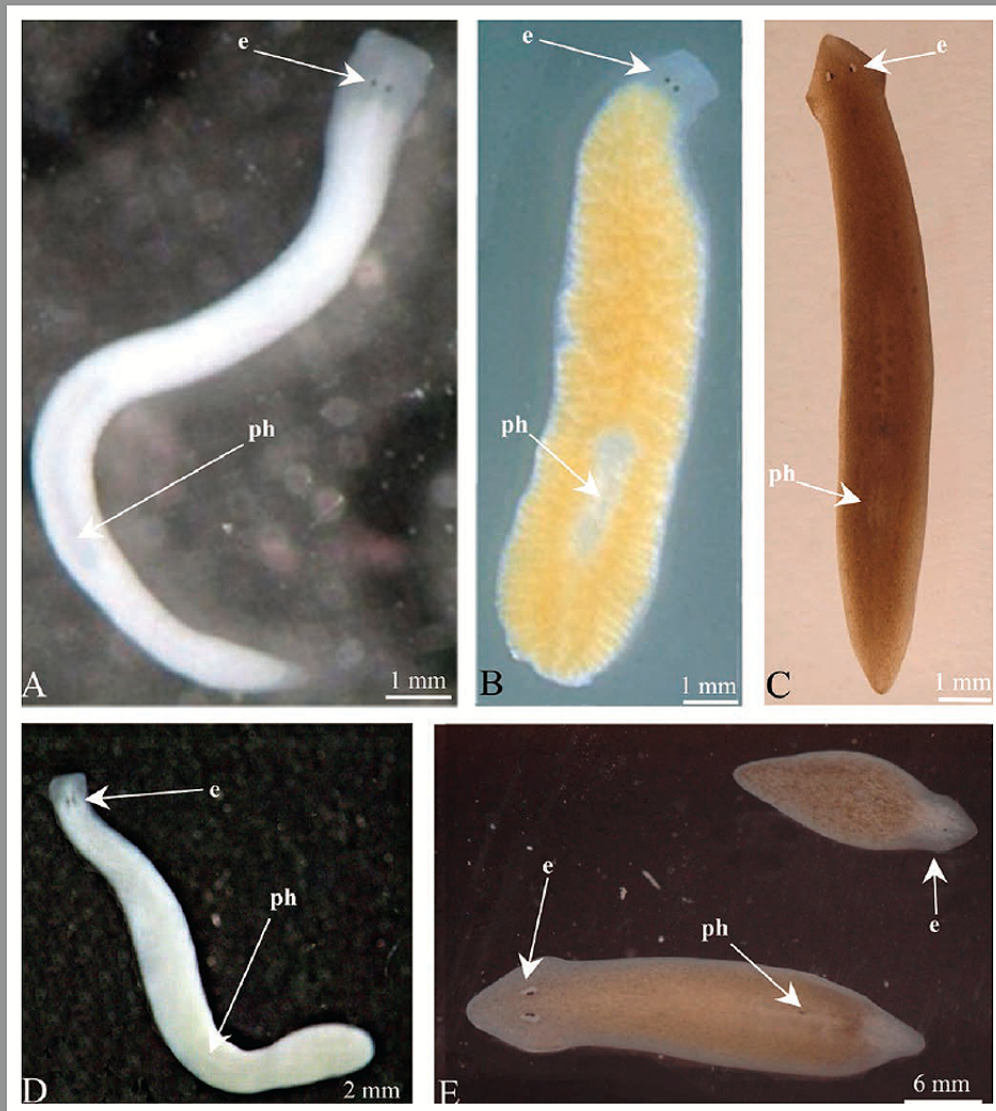


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Front cover: A collection of planarians from Sardinia. See Stocchino et. al. in this issue.



# SUBTERRANEAN AQUATIC PLANARIANS OF SARDINIA, WITH A DISCUSSION ON THE PENIAL FLAGELLUM AND THE BURSAL CANAL SPHINCTER IN THE GENUS *DENDROCOELUM* (PLATYHELMINTHES, TRICLADIDA, DENDROCOELIDAE)

GIACINTA ANGELA STOCCHINO<sup>1\*</sup>, RONALD SLUYS<sup>2</sup>, PAOLO MARCIA<sup>1</sup>, AND RENATA MANCONI<sup>1</sup>

**Abstract:** The paper provides the first detailed account on the taxonomic richness of the subterranean freshwater tricladids from Sardinia, including the description of four new species for the genera *Dendrocoelum* and *Phagocata*. New records for *Dugesia benazzii*, *Dugesia* sp., *Crenobia alpina*, and *Phagocata* sp. are also reported. The three new species of *Dendrocoelum* are the first reported for the island of Sardinia. These species display a bursal canal sphincter and a large adenodactyl with a characteristic anatomy with a zone of fine circular muscle fibers running through the mesenchyme of its papilla. A detailed analysis of the structure of the penial flagellum in the genus *Dendrocoelum* highlighted six main conditions, some of which have not been previously reported, in regard to the histology of the tip of the penis papilla and the extent of its inversion. The new species of *Phagocata* represents the first species recorded from Italy and the first anophthalmous species reported from Europe.

## INTRODUCTION

The Mediterranean region has been identified as a biodiversity hotspot on a global scale, with the island of Sardinia as one of its regional hotspots (Médail and Quézel, 1999; Myers et al., 2000). Together with part of coastal Tuscany, the island belongs to the Sardinian stygofaunistic province (Pesce, 1985). The presence of a greatly diversified and ancient karst, dating to the Palaeozoic and Mesozoic periods of the Sardinian-Corsica plate, gave rise to a wide range of underground aquatic systems, ranging from inland and coastal caves to underground rivers (Pesce, 1985). Although more than 3000 terrestrial and marine karstic caves are registered in the Regional Speleological Register, the groundwater biodiversity of Sardinia is largely unknown. The literature on the taxonomic richness in the caves, springs, and wells of Sardinia is mainly focused on Crustacea (Lindberg, 1956; Stella, 1957; Puddu and Pirodda, 1973; Ruffo and Vigna Taglianti, 1975; Cassola, 1982; Pesce and Maggi, 1983; Pesce, 1985; Cottarelli et al., 1996; Argano et al., 1997). A few papers refer to freshwater Oligochaeta (Martinez-Ansemil and Sambugar, 2008), Gastropoda (Giusti and Castagnolo, 1983), Trichoptera (Moretti and Cianficconi, 1983; Cianficconi et al., 1998), and marine cave-dwelling fauna (see Manconi et al., 2009 and references therein).

Faunistic investigations of Sardinian epigeal waters, promoted first by Benazzi (1938) and subsequently further developed by Lepori and co-workers, highlighted a considerable taxonomic richness and endemism of the Tricladida (Lepori, 1951; Pala et al., 1980a,b,c, 1981, 1995,

1999, 2000; Casu et al., 1982; Stocchino et al., 2005). In contrast, very few data are available on the Sardinian groundwater tricladids, dating back to 1938 when Benazzi recorded *Atrioplanaria* sp. from a hydropetric habitat on limestone in north-western Sardinia (Benazzi, 1938). Other specimens, discovered in 1952 from Su Coloru Cave (northern Sardinia) and from an old mine (Baccu Arrosu, southwestern Sardinia), were assigned by the same author to *Atrioplanaria* sp. (Benazzi, 1982). Pala et al. (1980c) reported on *Crenobia alpina* Dana, 1776 from some springs on the Gennargentu Massif (central-eastern Sardinia). Apart from these data no new records of subterranean tricladids from the island have become available for a long period of time. Only recently have a few stygobiological studies on Sardinian tricladids been performed (Stocchino, 2003; Stocchino et al., 2008). The present paper provides a detailed account on the taxonomic richness of the subterranean tricladids from Sardinia, including the description of four new species and new records for several others.

## MATERIALS AND METHODS

The specimens, collected during the period 2000–2010, were transferred to the laboratory, reared in shaded boxes in semi-dark conditions at  $18 \pm 2$  °C and fed with fresh beef liver. For morphological study, specimens were fixed

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for 24 hours in Bouin's fluid, dehydrated in a graded ethanol series, cleared in toluene, and embedded in paraffin. Serial sections of specimens of the Puntadella Scmunica Spring were made at intervals of 5, 6, or 7 µm and stained with haematoxylin-eosin (Harris), Hansen's haematoxylin and eosin-orange, Dane Herman's tetrachrome, Mallory-Cason, or Pasini's reagent.

Serial sections of specimens from the Monte Majore Cave were made at intervals of 5 or 8 µm and stained in Dane Herman's tetrachrome or Mallory-Cason. Serial sections of specimens from Sa Ucca 'e su Peltusu Cave were made at intervals of 8 µm and were stained with Mallory-Cason. Serial sections of the specimen from the Cuccuru Tiria Cave were made at intervals of 5 µm and stained in Dane Herman's tetrachrome. Serial sections of specimens from the Su Cantaru Spring were made at intervals of 5 µm and stained in haematoxylin-eosin (Harris). Serial sections of specimens from the Gennargentu Massif spring were made at intervals of 8 µm and stained in Harris's haematoxylin-eosin. The material is deposited in the Netherlands Centre for Biodiversity Naturalis (section ZMA) and in the Giacinta A. Stocchino collection (CGAS), University of Sassari.

#### SYSTEMATIC ACCOUNT

(All abbreviations for labels in Figures 2–9 are listed in Table 1)

Order TRICLADIDA Lang, 1884  
 Suborder CONTINENTICOLA Carranza, Littlewood, Clough, Ruiz-Trillo, Bagaña and Riutort, 1998  
 Superfamily PLANARIOIDEA Stimpson, 1857  
 Family DENDROCOELIDAE Hallez, 1892  
 Genus *Dendrocoelum* Örsted, 1844  
*Dendrocoelum mariae* Stocchino and Sluys sp. nov. (Table 2; Figs. 1, 2)

**Material examined.** All individuals (n = 20) were asexual at collection. The majority of the animals underwent a sexualization process after a year of rearing in the laboratory.

Holotype: ZMA V.Pl. 7100.1, sagittal sections on 17 slides, Punta della Scmunica, Asinara Island (41°05'35"N, 8°18'4"E), December 2000, coll. M. Piras and G.A. Stocchino.

Paratypes: ZMA V.Pl. 7100.2, ZMA V.Pl. 7100.3, ZMA V.Pl. 7100.4, *ibid.*, sagittal sections on 41 slides, 22 slides, 22 slides; ZMA V.Pl. 7100.5, *ibid.*, one set of transverse sections on 61 slides; CGAS Pla 1.1, *ibid.*, sagittal sections on 37 slides, immature specimen; CGAS Pla 1.2, *ibid.*, horizontal sections on 27 slides, not completely mature specimen.

Other material: ZMA V.Pl. 7101.1, ZMA V.Pl. 7101.2, two sets of sagittal sections on 5 slides and 19 slides, respectively, not completely mature specimens. Punta della Scmunica, Asinara Island (41°05'35"N 8°18'4"E) May 2005, coll. G.A. Stocchino; CGAS Pla 1.3, *ibid.* one

**Table 1. Abbreviations used in figures.**

Abbreviation	Term
<i>a</i>	adenodactyl
<i>ab</i>	bulb of the adenodactyl
<i>ap</i>	papilla of the adenodactyl
<i>bc</i>	bursal canal
<i>bg</i>	bulb glands
<i>ca</i>	common atrium
<i>cb</i>	copulatory bursa
<i>cm</i>	circular muscles
<i>co</i>	copulatory apparatus
<i>cod</i>	common oviduct
<i>cvs</i>	common vas deferens
<i>e</i>	eyes
<i>ed</i>	ejaculatory duct
<i>ep</i>	epithelium
<i>eep</i>	external epithelium
<i>f</i>	flagellum
<i>fcm</i>	fine circular muscles
<i>g</i>	gonopore
<i>h</i>	head
<i>ic</i>	intestinal caecum
<i>iep</i>	internal epithelium
<i>l</i>	lumen
<i>lm</i>	longitudinal muscles
<i>m</i>	musculature
<i>ma</i>	male atrium
<i>od</i>	oviduct
<i>pb</i>	penis bulb
<i>pg</i>	penis glands
<i>ph</i>	pharynx
<i>pl</i>	plug of cells
<i>pp</i>	penis papilla
<i>ppl</i>	penis papilla lumen
<i>s</i>	sphincter
<i>sg</i>	shell glands
<i>sp</i>	sperm
<i>spv</i>	spermiducal vesicles
<i>sv</i>	seminal vesicle
<i>vd</i>	vas deferens
<i>vdv</i>	vesicle of the vas deferens

set of sagittal sections on 12 slides; CGAS Pla 1.4–12 *ibid.*, nine sets of sagittal sections on 13 slides, 13 slides, 23 slides, 14 slides, 16 slides, 18 slides, 22 slides, 21 slides, 37 slides, respectively, not completely mature specimens. CGAS Pla 1.13–14, two sets of transverse sections on 21 slides and 46 slides, respectively, not completely mature specimens, Punta della Scmunica, Asinara Island (41°05'35"N 8°18'4"E) spring 2000, coll. M. Piras and G.A. Stocchino.

**Etymology.** The specific epithet refers to Professor emeritus Maria Pala (University of Sassari), in recognition of her contributions to the biology and systematics of freshwater triclads.

**Table 2. Checklist of groundwater triclads from Sardinia.**

Taxa	Caves	Springs	Locality	Coordinates		Reference
				Latitude	Longitude	
Dugesiidae Ball, 1974						
<i>Dugesia</i> Girard, 1850						
		1	Su Cantaru Spring, Monte Albo	41°34'30"N	9°40'31"E	Present paper
	1		Sa Ucca 'e su Peltusu Cave	40°27'00"N	8°40'43"E	Present paper
Dendrocoelidae Hallez, 1892						
<i>Dendrocoelum</i> Örsted, 1844						
		1	Punta della Scomunica Spring, Asinara Island	41°05'35"N	8°18'4"E	Present paper
	1		Monte Majore Cave	40°30'51"N	8°36'37"E	Present paper
	1		Sa Ucca 'e su Peltusu Cave	40°27'00"N	8°40'43"E	Present paper
Planariidae Stimpson, 1857						
<i>Atrioplanaria</i> de Beauchamp, 1932						
		1	Sassari surroundings	40°42'02"N	8°35'47"E	Benazzi, 1938
	1		Su Coloru Cave	40°49'01"N	8°48'46"E	Benazzi, 1982
	1		Baccu Arrosu Mine	39°9'36"N	8°45'15"E	Benazzi, 1982
<i>Phagocata</i> Leidy, 1857						
	1		Cuccuru Tiria Cave	39°19'25"N	8°34'29"E	Present paper
	1		Monte Majore Cave	40°30'51"N	8°36'37"E	Present paper
		1	Elighe Mannu Spring, Asinara Island	41°05'36"N	8°18'21"E	Present paper
		1	Alà dei Sardi	40°38'60"N	9°19'45"E	Present paper
<i>Crenobia</i> Kenk, 1930						
		4	East Gennargentu Massif	40°09'24"N	9°15'16"E	Pala et al., 1980c
		1	Su Sessene Spring, West Gennargentu Massif	40°01'40"N	9°12'1"E	Present paper

Habitat. From 2000 to 2005 (spring and winter) specimens of *D. mariae* were collected from a small perennial spring (at an altitude of ca. 350 m asl) on the eastern coast of Asinara Island (Fig. 1). The seasonal samplings highlighted the constant presence of planarians at this site. Substrate ranges from rocks to large boulders and sand. The spring is characterized by an extremely fluctuating flow, with minimum values during the summer. Water temperature ranges between 13 and 21.4 °C throughout the year; pH values range from 7 to 7.3. The water is rich in minerals (total dissolved solids ca. 600 mg L<sup>-1</sup>) and is characterized by high values of chlorides, due to the salty aerosol blown in by wind. Dendrocoelidae were associated with a diverse invertebrate fauna, consisting of Gastropoda, Tubificidae, Ostracoda, Cyclopoida, Asellidae, Amphipoda, Hydracnida, Chironomidae, and the endemic anuran *Discoglossus sardus* Tschudi, 1837.

Geographical distribution. Endemic to Asinara Island and only known from the type locality.

Diagnosis. *Dendrocoelum mariae* is characterized by an adenodactyl that is much larger than the penis papilla, a copulatory apparatus far behind the pharyngeal pocket, the presence of a sphincter in the terminal tract of the bursal canal, and by the penis being in dorsal position and the adenodactyl located ventrally.

Description. Live animals are unpigmented, typically whitish, with a body size of 8 to 10 mm in length and a width ranging from ca. 2 mm in the central part of the body to less than 1 mm at the level of the head. The anterior end is truncated, with the middle part of the frontal margin convex, and is provided with a pair of rounded lateral lobes. Just behind the eyes there is a slight but clear constriction or neck that sets off the head from the rest of the body (Fig. 2A).

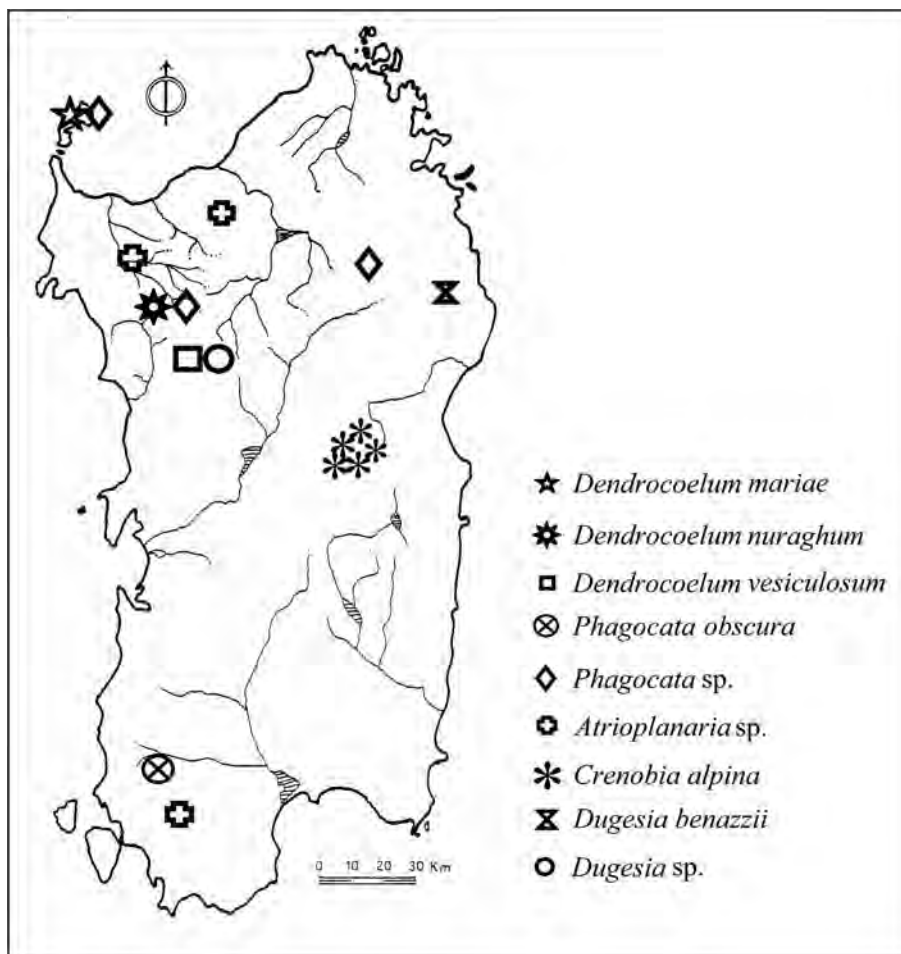


Figure 1. Geographic distribution of groundwater planarians in Sardinia. Data from Benazzi (1938, 1982), Pala et al. (1980c), and this study.

The two eyes are situated rather closely together. The distance between the eyes is slightly less than 1/4 of the width of the neck. Inter-ocular distance is smaller than the distance from each eye cup to the lateral margin, and the distance from the frontal margin to the eyes is greater than the distance to the lateral margin. Supernumerary eyes are often present, usually 2 or 3 on either side, sometimes arranged in a slightly crescent-shaped configuration.

The subterminal anterior adhesive organ, with a length comparable to the interocular distance, is moderately developed and consists of a shallow cup. The surface of the organ consists of a well-defined area of infranucleate epithelial cells pierced by numerous gland ducts. The cell bodies of the glands are scattered throughout the mesenchyme of the anterior part of the body. Two types of glands, strongly or weakly acidophilic, are recognizable with Dane Herman's tetrachrome, Pasini's reagent, and haematoxylin-eosin (Harris) stains. The musculature associated with this organ consists of a more strongly developed section of the ventral longitudinal body musculature.

The intestine is well visible, the anterior ramus reaches to a level just behind the neck and bears 13 to 15 branches on each side. The two posterior rami, each with 16 or 17 branches, usually converge behind the copulatory apparatus to form a common branch. The pharynx is located in the posterior half of the body and measures about 1/8 of the body length (Fig. 2A). Its internal muscle zone consists of a very thick layer of intermingled circular and longitudinal fibers. A subepithelial layer of longitudinal muscles followed by a layer of circular fibers forms the thin outer zone of muscles.

The two ovaries occur on the ventral side of the anterior region behind the brain. The ovaries are located at 1/5 of the distance between the brain and the root of the pharynx. The two oviducts originate from the dorsal part of the ovaries and are provided with a slight expansion at their anterior end, the tuba. The oviducts run posteriorly, fusing behind the copulatory apparatus to form a rather long common oviduct. The latter runs anteriorly to the right side of the opening of the bursal canal into the atrium. The common oviduct receives numerous openings of eosinophilic shell



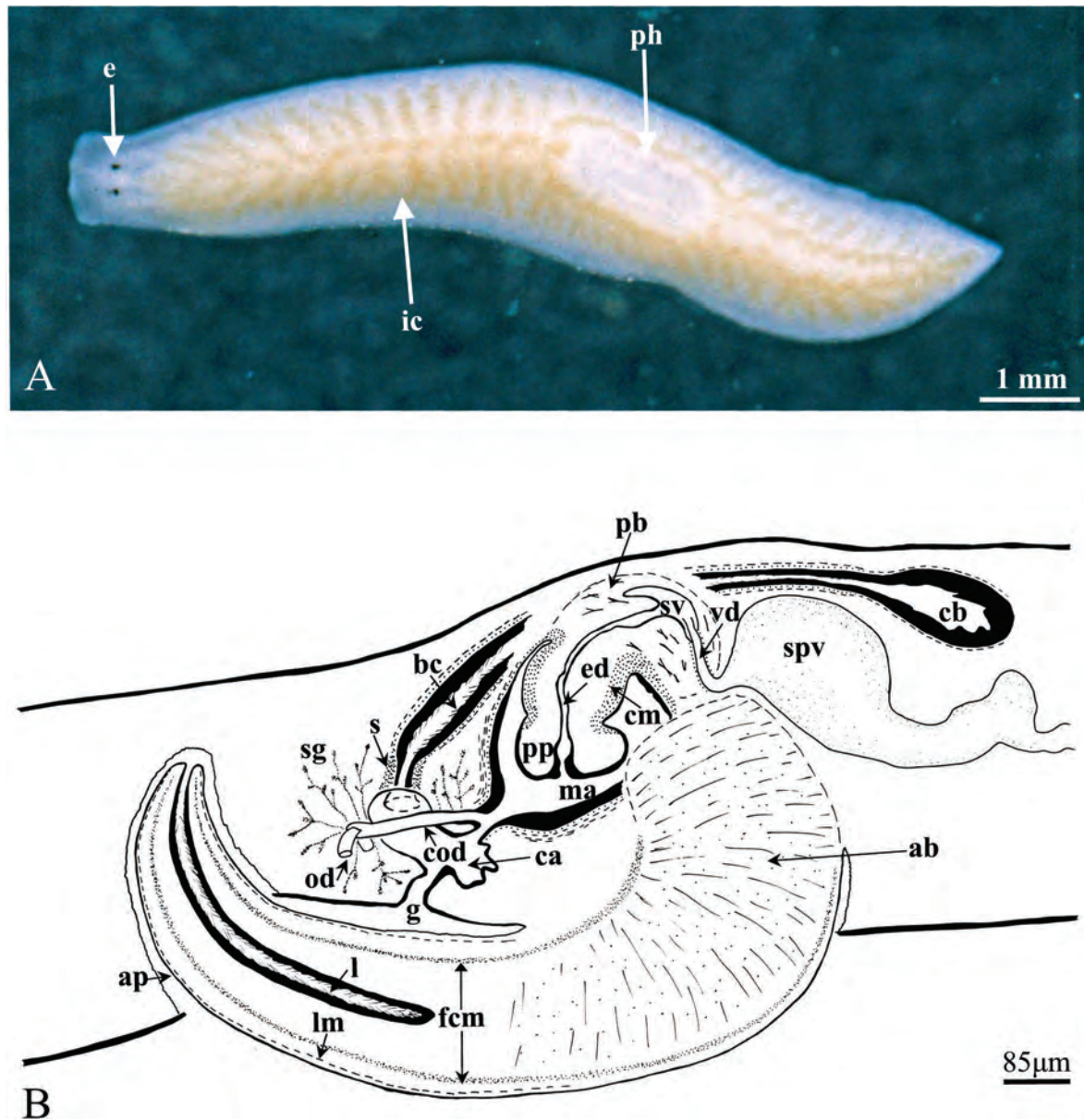


Figure 2. *Dendrocoelum mariae*. (A) habitus of a living specimen, (B) holotype ZMA V.Pl. 7100.1, sagittal reconstruction of the copulatory apparatus (anterior to the right).

glands along its entire length. The vitellaria are located between the intestinal diverticula and the testes.

The well-developed testes are numerous and essentially ventral in position (in one specimen some follicles are situated between the intestinal diverticula). The testes extend from a short distance behind the ovaries to the far posterior end of the body and are arranged on either side of the midline in two to three longitudinal zones. The sperm ducts form well-developed spermiducal vesicles, packed with sperm, between the mouth and the anterior level of the penis bulb (Fig. 2B).

The copulatory apparatus occupies the posterior half of the postpharyngeal region. The elongated, small copulatory

bursa is sac-shaped and situated in the dorsal part of the body. The bursa is lined with a high glandular epithelium and is surrounded by a layer of longitudinal muscles. The bursal canal runs posteriorly to the left of the penis and gradually widens, then turns ventrally and after narrowing again opens into the common atrium. The wall of the bursal canal consists of a nucleated epithelium with ciliated cells that gradually range from cuboidal in the proximal tract to cylindrical in the more distal section of the canal. The canal is surrounded by a subepithelial layer of circular muscles followed by a layer of longitudinal fibers. Just before the opening into the atrium the layer of circular muscles becomes thicker and thus forms a sphincter (Fig. 2B).

In the male copulatory apparatus the small, muscular penis bulb houses a seminal vesicle, devoid of a proper epithelium, that is surrounded by several interwoven layers of muscle. The vasa deferentia enter the seminal vesicle closely together from the antero-lateral sides and open symmetrically in its anterior part. The seminal vesicle communicates with the ejaculatory duct that opens at the tip of the small penis papilla. The penis papilla is slightly asymmetrical, with the ventral part somewhat larger than the dorsal one because of the dorsally displaced position of the ejaculatory duct. The penis papilla shows a division into two parts, a basal part with a thin epithelium underlain by a strong layer of circular muscles and a distal part covered with claviform epithelial cells with basal nuclei, but devoid of musculature. The two parts are separated by a constriction. The penis papilla has the same length as the penis bulb (Fig. 2B). In only one specimen (ZMA V.Pl. 7100.4) the penis papilla is twice as large as the penis bulb. In all examined specimens no penis glands were evident. The penis is located dorsally to the adenodactyl, while the bursal canal is situated to the left of the midline. The penis length is about 1/3 to 1/4 of the length of the adenodactyl depending on the state of contraction of the latter organ.

The adenodactyl is very large and consists of a free papilla and a well-developed bulbar part. The bulb is dorsal-ventral in orientation, and the adenodactyl makes a sharp posterior bend on the transition of the bulb to the papilla. In the majority of the examined mature specimens the adenodactyl is thrust out of the planarian body; in the holotype ZMA V.Pl. 7100.1 its tip is pushed into the parenchyma caudally to the copulatory apparatus (Fig. 2B). All of these conditions are very likely due to preservation artifacts. The bulb consists of rows of alternating longitudinal and circular muscles fibers. The lumen of the adenodactyl is lined by a layer of ciliated cells, and it is surrounded by a well-developed zone of glandular mesenchymatic tissue. Through this section of the mesenchyme runs a layer of circular muscle that is particularly evident in specimens ZMA V.Pl. 7100.4, ZMA V.Pl. 7100.5, and ZMA V.Pl. 7101.2. In the last-mentioned specimen, stained in Mallory-Cason, this layer of circular muscle is pale blue, whereas the intermingled muscles stain bright blue. Ectally to this zone of circular muscles runs a layer of longitudinal muscles fibers that stains red.

The male atrium is lined by a columnar epithelium, and it is surrounded by a subepithelial layer of circular muscles followed by three layers of longitudinal fibers. The male atrium communicates via a constriction with the common atrium. In this part it receives the opening of the common oviduct (Fig. 2B).

Discussion. The external morphology of *D. mariae* resembles that of *D. adenodactylosum* (Stanković and Komárek, 1927), both in the shape of the body and the frequent presence of supernumerary eyes. However, in *D. mariae* the eyes are arranged in two longitudinal, almost

straight rows, whereas in *D. adenodactylosum* they form two curved rows (see Kenk, 1978, p. 47).

*Dendrocoelum mariae* shares the presence of a very large adenodactyl, larger than the penis, with *D. adenodactylosum*, *D. maculatum* (Stanković and Komárek, 1927), *D. lacustre* (Stanković, 1938), *D. lychnidicum* (Stanković, 1969), *D. ochridense* (Stanković and Komárek, 1927), *D. sanctinaumi* (Stanković and Komárek, 1927), *D. minimum* Kenk, 1978, *D. nausicaae* (Stanković and Komárek, 1927), and *D. dani* Bromley, 1982. All of these species are endemic to the Lake Ohrid area, with the exception of *D. nausicaae* and *D. dani*. *Dendrocoelum nausicaae* is one of the most widely distributed species of the genus, together with *D. lacteum* Müller, 1774 and *D. album* (Steinmann, 1910), while *D. dani* is only known from northern Israel (Gourbault, 1972; Kenk, 1978; Bromley, 1982). Furthermore, *D. mariae* is similar to *D. adenodactylosum* and *D. maculatum* with respect to the anatomy of the adenodactyl. In the latter species the adenodactyl is characterized by the presence of a zone of fine circular muscle fibers that runs through the mesenchyme of the papilla. This particular adenodactyl anatomy, called the Balkan type, is also reported for *D. lacteum*, three recently described new species from Tunisia (Harrath et al., 2012), and the other two new species from Sardinia reported in the present paper. However, *D. mariae* differs from *D. adenodactylosum*, *D. maculatum*, *D. lacustre*, *D. lychnidicum*, *D. ochridense*, *D. sanctinaumi*, *D. nausicaae*, and *D. minimum* in the absence of the penis glands and the glandular field of tall epidermal cells surrounding the gonopore that is characteristic for these species. The glandular field around the gonopore and peculiarities in the histology of the oviducts (presence of numerous pear-shape cells) were considered by De Beauchamp (1931, 1932) to be distinguishing characteristics of the subgenus *Neodendrocoelum* Komárek, 1926. This subgenus comprises a homogeneous group of species with eyes, restricted to the Dinaric area and recorded chiefly from Lake Ohrid and its tributary streams and springs. According to Komárek (1926) all of these Balkan species are characterized by a large penis and a large adenodactyl, the latter being bigger than the penis, and by the presence of two atrial compartments. *Dendrocoelum mariae* differs from *D. dani* in the absence of a copulatory bursa in the latter that is replaced by bursal-intestinal communications (Bromley, 1982).

In most of the known species of the genus the copulatory apparatus is positioned just behind the pharyngeal pocket, while the penis and the adenodactyl in the majority of species are localized on the opposite sides of the body. In contrast, in *D. mariae* the copulatory apparatus is situated far behind the pharyngeal pocket, while the penis and the adenodactyl are always located dorsally and ventrally, respectively.

The new species bears a true sphincter in the terminal tract of the bursal canal. This prominent structure is mainly formed by several layers of circular muscle



surrounding a distinct narrowing of the bursal canal lumen (see separate discussion below).

*DENDROCOELUM NURAGHUM* STOCCHINO AND SLUYS SP. NOV.  
(Table 2; Figs. 1, 3)

**Material examined.** Holotype: ZMA V.Pl. 7102.1, Monte Majore Cave (40°30'51"N, 8°36'37"E), 13 May 2009, coll. B. Cadeddu and P. Marcia, sagittal sections on 4 slides.

Other material: CGAS Pla 2.1, Monte Majore Cave (40°30'51"N, 8°36'37"E), April 2005, coll. P. Marcia and G. Tomasin, sagittal sections on 24 slides.

**Etymology.** The specific epithet refers to nuraghe, the typical Bronze Age megalithic edifice symbol of Sardinia.

**Geographical distribution.** Endemic to the Monte Majore Cave and only known from the type locality.

**Habitat.** Specimens of *D. nuraghum* were found in the Monte Majore Cave at ca. 530 m asl, under pebbles in a small streamlet at ca. 130 meters from the cave entrance in completely dark conditions (Fig. 1). Planarians were associated with *Proasellus* sp. (Asellidae). Although several sampling campaigns (20 in total) were held in a period of five years (2005 to 2010) only 4 planarians were found on three occasions during the spring (April, May). Only three of the four individuals collected were sexual; one of these sexual specimens did not survive under laboratory conditions. The immature, asexual specimen was processed for karyological analyses (not reported in this paper).

**Diagnosis.** *Dendrocoelum nuraghum* is characterized by the presence of a single common vas deferens, a large penis papilla with introverted apical part, a very large adenodactyl located to the left of the penis papilla, the presence of a sphincter in the terminal tract of the bursal canal, and by the posterior extension of the testes to only the level of the root of the pharynx.

**Description.** Live adult animals had a body size of ca. 8 mm in length and a width ranging from ca. 2 mm in the central part of the body to ca. 0.6 mm at the level of the head. They are unpigmented and fairly transparent, so that the intestinal branches, pharynx, and copulatory apparatus are clearly visible. The anterior end is truncated, with a convex mid-frontal margin, and is provided with a pair of rounded lateral lobes (Fig. 3A).

The two eyes are situated rather closely together. There is a slight neck-like constriction just behind the eyes, after which the body gradually widens, attaining its maximum width at the level of the pharynx and the copulatory apparatus. The distance between the eyes is 1/3 to 1/4 of the width of the neck. Inter-ocular distance is smaller than the distance from each eye cup to the lateral margin, and the distance from the frontal margin to the eyes is greater than the distance to the lateral margin.

The subterminal anterior adhesive organ has a length slightly greater than the inter-ocular distance and consists of a shallow cup. The surface of the organ consists of an area of infranucleated epithelial cells pierced by numerous gland ducts. The cell bodies of the very abundant glands

are scattered throughout the mesenchyme of the anterior part of the body. They pass above the brain and then above and under the intestinal diverticula, reaching up to the anterior surface of the ovaries. The musculature of the adhesive organ consists of a more strongly developed section of the usual ventral longitudinal body musculature.

The intestine is well visible, the anterior ramus reaches to a level just behind the neck and bears 7 or 8 branches on each side. The two posterior rami bear 16 or 17 branches. The pharynx is located in the posterior half of the body and measures about 1/7 of the body length (Fig. 3A). Its internal muscle zone consists of a thick layer of intermingled circular and longitudinal fibers. A subepithelial layer of longitudinal muscles followed by a layer of circular fibers forms the thin outer zone of muscles.

The two ovaries occur on the ventral side of the anterior region behind the brain. The ovaries are located at less than 1/3 of the distance between the brain and the root of the pharynx. The oviducts originate from the latero-dorsal part of the ovaries and are provided with a slight expansion at their anterior end, the tuba. The oviducts run posteriorly, converging behind the copulatory apparatus to form a single common oviduct. The latter runs anteriorly to the right side of the opening of the bursal canal and opens into the end part of the male atrium. The distal part of the oviducts and the first tract of the common oviduct receive the openings of the shell glands (Fig. 3B).

The numerous well-developed testes are essentially ventral in position, but some are situated in a dorsal position or in the middle of the body. Some follicles are oval in shape and occupy almost the entire dorso-ventral space of the body. The testes extend from a short distance behind the ovaries to the level of the root of the pharynx. The sperm ducts form very large spermiducal vesicles, packed with sperm, between the mouth and the anterior level of the penis bulb (Fig. 3B).

In the holotype (ZMA V.Pl. 7102.1) the copulatory apparatus is localized just behind the pharyngeal pocket, while in CGAS Pla 2.1 specimen the copulatory apparatus occupies the posterior half of the postpharyngeal region. The penis is located to the right, the bursal canal and the adenodactyl to the left of the midline. The copulatory bursa has the shape of a large sack that occupies almost the entire dorso-ventral diameter of the body. The bursa is lined with a tall glandular epithelium and is surrounded by a layer of longitudinal muscle. The bursal canal runs posteriorly to the left of the penis and gradually widens, then turns ventrally to open into the common atrium. The wall of the bursal canal consists of a nucleated epithelium with ciliated cells. The canal is surrounded by a layer of circular muscle, followed by a layer of longitudinal fibers. At a short distance from the opening in the common atrium the canal lumen reduces, while the circular muscle layer becomes very thick; this tract of the bursal canal is enveloped by a sphincter (Fig. 3C).

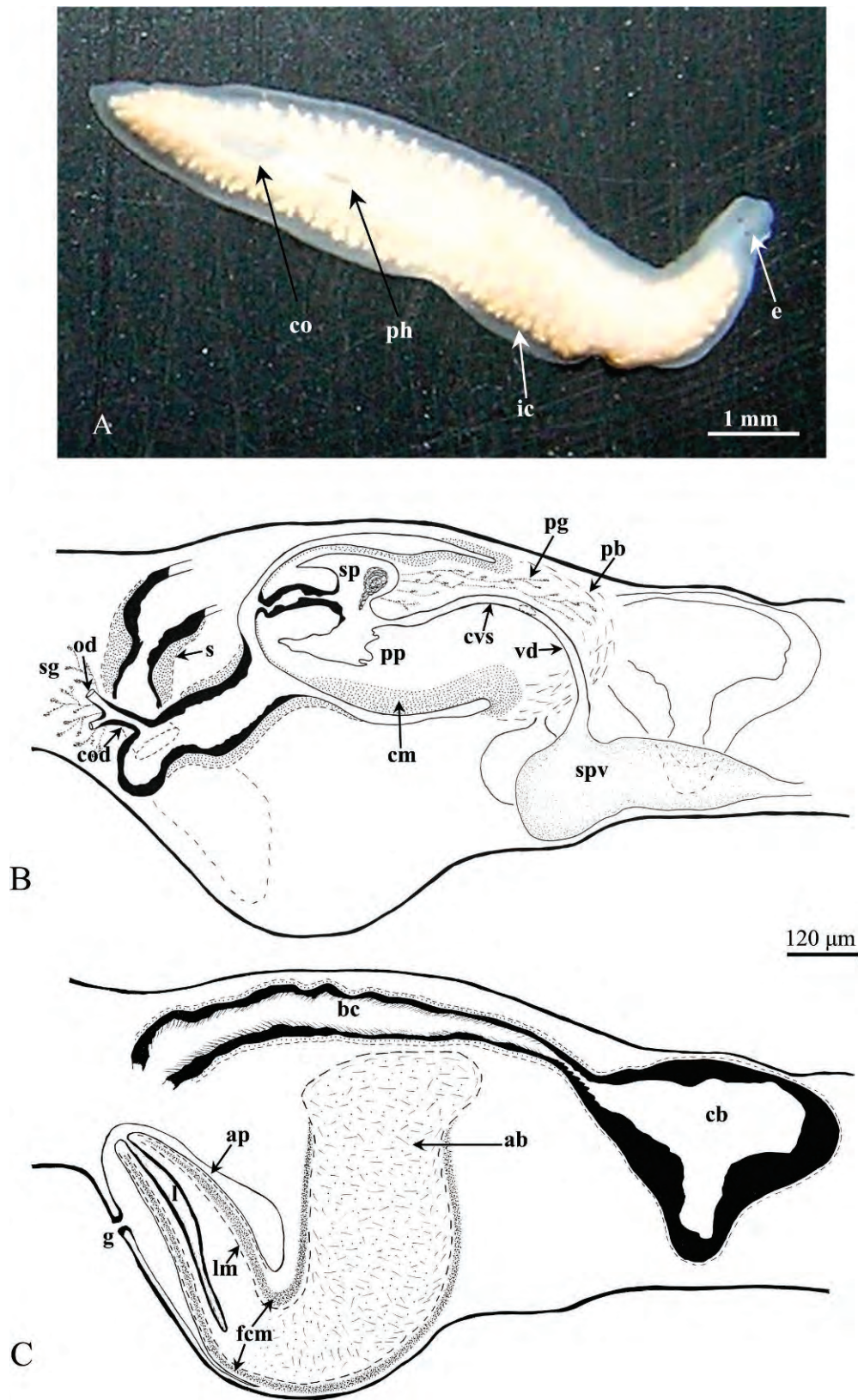


Figure 3. *Dendrocoelum nuraghum*. (A) habitus of a living specimen, (B-C), holotype ZMA V.Pl. 7102.1, sagittal reconstructions of the copulatory apparatus (anterior to the right).

In the male copulatory apparatus the muscular penis bulb is of moderate size. The vasa deferentia penetrate the penis bulb separately, but very close to each other, at its anterior part, and then they converge toward the midline of the penis bulb to form a common vas deferens that opens into the anterior part of the penis papilla lumen (Fig. 3B). The long penis papilla measures 3/4 of the total penis length. The penis length is ca. 1/2 of the adenodactyl length. The penis papilla is barrel-shaped in the holotype and finger-shaped in specimen CGAS Pla 2.1; the latter condition may be due to a preservation artifact.

The penis papilla is covered by a very thin epithelium at the basal part that becomes thicker at the apical part. The epithelium is underlined by a thick layer of circular muscle that is thicker in the ventral part and at the base of the papilla. In both specimens examined the apical part of the penis papilla lacks muscle layers and appears introverted into the very large papilla lumen like a pseudoflagellum (Fig. 3B; see discussion on flagellum below).

In the penis papilla lumen of the holotype a pack of sperm is present. The penis is rich in glands, which in the holotype are located in the penis bulb and in the dorsal part of the papilla. These glands open into the large penis lumen, which is full of secretion.

The adenodactyl is very large and consists of a free papilla and a well-developed, very muscular bulbar part (Fig. 3C). The free papilla is about 1/2 of the total adenodactyl length. The bulb starts in the dorsal part of the body and has a dorsal-ventral orientation. It consists of rows of intermingled longitudinal and circular muscles fibers covered by a thin layer of longitudinal fibers. Ectally to this thin longitudinal muscle layer runs a thick layer of fine circular muscle fibers that continues its course through the mesenchyme of the adenodactyl papilla (Fig. 3C). In specimen CGAS Pla 2.1, stained in Dane Herman's tetrachrome, this thick layer is grey and contrasts with the other muscular fibers, which stain bright yellow. In the holotype, stained in Mallory-Cason, this layer is pale blue, the longitudinal fibers stain red, and the intermingled muscles stain bright blue. The free papilla, which protrudes posteriorly and dorsally into the atrium, is characterized by a thin lining epithelium underlain by three layers of muscles: a subepithelial layer of longitudinal muscle, a thick layer of fine circular muscle fibers, and a thin inner layer of longitudinal muscle.

The male atrium consists of an anterior part that houses the penis papilla and a canal leading obliquely, in a postero-ventral direction, into the common genital atrium. This canal is lined by a thick epithelium and is surrounded by a strong layer of circular and longitudinal muscle fibers. The common atrium receives the opening of the bursal canal.

**Discussion.** *Dendrocoelum nuraghum* is characterized by the fusion of the two vasa deferentia into an intrapenial common duct. Within the genus *Dendrocoelum* this character is reported for only four other species: *D.*

*jablanicense* (Stanković and Komárek, 1927), *D. puteale* Kenk, 1930, *D. kenki* De Beauchamp, 1937, and *D. constrictum* Harrath and Sluys, 2012. *Dendrocoelum nuraghum* differs from *D. puteale* and *D. kenki* because these are both anophthalmous species. *Dendrocoelum puteale* is characterized by an extrabulbar seminal vesicle that receives in its right part a common vas deferens, originating from the fusion of the two vasa deferentia at a level just behind the copulatory bursa (De Beauchamp, 1932). In *D. kenki* the vasa deferentia unite just before entering the penis bulb. Moreover, in this species the penis is in the middle, while the adenodactyl is on the right side of the body (De Beauchamp, 1937), in contrast to *D. nuraghum* in which the penis is localized on the right and the adenodactyl on the left of the midline of the body.

*Dendrocoelum nuraghum* is similar to *D. jablanicense* because its vasa deferentia also separately penetrate the penis bulb and fuse to a common duct before opening into the penis papilla lumen. However, *D. nuraghum* differs from *D. jablanicense* in the absence of the glandular field of tall epidermal cells that in the latter surrounds the gonopore. Moreover, the adenodactyl in *D. nuraghum* is on the left of the midline of the body, whereas in *D. jablanicense* it is on the right. The last-mentioned species is also characterized by a double communication between the bursal canal and a common atrium (see Kenk, 1978), which is absent in *D. nuraghum*.

*Dendrocoelum nuraghum* is characterized by an irregular distribution of the testes (ventral, dorsal, and between the intestinal diverticula), which extend posteriorly to the level of the root of the pharynx. In contrast, in *D. puteale* and in *D. jablanicense* the testes extend to the tail end and are rather dorsal and ventral, respectively. In *D. kenki* the testes are mainly ventral and extend posteriorly to the level of the copulatory bursa.

*Dendrocoelum nuraghum* has a distinct narrowing in its bursal canal, surrounded by a sphincter. This character is shared with two other Sardinian dendrocoelids (see below).

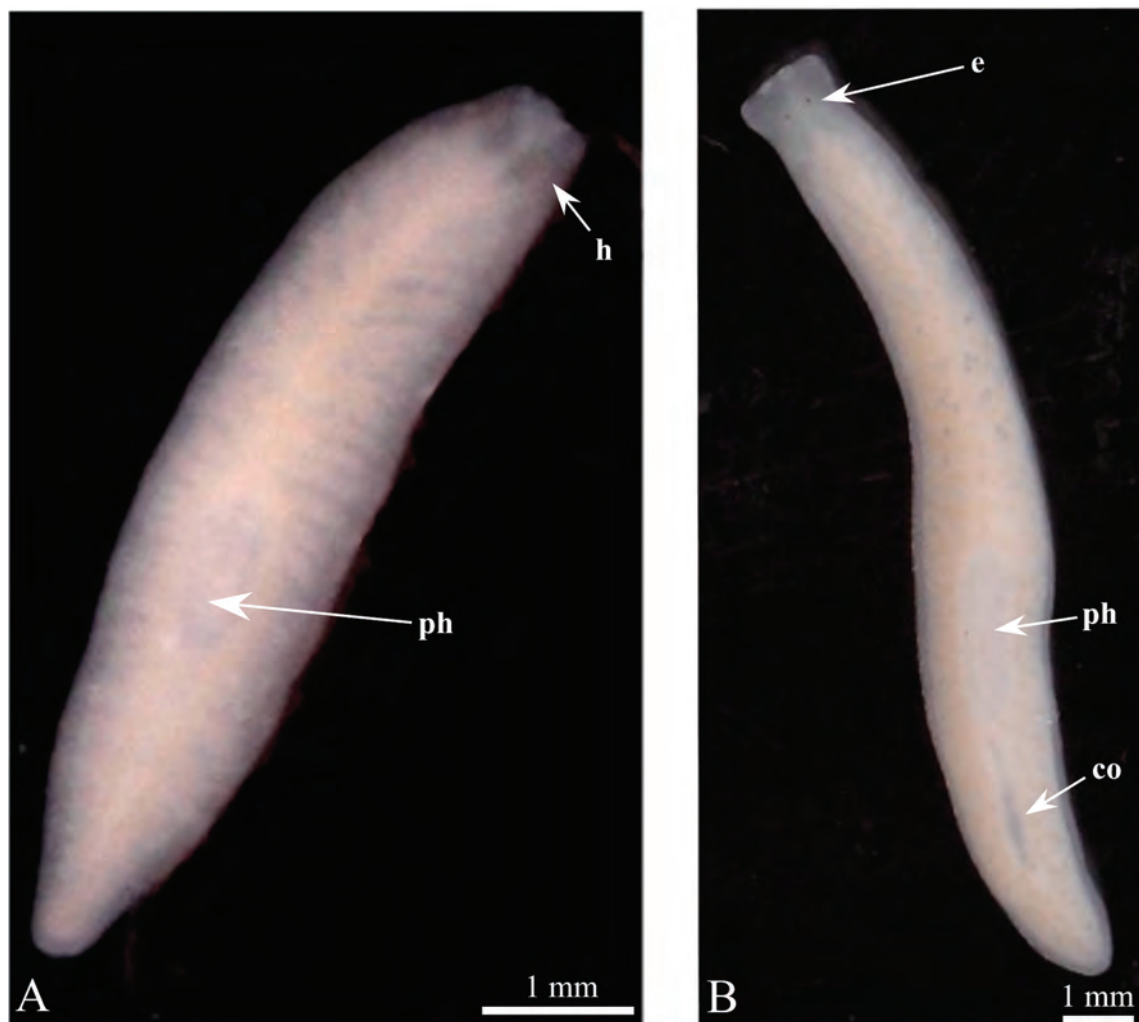
The large penis and large adenodactyl of *D. nuraghum* are shared with *D. adenodactylosum*, *D. maculatum*, *D. lacustre*, *D. lychnidicum*, *D. ochridense*, *D. sanctinaumi*, *D. minimum*, *D. nausicaae* and *D. dani*. *Dendrocoelum nuraghum* differs from *D. adenodactylosum*, *D. maculatum*, *D. lacustre*, *D. lychnidicum*, *D. ochridense*, *D. sanctinaumi*, *D. minimum*, and *D. nausicaae* in the absence of the glandular field around the genital pore that is present in all of these species.

*DENDROCOELUM VESICULOSUM* STOCCHINO AND SLUYS SP. NOV.  
(Table 2; Figs. 1, 4, 5, 6)

**Material examined.** The population is characterized by the coexistence of two morphotypes: anophthalmous (morphotype 1) and two-eyed (morphotype 2) individuals.

Holotype: ZMA V.Pl. 7103.1, sagittal sections on 4 slides of a morphotype-2 animal, Sa Ucca è su Peltusu Cave (40°27'00"N, 8°40'43"E), 6 May 2009, coll. P. Marcia, B. Cadeddu, F. Stoch and G. Tomasin.





**Figure 4.** *Dendrocoelum vesiculosum*. Habitus of living specimens, (A) morphotype 1 characterized by the absence of eyes, (B) morphotype 2 with eyes.

Paratypes: CGAS Pla 3.1, *ibid.*, sagittal sections on 3 slides of a morphotype-2 animal; ZMA V.Pl. 7103.2, *ibid.*, sagittal sections on 3 slides of a morphotype-2 animal, ZMA V.Pl. 7103.3, *ibid.*, sagittal sections on 4 slides of a morphotype-1 animal.

Other material: CGAS Pla 3.2, sagittal sections on 6 slides of a morphotype-2 animal, not completely mature, Sa Ucca 'e su Peltusu Cave (40°27'00"N, 8°40'43"E) 3 September 2007, coll. P. Marcia and M. Fois; CGAS Pla 3.3, *ibid.*, sagittal sections on 13 slides, of a morphotype-1 animal, not completely mature; ZMA V.Pl. 7104.1, sagittal sections on 5 slides of a morphotype-1 animal, F1 offspring; ZMA V.Pl. 7105.1, one not completely mature morphotype-1 animal, resulting from the F2 offspring, sagittal sections on 3 slides.

**Etymology.** The specific epithet is derived from the Latin adjective *vesiculosus*, full of vesicles, and alludes to the presence of an expansion or vesicle in each vasa deferens.

**Life cycle.** Specimens of *Dendrocoelum vesiculosum* were collected in September 2007 and May 2009. On the first occasion two juveniles were collected, one specimen being anophthalmous and the other with two very small eyes. The life cycle of these individuals was observed for about three years under laboratory conditions. In this species the breeding period follows a seasonal cycle. From May to July the specimens developed a copulatory apparatus, while in summer-autumn they laid cocoons. In May 2008, after 7 months of rearing, a sexualization process was displayed in both specimens. In the following June the two animals produced 2 cocoons. From the only fertile cocoon five young anophthalmous planarians (F1) hatched after five weeks of development. Only three of these worms survived after 7 months of rearing (January 2009), and they became sexual in July 2009 and produced three cocoons during October 2009. From the only fertile cocoon four young anophthalmous planarians (F2) hatched after 4 weeks. In June 2010 the only surviving planarian became sexually mature.

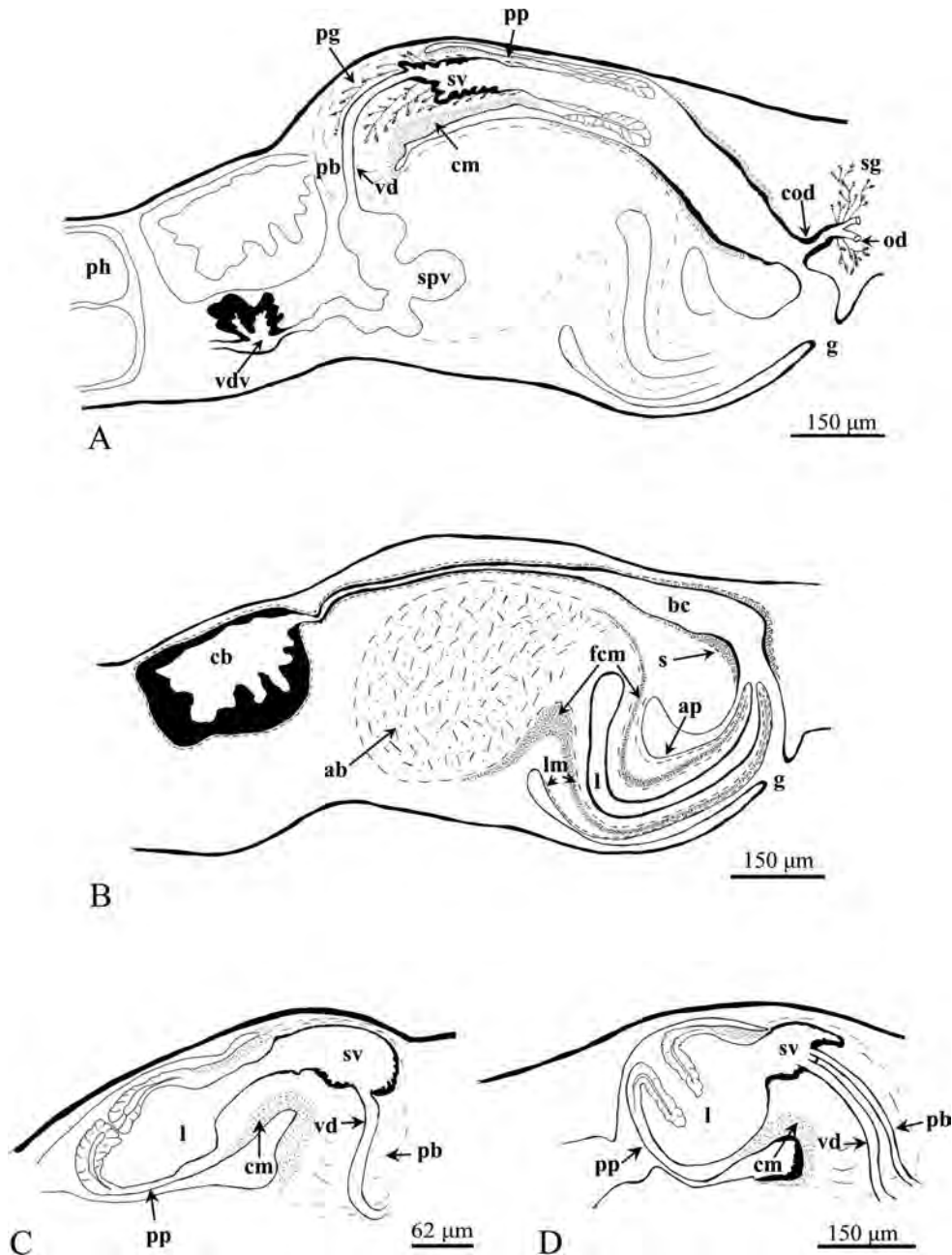


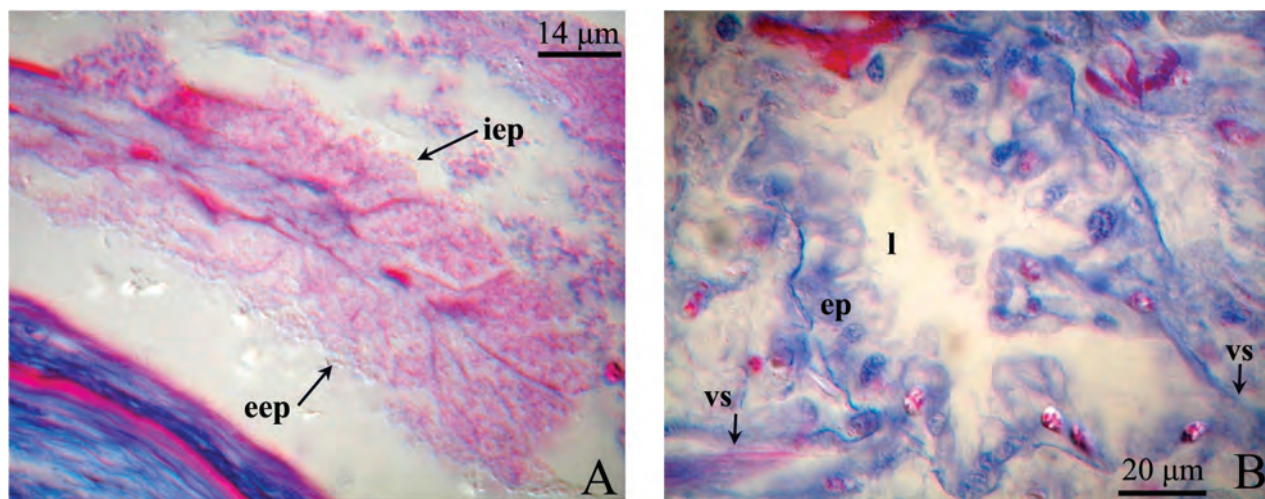
Figure 5. *Dendrocoelum vesiculosum*. (A-B) holotype ZMA V.Pl. 7103.1, sagittal reconstructions of the copulatory apparatus (anterior to the left), (C) CGAS Pla 3.1 sagittal reconstructions of the penis papilla (anterior to the right), (D) ZMA V.Pl. 7103.2, sagittal reconstructions of the penis papilla (anterior to the right).

The first two worms, i.e., the parents, were killed and preserved in July 2008, after the first offspring (F1) had been produced, one month after cocoon deposition. At that time, both gonads and the copulatory apparatus appeared to be in a state of regression, although abundant sperm were still present in the vasa deferentia. This condition of regression was particularly evident in the two-eyed specimen.

During the second collection, five specimens were captured, one sexual anophtalmous animal and four

with eyes, of which only two were sexually mature and eventually only one survived. The other two animals became sexually mature in January 2010, after eight months of rearing. All specimens were preserved at that time.

Habitat. Specimens of *Dendrocoelum vesiculosum* were found in totally dark conditions under pebbles in a small stream in the Sa Ucca 'e su Peltusu Cave at an altitude of ca. 560 m asl (Fig. 1). The dendrocoelids were associated with *Dugesia* sp.



**Figure 6.** *Dendrocoelum vesiculosum*. (A) photomicrograph of the penis papilla tip showing the vacuolate secretory epithelium, (B) photomicrograph of the expansion/vesicle in the vas deferens.

Geographical distribution. Endemic to the Sa Ucca ‘e su Peltusu Cave and only known from the type locality.

Diagnosis. *Dendrocoelum vesiculosum* is characterized by: the presence in the population of both anophtalmous and two-eyed individuals; a peculiar penis histology, with vacuolated cells lining the tip of the papilla; a convoluted adenodactyl; a sphincter in the terminal tract of the bursal canal; a very narrow horizontal tract of bursal canal; and the presence of a peculiar expansion in the vasa deferentia, different from the spermiducal vesicles.

Description. Live animals are unpigmented, typically whitish, with a body size of 8 to 10 mm in length and a width ranging from ca. 2 mm in the central part of the body to 1 mm at the level of the head. The anterior end is truncated with a pair of rounded lateral lobes (Fig. 4). In the ocular animals the eyes are small and placed not far from the frontal margin. Their distance from the frontal margin is slightly greater than the distance to the lateral margin. Inter-ocular distance is equal to the distance from each eye cup to the lateral margin. Just at level of eyes there is a slight constriction or neck that sets off the head from the rest of the body (Fig. 4B).

The anterior, subterminal adhesive organ, with a width slightly more than the inter-ocular distance, is feebly developed and consists of a small patch of epidermis with infranucleated cells that is pierced by numerous openings of glands ducts.

The pharynx is located in the posterior half of the body and measures somewhat less than 1/6 of the body length. The thin outer zone of muscles consists of a subepithelial layer of longitudinal muscle, followed by a layer of circular muscles. The inner epithelium is underlain by a thick layer of intermingled circular and longitudinal fibers.

The two ventral, paired ovaries are located at about 1/3 of the distance between the brain and the root of the

pharynx. The anterior-most sections of the oviducts are expanded to form a tuba. The oviducts run posteriorly, converge behind the copulatory apparatus, and unite to form a common oviduct that opens into the terminal tract of the male atrium. The common oviduct receives the openings of eosinophilic shell glands.

Well-developed rounded resorptive vesicles are present along the oviducts of all specimens examined. Each vesicle, characterized by the presence of vacuoles, communicates with the oviduct through a short, narrow duct (cf. Sluys, 1989). Sperm is present, both in the resorptive vesicles and in the short interconnecting ductules.

The well-developed, numerous testes are localized in both ventral and in dorsal position, while some follicles are situated in the middle of the body. The testes extend throughout the body from directly behind the ovaries to the tail end.

The sac-shaped copulatory bursa is situated just behind the pharynx. The bursa is lined with a tall glandular epithelium and is surrounded by a layer of longitudinal muscle. From the copulatory bursa the bursal canal runs posteriorly and horizontally to the left of the penis. This horizontal tract of the bursal canal is very narrow and is lined with a nucleated, ciliated epithelium, surrounded by only a thin layer of longitudinal muscle fibers. At the level of the insertion of the adenodactyl papilla the bursal canal widens, and from this point it is surrounded by a layer of circular muscle, followed by a layer of longitudinal fibers. Before opening into the atrium the bursal canal narrows again, while the circular muscles become thicker, thus forming a sphincter (Fig. 5B). This structure is most evident in those specimens in which the tip of the adenodactyl papilla is not deeply inserted into the bursal canal.

The well-developed penis bulb, formed by intermingled circular and longitudinal muscle fibers, houses a large



seminal vesicle that is lined by a vacuolated epithelium the cells of which protrude irregularly into the lumen (Fig. 5A).

In all specimens examined the two vasa deferentia run ventrally, and when the ducts arrive at the level of or just before the copulatory bursa they show a peculiar expansion of their dorsal wall, thus forming a large vesicle that can be either empty or full of sperm. The wall of this vesicle is lined by tall, claviform, vacuolated cells, very different from the rest of the ducts, which are lined with a thin epithelium (Figs 5A, 6B).

In specimen CGAS Pla 3.1 abundant sperm is present in the lumen of the two posterior intestinal branches. In the same specimen the right vas deferens shows at the level of the expansion a connection with the lumen of a posterior intestinal branch, most likely due to a preservation artifact.

After having given rise to an expansion, the vasa deferentia form well-developed spermiducal vesicles, packed with sperm. Thereafter the ducts curve to the dorsal side, in some cases (ZMA V.Pl. 7103.1, ZMA V.Pl. 7103.2, CGAS Pla 3.1) almost reaching the dorsal part of the body, and penetrate the penis bulb to open, closely together, into the antero-lateral section of the seminal vesicle. In specimens ZMA V.Pl. 7103.2 and ZMA V.Pl. 7103.3 the right vas deferens enters the penis bulb dorsally to the left one. The penis is rich in glands that open into the seminal vesicle and into the penis papilla lumen (Fig. 5A).

The penis papilla is characterized by a thick basal part and a thinner distal part. The basal part is covered with a flat epithelium that is underlain by a thick layer of circular muscle. The epithelium on the apical part consists of large, rounded, and vacuolated cells, with basal nuclei, very rich in secretion granules. This epithelium covers both the external and the internal distal part of the penis papilla (Figs 5A, 6A). In the holotype (ZMA V.Pl. 7103.1) sperm is present in the male atrium.

The penis papilla may assume different shapes. In the holotype and in ZMA V.Pl. 7103.3 it is considerably elongated and provided with a tunnel-shaped lumen (Fig. 5A). In CGAS Pla 3.1 the papilla is more contracted and therefore provided with a wider lumen (Fig. 5C). In ZMA V.Pl. 7103.2 the contraction and inversion of the tip is more advanced and thus forms a pseudo-flagellum (Fig. 5D).

The adenodactyl consists of a well-developed bulbar part and a free papilla. The bulb is very large and occupies almost the entire dorso-ventral space of the body (Fig. 5B). In all examined specimens the shape of the entire adenodactyl is sinuous, in that the bulb originates at the ventral region of the body and then gradually extends to the left with a dorso-lateral orientation. The adenodactyl papilla first has a dorso-ventral orientation and then makes a posteriorly directed bend, with the tip pointing into the terminal tract of the bursal canal. Due to the large size, the particular orientation, and the different states of contraction of the adenodactyl, the penis may be localized at

different positions. In specimens ZMA V.Pl. 7103.2, ZMA V.Pl. 7103.3, and ZMA V.Pl. 7104.1 the penis is located on the right side of the adenodactyl, whereas in CGAS Pla 3.1 and ZMA V.Pl. 7103.1 the penis is located on the left side of and dorsally to the adenodactyl, respectively.

The bulb of the adenodactyl consists of intermingled rows of longitudinal and circular muscle, bounded by a thin layer of longitudinal fibers. Ectally to this thin longitudinal muscle layer runs a thick layer of fine circular muscle fibers that continues its course through the mesenchyme of the adenodactyl papilla. Ectally to this zone of circular muscles runs a thin layer of longitudinal muscle fibers (Fig. 5B). In all examined specimens, stained in Mallory-Cason, this layer of fine circular fibers is pale blue, while the longitudinal fibers stain red and the intermingled muscles stain bright blue. No glands were observed to discharge into the lumen of the adenodactyl.

The male atrium is lined by a nucleated epithelium surrounded by a subepithelial layer of circular muscles, followed by a layer of longitudinal fibers. Just before opening in the common atrium the male atrium receives the opening of the common oviduct.

**Discussion.** In the course of our long-term study of the life cycle, during two generations (3 years), only anophthalmous offspring was observed. Partial or total loss of eyes may result from adaptation to the subterranean environment. A seasonal life cycle, as observed in *D. vesiculosum*, has been reported for only a few hypogean species, such as *Atrioplanaria delamarei* (Gourbault, 1972). The majority of subterranean triclads produce cocoons during the entire year (Gourbault, 1972).

The number of cocoons in *D. vesiculosum* is very low (2 or 3). Generally, the number of cocoons produced by hypogean species varies between species, but it is always considerably lower than in epigeic species (Gourbault, 1972). The fertility of the cocoons is in hypogean triclads also very variable between species (Gourbault, 1972). In *D. vesiculosum* the fertility was 30 to 50% and only 4 or 5 young hatched from each cocoon, in agreement with the general condition of hypogean triclads, in which generally fewer than eight young hatch from a cocoon.

The juveniles of *D. vesiculosum* became sexually mature 8 to 12 months after hatching, in contrast to *D. collini* (De Beauchamp, 1919), which needs 4 to 5 months, and *Phagocata vitta* juveniles that become sexual after 9 or 10 months (Gourbault, 1972). However, the low number of young hatching from the cocoons is counterbalanced by an increase of longevity. A specimen of *D. mariae* was still alive after 6 years, although laboratory conditions are less harsh than those in situ, for example, with respect to food availability. Our observations on the life history illustrate considerable slowing down of the cocoon deposition rhythm, as well as embryonic and post-embryonic development (K strategy) and are in agreement with the adaptive strategies typical of subterranean fauna (Juberthie

and Decu, 1994) and in particular of stygobiont triclads (Gourbault, 1994). This strategy ensures a more efficient management of a low energy budget.

*Dendrocoelum vesiculosum* shows a peculiar penis papilla histology, characterized by the presence of large, vacuolated cells that surround both the internal and the external wall of the tip of the penis papilla. This character is unique for this species (see discussion on flagellum below).

Another unique character for this new species is the presence of a peculiar dorsal expansion in the vasa deferentia. This expansion differs from the usual spermiducal vesicles and has not been described earlier for any other species of the genus *Dendrocoelum*.

*Dendrocoelum vesiculosum* is characterized by a large adenodactyl and by a sphincter in the terminal tract of the bursal canal. These characters are shared with two other Sardinian species (see discussion on sphincter below).

Family PLANARIIDAE Stimpson, 1857

Genus *Phagocata* Leidy, 1847

*Phagocata obscura* Stocchino and Sluys sp. nov.

(Table 2, Figs. 1, 7)

**Material examined.** Holotype: ZMA V.Pl. 7106.1, sagittal sections on 34 slides, Cuccuru Tiria Cave (39°19'25"N, 8°34'29"E), 28 March 2006, coll. P. Marcia.

**Habitat.** Specimens of *Phagocata obscura* were found under pebbles in a stream running in totally dark condition in the Cuccuru Tiria Cave (Fig. 1). Planarians were associated with *Stenasellus* sp. (Stenasellidae) and Oligochaeta.

**Etymology.** The specific epithet *obscura*, from the Latin adjective *obscurus* (dark, somber, hidden, invisible), alludes to both the cave habitat of the species and the fact that some details of its anatomy could not be discerned in the material available.

**Geographical distribution.** Endemic to the Cuccuru Tiria Cave and only known from the type locality.

**Diagnosis.** *Phagocata obscura* is characterized by the absence of eyes, a conical head, the presence of a well-developed penis bulb containing a spacious seminal vesicle, a plug of cells in the ejaculatory duct, a common vas deferens, and testes located mainly in prepharyngeal position.

**Description.** Live animals are unpigmented, typically whitish, with a body size in an elongated state of ca. 6 mm in length and a width ranging from 1 mm in the central part of the body to 0.5 mm at the level of the head. The anterior end is obtusely pointed and devoid of eyes (Fig. 7A). The pharynx is located in the posterior half of the body and measures about 1/5 of the body length.

The two ovaries are located at a short distance behind the brain; they are small and poorly developed. The oviducts run dorsally to the ventral cords and curve dorsally at the level of the penis bulb. The oviducts are still

visible at this point, but their continuation and subsequent communication with the female copulatory apparatus could not be discerned.

The numerous but poorly developed testes are closely packed together in ventral position. They begin at a considerable distance behind the ovaries and extend posteriorly to the level of the root of the pharynx. Some follicles may extend to halfway along the pharyngeal pocket.

The copulatory apparatus is located at some distance behind the pharyngeal pocket. The small sac-shaped copulatory bursa is lined with a tall epithelium and is surrounded by a layer of longitudinal muscle. From the bursa, a narrow bursal canal, lined with a squamous to cuboidal, nucleated epithelium, runs dorsally to the male atrium. Near the posterior end of the male atrium the canal curves ventrally to open into the common atrium (Fig. 7B).

The penis bulb is well-developed, with a length approximately equal to that of the penis papilla; its musculature is formed by intermingled longitudinal and circular muscles fibers.

A single vas deferens opens into the proximal, anterior section of the intrabulbar seminal vesicle. This duct may represent a common vas deferens, originating from the union of the two vasa deferentia in the anterior part of the penis bulb, but such could not be discerned in the sections.

The epithelium of the elongated, finger-shaped penis papilla is underlain by a thin layer of circular muscle fibers. The ejaculatory duct runs centrally through the penis and opens at the tip of the papilla. The ejaculatory duct is histologically differentiated into two sections. A short anterior portion is enlarged to form a relatively spacious seminal vesicle or intrabulbar cavity that is lined with tall secretory cells with basal nuclei and surrounded by a layer of longitudinal muscle. The posterior part of this cavity is lined with much taller cells, forming a plug that fills the entire lumen of the canal (Fig. 7B). The second, distal section of the ejaculatory duct is lined by an epithelium with cuboidal cells.

The male atrium is lined by tall nucleated cells, surrounded by a thick layer of circular muscle.

**Discussion.** *Phagocata obscura* is the first anophthalmous species of the genus reported in Europe. According to Kenk (1978) all European species of *Phagocata* are "two eyed planariids with a truncated head." This trait appears to be consistently present in all species that were subsequently described (cf. Sluys et al., 1995; Vila-Farrè et al., 2011).

The plug of cells in the ejaculatory duct is shared with a group of eleven species (cf. Sluys et al., 1995; Vila-Farrè et al., 2011): *P. albissima* (Vejdovský, 1883), *P. armeniaca*, *P. undulata*, *P. bosniaca*, *P. macedonica* (Stanković, 1926), *P. illyrica* (Komárek, 1919), *P. maculata*, *P. dalmatica* (Stanković and Komárek, 1927), *P. ochridana*, *P. stankovici*

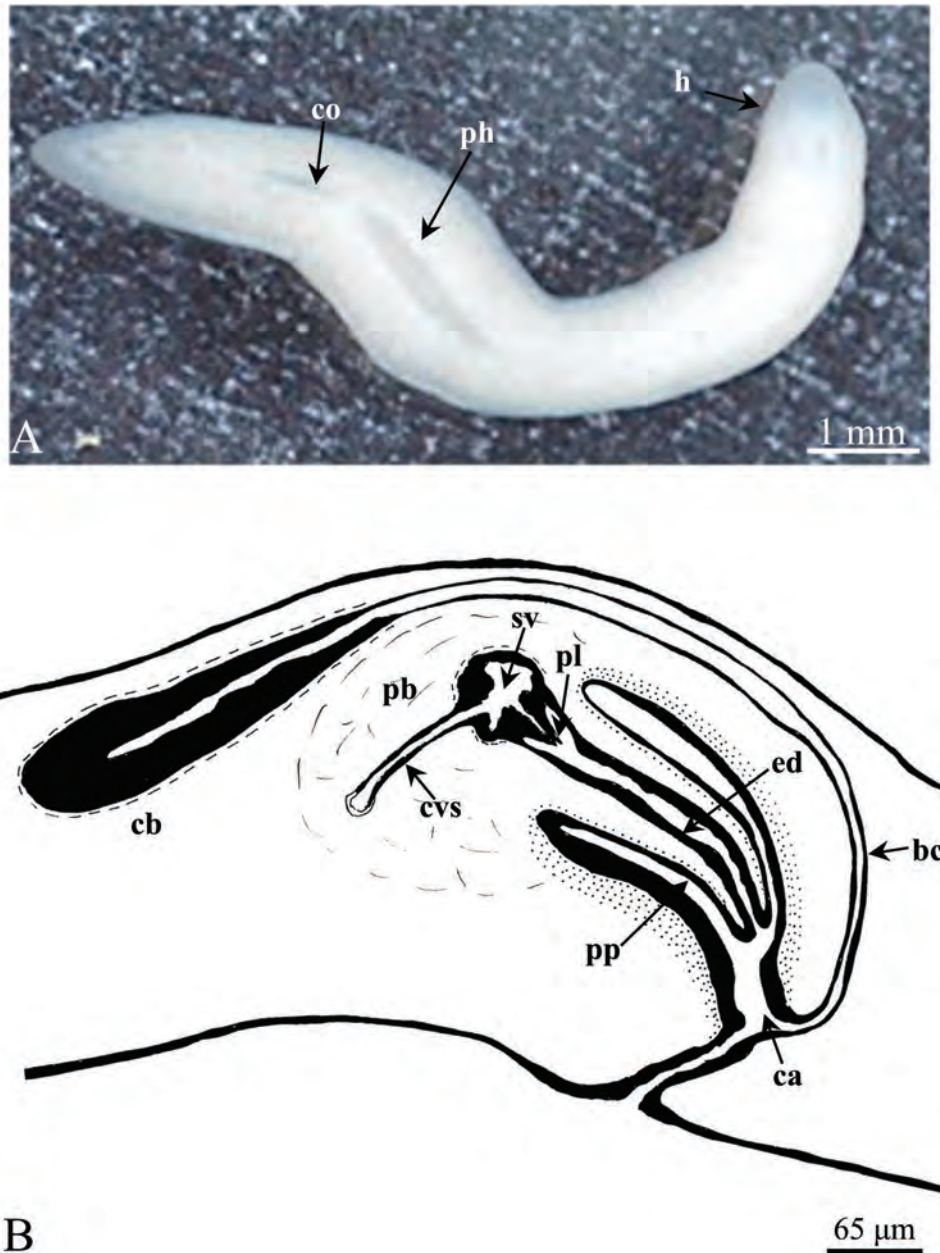


Figure 7. *Phagocata obscura*. (A) habitus of a living specimen, (B) holotype ZMA V.Pl. 7106.1, sagittal reconstruction of the copulatory apparatus (anterior to the left).

(Reisinger, 1960), and *P. hellenica* (Vila-Farré and Sluys, 2011). However, all of these species have two eyes.

*Phagocata obscura* shares its principally prepharyngeal testes with the North American species of *Phagocata*. In contrast to *P. obscura*, Palearctic species of *Phagocata* usually have their testes distributed through the entire body.

Although Stocchino et al. (2008) tentatively ascribed this material to *Atrioplanaria*, the present detailed morphological study highlighted that this is the first and only species of *Phagocata* recorded for Italy. Only *Phagocata* sp. was reported before now from Tuscany and Sardinia

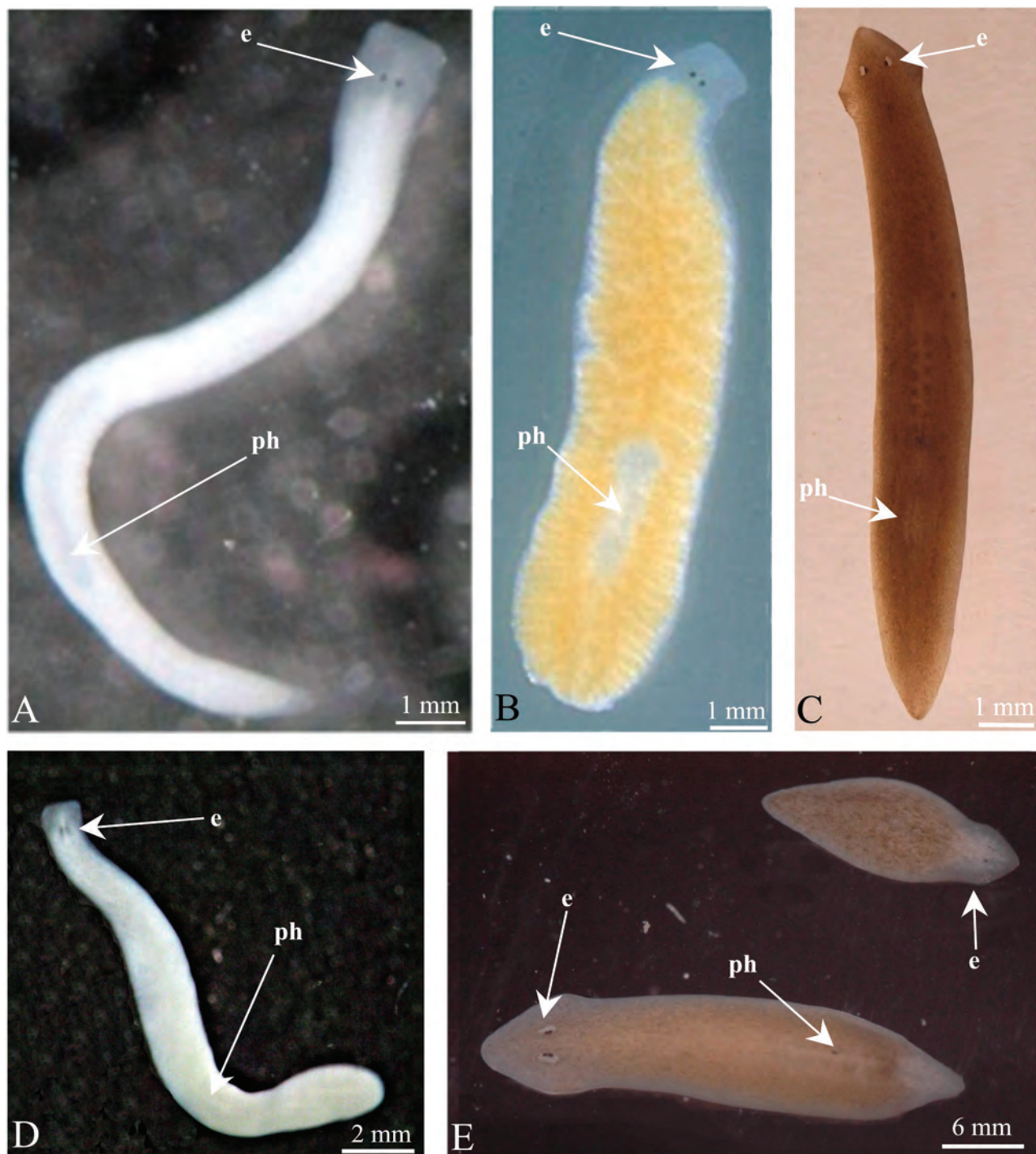
(Benazzi, 1982; Stocchino, 2003). The few records of *P. vitta* reported for the Italian fauna (Bello et al., 1995; Ferreri, 1995) are not supported by a morphological account and should therefore be considered doubtful.

*PHAGOCATA* SP.

(Table 2; Figs. 1, 8A, B, D).

Asexual individuals from three populations were collected from the following three sites; absence of a fully developed copulatory apparatus prevented a more detailed assessment of their taxonomic status:

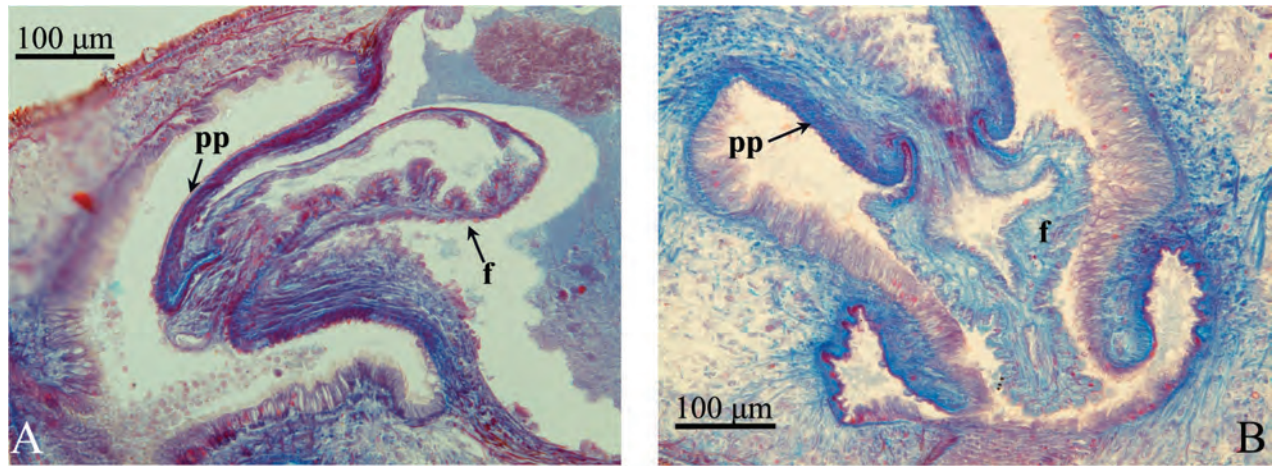




**Figure 8.** (A) *Phagocata* sp., habitus of a living specimen from the Monte Maggiore Cave, (B) *Phagocata* sp., habitus of a living specimen from the Elighe Mannu Spring, Asinara Island, (C) *Dugesia benazzii*, habitus of a living specimen from the Su Cantaru Spring (Monte Albo), (D) *Phagocata* sp., habitus of a living specimen from a spring at Alà dei Sardi, (E) *Dugesia* sp., habitus of live specimens from the Sa Ucca 'e su Peltusu Cave.

1) Monte Maggiore Cave (40°30'51"N, 8°36'37"E), coll. G.A. Stocchino and P. Marcia (Figs 1, 8A). Twenty-five specimens were collected during two occasions in June and July 2010. The specimens were found in a small pool with muddy bottom, originating from drip

water. The animals are whitish, very small and slender. The head is truncated, with two small eyes, situated close together and located far from the frontal margin. After more than one year of rearing they still did not show any sign of a sexualization process.



**Figure 9.** *Dendrocoelum lacteum* from the Ebro delta, Spain. (A) photomicrograph of an inverted, true flagellum (specimen RS 266 from the collections of NCB Naturalis-section ZMA), (B) photomicrograph of an everted, true flagellum (specimen RS 219).

- 2) Elighe Mannu Spring, Asinara Island (41°05'36"N, 8°18'21"E), December 2000–June 2002, coll. M. Piras and G.A. Stocchino (Figs 1, 8B). Eighteen asexual specimens were collected during eight collections made from December 2000 to June 2002. None of the specimens collected survived under laboratory conditions. The animals are unpigmented, with truncated head and with two small eyes located far from the frontal margin and situated close together. Planarians were associated with a diverse invertebrate fauna, including *Microdalyellia* sp. (Platyhelminthes, Dalyelliidae), *Ancylus* sp. (Gastropoda, Ancyliidae), Planorbidae (Gastropoda, Pulmonata), Nematoda, Oligochaeta, Ostracoda, Cladocera, Cyclopoida, Hydracnida (Arachnida), Ephemeroptera, Dytiscidae, Hydrophilidae, Chironomidae, Culicidae, Ceratopogonidae, Polycentropodidae, and Rhyacophilidae.
- 3) Alà dei Sardi (40°38'60"N, 9°19'45"E), February 2010, coll. M. Fois (Figs 1, 8D). Just one individual was collected from a spring. The animal is unpigmented, whitish, with a body size in elongate state of ca. 7 mm in length and 1 mm in width. The head is truncated, with two eyes located far from the frontal margin. Each eye is accompanied by one small supernumerary eye.

GENUS *CRENOBIA* KENK, 1930  
*CRENOBIA ALPINA* (DANA, 1766)  
 (Table 2, Fig. 1)

**Material examined.** CGAS Pla 4.1-4, sagittal sections on 11 slides, 13 slides, 13 slides, 16 slides, respectively, immature specimens, Su Sessene Spring, 1368 m asl, western face of the Gennargentu Massif (40°01'40"N, 9°12'1"E), February 2004, coll. R. Fadda and M. Deiana (water temperature 7°C).

Comparative discussion. Unfortunately, all specimens collected were immature, i.e. without copulatory apparatus. Therefore, the animals were identified solely on the basis of external appearance and presence of only one

pharynx. The latter condition rules out the polypharyngeal forms *Crenobia alpina montenigrina* (Mrázek, 1904), *C. alpina anophthalma* (Mrázek, 1907), and *C. alpina teratophila* (Steinmann, 1908). This new record indicates a wider geographic distribution than previously known, although the species is restricted exclusively to the Gennargentu Massif springs, as reported earlier by Pala et al. (1980c).

Family DUGESIIDAE Ball, 1974  
 Genus *Dugesia* Girard, 1850  
*Dugesia benazzii* Lepori, 1951  
 (Table 2; Figs. 1, 8C)

**Material examined.** ZMA V.Pl. 7107.1, one set of sagittal sections on 21 slides, ZMA V.Pl. 7107.2, one set of transverse sections on 37 slides, ZMA V.Pl. 7107.3, one set of horizontal sections on 7 slides, Su Cantaru Spring, Monte Albo (40°34'30"N, 9°40'31"E), 5 December 1998, coll. G.A. Stocchino and D. Salaris.

Comparative discussion. All specimens were sexual at collection. Identification of the species was based on histological study and karyological analysis. *Dugesia benazzii* is characterized by a finger-shaped penis papilla, a pointed diaphragm, a central course of the ejaculatory duct opening at the tip of the penis papilla, and symmetrical openings of the oviducts into the bursal canal. Specimens of *D. benazzii* from the Monte Albo Spring have a fold on the left of the penis papilla, like the majority of Sardinian populations (Lepori, 1951; Stocchino, unpublished data). The chromosomal number is  $2n = 16$ ;  $n = 8$ . This is the first population of *D. benazzii* recorded from a Sardinian spring.

*DUGESIA* SP.  
 (Table 2; Figs. 1, 8E)

Comparative discussion. Three fissiparous specimens were found under pebbles in a small stream running in the dark Sa Ucca 'e su Peltusu Cave (40°27'00"N, 8°40'43"E)



on 3 September 2007 (coll. P. Marcia and M. Fois), at an altitude of ca. 560 m asl (Fig. 1). Dugesiids were associated with *Dendrocoelum vesiculosum* (see above). After two months of rearing, 5 individuals were present, but they did not survive under laboratory conditions. This is the first record of dugesiids from a Sardinian cave.

## GENERAL DISCUSSION

### THE FLAGELLUM IN DENDROCOELID FLATWORMS

The term *flagellum* is often used *sensu lato* for the tip of a penis papilla that can be inverted, thus projecting into the lumen of the papilla. During the history of triclad taxonomy several attempts have been made to define the various states of inversion of the penis, notably how to differentiate between a so-called true flagellum and a pseudoflagellum. Komárek (1926) defined a true flagellum as a fold of the epithelium lining the internal surface of the penis papilla and mentioned as examples *Dendrocoelum lacteum* and *D. infernale* (Steinmann, 1907). Further, he mentioned *D. mrazeki* (Vejdovský, 1895) as an example of a flagellum that is merely formed by the invaginated distal part of the penis papilla. The same distinction had already been made by Steinmann (1909). He recognized that in most species the flagellum is “*nach innen gestülpt*” (folded inwards, inflected), whereas in *D. lacteum* and *D. infernale* the flagellum is “*umgestülpt*” (turned inside out). He also noted that in the flagellum of these two species the musculature is well-developed, notably the longitudinal muscles; the circular musculature is only weak.

Despite the studies of these workers, Hyman (1931, p. 318) considered presence or absence of an eversible flagellum to be an unreliable taxonomic character since “it has proved impossible to construct a precise morphological definition of the flagellum.”

Following up on the work of these earlier workers, however, we distinguish for the flagellum *s.l.* in the genus *Dendrocoelum* six main conditions in regard to the histology of the tip of the penis papilla and the extent of its inversion or invagination. Among our six conditions, the numbers 2, 3, and 4 correspond to the three main conditions distinguished earlier by De Beauchamp (1932).

- (1) Completely inverted penis papilla. In this case the entire penis papilla may be fully inverted or invaginated, e.g., *D. adenodactylosum*, *D. albidum* Kenk, 1978, *D. magnum* (Stanković, 1969), and *D. cf. beauchampi* (cf. Sluys and Benazzi, 1992).
- (2) Inverted tip of penis papilla. This condition refers to a histologically *homogeneous* penis (i.e., with no strong histological differences between the basal part and the apical part of the penis papilla) with an apical part that may be temporarily invaginated, as is the case in, for example, *D. mrazeki*.

- (3) Pseudoflagellum. This case is present in species with a penis papilla that can be divided into two parts, a basal part with a strong layer of circular muscle and a thin distal part with a few or no muscles and with an external epithelium (when it is everted) that is thicker than the basal part. In this case only the distal part of the papilla is invertible, while the basal part protrudes into the atrium. The pseudo-flagellum is more frequent when the apical part of the penis papilla is very thin with respect to the basal part. It is present, for example, in *D. maculatum*, *D. sanctinaumi*, *D. albidum*, *D. lacustre*, *D. minimum*, *D. translucidum* Kenk, 1978, *D. komareki* (Stanković, 1969), *D. nausicaae*, *D. remyi* De Beauchamp, 1926, *D. caspicum* Porfirjeva and Dyganova, 1973, *D. longipenis*, Komárek, 1916, and in the Sardinian *D. nuraghum* (this paper). A peculiar case is found in *D. tubuliferum* De Beauchamp, 1919, in which the apical part of the penis papilla has the shape of a tube, which may be considered to represent a pseudo-flagellum. A similar condition occurs in *D. hercynicum* Flösser, 1959 (cf. Sluys 2012).
- (4) True flagellum. This designates a tall, pleated section of the lining epithelium of the distal lumen of the penis papilla that may be everted or inverted, matching the definition of Komárek (1926) (Fig. 7). Moreover, the true flagellum is easily distinguished by the arrangement of its musculature, in that the circular fibers of the penis papilla reach only to the base of the flagellum and do not extend onto it. Therefore, the flagellum shows only longitudinal muscles underneath its tall, vacuolated epithelium. According to De Beauchamp (1932) and Gourbault (1972) the “true flagellum” is a character that only occurs in *D. lacteum* and *D. infernale*. Later, Kenk (1978) reported it also for *D. cruciferum* (Stanković, 1969). In *D. lacteum* the flagellum may become extraordinarily long, reaching the copulatory bursa of the partner and enveloping the sperms during copulation (cf. De Beauchamp, 1932, Fig. 36).
- (5) Inflated flagellum. The penis papilla is characterized by a basal thick part, with a squamous epithelium that is underlain by a strong layer of circular muscle, and a thinner distal part. The epithelium of this apical part is formed by large rounded vacuolated cells, with basal nuclei, very rich in secretion granules. This epithelium covers both the external and the internal apical part of the penis papilla. The penis papilla may assume different shapes, considerably elongated with a tunnel-shaped lumen, sac-shaped delimitating a wide lumen, or globular with its distal part inverted into a wider penis papilla lumen (like a pseudo-flagellum). This condition is present in the Sardinian species *D. vesiculosum* (this paper) and differs from the true flagellum because the vacuolated epithelium is present both on the outer and inner surface of the tip of the penis papilla (Fig. 6A).



- (6) Inflexible flagellum. The shape of this flagellum is independent of the degree of extension or contraction of the penis papilla and cannot be invaginated or inverted. It is present in the species *D. stenophallus* Codreanu and Bălcescu, 1967 and *D. nekoum* Sluys, 2012 (cf. Sluys, 2012).

#### SPHINCTER

All three new Sardinian *Dendrocoelum* species show around the terminal tract of the bursal canal a well-developed sphincter, i.e., an increase in the thickness of the circular muscle layer. Such a sphincter was first mentioned by De Beauchamp (1932) and, subsequently, by Kenk (1978) for *D. adenodactylosum*. However, examination of *D. adenodactylosum* material from the collections of the NCB Naturalis (section ZMA; specimens number RS157-1, 157-2), revealed that a well-developed coat of muscles is present on the vertically running part of the canal (i.e., after it has curved downwards to open into the atrium) consisting of alternating rows of longitudinal and circular muscle; the horizontal part of the bursal canal is merely surrounded by a layer of longitudinal muscle fibers. This was also correctly observed by De Beauchamp (1932, 1937), who described the vertical section to constitute a sphincter. However, it looks different from a proper sphincter, which probably induced Kenk (1978, p. 13) to refer to it as “sphincter”. Kenk (1978) reported the “sphincter” in *D. adenodactylosum* to be formed principally by circular muscles, contrary to our observations and those of De Beauchamp (1932). In our opinion, the difference between the musculature on the horizontal and vertical sections of the bursal canal merely gives the impression of the presence of a true sphincter. Furthermore, that this is not a true sphincter is also suggested by the fact that there is no distinct constriction of the lumen of the canal. All of this contrasts with the situation in the Sardinian species, which clearly show a true sphincter to be present in all three species, while the rest of the bursal canal is surrounded by a subepithelial layer of circular muscles, followed by a layer of longitudinal fibers.

A true sphincter is also present in, for example, *D. agile* De Beauchamp, 1932, *D. barbei* De Beauchamp, 1956, *D. atriostriatum* (Codreanu and Bălcescu, 1967), *D. caspicum*, *D. chujanum* Codreanu, 1943, *D. collini*, *D. cruciferum* (Stanković, 1969), *D. grimmi* Dyganova, 1983, *D. remyi*, and *D. romanodanubialis* (Codreanu, 1949).

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# OCCURRENCE OF TROGLOBITIC CLIVININES IN CHINA (INSECTA: COLEOPTERA: CARABIDAE)

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**Abstract:** A new genus, *Guiodytes* gen. nov., and two new species, *G. cavicola* sp. nov. and *G. bedosae* sp. nov., of the ground beetle tribe Clivinini (Coleoptera: Carabidae) are described from two caves of Guangxi, southern China. *Guiodytes* gen. nov. is characterized. It is the first genus of troglobitic clivinines discovered in Asia, and its relationships with other clivinines remain unclear.

## INTRODUCTION

There are numerous cavernicolous carabid beetles recorded in the world (Casale et al., 1998). Many anophthalmous species belong to the tribe Clivinini of Scaritinae, especially to the humicolous genus *Reicheia* Saulcy, 1863. But there are a few eyeless troglobitic clivinine species: *Italodytes stammeri* Müller, 1938 (from Italy), *Typhloreicheia monacha* Casale and Marcia, 2011, and *T. ilianae* Casale and Marcia, 2011 (from Sardinia, Italy), *Spelaeodytes mirabilis* Miller, 1863 (from Croatia), *Antroforceps bolivari* Barr, 1967 (from Mexico), and *Trogloclivina brehieri* Deuve, 2003 (from New Britain Island, Papua New Guinea). Another troglobitic species, *Clivina subterranea* Decu, Nitzu and Juberthie, 1994 (from Romania), is not blind.

In China, the first highly modified troglobitic carabid species, *Sinaphaenops mirabilissimus* Ueno and Wang, was described in 1991 (Ueno and Wang, 1991). All Chinese troglobitic ground beetles are members of either the tribe Trechini or the tribe Platynini. Trechini is the dominant group, represented so far by about eighty species of more than thirty genera (Chen et al., 2001; Tian, 2008). Although several species of the platynine genera *Jujiroa* Ueno, 1952 and *Xestagonum* Habu, 1978 have been recorded, all are microphthalmous, with reduced, but evident, eyes (Vigna Taglianti, 1995; Ueno and Kishimoto, 2001; Deuve, 2001, 2004; Ueno, 2007).

Recently, in collaboration with Dr. Louis Deharveng of the Muséum National d'Historie Naturelle in Paris, a biological survey on cave fauna was conducted in Guangxi, southern China, as part of research activities of the World Bank GEF-financed project Guangxi Integrated Forestry Development and Conservation. One of the most important discoveries during the survey was that of two interesting specimens of eyeless clivinines. This is the first record of blind carabid clivinines in China. Detailed study showed that they correspond to two different species of a new genus.

## MATERIALS AND METHODS

The specimens were collected by hand in caves of Guangxi and preserved in 60% ethanol solution before study. Other surface or soil species of clivinines, dry and

mounted specimens, were examined for comparison. All specimens are deposited in the insect collections of South China Agricultural University, Guangzhou, Guangdong Province.

Dissection and observation were conducted under a Leica S8AP0 microscope. Genitalia and related pieces of small structures were removed, put in 10% potassium hydroxide for 24 hours for cleaning, then stuck on paper-board and pinned beneath the associated specimen. Pictures were taken by means of a Leica Microsystem LAS V 3.6, and then processed using Adobe Photoshop CS5 software.

Abbreviations used in the text are as the same as described in Tian (2009): HW=head width; HL=head length; PW=pronotal width; PL=pronotal length; EW=elytral width; EL=elytral length. The terms for female genitalia are as in Deuve (1993).

## RESULTS

### *GUIODYTES*, GEN. NOV.

Type species: *Guiodytes cavicola* sp. nov. (Guangxi)

**Generic characteristics:** Description is based entirely on females. Habitus as in Figures 1 and 2. Body moderately elongate; upper side finely and sparsely punctate. Head stout, eyes effaced; wing of clypeus narrow, sharply projected anteriorly; both supraorbital setiferous pores present, located far from each other; supraorbital furrows wide and long; supra-antennal plates well defined, broad and strongly convex, much wider than clypeal wings; frons and vertex convex; frons without carina or tubercle; orbits well developed, genae well developed; labrum complete, mandibles quite stout, left mandible without median tooth; maxillary palpi ensiform; neck constricted by punctures. Antennomeres 1 and 2 glabrous, with a longer preapical seta on antennomere 1 and a short one on base of antennomere 2; antennomere 2 normally jointed to antennomere 1; antenna pubescent from antennomere 3; antennomeres 5–10 cylindrical (*G. cavicola* sp. nov.), or distinctly sub-moniliform (*G. bedosae* sp. nov.).

Pronotum (Figs. 8 and 9) peltate, median line not bifurcate anteriorly, with two normal setae at each side,





Figure 1. *Guiodytes cavicola* sp. nov., habitus of holotype.

one at anterior third, one near hind angle; median line well marked, moderately deep, not reaching base, but joining basal constriction; base of pronotum conspicuously depressed; side with three distinct teeth or projections before hind angle; hind angle nearly rectangular; basal border close to peduncle; anterior transverse impression free from median line, very close to anterior margin.

Elytra (Figs. 1 and 2) elongate-ovate, with base and shoulders serrate, lateral margin slightly undulate (*G. cavicola* sp. nov.) or distinctly crenulate (*G. bedosae* sp. nov.); striae deep, intervals of subequal width, strongly convex; stria 3 with four foveolate setiferous pores; umbilical setiferous punctures complete throughout, with several much longer setae apart from the short ones; base of stria 6 evidently carinate, striae 7 and 8 jointed at base, carinate at base and apex respectively. Hind wings reduced.

Proepisternum not tumid laterally, invisible from above; protarsomere 1 not enlarged; mesotibia with a conspicuous tuber-like spur at subapex.

**Female genitalia** (Figs. 12 and 13): Both gonosubcoxite IX and gonocoxite IX strongly elongate, gonocoxite IX deeply (*G. cavicola* sp. nov.) or slightly curved (*G. bedosae* sp. nov.), sharp (*G. cavicola* sp. nov.) or blunt (*G. bedosae* sp. nov.) at apex, with ensiform setae on outer margin.

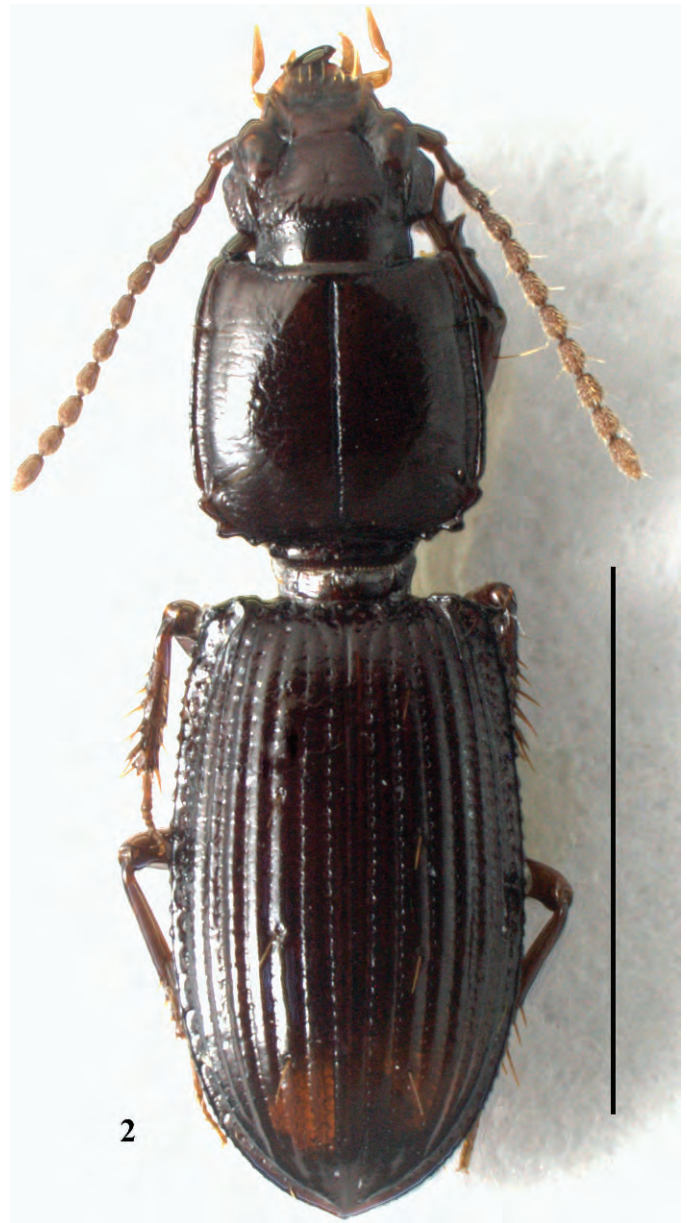


Figure 2. *Guiodytes bedosae* sp. nov., habitus of holotype.

**Male:** Unknown. In spite of our efforts, we were unable to catch another specimen in the caves, and no male is currently available. It is presumed that *Guiodytes* is as rare as many other cave dwelling species of Carabidae in China.

**Relationship of *Guiodytes* within Clivinini:** So far the known genera of anophthalmous clivinines are isolated. *Italodytes* Müller is endemic to Apulia, southern Italy, *Spelaeodytes* Miller, 1863 to Croatia, *Antroforceps* Barr, 1967 to Mexico, *Trogloclivina* to New Britain, Papua New Guinea, and *Guiodytes* to Guangxi, southern China. Jeannel (1957) was probably right to separate *Italodytes* from Clivinini, considering its peculiar morphological characteristics. *Guiodytes* is evidently closer to *Trogloclivina* than to *Italodytes* in appearance, but the relationships are not clear. In the absence of males, it is difficult to



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Figures 3–7. *Guiodytes* spp. nov.: 3. Head of *G. cavicola*, dorsal view. 4. Head of *G. bedosae*, dorsal view. 5. Head of *G. cavicola*, ventral view. 6. Right antenna of *G. cavicola*. 7. Right antenna of *G. bedosae*.



discuss the phylogenetic position of *Guiodytes* within the Chinese or Oriental fauna of Clivinini (Putzeys, 1866; Baehr, 1999; Balkenohl, 1999, 2001; Yang and Tian, 2008). The new genus might have affinities with *Clivina* Latreille, 1802. It differs from *Clivina* by its eyes completely reduced, anterior and posterior supraorbital setiferous pores located far from each other, orbits and genae well developed, each of antennomeres 5–10 longer than wide, pronotum with three toothed projections just before hind angle, and hind wings reduced.

**Adaptation of *Guiodytes* to subterranean environments:**

Apart from effaced eyes and reduced hind wings, no other evident morphological modifications were found in *Guiodytes* species for adapting to a subterranean environment. The appendages, such as palpi, antennae, and legs, are not more slender than those in surface species, though antennomeres 5–10 are more or less elongate. The body of *Guiodytes* is moderately elongate for Clivinini, and the elytral chaetotaxal pattern is not so special, as trichobothria are not very long. Although they look slightly lighter in color than surface or soil species of Clivinini, both species of *Guiodytes* are not depigmented.

**Etymology:** “*Gu*” is the abbreviation of Guangxi Zhuang Minority Autonomous Region in Chinese. The generic name, *Guiodytes*, refers to the distribution of the new genus.

**Distribution:** Southern China (Guangxi).

*GUIODYTES CAVICOLA* SP. NOV.

**Holotype:** Female, Nongshui Cave, Shangjia Cun, Disu Xiang, Du’an Xian, Hechi City, Guangxi, 23°84.066’ N / 108°00.201’ E, 2010-IV-24, Mingyi Tian leg.

**Description:** Length (from apex of left mandible to end of elytra) 7.0 mm; width 2.2 mm.

Light dark brown; tarsi and antennae light brown, palpi yellow, apex of mandibles dark brown. Habitus as in Fig. 1.

Head (Figs. 3–5) stout, slightly longer than wide, HL/HW = 1.17; narrower than pronotum (HW/PW = 0.71); clypeus wide, rather flat, bisetose, slightly emarginate at anterior margin; lateral wings narrow, projected anteriorly, extended distinctly beyond anterior margin of clypeus, separated from clypeus by an indistinct notch; supra-antennal plates well defined, clearly separated from clypeal wings by obvious notch, more or less rounded, strongly convex, smooth and glabrous; frons and vertex convex; supraorbital furrows very deep and wide, extended to the level of hind supraorbital pore, subparallel along frons, strongly divergent afterwards; supraorbital carina absent; frons separated from clypeus by inconspicuous frontoclypeal suture; eyes completely reduced and disappeared; both anterior and posterior supraorbital pores present, located far from each other; genae well developed; neck constriction distinct, beginning at level of hind supraorbital pore; labrum conspicuously wide, as wide as clypeus excluding clypeal wings, 5-setose, and ciliate on both sides, almost straight at anterior margin; mandible stout, more or less

widened, right mandible without median tooth; palpi ensiform, glabrous, apical segments of both maxillary and labial palpi much longer than penultimate ones respectively; labial palpomere 2 bisetose; ligula thin, unisetose at apex; mentum well developed, with two pairs of setae, one pair situated beneath mental tooth, other at base, close to side margins of mentum; median tooth pointed, lateral lobes wide, almost truncate at apex; submentum distinctly separated from mentum, quadrisetose. Antennae filiform (Fig. 6), comparatively long for Clivinini, extended to scutellum; antennomeres 1 and 2 glabrous and smooth, antenna pubescent from antennomere 3; antennomeres 2 and 3 subequal in length; antennomeres 5–10 subcylindrical, somewhat flat, distinctly longer than wide.

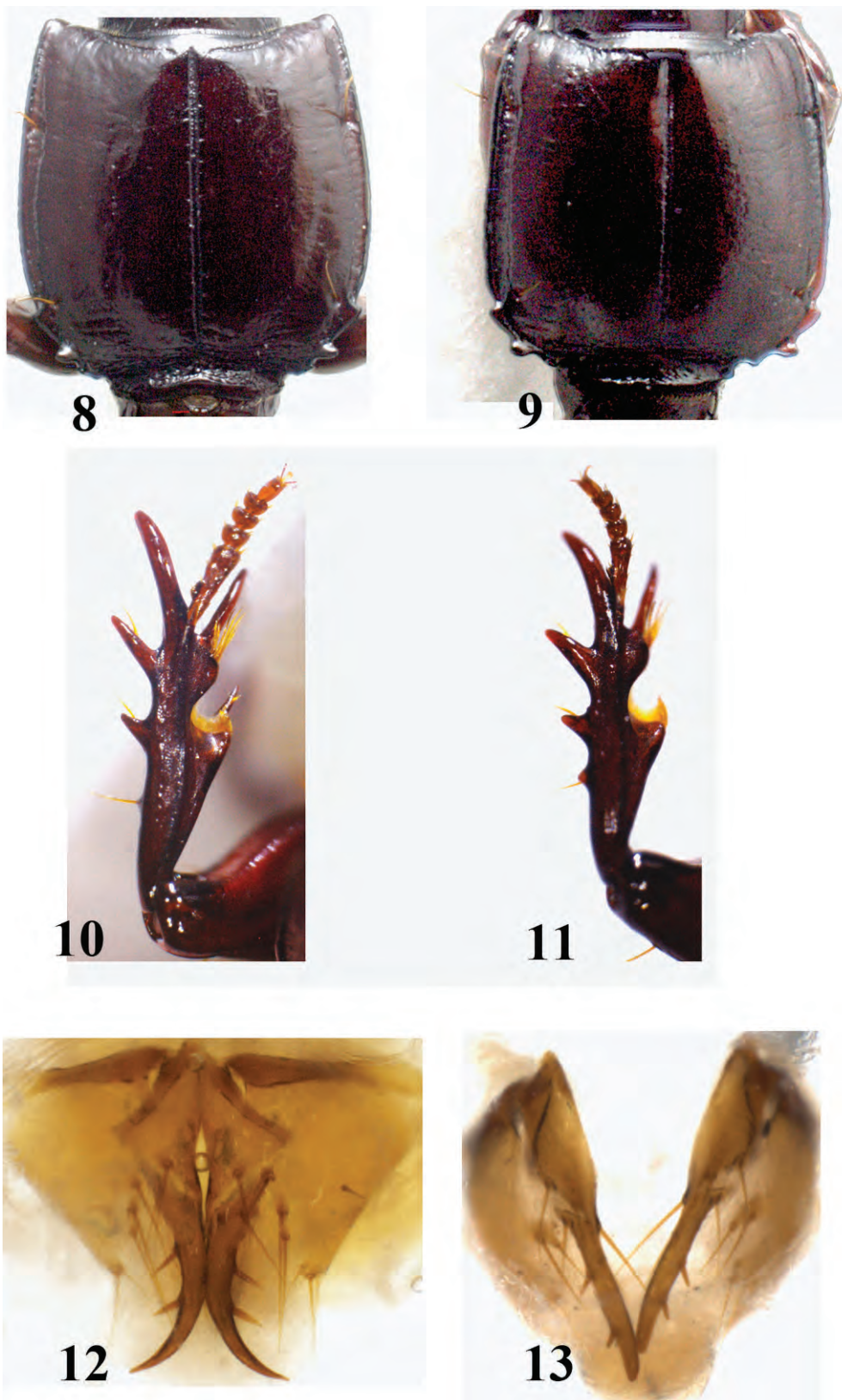
Pronotum (Fig. 8) peltate in form, much wider than head; disc smooth, moderately convex, slightly longer than wide (PL/PW = 1.09); apex deeply concave, marginated in median portion, anterior angles strongly protruded, nearly rectangular, widest at about middle; side strongly serrated just before hind angle, with three conspicuous toothed projections; marginal channel wide and uneven, widest near anterior lateral setiferous pore, ended before posterior lateral setiferous pore; anterior setiferous pore situated at about apical fourth, posterior one at about basal third, just before hind angle; base unbordered; median line clear and deep; basal transverse impression deep and wide, with several transverse striae behind and a small marginal denticle; anterior transverse line free from median line, very close to anterior margin. Peduncle distinct.

Elytra wider than pronotum (EW/PW = 1.10), elongate-ovate, EL/EW = 1.85; disc strongly convex; lateral margin slightly expanded, widest at about middle; base more or less straight; shoulders broadly obtuse, with three large serrated teeth; lateral margin more or less smooth but undulate near base; striae deep, intervals strongly convex; interval 1 with a small but distinct tubercle at base, just before scutellar pore; intervals 5–9 bordered at base; intervals 6–8 carinate at base, base of intervals 7 and 8 joined; interval 3 with four setiferous pores, setae moderate in length for clivinines; marginal channel with uninterrupted series of small setiferous pores and several large pores with much longer setae; scutellar stria and scutellar pore present; hind wings reduced.

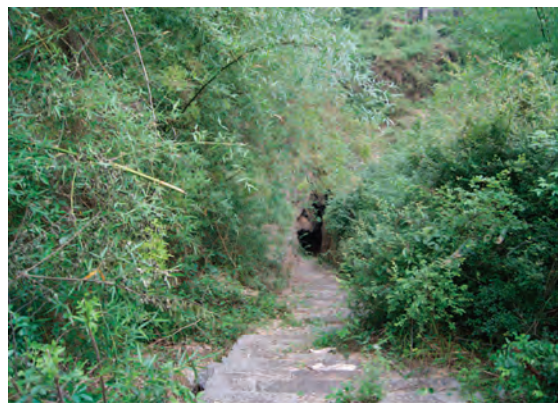
Underside of head and proepisternum with dense isodiametric punctures, without wrinkles; prosternum smooth, with sparse punctures; abdominal sterna more or less punctured; sternum VII of female with two pairs of subapical setae; epipleuron with a few coarser punctures near base.

Legs not modified compared with surface clivinines; fore leg stout, profemur moderately dilated, smooth, without carina or ruga ventrally; protibia (Fig. 10) well developed, quadridentate, with distinct and complete carina dorsally, sulcus indistinct; lateral upper spine elongate-ensiform, blunt at apex, much longer and stouter than subapical spur; protarsi slender, tarsomere 1 very





Figures 8–13. *Guiodytes* spp. nov.: 8. Pronotum of *G. cavicola*. 9. Pronotum of *G. bedosae*. 10. Protibia of *G. cavicola*, dorsal view. 11. Protibia of *G. bedosae*, dorsal view. 12. Female genitalia of *G. cavicola*, ventral view. 13. Female genitalia of *G. bedosae*, ventral view.



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Figures 14–17. Habitat caves of *Guiodyte*: 14 and 15. Entrance of Nongshui Cave. 16 and 17. Entrance of Paoma Cave.

long, much longer than tarsomeres 2–4 combined; middle and hind legs slender, mesotibia gradually dilated towards apex, with a conspicuous tuber-like spur at subapex.

**Female genitalia** (Fig. 12): Lateralotergite IX well developed, nearly reverse triangular in form, with four long setae at subapex and apex; gonosubcoxite IX triangular, with one long seta at median portion of outer margin; gonocoxite IX very long and slender, gradually and evenly curved, very sharp at apex, with one long seta near base, and two strong and long ensiform setae on outer margin.

**Male:** Unknown.

**Etymology:** The name of this new species refers to its cave habitat.

**Distribution:** China (Guangxi: Du'an Xian). Known only from the type locality. It is a cave with torrential flooding just past the entrance (Figs. 14 and 15). Near the entrance there is a pumping station.

*GUIODYTES BEDOSAE* SP. NOV.

**Holotype:** Female, Paoma Cave, Xiadong Xiang, Longzhou Xian, Southern Guangxi, 106°86.903' E / 22°37.422' N, 2010-IV-15, Mingyi Tian leg.

**Description:** Length (from apex of left mandible to end of elytra) 5.2 mm; width 1.8 mm.

Light dark brown; tarsi and antennae light brown, palpi yellow, apex of mandibles dark brown. Habitus as in Figure 2.

Head (Fig. 4) stout, slightly longer than wide, HL/LW = 1.20, slightly narrower than pronotum (HW/PW = 0.74); clypeus wide, rather flat, bisetose, distinctly emarginated at anterior margin; lateral wings narrow, projected anteriorly, extended clearly beyond anterior margin of clypeus, separated from clypeus by an indistinct notch; supra-antennal plate well defined, clearly separated from clypeal wing by an obvious notch, elongate-rounded, strongly convex, smooth and glabrous; frons and vertex convex; supraorbital furrow deep and wide, extended to level of hind supraorbital pore, slightly divergent along frons, posteriorly strongly divergent; supraorbital carina absent; frons separated from clypeus by inconspicuous frontoclypeal suture; eyes completely reduced; both anterior and posterior supraorbital pores present, located far from each other; genae well developed; neck broad, constriction distinct, beginning at level of hind supraorbital



pore; labrum conspicuously wide, as wide as clypeus excluding clypeal wings, 7-setose, and ciliate on both sides, almost straight at anterior margin; mandible stout and widened, right mandible without median tooth; palpi ensiform, glabrous, stout, dilated at about basal third; apical segments of both maxillary and labial palpi much longer than penultimate ones respectively; labial palpomere 2 bisetose; ligula thin, unisetose at apex; mentum well developed, with two pairs of setae, one pair situated beneath mental tooth, other at base close to side margins of mentum; median tooth very sharp, lateral lobes wide, almost truncate at apex; submentum distinctly separated from mentum, quadrisetose. Antennae sub-moniliform (Fig. 7), moderate in length for Clivinini, shorter than above species, extended to base of pronotum; antennomeres 1 and 2 glabrous and smooth, antenna pubescent from antennomere 3; antennomeres 2 and 3 subequal in length; antennomeres 5–10 somewhat flat, slightly longer than wide.

Pronotum (Fig. 9) peltate in form, much wider than head; disc smooth, moderately convex; slightly longer than wide (PL/PW = 1.04); anterior margin deeply concave, anterior angles protruded, nearly rectangular; widest at about middle; side strongly serrated just before hind angle, with three conspicuous toothed projections; marginal channel wide, ended before posterior setiferous pore; anterior setiferous pore situated at about apical fourth, posterior one at about basal third, just before hind angle, which is more obtuse than that of *G. cavicola*; base unbordered, almost straight; median line deep and distinct; basal transverse sulcus wide and deep, narrower than *G. cavicola*, with several transverse striae behind; anterior transverse impression free from median line, very close to anterior margin. Peduncle distinct.

Elytra wider than pronotum (EW/PW = 1.21), elongate-ovate, EL/EW = 1.73; disc strongly convex; lateral margin gently expanded, widest at about middle; base more or less straight, little indentations corresponding to the intervals; shoulders with two evident serrated teeth, lateral margin distinctly crenulated throughout; striae punctate, deep, intervals strongly convex; intervals 6–8 carinate at base, base of intervals 7 and 8 separate; interval 3 with four setiferous pores, setae moderate in length for clivinines; marginal channel with uninterrupted series of small setiferous pores and several large pores with much longer setae; scutellar stria long and distinct, scutellar pore present. Hind wings reduced.

Underside of head and proepisternum with dense isodiametric punctures, without wrinkles; prosternum smooth; abdominal sterna more or less punctured; sternum VII of female with two pairs of subapical setae; epipleuron with a few coarser punctures near base.

Legs normal for clivinines; fore leg stout, profemur moderately dilated, smooth; protibia (Fig. 11) well developed, quadridentate, with distinct and complete carina dorsally, but without sulcus; lateral upper spine elongate-

ensiform, blunt at apex, much longer and stouter than subapical spur; protarsi slender, tarsomere 1 long, slightly longer than tarsomeres 2–4 combined; middle and hind legs slender, mesotibia gradually dilated towards apex, with a conspicuous tuber-like spur at subapex.

**Female genitalia** (Fig. 13): Lateralotergite IX weakly sclerotized, with four long setae at inner margin; gonosubcoxite IX triangular, with one long setae at median portion of outer margin; gonocoxite IX long and slender, slightly curved, apex somewhat blunt, each side of base with one long seta, and two small and short ensiform setae on outer margin.

**Male:** Unknown.

**Remarks:** This new species is similar to *G. cavicola* sp. nov., but it is smaller, head stouter, labrum 7-setose, palpi slightly stouter, antennae shorter and sub-moniliform, lateral margin of elytra distinctly crenulate throughout, and the female genitalia with gonocoxite IX slightly curved, and blunt at apex.

**Etymology:** This new species is named in honor of Dr. Anne Bedos (Muséum National d'Historie Naturelle), a well known biospeleologist.

**Distribution:** China (Guangxi: Longzhou Xian). The unique specimen was caught by hand beneath a piece of soil in Paoma Cave, at about 150 m from the entrance. The cave is as long as 4 km, according to local villagers, but we had only explored a small part of it because of flooding (Figs. 15 and 16).

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# THE FIRST CAVERNICOLOUS NICOLETIIDAE (INSECTA: ZYGENTOMA) FROM THE UNITED ARAB EMIRATES

LUIS ESPINASA<sup>1</sup> AND LUIS F. MENDES<sup>2</sup>

**Abstract:** *Lepidospora mazyadi*, n. sp., is described and differentiated from all the other known *Lepidospora* s.s. The new species was collected from a cave in Jebel Hafeet in the United Arab Emirates. Morphology and preliminary analyses of 16S rRNA DNA sequences are described. The new species is unique because, unlike other species in the genus, it lacks sexually dimorphic terminal filaments. It is also the first nicoletiid reported from the United Arab Emirates and the second cave-adapted species from Arabia.

## INTRODUCTION

In 1997, a cave was encountered during construction work on the top of Jebel Hafeet, near the village of Mazyad in the United Arab Emirates. Subsequent exploration of the cave showed it to have 450 meters of passage and a depth of 96 meters, becoming the longest cave in the UAE. During the original explorations, nicoletiid insects were observed and collected, but samples were too degraded to be identified. In 2011, another exploration trip was conducted during which six specimens were successfully collected. A description of this new species of nicoletiid and preliminary DNA sequence data are provided here.

## MATERIALS AND METHODS

Dissections were made with the aid of a stereo microscope. Illustrations were made with the aid of a camera lucida attached to a microscope. Specimens will be deposited in the collection of the American Museum of Natural History, New York.

Genomic DNA from the holotype and a female paratype were extracted using Qiagen's DNEasy Tissue Kit by digesting a leg in lysis buffer. Amplification and sequencing of the 16S rRNA fragment was done as in Espinasa and Giribet (2009) following standard protocols with primers used in the past for nicoletiids. Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software Sequencher 3.0. External primers were excluded from the analyses. Sequences were aligned and neighbor-joining analysis was performed with ClustalW2.

## RESULTS AND DISCUSSION

Molecular data were obtained from two individuals. A partial 16S rRNA fragment 486 bp in length was obtained (Fig. 1). Both specimens had identical sequences. Nucleotide alignment of the UAE specimen with other species of Nicoletiids from which 16S rRNA sequence are available showed considerable sequence differences. A neighbor-joining tree showed the new species distantly related to

*Australiatehura*, an inquiline species of the subfamily Atelurinae from Australia and New Zealand (111 bp differences; 22.8%). Nevertheless, these results should be considered as preliminary. With the exception of the American nicoletiids of the subfamily Cubacubanae, very few studies on the Old World nicoletiids have used DNA sequencing, and it is likely that other unsequenced species are more closely related.

*LEPIDOSPORA MAZYADI*, ESPINASA AND MENDES  
NEW SPECIES  
(Figs. 2A–H, 3A–G)

**MATERIAL:** Holotype male, body 10 mm, tarsus third leg 1.1 mm. Paratypes: one male (9 mm), three females (13, 13, and 12 mm), and one juvenile (5.5 mm). In the vicinity of 40R 375360 E / 3661550 N, Magharet Qasir Hafeet Cave, Jebel Hafeet, near the village of Mazyad and the city of Al Ain, Emirate of Abu Dhabi, United Arab Emirates. 6/17/2011 and 8/8/2011. Collected by Tim Fogg, Pam Fogg, Angus Tillotson, and Abdul Raheem Abdul Rahman Iqtadar Al Marzooqi.

**DESCRIPTION:** Body proportions as in Figure 2A. Maximum body length of male 10 mm, of female 13 mm. Maximum conserved length of antennae and caudal appendages 8 mm. Head and body with typical scales (Figs. 2B, 2G, 3B–C, and 3F). General color light yellow to white. Head with scales, macrochaetae, and microchaetae as shown in Figure 2B. Pedicellus of male slightly shorter than first article and with a distinct protuberant apophysis that extends inwards and ventrally (Fig. 2B–C). On the apophysis basally there are multiple microchaetae and distally a couple of macrochaetae and a distinct small spiniform chaeta on its apex (Fig. 2C). Basal articles of antennae of female simple.

Appendages long, as in other cave-adapted nicoletiid species. This is most evident in the maxillary palp, which is particularly long (Figs. 2A and 2E). Mandible chaetotaxy as in Figure 2H, with about five macrochaetae on central-distal

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GTCAGGCCTGCTCGATGATTGTTTTAAATGGCCGCGGTATTTTGACCGTGCAAAGGTAGCATAATCATTAGTCTTTTA  
 AATGGGGGCTTGTATGAATGGTTAGACGAAAGGGGCTTTTCTTATAAGAATAGTTTGTATGAATTTTACCTTTTGG  
 TGAAAAGGCTGAAATAATTTAARGGGACGATAAGACCCTATAGATCTTTATGGTTGATTGATCTTATAATCTACTAG  
 GATTTGTATAATTTTATCAATTGTTTAGCTGGGGCGGTTGGGAGATATATTTAACTCTCTTTAGGGTATTTATTTA  
 TTTATATTTTTTGTATCCTTATTTAGTGGATAGAAGACTAAGTTACCTTAGGGATAACAGCGTAATCTTTTTGGAGAG  
 TTCTTATCGATAGAAGGGTTTGGACCTCGATGTTGGATTAAGGTTACTTTCTGGTGAAGGAGCTGGAGAAGTTAGT  
 CTGTTTCGACTATTAACCTTACA

**Figure 1. 16S rRNA fragment sequence of *Lepidospora mazyadi* n. sp.**

portion. Maxilla as shown in Figure 2E. Last article of subequal length to penultimate article. Apex of galea with two subequal conules and two teeth on lacinia (Fig. 2F). Labial palp as in Figure 2D, with apical article distinctly longer than wide and longer than the penultimate article.

Pro-, meso-, and metanota with several macrochaetae on postero-lateral margins, apart from several setae of varied sizes (Fig. 2G). Legs long; hind tibia about 8× longer than wide and one fifth shorter than tarsus (Fig. 3A).

Abdominal tergites as in Figure 3F, with robust macrochaetae on their posterior edges. Urotergite X protruding, very deeply emarginated in both sexes, but more so in males. In males multiple macrochaetae plus two rows of sclerotized pegs, one lateral (Fig. 3F) and the other ventral (Fig. 3G). The tips of urotergite X also with sclerotized pegs (Fig. 3F). Depth of emargination almost as deep as the macrochaetae and slightly wider (Fig. 3F–G). In females urotergite X slightly less deep, without lateral and ventral pegs, but instead of the pair of pegs at the tips they have a distinct large macrochaetae.

Abdominal sternites as in Figures 3B–D. Urosternite I divided into a median sternite and 1 + 1 lateral coxites. Urosternites I–VIII entire. Stylets in urosternites II–IX. Stylets have a terminal spine with small teeth and ventrally with two macrochaetae plus another distal pair (Fig. 3B–D). Stylets IX larger than others (Fig. 3C–D). Posterior margin of urosternite VIII of male prominent, straight, and with a couple of macrochaetae (Fig. 3C). Penis and paramera as in Figure 3C. Paramera long, almost 8× longer than wide. Paramera attain about two thirds the length of stylets IX in the largest male (10 mm). Subgenital plate of female triangular to sub-parabolic (Fig. 3D). In the largest female (13 mm), the ovipositor surpasses apex of stylets IX by two thirds the length of stylets (Fig. 3D). Gonapophyses with about 14 to 15 annuli. Male and female cerci and appendix dorsalis simple. Cerci with trichobothria. (Fig. 3E).

**POSTEMBRYONIC DEVELOPMENT:** Only partially understood due to sample size. Both males 10 and 9 mm share equal characteristics: pedicellus with distinct protuberant apophysis and parameres attain about two thirds the length of stylets IX. In the three females (13, 13, and 12 mm) the ovipositor surpasses apex of stylets IX by two thirds to one half the length of stylets and gonapophyses with about 14 to 15 annuli. The species appears to share with other cavernicolous species a rather late sexual development. The single juvenile is quite large, and at

5.5 mm long it has yet to develop either male or female character, and paramera or ovipositor are absent.

**ETYMOLOGY:** *mazyadi*. From the nearby village of Mazyad, where the cave is located.

**REMARKS:** The new species belongs within the subfamily Coletiniinae because it has urosternite I divided, II–VIII entire, and coxites IX of male free, male pedicellus with an apophysis without apparent glands, and parameres not divided (Mendes, 1988). It belongs within the genus *Lepidospora* because stylets are present on urosternites II–IX and they have typical scales (Wygodzinsky, 1980). It belongs to the nominate subgenus because there are scales on both the body and on the cephalic capsule. It can be differentiated from other species within the genus by the complete lack of sclerotized pegs on all three terminal filaments in the male (cerci and paracercus). It is immediately distinguishable from the only other *Lepidospora* known from the Arabian Peninsula, *L. (L.) angustotergum*, a troglobiont or deep edaphic species from southeastern Oman (Mendes, 2002a), by the quite different urotergite X, shorter and less delicate paramera, and lack of submedian macrochaetae on urosternite VIII in the male. Females are differentiated by the distinct subgenital plate and much longer ovipositor.

The new species enters the identification keys previously proposed for the genus (Mendes, 2002b) at point 3 for the males, as all remaining known *Lepidospora* have pegs at least on the cerci, as reported earlier. The female runs to couplet 21, and is quite different from *L. (L.) angustotergum*, as mentioned before. It is not possible to enter the other option (21') due to the adult size and the elongation of the appendages. The two most recently described species, both epigean from the Socotra Islands (Mendes, 2004), were described after the key was proposed. They can be differentiated from the new species because males have sclerotized pegs on the cerci and paracercus, by the elongation of legs, maxillary and labial palps, by the size and slenderness of paramera, and by the shape of both the antennal pedicellus and of urotergite X. Females are unknown in these species.

**DISTRIBUTION:** Known only from type locality.

#### ACKNOWLEDGMENTS

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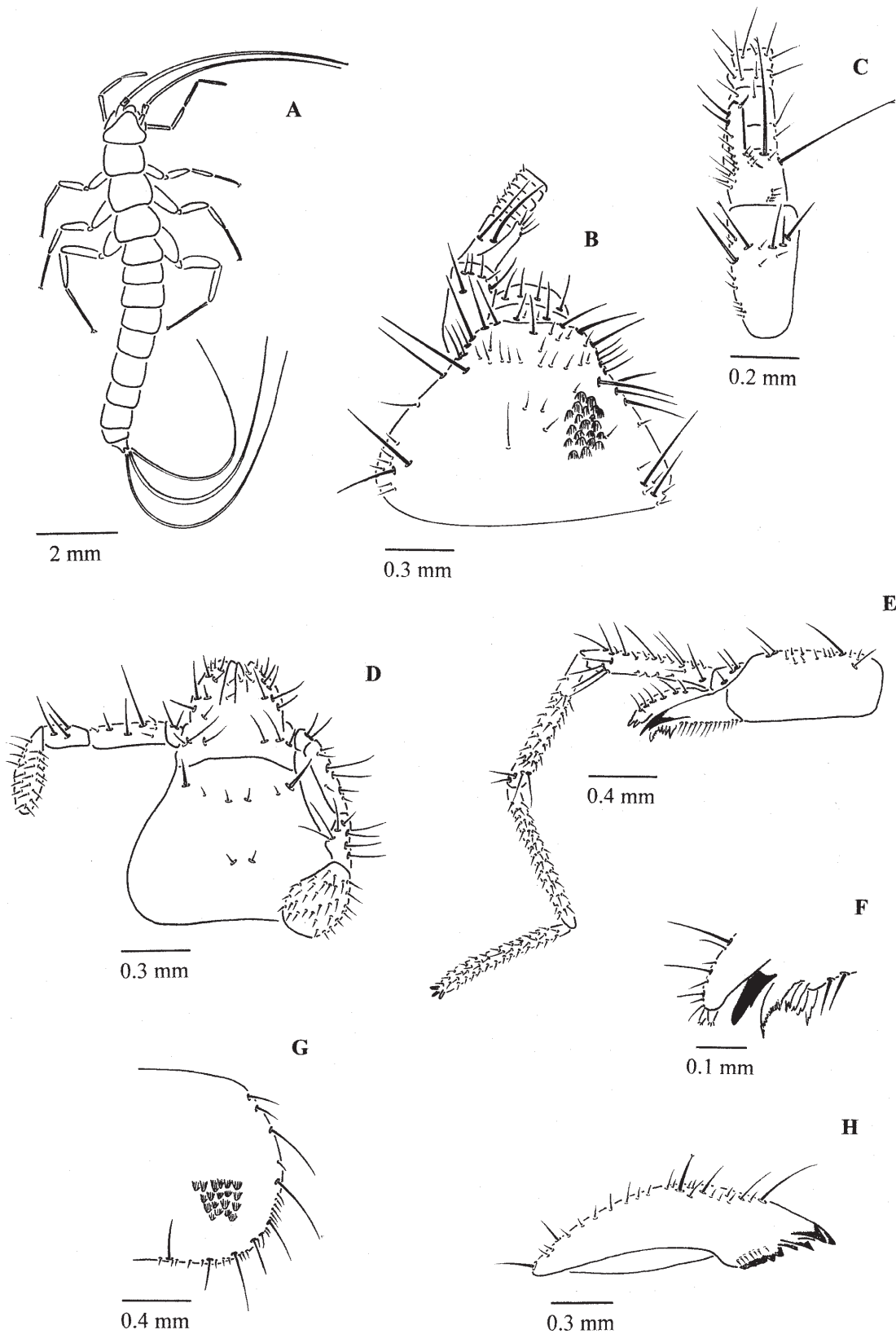


Figure 2. *Lepidospora mazyadi* n. sp. Scales and microchaetae partially shown. A, Body. B, Head and antennae. C, Ventral view of basal portion of antennae. D, Labium. E, Maxilla. F, Apex of maxilla. G, Thoracic nota. H, Mandible.

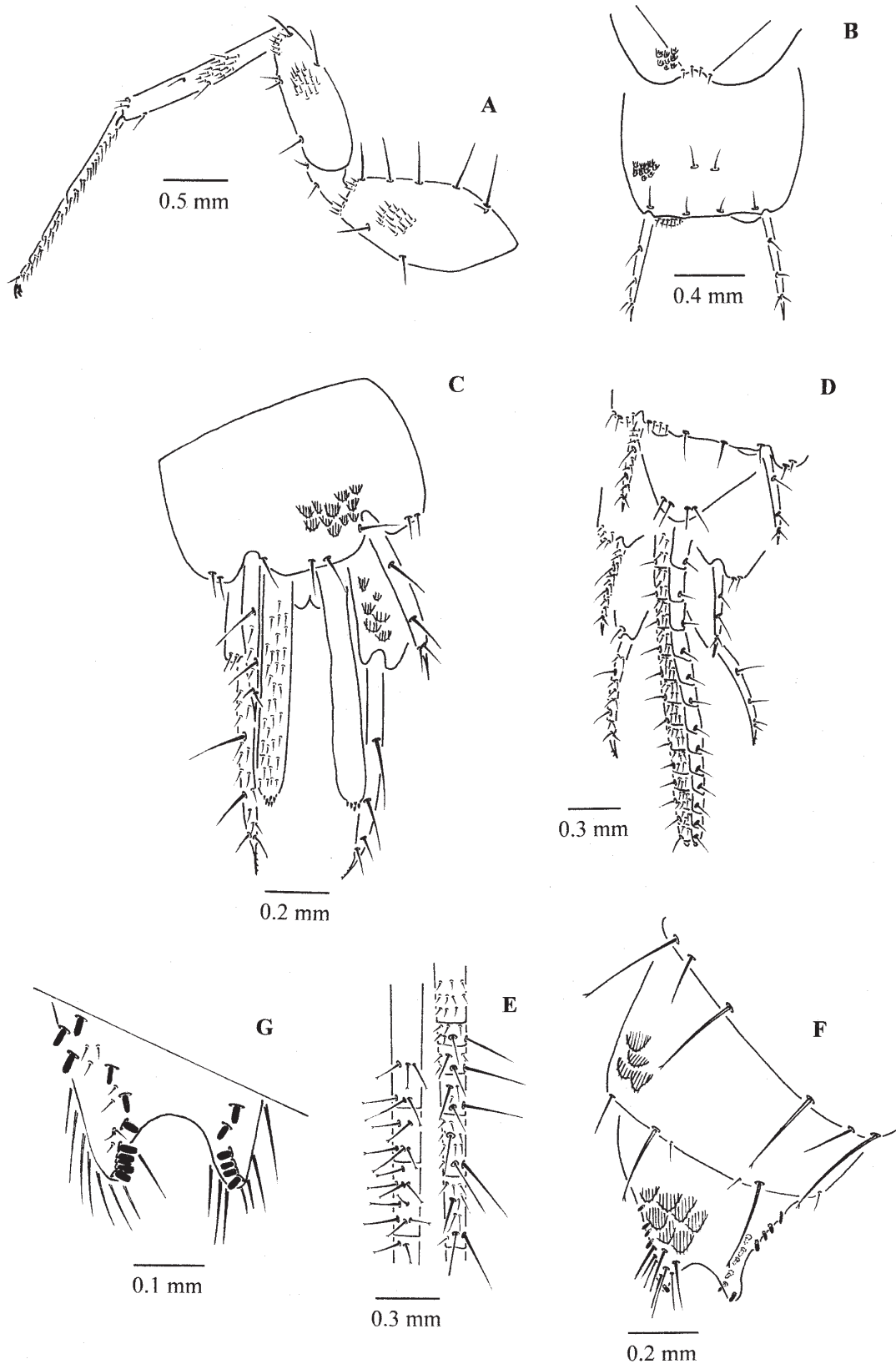


Figure 3. *Lepidospora mazyadi* n. sp. Scales and microchaetae partially shown. A, Hind leg. B, Urosterna I and II. C, Genital area of male. D, Genital area of female. E, Caudal appendages. F, Dorsal view of uroterga VIII-X (ventral pegs represented with dots on one single side). G, Ventral view of urotergite X.

Hafeet cave from which the specimen was identified. H.E. Mubarak Sa'ad al-Ahbabi, Chairman of the Department of The President's Affairs, Nick Cochrane-Dyett, and Peter Hellyer for facilitating the study of the nicoletioid specimens. Pam and Tim Fogg were both collectors and our liaison for sources of information. DNA was sequenced by students of the genetics course (BIOL320) at Marist College. We thank Graeme Smith and Julian J. Lewis for reviewing the manuscript.

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# THE VIEW OF MAYA CAVE RITUAL FROM THE OVERLOOK ROCKSHELTER, CAVES BRANCH RIVER VALLEY, CENTRAL BELIZE

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**Abstract:** Archaeological investigations of the Overlook Rockshelter in the Caves Branch River Valley of central Belize offer a unique view of ancient Maya cave ritual through the complete recovery and analysis of all artifacts within the site's two small activity areas. In general, the assemblage contains many of the same types of objects documented from other nearby caves and rockshelters. However, the nearly 1700 ceramics sherds showed almost no refits, demonstrating that sherds were deposited at the site individually, rather than as complete vessels. The human bone assemblage represents three or four individuals, with the majority of the bones comprising a single individual, and all of these were deposited as incomplete secondary interments. Analogies for this depositional behavior based on archaeological and ethnographic studies suggest that this rockshelter may represent a waypoint within a ritual circuit composed of multiple locations over which fragments of complete items such as ceramic vessels and secondary burials were spread.

## INTRODUCTION

Maya cave archaeology has gained much momentum within the last two decades, supplementing traditional archaeological approaches by expanding discussions of the roles of ideology and ritual within Maya sociopolitical structure. While ethnographic and ethnohistoric studies link caves, rockshelters, and sinkholes to rain and fertility rituals, iconographic and archaeological data from pre-contact contexts often demonstrate the further use of these natural features in rites of political accession, aggrandizement, legitimization, and social incorporation (Bassie-Sweet, 1996; Prufer and Brady, 2005; Vogt and Stuart, 2005). Thus, an important aspect of Maya speleoarchaeology is the contextualization of cave use, which has been accomplished by identifying the nature and timing of ritual activities performed in caves through the analysis and interpretation of artifact assemblages. The current study focuses on a single, small rockshelter site, the Overlook Rockshelter (OVR), that was investigated as one component of a broad regional cave survey currently being conducted by the Central Belize Archaeological Survey project.

Increasingly, cave studies in the Maya area have taken the form of broad regional surveys that have sought to articulate data from a variety of subterranean sites into more traditional archaeological research designs, actively incorporating data from sites of different size, morphology, and proximity to other archaeological contexts (Awe, 1998; Bonor Villarejo, 1987; Brady et al., 1997; Hardy, 2009; Peterson, 2006; Prufer, 2002; Risollo, 2003; Wrobel et al., 2009). In contrast to the generalized picture of ancient Maya cave use previously available, which was generally based on single sites, these regional approaches are

designed to paint a holistic picture of the range of cave rituals practiced by specific groups living in a particular area. Furthermore, such studies can help illuminate broader patterns of cave use that are not necessarily restricted to rituals performed in a single locale. Such patterns would be invisible in cases where researchers treated cave contexts as isolated and bounded ritual contexts. Thus, the data from OVR can be compared to and contrasted with other related sites, providing contextualization for identified ritual behaviors. Furthermore, ethnographic analogy is often utilized as a means of linking artifact assemblages to specific types of rituals, as well as the nature of the ideological significance caves held within Maya belief systems (see Brady, 1989, and Brady and Prufer, 2005 for extensive reviews and examples). As an example, our discussion of OVR as a cave stems from ethnographic and ethnohistoric studies suggesting that rockshelters were conceptualized and appropriated by Mesoamerican peoples as caves (see Risollo, 2005, p. 354–356). However, ethnographic analogy does not provide all the answers, and as Risollo (2005, p. 354) points out, “while semantic analyses among the modern Maya are important in steering the direction of research, it remains for archaeologists to document the palpable evidence of underground utilization in order to define boundaries of the ancient Maya concept of ‘cave’.”

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Figure 1. Elmer “Neko” Medrano at the Overlook Rockshelter. View toward the north.

Overlook Rockshelter was targeted for excavation in 2009 because it possessed a relatively unique combination of attributes not found at other sites in our survey. OVR is a small overhang with no associated dark zone and located on a sheer limestone cliff face hidden by vegetation high above the Caves Branch Valley (Fig. 1). Surface collection had revealed an assemblage consisting primarily of heavily fragmented ceramics. In particular, the unexpected presence of human remains at such a small and isolated site was intriguing; rockshelter burials have been noted at other nearby sites, such as Caves Branch Rockshelter (Glassman and Bonor, 2005; Wrobel et al., 2007), Sapodilla Rockshelter (Wrobel and Shelton, 2011), Actun Nak Beh (Halperin, 2005), and Uayazba Kab (Gibbs, 2000), but these sites possess generally larger activity areas with an associated dark-zone cave (Fig. 2). Furthermore, unlike the OVR, these other examples are easily accessible and seem to be directly associated with nearby settlement. Because of the small size of the activity areas and the fact that this site is not actively used for tourism, we decided to excavate the cultural features completely, collecting all artifacts. While taphonomic processes have likely played their part, we feel

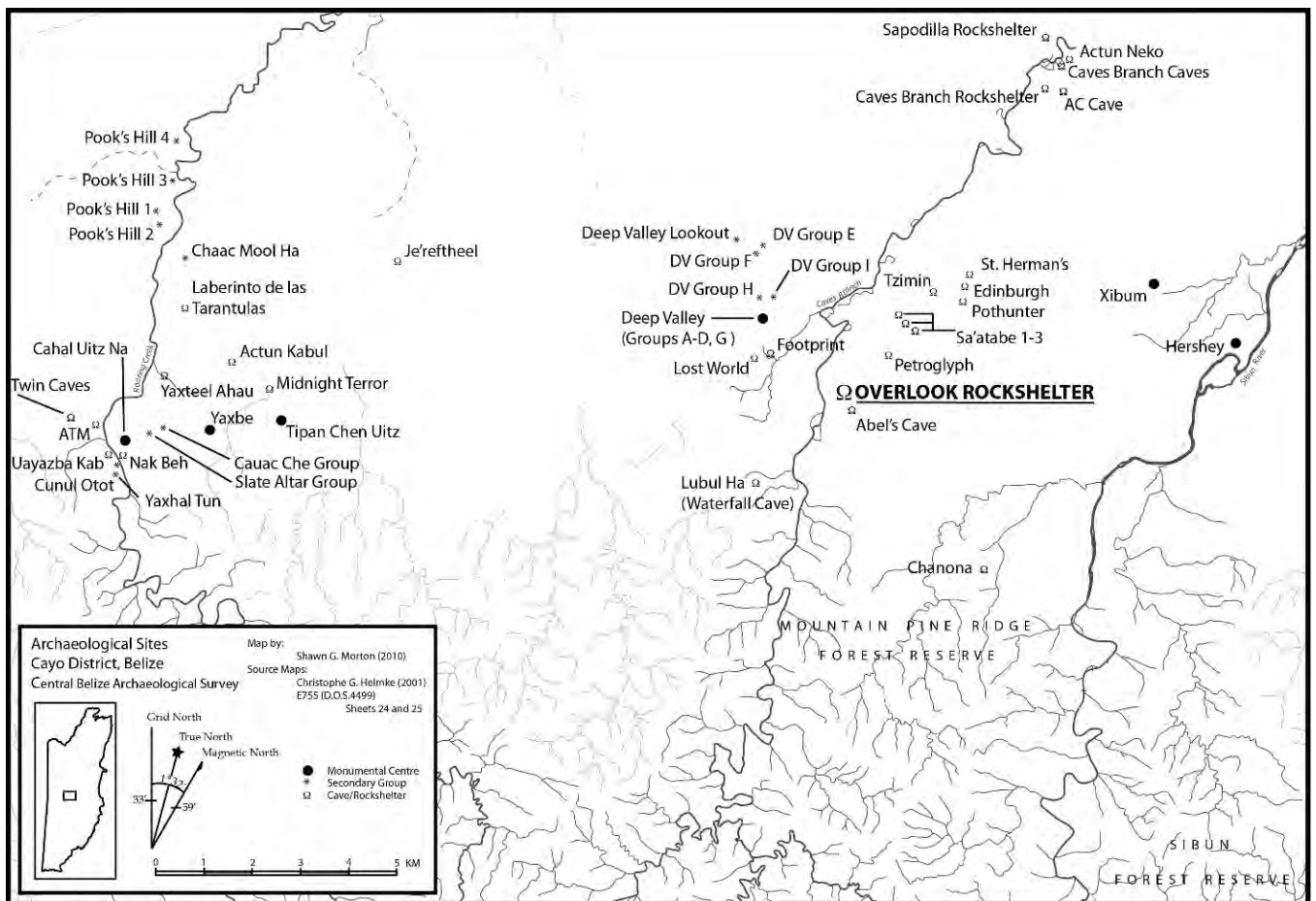


Figure 2. Map of study area, showing nearby sites.



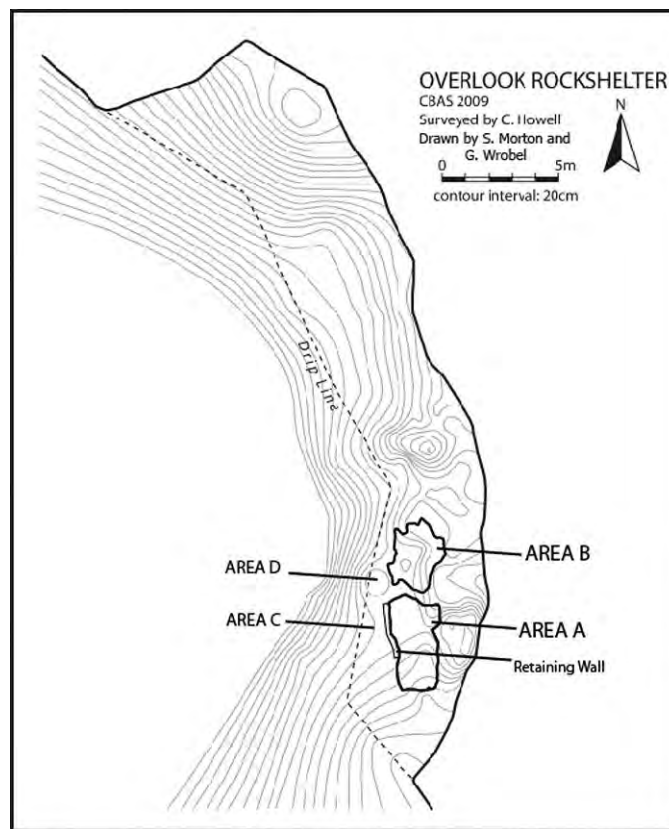


**Figure 3.** Area A, showing recently piled bones and artifacts to the left, and the area of disturbance to the right.

reasonably confident in stating that the vast majority of the artifacts deposited in the past remained in context. There are no previous attempts to collect and study a complete artifact assemblage from a rockshelter, and for this reason this study represents a unique and invaluable source of data with which to interpret the ritual activities performed at such sites.

#### DESCRIPTION OF THE SITE AND THE EXCAVATIONS

Members of the Belize Valley Archaeological Reconnaissance project and guides from Ian Anderson's Caves Branch Adventure Company and Jungle Lodge first reported Overlook Rockshelter after a brief reconnaissance in the summer of 2008. The ground surface is uneven, with only two discrete areas that contained scattered artifacts and human bone. In both areas, bones and sherds had been stacked (probably recently) on some of the loose stones on the surface (Fig. 3). Ceramic sherds accounted for most of the artifacts visible on the surface, though freshwater *jute* shells and several river cobbles were also noted. Typical of most other cave and rockshelter sites in the area, the *jute* had broken, or spire-lopped, tips to facilitate consumption of the animal inside (see Halperin et al., 2003 for discussion of the ritual significance of *jute* among the Maya). The rest of the surface does not contain soil or any traces of human activity and is composed of flowstone and large boulders of breakdown from the cliff face above. The rockshelter is oriented roughly north to south, facing east with a spectacular view of the valley floor. By late afternoon the sun clears the overhanging roof and the entire rockshelter is exposed to direct sunlight. The wall of the OVR curves slightly outward toward the northernmost



**Figure 4.** Topographic map of the Overlook Rockshelter.

end of the shelter. A short climb up the limestone wall of the elevated northern corner of the OVR reveals another small rockshelter; it has no artifacts visible on the surface.

Excavation units were set up in the only two areas with noticeable cultural material (Fig. 4). These areas were the only areas within the rockshelter that contained loose soil, while all other surfaces were composed either of solid or eroding limestone. Furthermore, the boundaries of both were naturally lined by large stones and thus were relatively easy to determine. The soil within these areas matched the matrix found in other rockshelters in the surrounding area—loose, dark, and composed mainly of limestone breakdown from the cliff walls (Hardy, 2009, p. 62). All of this loose soil from both areas comprised a matrix containing ceramic sherds, as well as a few other pieces of material cultural, that were mixed throughout it. The surface of these areas contained recent breakdown from the rockshelter ceiling in the form of several large rocks that were removed prior to excavation. All visible scattered or stacked artifacts and bones were collected as a single surface context. Based on prior experience with rockshelters, we did not anticipate finding clear stratigraphy, so arbitrary 20 cm levels were used.

Area A is located in the southernmost portion of the rockshelter and was defined by rock collapse (north and



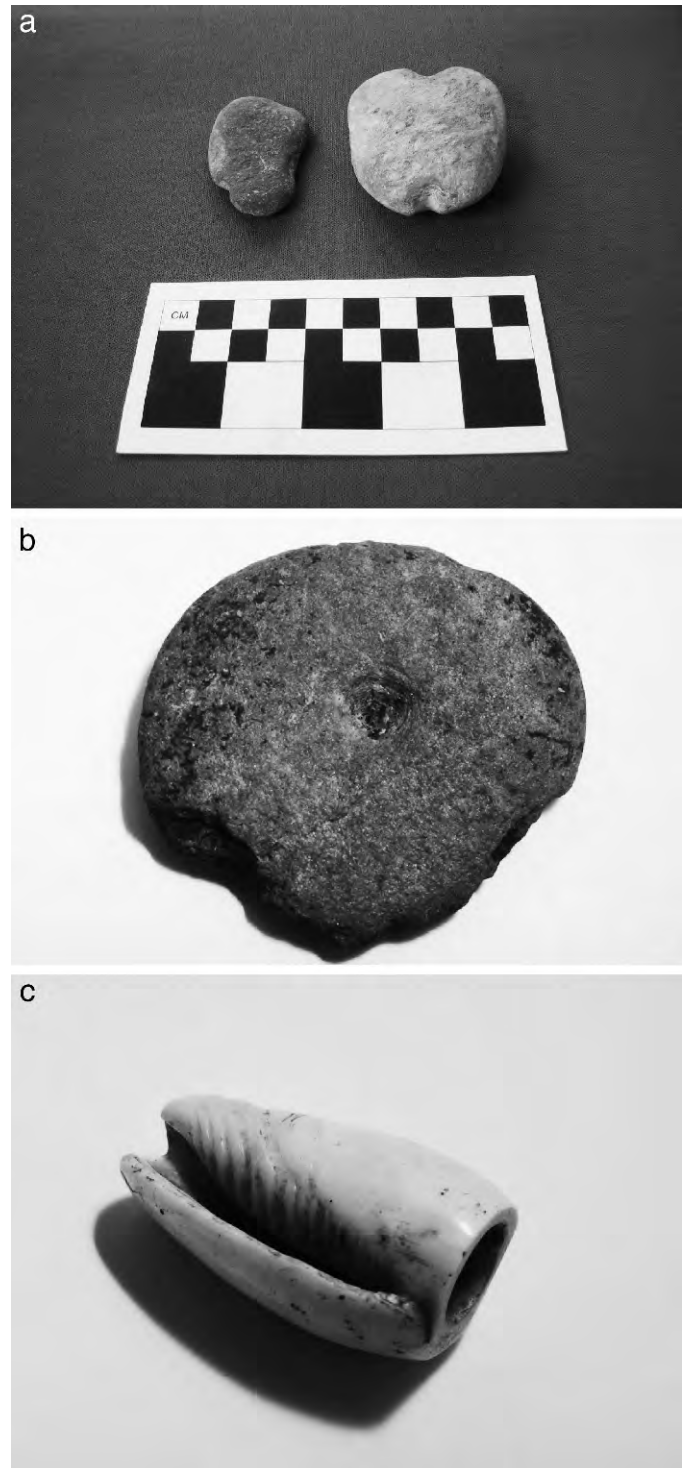
south), the natural cave wall (west), and a crudely constructed retaining wall (east). It measured 202 cm (E-W) by 384 cm (N-S) at its largest. There was a narrow trench, initially hypothesized to be a small looter's pit, that upon further investigation turned out to be an area of natural collapse into a narrow fissure beneath the soil. Area B was the other main focus of excavation efforts. It measured 220 cm (E-W) by 302 cm (N-S) at its largest and was separated from Area A by a loose pile of collapsed rock. Excavations within both areas, as expected, did not reveal any clear stratigraphy (i.e., the soil matrix was relatively uniform in color and artifact density throughout) and sterile soil was reached at a maximum depth of approximately 60 cm within most of both units. Artifacts found in the excavations from both areas included numerous pottery sherds, *jute* shells, and a few pieces of obsidian. Area A contained a small, drilled slate disk and a carved marine shell bead, as well as a higher concentration of human bone (Fig. 5). Area B contained a fish-net weight and a relatively higher concentration of *jute* shells.

A small flat area of ground that runs from the base of the eastern stone wall boundary of Area A to the edge of the rockshelter also contained a few artifacts, including small ceramic sherds and a few faunal remains. These remains were collected as Area C. The shallow soil was very dense, and it is very likely that the cultural remains spilled over from Area A. Also, some of the soil and artifacts from Area B were washing out between the large boulders defining the eastern edge of the context. These artifacts, which included some of the largest sherds from the assemblage, were collected as Area D, though these clearly originate from Area B.

#### OSTEOLOGICAL ANALYSIS

The inventory of the skeletal remains shows that most bones likely come from a single incomplete individual, based on similar taphonomic characteristics, the generally consistent size and robusticity of elements, and in some cases corresponding articular surfaces of adjacent bones (in the left arm and the feet). However, the majority of the skeleton is absent, and the small size of the fissure in Area A is not sufficient to explain the missing elements. Most of the bones are complete and, apart from the ribs, no fragments were found. Where available, sex and age indicators consistently indicate that the primary individual is an older adult female. The age is based on the presence of osteophytes visible on the lumbar vertebra and on discrete patches of arthritis noted on the glenoid surface and on the pelvis around the auricular surface. In addition, the individual displays a pronounced preauricular sulcus and pubic pitting, which are typically interpreted as stress resulting from childbirth.

Each segment of the spinal column is represented by at least one vertebra. Few of these appear to be consecutive, however, and thus the vertebral bones included in the



**Figure 5.** Photograph of (a) slate fragment and net weight, (b) drilled slate fragment, and (c) shell bead from Lots 4 and 5, Overlook Rockshelter, Belize.

assemblage were likely selected individually rather than as segments (i.e., upper or lower body). The pelvis includes small fragments of the sacrum and segments of the left os coxae, including the ischium, pubis, acetabulum, and a

portion of the left auricular surface. Approximately half of the ribs were present, though many of these had fragmented. The most consistently represented portion of the skeleton is the left arm, which includes the clavicle, humerus, ulna, radius, and much of the scapula. The left hand is represented by all five metacarpals, as well as the lunate, capitate, and hamate. The right hand has only metacarpals 1, 3, and 4, though five phalanges were identified that may belong to either hand. Foot bones include several tarsals from each foot (left = navicular, talus, and calcaneus; right = navicular, talus, and 3rd cuneiform) and only a fragment of a single metatarsal. Toe phalanges present included all proximal phalanges except the first left, no middle phalanges, and only a single distal phalange of the right first toe. No fragments of the leg bones were present, though both patellas were found. An AMS analysis (Beta-279539;  $1640 \pm 40$  BP) of a rib gave a calibrated two-sigma range of AD 330–540, placing the individual securely within the Early Classic (AD 300–600) period.

Within the bone assemblage are also at least a few random elements from other individuals, including an unfused coracoid process from a juvenile's scapula. Also, the only four teeth found at the site, all of which were permanent, represent at least two different individuals based on differences in attrition. An upper left first premolar had a nearly complete root, suggesting an approximate age around 10 to 12 years. A lower left first premolar had a complete root, but displayed a similar amount of wear, and thus could represent the same individual. The remaining two teeth, a lower right lateral incisor and canine, were from a slightly older individual and displayed minimal amounts of attrition and similarly polished surfaces. Since no cranial elements were found, and all teeth seem to come from relatively young individuals, it is likely that none belong to the primary individual. We have calculated a minimum number of three individuals on the basis of the different ages represented by the teeth and bones, though it is certainly conceivable that some of the teeth or smaller bone fragments belong to additional individuals.

#### CERAMIC ANALYSIS

Excavations revealed approximately 1700 ceramic sherds, which demonstrated diagnostic attributes spanning the Late Preclassic (300 BC to AD 250) through the Late Classic (AD 600–900) periods; identification was based on the type-variety method of Gifford, 1976. The relatively small assemblage contained a wide range of types within four wares: Uaxactun Unslipped, Peten Gloss, Pine Ridge Carbonate, and Paso Caballo Waxy Ware. The Late Preclassic diagnostic specimens were limited to two Sierra Red rim sherds and possibly one Sapote Striated: Unspecified (red rim) variety. Early Classic sherds included ring bases of unspecified types and one Actuncan Orange

Polychrome: Actuncan Variety rim sherd. In addition, a number of decorated body sherds appear to be of the Protoclassic–Early Classic Cocay Appliquéd type, which is also known as Tzimin Appliquéd (Reents, 1980, p. 168–186, 60). The Late Classic is represented by several Dolphin Head Red: Dolphin Variety rim sherds, which date to the early facet Spanish Lookout phase. Over 86% of the sherds were unslipped, with 30% of these being striated. Less than 10% were slipped, and only three polychrome sherds were present. There were no apparent differences in the ceramics between the sub-assemblages from Areas A and B. Furthermore, comparisons within the units did not reveal expected temporal differences in the ceramics found in the upper and lower levels of the deposits. This has implications for the site's formation processes. Mixing of sherds throughout vertical levels was also noted at other nearby rockshelters (Hardy, 2009; Wrobel et al., 2007). At Caves Branch Rockshelter, mixing was explained by the constant intrusion of new burials. As this behavior is not evident at Overlook Rockshelter, it is possible that the depositional pattern observed is the result of extensive bioturbation in the rockshelter's unconsolidated matrix (see Haynes and Agogino, 1986). Alternative cultural hypotheses include that this depositional pattern reflects an as yet unidentified cultural process that would have acted to churn the matrix or that these sites may have been used over a relatively protracted period in the Late Classic, in which case deposits would include old sherds moved from other locations on the landscape. In reference to this second possibility, Joel Palka (personal communication, March 2012) has noted that among the modern Lacandon Maya at Mensabak, Chiapas, sherds and other ancient objects that are found in fields are thought to be associated with the past or with gods or ancestors and are picked up and redeposited within rockshelters. This possibility is especially intriguing because it would also explain the lack of stratigraphy in the deposits at OVR, and it thus deserves further consideration in future studies.

Overall, the broad time scale and the types of vessel forms represented at Overlook Rockshelter fit relatively well within established regional patterns for rockshelters and caves (Awe et al., 1998; Graham et al., 1980; Hardy, 2009; Helmke, 2009; Reents, 1980). As expected, OVR (like other rockshelters) does not show the full variety of ceramic forms often noted in dark-zone caves, which, in addition to utilitarian vessels, often include vases, shoe-shaped ollas, censor drums, ocarinas, comales, and disks or lids (Helmke, 2009, p. 494–95; Reents, 1980). Similarly to both caves and other rockshelters, OVR's assemblage has a relatively large percentage of sherds from unslipped and striated jars (ollas) and bowls. The forms of the plainware vessels from which the sherds originate are consistent with those typically identified as utilitarian when they are found in household or other non-cave contexts. Unfortunately, we cannot make specific inferences about which of the many possible ritual or domestic activities these vessels

were actually used in prior to their fragmentation and the subsequent deposition of their pieces. However, the consistency of OVR's assemblage does appear to be somewhat distinct from other nearby documented rockshelters that generally contain a very large percentage of the same plainwares, but also tend to include individual sherds from a much wider variety of specialized vessel forms, including polychrome dishes, column or pyriform vases, miniatures, ceramic spindle whorls, and net weights (Hardy, 2009). Additionally, other assemblages often contain a larger variety of decorated sherds from specialized vessels such as Terminal Classic model-carved sherds and Daylight-Darknight vessels (Hardy, 2009; Wrobel and Shelton, 2011). Thus, it appears that this aspect of depositional behavior typical of cave and rockshelter ritual was absent at OVR.

The ceramic analysis implies some specific depositional behaviors, which also can be used to inform reconstructions of ritual use of the rockshelter. Perhaps the most significant discovery during the analysis of the ceramics was that the assemblage contained very few refits between sherds, with no evidence for the presence of partial or complete vessels. Only 35 of the nearly 1700 sherds were rim sherds representing over 30 vessels with no refits. These vessels represent a variety of forms, including jars, dishes, and bowls. Despite the number of Peten Gloss body sherds, there were no rim sherds that refit to the incised Peten Gloss neck sherds in the assemblage. The appliqué decorations on the limited number of decorated body sherds are typically found around the circumference of Cocay Appliqué jars, yet if there were whole vessels in the assemblage, there would have been a much larger number of decorated sherds. Based on the overall small size of the sherds, their eroded condition, and the lack of refits, it appears that the ceramics deposited at OVR were left as individual sherds instead of whole vessels.

In her regional rockshelter study of the Caves Branch River Valley, Hardy (2009) identified distinctly different depositional patterns of ceramics. For instance, the sixteen whole vessels found at the Caves Branch Rockshelter (CBR), all of which dated to around the Protoclassic period, appeared to have been interred intact with burials. However, the broader temporal diversity and variation in vessel types and the general lack of refits of the sherds found throughout the matrix at the CBR and the other rockshelters in the study suggested to Hardy (2009, p. 103) that they were instead deposited as fragments. Because these sites were only sampled using test-pits, the lack of refits in her sample could possibly be explained by ritual behavior in which complete vessels were smashed and then spread across an entire single site. However, the data from OVR suggest otherwise. In the neighboring Sibun River Valley and the Maya Mountains, deposition of complete vessels left whole or ritually killed/smashed and of scatters of individual sherds, have been recorded (Peterson, 2006, p. 57, 64, 126; Prufer, 2002). At Actun Toh in Quintana

Roo, Rissolo (2003, p. 47–54) notes that it appears as if ceramics were brought into the cave as sherds and were scattered along the surfaces so as to mark them as paths (see also Moyes, 2005, p. 281). Recent salvage operations at the Sapodilla Rockshelter found similar patterns to Caves Branch Rockshelter, with whole vessels likely associated with burials, while the surface and matrix were littered with seemingly random ceramic sherds (Wrobel and Shelton, 2011).

## DISCUSSION

As mentioned above, the rockshelter was excavated completely, and thus the analyses of the artifacts and bones are unique in that they are based on a nearly complete assemblage, assuming that only a small percentage of the assemblage washed out over the edge of the steep cliff face. The importance of this approach lies in being able to rule out the possibility that missing elements from the skeleton or from ceramic vessels were initially deposited at the site but are buried elsewhere in it. While looting may also account for some missing elements of the assemblage, we suspect this is minimal, given the general lack of surface disturbance. In our experience, artifacts worth removing found lying on the surface of a site generate at least enough enthusiasm by looters to dig small pits, none of which were evident at OVR.

Two other ritual behaviors that are often documented ethnographically may also be responsible for the specific composition of ceramics within the Overlook Rockshelter assemblage. The first relates to the collection of ritually-charged objects (Brown, 2000; Scott, 2009, p. 91), as well as relocation of objects as caches in other ritual locations (Brady, 1989, p. 109). In this case, it is conceivable that individuals visiting OVR removed ceramics from piles of complete smashed vessels as keepsakes or for use in rituals performed elsewhere. The second ritual behavior is sweeping, which has been documented extensively in ethnographic contexts (Tozzer, 1941, p. 151–2; Vogt, 1976, p. 99; Brown and Emery, 2008, p. 317; Scott, 2009, p. 97) and has been hypothesized in a number of archaeological settings at both cave (Halperin, 2005; Prufer, 2002, p. 620) and surface sites (Coggins, 1987; Glassman et al., 1995, p. 60; Mathews and Garber, 2004, p. 52–53). Indeed, it is possible that the deposits at OVR may have accumulated as the result of sweeping clean a nearby activity area. If the ritual area requiring sweeping was located within OVR, it is also possible that the majority of ceramics initially deposited there were swept over the edge of the cliff and thus not recovered. While these remain possibilities, we suggest that they are unlikely, since both of these processes would result in a rather random assortment of ceramic sherds, and among the 1700, we would expect that if each began as a whole vessel that at least some of those vessels would be represented by more than two pieces. Careful examination of the



assemblage demonstrated through comparisons of variables like thickness, color, shape, decorations, inclusions (temper), and form that almost none of the pieces could belong to the same vessel. It seems unlikely that post-depositional collection of objects or sweeping would result in such a consistent pattern. Furthermore, periodic cleaning over an extended period of time, presumably at the initiation of a new rite, has been demonstrated elsewhere to produce a distinctive distributional pattern (Helmke et al., in press). This pattern is characterized by marked disparity in sherd density between activity areas and the peripheral areas of secondary deposition that such "sweeping" produces. No such pattern was observed at OVR. Thus, we are convinced that this assemblage represents a non-random assortment of sherds, and that individual vessels are only represented by one or two sherds, which is more consistent with the hypothesis that sherds were deposited at OVR individually.

The single partial secondary burial found at Overlook Rockshelter is unusual, but does have some correlates that may help to explain its significance. For instance, the three burials documented at Actun Nak Beh are also thought to be secondary partial burials. Halperin (2005) suggests that they may be the remains of elites who were incorporated into a specific type of cave ritual intended to legitimize authority and power of their relatives. Unlike OVR, however, the rockshelter entrance of Actun Nak Beh likely would have served as an area for public ritual, because it is a flat open space, it is directly connected to the monumental center of Cahal Uitz Na by a sacbe, and there is a small and restrictive dark zone that could have been used for private ceremonies. Furthermore, the OVR artifact assemblage consisted primarily of dense deposits of individual ceramic sherds, while Actun Nak Beh had very few sherds, perhaps as a result of ritual sweeping or periodic looting (Halperin, 2005, p. 80). So, while the form of the burials at Actun Nak Beh (i.e., secondary and partial) is similar to that at OVR, the contexts at the two sites are quite different, especially in regard to the relative isolation and difficult access of OVR, likely suggesting that the rituals performed at the two sites were distinctive.

A recent report on a solitary walled burial from Actun Bats'ub in southern Belize also shows some basic correlates with OVR, as well as a similar Early Classic date (Prufer and Dunham, 2009). Based on its isolated location and the likely postmortem manipulation of skeletal elements, the authors argue that the artifacts within Actun Bats'ub reflect a single specialized ritual specific to the individual based on the importance of his or her social role as a shaman. While the solitary nature of the OVR burial may also be argued to imply individualized treatment, the case for the Bats'ub burial is based on many contextual aspects that are not evident in the OVR interment. Bats'ub is a disturbed primary burial, it contains elaborate grave goods, and it is sealed within a dark-zone environment. In other words, the Bats'ub burial clearly parallels the

reverential mortuary behavior characteristic of tombs, while the scattered and almost random bones of the OVR burial are treated in a way similar to the rest of the artifact assemblage within the general matrix. Furthermore, unlike at OVR, the diagnostic artifacts and radiocarbon dates from the Actun Bats'ub are all temporally homogenous, supporting the argument that this assemblage represents a single, though possibly protracted, ritual event.

The inventory and analysis of the remains at OVR provides some clues for deciphering the mortuary pathway leading to the interment. While the left arm is relatively complete, the almost random assortment of other elements, such as non-continuous elements of the spinal column and feet, for instance, and the complete lack of cut marks rules out peri-mortem mutilation of the body as an explanation for its incomplete state. Clearly, elements of the initial primary burial were removed after decomposition. Furthermore, while the presence or absence of some elements appears random (e.g., carpals), the suspiciously complete absence of leg long bones and the skull seems deliberate. (See Tiesler, 2004 for a discussion of natural and cultural influences on decomposition leading to differential preservation and representation of bones in Maya burials.) What is less clear is whether the elements of the OVR interment were moved to the rockshelter as a secondary burial, or whether the OVR was the site of the primary burial and the missing elements were subsequently removed. (See Fitzsimmons, 1998 for a description of prolonged mortuary practices among the Classic period Maya elite.) The complete lack of articulation supports the former hypothesis, while the presence of many of the smallest bones would tentatively support the latter. (See Prufer, 2002, p. 613, who discusses how assemblages can be affected by the behavior of ritual specialists, who pick up previously discarded objects for reuse in ceremonies.) However, the extra bones (teeth and infant clavicle) certainly must have been moved from elsewhere as individual elements, or one would expect to have found more elements of those individuals.

The nature of the artifact assemblage at Overlook Rockshelter may offer the best analogy for explaining the presence of the scattered bones of a partial skeleton. As noted, few of the sherds could be fitted together, suggesting that almost all came from different vessels. Furthermore, the presence of diagnostic forms from a span of nearly a millennium is certainly curious, since it may imply repeated and continuous use. While this pattern has been documented at a number of sites like the larger rockshelters and caves in the region (Hardy, 2009), it is surprising to find such consistent devotion to such a small, hard-to-reach, and generally unimpressive site as the OVR. It may be, however, that the single cave was not the focus of the ritual. Several researchers have argued that sites and even specific areas within sites were sometimes seen as connected parts within a ritual landscape and formed a series of way points along a ritual circuit (Garza et al., 2001, p. 22;

Kenward, 2005; Moyes, 2005; Peterson, 2006, p. 120; Prufer, 2002, p. 639; see Adams and Brady, 2005 for an ethnographic discussion of ritual circuits). Ethnographically, this behavior is common to near ubiquitous and can be seen as an act in rituals of the hunt (Brown, 2005), of the house, of the field, and of the healing of the sick (Vogt, 1976, p. 9–10, 54–55, 75), as well as in rituals of socio-political investment (Tedlock, 1982; see also Freidel et al., 1993, p. 419) and boundary maintenance (Tozzer, 1941; Vogt, 1969, p. 391). At each of these sites, individuals would leave a small offering.

In the ethnographic record, offerings are typically composed of what would be considered whole objects in a material sense. That is, ritualists deposit whole and intact arrangements of candles, plants, and other paraphernalia (see Tedlock, 1982; Vogt, 1976, p. 9–10, 54, 55). In our archaeological hypothesis, the difference is thus striking as we propose that the lack of an entire vessel in our assemblage may suggest that its individual pieces were dispersed across the landscape instead of being deposited together. However, while the material signature of the assemblage varies from our ethnographic model, it remains conceptually analogous: The ritual functions because offerings and locations are inexorably bound as one object open to manipulation (as in the Zinacanteco healing ritual described by Vogt, 1976, p. 9–10); in this way, the ritual is no less than the sum of its parts, and individual offerings are but fragments of this greater whole.

The paucity of rim sherds and the absence of larger sherds and partial vessels, as well as the rather mundane nature of most of the non-ceramic offerings, may reflect the small and marginal nature of the site compared to others on the circuit. If this scenario is accurate, the incomplete skeleton may represent an analogous situation in which the parts of the individual were also similarly dispersed. The largest and most distinctive bones of the primary individual—the leg bones and the cranium—may have been reserved for another more meaningful or significant location along the circuit. In other contexts, crania and longbones are often missing from elite burials, likely as a result of removal for use in veneration rituals (McAnany, 1995, p. 60–63). Finally, the Early Classic date of the bones falls securely between the earliest (~AD 1) and latest (~AD 800) ceramic dates. While this suggests that the interment likely does not represent a dedication ritual, the secondary nature of the burial leaves open the possibility that it was placed as part of a termination ritual despite the nearly 400 year interval (see Kunen et al., 2002).

#### CONCLUSIONS

The Overlook Rockshelter was excavated completely, resulting in the collection of a complete artifact assemblage. An Early Classic partial secondary burial of an adult female had been placed within the rockshelter, and was found scattered within the matrix. The artifact assemblage

was generally typical of those from other rockshelters excavated in the area. Diagnostic ceramics showed a variety of forms similar to those reported in other cave contexts and also showed a range of dates spanning the Late Preclassic through Late Classic periods, which were mixed throughout all levels of the matrix. The lack of fits between the sherds showed that no whole vessels had been placed in the rockshelter, though it is certainly possible that looters removed whole vessels left on the surface. However, the general nature of the deposit suggests that the assemblage was created over a long period of time through the repeated deposition of single sherds, and occasionally other small, common, and inexpensive (though perhaps symbolically meaningful) objects, such as river cobbles, small used obsidian blade fragments, and *jute* shells. The documentation of the complete artifact assemblage at OVR has important implications for the interpretation of other cave contexts. Most cave sites contain ubiquitous single sherds in isolation or within clusters that are often hypothesized to be the remnants of complete smashed vessels whose fragments were subsequently moved by water or human activity. Instead, sites like these may represent part of a ritual circuit composed of many such sites, where an individual leaves small offerings at each. Similarly, this pattern may explain the incompleteness of the skeleton, which, like the vessels, could have been spread across the landscape. Future research focused on answering questions related to the nature of such deposits would benefit from detailed comparisons of assemblages at multiple sites across the landscape.

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# FLOW CHARACTERIZATION IN THE SANTEE CAVE SYSTEM IN THE CHAPEL BRANCH CREEK WATERSHED, UPPER COASTAL PLAIN OF SOUTH CAROLINA, USA

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**Abstract:** Karst watersheds possess both diffuse and conduit flow and varying degrees of connectivity between surface and groundwater over spatial scales that result in complex hydrology and contaminant transport processes. The flow regime and surface-groundwater connection must be properly identified and characterized to improve management in karst watersheds with impaired water bodies, such as the Chapel Branch Creek (CBC), South Carolina watershed, which has a long-term sampling station presently listed on an EPA 303(d) list for phosphorous, pH, and nitrogen. Water from the carbonate limestone aquifer of the Santee Cave system and spring seeps in the CBC watershed were monitored to characterize dominant flow type and surface-groundwater connection by measuring dissolved calcium and magnesium, total suspended solids, volatile suspended solids, alkalinity, pH, specific conductance, and stable isotopes ( $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ). These measurements indicated that the conduit flow to Santee Cave spring was recharged predominantly from diffuse flow, with a slow response of surface water infiltration to the conduit. Qualitative dye traces and stage elevation at Santee Cave spring and the adjacent Lake Marion (equal to the elevation of the flooded portion of CBC) also indicated a relation between fluctuating base level of the CBC reservoir-like embayment and elevation of the Santee Limestone karst aquifer at the spring. Methods described herein to characterize the flow type and surface-groundwater connection in the Santee Cave system can be applied not only to watershed management in the Chapel Branch Creek watershed, but also to the greater region where this carbonate limestone aquifer exists.

## INTRODUCTION

This study uses measurements of dissolved calcium and magnesium, total suspended solids, volatile suspended solids, alkalinity, pH, specific conductance, stable isotopes ( $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ), dye tracing, and stage elevation measurements from both the Santee Cave spring and Lake Marion to characterize the flow regime and surface-groundwater connection in the Santee Limestone (SL) aquifer in the Chapel Branch Creek (CBC) watershed in South Carolina. The hydrology and water quality of the CBC watershed is significantly impacted by its karst terrain and the shallow carbonate aquifer of the regional SL formation. The karst terrain provides a potential for rapid and substantial loss of surface water to the groundwater in the aquifer. The anisotropic porosity of the carbonate bedrock, created by bedding planes, fractures, and ongoing dissolution within the matrix of the carbonate rock, produces groundwater movement from zones of recharge to discharge through a network of voids of diverse sizes and various degrees of vertical and horizontal connectivity. The result is a watershed dominated by subsurface flow, in which surface water is commonly lost to the subsurface, and groundwater is stored or discharged via springs to CBC at different rates and flow regimes throughout the aquifer (Williams et al., 2012).

The two main types of flow applied to karst aquifers are diffuse and conduit (Shuster and White, 1971; Atkinson, 1977; Gunn, 1986), also referred in the literature as slow and fast flow, respectively. Diffuse flow occurs when the voids in the aquifer matrix are poorly connected and typically result in a system with slow groundwater movement and longer periods of water storage (Fiorillo, 2009). Conduit flow results when voids in the aquifer become well developed and possess high connectivity, thus having less water storage and a more rapid response of recharge to discharge (Fiorillo, 2009). Atkinson (1977) concluded that most flow in karst aquifers occurs in conduits, whereas most water is stored in the matrix. A study by Worthington et al. (2000) analyzed spring and well data from limestone and dolostone aquifers and found that 96% of storage occurred in matrix, while 94% of flow occurred in conduits. The SL unit, in the CBC watershed, is

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the northern portion of the Floridan aquifer. Several studies of the Floridan aquifer including the southern part of the Floridan aquifer in Florida found spring discharge as primarily diffuse recharge from aquifer matrix (Martin and Gordon, 2000; Martin and Dean, 2001; Katz, 2004; Florea and Vacher, 2006; Moore et al., 2009; Ritorto et al., 2009). Even in conduit dominated systems of the Floridan aquifer, the matrix contributes heavily to aquifer discharge (Moore et al., 2009).

Geochemical and isotopic data from springs have been used in numerous studies in different karst regions to characterize flow in carbonate aquifers and degree of surface-groundwater connections (e.g. Jakucs, 1959; Ashton, 1966; Shuster and White, 1971; Jacobson and Langmuir, 1974; Atkinson, 1977). The discharge from springs represents a composite of all the upbasin waters in an aquifer, which show variations in hydrology and water chemistry depending on contributions from diffuse and conduit flow components (White, 2003). Springs dominated by diffuse flow recharge have fewer variations in flow and water chemistry (Shuster and White, 1971). The water is typically saturated with dissolved ions (e.g. calcium and magnesium), because diffuse flow has longer residence time in the aquifer; and therefore, longer contact with the bedrock (White, 1988). In contrast, systems dominated by conduit flow respond more quickly to aquifer recharge and produce greater variations in spring water chemistry (Shuster and White, 1971; White, 1988). This more rapid infiltration and transit of recharge waters also leaves conduit springs under-saturated (White, 1998), and high suspended solids at springs indicate arrival of surface waters coming from sinkholes and direct surface recharge (Mahler and Lynch, 1999; Massei et al., 2002; Vesper and White, 2004). Stable isotopic analysis is commonly employed to infer the flowpaths and sources of spring waters by using end-member mixing analysis (Katz et al., 1997; Plummer et al., 1998; Criss et al., 2000).

Dye tracing is an established method used to determine connections between recharge and discharge areas in karst aquifers and was used in this study to obtain information about the response of the SL aquifer at the Santee Cave system during times of low and high lake elevations.

The flow type and surface-groundwater connections in the SL carbonate aquifer are clearly important factors in water movement; and thus, also contaminant transport and water quality of the CBC watershed. The goal of this study is to characterize flow in the watershed using hydrologic, water chemistry, dye tracer, and stable isotope data collected in the Santee Cave system and nearby spring seeps during 2008–2009. The specific objectives of this study are to test two hypotheses: (1) the discharge from SL to CBC is predominantly a diffuse flow component from the matrix with slow surface-groundwater connection and (2) the aquifer water table at Santee Cave is influenced by stage elevations of the lower flooded portion of CBC.

## SITE DESCRIPTION

The region, south of Lake Marion, SC is underlain by the Santee Limestone (SL), a middle Eocene (40 ma), microfossiliferous lime mudstone to slightly shelly wackestone (Willoughby, 2002). The SL is blanketed by the Upper Duplin Formation, a combination of clay and quartz pebble beds, with sinkhole, modern alluvium and swamp deposits throughout the region (Willoughby, 2002). The SL is part of the northern end of the multi-state Floridan aquifer system and a major groundwater aquifer in coastal South Carolina for industrial, agricultural, and public purposes (Hockensmith, 2009). Recharge to SL is by precipitation and surface runoff that infiltrates into the subsurface voids via soil and karst topography (e.g. sinkholes, disappearing streams) and discharges via springs like the Santee Cave spring in Santee, SC along the CBC and the southern shore of Lake Marion. Tritium and radiocarbon dating from a spring northwest of the CBC watershed and from regional wells for a SC Department of Health and Environmental Control (DHEC) recharge and aquifer vulnerability study found that groundwater was a combination of younger waters from recharge along the southern shore of Lake Marion and older waters from lateral flow from aquifer up dip areas, such as the city of Orangeburg, SC (Stone, per. comm., 2009).

The surface watershed for Chapel Branch Creek is 1,555 hectare (3,840 acres) and comprises multiple land uses. The Santee Cave system lies in the forested Santee State Park in the lower part of the watershed. The upper watershed land uses are crop agriculture, roadways and the major interstate I-95, a commercial strip off the I-95 exit, low and medium-density residential areas, two golf courses, and forest. The surface soils in the CBC watershed are sand or sandy loam and vary from sand to clay in the subsurface (SCS, 1988). The upper watershed is relatively flat and around 36.6 m (120 ft) above mean sea level (a.m.s.l.), while the lower watershed at the edges of CBC and Lake Marion have steeper topography of around 25.9 m (85 ft) a.m.s.l.

The CBC watershed drains directly to Lake Marion (Fig. 1). Lake Marion and a lower portion of the CBC are shallow reservoirs created when the Santee River was dammed by the Corps of Engineers for production of hydroelectric power in 1941. The CBC was a spring-fed surface stream before construction of the dam, but afterwards, the lower section became a part of the Lake Marion reservoir. A stretch of the uppermost surface reach of CBC was dammed in 1989 (ERC, 1999) to create a pond for a golf course that releases water downstream by an overflow culvert at location SL7 (Fig. 1). Water from the culvert flows in a channel and remains surface water until it becomes a disappearing stream where it enters the Santee Cave system at a point labeled SS (surface stream) in Figures 1 and 2. During periods of heavy rainfall, the surface water exceeds the capacity of the SS conduit and a



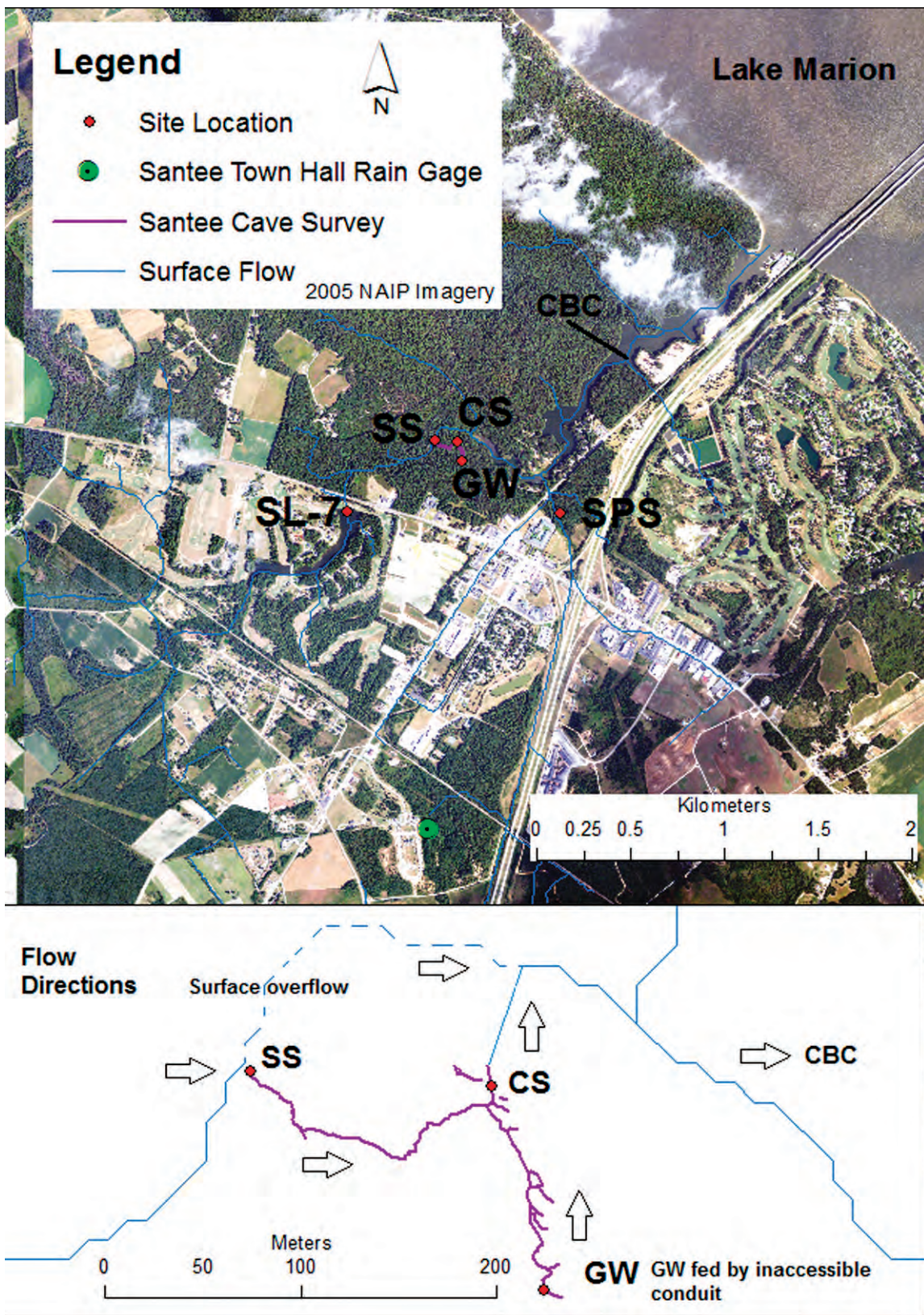


Figure 1. Generalized flow directions for the study site sampling locations in the Chapel Branch Creek watershed.





**Figure 2. Images of sampling locations SS, GW, CS, and SPS, providing typical conduit pattern and relative scale. Researchers provide scale for these karst features.**

portion of flow continues as surface water via a by-pass channel (seen as dashed blue line in Figure 1) to combine with CS (Santee Cave spring) discharge about 175 m downstream. The flow then enters the lower section of CBC, a shallow water body shown in 2009 bathymetry data to have an average width of 200 meters and depth of 3 meters (Amatya et al., 2011).

The Santee Cave system is a stream passage cave with approximately 300 meters of surveyed passage (Holler, 2000). The flow at CS occurs after the convergence of two main stream passages, one originating at GW and the other at SS (Figs. 1 and 2). Based on the cave survey map, the stream passage starting at the SS location drops 3 m (10 feet) along the passage to CS. The GW passage is steeper, dropping 6 m (20 feet) to CS. These two passages combine before emerging at CS at 26 m (85 feet) MSL. The SS location was sampled where the surface stream disappears into the cave. The GW location was sampled where the groundwater conduit emerges at the bottom of a sinkhole. The inaccessible upstream conduit passage feeding GW most likely continues in a southeastern direction, in line with a 2009 sinkhole collapse, a sinkhole pond, and two other sinkholes detected in LiDAR images (Edwards and Amatya, 2009; 2010). A karst window between GW and CS exposes the cave stream and the cave system lies within a forested nature preserve managed by the South Carolina Department of Natural Resources.

Several small spring seeps (SPS) (Figs. 1 and 2) around 30 mm diameter at higher elevations than CS, and not connected to the Santee Cave system, were not monitored for flow, but were sampled in August 2009 for dissolved calcium and magnesium, pH, alkalinity, and specific conductance for comparison to GW, CS, and SS. Several of the higher elevation seeps dried up during a period of

low precipitation and low lake levels in 2009 (Amatya et al., 2011).

Although water is discharged from the SL aquifer into the CBC at numerous small springs along the shoreline, the only spring above the shoreline with substantial flow is CS. A water budget using rainfall data from the watershed and flow data at CS indicated that the source of CS discharge is not solely accounted for by runoff collected by the upstream surface-defined watershed, but also includes groundwater contributions from a larger subsurface watershed (Williams et al., 2012). The estimated baseflow for the region, using data collected at CS, is  $0.09 \text{ m}^3/\text{s}$  (90 l/s). Using these data with a reference value of  $2.3 \text{ L}/(\text{s}/\text{km}^2)$  assumed from the method by Quinlan and Ray (1995), the approximate subsurface drainage area for CS is  $39.1 \text{ km}^2$ . This water budget-based calculated area is  $\sim 3.5$  times the surface drainage area of  $10.9 \text{ km}^2$  (1090 ha) delineated at the CS location in the CBC watershed study. This result was plausible given that surface and subsurface watersheds often do not overlap in karst watersheds (White, 1988). This conclusion was further substantiated by the results from hydrologic computer modeling for the CBC watershed study, in which modeling results predicted more surface flow at CBC subwatershed outlet monitoring stations than measured (Amatya et al., 2011). This “missing” flow at the subwatershed outlets was assumed to be lost from the surface to the groundwater aquifer matrix, eventually discharging downstream into the CBC via CS or other springs (Williams et al., 2012). Also during the watershed study, flow at CS was found to be yielded predominantly by the GW contribution, with peak flows in the hydrograph occurring from rain events due to influx of surface water from the disappearing stream at SS (Williams et al., 2012). A water balance approach indicated flow from the GW passage to CS was nearly continuous at  $0.08 \text{ m}^3/\text{s}$ ; over 60% of the flow, nearly 50% of TP loading, and over 70% of total nitrogen loading into the CBC reservoir (Williams et al., 2012).

These hydrologic measurements from the watershed study indicate a dominant subsurface source in the CS discharge and likely other springs discharging into the CBC. The nature of this complex watershed hydrology, combined with the karst topography and multiple land uses, make this watershed susceptible to water quality impairment. A long-term sampling station in the CBC has been on the U.S. EPA 303(d) list for impaired water bodies for various combinations of parameters for years, and is presently listed for phosphorous, pH and nitrogen. Past incidences of pollution include a waste lagoon at a sewage treatment plant in the CBC surface watershed that disappeared overnight in the 1990s, and the fumigant EDB found in discharge from a spring outside the CBC surface watershed (Stone, per. comm., 2009). Considering this past and ongoing water quality impairment, characterizing the carbonate aquifer for flow type and surface-groundwater connectivity is relevant for improving water

quality in the CBC watershed and the region south of Lake Marion.

## SITE MONITORING

### *Dye Traces*

Three qualitative dye traces based on visual inspection were conducted in the Santee Cave system. The first two traces were carried out on August 28, 2008. A 16 FL. OZ. bottle of Bright Dyes Liquid Concentrate Fluorescent Red was injected into the disappearing stream at location SS, and a 16 FL.OZ. bottle of Bright Dyes Liquid Concentrate Fluorescent Green/Yellow was injected at location GW. Arrival time from GW and SS locations to location at CS (Fig. 1) and the predicted flow path according to the cave survey were recorded. Since a visible amount of organics enters the disappearing stream, and red dye reacts with organics to decompose, a third trace was conducted on April 2, 2009 at location SS using a 16 FL. OZ. bottle of Bright Dyes Liquid Concentrate Fluorescent Green/Yellow dye.

### *Hydrologic Data*

Stage data at CS were collected using an Infinities Water Level Data Logger at 15-minute intervals. Procedures for detailed instrument calibration, data collection and calculations are found in Amatya et al. (2010). The elevation of the CS water level was determined by adding the stage in feet measured for stage discharge relationship to the cave survey elevation for comparison with Lake Marion elevation, which was used as a proxy for CBC elevation. Lake Marion maximum elevation data (datum NGVD1927) was obtained from USGS gaging station #02169921 at Lake Marion near Elloree, SC (USGS, 2009).

### *Water Quality and Isotope Sampling and Analysis*

Water quality measurements were taken on a predominantly monthly basis during various flow conditions. Measurements for pH and specific conductance were taken in the field using Oakton pH/conductivity/°C 10 series hand held meters, which were calibrated with Oakton buffers (4.01, 7.00, 10.0, 84  $\mu$ S and 447  $\mu$ S) during every field visit. A LaMotte Alkalinity kit, Model DR-A was used in the field to titrate for alkalinity. Grab samples were collected using protocols and containers dependent on the desired analysis according to Standard Methods (Clesceri et al., 1998). Samples for dissolved calcium and magnesium concentrations were analyzed by the Santee Cooper Analytical and Biological Laboratory located in Moncks Corner, SC. A detailed description of Standard Operating Procedures is included in the TMDL Quality Assurance Project Plan and Final Draft Report (Amatya et al, 2010). Samples for Total (TSS) and Volatile (VSS) Suspended Solids were analyzed by project personnel at the USDA Forest Service Center for Forested Wetlands

Research using Standard Methods 2540 D and E (1988). Water chemistry data on TSS, VSS, pH, specific conductance, and alkalinity were statistically analyzed for their means and distributions and plotted using a program in MATLAB software (Mathworks, 2009). The Tukey's test for significant difference in multiple mean concentrations of each of the parameters between the locations was used at  $\alpha = 0.05$  level using a standard error bar representing 95% confidence intervals.

Samples collected for stable isotope ( $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ) analyses were filtered and stored away from light in glass vials with no head space. Analysis was performed with a Picarro L2120-i high-precision isotopic water analyzer, using Cavity Ring-Down Spectroscopy (CRDS), in the Department of Geography at Nipissing University, Ontario, Canada. Sample precision (maximum standard deviation of all injected samples) for  $\delta^{18}\text{O}$  and  $\delta\text{D}$  was  $\leq 0.12\text{‰}$  and  $\leq 0.26\text{‰}$ , respectively. Accuracy (average deviation from true value or 'Big Delta') for  $\delta^{18}\text{O}$  and  $\delta\text{D}$  was  $\leq 0.24\text{‰}$  and  $\leq 0.81\text{‰}$ , respectively. Accuracy was evaluated using secondary lab standards and IAEA standard VSMOW2. Samples were tested for chemical contamination with potential to affect CRDS results using Picarro's ChemCorrect™ software.

## RESULTS

### A. HYDROLOGICAL AND DYE TRACE DATA

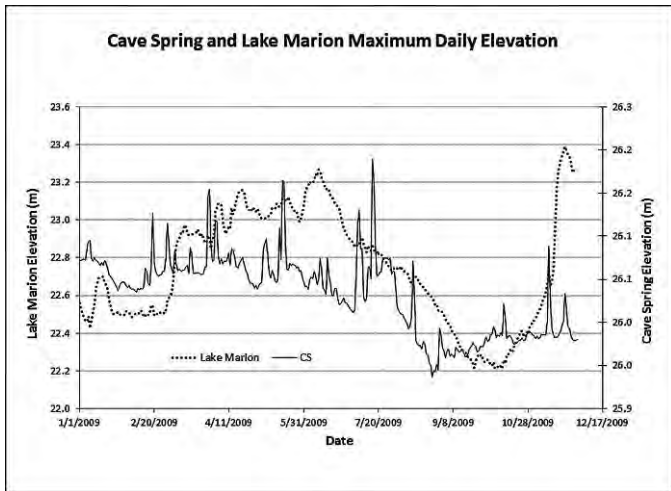
The impact of fluctuating lake levels on the limestone aquifer, as observed at CS, is shown in Figure 3. The daily maximum elevation of CS had an overall trend that matched the rise and fall of Lake Marion daily maximum elevation in 2009.

Two qualitative dye tracing events were conducted at SS during low and high flow conditions. Approximate arrival of dye at CS was observed using visual inspection only. The red dye injected at SS on August 28, 2008 was expected, but not observed, at CS, even though depth of water at the SS location was 0.19 m, with an average velocity of 0.73 m/s (2.4 ft/sec). It was hypothesized that the organics present in the surface stream at SS could be interfering with the red dye tracer. A second tracer was injected using green dye at SS on April 2, 2009, a wet antecedent condition. This time, the dye arrived at CS in 15 minutes. The depth and velocity at SS were not measured, but the depth was noticeably greater and flow volume much higher on April 2, 2009 than on the previous tracer date on August 28, 2008, a relatively dry period. Another tracer test conducted on August 28, 2008 resulted in the green dye taking 18 minutes to arrive from GW to CS, and dye at this location was not repeated on the April 2, 2009 trace.

### B. GEOCHEMICAL DATA

Tukey's multiple means testing for geochemical data (Fig. 4) showed significant ( $\alpha = 0.05$ ) differences in TSS





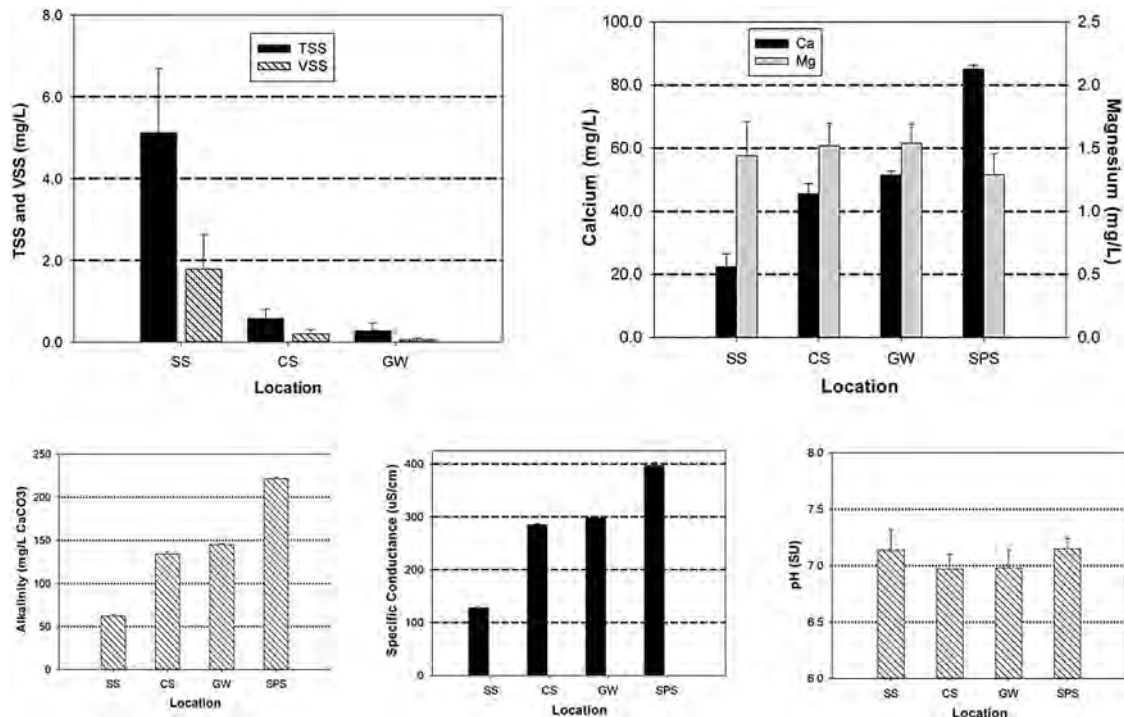
**Figure 3.** Daily maximum elevations of CS and Lake Marion (proxy for CBC) demonstrate a relationship of increasing and decreasing elevation in tandem (elevation scales on separate axes and vary by a factor of 4).

and VSS between locations SS, CS, and GW. The highest values were measured at SS, which varied based on surface water conditions and averaged 5.0 mg/L for TSS and 1.5 mg/L for VSS. Significantly ( $\alpha = 0.05$ ) lower values

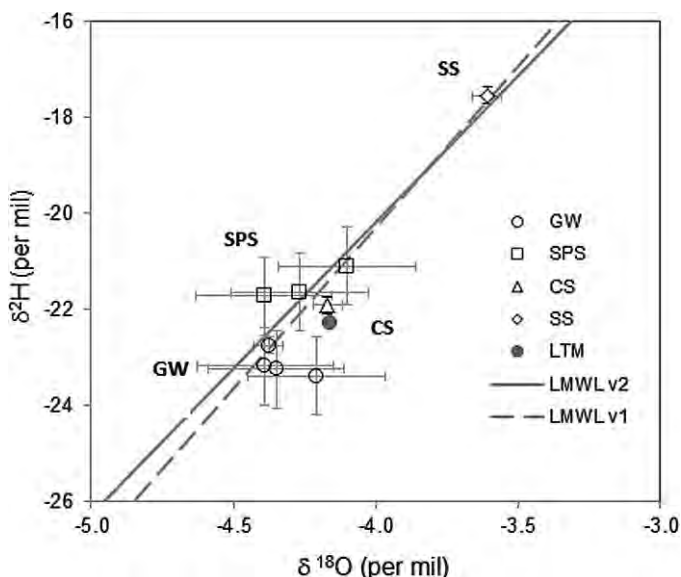
were measured at GW and CS. CS had an average TSS value of 0.5 mg/L and an average VSS value of 0.2 mg/L. GW had an average TSS value of 0.3 mg/L and an average VSS value of 0.1 mg/L. The high TSS and VSS values at GW measured on the day of a sinkhole collapse in the suspected upstream portion of the conduit were not included in the averages. The extremely high TSS and VSS values were 18.6 and 2.5 mg/L, respectively, a 6100% and 2400% increase in the average values.

Significant differences ( $\alpha = 0.05$ ) were also found between the sampling locations for dissolved calcium. The measurements at SPS were significantly higher than GW and CS, which, in turn, were significantly higher than SS. The average dissolved calcium value at SPS was 85.1 mg/L. The average value was 53.3 mg/L for GW, 46.1 mg/L for CS, and 23.7 mg/L for SS. The lowest measured value (15.6 mg/L) at SS was 83% lower than the highest measured value (91.7 mg/L) at SPS.

Significant differences ( $\alpha = 0.05$ ) for alkalinity were also observed between the sampling locations. The measurements at SPS were significantly higher than at GW and CS, which, in turn, were significantly higher than at the SS. The average alkalinity value at SPS was 221.3 ppm. The average value was 146.7 ppm for GW, 131.8 ppm for CS, and 61.2 ppm for SS. The lowest



**Figure 4.** Mean water quality data and 95% confidence interval shown by vertical bar for (a) total suspended solids (TSS) and volatile suspended solids (VSS) ( $n = 12$ , no samples from SPS), (b) dissolved calcium and magnesium ions ( $n = 15$  except at SPS where  $n = 5$ , and SS where  $n = 14$ ), (c) specific conductance ( $n = 24$  except SPS where  $n = 5$ ) for the designated locations (Fig. 1), (d) alkalinity, and (e) pH (both have  $n = 19$  except for SPS where  $n = 3$ ). SPS was sampled less often due to low flow volume at location, likely due to drought during sampling period. GW and SS flow combine together right before discharging at CS; SPS is separate from the Santee Cave system.



**Figure 5.** Stable isotope signatures of SS, CS, SPS, and GW from Mar-09 and Aug-09 sampling events. LMWL for the Cape Hatteras GNIP station (includes two versions of regression fit) and the long-term weighted mean precipitation signature (LTM) are also included.

measured value (46.0 ppm) at SS was 81% lower than the highest measured value (236.0 ppm) at SPS.

Significant differences ( $\alpha = 0.05$ ) were also found between the sampling locations for specific conductance. The measurements at SPS were significantly higher than GW and CS, which, in turn, were significantly higher than at SS. The average specific conductance value at SPS was 397.2  $\mu\text{S}$ . The average value was 292.8  $\mu\text{S}$  for GW, 274.7  $\mu\text{S}$  for CS, and 129.3  $\mu\text{S}$  for SS. The lowest measured value (104.7  $\mu\text{S}$ ) at SS was 76% lower than the highest measured value (445.0  $\mu\text{S}$ ) at SPS.

Dissolved magnesium and pH values show little variation between the four locations SS, GW, CS, and SPS, as indicated in difference of means testing in Figure 4. Values for dissolved magnesium were similar between sites and ranged from 1.2–1.8 mg/L with a mean concentration of 1.5 mg/L at all locations except SPS, which had a mean concentration of 1.3 mg/L (Figure 4). The pH values ranged from 6.6–7.6 SU with means of 7.1, 7.0, 6.9, and 7.2 at SS, CS, GW, and SPS, respectively (Fig. 4).

### C. STABLE ISOTOPE ANALYSES

Stable isotopes of water (Fig. 5) plot along the Cape Hatteras Local Meteoric Water Line (LMWL), used here to approximate the range of signatures for precipitation in the CBC watershed region; this data is available through the IAEA’s Global Network of Isotopes in Precipitation (GNIP) program and includes two different versions of line regression fits (IAEA/WMO, 2006). The long-term weighted mean isotopic signature of precipitation from the Cape Hatteras GNIP station is also included in this figure.

Spring waters (SPS), GW and CS plot significantly more negative (depleted of the heavier isotopes) and closer together than SS. They also plot closer to the long-term weighted mean precipitation signature, expected for groundwater recharged by contemporary precipitation. GW plots more negative than SPS, suggesting that SPS, with less updip aquifer area, may be slightly more influenced by surface recharge. This also appears consistent with earlier reference to Stone (pers. comm., 2009) where GW has been shown to be a combination of recharge and old water.

On 3-18-09, samples from all three locations (GW, CS and SS) were collected during baseflow conditions 2 days after a 25–30 mm rain event. Using a simple linear mixing model approach (e.g. Sklash and Farvolden, 1979), with contributing end-members of GW ( $\delta^{18}\text{O}$  of  $-4.38\text{‰}$ ) and SS ( $\delta^{18}\text{O}$  of  $-3.61\text{‰}$ ), diffuse groundwater contributions to CS ( $\delta^{18}\text{O}$  of  $-4.17\text{‰}$ ) are estimated to be 73% of discharge, with 23% derived from upstream surface water SS.

### DISCUSSION

Results from this study indicate that the flow at CS and the spring seeps was recharged predominantly from diffuse flow from the matrix, with a slow response of surface water infiltration to the conduit. The term “slow” is relative. In this study, “slow” implies that surface water did not respond with immediate (storm event) movement from the surface, into the aquifer matrix, and then discharged from spring seeps in the CBC watershed.

Springs dominated by diffuse flow with slow surface water recharge have fewer variations in flow than springs dominated by conduit flow. Data analysis from Williams et al. (2012) indicated that hydrograph peaks at CS were not from GW baseflow but mainly SS inputs from surface water runoff during rain events. Independent stable isotope analysis additionally suggested that discharge at CS is dominated by diffuse flow from the aquifer matrix (slow moving water), contributed heavily from the GW conduit. A linear mixing model using  $\delta^{18}\text{O}$  and assuming contributing end-members of GW and SS estimated diffuse groundwater contributions to CS to be 73% GW and 23% SS.

The TSS and VSS measurements also substantiated the claim of a dominant GW contribution from diffuse flow to CS. TSS and VSS values at GW during the study period were consistently small values and had a small standard deviation. This was likely due to sinkholes in the upbasin of the surface watershed routinely displaying no obvious direct openings to the karst below. The only anomalously large TSS and VSS values occurred after a sinkhole collapse in a suspected upstream portion of the conduit serving GW (Edwards and Amatya, 2010). The otherwise low TSS and VSS values indicated a typically slow arrival time from surface to groundwater inputs to GW. The TSS and VSS values at CS had only slightly larger average than

GW, but significantly smaller average than SS, indicating the dominant flow to CS during baseflow was clearly from GW and not SS. The average TSS and VSS values were significantly larger at SS than either GW or CS, and likely contributed to the slight increase of TSS and VSS values at CS.

Alkalinity measurements also substantiated this claim of a dominant GW contribution from diffuse flow to CS. The alkalinity measurement at GW during the April 2, 2009 storm event was 148.0 ppm, very close to the 146.7 ppm (5.0 ppm standard deviation) average measurement at that location. During the same storm event, the alkalinity measurement at CS was 70 ppm, almost half as much as the average measurement of 131.8 ppm (20.4 ppm standard deviation) at that location. The minimal change in average value, with a small standard deviation, at GW during a storm event indicated diffuse flow. The flow at CS showed a significant decrease in alkalinity. The CS flow was likely diluted with the surface waters of significantly lower alkalinity from SS (average alkalinity of 61.2 ppm with a 6.4 standard deviation).

The alkalinity, dissolved calcium and specific conductance measurements from the four sampling locations SS, CS, GW, and SPS all represented varying degrees of diffuse flow and surface-groundwater connections in the Santee Limestone matrix in the CBC watershed. The sampling location SPS, with the highest of these measurements, represented the discharge with the longest residence time in the aquifer; and therefore, longest contact with the bedrock and slowest flow. The seeps were not hydrologically connected to the Santee Cave system. More likely, these seeps were impacted by the fluctuating CBC and Lake Marion water elevation, as depicted in Figure 3 (discussed below). The conduit serving Santee Cave at GW and CS had significantly lower alkalinity, specific conductance, and dissolved calcium than SPS, indicating shorter residence time of water in the aquifer; and therefore, faster through or conduit flow than SPS. GW had similar, but slightly larger, alkalinity concentrations than CS due to the contribution to CS from not only the predominant CS baseflow component of GW, but also SS baseflow and stormflow components to CS. The lowest values, as expected, were measured at the surface waters of a disappearing stream at SS. The difference in the chemistry in spring seeps and GW could be due to several factors. The noticeably larger conduit size at GW could move water faster through the aquifer to reduce contact time with the limestone. The groundwater conduit feeding GW also had a larger surface watershed recharge area with sinkholes that may allow for quicker infiltration of water into the matrix and to the conduit. Once in the matrix, the aquifer recharge could flow diffusely over a larger area for GW than SPS, until it reached the GW conduit. The seeps were also higher in elevation within the Santee Limestone unit, giving them potentially less volume of the aquifer matrix above to yield a discharge.

The stage elevation at CS and adjacent Lake Marion (equal to the elevation of the flooded portion of CBC) also indicated a relation between fluctuating base level of CBC and discharge of the Santee Limestone karst aquifer at CS. If we ignore the peaks in Figure 3, which were surface water contributed from SL7, the baseflow stage elevation at the spring broadly increased and decreased with the changing lake levels. If the surface-groundwater connections in GW were quick, one would not expect the broad several month long correspondences, but rather would expect rapid changes into the conduit that serves GW and CS. The correspondence suggested the GW source reflected a rainfall response at a rate more similar to the large reservoir. Geochemical data also indicated a slow surface-groundwater connection typical for GW and CS (minus the stormflow contributions from SS), and the hydrologic data in Figure 3 were collected during drought years, as indicated from measured precipitation compared to long-term precipitation records (Williams et al., 2012).

These results suggested that during low lake levels, such as at the time of first dye trace, the water table in the limestone might be low and the surface water going into SS infiltrates downward into the empty conduits. The water may then either be stored until the water table increases or perhaps continues draining to CBC through the aquifer at a lower elevation than the cave stream passage, which was hypothesized earlier by Amatya et al. (2011). For the first dye trace, when no dye was observed from SS to CS, the average lake level on August 28, 2008 was 21.79 meters (71.49 feet) (USGS, 2009). The average lake level on April 2, 2009, when the dye took only 15 minutes to reach CS, was 22.96 m (75.34 feet) (USGS, 2009). Thus, a 1.17 m (3.85 foot) fluctuation in lake level showed an impact on the water table in the limestone aquifer and cave stream. As lake levels decrease, the aquifer may empty at lower elevation springs and more surface water in the basin is lost to groundwater, as was also argued by Amatya et al. (2011).

The trace conducted on August 28, 2008 resulted in the green dye taking 18 minutes to arrive from GW to CS. The conduit from GW to CS can be mostly traversed and there was no indication of loss to a lower conduit. In fact, conduit baseflow discharge at GW and CS was witnessed on weekly field visits during 2009 as always continuous and steady. Because 2007–08 were drought years, the Santee Limestone in this region appeared to have significant storage capacity, characteristic of diffuse flow, to maintain steady flow at the spring after a two-year drought. Even though GW may result from flow in multiple conduits from an unknown upstream area, the recharge was likely more diffuse than direct recharge from surface water and precipitation. A conceptual model for groundwater flow in the CBC watershed is shown in Figure 6.

The lack of significant difference between the locations for pH parameter indicated a limited value for measuring these individual parameters to identify flow type and



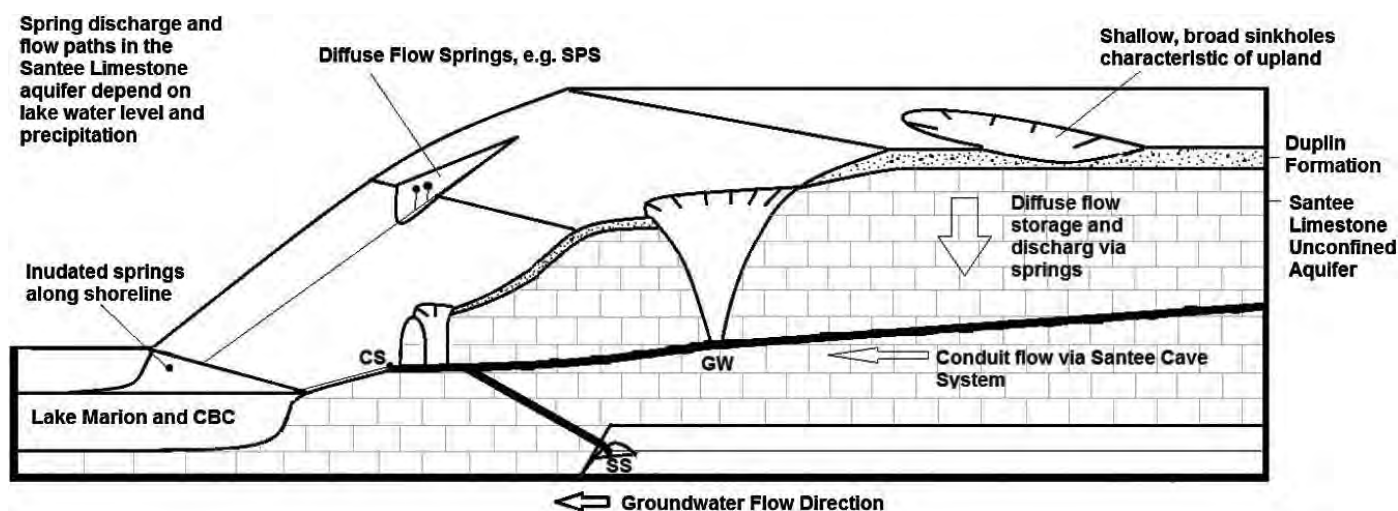


Figure 6. Conceptual model of groundwater flow (diffuse and conduit) between Santee Limestone karst aquifer and Chapel Branch Creek discharging to Lake Marion.

surface-groundwater connections in this particular watershed. However, the comparison between variable dissolved calcium and non-variable dissolved magnesium between locations provided insight into karst subsurface flow dynamics given the contact time with limestone.

#### CONCLUSIONS

The analysis of hydrologic, geochemical and stable isotopic data collected within the Chapel Branch Creek watershed in 2009 confirmed the first hypothesis that the discharge via CS from the Santee Limestone to CBC is predominantly recharged by a diffuse flow component from the matrix with slow surface-groundwater connection. The Santee Limestone in the upper Floridan aquifer, therefore, behaves like the karst in the lower Floridan aquifer, with the matrix (diffuse flow) contributing heavily to aquifer discharge via conduits to springs. Analysis and interpretation of these data also confirmed the second hypothesis that the fluctuating CBC stage elevations influence the aquifer water table at Santee Cave. Studies in the Floridan aquifer have likewise found hydrologic connections between the surface water in lakes and groundwater levels (Lee, 2000; Watson et al., 2001; Lin, 2011).

Characterizing the hydrology of the Chapel Branch Creek watershed is essential for water resource protection. This reservoir-like embayment has been on the EPA 303(d) list for impaired water bodies for several years, and both point and non-point sources of water pollution are abundant in this multiple land-use watershed. Further studies to elucidate surface-groundwater connections and water quality at the springs along CBC would help with future Best Management Practices for Chapel Branch Creek and the greater karst region.

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# NEW SPECIES AND NEW RECORDS OF SPRINGTAILS (HEXAPODA: COLLEMBOLA) FROM CAVES IN THE SALEM PLATEAU OF ILLINOIS, USA

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**Abstract:** The springtail (Hexapoda: Collembola) fauna of eight caves (Wizard Cave, Pautler Cave, Spider Cave, Wanda's Waterfall Cave, Illinois Caverns, Stemler Cave, Hidden Hand Cave, and Bat Sump Cave) in the Salem Plateau of southwestern Illinois (Monroe and St. Clair counties) was surveyed in 2009 using a combination of methods, including pitfall traps, Berlese-funnel processing of litter, and hand collections by quadrat, on drip pools, free standing bait, and random locations. In total, forty-nine species of springtails were found. Four are described as new to science (*Onychiurus pipistrellae* n. sp., *Pygmarrhopalites fransjanssens* n. sp., *P. incantator* n. sp., and *P. salemensis* n. sp.), four may represent new species but there is insufficient material available to prepare full descriptions (two species in the genus *Superodontella*, one in *Pseudachorutes*, one in *Sminthurides*), and three others (*Ceratophysella* cf. *brevis*, *C.* cf. *lucifuga*, and *Folsomia* cf. *bisetosa*) are identified to species, but differences from the nominal species suggest further studies may indicate the Illinois populations represent distinct forms. In addition, five other species represent new records for Illinois, and eighteen are new cave records for the species in North America. The new records more than double the number of springtails species known from caves in the Salem Plateau region. More than half (twenty-nine) of the species reported are ranked as rare (S1–S2) at the state level. The total number of springtail species in Salem Plateau caves could be more than twice what is recorded in the present study, and more new species and state records should be found when caves in other Illinois karst regions are more thoroughly examined.

## INTRODUCTION

Illinois's karst is distributed across five regions (Fig. 1) that contain numerous sinkholes, springs, and shallow groundwater conduits. Four of these regions—Driftless Area, Lincoln Hills, Salem Plateau, and Shawnee Hills—contain caves accessible to humans. In addition to their hydrological, recreational, geological, and cultural values, these caves contain fascinating assemblages of life. The fauna most familiar to the public are bats and salamanders, but caves also contain a wide variety of invertebrates. Among these are the Illinois cave amphipod, *Gammarus acherondytes* Hubricht and Mackin, which is federally listed as endangered, the enigmatic cavesnail, *Fontigens antrochetes* (Hubricht), a state-listed species, and a single-site endemic Illinois cave beetle *Pseudanophthalmus illinoisensis* Barr and Peck. One cave springtail, *Pygmarrhopalites madonnensis* (Zeppelini and Christiansen), is listed as state-endangered in Illinois. Numerous other invertebrate species occur in Illinois caves, including a variety of other springtails (Collembola).

Springtails are small hexapods characterized by the presence of four-segment antennae, a six-segment abdomen, a large vesicle (the ventral tube) on the ventral part of the first abdominal segment, and, in many species, a

jumping-organ complex formed by the tail-like furcula and the furcula catch or retinaculum. Springtails are most commonly found in soil and leaf litter, but they have invaded other specialized habitats, including caves. Many soil or leaf-litter species are commonly found in caves as xenobionts, but some species are cave-adapted or cave-limited and do not sustain surface populations (Christiansen and Culver, 1987).

In Illinois, the most common families of Collembola reported from caves are Hypogastruridae, Onychiuridae, Oncopoduridae, Tomoceridae, Isotomidae, Entomobryidae, and Sminthuridae *sensu lato*. Of the forty-three species of springtails previously recorded from Illinois caves, slightly more than 25% (eleven species) are either troglionts (obligatorily permanent residents of subterranean habitats) or eutroglophiles (facultatively permanent residents of subterranean habitats). The genera of eutroglophiles or troglionts reported from Illinois prior to our study are *Typhlogastrura*, *Lethemurus*, *Oncopodura*, *Pseudosinella*, *Sinella*, *Pygmarrhopalites*, and *Arrhopalites*. *Pygmarrhopalites sapo* (Zeppelini and Christiansen) and *Pygmarrhopalites madonnensis* (Zeppelini and Christiansen) are the only species of cave springtails currently known to be endemic to Illinois. The number of endemics is probably higher than current inventories would suggest,





Figure 1. Illinois karst areas, adapted from Weibel and Panno (1997) and Panno et al. (1997).

as most groups have not been studied in detail. For example, the genera *Typhlogastrura* and *Pseudosinella* have diversified extensively in caves elsewhere in North America and Europe, and although both are reported from Illinois caves, no species from either genus has been identified for the state. In addition, some eutroglophiles and troglobionts first thought to be widely distributed across karst regions are now known to represent complexes of species endemic to just a few caves. The most striking example of this is the *Onychiurus relictus* species complex, in which five species were recently identified (Pomorski et al., 2009).

This report presents findings from an inventory of springtails collected in eight caves in Illinois's Salem Plateau.

## METHODS

### FIELD SAMPLING

At each cave, the dominant habitat types in which springtails might occur were sampled. Collections were made using Limburger cheese-baited pitfall traps partially filled with 95% ethanol, Berlese-funnel processing of litter samples, and hand sampling using an aspirator either in a



Figure 2. Distribution of eight Illinois caves where springtails were collected during 2009. Gray shading indicates approximate boundaries of karst based on sinkhole mapping (Illinois State Geological Survey). Cave locations are approximate.

structured fashion (timed search of drip pools and quadrat searches on cave floors and walls) or by general inspection around haphazardly placed cheese smears and other potential sources of energy such as animal scats and rotting logs. All specimens were preserved in 95% ethanol.

Eight caves were selected for study in the Salem Plateau (Fig. 2). All necessary permits required to conduct the research were obtained prior to the beginning of fieldwork. The collection dates given below include the period during which pitfall traps were exposed in the field. Litter samples to be processed in Berlese funnels in the laboratory were usually collected during the second visit to a cave. Collection information for each cave is as follows:

USA: IL: St. Clair Co.: Wizard Cave, Dupo, near Falling Spring, 15–17 June 2009, SJ Taylor, FN Soto-Adames, and CA Phillips.

USA: IL: Monroe Co.: Pautler Cave, 3.0 mi WSW of Waterloo, 14–16 September 2009, SJ Taylor, and FN Soto-Adames.

USA: IL: Monroe Co: Wanda's Waterfall Cave, 7.4 mi SE of Valmeyer, 15–17 September 2009, SJ Taylor and FN Soto-Adames.

USA: IL: Monroe Co: Spider Cave, 6.5 mi S of Waterloo, 15–17 September 2009, SJ Taylor and FN Soto-Adames.

USA: IL: Monroe Co: Illinois Caverns, 24–26 September 2009, SJ Taylor, FN Soto-Adames, A Kuhns, E Zaborski, J Jacoby, A Paprocki, and M Pessino.

USA: IL: St. Clair Co: Stemler Cave, 2.7 mi NE of Columbia IL, 28–30 September 2009, SJ Taylor and FN Soto-Adames.

USA: IL: Monroe Co: Hidden Hand Cave, 3 mi W of Waterloo, 14–16 October 2009, SJ Taylor and FN Soto-Adames.

USA: IL: Monroe Co: Bat Sump Cave, 6.6 mi WSW of Red Bud, 3–5 November 2009, SJ Taylor and FN Soto-Adames.

#### NOTES TO SPECIES DESCRIPTIONS, ECOLOGICAL CLASSIFICATION AND DISTRIBUTIONAL RANKING

The nomenclature of the chaetotaxy in *Ceratophysella* and *Superodontella* follows Fjellberg (1985) and Jordana et al. (1997), respectively. The chaetotaxy of *Pseudosinella* follows Szeptycki (1979) and Soto-Adames (2010).

Nomenclature of the chaetotaxy of the head and small abdomen in *Pygmarrhopalites* follows Christiansen and Bellinger (1998). The identity of head vertical setae M4 and M5 appears confusing in the literature. We consider M5 to form a row with L2 and IL3 (row D in Betsch and Waller, 1994), whereas M4 forms a row with L1 and IL2 (row C in Betsch and Waller, 1994). None of the species of *Pygmarrhopalites* reported here carries seta M5, but the clarification is noted because *P. hirsuta* (Christiansen) has been described as having M5 and lacking M4, but an individual identified as representing this species (Zeppelini et al., 2009) examined by us carries M4 (by our convention) instead of M5. The nomenclature of distal setae in rows D and E on the lateral valve of the small abdomen is confusing. We have identified as E7 the seta labeled E8 in Christiansen and Bellinger (1998) because in all individuals examined by us the socket of this seta aligns with D7 (when present) and C7 instead of D8 and C8 (Figs. 15C, F). Seta D9 is always longer than setae D7–D10 and can be used as a reference point when some setae in the series are absent.

Abbreviations used throughout the descriptions are Ant., PAO, Th., and Abd. for antennal segment, post antennal organ, thorax, and abdomen, respectively.

Types of the new species described here and vouchers for all previously named species are deposited in the Illinois Natural History Survey Insect Collection.

We assigned each species to an ecological classification following Culver and Pippan (2009), and Sket (2008), instead of the system utilized by Barr (1963, 1968), which is more familiar to North American speleologists. The ecological categories considered are: troglobiont (TB – obligate and permanent residents of subterranean habitats), eutroglophile (EU – facultatively permanent residents of subterranean habitats also found in other habitats), subtroglophile (SU – obligate or facultative residents of subterranean habitats that utilize other habitats for some portion of life cycle) and troglonexene (TX – sporadic

residents of subterranean habitats [“accidental” of Barr 1963, 1968]).

Rankings of the species below provide a measure of the extent of a species distribution at the state (S) and global (G) level. Rankings are circumscribed following Nature-Serve conservation status ranks (Master et al., 2009): For state and global imperilment, species reported in five or fewer localities are ranked S/G1; six to twenty localities, S/G2; twenty-one to one hundred localities, S/G3; uncommon but not rare, with some cause for long-term concern due to declines or other factors, S/G4; and widespread and common, S/G5. Species introduced from other biogeographic regions are indicated with IN. These rankings are based on Illinois and North American records obtained from either the Collembola of North America (Christiansen and Bellinger, 1998) or the database of North American Collembola records (Christiansen, 2012). Additional sources of distributional information are listed under individual species accounts.

#### SPECIES ACCOUNTS

##### HYPOGASTRURIDAE

*Hypogastrura pannosa* (Macnamara), 1922 — TX S1/G5; New Illinois Record

Localities: Wanda’s Waterfall Cave, Stemler Cave

*Hypogastrura pannosa* is part of a species complex that includes *H. essa*, *H. matura*, and the Palearctic *H. assimilis*. *Hypogastrura pannosa* may be a junior synonym of *H. assimilis* (Babenko et al., 1994; Thibaud et al., 2004), but we follow previous workers (Fjellberg, 1985; Christiansen and Bellinger, 1998) in assigning the epithet *pannosa* to North American populations having a PAO with four papillate arms, mucronal lamella ending abruptly subapically, and Abd. 4 with sensillum-like seta p5.

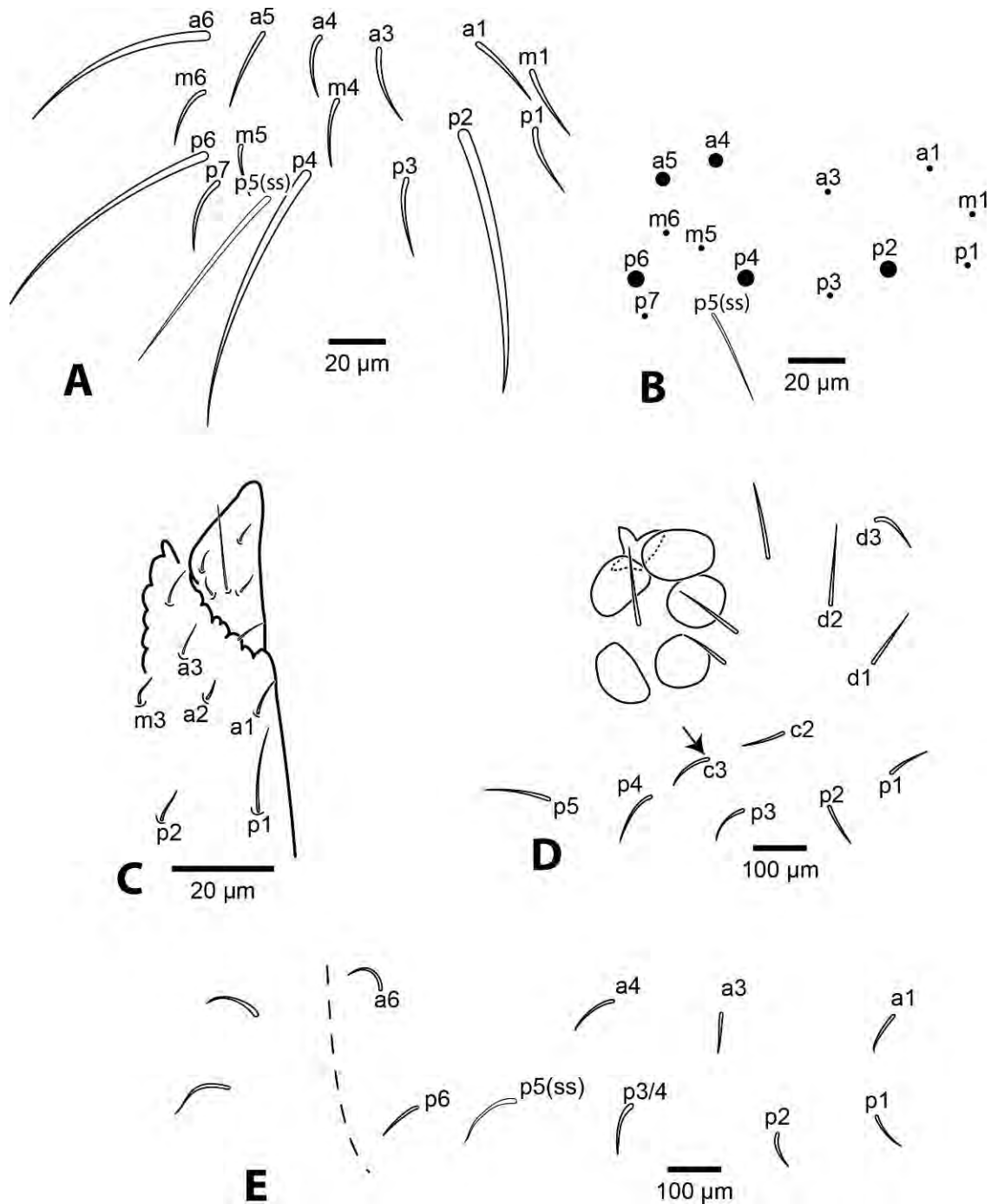
This species is widespread in North America, but this is the first report from Illinois. It is possible that previous Illinois reports of either *H. matura* or, more likely, *H. essa* actually refer to *H. pannosa*. There is one previous report from Kentucky caves.

*Ceratophysella boletivora* (Packard), 1873 — TX S2/G5

Localities: Wanda’s Waterfall Cave, Illinois Caverns, Bat Sump Cave

As circumscribed by Christiansen and Bellinger (1980), based on the lectotype, this species is characterized by having Abd. 4 seta p2 clearly longer than p1 and with 7–9 (depending on the position of a5) microsetae internal to a line drawn across a6–p4 (Fig. 3A), tenent hairs capitate, Yoshii “a” measure 16–18, Ant. 4 apical bulb bilobed, and distal inner dental setae only weakly enlarged basally. The species is easy to identify in alcohol by having rust-red head and antennae, and, usually, patternless dark blue to black body.

*Ceratophysella boletivora* was previously reported from non-cave sites in Cisco, Markham, and Cook counties in



**Figure 3.** Dorsal chaetotaxy of fourth abdominal segment: (A) *Ceratophysella boletivora*; (B) *Ceratophysella* cf. *brevis*, filled-in circles represent macrosetae, dots represent regular acuminate setae. *Superodontella* sp. 1: (C) labium and postlabium; (D) dorsal chaetotaxy of left side of head, arrow points to seta absent in *S. substriata*; (E) dorsal chaetotaxy of first abdominal segment.

Illinois. This species has been reported from caves in Missouri, but this is the first record for the species in Illinois caves.

*Ceratophysella* cf. *brevis* Christiansen and Bellinger, 1980  
— EU S1/G5; New Illinois Record

Localities: Wanda's Waterfall Cave, Bat Sump Cave

We identify as *C.* cf. *brevis* all individuals showing characters of the *C. denticulata* species complex as defined by Christiansen and Bellinger (1980), but with anterior lobes of PAO less than 1.5× the length of posterior lobes and inner distal dental setae not sharply bent or basally enlarged. Our specimens are light blue dorsally and white ventrally instead of dark blue to black as pointed out by



Christiansen and Bellinger (1980) and Fjellberg (1985) for Alaskan populations. We examined eleven specimens, six from Wanda's Waterfall and five from Bat Sump, and all have acuminate serrate macrosetae, seta m6 present on Th. 2–3, and lack seta m3 on Abd. 1–4 (Fig. 3B). Most individuals also lack seta m4 on Abd. 1–3, although four specimens (two from each cave) carry this seta on at least one of the abdominal segments.

Fjellberg (1985) reported two forms of *C. brevis* from Alaska: one form should be easy to identify as *C. brevis* by the absence of coarsely serrated setae and absence of setae m6 on Th. 2–3, and m3–4 on Abd. 1. The second form has coarse body setae, and all the thoracic and abdominal setae mentioned above are present. This second form would differ from *C. engadinensis* only in the proportion of anterior to posterior PAO lobes and in not having sharply bent setae on the dens. Our specimens do not match either of Fjellberg's forms or the original description of the species in details of the dorsal chaetotaxy of the body and may represent a different species altogether. However, the significance of the morphological variation observed among populations included in the *C. denticulata* species complex remains unclear, and some other source of information may be required to sort discrete units within the group.

This report represents the first record of *Ceratophysella* cf. *brevis* from Illinois. *C. brevis* was originally described from surface habitats in Wyoming, but now it is also known from Alaska (Fjellberg, 1985) and from caves in Kentucky, California, and Indiana. In addition to the North American records, the species has been reported from Siberia (Babenko et al., 1994).

*Ceratophysella denticulata* (Bagnall), 1941 — EU S5/G5

Localities: Illinois Caverns, Stemler Cave

The individuals sampled in Illinois Caverns have a well-developed antennal file and coarsely serrate, capitate macrosetae. *Ceratophysella denticulata* is a common species, widely distributed throughout the Northern Hemisphere (Christiansen and Bellinger, 1998; Thibaud et al., 2004). In Illinois *C. denticulata* has been previously identified from at least seven caves in Calhoun, Johnson, Union, and Monroe counties.

*Ceratophysella engadinensis* Gisin, 1949 — EU/TX S5/G5; New Illinois Record

Locality: Illinois Caverns

This is another species widely distributed throughout the temperate zone of the Northern Hemisphere. *Ceratophysella engadinensis* differs from *C. denticulata* only in the absence of seta a' on Abd. 5 (Christiansen and Bellinger, 1998; Fjellberg, 1998). Although the species has not been previously reported from Illinois, it is likely that in the past it has been confused with or included as part of *C. denticulata*; the individuals collected in Illinois Caverns were found mixed-in among many specimens of *C. denticulata*. However, we recognize both species based on

recent hybridization studies that suggest the two forms are reproductively isolated by postembryonic isolation mechanisms (Skarżyński, 2005).

*Ceratophysella* cf. *lucifuga* (Packard), 1888 — TB S1/G1; New Illinois Record

Locality: Wanda's Waterfall Cave

The species lacks pigment, but retains all eyes, and there is little change in the relative length of antennae and claw, or in the development of sensory organs.

One subadult male (1.2 mm long) and two juveniles (0.89 and 0.69 mm) were examined, and they differ from *C. lucifuga*, as redescribed by Skarżyński (2007), in not having pigment on the eye patch (individuals appeared to be blind under the dissecting microscope), in having an undivided apical lobe on Ant. 4 (trilobed in *C. lucifuga*), in having 4–6 differentiated sensilla on the ventral file of Ant. 4 (15 in *C. lucifuga*), and in the presence in the Illinois specimens of a supplementary seta between p5 and p6 on Abd. 4. The male does not have inner unguis teeth, but the two juveniles have a clear inner unguis tooth on all legs. Seta m4 is absent on Abd. 1–3 and present on Abd. 4 of the subadult; m4 is present on all abdominal segments in the juveniles.

Up to the present report, *C. lucifuga* was known only from three caves in Crawford and Harrison counties, Indiana. The physical distance (≈330 km) and morphological differences between the Illinois and Indiana populations suggest they may represent distinct species, but additional material from Illinois will be needed to determine if the morphological differences noted here are fixed.

*Xenylla welchi* Folsom, 1916 — TX S5/G5

Locality: Stemler Cave

This cosmopolitan species occurs throughout North America and Eurasia in surface leaf litter (Thibaud et al., 2004).

#### ODONTELLIDAE

*Superodontella cornifer* (Mills), 1934 — TX S1/G5

Locality: Bat Sump Cave

This species is widely distributed throughout North America and was previously reported from Hardin County, Illinois. However, this is the first record of the species from an Illinois cave. This individual is a small juvenile with only 4 dental setae.

*Superodontella substriata* (Wray), 1953 — TX S2/G2

Localities: Wanda's Waterfall Cave, Illinois Caverns

This species is characterized by having anal spines, body tubercles on head to Abd. 5 circular to somewhat polygonal in cross section, but forming ridges and valleys on Abd. 6; Ant. 4 with 7–8 blunt sensilla; and 2 clavate tenent hairs on each leg. In addition, the individuals from Illinois have dorsal head setae c3 present and c2 absent (arrow in Fig. 3D); 3 labial and 7 postlabial setae (Fig. 5A); chaetotaxy of Th. 2–3 as in *S. shasta* but with

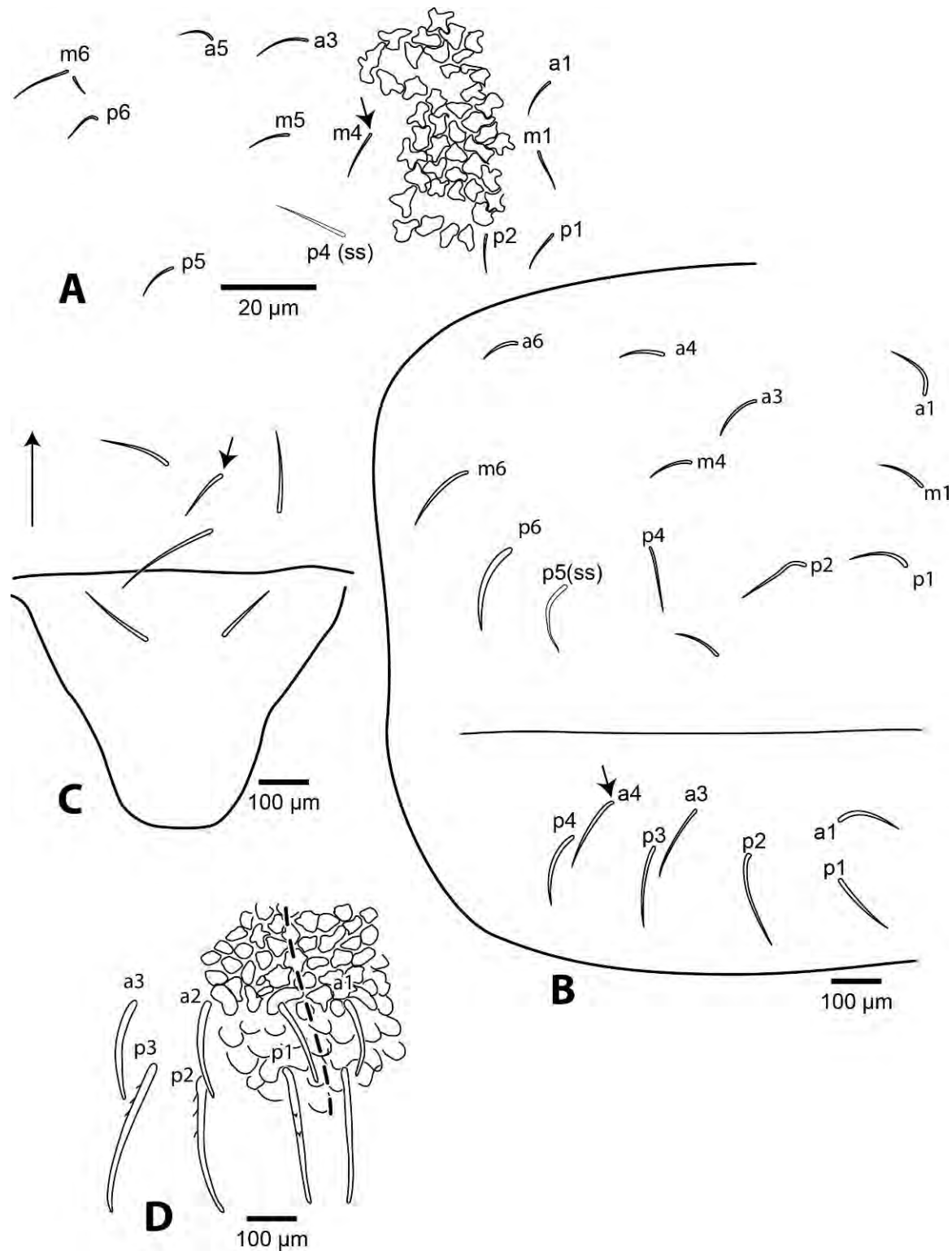
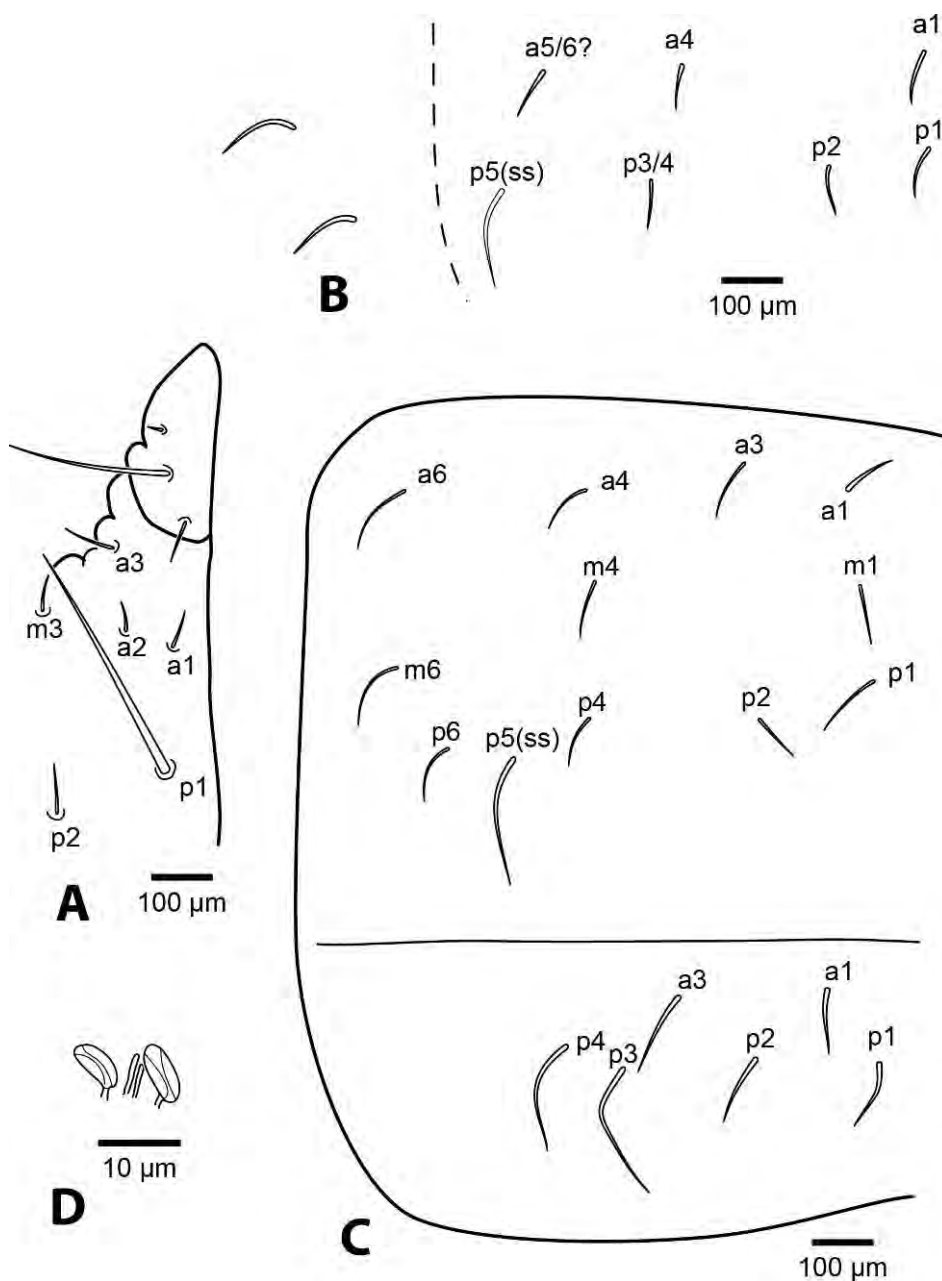


Figure 4. Chaetotaxy of *Superodontella* sp. 1, short arrows point at setae absent in *S. substriata*: (A) mesothorax; (B) abdomen 4-5; (C) sterna of abdomen 3-4, long arrow points anteriorly; (D) cuticle sculpturing and structure of setae on abdomen 5.



**Figure 5.** Chaetotaxy of *Superodontella substriata*: (A) labium and postlabium; (B) abdomen 1; (C) abdomen 4–5. *Onychiurus pipistrellae* n. sp.: (D) sensilla of third antennal segment sense organ.

seta m4 absent (arrow in Fig. 4A); Abd. 1–3 chaetotaxy reduced to 3 anterior and 4 posterior setae (Fig. 5B); Abd. 4 (Fig. 5C) with p5 clearly longer than p6; Abd. 5 with 2 anterior setae (Fig. 5C); sterna of Abd. 3–4 with 3 anterior and 2 posterior setae (arrow in Fig. 4C points at seta absent).

This is a forest leaf litter species originally described from Illinois, and it has been reported from Champaign, Clark, Jackson, Logan, and Pope counties. Additional records for the species include North Carolina, Connecticut, Quebec (Therrien et al., 1999), and British Columbia. This is the first record of this species from caves in Illinois.

*Superodontella* sp. 1 — TX? S1?/G1?  
Locality: Wanda’s Waterfall Cave

The single individual collected is similar to *S. shasta* in lacking anal spines, in having body tubercles angulate or polygonal in cross section dorsally on head and body, although on the posterior part of Abd. 5 the tubercles become rounded or circular (Fig. 4D), in having relatively short unguis, and in the presence of 6 blunt sensilla on Ant. 4. However, it differs from *S. shasta* in having a relatively blunt mouth cone (Fig. 3C), in having 1, 2, 2 clavate tenent hairs on the pro-, meso-, and metathoracic legs, respectively, and in that the longest posterior setae on Abd. 4



are apically expanded to weakly blunt and serrate. Other potentially informative characters observed in the individual from Illinois, but unknown in *S. shasta* are 6 labial and 7 postlabial setae (Fig. 3C); dorsal head setae c2 and c3 present (Fig. 3D); Th. 2–3 setae a2 and a6 absent, and m5 present (Fig. 4A); Abd. 1–3 with 4 anterior and 5 posterior setae (Fig. 3E); Abd. 4 with seta a3 displaced posteriorly, and p6 as long as p5, but clearly thicker (Fig. 4B); Abd. 5 with 3 anterior setae (Fig. 4B); and sterna of Abd. 2–4 with 3, 4 and 2 setae, respectively (Fig. 4C, only Abd. 3–4 shown).

*Superodontella* sp. 2— TX? S1/G1?

Locality: Wanda's Waterfall Cave

The single individual collected in Wanda's Waterfall Cave is a juvenile and appears to represent a new species characterized by a very short furcula with only two dental setae, PAO with three arms, tenent hairs on all legs acuminate, anal spines well developed, body tubercles round or oval in cross section, and Ant. 4 with 7 well-differentiated blunt sensilla and dorsal head row c only with seta c3 present. The species is dark blue and does not show characters typically present in cave-adapted species.

#### NEANURIDAE

*Pseudachorutes aureofasciatus* (Harvey), 1898 — TX S5/G5

Locality: Stemler Cave

This widespread species is found in forest leaf litter from British Columbia and California to Pennsylvania and Florida. In Illinois the species has been reported from Calhoun, Johnson, and Union (Guthrie Cave) counties. *Pseudachorutes aureofasciatus* is also known from Hunter's Cave, Jackson County, Iowa.

*Pseudachorutes* sp. — TX? S1/G1?

Locality: Wanda's Waterfall Cave

The single individual collected in Wanda's Waterfall Cave appears to represent an undescribed species. This is a black species, without morphological adaptations to subterranean life. This is likely a surface leaf litter form accidentally found inside the cave. After this individual was identified as a potential new species, several collecting trips were made to Wanda's Waterfall, but no additional specimens were found.

*Vitronura giselae* Gisin, 1950 — TX IN?

Locality: Wanda's Waterfall Cave

This is probably an introduced species now common in moist woodchip beds in central Illinois. *Vitronura giselae* was first reported from North America by Christiansen and Bellinger (1998), although the species was known to be established on the continent before that (Richard Snider, Michigan State University, personal communication). In Illinois the species was previously observed in Champaign County by the senior author. This is the second record of *V. giselae* from North American caves; the species was already reported from Swamp River Cave, Tennessee.

Although this species has a reduced number of eyes and is white when preserved in alcohol, it is not a sub- or eutroglophile. Living specimens are bright orange and the reduced number of eyes is characteristic of the lineage.

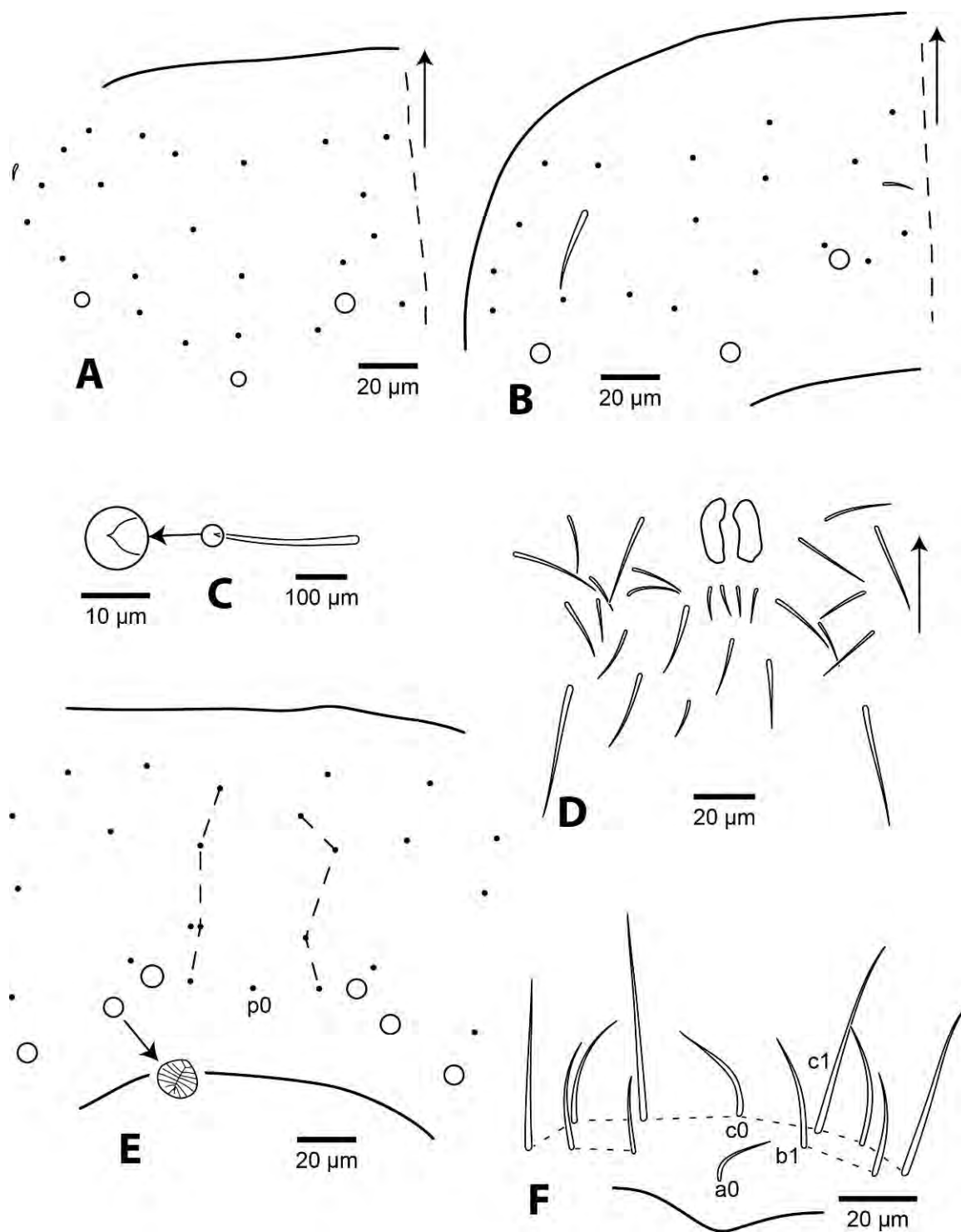
#### ONYCHIURIDAE

*Onychiurus pipistrellae* n. sp. — TB? S1?/G1?

Material Examined: Holotype, INHS Collection Number: 551,651; USA, IL, Monroe Co, Bat Sump Cave, 6.6 mi WSW of Red Bud, hand collected on cave floor, swarming around cheese bait, 3–5 November 2009, SJ Taylor and FN Soto-Adames; Female, slide mounted. The slide with the holotype also includes one individual of *Heteraphorura subtenua* (Folsom) and one individual of *Folsomia stella* Christiansen and Tucker. Paratypes, INHS Collection Number 551,652–56; 9 males and females on slides and 69 adults and juveniles of both sexes in alcohol with same collection information as holotype. Additional paratypes, INHS Collection Number 551,657–60; USA, IL, St. Clair Co, Stemler Cave, 2.7 mi NE of Columbia IL, hand collected on cave floor and drip pool, 28–30 September 2009, SJ Taylor and FN Soto-Adames; 3 mounted on slides and 5 in alcohol.

Etymology: The epithet refers to the type locality, Bat Sump Cave.

Description: Largest male and female 1.7 mm and 2.2 mm long, respectively. Subapical sensilla of Ant. 4 smooth, rounded and resting in a shallow pit; subapical invagination as is *O. reluctus*; basal sensilla longer than subapical. Ant. 3 sense organ with five guard setae, five papilla, two rods, and two smooth sensilla distally expanded and bent, with middle groove (Fig. 5D); basal sensilla shorter than basal sensilla on Ant. 4. Postantennal organ with 12–15 compound vesicles. Apical seta of outer maxillary lobe unilaterally coarsely serrate on apical third, sublobular plate with two appendages. Labial palp with 6 proximal setae; thickened-blunt terminal seta present only on papilla A and B; papilla E with 3 guard setae. Dorsal pseudocelli 32/033/33353; ventral 2/000/1112; subcoxae 1–3 with 222 pseudocelli. Ventral parapseudocelli 00/000/11100? (view of the dorsal anal valve is obstructed in all individuals examined and the presence or absence of a parapseudocellus cannot be established). Ventral thoracic setae absent. Prothorax with eight setae. Dorsal chaetotaxy of Th. 2 and Abd. 1 as in Figures 6A and 6B; tip of macro- and mesosetae acuminate and wide (Fig. 6C); medial seta absent on Abd. 1 (Fig. 6B) and present on Abd. 4 (Fig. 6E); Abd. VI seta a0 absent and p0 present, spines strongly conical. Subcoxae 1–3 with 555 setae. Ventral tube with 6–7+6–7 setae. Furcula scars with 4 short posterior setae in a single row (Fig. 6D), homology of other furcula-associated setae difficult to assess, as their arrangement varies asymmetrically within individuals. Dorsal anal valve with setae a0 and c0 present, seta b0 absent (Fig. 6F); lateral anal valves with 4 hr, 6 inner, 4 medial and 5 outer setae (Fig. 7A). Pro- and metathoracic



**Figure 6.** *Onychiurus pipistrellae* n. sp., dots represent setae and circles pseudocelli, long arrows point anteriorly: (A) dorsal chaetotaxy of mesothorax; (B) dorsal chaetotaxy of abdomen 1; (C) typical macroseta, arrow points at detail of apical end; (D) furcula scar and associated setae, arrow points to anterior end; (E) chaetotaxy of medial section of abdomen 4, arrow points at detail of pseudocelli ornamentation; (F) upper anal valve.

tibiotarsi with 9, 8, 1 setae in whorls A–C, respectively; mesothoracic tibiotarsus with 9, 8, 2 setae in whorls A–C (Figs. 7B, 7C); seta M present; tenent hair acuminate. Unguis with outer teeth, inner teeth absent. Unguiculus

without basal lamella and as long as, to marginally longer than, inner edge of unguis.

Remarks: Table 1 summarizes differences between the new species and other North American members of the *O.*

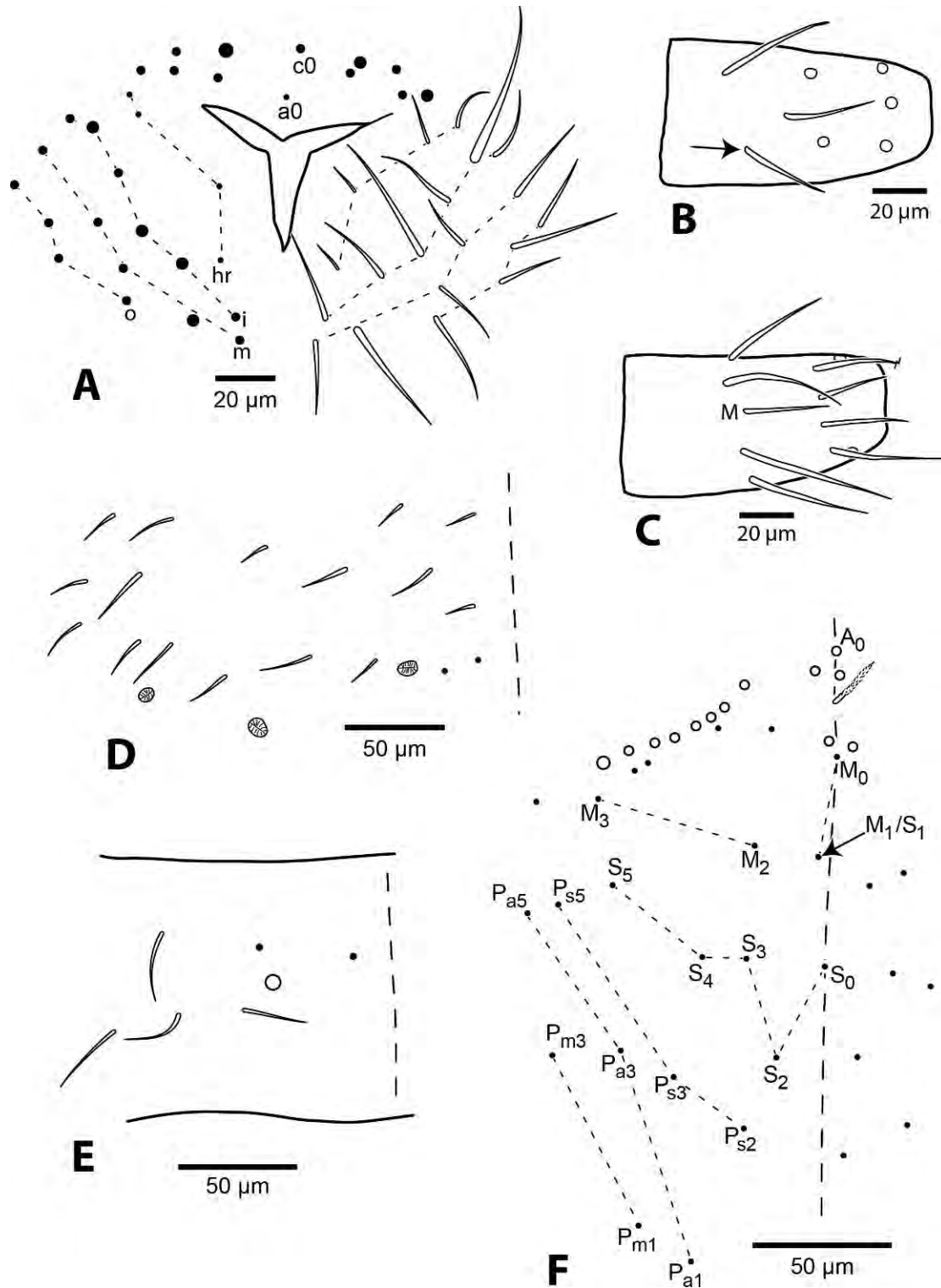


Figure 7. Chaetotaxy of *Onychiurus* spp., dots represent regular acuminate setae, dot diameter is roughly proportional to seta length: (A) *O. pipistrellae* n. sp., anal valves; (B) *O. pipistrellae* n. sp., mesothoracic tibiotarsus, anterior view, inner is toward top of figure, arrow indicates seta absent from pro- and metathoracic tibiotarsi; (C) *O. pipistrellae* n. sp., mesothoracic tibiotarsus, posterior view, inner is toward top of figure; (D) *O. wilchi*, abdomen 1, syntype deposited at the Illinois Natural History Survey; (E) *O. wilchi* as above, but prothorax, circle represents pseudocellus; (F) *Pseudosinella aera*, dorsal head chaetotaxy, dots and open circles represent micro- and macrosetae, respectively.



**Table 1. Diagnostic characters for species in the *Onychiurus relictus* species group. Characters for *O. wilchi* taken from Wray (1950) and from three individuals of the syntypic series deposited at the Illinois Natural History Survey (INHS). Characters for all other previously named species follow Pomorski et al. (2009).**

Species	Number of PAO Vesicles		Number of Dorsal Pseudocelli		Number of Ventral Pseudocelli		Shape of Macrosetae tip		Number of Setae		Number of Setae		Unguicular Lamella
	12-15	15-16	32/033/33353	32/133/33353	2/000/1112	2/000/1112	broadly acuminate	truncate	Ventral Tube	Subcoxae Legs 1-3	Subcoxae Legs 1-3	Unguicular Lamella	
<i>O. pipistrellae</i> n. sp.	12-15	15-16	32/033/33353	32/133/33353	2/000/1112	2/000/1112	broadly acuminate	truncate	6-7	555	555	absent	
<i>O. relictus</i> Christiansen and Bellingier	15-16	15-16	32/133/33353	32/133/33353	2/000/1112	2/000/1112	truncate	truncate	10	555	555	absent	
<i>O. furcisetosus</i> Pomorski, Furgol and Christiansen	16-17	16-17	32/133/33353	32/133/33353	2/000/1212	2/000/1212	bifurcate	bifurcate	8	555	555	absent	
<i>O. nathanieli</i> Pomorski, Furgol and Christiansen	15	15	32/033/33354	32/033/33354	2/000/1112	2/000/1112	acuminate	acuminate	6	455	455	present	
<i>O. relictoides</i> Pomorski, Furgol and Christiansen	14-15	14-15	32/133/33354	32/133/33354	2/000/1212	2/000/1212	acuminate	acuminate	9	555	555	absent	
<i>O. steinmanni</i> Pomorski, Furgol and Christiansen	16-17	16-17	32/022/33353	32/022/33353	2/000/1112	2/000/1112	acuminate	acuminate	9	555	555	absent	
<i>O. wilchi</i> Wray (Fig. 18 in original description)	18-20	18-20	32/022/02233	32/022/02233	..	..	..	..	..	..	..	absent	
<i>O. wilchi</i> INHS cotypes	..	..	32/133/3???	32/133/3???	..	..	acuminate?	acuminate?	..	..	..	absent	

*relictus* species complex. Most individuals of *O. pipistrellae* n. sp. will key out to *O. steinmanni* Pomorski, Furgol, and Christiansen, 2009 in Pomorski et al. (2009), but the new species is easily distinguished from *O. steinmanni* by the number of pseudocelli on Th. 2-3, the number of setae on ventral tube, the number of PAO vesicles, and, apparently, the number of proximal setae on the labial palp. The new species is most similar to *O. relictus* Christiansen, 1961, from which it differs in lacking pseudocelli on Th. 1, the number of setae on the ventral tube, the shape of the macrosetal tip, and possibly the number of PAO vesicles. An adult female from Bat Sump cave has 1+1 pseudocelli on Th. 1, but it retains the low number of setae on the ventral tube, the shape of the macrosetae, and a PAO with only 13 vesicles.

*Onychiurus pipistrellae* n. sp. was collected about 50 to 60 km southwest of the type locality of *O. wilchi* Wray, 1950. Following Wray's (1950) original description, the two species should be easy to distinguish by the combined absence of dorsal pseudocelli on Th. 1 and Abd. 1 in *O. wilchi* and the number of PAO vesicles. However, the syntypes of *O. wilchi* deposited at the Illinois Natural History Survey examined carry 1 and 3 pseudocelli on Th. 1 (Fig. 7E) and Abd. 1 (Fig. 7D), respectively. This suggests that *O. wilchi* is a senior synonym of either *O. relictus* or *O. pipistrellae* n. sp. Unfortunately, the characters needed to decide between the alternatives (i.e., number of seta on the ventral tube, number of vesicles on the postantennal organ, and shape of the tip of the macrosetae) are not visible in the types studied, and the status of *O. wilchi* remains unresolved until fresh topotypic material is obtained.

The distribution of *O. pipistrellae* is unclear. The populations from Bat Sump and Stemler caves were previously sampled and identify as *O. relictus* (Lewis et al., 2003). However, the redescription of *O. relictus* by Pomorski et al. (2009) makes it clear that the population in Bat Sump and Stemler Cave represent a new species. It is possible that other records of *O. relictus* in caves from southern Illinois (e.g., Fogelpole Cave, Hidden Hand Cave, Fults Saltpeter Cave), Missouri, and Kentucky may be referable to the new species. The five other described species in the *O. relictus* species complex are found in caves in Virginia, Indiana, Wisconsin, Iowa, and Colorado.

*Thalassaphorura encarpata* (Denis), 1931 — EU S2/G5

Localities: Bat Sump Cave, Stemler Cave

This common species is widespread across North America. *Thalassaphorura encarpata* is known from Cumberland, Jackson, Johnson (Firestone Creek Cave), Piatt, and Wayne counties in Illinois. This species is relatively common in subterranean habitats, as it has been reported from caves in Missouri, Indiana, Texas, and Hawaii.

*Heterophorura subtenua* (Folsom), 1917 — EU S2/G5

Locality: Wanda's Waterfall Cave

This is part of a complex that includes three other species (*H. bima*, *H. casa*, and *H. tala*). The actual distribution of *H. subtenua* is unclear, as old identifications might have conflated the identity of all four forms. Confirmed records indicate the species ranges from Alaska and British Columbia to Maine and North Carolina, although it appears to be absent in the region between Iowa-Missouri-Arkansas and British Columbia. In Illinois the species has been reported from Alexander, Champaign, Coles, Jackson, La Salle, Union, Vermillion, and Washington counties. *Heterophorura subtenua* has been previously reported from caves in Alaska, Illinois, Indiana, North Carolina, Texas, and West Virginia.

#### TULLBERGIDAE

*Mesaphorura silvicola* (Folsom), 1932 — TX S1/G5

Localities: Wanda's Waterfall Cave, Bat Sump Cave

*Mesaphorura silvicola* is widespread in North America and common in surface leaf litter. In Illinois, *M. silvicola* is known from Jackson, Monroe, and Vermillion counties. The species was previously reported from a cave in Indiana.

#### ISOTOMIDAE

*Hemisotoma thermophila* (Axelson), 1900 — TX S1/G5

Localities: Wanda's Waterfall Cave, Stemler Cave

This species is commonly found on surface leaf litter across temperate and tropical regions of the world (Potapov, 2001; Mari Mutt and Bellinger, 1990), and in Illinois it was previously known from Champaign County. This appears to be the first record for this species from Illinois caves.

*Desoria trispinata* (MacGillivray), 1896 — EU S5/G5

Localities: Wanda's Waterfall Cave, Illinois Caverns

This common surface species is widely distributed across temperate regions of the North Hemisphere (Potapov, 2001) and relatively common in cave leaf litter in North America. The individuals from the Salem Plateau have three instead of four labral papillae, a character shared with some populations from Missouri caves (Christiansen and Bellinger, 1998). The labral character may define an isolated population restricted to the Salem Plateau region on both sides of the Mississippi river.

*Folsomia* cf. *bisetosa* Gisin, 1953 — TX S1/G5; New Illinois Record

Locality: Stemler Cave

The single individual collected deep in Stemler Cave agrees with the description of *F. bisetosa* provided by Fjellberg (2007) in all characters except in having long unilaterally serrate macrosetae on all segments (macrosetae are smooth in *F. bisetosa*) and in having dorsal manubrial seta m11 (m11 absent in *F. bisetosa*). This may be the same form identified as *F. cf. bisetosa* from Indiana by Waltz and Hart (1996). *Folsomia bisetosa* is distributed across the Old World arctic and subarctic regions, and its presence in an

Illinois cave seems unlikely. Additional material is needed to confirm the identity of the species.

*Folsomia candida* Willem, 1902 — EU S5/G5

Localities: Wanda's Waterfall Cave, Illinois Caverns, Bat Sump Cave, Stemler Cave

This is a common litter and soil species widespread in North American caves.

*Folsomia prima* Mills, 1931 — EU S2/G5

Localities: Illinois Caverns, Bat Sump Cave

This common surface leaf litter species is often found in caves. In Illinois the species has been reported from caves in Hardin County and from surface leaf litter in Champaign, Vermillion, and Williamson counties.

*Folsomia stella* Christiansen and Tucker, 1977 — EU S5/G5

Localities: Bat Sump Cave, Stemler Cave

This species is distributed across temperate North America and Eurasia (Potapov, 2001). In North America, *F. stella* is often found in caves. In Illinois, the species is previously known from Alexander, Champaign, Monroe, and Vermillion counties.

*Folsomia variabilis* Fjellberg, 1984 — TX S1/G5; New Illinois Record

Localities: Wanda's Waterfall Cave, Stemler Cave

This is the first record for *F. variabilis* from Illinois and from caves. The species was originally described from populations found in mosses at 11,000 ft (3,400 m) of elevation in Colorado (Fjellberg, 1984).

*Isotomiella minor* Schäffer, 1896 — EU S5/G5

Locality: Wizard Cave

This species is common in surface and cave habitats throughout North America.

*Parisotoma notabilis* (Schäffer), 1896 — EU S5/G5

Localities: Wanda's Waterfall Cave, Illinois Caverns, Hidden Hand Cave

*Parisotoma notabilis* is a common surface species, widely distributed across temperate regions of the Northern Hemisphere and probably the most common species of springtail in surface leaf litter in North America. The species is often found in caves.

*Proisotoma sepulchralis* Folsom, 1902 — TX S1/G1; New Illinois Record

Locality: Stemler Cave

This is the first record from Illinois and from caves for this uncommon species. *Proisotoma sepulchralis* was previously known only from Washington D.C., Michigan, and Pennsylvania.

#### TOMOCERIDAE

*Lethemurus missus* Mills, 1949 — TB S2/G2

Locality: Pautler Cave

This troglobiont is widespread in caves in the Salem Plateau. *Lethemurus missus* was originally described from

Jersey County Illinois, but is now known to occur in Kentucky, Missouri, Alabama, Indiana, Virginia, and Colorado.

*Pogonognathellus flavescens* (Tullberg), 1871 species complex — EU S5/G5

Localities: Wizard Cave, Pautler Cave, Wanda's Waterfall Cave, Spider Cave, Illinois Caverns, Bat Sump Cave, Hidden Hand Cave, Stemler Cave

Species in the *P. flavescens* complex are the most common members of the family Tomoceridae in surface leaf litter and in caves in North America. Members of the *P. flavescens* complex appear to be widespread in North America and Europe. Until recently, all forms in the complex in North America were assigned to the nominal species, although the large amount of morphological variation reported from throughout its range suggested that it represented a species complex (Christiansen, 1964). Recently, Felderhoff et al. (2010) and Park et al. (2011) concluded that, based on molecular evidence, *P. flavescens* does not occur in North America and all or most forms previously identified as such are endemic to North America. Most species in the complex have been identified only by using molecular data, and just a handful of them have been diagnosed using morphological characters. Thus, the identity of the forms collected in the Salem Plateau caves will remain unclear until their DNA can be studied.

*Pogonognathellus nigritus* (Maynard), 1951 — TX S5/G5?; New Illinois Record

Localities: Spider Cave, Illinois Caverns, Bat Sump Cave

This is a common surface leaf litter species in wooded areas of the Salem Plateau. The actual distribution of *P. nigritus* in North America is not known, because in the past it has been included as part of the variation of *P. elongatus* Maynard (Felderhoff et al., 2010), hence the question mark for global ranking. This is the first record for the species from caves.

#### ONCOPODURIDAE

*Oncopodura iowae* Christiansen, 1961 — TB/EU S2/G2

Localities: Wizard Cave, Wanda's Waterfall Cave, Illinois Caverns, Hidden Hand Cave, Stemler Cave

This is the most common species of *Oncopodura* found in Illinois caves. Recent collections of this species on surface leaf litter (IL: Monroe Co., Valmeyer, Salt Lick Point, N 38.30644 W 90.30475, leaf litter between rock outcrops on bluff top, 13 May 2011, F.N. Soto-Adames) indicate the species can move freely above ground under appropriate conditions. The species has been previously reported from other localities in Monroe County, Illinois, and in caves in Iowa, Missouri, and South Dakota.

#### ENTOMOBRYIDAE

*Pseudosinella aera* Christiansen and Bellinger, 1980 — EU S2/G5

Locality: Wizard Cave

Complement to the description of *P. aera* based on individuals collected in Wizard Cave: Freshly killed individuals uniformly light blue, pigment more intense on eye patch. Scales present only on head, trunk, and coxae. Apical bulb of Ant. 4 absent; subapical sense organ "Y" shaped (Fig. 8A), appearing capitate in side view and in small individuals. Sense organ of Ant. 3 with main sensilla (i.e., numbers 2 and 3 in Chen and Christiansen, 1993) slightly bent, laterally expanded with dense central rachis (Fig. 8B); sensilla 1 and 4 longer, and sensillum 5 shorter, than sensilla 2–3; at least one lenticular organ present. Eyes 2+2. Dorsal head chaetotaxy as in (Fig. 7F): macrosetae A0, A2 and A3 present; A1 coarsely ciliate, but not a macroseta; other dorsal head setae narrow and smooth; M0 present, inserted almost between A3; row S with 4+1 setae, S0 in line with S3; row Pa with 3 setae and bothriotrix Pa6, Pa5 a microseta. Prelabral setae ciliate, all other setae smooth, labral intrusion and papillae not seen. Maxillar capitulum with four teeth. Sublobular plate of outer maxillary lobe with three seta-like appendages; subapical and apical setae smooth and subequal. Labial papilla E with 4 guard setae, lateral appendage straight, blunt and reaching tip of papilla. Labial palp proximal setae Y and Z subequal. Labial triangle chaetotaxy with anterior setae smooth, posterior setae coarsely ciliate, except for r, which is acuminate, short and smooth; formula as  $\overline{M1M2rEL1L2A1-5}$  (Fig. 8C). All postlabial setae, except  $\overline{L2}$  and O1, coarsely ciliate; columns I, C, E, L and O with 4, 1, 2, 3, 2 setae, respectively; setae  $\overline{L2}$  and O1, smooth and acuminate as r; one additional seta similar to r external to column O. All pleural and peristomal setae coarsely ciliate, pss1-2 bothriotrix-like. Trunk macrosetae formula 00/0100+2. Mesothoracic hood not developed, collar formed by 2–3 rows of acuminate to weakly apically bent macrosetae. Mesothorax not polychaetotic. Metathorax without p2p, but otherwise with a full complement of setae; setae p2 less than 2× the length of a3. Abd. 1 with 10 posterior setae (Fig. 8D) arranged in a single row, but not evenly distributed along row, seta a3 inserted very close to m3 and a5 to m4; a6 present, not paired to sensilla as. Abd. 2 (Fig. 8E) with mi, ml, Lm, and L1 fan-shaped; a2, a3, a2p, m4, and p5p ciliate, all other setae either denticulate or smooth; a3 inserted near and reaching as; m3 and m5 macrosetae, a6 present. Th. 3 (Fig. 8F) with a2, a6, am6, and all supplementary setae fan-shaped; a3 not reaching as; as half the length of m3; d2 present, a7 inserted anterior to and reaching am6; m7 smooth microseta inserted in row with p5; p7 a ciliate microseta; m7 enlarged. Bothriotrichal complex of Abd. 4 (Fig. 9A) with seta s present and fan-shaped; a, m, D1, Pi, and Pe fan-shaped; C1p, T3, and D1p ciliate. Other chaetotaxy of Abd. 4 as in Figure 9B: B5 and B6 macrosetae, B5 inserted just anterior or on the line between A5-C2; C1 a smooth microsetae; D3, E2, E3, F1, and F3 large macrosetae; T6, T7, D2, and E1 small macrosetae; F2 a microsetae; microsetae posterior to E3 present; posterior setae 3+3. Trochanteral organ with 23



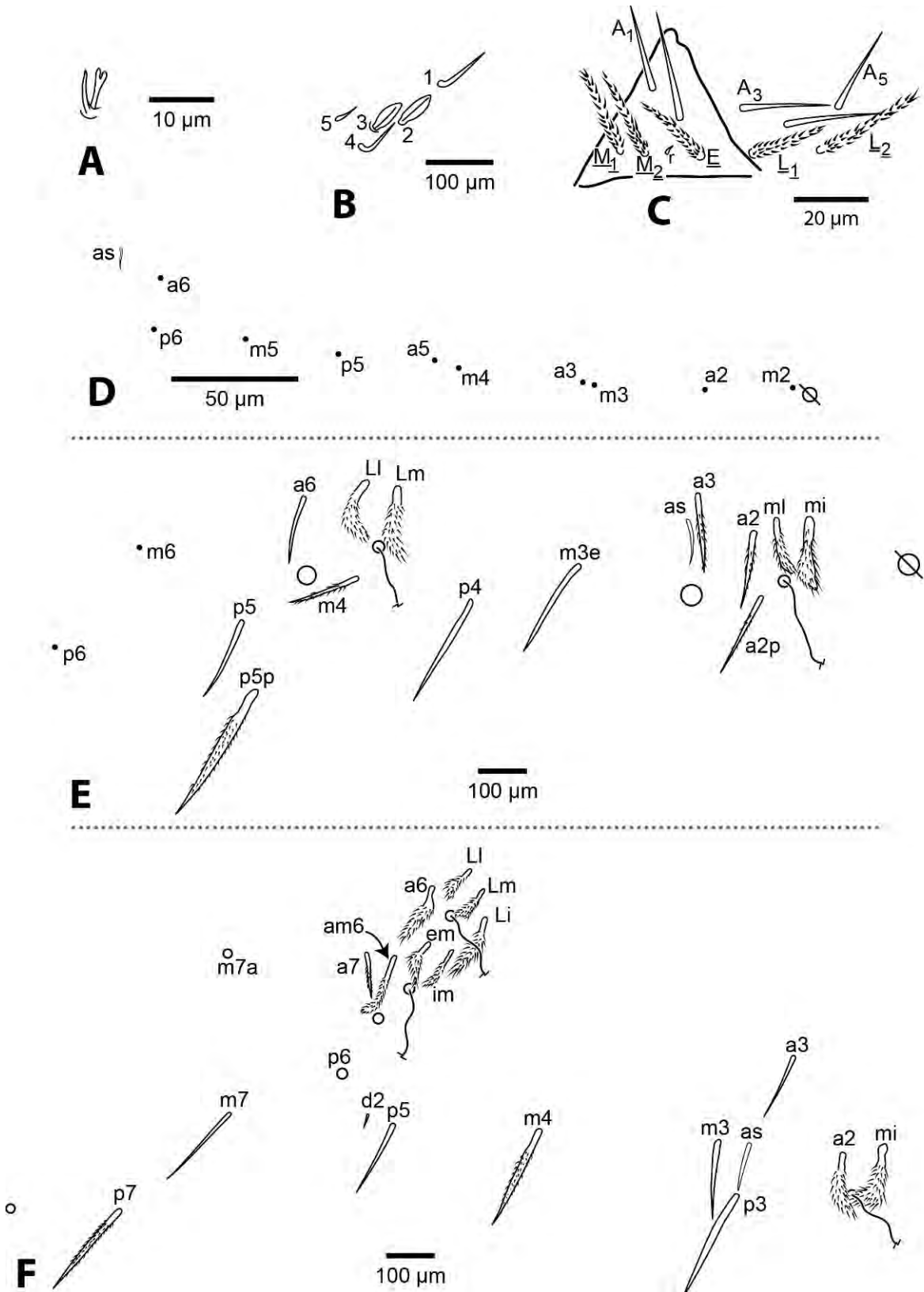
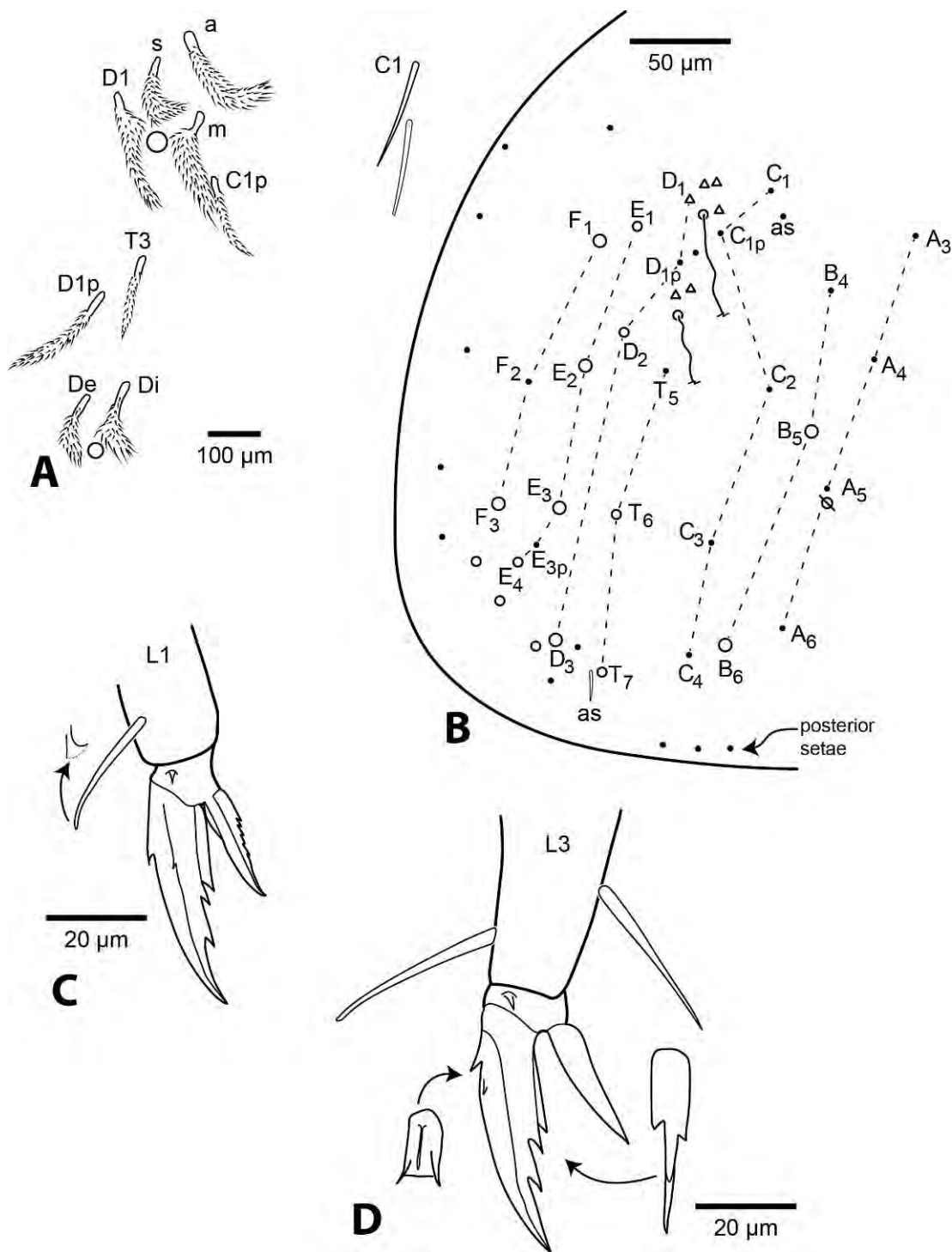


Figure 8. *Pseudosinella aera*: (A) supapical organ of fourth antennal segment; (B) sense organ of third antennal segment and associated sensilla; (C) labial triangle. Tergal chaetotaxy, dots, open circles and crossed circles represent micro- and macrosetae and pseudopores, respectively; (D) abdomen 1; (E) abdomen 2; (F) abdomen 3.



**Figure 9.** *Pseudosinella aera*, dots, open circles, triangles and crossed circles represent micro- and macrosetae, fan-shaped setae, and pseudopores, respectively: (A) abdomen 4 bothriotrichal complex; (B) complete chaetotaxy of abdomen 4; (C) prothoracic claw complex; (D) metathoracic claw complex.

setae in females, 15 in the single adult male studied, and 4 in one small juvenile. Internal face of all femora with 5 conic setae. All tibiotarsi with one posterior blunt or acuminate macroseta inserted near basal third of segment. Tenent hair weakly capitate, appearing acuminate or blunt

at low magnification (Figs. 9C, D). Proportion of unguiculus: tenent hair: posterior smooth seta on hind legs as 1:1.3:1.3. Unguiculus lanceolate, posterior lamella serrate on forelegs, apparently smooth on hind legs. Unguis (Figs. 9C, D) with 4 internal teeth; basal teeth not aligned,

one tooth slightly larger than other, basal tooth ending at  $\approx 46\%$  (range 43–49%;  $n=3$ ) of inner claw length; distal unpaired tooth as large as basal teeth and ending at  $\approx 74\%$  (range 70–77%;  $n=3$ ) of inner claw length; external teeth conspicuous. Anterior face of ventral tube with 9 to 14 setae, 4 of them inserted along ventral groove, with 1 distal margin macroseta; lateral vesicles with 4 smooth and 5 to 10 strongly ciliate setae; distal margin of posterior face with 3 medial and 3 or 4 lateral setae, basal ventral chaetotaxy not clearly seen on any specimen, but apparently with 13 setae (5+5 and 3 unpaired). All manubrial setae ciliate; dorsal manubrial plate with 2 internal and 5 external, coarsely ciliate setae. Dens tubercle absent. Mucronal teeth subequal; mucronal spine with minute basal tooth.

Remarks: The individuals from Wizard Cave differ from the original description of the species only in having dorsal head macrosetae A3 present. *Pseudosinella aera* is very similar to *P. argentea* Folsom, *P. flatua* Christiansen and Bellinger, and *P. granda* Christiansen and Bellinger, but it is easily distinguished from all by the presence of 2+2 eyes. In addition, it can be distinguished from *P. flatua* by the presence of ciliate prelabral setae, from *P. argentea* by the absence of seta m4i on Abd. 2, and from *P. granda* by the presence of only one head macroseta A0, by the presence of short acuminate labial seta r, and by having a ciliate a3 on Abd. 2. Additional differences between the four species are listed in Table 2.

In Illinois *Pseudosinella aera* has been previously reported from caves in Gallatin and Johnson (Firestone Creek Cave) counties. *P. aera* appears to have a mostly southern distribution; the localities in Illinois are at or near the northernmost limit of the species. This species does not show strong adaptations to cave habitat, but almost all records, from Mexico to Illinois, are from caves.

*Pseudosinella argentea* Folsom, 1902 — EU S2/G5?

Localities: Spider Cave, Illinois Caverns

As currently circumscribed, this species shows considerable morphological variation throughout its distribution, suggesting it likely represents a species complex (Christiansen and Bellinger, 1998). The specimens from the Salem Plateau have 4+4 posterior setae on Abd. 4 (Fig. 10A), and the tenent hair on all legs is acuminate. This is consistent with the population of *P. argentea* reported from Maiden Cave, West Virginia (Soto-Adames, 2010).

This is a common cave species in Illinois. It has been previously reported from Carroll, Hardin (Brown's Hole Cave), and Monroe (Fults Saltpeter, Fogelpole, Spider and Wanda's Waterfall caves) counties. The species also appears to be common in caves in Kentucky, Missouri, Arkansas, Indiana, Alabama, Tennessee, Washington, Virginia, West Virginia, Pennsylvania, and Connecticut. Surface populations in Illinois have been reported from Champaign, Clark, Edgar, Johnson, and Union counties.

*Coecobrya tenebricosa* (Folsom), 1902 — EU S5/G5 (IN?)  
Localities: Wizard Cave, Pautler Cave, Wanda's Waterfall Cave, Illinois Caverns

This is a species commonly found in caves in North America. Previous reports from Illinois caves include Rose Hole and Pautler Cave, both in Monroe County.

This species was originally described from the Washington, D.C., area, but currently it is known to occur around the world in protected habitats such as greenhouses and caves (Chen and Christiansen, 1997). The genus *Coecobrya* is almost exclusively East Asian in distribution (Chen and Christiansen, 1997), and it is likely that *C. tenebricosa* represents an early introduction, during historic times, into North America.

*Entomobrya* sp.

Locality: Wizard Cave

The single individual collected is in an early instar and not identifiable to species.

*Homidia socia* Börner, 1909 — TX IN

Locality: Spider Cave

This is an introduced species. Almost all species of *Homidia* are restricted to East Asia and Oceania; only two (*H. socia* and *H. sauteri*) are known from North America. The oldest record of *H. socia* in North America appears to be from 1970, from Georgia (K. Christiansen, Collembola records database). The historical collection of springtails at the Illinois Natural History Survey that goes back to the second half of the 1800s, does not include representatives of this species, despite it now being the most common form found in grasses growing along country roads in Champaign County. *Homidia socia* was first noticed in a cave in Johnson County, southern Illinois, in 1973 (Christiansen and Bellinger, 1980, 1998; K. Christiansen, Collembola records database) and in Champaign County by the senior author in 1988. The species is also known from caves in Harrison and Crawford counties, Indiana.

NEELIDAE

*Megalothorax minimus* (Willem), 1900 — EU S5/G5

Locality: Wanda's Waterfall Cave

This is a common surface leaf litter species frequently found in caves. The species has been recorded from Grundy, Lawrence, and Washington counties in Illinois. This appears to be the first record for the species from Illinois caves.

*Megalothorax tristani* (Denis), 1933 — TX S1/G1

Locality: Wizard Cave

This is either a rare species or it has been generally confused with the more widely distributed *M. incertus*. *Megalothorax tristani* was previously reported from Illinois by Bonet (1948).

*Neelides minutus* (Folsom), 1901 — EU S5/G5

Locality: Wanda's Waterfall Cave

This is a common surface leaf litter species often seen in cave samples. In Illinois the species has been previously



**Table 2. Comparison of *Pseudosinella aera* and *P. argentea* from Salem Plateau to *P. flatua* and *P. granda*.**

Character	Species (Caves)			
	<i>P. argentea</i> (Spider Cave and Illinois Caverns)	<i>P. aera</i> (Wizard Cave)	<i>P. granda</i>	<i>P. flatua</i>
Eye number	0	2	0	0
Head macroseta A0	1	1	2	1
Prelabral setae	ciliate	ciliate	ciliate	smooth
Size of labial seta m1 & m2 <sup>a</sup>	<u>M1 ≈ M2</u>	<u>M1 ≈ M2</u>	<u>M1 ≈ M2</u>	<u>m1 &lt; M2</u>
Labial seta r	smooth, thin walled blunt microseta	smooth acuminate microseta	absent or smooth conic reduced	smooth acuminate microseta
Head ventral groove setae	4 ciliate	4 ciliate	4 ciliate	3 smooth 1 ciliate
Abd. 2 seta a3	ciliate	ciliate	smooth	smooth
Abd. 2 seta a2p	ciliate & ≈ a2	weakly ciliate & > a2	ciliate & > a2	ciliate & > a2
Abd. 2 seta 4mi	present	absent	absent	absent
Abd. 4 posterior setae	4	3	...	...
Tenent hair	acuminate	weakly truncate	acuminate	acuminate
Ventral tube anterior setae	9	9-14	9 <sup>b</sup>	11-13
Ventral tube posterior setae (total/distal margin)	19/9	22-24/9-11	13/7	14/?
Ventral tube laterodistal setae	7-8; 3-4 ciliate	10-14; 5-10 ciliate	8-9; 4-5 ciliate	11-13

<sup>a</sup> Underline and without underline indicate the setae is ciliate or smooth, respectively. Upper- and lower-case 'm' corresponds to macro- or microsetae, respectively.

<sup>b</sup> The original description of *P. granda* states there are 6-7 setae on the anterior face of the ventral tube (Christiansen and Belling, 1996), but their figure 46 shows 9 setae.

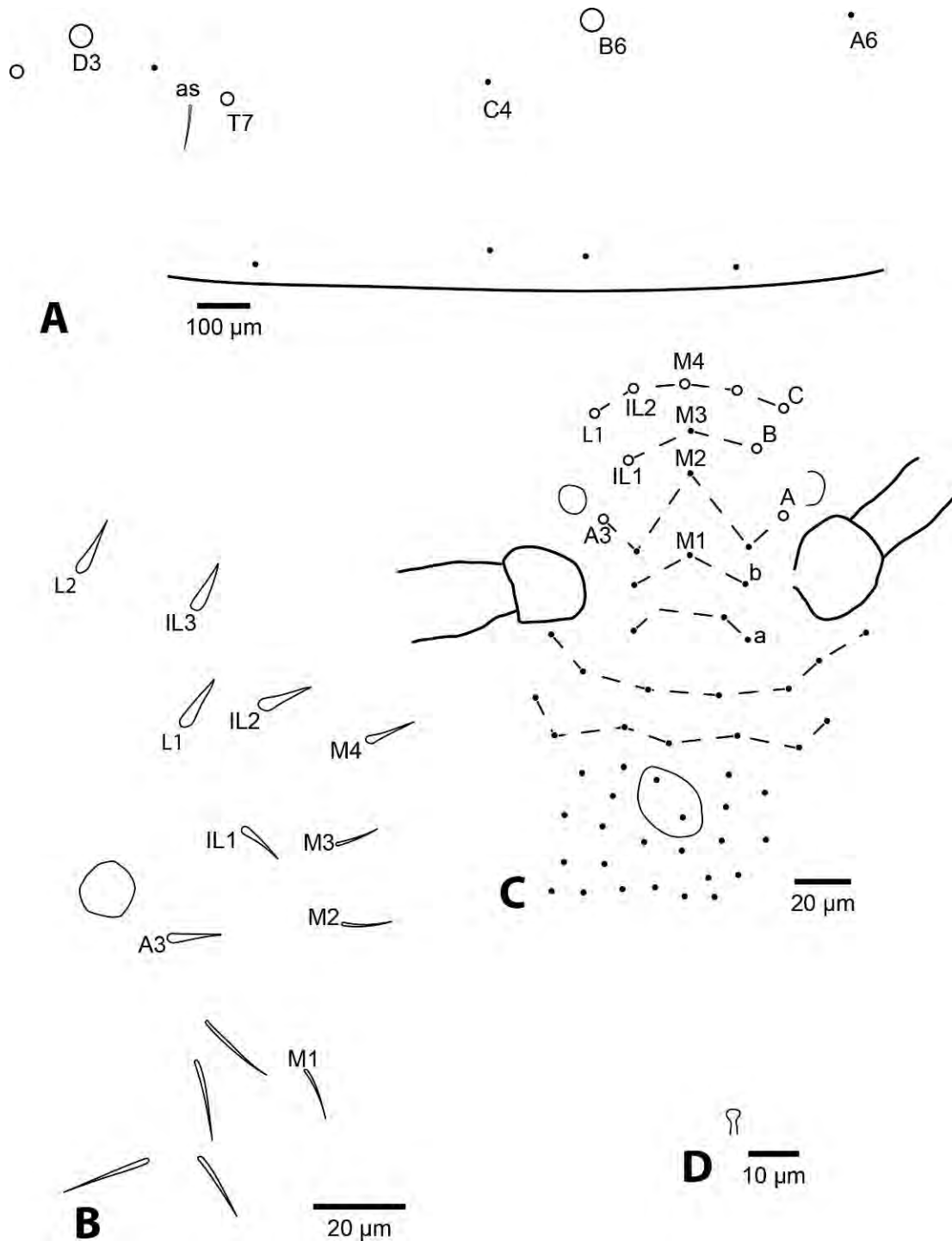


Figure 10. *Pseudosinella argentea*, dots, and open circles represent micro- and macrosetae, respectively: (A) posterior chaetotaxy of abdomen 4. *Pygmarrhopalites fransjanssens* n. sp., dots, and open circles represent microsetae and spine-like setae, respectively: (B) detail of vertical chaetotaxy of the head, left side; (C) general chaetotaxy of face; (D) subapical sensilla of fourth antennal segment.

reported from Calhoun, Cook, Gallatin, Jackson, Lake, La Salle, Lawrence, and Wayne counties.

SMINTHURIDIDAE

*Sphaeridia serrata* (Folsom and Mills), 1938 — TX S1/G3

Locality: Wanda's Waterfall Cave

This is an uncommon species previously reported from Herod and Pope counties in Illinois. This is the first record of this species from Illinois caves.

*Sminthurides* sp. — TX S1/G1

Locality: Bat Sump Cave

This species has a unique combination of characters not seen in other Nearctic species. This is not a cave-adapted form. One male and one female were seen, and formal description of the species must await the study of further material.

#### ARRHOPALITIDAE

*Pygmarrhopalites fransjanssens* n. sp. — TB/EU? S1/G1?

Material Examined: Holotype, INHS Collection Number 551,634; USA, IL, St. Clair Co., Wizard Cave, Dupo, near Falling Spring, adult female, slide-mounted, collected in pitfall trap in twilight zone, 15–17 June 2009, SJ Taylor, FN Soto-Adames and CA Phillips. Paratypes, INHS Collection Number 551,635-36; 2 slide-mounted adult females on individual slides and 8 other individuals of undetermined sex in alcohol with same collection locality as holotype; 2 paratypes of undetermined sex collected with an aspirator under rotting log in twilight zone; 8 paratypes collected in pitfall traps in both dark and twilight zones.

Etymology: This species is dedicated to Frans Janssens, Department of Biology, University of Antwerp, Antwerp, Belgium, in recognition of his contributions to springtail taxonomy through the development of collembola.org.

Description: Largest individual 0.65 mm. Background color white, with dark brown scattered over head, body, and all appendages. Ant. 4 with 5–6 subsegments in proportions as 26–32:10–11:10–11:10–11:27–29. Ant. 4 subapical sensilla capitate (Fig. 10D), each short preapical subsegment with 11 setae. Ant. 3 with a weak basal bulge (Fig. 11F); sense organ (Fig. 11G) with two rod sensilla in a shallow depression, setae *Api* and *Ape* appearing thin walled and with drawn out apices, but otherwise normally acuminate, *Aai* a rod sensilla. Eyes 1+1 in a dark patch. Dorsal head chaetotaxy as in Figures 10B and C, with vertical setae M4, IL1-3, L1-2, and A3 short spine-like; M5 absent. Apical setae of outer maxillary lobe bifurcate, sublobular plate with three appendages (Fig. 11A). Labial palp papilla E with 3 guard setae. Latero-posterior setae (*lp1*) 2.5–3.5× seta *c2* (Fig. 11B). Small abdomen without denticles or spines, chaetotaxy as in Figure 10C: C1 bifurcate, C2-6 and C8 smooth, enlarged at base but without tunica, C7 and C9 long but not basally enlarged; seta C2 ≈1.3× B2 and C3 ≈2.2× D3; setae D7–8 absent, D9 typically long; female appendage (Fig. 11D, E) palmate, with smooth stem and shallow branches, sitting on a circular papilla. Metatrochanter longer than it is wide (Fig. 12A), with 5 setae. All claws with 1 inner tooth (Figs. 12B, C); dorsal tunica smooth, covering distal half of L1–2, and 4/5 of L3. Unguiculus of all legs with inner tooth, outstanding on L1 but so minute on L3 as to be visible only in some perspectives; apical unguicular filament surpassing length of unguis on all legs. Tenaculum with 2 setae. Manubrium with 4+4 dorsal setae. Dens dorsally (Fig. 12D) with 3 inner (L), 6 dorsal (D1–2, ID1–4) and 7 external (E) setae; setae L1–3, E1 and E3

spine-like. Dens ventrally with 2 unpaired setae. Mucro with spatulate apex.

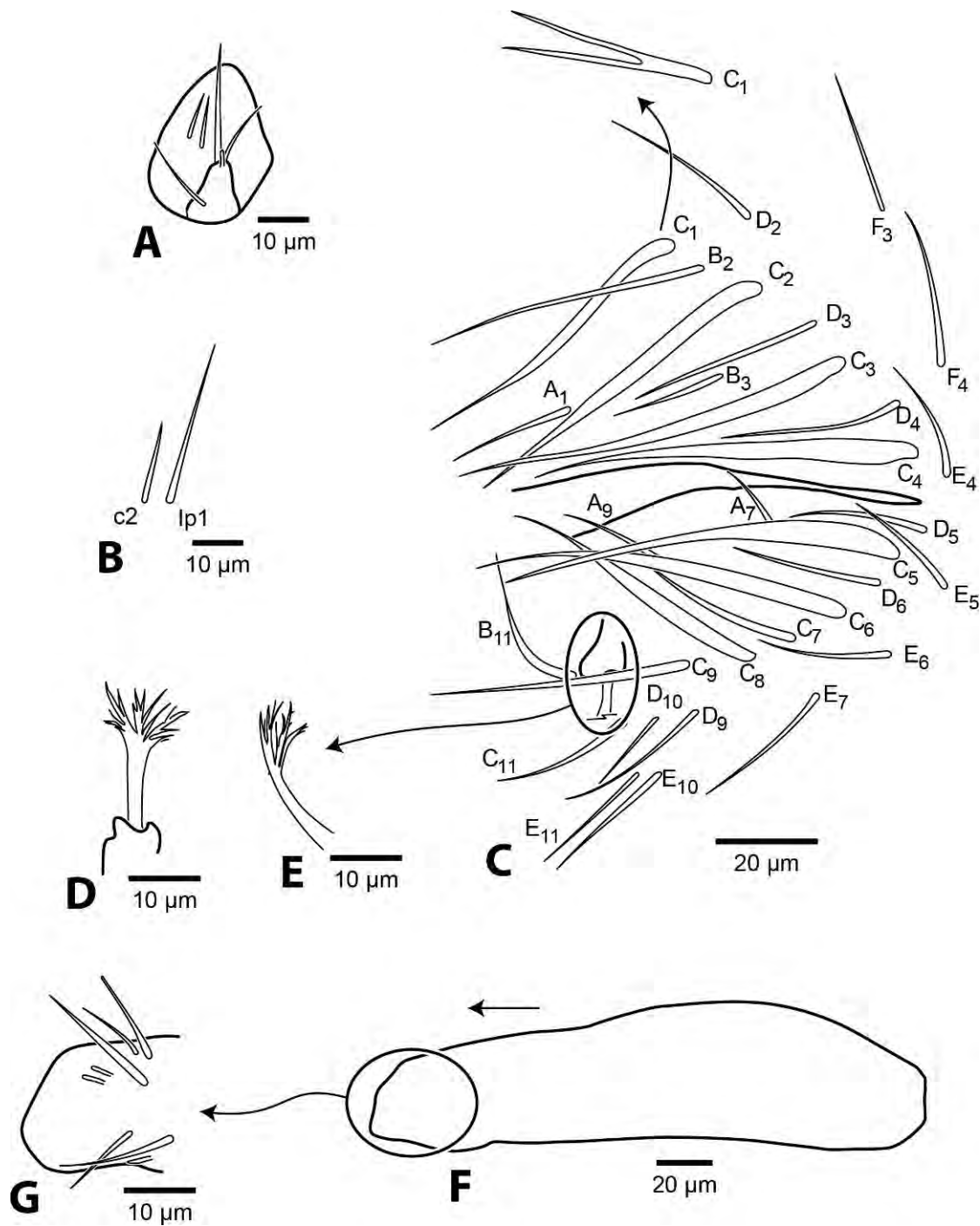
Remarks: This species is characterized by the combined presence of 3 sublobal setae on the maxillary palp, 4+4 dorsal manubrial setae, a palmate female appendage, and small abdomen with setae C1 bifurcate and setae D7–8 absent. Table 3 lists varying characters for the group of species with palmate female appendage, small abdomen seta C1 bifurcate, and two unpaired ventral seta on dens. *Pygmarrhopalites fransjanssens* n. sp. is most similar to *P. incantator* n. sp., from which it differs in the number of sublobal setae on the maxillary palp, the number of guard setae on labial papilla E, the number of dorsal manubrial setae, the absence of D7–8 on the small abdomen, and the shape of metathoracic unguiculus.

The new species keys out to *P. principalis* (Stach) in Bretfeld (1999), but the differences between the two forms are not clear because the state of some characters in the European form remain in dispute. Stach's (1945) original description of *A. principalis* does not mention the condition of small abdomen seta C1. Vargovitsh (2009) points out that Gisin's (1947) Figure 2 shows C1 as bifurcate, the mucro as pointed instead of spoon shaped, and the small abdomen setae in series C as basally expanded instead of enlarged but simple. In addition, Gisin (1947) depicts head vertical setae A3, IL 1–2, and L1 as strongly spine-like and distinct from those in series M, and seta M4 is absent. Fjellberg (1984) first reported *P. principalis* from North America, but the condition of C1 is not mentioned, and his Figure 9C shows what appears to be vertical head seta M5 present and M4 absent. Fjellberg (2007) reported that Fennoscandian populations of *A. principalis* have vertical cephalic M1-3, but not M4–5, maxillary palp with two sublobal hairs, and labial palp papilla E with 3 guard setae. Fjellberg (2007) does not mention the number of manubrial setae or the condition of C1. Vargovitsh (2009) described a new subspecies, *P. principalis skelicus*, which he diagnosed based on the relative length of antennae to cephalic diagonal and the presence of annulations on Ant. 4 of males. Vargovitsh (2009) presented the most complete description for *P. principalis* so far published, but he does not mention the number of setae on the maxillary palp or labial papilla E. In view of the differences between *P. fransjanssens* n. sp., *P. principalis skelicus*, and *P. principalis* in the sense of Gisin (1947) and Fjellberg (2007), we opted to describe the form collected at Wizard Cave as a new species.

*Pygmarrhopalites incantator* n. sp. — EU/TB? S1/G1

Material Examined: Holotype: INHS Collection Number: 551,638; USA, IL, St. Clair Co., Wizard Cave, Dupo, near Falling Spring, adult female, slide-mounted, collected in pitfall trap in twilight zone, 15–17 June 2009, SJ Taylor, FN Soto-Adames and CA Phillips. Paratypes, INHS Collection Number 551,639-40; 1 adult female and 1 adult





**Figure 11.** *Pygmarrhopalites fransjanssens* n. sp.: (A) dorsal view of outer maxillary palp and lobe; (B) big abdomen setae c2 and lp1; (C) lateral view of complete chaetotaxy of female anal valves, inset dorsal view of seta C1 from another individual; (D) dorsal view of female anal appendage; (E) lateral view of female anal appendage; (F) third antennal segment; (G) detail of apical sense organ of third antennal segment.

male mounted on individual slides with same collection information as holotype.

**Etymology:** The epithet of the new species refers to Wizard Cave, the type locality.

**Description:** Largest individual 1.0 mm. Background color white, with orange spots scattered over head, body,

and all appendages. Ant. 4 with 6 subsegments (Fig. 12E) in proportions as 36–37:11:10:10:10:25. Ant. 4 subapical sensilla capitate as in *P. fransjanssens* n. sp., each short subterminal subsegment with 11 setae. Ant. 3 without basal bulge (Fig. 12F); sense organ as in *P. fransjanssens* n. sp., with 2 rod sensilla in independent shallow depressions,

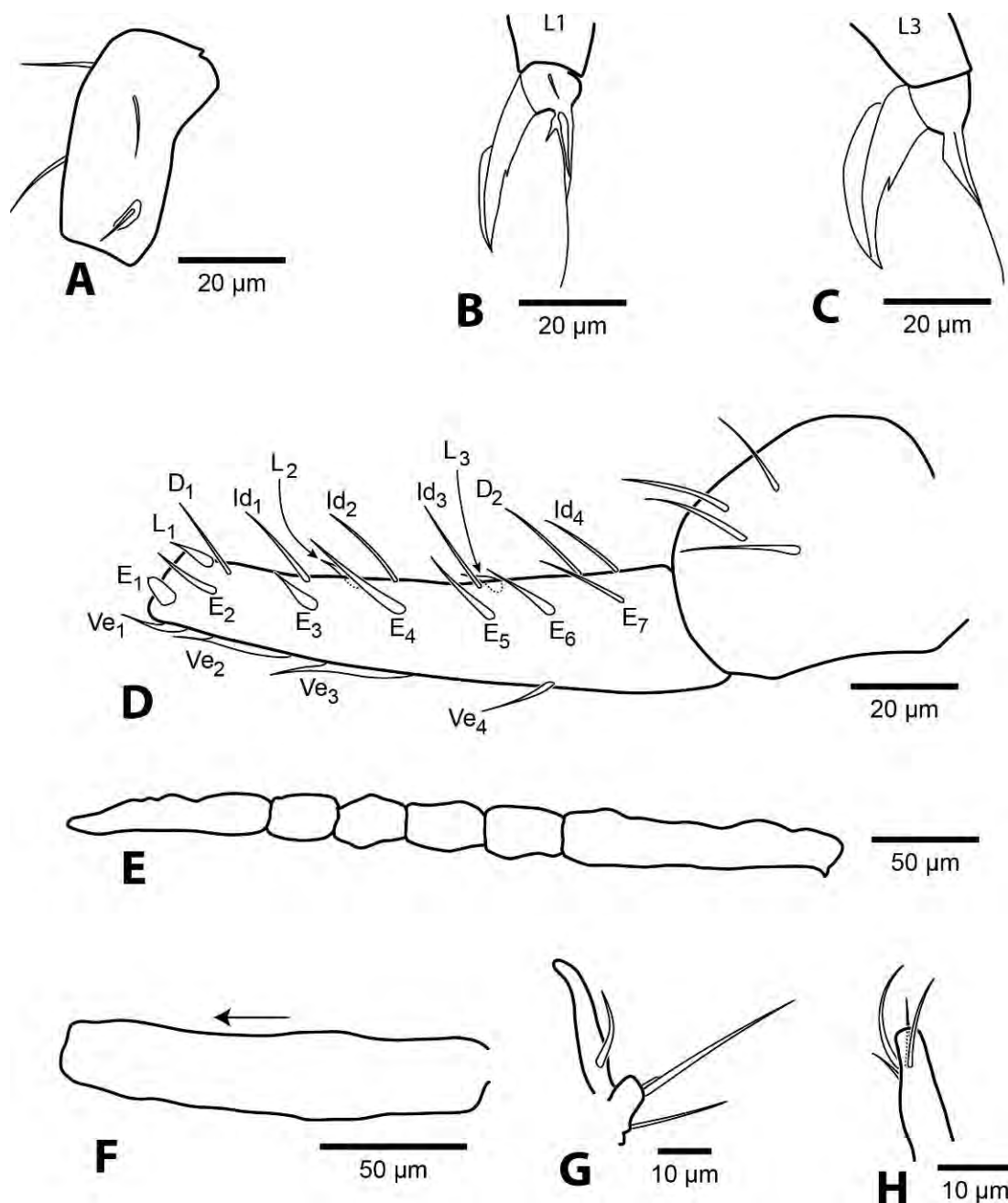


Figure 12. *Pygmarrhopalites fransjanssens* n. sp.: (A) metatrochanter; (B, C) pro- and metathoracic claw complexes, respectively; (D) complete chaetotaxy of furcula, L = inner column, D = dorsal, Id = laterodorsal, E = outer column. *Pygmarrhopalites incantator* n. sp.: (E) fourth antennal segment; (F) third antennal segment; (G) lateral view of outer maxillary palp and lobe; (H) ventral view of labial palp papilla E, terminal seta omitted.

setae Api and Ape appearing thin walled and with drawn out apices, but otherwise normally acuminate, Aai a rod sensilla. Eyes 1+1 in a dark patch. Dorsal head chaetotaxy (Figs. 13A, B) with setae A3, IL1, M4, and L1–3 weakly spine-like; M5 absent. Apical setae of outer maxillary lobe bifurcate (Fig. 12G), but basal spine closely appressed against apical setae and visible only in some perspectives; sublobular plate with one appendage. Labial palp papilla E with 4 guard setae (Fig. 12H). Small abdomen without

denticles or spines; chaetotaxy as in Figure 13C: C1 bifurcate, C2–6 smooth, enlarged at base but without extensions, base of C7–8 not enlarged; seta C2  $\approx 1.2 \times$  B2 and C3  $\approx 1.7 \times$  D3; setae D7–D8 present; female appendage (Fig. 13D) palmate, with smooth stem and deep branches, some of which originate close to the middle of the stem, appendage sitting on a circular papilla. Metatrochanter rectangular, with 4 anterior and 1 posterior setae. All claws with 1 inner tooth (Figs. 13E, F), tooth strongest on L3;

Table 3. Diagnostic characters for species of *Pygmarrhopalites* with bifurcate C1, palmate female appendage and two unpaired ventral setae on dens.

Species	Ant. 4 Subsegments	Head Vertical Seta M5	Outer Plate Maxillary Palp Setae	Labial Palp E Guard Setae	Small Abdomen C1	Small Abdomen D Series Posterior to D6	Female Appendages Branches / Stem	Dorsal Manubrial Setae	Number of Setae on Dens
<i>P. fransjanssens</i> n. sp.	6	—	3	3	bifurcate	2	shallow / smooth	4	7
<i>P. incantator</i> n. sp.	6	—	1	4	bifurcate	4	deep / smooth	5	6–7
<i>P. hirtus</i> (Christiansen)	6	+	...	...	bifurcate	4	shallow / smooth	...	6
<i>P. arcus</i> (Zeppelini and Christiansen)	7	—	...	...	bifurcate	3	shallow / smooth	...	7
<i>P. hubbardi</i> (Zeppelini and Christiansen)	7	+	...	...	bifurcate	4	shallow / denticulate	...	7
<i>P. principalis skelicus</i> Vargovitsh <sup>a</sup>	6	—	...	2?	normal bifurcate?	3–4	shallow / smooth	7	7

<sup>a</sup> Although *P. principalis skelicus* has unbranched C1 it is included in the table because it seems most similar to *P. fransjanssens* n. sp. from among Palearctic forms, and because *P. principalis* is sometimes described as having bifurcate C1.

dorsal tunica on all legs smooth, covering apical third of claw on L1-2 and 4/5 of L3 claw. Unguiculus of L1 with a minute inner tooth, unguiculus of L2–3 toothless, apical unguicular filament surpassing length of unguis on all legs. Tenaculum with 2 setae. Manubrium with 5+5 dorsal setae. Dens dorsally (Fig. 14A) with 3 inner (L) and 6 dorsal (D1-2, 1d1–4) setae; series E with a maximum of 7 setae in females and 6 in male; setae L1–3, E1, and E3 spine-like. Dens ventrally with 2 unpaired setae. Mucro with spatulate apex.

Remarks: Both females have one dens with 7 E setae and one with 6 setae. In both cases the missing seta is E6. In one female, the dens without E6 also has only 5 dorsal setae.

This species is distinguished from *P. hirtus* as described by Christiansen and Bellinger (1998) and Zeppelini and Christiansen (2003) by the absence of head vertical seta M5, by the shape of the female appendage and, perhaps, by the presence of a smooth tunica on all claws. One individual from Wisconsin deposited at the INHS and identified as *P. hirtus* (Zeppelini et al., 2009) is identical to the specimens from Wizard cave in having only four vertical head setae in series M (M5 absent), the number of dorsal manubrial setae, general shape of female appendage, and the number of maxillary and labial palps setae.

*Pygmarrhopalites sapo* (Zeppelini and Christiansen), 2003 — TB S1/G1

Locality: Pautler Cave

The individuals studied have 6+6 dorsal manubrial setae, maxillary palp with bifurcate apical seta and 3 sublobal appendages, and labial papilla E with 3 guard setae.

This species is endemic to Monroe County, and it previously was reported from Frog, Pautler, Jacobs, and Rose Hole caves (Zeppelini and Christiansen, 2003).

*Pygmarrhopalites salemensis* n. sp. — TB S1/G1

Material Examined: Holotype: INHS Collection Number 551,641; Illinois, St. Clair Co, Stemler Cave, 2.7 mi NE of Columbia IL, adult female, slide-mounted, hand collected on cave floor in dark zone, 28–30 September 2009, SJ Taylor and FN Soto-Adames. Paratypes on slides with INHS Collection Numbers 551,642–50; same locality as holotype, 1 slide-mounted adult female and 3 adults of undetermined sex in alcohol, hand collected on drip pool, dark zone; Illinois, Monroe Co., Wanda's Waterfall Cave, 7.4 mi SE of Valmeyer, 2 females and 1 male adults on individual slides and 1 adult of undetermined sex in alcohol collected in pitfall traps and by hand on cave floor 15–17 September 2009, SJ Taylor and FN Soto-Adames; Illinois, Monroe Co., Illinois Caverns, 1 adult female mounted on a slide, pitfall trap in dark zone, 2 additional adults and 1 juvenile of undetermined sex in alcohol, pitfall and leaf litter, 24–26 September 2009, SJ Taylor, FN Soto-Adames, A Kuhns, E Zaborski, J Jacoby, A Paprocki, and M Pessino; Illinois, Monroe Co., Spider Cave, 6.5 mi S of Waterloo, 1 slide-mounted adult male, pitfall trap, 15–17 September 2009, SJ Taylor and FN Soto-Adames; Illinois,



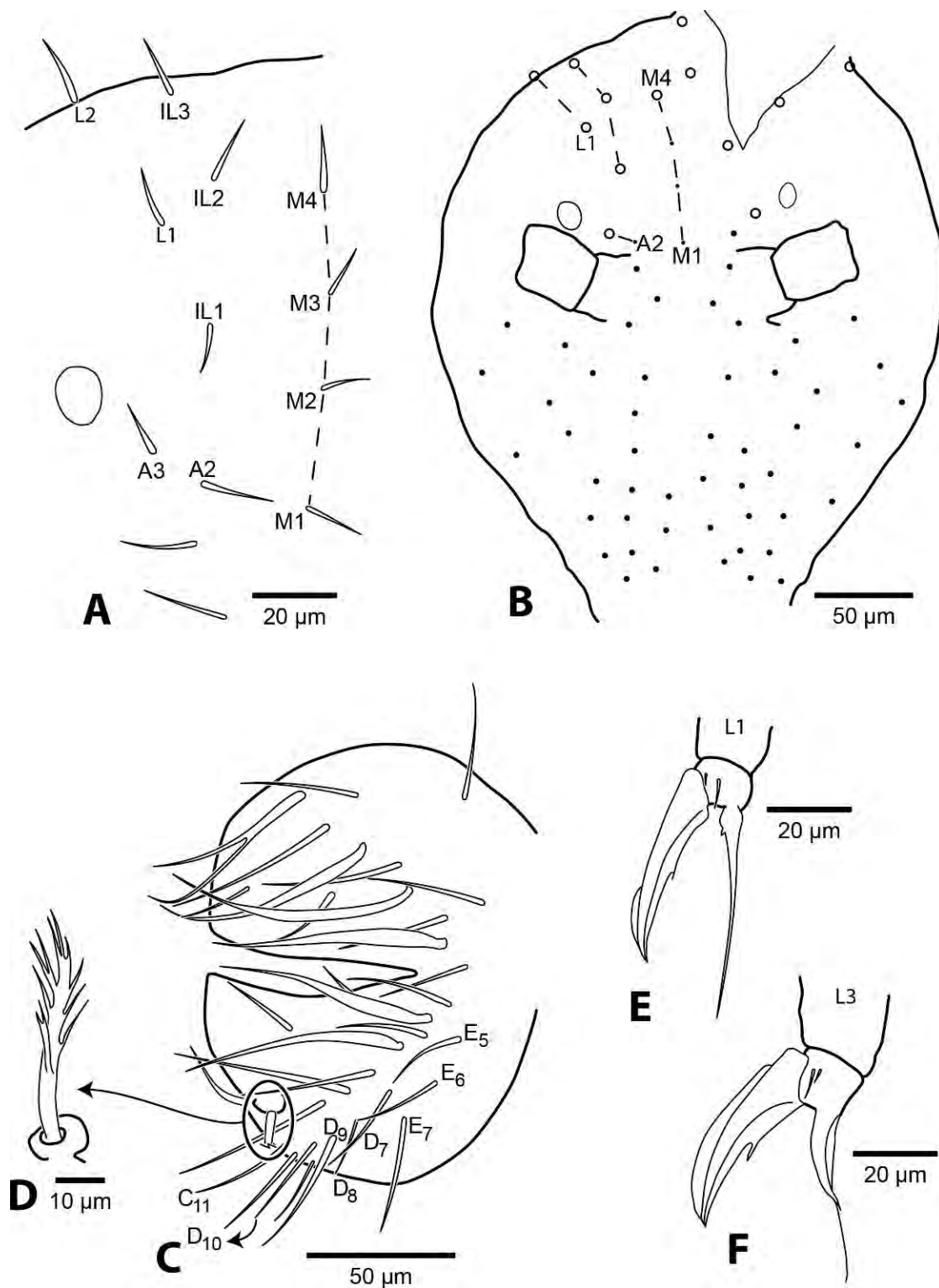


Figure 13. *Pygmarrhopalites incantator* n. sp., dots, and open circles represent microsetae, and spine-like setae, respectively: (A) detail of vertical setae of the head; (B) general chaetotaxy of face (C) complete chaetotaxy of small abdomen, lateral view; (D) female anal appendage; (E, F) pro- and metathoracic claw complex, respectively.

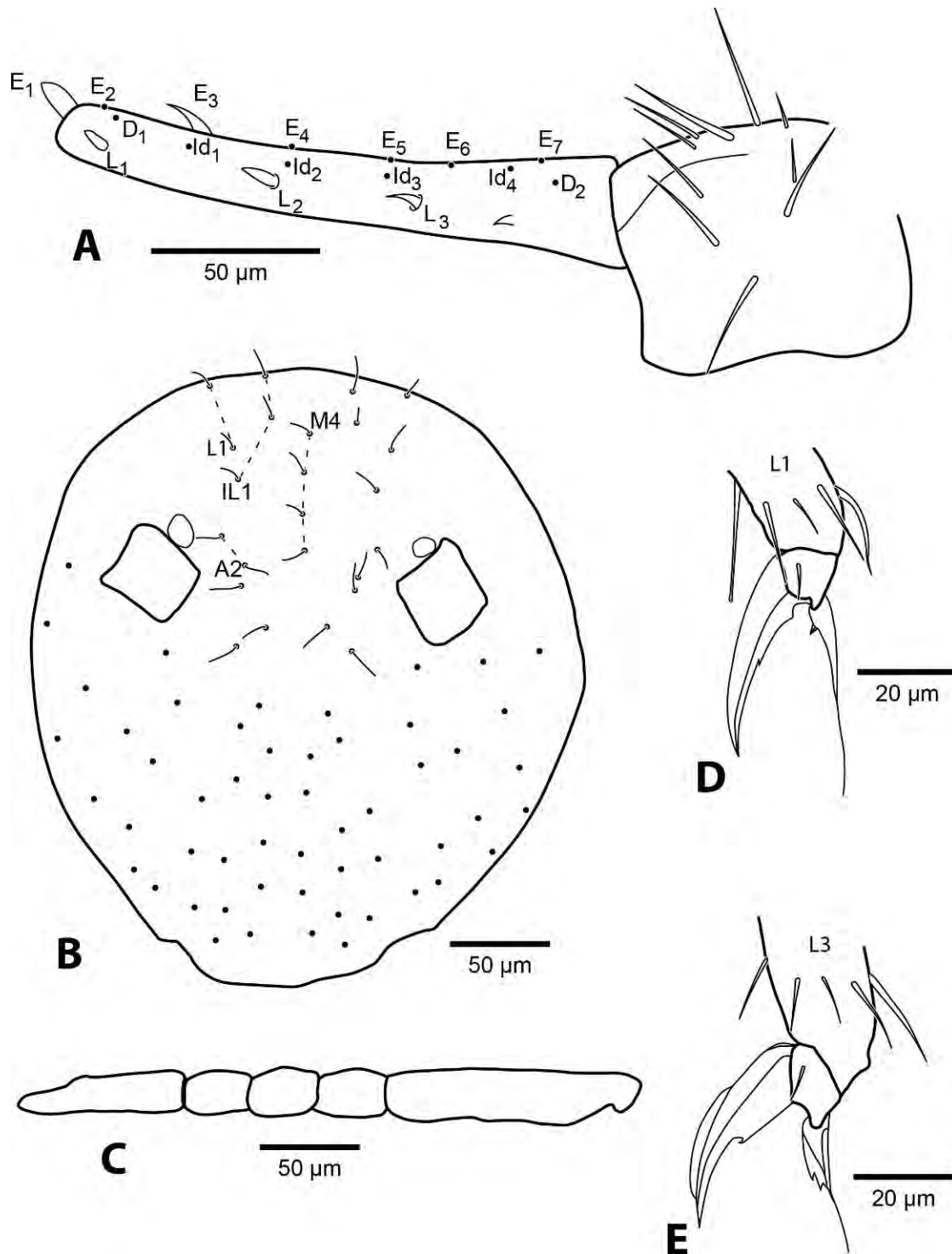


Figure 14. *Pygmarrhopalites* spp., dots represent regular acuminate setae: *Pygmarrhopalites incantator* n. sp.: (A) complete dorsal chaetotaxy of furcula, L = inner column, D = dorsal, Id = laterodorsal, E = outer column. *Pygmarrhopalites salemensis* n. sp.: (B) complete chaetotaxy of face; (C) fourth antennal segment; (D, E) pro- and metathoracic claw complex, respectively.

Monroe Co., Hidden Hand Cave, 3 mi W of Waterloo, 2 adult females and 1 juvenile, slide-mounted, 7 additional adults and juveniles in alcohol, pitfall traps in dark zone, 14–16 October 2009, SJ Taylor and FN Soto-Adames.

**Etymology:** The new species is named after the Salem Plateau region, where it seems to be widespread in caves.

**Description:** Largest individual 0.89 mm. Background color white, with dark orange spots on head covering

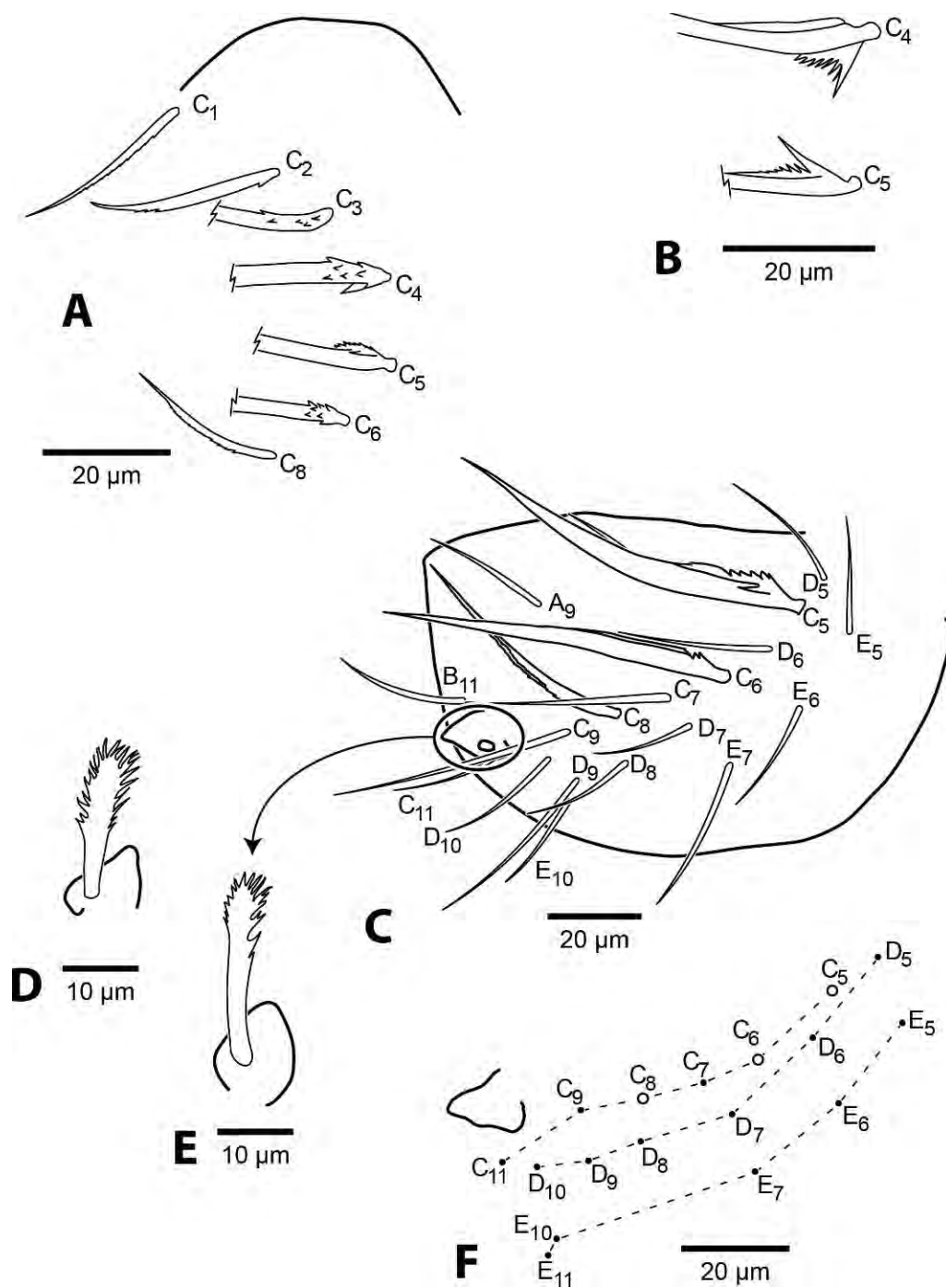
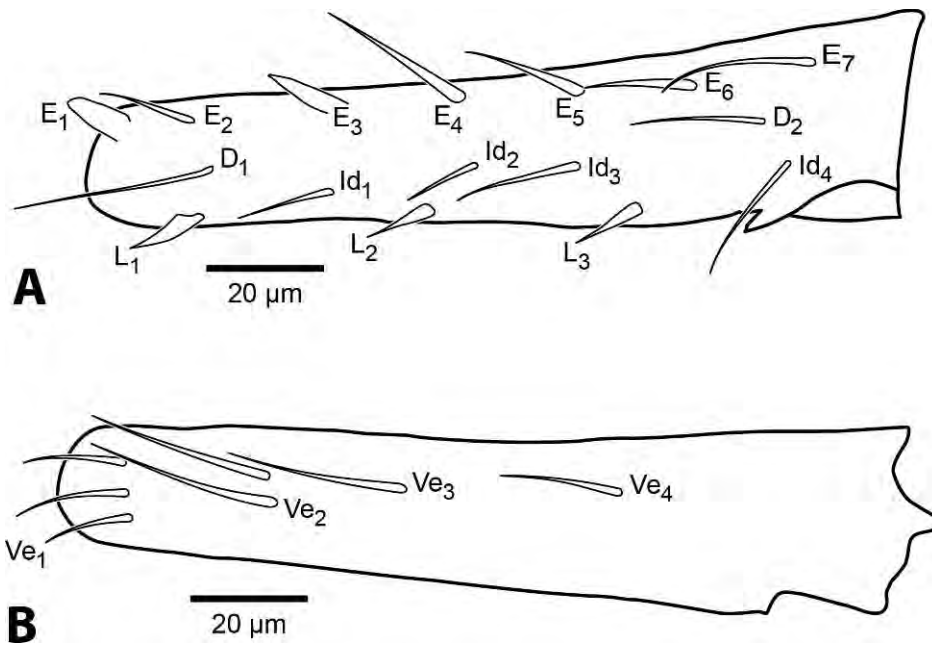


Figure 15. *Pygmarrhopalites salemensis* n. sp., chaetotaxy of female small abdomen, dots and open circles represent micro- and macrosetae, respectively: (A) series C setae of holotype, Stemler Cave; (B) setae C<sub>4</sub> and C<sub>5</sub>, female paratype from Stemler Cave; (C) complete chaetotaxy of lower valve, paratype from Spider Cave; (D, E) detail of female anal appendage, paratypes from Wanda's Water Fall and Stemler Cave, respectively; (F) organization of complete chaetotaxy of lower valve in holotype.

clypeus, area between antennal bases and vertex; genal and labral areas white; large abdomen with scattered orange spots dorsolaterally, sometimes forming ill defined stripes; small abdomen and all appendages white. Ant. 4 with 5 subsegments (Fig. 14C) with proportions as 40:10:10:10:30 of the total Ant. 4 length. Ant. 4 with 4 well defined whorls

of setae corresponding to the apical whorl on subsegment I and each one of subsegments II–IV; the number of setae/sensilla on each whorl as 10/10/12/12; basal whorls missing 2 sensilla present on distal whorls. Ant. 4 subapical sensilla capitate. Ant. 3 without basal bulge; sense organ with two rod sensilla in independent shallow depressions, setae Api





**Figure 16.** *Pygmarrhopalites salemensis* n. sp., complete dorsal chaetotaxy of dens: (A) dorsal, L = inner column, D = dorsal, Id = laterodorsal, E = outer column; (B) ventral.

and Ape appearing thin walled, basally swollen and with drawn out apices, but otherwise normally acuminate, Aai a rod sensilla. Eyes 1+1 in dark orange patch. All vertical head setae normal (Fig. 14B), M5 absent. Apical setae of outer maxillary lobe bifurcate; sublobular plate with three hairs. Labial papilla E with 4 guard setae. Small abdomen without denticles or spines, chaetotaxy of series C as in Fig. 15A: C1 simple and smooth or very finely serrate; C2–6 and C8 with basal serrations, denticles or teeth, and lateral extensions variously developed according to individuals and localities (Figs. 15B, C); setae C7 and C9 long but not basally enlarged; seta D2/F3=1.2 to 1.8 (mode=1.6; 3/7) C2/B2=0.9 to 1.1 (mode=1.1; 5/7) and C3/D3=1.8–3.1 (mode=2.0; 2/7); setae D7–10 present (Figs. 15C, F); female appendage apically square or rounded (Figs. 15D, E), with short serrations covering apical 1/3–2/3 and sitting on a heart-shaped papilla. Metatrochanter rectangular, with 4 anterior and 1 posterior setae. All claws with 1 inner tooth. Unguiculus with inner tooth large, single and basal on L1 and L2 (Fig. 14D), and absent or small, duplicated, and distal on L3 (Fig. 14E); apical unguicular filament acuminate and surpassing length of unguis on all legs. Tenaculum with 2 setae. Manubrium with 6+6 dorsal setae. Dens dorsally (Fig. 16A) with 3 inner (L), 6 dorsal (D1–2, Id1–4) and 7 external (E) setae; setae L1–3, E1 and E3 spine-like. Dens ventrally (Fig. 16B) with 2 unpaired setae. Mucro apically acuminate and 0.6–0.7× (mode=0.7; 4/7) as long as dens.

Remarks: One individual from Wanda's Waterfall Cave has 10/11/11/11 setae/sensilla on whorls I–IV. The two individuals from Stemler Cave are missing dental seta E6

on one dens but not the other. One individual from Stemler Cave has one proximal and one distal inner unguicular tooth on L1–2, but only the distal tooth on L3. One individual from Hidden Hand Cave has 3 tenacular setae.

*Pygmarrhopalites salemensis* n. sp., belongs to a group of Midwestern species characterized by having five subsegments on Ant. 4 and small abdomen series C setae sculptured or with lateral extensions. The five species in the group differ in details of the sculpturing of setae in series C, shape of the female appendage, number of dental spines, number of ventral unpaired setae on dens, and number of head vertical setae in series M (Table 4). The new species seems intermediate between the recently described *P. sapo* and *P. leonardwoodensis* Zeppelini, Taylor and Slay, 2009. The three species can be distinguished by dens chaetotaxy, pattern of sculpturing of small abdomen setae C3–6, and female appendage according to Table 4. In addition, in *P. leonardwoodensis* the inner tooth on the prothoracic claws is basal, while in *P. salemensis* the tooth is insert near the middle of the claw; *P. sapo* carries 3 guard setae in labial papilla E, whereas *P. salemensis* has 4 guard setae. Differences with other species are detailed in Table 4.

#### KATIANNIDAE

*Sminthurinus henshawi* (Folsom), 1896 — EU S5/G5

Locality: Bat Sump Cave

This is a common surface species widespread across North America. In Illinois, the species has been previously reported from Jackson, Champaign, Coles, Cook, DuPage, Kane, Lake, Randolph, Richland, and Woodford counties.

**Table 4. Diagnostic characters for species of *Pygmarhopalites* from the midwestern USA states having five subsegments on the fourth antennal segment and small abdomen setae in series C either sculptured or with lateral extensions.**

Species	Color	Number of Head Vertical M Seta	Small Abdomen Series C Setae Ornamentation	Small Abdomen Series C Setae Extensions		Female Appendage	Female Appendage Papilla	Number of Unpaired Ventral Setae on Dens	Spines on Dens <sup>a</sup>
				present	absent				
<i>P. salemensis</i> n. sp.	white, with extensive orange pattern	4	basally serrate	present	narrow paddle, short teeth symmetrical on apical 1/3–2/3	narrow paddle, heart-shaped	heart-shaped	2	5
<i>P. leonardwoodensis</i> Zeppelini, Taylor and Slay	white	4	single basal tooth or smooth	present or absent	narrow paddle, short teeth asymmetrical on apical 1/3	narrow paddle, heart-shaped	heart-shaped	2	5
<i>P. sapo</i> (Zeppelini and Christiansen)	white	4	denticulate	absent	narrow paddle, short teeth symmetrical on apical 1/3	narrow paddle, heart-shaped	heart-shaped	2	1
<i>P. madonnensis</i> (Zeppelini and Christiansen)	...	4	smooth	present	palmate, long apical teeth	narrow paddle, heart-shaped	heart-shaped	2	5
<i>P. lewisi</i> (Christiansen and Bellinger)	white	5	basally serrate	absent	narrow paddle, short teeth symmetrical on apical 1/3	narrow paddle, heart-shaped	heart-shaped	1	2
<i>P. ater</i> (Christiansen and Bellinger)	white	4	basally serrate	present	serrate, seta-like acuminate	narrow paddle, heart-shaped	elongate, pointed	2	1
<i>P. pygmaeus</i> (Wankel)	reddish brown	4	smooth	absent	narrow paddle, short teeth symmetrical on apical 1/3	narrow paddle, heart-shaped	circular	2	5

<sup>a</sup> When five dental spines are present they are always L1-3, E1 and E3; when two spines are present they are L1 and E1; when one spine is present it is always E1.

**Table 5. Comparison of springtail species recorded from caves in the Salem Plateau (Monroe and St. Clair counties, Illinois) by Lewis et al. (2003) relative to the number recorded in the present study.**

Cave	Lewis et al. (2003)	Present Study	Species in Common	Lewis et al. (2003) Species not Found in Present Study
Pautler Cave	2	4	2	0
Wanda's Waterfall Cave	1	22	0	<i>Pseudosinella</i> sp. nr. <i>argentea</i>
Spider Cave	1	7	1	0
Stemler Cave	1	15	1	0
Illinois Caverns	3	13	2	<i>Lethemurus missus</i>
Hidden Hand Cave	3	4	1	<i>Pygmarrhopalites carolinae</i> <i>Onychiurus "reluctus"</i>
Bat Sump Cave	2	12	1	<i>Sensillanura illina</i>
Total	13	77	8	5

*Sminthurinus henshawi* is common in caves, but this is the first record from this habitat in Illinois.

#### BOURLETIELLIDAE

*Bourletiella* sp.

Locality: Spider Cave

This is an early instar individual not identifiable to species. Members of this genus are not commonly found in caves. This is likely an accidental.

#### DICYRTOMIDAE

*Ptenothrix* sp.

Locality: Spider Cave

The single juvenile collected is not identifiable to species. Several species of *Ptenothrix* frequent caves, including *P. atra*, *P. marmorata*, and *P. maculosa*. In Illinois, *P. atra* is the species most commonly reported from caves.

#### DISCUSSION

Sampling of eight caves in the Salem Plateau region using a combination of methods yielded forty-nine species, sixteen of which represent new records for Illinois. The new records include four species described as new, four species that are likely new, but with insufficient material for proper descriptions, and three other forms that were assigned names but are sufficiently distinct that a study of additional material may show them to also represent new species. Eighteen species are reported for the first time from Illinois caves. Seventeen species are ranked as rare for the state (S1), but eight of these are widely distributed across North America and the state ranking is either an artifact of the relatively poor knowledge of the fauna of the state or the result of unresolved taxonomic issues (e.g., *Onychiurus pipistrellae* n. sp.). Some of the other species ranked as rare are probably truly rare in the state, even if they are widespread elsewhere on the continent, because they represent the limit of the distributional range for the species (e.g., *Pseudosinella aera*, *Folsomia bisetosa*, *Proisotoma sepulcralis*, and *Megalothorax tristani*). Others, such

as *Pygmarrhopalites* spp., are probably truly rare and endemic to the region.

Twelve species have morphological characters that suggest some level of adaptation to caves and are classified as either troglobionts, or eu- or subtroglophiles. Seven species (*Ceratophysella lucifuga*, *Onychiurus pipistrellae* n. sp., *Lethemurus missus*, *Pygmarrhopalites fransjanssnes* n. sp., *P. incantator* n. sp., *P. sapo*, and *P. salemensis* n. sp.) are currently known only from caves, although *L. missus* and perhaps *O. pipistrellae* n. sp. are widely distributed across unconnected cave systems, suggesting they are able to migrate through protected surface habitats. Most collections of *Oncopodura iowae* have been made in caves, but recent surface collections in cave bearing areas suggest a mechanism to explain its widespread distribution. It is possible that similar mechanisms may support the movement and dispersal of *L. missus* and *O. pipistrellae* n. sp. through surface leaf litter. *Folsomia candida*, *F. stella*, *Pseudosinella argentea*, *P. aera*, and *Coecobrya tenebricosa* show weak morphological adaptations to caves, all are widely distributed across North America and surface populations are not rare.

Seven of the caves surveyed were previously sampled by Lewis et al. (2003). They published findings relating primarily to troglobionts, whereas the present study reports on findings for springtails of all ecological classifications found in caves. In addition, our study focused only on springtails, whereas Lewis et al. (2003) surveyed all cave invertebrates. In all instances each cave sampled in the present survey yielded more springtail species than reported by Lewis et al. (2003) (Table 5). Most species reported by Lewis et al. (2003) were collected again during the present survey. Five records listed by Lewis et al. (2003) were not confirmed by the present study (Table 5). The absences of collections of *Pseudosinella* from Wanda's Waterfall Cave, of *Pygmarrhopalites carolinae* (Christiansen and Bellinger), of *Onychiurus "reluctus"* from Hidden Hand Cave, and of *Sensillanura illina* (Christiansen and Bellinger) from Bat Sump Cave during the present survey is curious, given that these were the



shortest cave included in the present study and, with the exception of Wanda's Waterfall Cave, we sampled nearly the full length of the accessible passages at these sites. *Pygmarhopalites carolynae* and *P. salemsis* n. sp., the only *Pygmarhopalites* collected in Hidden Hand Cave, differ sharply in color pattern, small abdomen setae ornamentation and female anal appendage, and hind claw morphology, and it is not likely the two species could be confused. *Sensillanura illina* is probably a troglaxene or subtroglophile, and although the species was not collected inside Bat Sump Cave during our visits, it was taken in surface leaf litter sampled near the cave entrance on the same day the cave was visited.

The absence of *Lethemurus missus* in samples from Illinois Caverns is not surprising. Illinois Caverns is a large, complex system, and most areas of the system were not sampled during the present study due to time constraints. Sampling effort focused on those sections of the cave most impacted by the large number of visitors that tour this system every year; the cave has since been closed to the public in an attempt to help manage white nose syndrome of bats.

It is telling about the general state of our knowledge of the springtail fauna of Illinois that leaf litter samples collected inside caves at the entrance, have yielded three new species and six new state records of what are clearly surface leaf litter species.

It is of some concern that *Coecobrya tenebricosa*, the only member of the *Sinella-Coecobrya* genera complex, a complex of typically eu/subtroglophile or troglobiont species, is an invasive species. It is not clear what the role of this introduced springtail species might be in fragile cave ecosystems. Caves in the Shawnee Hills, south of the Salem Plateau region, harbor three native species of eutroglophiles/troglobionts in the genus *Sinella* (Christiansen and Bellinger, 1998). It is possible that the introduced form could move south, invade caves, and extirpate the native springtail species.

The relatively large number of new species and records for the state and the potential threat to native eutroglophiles and troglobionts by introduced species point to the need for continued detailed, taxon-focused sampling of cave systems in Illinois. Only through intense sampling will we be able to identify communities under threat and have the information needed to make more effective management and conservation decisions.

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