Distribution. Galápagos (new record), Puerto Rico, Colombia, Jamaica (Mari Mutt and Bellinger 1990), St. Thomas US Virgin Islands (Soto-Adames 2002a, 2002b), Brazil (Bellini and Zeppelini 2009), Nevis (Soto-Adames and Anderson in press).

Material examined. Ecuador, Galápagos, Santa Cruz Island: 1♀ on slide, Cueva Cascajo, wet breakdown with leaf litter on entrance floor, 9.iii.2014 (S. Taylor, J. Jacoby and M. Sutton), GLP-030, INHS Acc. 567,402; 1♀ on slide, Cueva Cañón, mossy breakdown near entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-032, INHS Acc. 567,403; 1 on slide, Cueva Aguirre, leaf litter, entrance, 10.iii.2014 (G. Hoese), GLP-046, INHS Acc. 567,404; 1♀ on slide, Cueva Chato 1, on wet soil near entrance, 8.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R.

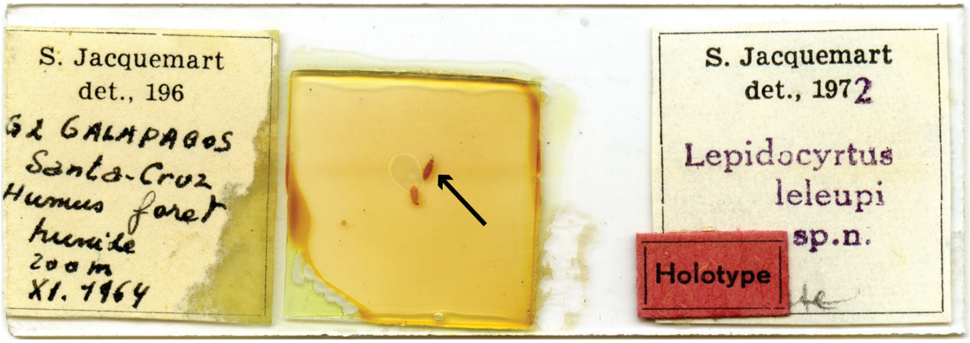

Figure 12. *Lepidocyrtus leleupi* holotype (arrow).

Toomey), GLP-075, INHS Acc. 567,405; 1 on slide, Cueva Chato 1, on wet soil near entrance, 8.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-075, CDRS; 1♂ on slide, Cueva Chato 2, leaf litter at entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-086, INHS Acc. 567,406; 1 on slide, Cueva Chato 2, leaf litter at entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-086, CDRS; 2 on slides, Cueva Chato 2, leaf litter at entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-086, INHS Acc. 567,407 & 567,408.

Lepidocyrtus leleupi Holotype, Galápagos, Santa Cruz, humus forêt humile, 200m, xi.1964; 11 paratypes with same collection information as holotype; 2 other slides with same collection locality, but 22.x.1964; 2 slides Galápagos, Santa Cruz, Station 92B, 17.ii.1974, I.G. 24.965, RBINS.

Pseudosinella intermixta **(Folsom, 1924)**

Fig. 13

Descriptive notes of type specimen. Slide mounted syntype is 0.63 mm in length (Fig. 13A). Unguis with 3 inner teeth: 2 minute basal paired teeth and 1 large unpaired distal tooth (Fig. 13B). Dorsal head Mc A0, A2, A3, and M2 (S) present; Pa5 absent. Th. II with 1 Mc. Th. III without Mc.

Remarks. *Pseudosinella intermixta*, originally described by Folsom (1924) from material collected on Baltra Island (as South Seymore Island), is the only member of the genus with 3+3 eyes, an apical Ant. IV bulb, with head Mc M2, 1 Mc on Th. II, and without Mc on Th. III. Folsom's species is very similar to *P. stewartpecki* sp. n. described below, but can be separated by the presence in *P. inermixta* of head Mc M2 and an enlarged unpaired ungual tooth that is larger than both inner paired teeth.

Folsom's (1924) description of *P. intermixta* is relatively incomplete, lacking details for many important characters, thus it was necessary to revisit the type material to determine if our specimens (described below as *P. stewartpecki* sp. n.) differed from

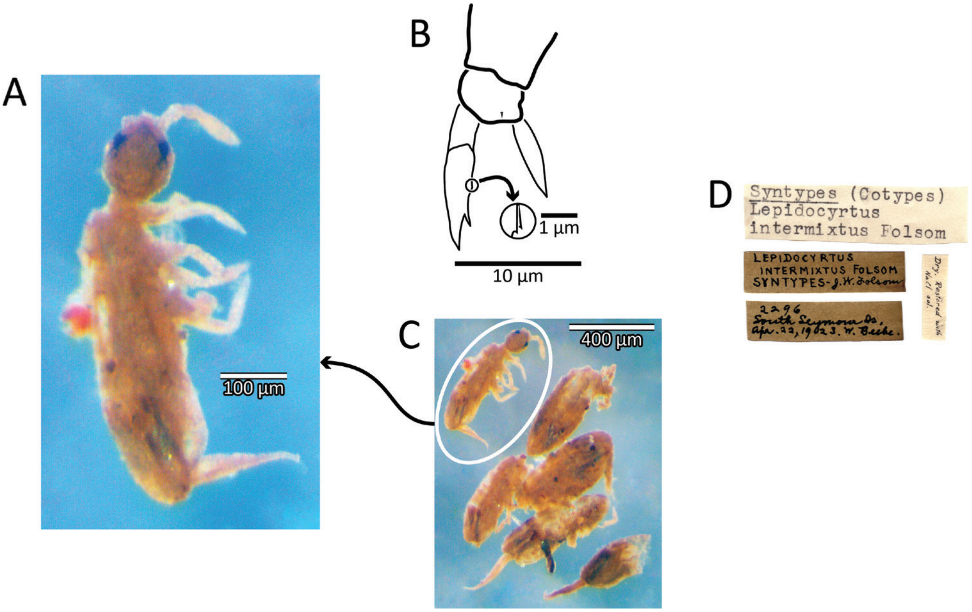

Figure 13. *Pseudosinella intermixta* syntypes. **A** syntype specimen chosen for slide mount (INHS Acc. 567,409) **B** hind claw complex **C** detail of preservation condition of syntypes in ethanol **D** original labels in vial.

P. intermixta. The syntypes, stored in ethanol with labels (Fig. 13C, D), were in extremely poor condition. Despite their poor condition, after slide mounting the most complete specimen (Fig. 13A) we were able to supplement Folsom's (1924) description with the few additional characters provided above.

Folsom (1924) described and illustrated *P. intermixta* with only 2 subequal inner teeth on the unguis. However, the mounted syntype clearly has 3 inner teeth: 2 minute paired teeth and 1 large unpaired distal tooth (Fig. 13B). Due to old age and poor preservation, dorsal chaetotaxy is mostly obscured on the mounted type specimen, but the head clearly carries Mc M2.

Distribution. Baltra Island, Galápagos, Ecuador (Folsom 1924).

Material examined. *Syntypes*, 1 on slide, 5 in vial, Ecuador, Galápagos, Baltra (South Seymour) Island, Apr. 22, 1923, coll. W. Beebe, 2296, dry restored with NaCl sol.; INHS Acc. 567,409 (slide mounted syntype) & 567,410 (syntypes in alcohol).

Pseudosinella stewartpecki **Katz, Soto-Adames & Taylor, sp. n.**

http://zoobank.org/A1A125AB-B9B2-41E5-863F-7A17BBA70C63

Figs 14–17

Etymology. A patronym honoring Stewart B. Peck (Carleton University, Ottawa) whose work in caves and other habitats has done much to increase our understand-

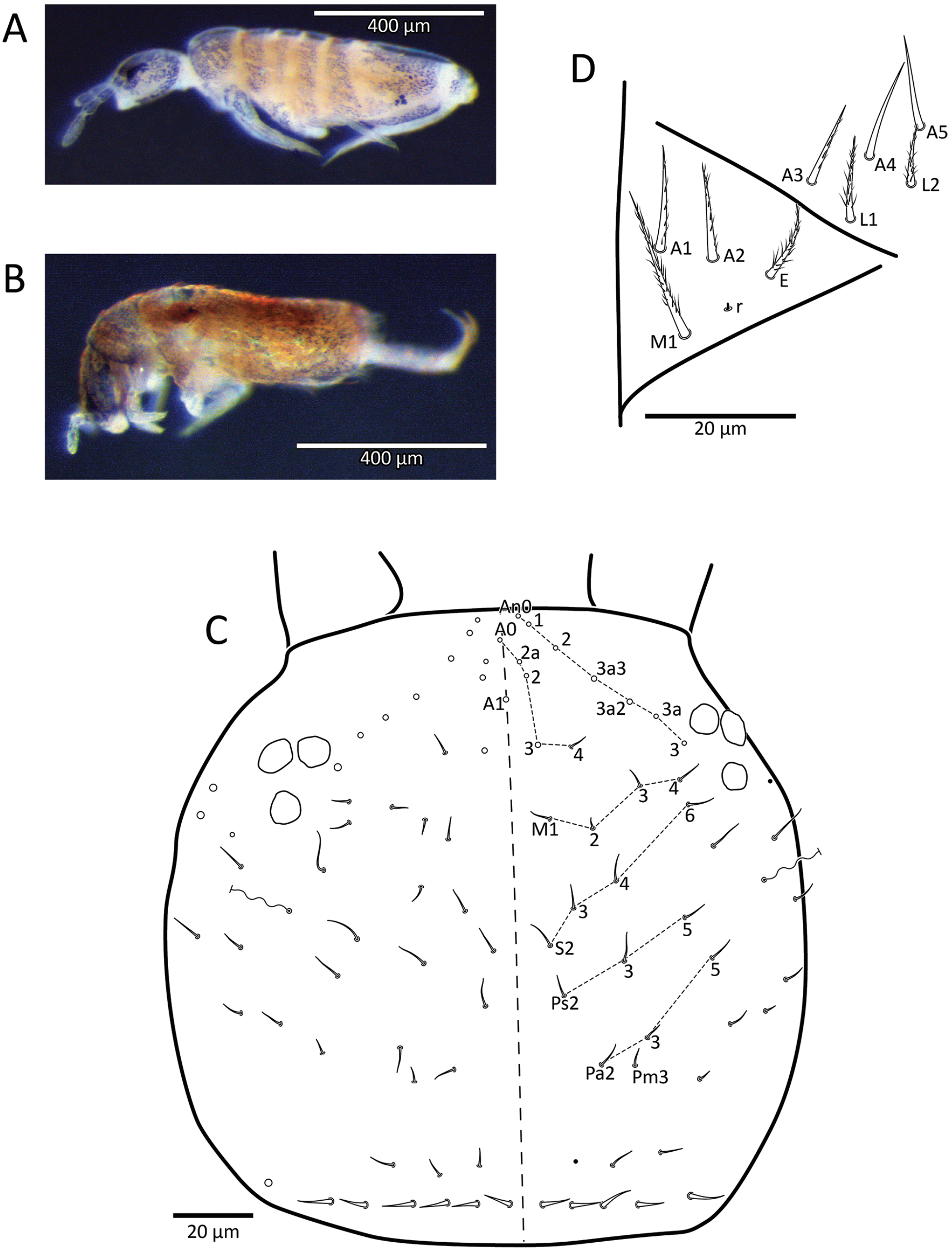

Figure 14. *Pseudosinella stewartpecki* sp. n. **A–B** habitus (INHS Acc. 567,418 & 567,419) **C** dorsal chaetotaxy of head **D** labial triangle.

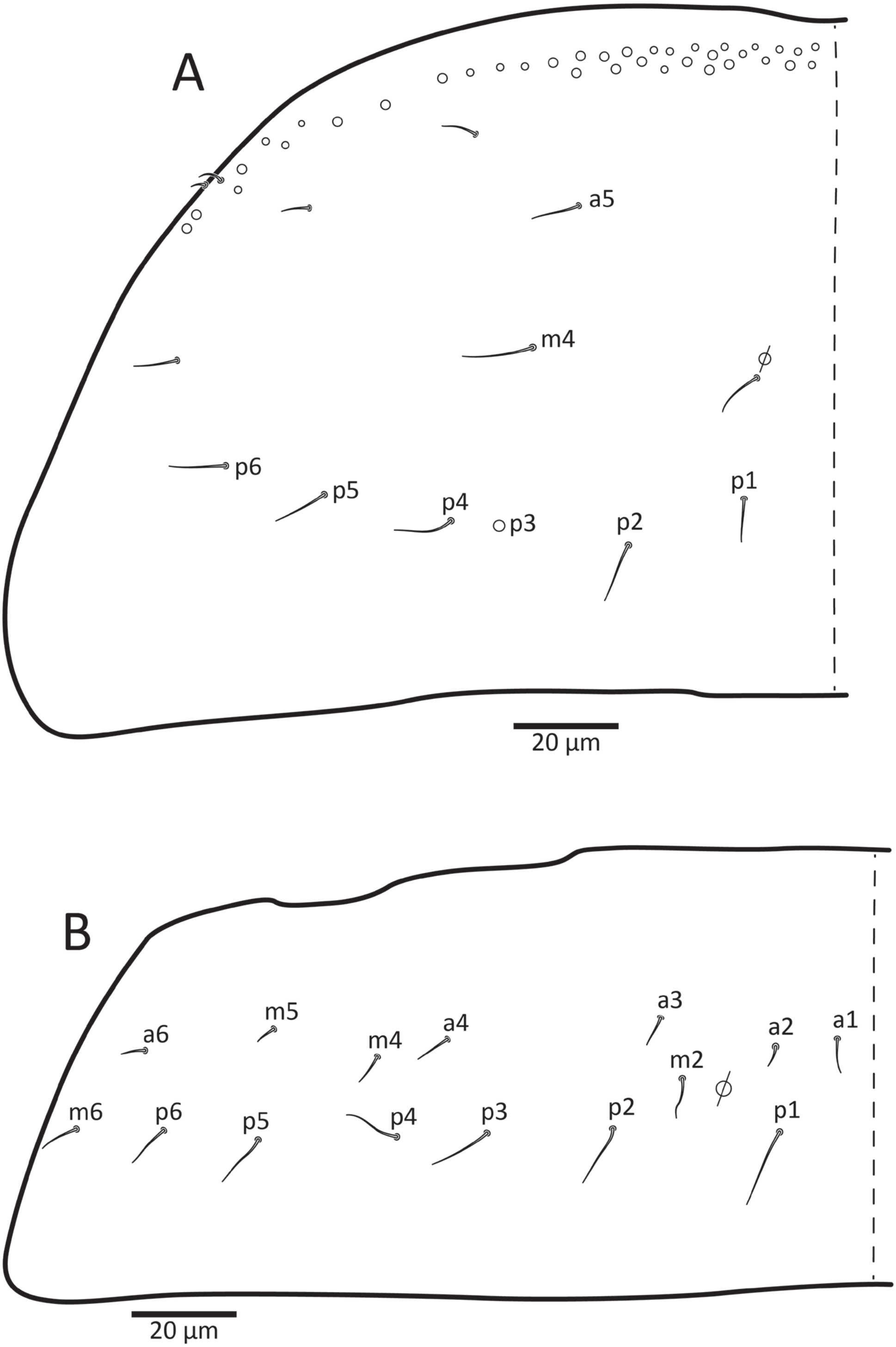

Figure 15. *Pseudosinella stewartpecki* sp. n. **A–B** dorsal chaetotaxy: **A** Th. II **B** Th. III.

ing of invertebrate biodiversity in the Galápagos Islands and throughout the Western Hemisphere.

Type material. *Holotype*, ♂ on slide, Ecuador, Galápagos, Santa Cruz Island: La Llegada, leaf litter from entrance, 12.iii.2014 (C. Plowman, D. Butler and G. Hoese), GLP-095, INHS Acc. 567,414.

Paratypes, Ecuador, Galápagos, Santa Cruz Island: 2♂ on slides, La Llegada, leaf litter from entrance, 12.iii.2014 (C. Plowman, D. Butler and G. Hoese), GLP-095, INHS Acc. 567,415 & 467,416; 2♀ on slides, La Llegada, leaf litter from entrance, 12.iii.2014 (C. Plowman, D. Butler and G. Hoese), GLP-095, INHS Acc. 567,417 & 467,418; 2 on slides, La Llegada, leaf litter from entrance, 12.iii.2014 (C. Plowman, D. Butler and G. Hoese), GLP-095, INHS Acc. 567,419 & 467,420; 1 on slide, La Llegada, leaf litter from entrance, 12.iii.2014 (C. Plowman, D. Butler and G. Hoese), GLP-095, CDRS.

Description. *Body shape and color pattern*. Maximum body length 1.14mm (♀) and 0.85mm (♂). Body with uniformly light blue pigment and white (rarely light orange) background (Fig. 14A, B).

Appendicular scales distribution. Scales present on head, body and ventral face of furcula. Antennae, legs, ventral tube and dorsal face of furcula without scales.

Head. Apical bulb of Ant. IV simple, membranous. Subapical sense organ acuminate; length subequal to guard sensillum. Sense organ of Ant. III with 2 normal rods; at least 3 additional short, blunt sensilla. Eyes 3+3. Dorsal head Mc (Fig. 14C) A0, A2a, A2 and A3 present, An series with 6+6 Mc (7+7 including An0). Pa5 absent. Prelabral setae weakly ciliate. Proximal labral setae ciliate, medial and distal labral setae smooth. Distal margin of labrum smooth. Outer maxillary lobe with basal and distal setae smooth and subequal. Sublobal plate with 3 appendages, subequal in length. Lateral appendage of labial papilla E reaching tip of papilla. Proximal labial setae smooth. Labial triangle setae formula: M1rEL1L2A1-3A4-5; A1-3 serrate, A4-5 smooth; r minute, smooth and conical; all other posterior setae ciliate (Fig. 14D). Postlabial setae ciliate; 4 setae and 4 scales along ventral groove; modified setae absent.

Body dorsal chaetotaxy. Th. II with Mc P3 present (Fig. 15A): polychaetosis absent; hood not developed. Th. III without Mc (Fig. 15B). Abd. I with 11 posterior mc, a6 present (Fig. 16A). Abd. II (Fig. 16B) with 2 Mc (m3, m5); all supplementary mc associated with bothriotricha acuminate and smooth; mc a2p, m4i, p5p, Lm, and Ll absent; seta a3 anterior to and reaching sensillum as. Abd. III (Fig. 16C) with 2 Mc (pm6, p6); all supplementary mc associated with bothriotricha acuminate and smooth; sensillum d2 and mc c3 and Ll absent; seta a3 anterior and reaching sensillum as; as less than half the length of m3; seta a7 anterior to im and em, reaching am6. Abd. IV (Fig. 17A) with 2 inner (B5, B6) and 7 lateral Mc (T6, D2-3, E2-4, F1); E1 a mc; 1 additional posterior-lateral Mc of uncertain homology present; supplementary mc associated with bothriotricha acuminate and smooth, mc s, a, and Pe absent; mc T3 anterior to and not reaching D1p; mc F2 in row with D2 and E2; posterior setae absent. Scales present on ventral face of furcula. Dens tubercle absent. Mucro with sub-apical tooth slightly longer than apical; basal spine smooth.

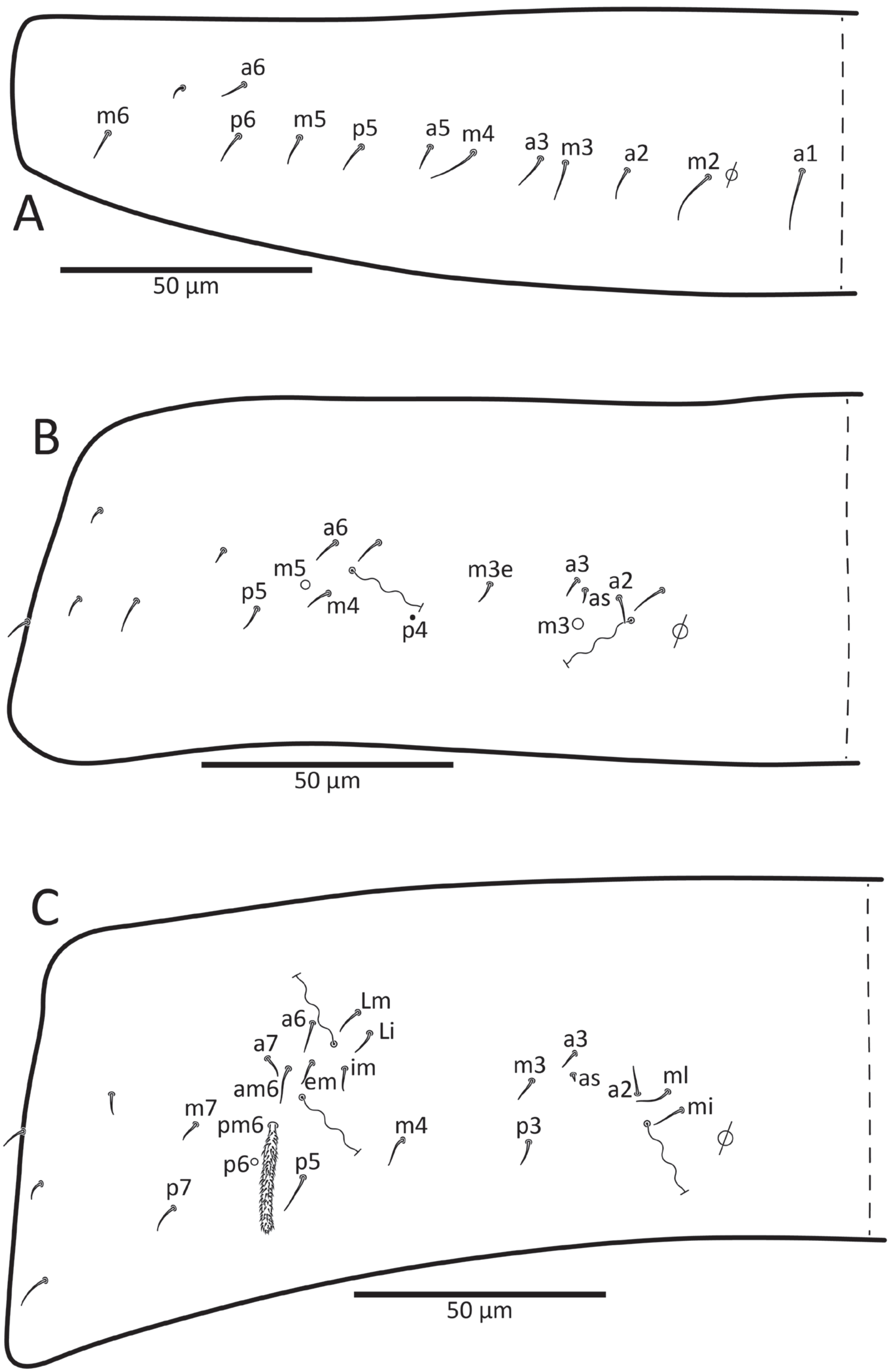

Figure 16. *Pseudosinella stewartpecki* sp. n. **A–C** dorsal chaetotaxy: **A** Abd. I **B** Abd. II **C** Abd. III.

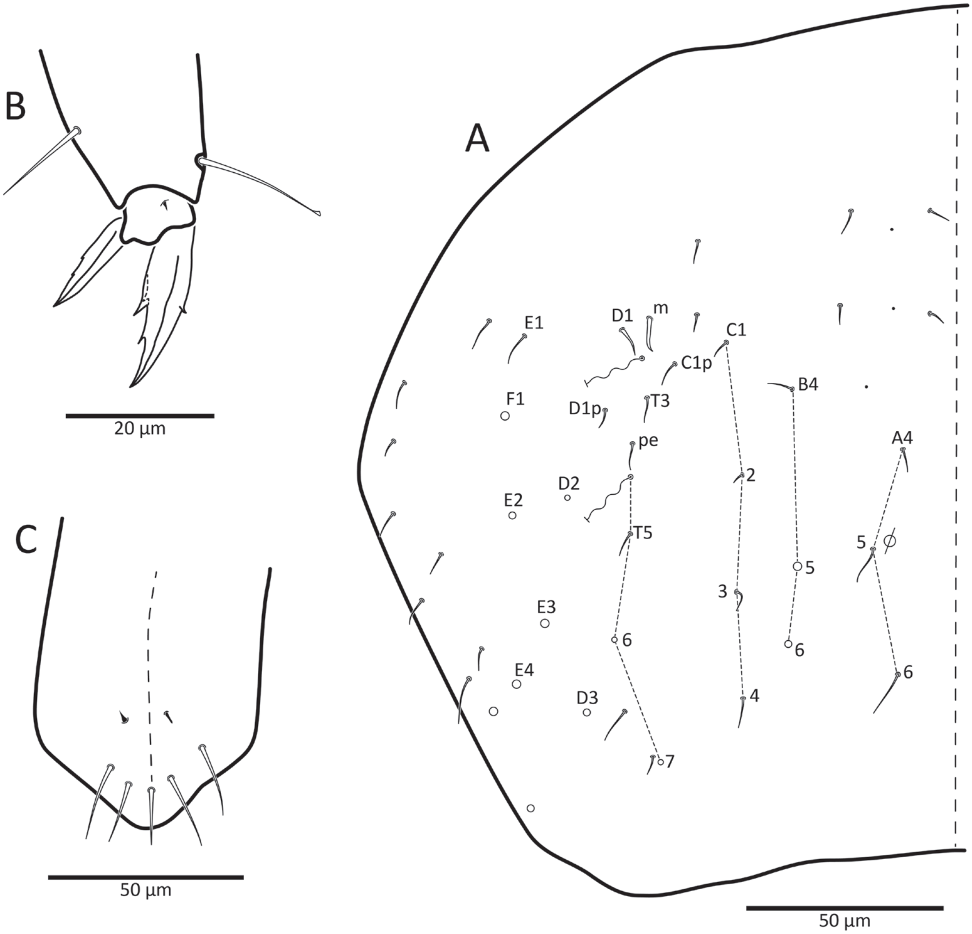

Figure 17. *Pseudosinella stewartpecki* sp. n. **A** dorsal chaetotaxy of Abd. IV **B** hind claw complex **C** posterior face of collophore.

Legs. Trochanteral organ with 5 setae on all specimens when visible. Metatibiotarsi without outstanding posterior blunt setae. Tenent hair spatulate. Unguis with 3 inner teeth: 1 distal unpaired tooth and 2 basal paired teeth with 1 large tooth and 1 small tooth, the latter significantly smaller than distal unpaired tooth. Unguiculus lanceolate with at least 2 or more minute teeth on all legs (Fig. 17B).

Ventral tube. Anterior face with 4+4 or 5+5 ciliate setae; lateral flaps with 5+5 or 6+6 smooth setae; posterior face (Fig. 17C) with 2+2 smooth lateral setae, 1 smooth medial seta, and 1+1 minute conical microsensilla.

Remarks. *Pseudosinella stewartpecki* sp. n. is the only member of the genus with 3+3 eyes, an apical antennal bulb, head series M and S without Mc, with 1 Mc on Th. II, and without Mc on Th. III. This new species is most similar to *P. intermixta*, also described from the Galápagos Islands (Folsom 1924). However, *P. stewartpecki* sp. n. lacks head Mc in rows M and S, and the unpaired inner tooth of the unguis is smaller than the largest of the two inner paired teeth (Fig. 17B), whereas in *P. intermixta*, head Mc M2 is present and the unpaired inner tooth is substantially larger than both paired teeth (Fig. 13B). *Pseudosinella intermixta* was collected on Baltra Island, which can be characterized as dry, lowland (maximum elevation of 100m) habitat. *Pseudosinella stewartpecki* sp. n. was collected in relatively moist, upland (251 m) habitat on Santa Cruz Island. These differences, in morphology and habitat, are sufficient for the separation *P. intermixta* and *P. stewartpecki* sp. n.

Distribution. Santa Cruz Island, Galápagos, Ecuador.

Pseudosinella vulcana Katz, Soto-Adames & Taylor, sp. n.

http://zoobank.org/93AB0B74-D429-4E08-A33E-B4D8E922E222
Figs 18–20

Etymology. Latin, feminine form, alludes to the shield volcanoes of the Galápagos Islands.

Type material. *Holotype*, ♂ on slide, Ecuador, Galápagos, Santa Cruz Island: Cueva Chato 2, leaf litter at entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-086, INHS Acc. 567,411.

Paratypes, Ecuador, Galápagos, Santa Cruz Island: 1♀ on slide, Cueva Chato 2, leaf litter at entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R Toomey), GLP-086, INHS Acc. 567,412; 1♀ on slide, Cueva Chato 2, drip pool, dark zone, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-090, INHS Acc. 567,413; 1♀ on slide, Cueva Chato 2, drip pool, dark zone, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-090, CDRS.

Description. *Body shape and color pattern*. Maximum body length 0.74mm (♀) and 0.68mm (♂). Body white, without pigment, except for minute black eye spot (Fig. 18A).

Appendicular scales distribution. Scales limited to head, body and ventral face of furcula. Antennae, legs, ventral tube and dorsal face of furcula without scales.

Head. Apical bulb of Ant. IV absent; subapical sense organ clubbed, as large as guard sensillum; additional bulb-like sense organ present within a deep pit. Sense organ of Ant. III with 2 normal rods; at least 2 additional short, blunt sensilla present. Eyes 1+1, each within a minute eye spot. Dorsal head chaetotaxy (Fig. 18B) with 5+5 Mc in An series, in addition to Mc A0, A2, A3, and Pa5. Prelabral setae acuminate and weakly ciliate. Proximal labral setae ciliate, medial and distal labral setae smooth. Distal margin of labrum smooth. Outer maxillary lobe with basal and distal setae smooth and subequal; sublobal plate with 3 seta-like appendages, middle appendage 2′ longer and 2′ thicker than outer appendage. Lateral appendage of labial papilla E S-shaped, not reaching tip of papilla. Proximal labial setae smooth. Labial triangle setae formula: M1rEL1L2A1-5; A1-5 smooth; r minute, smooth, and conical; all other posterior setae ciliate (Fig. 18C). Postlabial setae ciliate; 3 setae and 4 scales along each side of ventral groove; modified setae absent.

Dorsal body chaetotaxy. Th. II without Mc (Fig. 19A): polychaetosis absent; mesothoracic hood not developed. Th. III without Mc (Fig. 19B). Abd. I (Fig. 19C) with 9

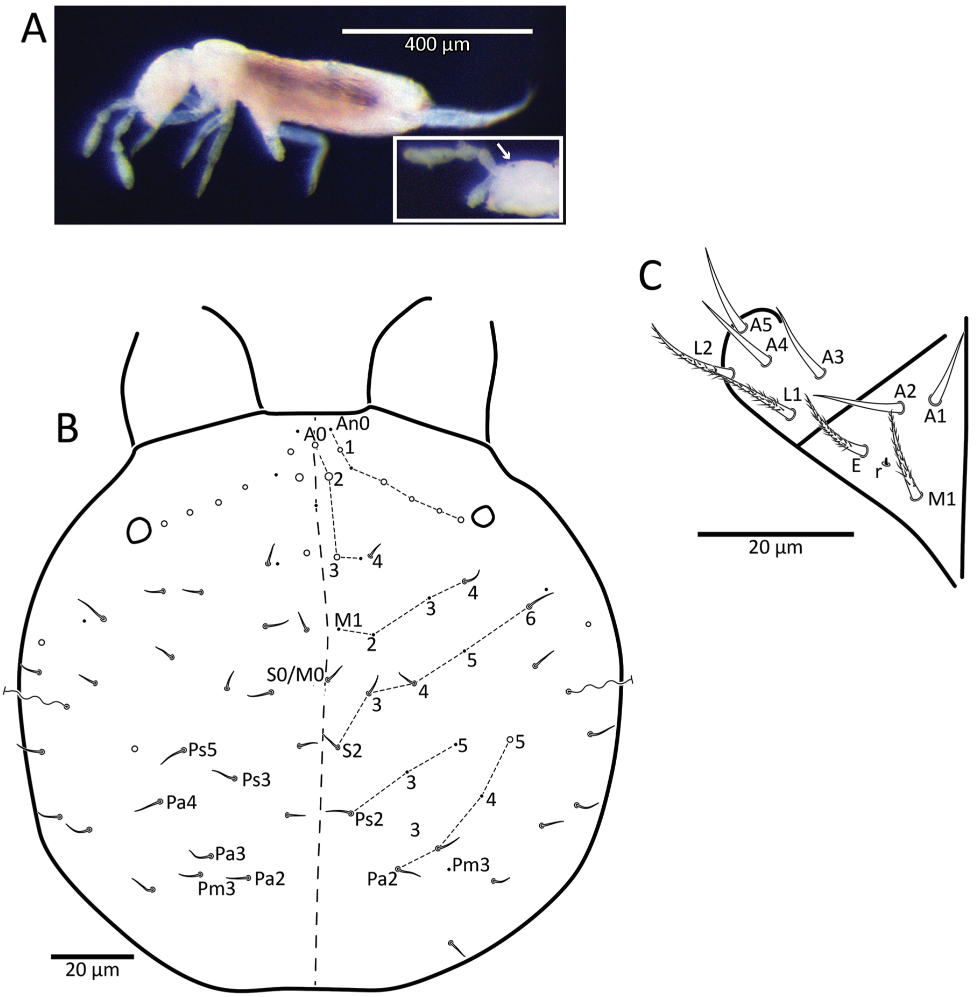

Figure 18. *Pseudosinella vulcana* sp. n. **A** habitus (INHS Acc. 567,412) with detail of eyespot (arrow) on another specimen **B** labial triangle **C** dorsal chaetotaxy of head.

posterior mc; seta a6 absent. Abd. II (Fig. 19D) with 3 Mc (m3, m5, a2); Mc a2 short, thickened distally, with relatively small socket; supplementary setae mi, Li, Ll and m4i associated with bothriotricha m2 and a5 ciliate and weakly fan-shaped/truncate; all other mc acuminate and smooth; mc a2p and Lm, absent; seta a3 external to and half the length of as. Abd. III (Fig. 20A) with 2 Mc (pm6, p6); supplementary mc mi, ml, a2, Li, Lm, im and a6 weakly fan-shaped and ciliate, all other mc smooth; sensillum d2 and mc c3 and Ll absent; seta a3 anterior to and nearly reaching sensillum as; as twice as long as m3; seta a7 posterior to im and em and external to am6. Abd. IV (Fig. 20B) with 2 inner (B5, C1) and 4 lateral Mc (D3, E2, E3, F1); at least 1 additional posterior-lateral Mc of uncertain homology present; supplementary mc associated with bothriotricha weakly fan-shaped and ciliate; seta s present; mc T3 anterior to and not reaching D1p; posterior setae absent.

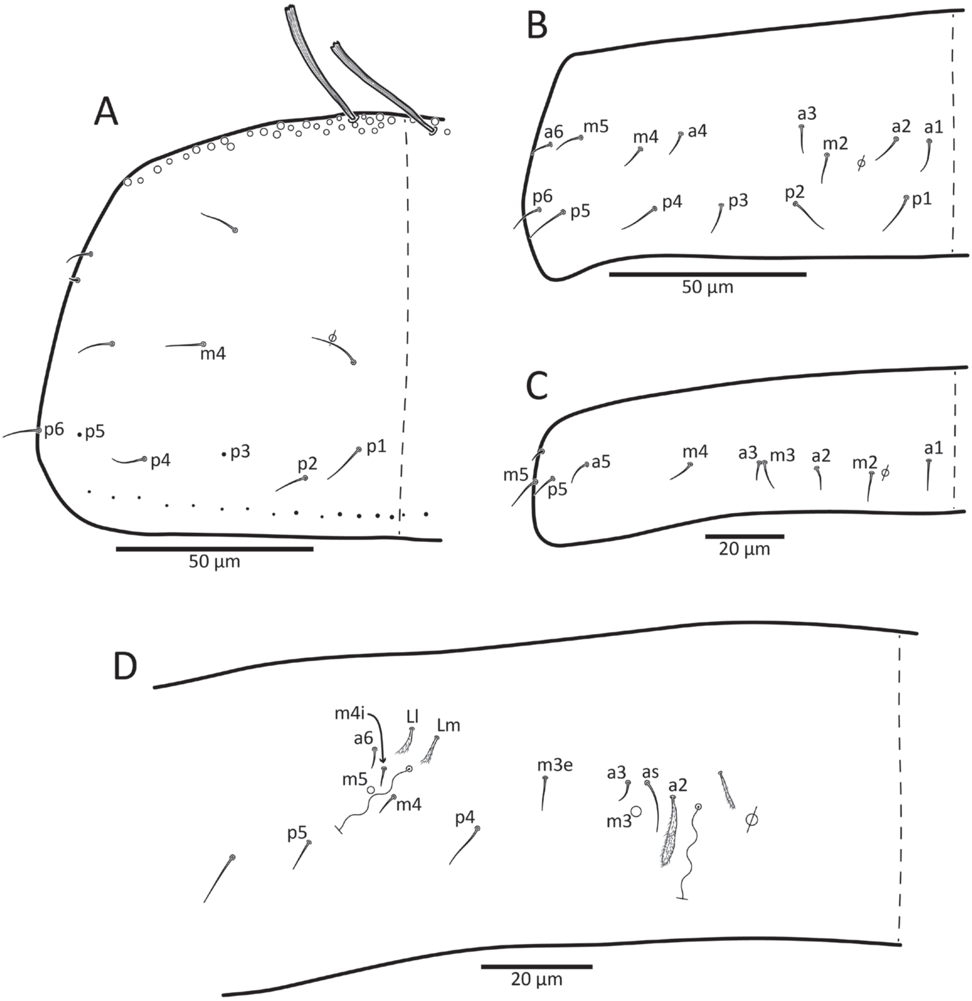

Figure 19. *Pseudosinella vulcana sp. n.* **A–D** dorsal chaetotaxy: **A** Th. II **B** Th. III **C** Abd. I **D** Abd. II.

Legs. Trochanteral organ with up to 9 setae. Metatibiotarsi with 2 outstanding posterior blunt seta. Tenent hair short and acuminate. Unguis with 4 inner teeth; 1 large wing-like inner tooth with 2 basal minute paired lateral teeth, and 1 unpaired proximal tooth. Unguiculus basally swollen on all legs; with 1 large outer wing tooth (Fig. 20C).

Ventral tube. Lateral flaps with 4+4 or 5+5 smooth setae, anterior face with 4+4 ciliate setae, and posterior face (Fig. 20D) with 1+1 smooth setae and 1+1 minute conic microsensilla.

Furcula. Dens tubercle absent. Mucro with sub-apical tooth larger than apical tooth; basal spine smooth.

Remarks. *Pseudosinella vulcana* sp. n. is the only member of the genus with 1+1 eyes and with a wing tooth on both the unguis and unguiculus. This new species is most similar to *P. biunguiculata* Ellis, 1967, *sensu* Mari Mutt (1986), but differs by

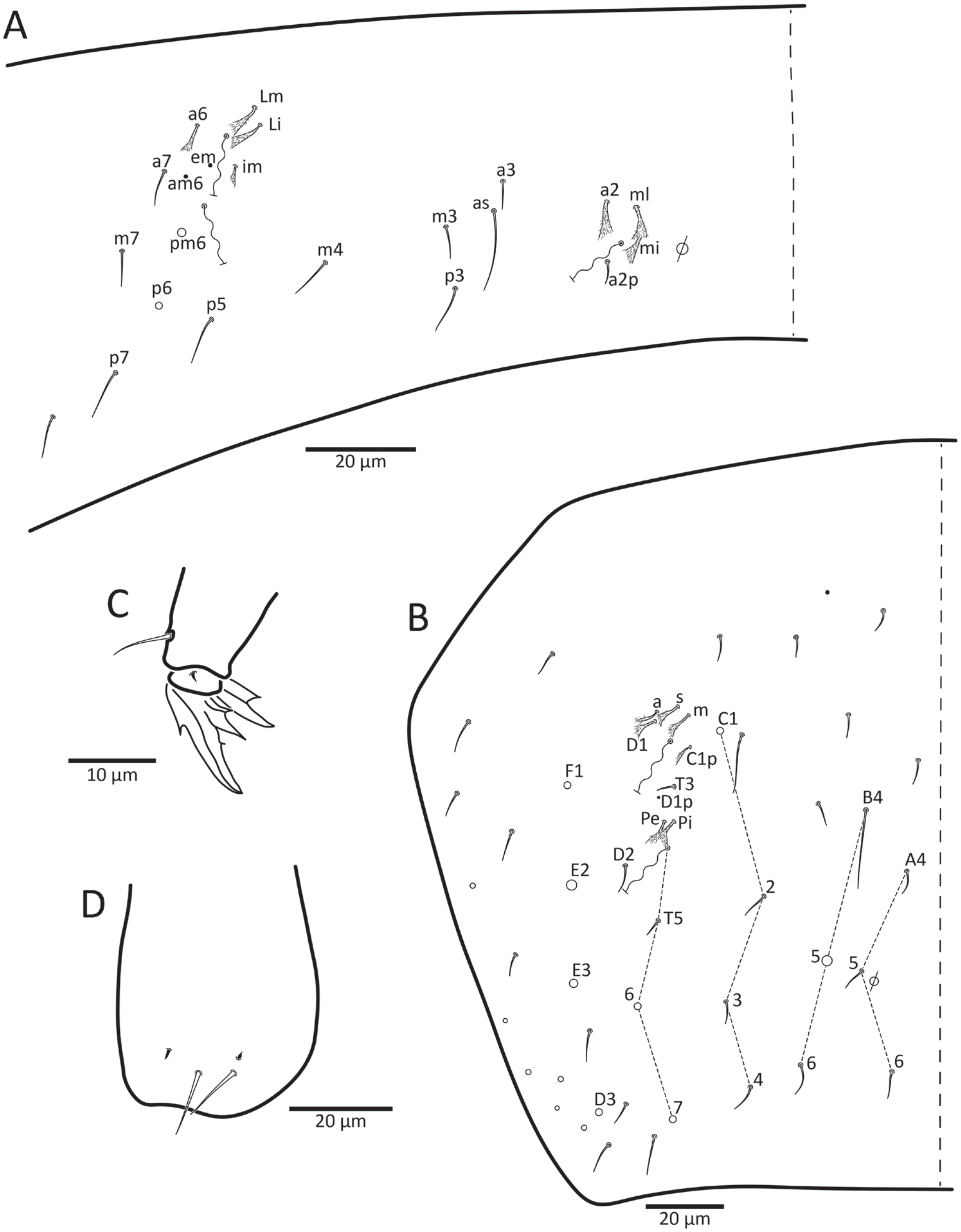

Figure 20. *Pseudosinella vulcana* sp. n. **A–B** dorsal chaetotaxy: **A** Abd. III **B** Abd. IV **C** prothoracic claw complex **D** posterior face of collophore.

having 1+1 eyes, Abd. IV supplemental seta s present (Fig. 20B), and only 1+1 paired setae on the posterior face of the collophore (Fig. 20D), where as in *P. biunguiculata* eyes and supplemental seta s are absent and the posterior face of the collophore has 2+2 paired setae and 1 unpaired medial seta.

Pseudosinella vulcana sp. n. has 2 thickened, apically blunt metatibiotarsal setae, which was originally thought to differ from *P. biunguiculata* since Mari Mutt (1986) described only 1 blunt metatibiotarsal seta. However, we observed 2 metatibiotarsal setae on *P. biunguiculata* from Puerto Rico (Guajataca Commonwealth Forest, at end of trail #10, leaf litter, 19.v.2009, F. Soto, coll.). *Pseudosinella caoi* Chen, Wang & Christiansen, 2002 and *Pseudosinella fujiokai* Yosii, 1964, *sensu* Christiansen and Bellinger (1992), also have blunt metatibiotarsal setae, ungual wing teeth, Head Mc A3 (R2), and lack Mc on Th. II and Th. III, but can be differentiated from *Pseudosinella vulcana* sp. n. by characters outlined in Table 2.

Mari Mutt (1986) described 3 morphologically distinct forms among and within populations of *P. biunguiculata* in Puerto Rico that differ in dorsal chaetotaxy (presence/absence of head Mc A3 (R2)) and tenent hair morphology (clavate/acuminate). He also noted differences between the Puerto Rican forms and Ellis' (1967) type specimens; primarily the absence of head Mc A2 (R1), A3 (R2), and Pa5 (Po) on the holotype. Furthermore, the original description of *P. biunguiculata* does not show the presence of m4i on Abd. II, which is distinctly present in the Puerto Rico populations. The high levels of morphological variation exhibited among these forms suggest that *P. biunguiculata* represents a species complex: small and seemingly insignificant differences in morphology have been shown to correlate with large genetic distances among populations, indicating the presence of species complexes (Porco et al. 2012, Cicconardi et al. 2013, Katz et al. 2015a). In fact, Soto-Adames (2002b) observed large genetic differences between sympatric individuals of Puerto Rican *P. biunguiculata*, but these differences could not be correlated with differences in morphology due to destructive DNA extraction methods. Additional investigations utilizing morphological and molecular data may clarify species-level relationships among populations of *P. biunguiculata*, a species with a widespread neotropical distribution. See Table 2 for a list of diagnostic characters separating the different forms and descriptions of *P. biunguiculata*.

Pseudosinella vulcana sp. n. was collected from entrance and from the surface of a drip pool (Fig. 2A) within the dark zone, in generally cool, moist, low light conditions (Table 3), suggesting the species may be a troglophile.

Distribution. Santa Cruz Island, Galápagos, Ecuador.

Coecobrya sp. A

Fig. 21A

Remarks. A single juvenile male with 1+1 eyes was collected on the surface (Table 3) at Cueva Gallardo. Four species of *Coecobrya* with 1+1 eyes have been described (Xu and Zhang 2015): *C. boneti* (Denis, 1948), *C. indonesiensis* (Chen & Deharveng, 1997), *C. sanmingensis* Xu & Zhang, 2015, and *C. tukmeas* Zhang, Deharveng & Chen, 2009. Our specimen is very similar to the four species listed above, but the combination of characters on the labial triangle, Abd. III, and unguiculus suggest our specimen represents an undescribed species. Unfortunately, a single juvenile is insufficient material on

Table 2. Diagnostic characters of *Pseudosinella vulcana* sp. n. in relation to *P. biunguiculata* (4 morphological forms from Puerto Rico with locations(s) indicated in parentheses, and the holotype), *P. fujiokai*, and *P. caoi*. Present (+), absent (-), not reported (?).

Species/forms	Eyes	Labial triangle seta r	Number of blunt setae on metatibiotarsi	Hd A2 (R1)	Hd A3 (R2)	Hd Pa5 (Po)	Abd. 2 m4	Abd. 2 m4i	Abd. 4 suppl. seta s	Tenent hair	Unpaired ungual tooth
Pseudosinella vulcana sp. n.	1+1	+	2	+	+	+	+	+	+	acuminate	+
P. biunguiculata (Guajataca)	0	+	2	+	+	+	+	-	-	acuminate	+
P. biunguiculata (Mayagüez, Caguas)	0	+	1	+	+	+	+	+	-	acuminate	+
P. biunguiculata (Mayagüez, Manatí)	0	+	1	+	-	+	+	+	-	clavate	+
P. biunguiculata (Utuado)	0	+	1	+	-	+	+	+	-	acuminate	+
P. biunguiculata Holotype Ellis, 1967	0	+	1	-	-	-	?	-	-	acuminate	+
P. fujiokai Yosii, 1964	0	+	2	+	+	?	?	-	?	acuminate	- / +
P. caoi Chen et al., 2002	0	-	2-3 (rarely 4)	+	+	+	-	-	-	acuminate	-

Table 3. Environmental conditions associated with March 2014 collections of Entomobryoidea from lava tube sites on Santa Cruz and Isabela islands, Galápagos Islands, Ecuador.

Species	Light (Lux)	Relative humidity (%)	Air temperature (°C)	Soil temperature (°C)	Elevation (m)	Cave zone
Heteromurus (*Heteromurtrella*) *nitens*	-	-	-	-	379	-
Lepidocyrtus nigrosetosus	19–794	75.7–85.6	21.9–27.1	21.3–22.9	275–373	Entrance, Twilight
Pseudosinella stewartpecki sp. n.	-	-	-	-	251	Entrance
Pseudosinella vulcana sp. n.	0–702	75.7–92.3	21.9–25.5	21.3–21.8	373	Entrance, Dark
Coecobrya sp. A	13,260	71.7	28.6	26.7	213	Surface
Entomobrya darwini sp. n.	702–13,260	71.7–75.7	25.5–28.6	21.3–26.7	213–275	Surface, Entrance
Cyphoderus cf. *agnotus*	-	-	-	-	251	Entrance
Salina sp. A	13,260	71.7	28.6	26.7	275	Surface

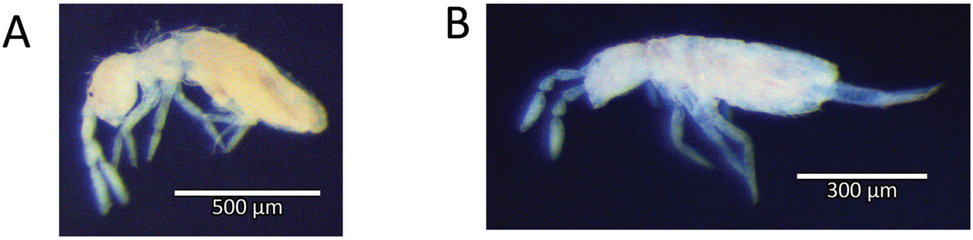

Figure 21. A *Coecobrya* sp. A habitus (INHS Acc. 567,421) **B** *Cyphoderus* cf. *agnotus*, habitus.

which to base a new species description. However, this is the first record of the genus *Coecobrya* from the Galápagos Islands.

Material examined. Ecuador, Galápagos, Santa Cruz Island: 1 juvenile ♂? on slide, Cueva Gallardo, leaf litter near entrance, 8.iii.2014 (S. Taylor and J. Jacoby), GLP-047, INHS Acc. 567,421.

Entomobrya darwini Katz, Soto-Adames & Taylor, sp. n.

http://zoobank.org/12CC806B-39AD-4F2A-B7E8-3535CE594FDD
Figs 22–24

Etymology. A patronym honoring the naturalist Charles R. Darwin (1809–1882) for his work on the Galápagos Islands, which helped inspire his contributions to evolutionary theory. We believe Charles Darwin would have overcome his views on Collembola had he seen the color patterns of this new species: “They [Collembola] are wingless, dull-coloured, minute insects with ugly, almost misshapen head and bodies” (Darwin 1871).

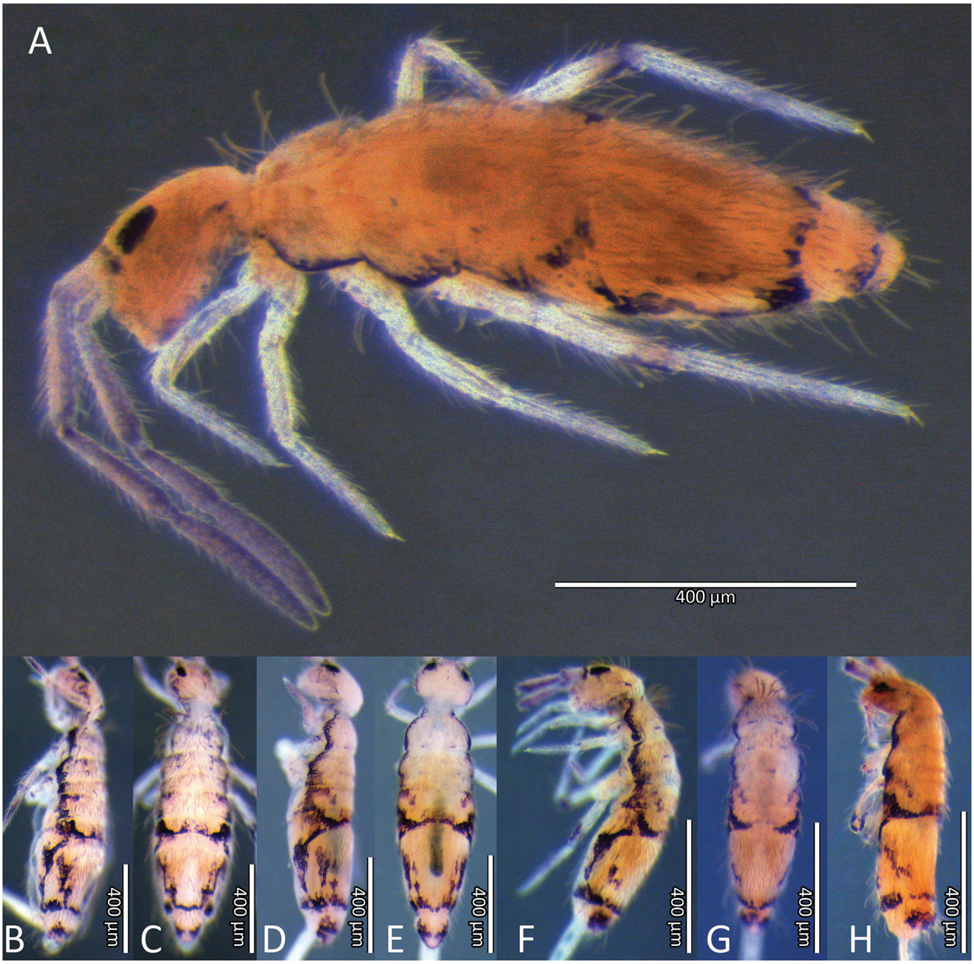

Figure 22. *Entomobrya darwini* sp. n. **A** habitus (INHS 567,423) **B–H** dorsal and lateral color pattern variation of four individuals: **B–C** (INHS Acc. 567,422) **D–E** (INHS Acc. 567,425) **F–G** (INHS Acc. 567,424) **H** (deposited at CDRS).

Type material. *Holotype*, ♀ on slide, Ecuador, Galápagos, Santa Cruz Island: Cueva Chato 2, leaf litter at entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-086, INHS Acc. 567,422.

Paratypes, Ecuador, Galápagos, Santa Cruz Island: 1 on slide, Cueva Cascajo, leaf litter from skylight entrance, 9.iii.2014 (S. Taylor, J. Jacoby and M. Sutton), GLP-031, CDRS; 2♂ on slide, Cueva Cascajo, leaf litter from skylight entrance, 9.iii.2014 (S. Taylor, J. Jacoby and M. Sutton), GLP-031, INHS Acc. 567,423 & 567,424; 1♀ on slide, Cueva Gallardo, leaf litter near entrance, 8.iii.2014 (S. Taylor and J. Jacoby), GLP-047, INHS Acc. 567,425; 1♀ on slide, Cueva Chato 2, leaf litter at entrance, 15.iii.2014 (S. Taylor, J. Jacoby, S. Hagan and R. Toomey), GLP-086, INHS Acc. 567,426.

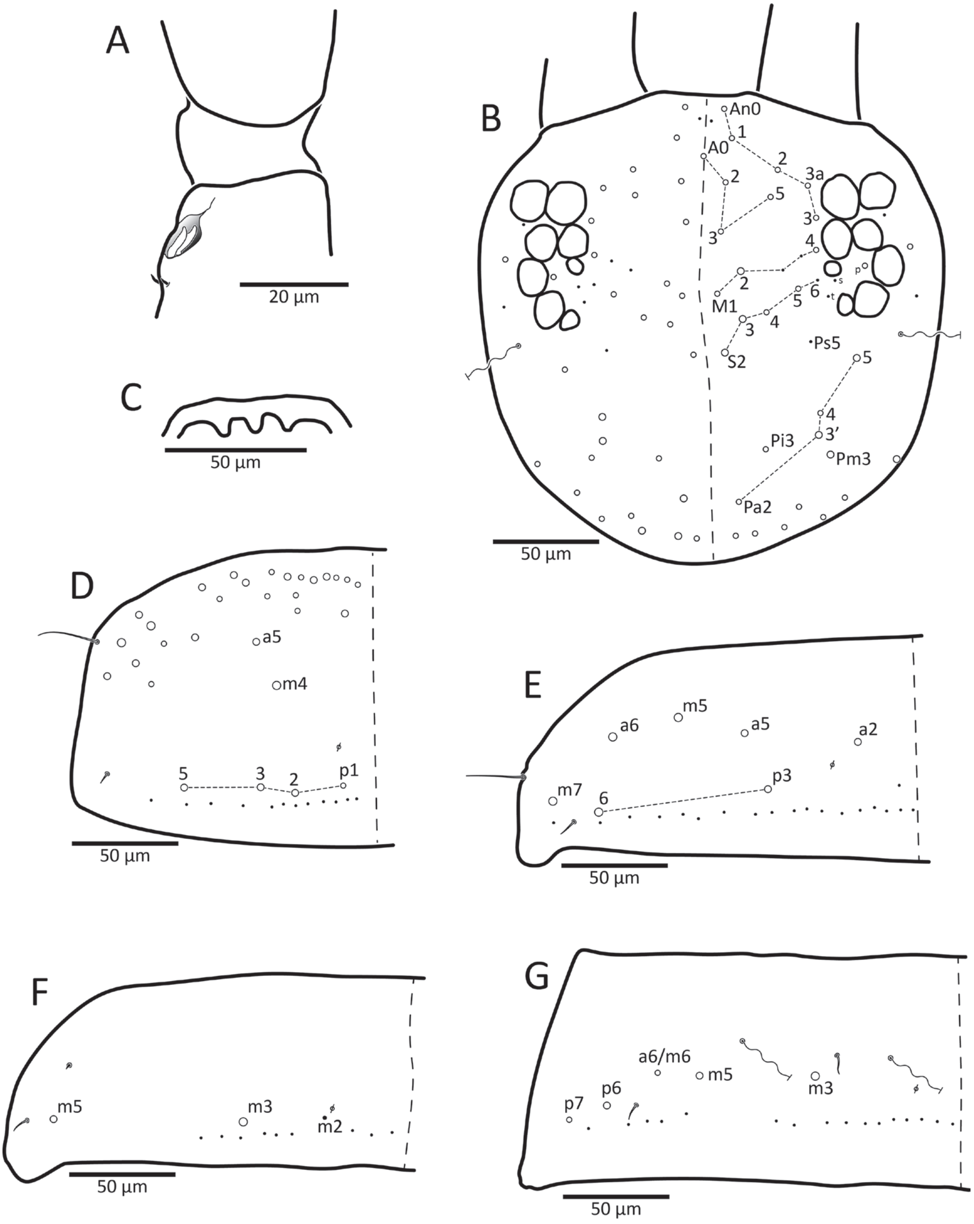

Figure 23. *Entomobrya darwini* sp. n. **A** apical sense organ of Ant. III **B** dorsal chaetotaxy of head **C** labral papillae **D–G** dorsal chaetotaxy: **D** Th. II **E** Th. III **F** Abd. I **G** Abd. II.

Description. *Body shape and color pattern.* Body slightly dorso-ventrally flattened. Length up to 1.57mm (♀) and 1.23mm (♂). Males and females with no obvious difference in color pattern. Color pattern with slight variation (Fig. 22): light orange/tan background with black or dark purple pigment forming two irregular lateral triangles or sometimes broken angled bands on the posterior margin of Abd. III; an additional pair of lateral angled broken bands on Abd. II; dark pigment present along lateral margins of Th. II through Abd. IV, sometimes broken along lateral margin of Abd. III; dark irregular/broken transverse band along posterior margin of Abd. III – Abd. V. Abd. IV usually with an irregular U- or "11"-shaped pattern connecting basally with band along posterior margin. Antennae usually with uniform purple pigment. Legs white, with purple patches on apical end of femora and tibia.

Head. Apical bulb of Ant. IV simple. Apical sense organ of Ant. III enlarged and recessed in shallow pit (Fig. 23A). Apical sense organ on Ant. II with single modified seta. Eyes G and H small and subequal. Eye patch with 3 setae; s, t, and p. Dorsal head chaetotaxy reduced and fixed, no Mc variation observed (Fig. 23B): A6, M3, S'0, S0, S1, S4i, S5i, Ps2, Ps3, and Ps5 always absent; Ps5 present as micro- or mesoseta. Labral setae smooth. Prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae smooth (Fig. 23C). Labial lateral appendage of labial papillae slightly curved, relatively thin, length subequal to apex of labial papilla E. Labial triangle chaetotaxy formula: M1rEL1L2A1-5.

Thorax. Dorsal chaetotaxy of mesothorax reduced and stable, no Mc variation observed (Fig. 23D): a5, m4, and posterior Mc p1, p2, p3, and p5 present; anterolateral sensilla straight and extremely elongated; ms and posterior sensilla present. Dorsal chaetotaxy of metathorax reduced and fixed (Fig. 23E): a2, a5, a6, m5, m7, p3, and p6 present; anterolateral sensilla straight and extremely elongated; additional sensillum observed just internal to P3 in one individual.

Abdomen. Abdominal chaetotaxy reduced and stable; no Mc variation observed. Abd. I with 2 Mc (m3 and m5) present; m_2 present as mesoseta (Fig. 23F). Abd. II with 5 Mc (m3, m5, a6, p6, and p7) (Fig. 23G). Abd. III with 3 Mc (m3, pm6, p6) (Fig. 24A). Abd. IV with 2 inner Mc and up to 11 outer Mc (Fig. 24B). Mucronal sub-apical tooth larger than apical tooth (Fig. 24C).

Legs. Trochanteral organ (Fig. 24D) with up to 9 setae in a triangular pattern; setae thick and apically recurved, increasing in size toward distal margin of trochanter. Unguis (Fig. 24E) with 4 inner teeth: 2 paired basal teeth located approximately middle of inner claw length, and 2 unpaired distal teeth; 3 short, basal outer teeth: 1 dorsal, 2 lateral. Unguiculus lanceolate and serrated. Tenent hair spatulate.

Remarks. *Entomobrya darwini* sp. n. is the only member in this genus with the combination of color pattern and chaetotaxy presented in the description above. In addition, *E. darwini* sp. n. has some unique diagnostic characters that, to our knowledge, have not been previously documented: the conspicuously long lateral sensilla on Th. II and Th. III (Fig. 23D, E) and the spine-like setae on the trochanteral organ are uncharacteristically enlarged, stout, and slightly truncated (Fig. 24D)

This species shares a similar color pattern with *Entomobrya litigiosa fasciata* Denis, 1931 described from Costa Rica, but *E. darwini* sp. n. has two dark broken/irregular triangles or angled bands along the lateral margins of Abd. III that are always absent in *E. litigiosa fasciata*, in addition, the unguiculus is lanceolate in *E. darwini* sp. n., whereas in *E. litigiosa fasciata*, it is truncate. The color forms of the Nearctic species

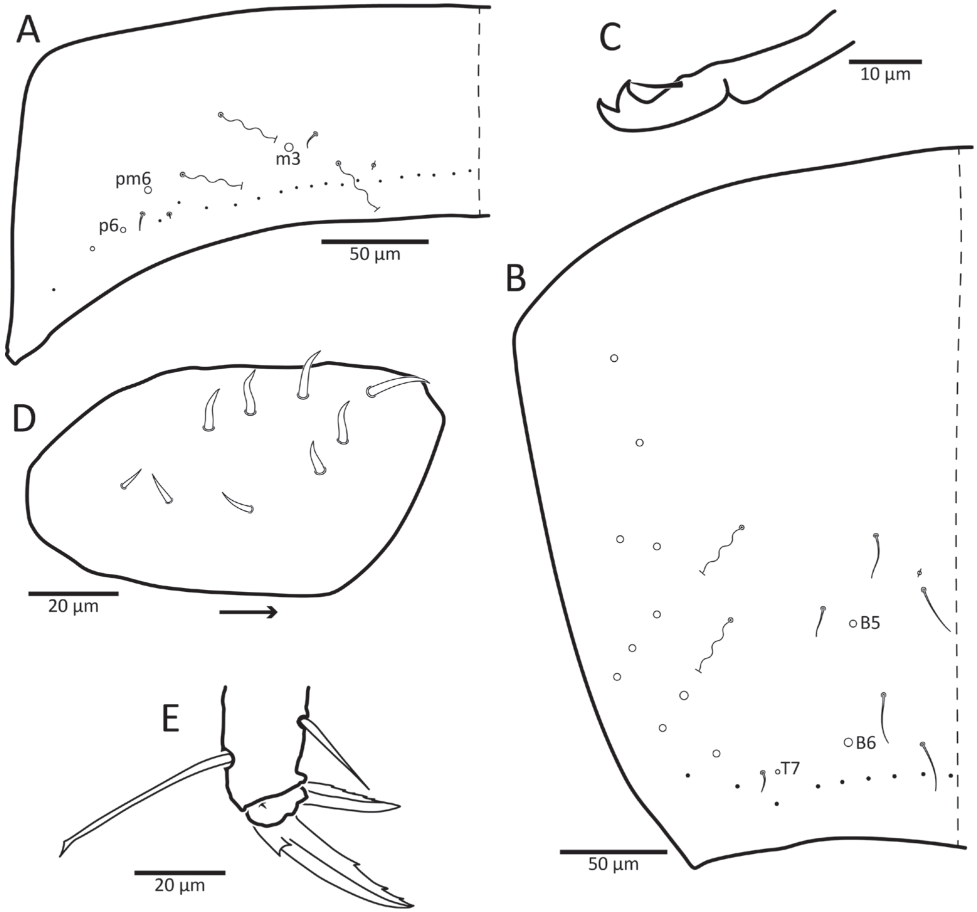

Figure 24. *Entomobrya darwini* sp. n. **A–B** dorsal chaetotaxy: **A** Abd. III **B** Abd. IV **C** mucro **D** trochanteral organ **E** hind claw complex.

Entomobrya decemfasciata (Packard, 1873), *sensu* Katz et al. (2015b), also include angled bands on Abd. III that superficially resemble those exhibited by *E. darwini* sp. n. However, these species can easily be separated by chaetotaxy: *E. decemfasciata* is characterized by having an extreme abundance of dorsal Mc, where as in *E. darwini* sp. n., the dorsal Mc are generally reduced (Figs 23B, D–G; 24A, B). *Entomobrya nicoleti* (Lubbock, 1871) also shares a similar color pattern with *E. darwini* sp. n. that includes forms with angled lateral bands on Abd. III, but differences in dorsal chaetotaxy (e.g., *E. nicoleti* has a1 on Abd. III that is absent on *E. darwini* sp. n.) easily separates these two species.

Entomobrya darwini sp. n. was collected from both surface and entrance habitats (Table 3) at three caves. This is the first record of the genus *Entomobrya* from the Galápagos Islands.

Distribution. Santa Cruz Island, Galápagos, Ecuador.

Family Paronellidae

Cyphoderus cf. *agnotus* Börner, 1906

Fig. 21B

Remarks. A single individual was collected in the entrance area of a lava tube. Our specimen has a bidentate mucro and the unguis lacks unpaired ungual distal teeth, distinguishing it from *Cyphoderus* galapagoensis Jacquemart 1976 while matching the description of *Cyphoderus agnotus*, *sensu* Cassagnau (1963), albeit Cassagnau's (1963) species description is relatively vague and lacks details about many important characters. Unfortunately, the material available is insufficient for a more complete redescription.

Distribution. Santa Cruz Island, Galápagos, Ecuador; widespread throughout Neotropics.

Material examined. Ecuador, Galápagos, Santa Cruz Island: 1 on slide, La Llegada, leaf litter from entrance, 12.iii.2014 (C. Plowman, D. Butler and G. Hoese), GLP-095, INHS Acc. 567,427.

Salina sp. A Soto-Adames, 2010b

Remarks. A single female, likely a juvenile, was collected. Our specimen keys out to *Salina thibaudi* Soto-Adames, 2010b according to the preliminary key to American *Salina* (Soto-Adames 2010b). However, evaluation of additional characters listed in Soto-Adames (2010b) indicates that this is a new species, similar or identical to *Salina* sp. A from Panama reported in Table 1 in Soto-Adames (2010b). The material available is insufficient to make a complete description and provide an unambiguous diagnosis. Nevertheless, this is the first record of the genus *Salina* from the Galápagos Islands.

This species was collected from a surface habitat adjacent to the entrance of a lava tube (Table 3).

Material examined. Ecuador, Galápagos, Santa Cruz Island: 1♀ on slide, Cueva Cascajo, surface leaf litter near skylight entrance, 9.iii.2014 (S. Taylor, J. Jacoby and M Sutton) GLP-031, INHS Acc. 567,428.

Updated checklist of Superfamily Entomobryoidea (Collembola) of the Galápagos

The following checklist includes all valid entomobryoid species previously reported from the Galápagos Islands, as well as new species records, new genus records, and the three newly described species presented in this paper.

Family Entomobryidae

1. *Coecobrya* sp. A, Santa Cruz Island (new genus record).

2. *Entomobrya darwini* Katz, Soto-Adames and Taylor, sp. n., Santa Cruz Island (new genus record).
3. *Heteromurus* (*Heteromurtrella*) *nitens* Yosii, 1964, Isabela Island (new record).
4. *Lepidocyrtus nigrosetosus* Folsom, 1927, *sensu* Mari Mutt (1986) = *Lepidocytrus leleupi* Jacquemart, 1976, Santa Cruz Island (Jacquemart 1976; and this report).
5. *Pseudosinella intermixta* (Folsom, 1924), Baltra Island (as South Seymour Island) (Folsom 1924).
6. *Pseudosinella vulcana* Katz, Soto-Adames & Taylor, sp. n., Santa Cruz Island.
7. *Pseudosinella stewartpecki* Katz, Soto-Adames & Taylor, sp. n., Santa Cruz Island.
8. *Seira dowlingi* (Wray, 1953), *sensu* Christiansen and Bellinger (2000) = *Seira caheni* Jacquemart, 1976, Marchena (Peck 2001), San Cristóbal (Peck 2001), and Santa Cruz (Jacquemart 1976) Islands.
9. *Seira galapagoensis* Jacquemart, 1976, Pinzón (Peck 2001), San Cristóbal (Peck 2001), Santa Cruz (Jacquemart 1976), and Santiago (Peck 2001) Islands.

Family Paronellidae

10. *Cyphoderus agnotus* Börner, 1906, Santa Cruz Island (new record).
11. *Cyphoderus galapagoensis* Jacquemart, 1976, Isabela (Peck 2001) and Santa Cruz (Jacquemart 1976) Islands.
12. *Salina* sp. A Soto-Adames, 2010b, Santa Cruz Island (new genus record).

The island records cited above from Peck (2001) were gathered from unknown sources (pers. comm. Peck December 10th, 2015) and need additional confirmation. The following species are listed in Peck and Jacquemart (2013), but are excluded from the checklist above: *Cyphoderus innominatus* Mills, 1938 and *Lepidocyrtus* cf. *lanuginosus* (Gmelin, 1788) do not have formally published records of Galápagos distributions; five additional species names in the genera *Cyphoderus*, *Entomobrya*, *Pseudosinella*, and *Seira* listed in the Charles Darwin Foundation online checklist (Peck and Jacquemart 2013) have not been formally published.

Discussion

Seven species were identified from the collections, including three new species, raising the total number of Galápagos entomobryoids from five to twelve species. Four genera are reported from the Galápagos for the first time: *Coecobrya*, *Entomobrya*, *Heteromurus*, *and Salina*. While none of the entomobryoid species other than *P. vulcana* sp. n. seem to show a particular association with lava tubes, the relatively cool moist conditions found in the entrance and twilight zones of these caves (Fig. 3) provide both a refuge from harsh surface conditions for these and many other organisms, and a window into subsurface volcanic habitats which would otherwise be under-sampled by biologists.

Remarkably, the specimens studied for this paper represent the first new collections of Galápagos Collembola in almost 40 years to be identified and subsequently reported and described. In spite of numerous scientific studies of invertebrates in the Galápagos Island, the new records and new species descriptions presented in this paper and the long gap between new collections being represented in the Galápagos springtail literature suggest that our understanding of the species richness of springtails and other microarthropods in the Galápagos Islands is still incomplete. Additionally, springtail collections from the Galápagos Islands made by Heinrich Schatz (University of Innsbruck) contain dozens of species not presently recorded from the islands (Palacios-Vargas, Pers. Comm. January 2016). Thus, further biological sampling in Galápagos lava tubes and lava tube entrances, sampling of microarthropods from other microhabitats, as well as further taxonomic studies of other material from our 2014 collections, should yield more additions to the fauna of this unique archipelago.

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RESEARCH ARTICLE

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Mexistenasellus floridensis sp. n., the first stenasellid isopod discovered from the Floridan aquifer (Crustacea, Isopoda, Asellota)

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Abstract

Mexistenasellus floridensis sp. n. is described from Hole in Wall Cave, Jackson County, Florida. The discovery of this unique isopod nearly doubles the range of the Family Stenasellidae in North America, which was previously known from Oaxaca, Mexico to southern Texas, USA. This is the first stenasellid reported swimming in the water column of a cave, a curious finding for an isopod that belongs to a group that is generally more adapted to inhabiting interstitial spaces. *Mexistenasellus floridensis* occurs syntopically with the subterranean asellid *Caecidotea putea*. Both species have been observed swimming in the water column, although neither has apparent anatomical adaptations for swimming as compared to the natatory pereopods of *Remasellus parvus*, an asellid that occurs in caves in Alachua, Madison and Wakulla counties, Florida.

Keywords

Isopoda, Stenasellidae, *Mexistenasellus floridensis*, *Remasellus*, *Caecidotea*, Florida, cave

Introduction

The northern edge of the range of stenasellid isopods in North America was previously the Balcones Fault Zone in southcentral Texas, with the eight known species distributed from approximately 30° north latitude in Texas southward along eastern Mexico to Oaxaca at about 17° (Bowman 1982). The extraordinary discovery of a stenasellid in Florida nearly doubles the Nearctic range of the family (Figure 1), which is otherwise known from across the Palaearctic (Magniez 1999). The zoogeographic implications of this discovery are immense as there now is an area stretching over a thousand kilometers between Texas and Florida where other stenasellids might be discovered, particularly given the penchant of these isopods for inhabiting the interstices of unconsolidated sediments. Needless to say, this also opens the door to the possibility of new discoveries in Florida, where the karst is replete with large water-filled cave systems.

The history of the discovery of stenasellids in the Nearctic is relatively brief. The first species described from the New World was *Mexistenasellus coahuila* Cole & Minckley (1972) discovered along with other crustaceans in the remarkable Cuatro Ciénegas basin of northern Mexico (Cole 1984). Magniez (1972) described *M. wilkensi* and *M. parzefalli* from Cueva del Huisache, San Luis Potosi, the latter from a single female specimen. Males of *M. parzefalli* were subsequently collected and described (Magniez 1973). Argano (1974) described *M. magniezi* from Veracruz. A second genus of Nearctic stenasellid, *Etlastenasellus*, was erected by Argano (1977) to receive the new species *E. mixtecus* from Oaxaca. Bowman (1982) described a second species in this genus, *E. confinis*, from Cueva del Guayabo, in Oaxaca, as well as *Mexistenasellus colei* from Cueva de Infiernillo, Tamaulipas, and *M. nulemex* from a mine below Cueva de la Boca, in Nuevo León. By 1982 eight Nearctic species of stenasellid isopods, divided among the two genera, were known. *Mexistenasellus floridensis* is the first new Nearctic stenasellid to be described since that time.

Bowman (1982) alluded to the presence of stenasellids in the United States in reference to an unidentified collection in Texas. This was confirmed by Bowman (1992) as a range extension of *Mexistenasellus coahuila* in springs and artesian wells in Bexar County and a hyporheic habitat in Medina County, Texas (Figure 1). Little karst and few caves are present in the states between the Texas and Florida populations. However, evidence of the presence of groundwater habitats in the intervening area exist in records of the subterranean asellid *Caecidotea paurotrigonus* from a drain tile in Mississippi (Fleming 1972), a hole dug in soil in Louisiana (Lewis 2000), and the possibly conspecific *Caecidotea dauphina* from a drain tile outlet on Dauphin Island, Alabama (Modlin 1986). With the description of *Caecidotea phreatica* from saturated soil habits in coastal Virginia, Lewis and Holsinger (1985) discussed other species of *Caecidotea* occurring in similar habitats in the Gulf Coastal Plain physiographic province. These included *C. hobbsi* from crayfish burrows in Florida (Steeves 1966) and *C. beattyi* from drain tiles in southern Illinois (Lewis and Bowman 1981). There clearly remains a large geographic area where stenasellids might occur in the southeastern US.

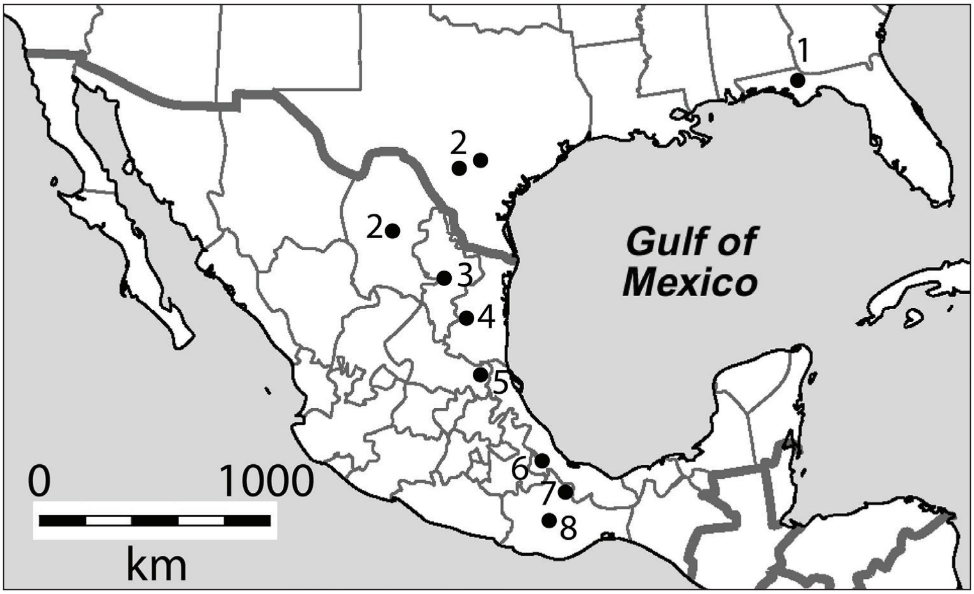

Figure 1. Distribution of Stenasellid isopods in the Nearctic Region: **1** *M. floridensis* **2** *M. coahuila* **3** *M. nulemex* **4** *M. colei* **5** *M.parzefalli* and *M. wilkensi* **6** *M. magniezi* **7** *Etlastenasellus confinis* **8** *E. mixtecus*.

Systematic part

Family Stenasellidae Dudich, 1924
Mexistenasellus Cole & Minckley, 1972

Mexistenasellus floridensis sp. n.

http://zoobank.org/F45C7937-CE7A-45E8-A295-AA1244B4CCBE
Figures 1–5

Material examined. USA: FLORIDA: Jackson County, Hole in Wall Cave, approximately 7 km east of Marianna (N30.78334 W85.15671), male holotype, 3 male and 2 female paratypes, collected 19 October 2013, Thomas R. Sawicki and Michael Stine; same locality, 3 male and 1 female paratypes, 2–3 January 2009, Thomas R. Sawicki. The specimens are deposited in the collection of the US National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Description. Eyeless, unpigmented, longest male approximately 9.0 mm, female 9.4 mm. Body slender, linear, about 4.3× as long as wide. Head about 1.4× as wide as long, rostrum and lateral incisions absent. Coxae not visible in dorsal view. Body becoming more dorsally spinose on pereonites 6–7 and pleon. Pleotelson about 1.65× as long as wide, caudomedial lobe moderately produced, broadly rounded.

Antenna 1 of 20 articles, distal 6 articles each with one esthete, then alternate articles with one esthete (8 esthetes total). Antenna 2 broken or detached in most specimens, flagellum 57–58 merous, esthetes absent. Mandibles with 4-cuspate incisors

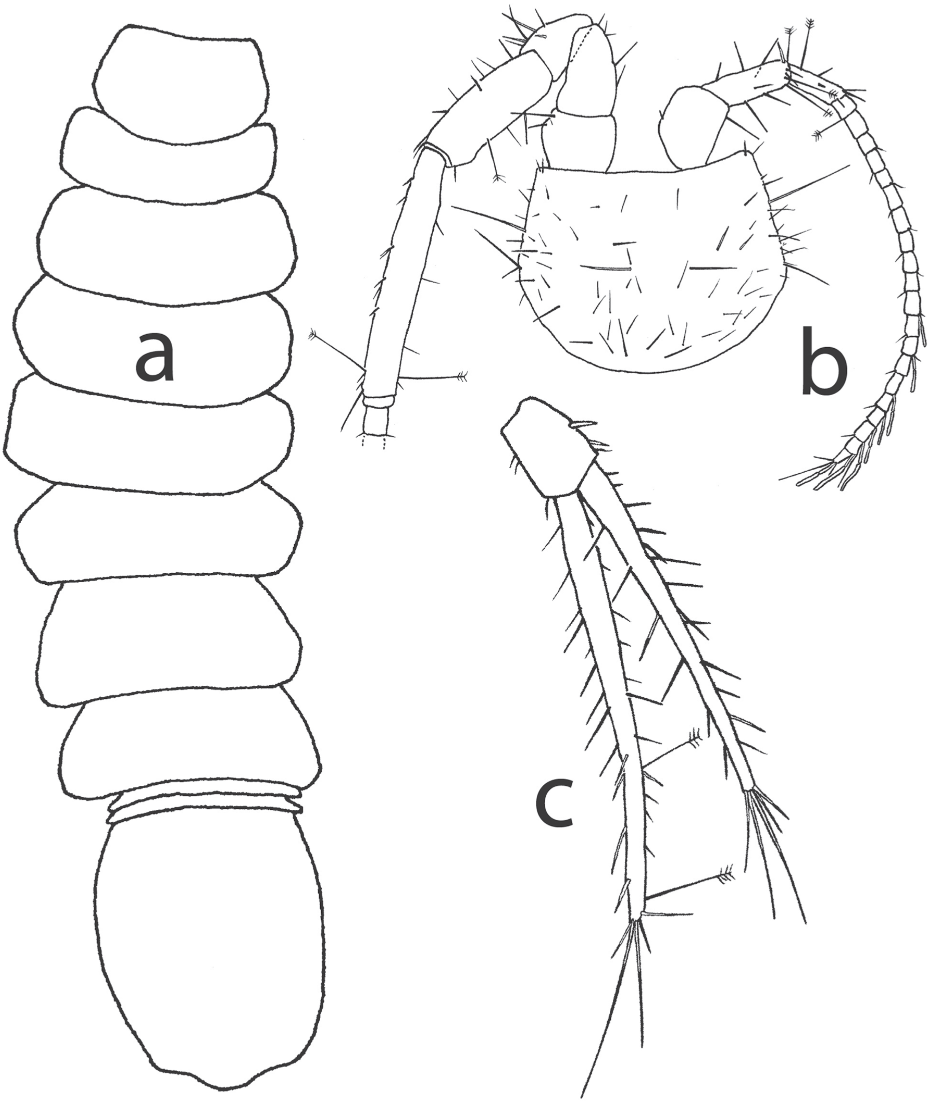

Figure 2. *Mexistenasellus floridensis* sp. n., male: **a** habitus **b** head, antenna 1, antenna 2 peduncle **c** uropod.

and lacinia; spine row with 5 spines on left, 4 spines on right, both with simple stout spines adjacent to incisors that resemble the cusps of the incisors and spines distad from incisors with complex plumosity; left molar with row of 15 plumose setae, right molar with row of 12 plumose setae. Mandibular palp 3-merous, with plumose setae on distal articles. Maxilla 1, inner lobe with 4 apical stout plumose spines; outer lobe with 12 dentate apical spines. Maxilliped without retinaculae.

Pereopods with sexual dimorphism not apparent. Pereopod 1, dactyl with elongate spine resembling accessory claw, 0.8× length of claw; propodus about 2.1× as long as

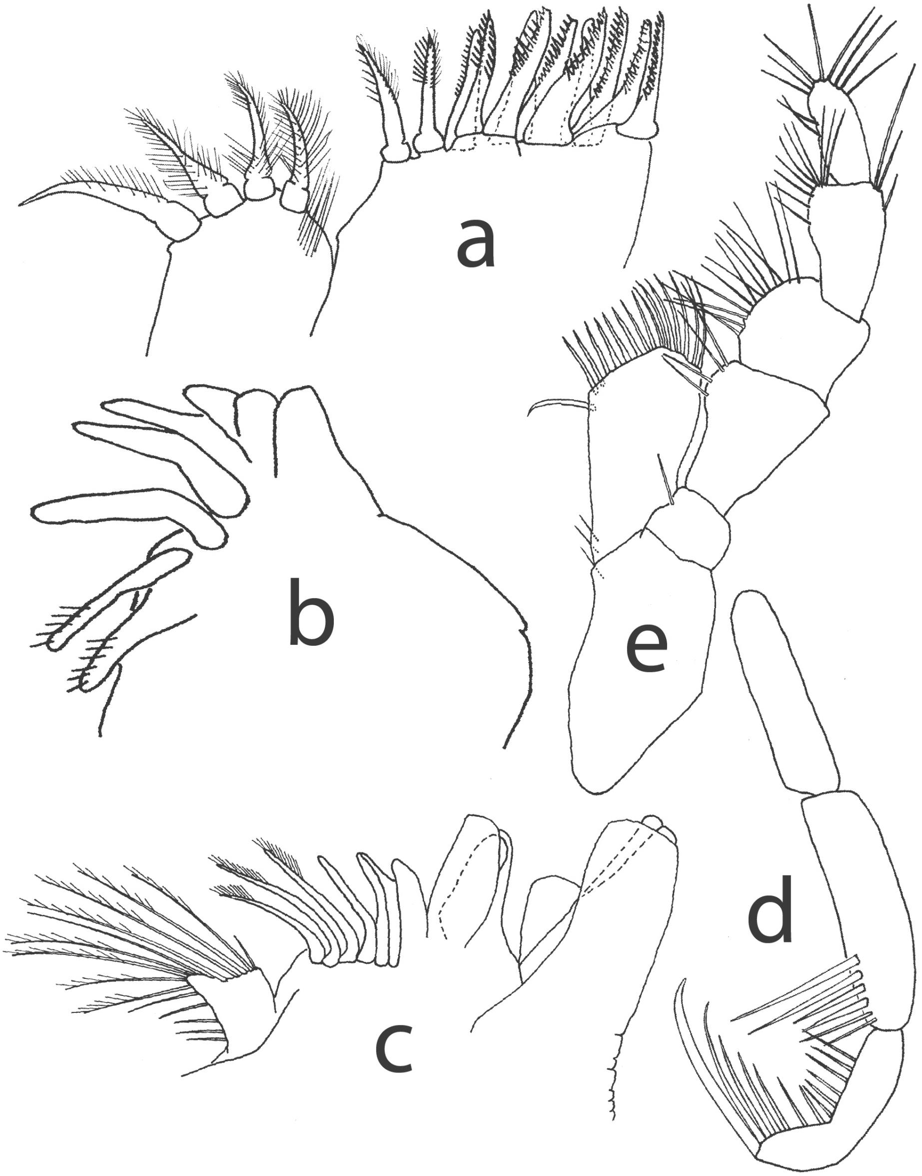

Figure 3. *Mexistenasellus floridensis* sp. n., mouthparts: **a** maxilla 1 **b** mandible, right, incisors **c** mandible, left, incisors and lacinia **d** mandibular palp **e** maxilliped.

wide, palmar margin with 4–6 robust plumose spines; carpus with 3–4 spines continuing from propodus. Pereopods 2–7 increasing in length, pereopod 7 longest; dactyls with prominent elongate claw-like spine parallel to claw.

Male pleopod 1, protopod with retinaculae absent; exopod oval with 5 elongate disto-lateral setae. Female pleopod 2 triangular, about 2.1× as long as wide, with 3 setae inside mesial margin, 1 distal seta and 1 lateral seta at mid-point. Male pleopod 2, protopod elongate, about 1.7× as long as wide; exopod, proximal article sub-equal in length to distal article, setae absent, distal article broadly rounded apically, with 5 lateral setae; endopod, setation absent, distal article about 2× length of proximal article, bent at approximately 60 degree angle to proximal article, tip bi-lobed, separated by endopodial groove terminating in sub-conical stylet (cannula). Pleopod 3, exopod with transverse suture, distal area slightly longer than proximal, with submarginal spines in row along mesial margin, spines and setae along distal and lateral margins; endopod about 0.6× length of exopod, bifurcated distally. Pleopod 4, exopod with oblique suture, about 2.5× as long as wide, area distal to suture about 0.67× length of proximal area, with about 32 marginal setae; endopod about 0.6× length of exopod, bifurcated distally. Pleopod 5, exopod with oblique suture, setae absent, about 2.5× as long as wide, area distal to suture about 0.3× length of proximal area; endopod sub-equal in length to exopod, bifurcated distally.

Uropods about 2.4mm in length, equal to pleotelson; rami slender, linear, endopod 5× length of protopod, 1.25× length of exopod.

Etymology. Named for the state of Florida, in recognition of the first stenasellid discovered in the United States east of the Mississippi. Suggested vernacular name is the Florida cave isopod.

Relationships. Magniez (1981) believed that the North American stenasellidae would prove to be multi-generic, although the paucity of collections has so far supported the partitioning of the New World taxa into only two genera (Magniez 1999; 2008). Within the genus *Mexistenasellus*, *M. floridensis* seems to most closely resemble *M. coahuila* and *M. colei*. The male pleopod 2 endopodite of all three species is an elongate, sub-rectangular structure terminating in a short stylet that is presumably the sperm transfer cannula, surrounded by the rounded terminal lobes of the endopodial groove. The exopod of each is divided by a suture into two sub-equal parts, with sparse setation along the distal margin. The first maxilla of all three species also share an inner lobe with 4 setae and outer lobe with 12 apical spines.

Nearctic *Mexistenasellus* species can be tentatively identified by their ranges (Fig. 1). From a morphological standpoint, *Mexistenasellus floridensis* is separated from the most similar species (*M. colei*, *M. coahuila*) by the structures of the male second pleopod, namely the more slender, elongate endopodite and the presence of five setae along the margin of the exopod, as compared to one seta in the other species. Other unique morphological aspects of *M. floridensis* include the absence of retinaculae on the maxilliped and the distally bifurcate endopods of pleopods 3–5.

When Cole and Minckley (1972) described the first stenasellid discovered in the Nearctic Region they noted the morphological resemblance between *Mexistenasellus coahuila* in northeastern Mexico and *Parastenasellus* from northern Africa. Magniez (1981) concurred with Cole and Minckleys' observation, and further compared the morphology of *M. coahuila* with the African stenasellid genera *Parastenasellus*, *Magniezia* and *Metastenasellus*. The male second pleopods of all of these genera are similar, excluding *Metastenasellus*, which possesses a spiraled-appearing endopodite that is quite dissimilar.

The hypothesis of Magniez entails colonization of groundwater by the stenasellid ancestors prior to the separation of Africa and South America during the early

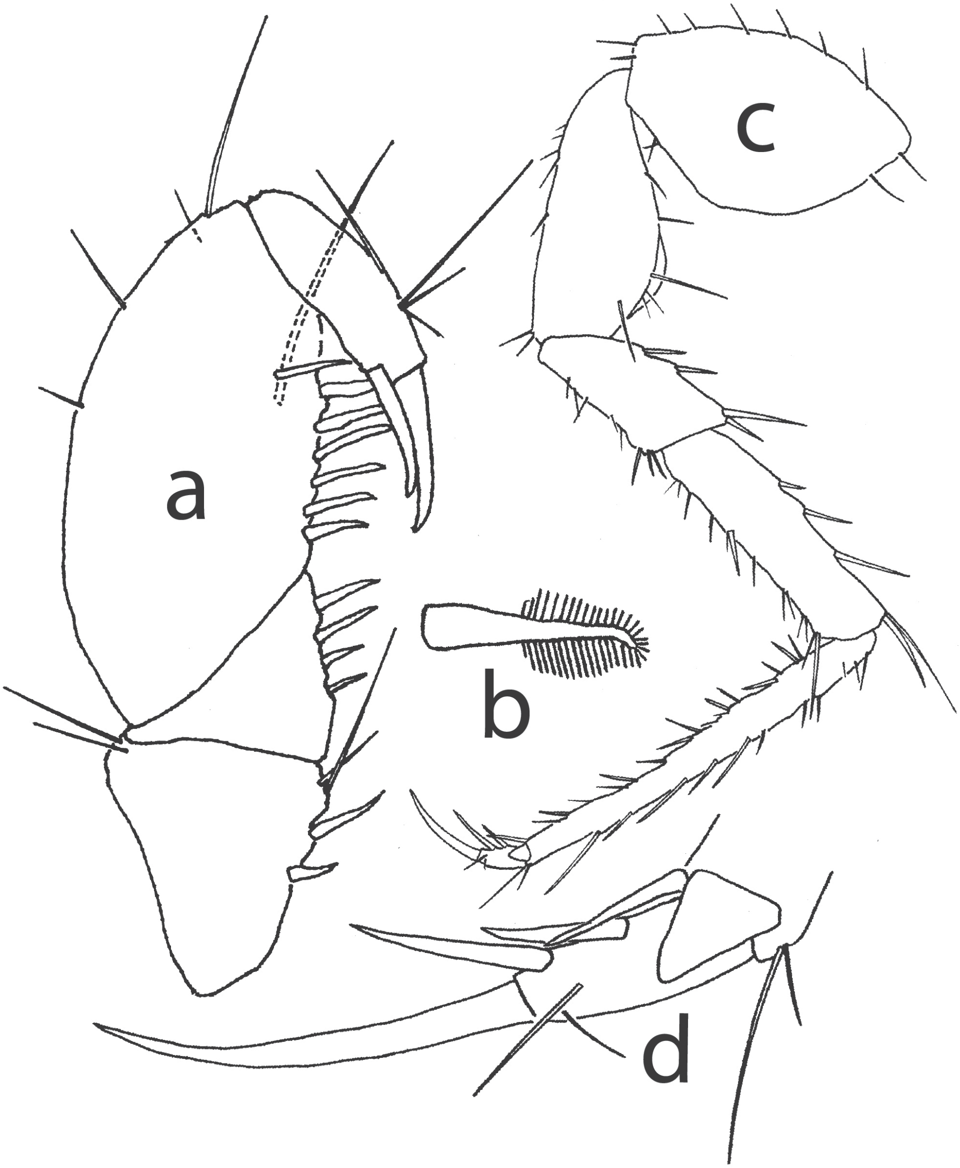

Figure 4. *Mexistenasellus floridensis* sp. n., pereopods: **a** pereopod 1 distal articles, female **b** spine, pereopod 1, palmar margin of proprodus **c** pereopod 7, male **d** same, dactyl.

Cretaceous (140–150 million years before present). This idea requires the presence of stenasellids in South America that remain to be discovered, and subsequent dispersal to North America during the Cenozoic. Magniez acknowledged the flaw in the hypothesis presented by the fact that the North and South American continents were separated during the majority of the Cenozoic. This problem is avoided by pushing back the invasion of freshwater habitats by stenasellids even further, to a time prior to the rifting of Pangea during the Jurassic (about 175 million years b.p.).

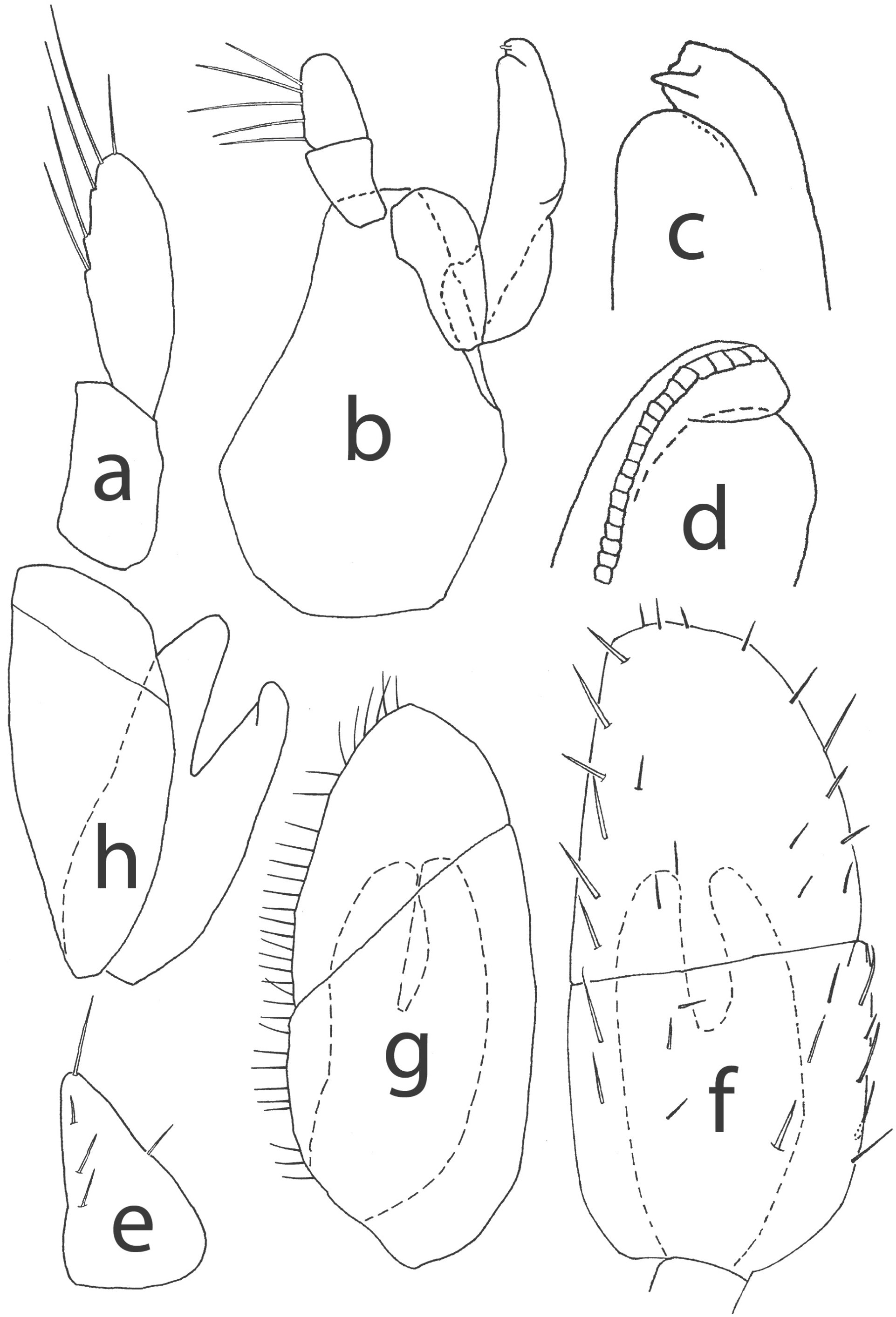

Figure 5. *Mexistenasellus floridensis* sp. n. pleopods, male except 4e: **a** pleopod 1 **b** pleopod 2 **c** same, tip of endopodite, anterior **d** same, posterior **e** pleopod 2 **f** pleopod 3 **g** pleopod 4 **h** pleopod 5.

The molecular genetic analysis of stenasellids by Morvan et al. (2013) supports the hypothesis that stenaselllids were present on Pangaea. Their data indicates that *Mexistenasellus* and *Magniezia* are sister groups and form a group separate from *Stenasellus*, with divergence possibly dating to the late Paleozoic. If one assumes that the African precursors were present prior to the breakup of Pangea, the theoretical presence of stenasellids in South America is no longer necessary. On the other hand, the molecular data do not support the argument of Bowman (1982) that supposed the invasion of groundwater in Mexico by the marine ancestors of *Mexistenasellus* as the shallow marine embayment regressed during the early Cenozoic.

Ecology. The Hole in Wall Cave is a water-filled cave system that is a popular dive site located in Merritt's Mill Pond, east of Mariana, Florida. The cave was mapped by Exley (1978) with the current surveyed length of approximately 8.5 km and maximum depth of 42.3 m. The isopods were collected as they swam, mid-water, at an average depth of approximately 24 m.

This observation of the isopods swimming is curious since the pereopods of *Mexistenasellus floridensis* do not exhibit the dense rows of setae present on the legs of *Remasellus parvus* that appear to be natatory adaptations. Regardless of how ill-prepared the isopods were for swimming that was precisely what they appeared to be doing each time specimens were collected. The asellid *Caecidotea putea* Lewis (2009) was also present in the water column with *M. floridensis*. This isopod seems no more morphologically adapted for swimming than the stenasellid.

On 17 July 2015 and 31 July 2015, dives were conducted in Hole in the Wall Cave to conduct careful behavioral observations of the isopods as well as to collect physicochemical data from the cave and surface pond. During these dives isopods were observed crawling on the floor, walls, and ceiling of the cave, as well as swimming in the water column. The swimming behavior observed in these animals may be in response to physical disturbance. This hypothesis is based on the fact that isopod swimming behavior was observed more frequently by the second diver than the lead diver as the team swam through the cave. Both *Mexistenasellus floridensis* and *Caecidotea putea* exhibited a fluid, graceful swimming motion. This fact at least suggests that although fin kicks and bubbles exhaled from SCUBA equipment may have induced their movement, it is not an unfamiliar, artificial behavior. It is easier to visually spot the isopods as they swim in the water column, where their unpigmented bodies are set against the dark cave background, than against the white limestone walls and ceilings or the silt-covered bottom. Due to this fact divers were focused on collecting animals that were swimming in the water column, and therefore likely missed many isopods that may have been crawling on surfaces within the cave. In total, these facts may explain why animals, seemingly poorly adapted for swimming, were collected solely from the mid-water column.

This swimming behavior may be an adaptive flight response to escape predation from the crayfish *Cambarus cryptodytes*, the Georgia cave salamander *Eurycea wallacei* or the trogloxene yellow bullhead catfish *Ameiurus natalis*. Other members of the community, a subset of the Florida subterranean fauna discussed by Franz et al. (1994),

Table 1. Physicochemical data correlated with depth. The 2 m reading was outside of the cave in the open water of Merritt's Mill Pond. The deepest sections of the cave correlated with the lowest temperature, pH, and DO, and highest specific conductivity.

Depth m	Temperature °C	pH	Dissolved Oxygen		Conductivity µS/cm
			mg/l	% Saturation	
2,21	20,58	7,37	5,82	64,7	315
19.57–29.5	20.08–20.22	7.36–7.39	5.22–5.28	57.6–58.3	318–327
30.0–31.53	18.73–19.97	7.31–7.32	3.01–4.88	32.2–53.6	333–359

were the stygophilic amphipod *Crangonyx floridanus* and other (probably undescribed) stygobitic amphipods.

During the 31 July 2015 dive, physicochemical data was taken using a Hydrolab HL4 sonde. Readings of depth, temperature, pH, conductivity, and dissolved oxygen (DO) were recorded every 20 seconds continuously during the dive. Dive bottom time (time spent swimming through cave passages and not entering and exiting the cave) was 51 minutes, and 155 separate readings were taken with depth varying between 19.57 and 31.53 meters. Readings were also taken in the open water of Merritt's Mill Pond. For each parameter measured, the shallowest regions of the cave most closely approximated the pond water; however, temperature, pH, and DO progressively decreased with depth and specific conductivity increased with depth (Table 1). The lowest temperature, pH and DO, and highest conductivity were measured below 31 meters. The cold, low DO, high conductivity water entered the main cave passage from deep side tunnels at approximately 600 meters from the cave entrance. One of us (TRS) has explored these cave passages for the past six years and has noted seasonal variation in cave water temperature at depths between 19 and 30 meters. In total, these data suggest that the cold water entering the primary passage comes from deep water sources, less influenced by seasonal variation. While no isopods were observed in this colder water, stygobitic amphipods were collected.

Concerning reproduction, a 7.4 mm female *M. floridensis* was ovigerous with a brood pouch containing eggs approximately 0.5 mm in diameter. Another post-ovigerous female released 32 juveniles about 1.4 mm in length.

Acknowledgements

The senior author gratefully acknowledges conversations and suggestions regarding stenasellids with his friend and colleague, the late Dr. Guy Magniez. The authors would like to thank Dr. Michael Stine for sharing his time and cave diving expertise in assisting with the collection of specimens for this project. We would also like to thank the staff at Cave Adventurers for their professionalism and sharing their local knowledge. Funding for this project was provided by the American Public University System Faculty Research Grant.

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SHORT COMMUNICATION

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First report of cave springtail (Collembola, Paronellidae) parasitized by mite (Parasitengona, Microtrombidiidae)

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Abstract

Although mites and springtails are important components of cave fauna, until now there was no report about host-parasite associations between these groups in subterranean ecosystem. Here we present the first record of mite parasitism in *Trogolaphysa* species (Paronellidae), and the first known case of parasitism in the Brazilian cave springtail. The Microtrombidiidae mite was attached on the head of the Collembola by the stylostome. Collembola is not a usual host for Microtrombidiidae mites but it may be related to the lack of reports by researchers or few samplings specific to parasitism studies in these invertebrates. Another possibility relates to the cave environment itself. The oligotrophic condition of these ecosystems could limit the occurrence of the main hosts for these mites and the parasitism in unusual groups, such as Collembola, may have been favored.

Keywords

Parasitengona, Collembola, host-parasite associations, subterranean fauna, parasitism

Introduction

Mites and springtails are important components of soil fauna, being widely distributed throughout terrestrial environments and found in almost all known habitats (Hopkin 1997, Walter and Proctor 2013). In some places, such as tropical rainforests, these groups can represent up to 89% of the species found in soil and leaf litter (Franklin and Morais 2006). Besides being common in epigean ecosystems, they are often found in subterranean environments (e.g. Sharrat et al. 2000, Simões et al. 2014, Prous et al. 2015), including many species restricted to caves (e.g. Culver et al. 2003, Lewis et al. 2010, Niemiller and Zigler 2013, Zeppelini et al. 2014).

The high abundance of these groups and the coexistence of their species in the soil allows the occurrence of a series of interactions between these organisms, such as predation and competition, besides parasitism. According to Christiansen (1964), natural enemies of collembolans include different predatory arthropods, primarily mites. Other records indicate that predatory mites that inhabit the soil often feed on springtails (Berg et al. 1998, Ferguson 2001). Ferguson and Joly (2002), in a study of population dynamics of mites and springtails in Canadian prairie areas, showed that the populations of these groups are more influenced by endogenous factors (e.g. population growth dependent on the density related to resource availability) than exogenous factors such as predation (of mites on Collembola) or climatic conditions. However, alterations in mites and springtail abundance in soil can also be explained by competition for food, since these groups have many scavenging species with similar food preferences (Rieff et al. 2014, Kaneko et al. 1998). Another curious interaction between these groups was reported by Norton and Ryabinin (1994), in which some Oribatid mite nymphs carried disposed Collembola exoskeletons on their backs, presumably as camouflage.

The records of parasitism between mites and springtails relates mainly to the Erythraeidae family with species of the genus Erythrites (*E. womersleyi*) parasitizing *Corynephoria* (Collembola: Bourletiellidae) (Greenslade and Southcott 1980), *Leptus trimaculatus* in species of Arthropleona and Symphypleona (Wendt et al. 1992) and a *Leptus* species in unidentified Collembola (Wohltmann 2001). Some Acaridae family species have also been observed in Collembola: *Michaelopus sminthurus*, *Congovidiella collembolicola* and *Calvolia waldorfae* in specimens of *Sminthurus fuscus* (Collembola: Sminthuridae) (Fain and Johnsthon 1974). However, in the latter case, it was not shown that the interaction between the species was only phoretic or promoting damage to the host.

Despite the large number of parasitic mites among those found in the soil, especially the Parasitengona cohort, which feature a wide range arthropods among their hosts, Collembola are not a frequent host (Gabrys 2011, Wohltmann 2001, Zhang 1998). Therefore, we present the first record of mite parasitism in *Trogolaphysa* species (Paronellidae), and the first known case of parasitism in the Brazilian cave springtail.

Methods

The specimens evaluated in this study were sampled in the Clarabóias Cave (20°06.63'S, 43°39.45'W), in the municipality of Rio Acima, Minas Gerais, Brazil. This cave comprises a small iron ore cavity (28 meters of linear development and 58 square meters in area) inserted in the Atlantic Forest domain, in an important mineral production region known as the Iron Quadrangle (Figure 1). Collembola specimens were fixed in 70% ethanol and slide-mounted in Hoyer's solution for identification. The specimen was identified according to Bellinger et al. (1996–2016). Before being removed from the host, the mite was photographed using the Leica S8APO stereomicroscope with attached Leica DFC camera (Leica, Germany). The specimen was then cleared in Nesbitt's fluid and mounted on slides in Hoyer's solution (Walter and Krantz 2009). The specimens were identified with the Leica MDLS phase-contrast microscope (Leica, Germany).

The Collembola is deposited in Collembola and Conservation Systematics Laboratory Collection of the State University of Paraíba (CRFS-UEPB # 4824), and the mite in Subterranean Invertebrate Collection of the Federal University of Lavras (ISLA-UFLA # 11710).

Results

The springtail comprises an undescribed species of *Trogolaphysa*, and the mite was determined as an unidentified genus and species of Microtrombidiidae. This is the first report of this mite family in Brazilian caves. Unfortunately, the only mite specimen collected had some deformities in the legs, and due to uncertainty about the number

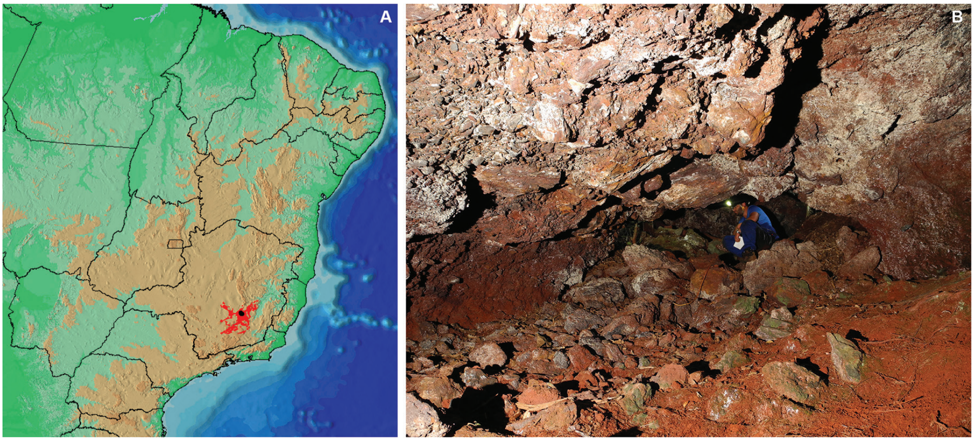

Figure 1. A Location of the Clarabóias Cave (black point) in the Brazilian ferruginous area denominated Iron Quadrangle (red area) **B** General aspect of the cave interior.

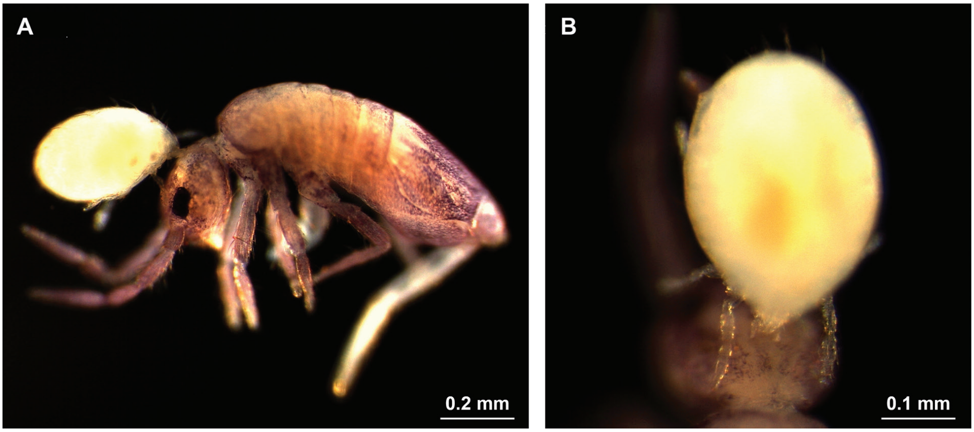

Figure 2. A *Trogolaphysa* sp. n. (Collembola: Paronellidae) parasitized by larvae of an unidentified Microtrombidiidae (Trombidiformes) species **B** Dorsal view of the mite on the springtail's head.

of setae on some of its segments, it was not possible to determine the genus. However, the possibility of this belonging to an as yet undescribed genus in not yet ruled out.

The Microtrombidiidae mite was attached on the head of the Collembola by the stylostome (Figure 2). The estimated mite size is 0.39 mm, almost one third of the springtail body size (20.01 mm).

Discussion

The diversity of habitats and wide distribution of the *Trogolaphysa* genus qualifies it as potential host for mites, although this is the first report of parasitism for this group. The genus consists of 40 species worldwide, and 37 of these are present in the Americas (Soto-Adames and Taylor 2013). In Brazil, it is the richest genus of Paronellidae, with five described species, which corresponds to one third of the species known for this family in the country (Da Silva and Bellini 2015). It is widely distributed in Brazil, being present in areas of the Atlantic Forest, Cerrado and the Amazon rainforest. In these biomes they are often found in leaf litter, soil, forest humus and near flooded areas (Oliveira 2013), habitats similar to those described for the parasitic larvae of Parasitengona (Wohltmann 2001). According to Palacios-Vargas and Thibaud (1997), *Trogolaphysa* can be considered one of the most popular genera in Collembola, due to morphological adaptations (troglomorphisms) often observed in species in the subterranean environment. There are at least nine species of troglomorphic and subterranean environment-restricted *Trogolaphysa* (Palacios-Vargas 2002). In addition, new species have been sampled in South American caves, mainly in Brazil, which will further increase the representation of this group in the hypogean environment (Brito et al. unpublished results).

For Microtrombidiidae, there are the no reports of parasitism on springtails. Some groups of arthropods, such as Hemiptera, Orthoptera and Diptera, are the most common host for larval stages of this family (Heath and Snell 2006, Stroiński et al. 2013). The absence of Collembola and other potential groups amnog the hosts may be related to the lack of reports by researchers or few samplings specific to parasitism studies in invertebrates, especially in tropical areas. The absence information can also be related to the duration of the parasitic larval phase of Parasitengona, ranging from 3-14 days in most species, temporally limiting sampling of parasitized individuals (Wohltmann 2001). Another possibility relates to the cave environment itself, which may be the result of a characteristic oligotrophic association condition of these ecosystems. Since the absence of primary production in caves limits the occurrence of the main hosts for these mites, the parasitism in unusual groups, such as Collembola, may have been favored. However, these assumptions are speculative, and future studies and observations are needed to confirm or refute these hypotheses.

The presence of the mite on the head of the Collembola probably facilitated the development of the parasite. According to Wohltmann (2001), field observations of some Microtrombidiidae have indicated their preference to parasitize on the dorsal parts and the first abdominal segments of the hosts. However, subsequent observations in the laboratory have shown that, indeed these mites anchor on all parts of the body, but most are removed by cleaning movements of the host. The exception are those present in dorsal areas not reached by the host appendages (Wohltmann 2001).

The successful development of the larval stage allows the mite a 10 to 576 fold freshmass increase (Wohltmann 1999), possibly even exceeding the host size (Wohltmann 2001). Despite the excessive growth, there are few studies about the influence of Parasitengona parasitism on their hosts. For some aquatic species of this cohort late ontogenetic development besides reduced host fertility and longevity were demonstrated (see Wohltmann 2001). In aphids, the presence of a few Parasitengona larvae can be lethal to parasitized individuals (Zhang 1992).

This work revealed an interesting association between springtails and Microtrombidiidae mites, indicating that the chances of hosts for this family should be much more diverse than is currently known. New reports on host-parasite interactions will be important to improve the understanding of the group and its spectrum of host. For subterranean environments such research is even more relevant, since data on the effect of these environments on the parasitism relationships is nonexistent.

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