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Tardigrades of the Tree Canopy: *Milnesium swansoni* sp. nov. (Eutardigrada: Apochela: Milnesiidae) a new species from Kansas, U.S.A.

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Abstract

Milnesium swansoni **sp. nov.** is a new species of Eutardigrada described from the tree canopy in eastern Kansas, USA. This species within the order Apochela, family Milnesiidae, genus *Milnesium* is distinguished by its smooth cuticle, narrow buccal tube, four peribuccal lamellae, primary claws without accessory points, and a secondary claw configuration of [3-3]-[3-3]. The buccal tube appears to be only half the width of the nominal species *Milnesium tardigradum* for animals of similar body length. The species adds to the available data for the phylum, and raises questions concerning species distribution.

Key words: Four peribuccal lamellae, Thorpe morphometry, Tardigrada, Canopy diversity

Introduction

Milnesium Doyère, 1840 is a genus of predatory limno-terrestrial tardigrades within the Order Apochela and the Family Milnesiidae with unique morphological characteristics (Guil 2008). The genus is distinct within the phylum Tardigrada for lacking placoids, but having peribuccal papillae, lateral papillae, peribuccal lamellae, a wide buccal tube, and separated double claws (Kinchin 1994). The genus was considered monotypic and cosmopolitan, receiving little attention since the first species, *Milnesium tardigradum* Doyère, 1840 was described. Even though Rahm (1931) reported *Milnesium tardigradum trispionosa* Rahm, 1931 as having three spines at the end of the body, and Ramazzotti (1962) described *Milnesium tardigradum granulatum* Ramazzotti, 1962 with a granulated cuticle, Ramazzotti and Maucci (1983) declined to raise either to even subspecies level. In 1975, Schuster *et al.* speculated there may be more than one species of *Milnesium* and presented a scanning electron micrograph showing a specimen with four large and two small buccal lamellae from Tanzania. In New Zealand examples attributed to *Milnesium tardigradum* were reported as having a cuticle that was uniformly pitted by shallow depressions (Horning *et al.* 1978).

The first new species was described by Binda and Pilato (1990), *Milnesium brachyungue* Binda & Pilato, 1990 from Tierra del Fuego, Chile, based on significantly different proportions of the buccal apparatus. Currently, the genus has been expanded to include 26 species (Degma *et al.* 2015).

Milnesium species with only four peribuccal lamellae (instead of six) were first described by Pilato and Binda (1991) with *Milnesium tetralamellatum* Pilato & Binda, 1991 from Tanzania. The range of this species has since been extended to include the Seychelles Islands, Indian Ocean and Spain (Pilato *et al.* 2004; Guil 2008). A second species with four peribuccal lamellae from the Seychelles Islands is *Milnesium reticulatum* Pilato, Binda & Lisi, 2002 (Pilato *et al.* 2002), and more recently, Meyer *et al*. (2013) reported the first new world species, *Milnesium lagniappe* Meyer, Hinton & Dupre, 2013.

Many factors have contributed to the recent increased reporting of new *Milnesium* species including the use of a number of different microscopy techniques and re-assessment of the morphological characters. A re-assessment

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of the morphology was provided by Tumanov (2006) who used modern morphometric analysis and proposed novel measurement rules for the claws, when he described five new species. This was followed by Michalczyk *et al.* (2012a, 2012b) who used modern morphological characters to re-describe the *Milnesium* type species, *M. tardigradum*, and provided a neotype series from Germany, though the original holotype was collected from Saint-Maur, France. They defined a suite of characteristics to differentiate species within the genus declaring that the difference in the number and size of sub-branches of the secondary claw should be used as a species character. As a result they have challenged the validity of all reports of *M. tardigradum* outside of Europe. To support the decision process for separating a growing number of species, Michalczyk *et al.* (2012) have divided the genus into two groups, those with smooth cuticle (*tardigradum*) and others with a reticulate cuticle (*granulatum*) and developed an excellent key to the species.

This report adds the second species outside Africa with only four peribuccal lamellae. A population of both sexes of the new species was found in the forest canopy in a transitional deciduous forest on the edge of the great tall grass prairie in north eastern Kansas, U.S.A.

TABLE 1. Location, substrate and habitat of *Milnesium swansoni* **sp. nov.** collections.

Locations		Substrates		Habitats	
Sites	Count	Species of Tree	Count	Moss	Lichen
Baker Prairie	9	Shagbark Hickory (*Carya ovate*)	6	2	28
Black Jack Battlefield	4	Green Ash (*Fraxinus pennsylvanica*)	1	3	12
Baker Campus	19	Virginia Creeper (*Parthenocissus quinquefolia*)	2	0	11
Baker Woods	32	Honey Locust (*Gleditsia triacanthos*)	6	2	9
Baker Wetlands	27	American Elm (*Ulmus americana*)	2	2	6
KU Rice Woods	12	Red Oak (*Quercus rubra*)	2	2	7
KU Field Station	41	Hackberry (*Celtis* sp.)	5	0	8
Konza Prairie LTER	3	Cottonwood (*Populus* sp.)	3	0	7
		Ponderosa Pine (*Pinus ponderosa*)	1	0	5
		Red Mulberry (*Morus rubra*)	1	2	2
		Black Walnut (*Juglans nigra*)	4	0	3
		Chinquapin Oak (*Quercus muehlenbergii*)	3	1	1
		Sugar Maple (*Acer saccharum*)	1	0	1
		Sycamore (*Acer pseudoplatanus*)	1	1	0
		Burr Oak (*Quercus macrocarpa*)	1	0	1
		Post Oak (*Quercus stellata*)	1	0	0
		Eastern Red Cedar (*Juniperus virginiana*)	1	0	0
Totals	115		41	15	100

Material and methods

Specimens were extracted from small habitat samples of moss or lichen (Miller 1997) and collected from different heights on substrate (tree) species throughout mixed deciduous canopies in north eastern Kansas (Table 1). Researchers ascended into the canopy using double rope technique (Haefke *et al.* 2013), allowing collections up to 20 meters and samples to be stratified at 3 meter increments. Samples were hydrated for 24 hours in filtered, commercial bottled spring water and examined using a dissecting microscope at 30x with reflected light. Three 1

ml subsamples were searched for tardigrades and density was measured as the number of specimens per sample. Tardigrades were captured with an Irwin loop (Schram & Davidson 2012) and preserved on glass slides using PVA (Salmon 1951). Tardigrades were identified and imaged using an Olympus BX60 DIC (differential interference contrast) microscope equipped with a 12 megapixel camera. Digital images have not been enhanced except to adjust contrast and brightness to achieve a printable image (McInnes 2001).

Morphological measurement. Morphometric values (Tables 2, 3) were measured with the Olympus cell Sense Standard® ver. 1.6 software. All linear measurements are in micrometers (µm) and *pt* ratios (Pilato 1981) are expressed as the percent ratio of a trait to the buccal tube length. Ten tardigrades of each sex were measured. Only fully elongated tardigrades were measured for body length, excluding legs IV. Trait values were only measured when in the horizontal plane of the slide and according to the protocols of Tumanov (2006). Morphometric data follow the Apochela ver. 1.1 template from the Tardigrada Register (Michalczyk & Kaczmarek 2013); which includes the anterior and posterior buccal tube widths, and the configuration of the number of secondary claw points as described by Michalczyk *et al*. (2012). We have added the measurement of the basal thickening for each claw. We have followed the precedent of Bartels *et al.* (2014) in presenting allometry (a*) data in Tables 2 and 3 to remove the effects of body size in the morphometric analysis (Bartels *et al*. 2011).

Identification was based on Ramazzotti and Maucci (1983), Pilato and Binda (2010), and Michalczyk and Kaczmarek (2012). Nomenclature was based on Guidetti and Bertolani (2005), Degma and Guidetti (2007), and Degma *et al.* (2009-2015).

Ecological measurements. Miller *et al*. (2013) have provided considerable support for the presence of tardigrade communities in the canopy. The ecological relationships of habitat, substrate, and level selection of the new species were tested by comparing the actual observations against an expectation of uniformity with the number of individuals found at the base level of the trees where sampling has been conducted for 200 years A chi-square value greater than the critical value of 3.84 was interpreted to be significantly different ($P < 0.05$, 1 df) from normal variation in distribution.

Taxonomic account

Phylum: Tardigrada (Spallanzani, 1777)

Class: Eutardigrada Richters, 1926

Order: Apochela Schuster, Nelson, Grigarick & Christenberry, 1980

Family: Milnesiidae Ramazzotti, 1962

Genus: *Milnesium* Doyère, 1840

***Milnesium swansoni* sp. nov.**
Figure 1, Tables 2–4

Diagnosis. *Milnesium* without eyes, with a smooth cuticle, with short, narrow buccal tube, and four peribuccal lamellae. Main branch of claw long, without accessory points. Basal thickening wide, basal spurs small but present with a secondary branch configuration of [3-3]-[3-3].

Description of the holotype. Female *Milnesium swansoni*, body transparent, length 330 µm (BL *pt = 1,430%*), eye spots not found. Cuticle smooth without ornamentation, pores, reticulations, or gibbosities. Mouth with six peribuccal papillae, four peribuccal lamellae and two lateral papillae. Buccal tube short, narrow, and cylindrical; length 23.1 µm, width 9.2 µm (BTW *pt = 39.7*). Stylet support attachment at 15.5 µm (SSA *pt = 67*). Pharyngeal bulb elongated, pear-shaped, without apophyses, placoids, or septula. Claws of *Milnesium* type, main branch separated from secondary branch without accessory points. Secondary branch with base and primary, secondary, and basal spurs [3-3]-[3-3]. Basal thickening wide. Length of primary branches, secondary branches, basal spurs and the width of basal thickenings are given in Table 2, with all measurements and ranges for paratypes. Individual measurements for all type material is provided in the Supplementary Data.

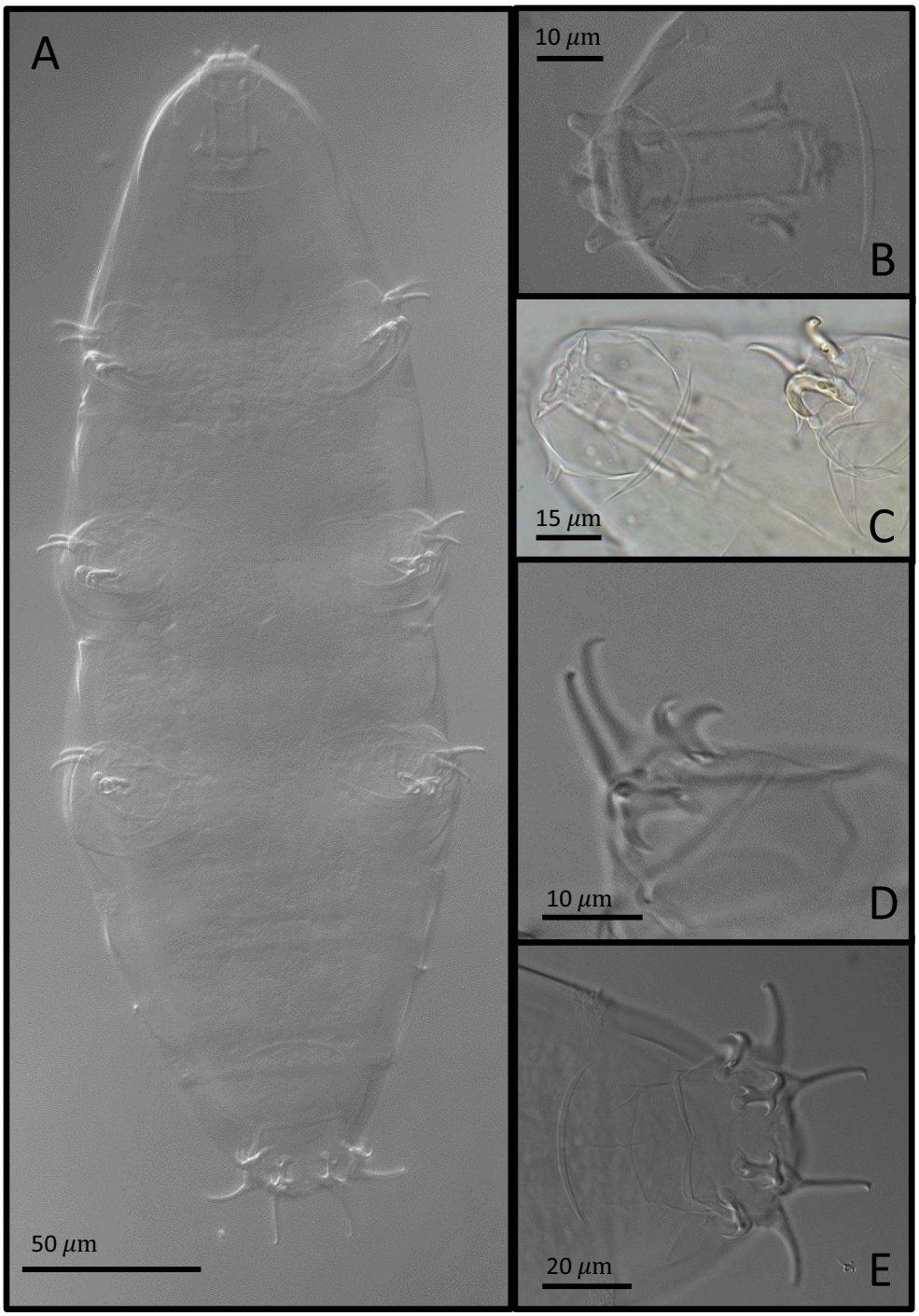

FIGURE 1. *Milnesium swansoni* **sp. nov.** A. Body of holotype showing smooth cuticle, B. Mouth showing holotype with narrow buccal tube and four peribuccal lamellae, C. showing secondary characteristic of male paratype, D. Claw I of paratype showing [3-3] claw formation, E. showing claw IV with [3-3] claw.

Type location. Collected from between 9 and 12 meters high on 20th June 2014, by Alexander Young: lichen on bark of a Virginia creeper (*Parthenocissus quinquefolia*) in the Baker University Wetlands (38.91426N, -95.22858W) Douglas County, Kansas, U.S.A.

Etymology. The new species is named in honour of Kent Swanson Jr. whose family established the Swanson Family Scholarship, for which author AY was a recipient.

Holotype. Deposited at the California Academy of Science, San Francisco, California, U.S.A. Slide Collection number: CASIZ-198191.

TABLE 2. Female morphometric measurements for *Milnesium swansoni* **sp. nov.**

Female Character	N	RANGE		MEAN		SD		Holotype		a*
		µm	*pt*	µm	*pt*	µm	*pt*	µm	*pt*	µm
Body length	5	329–404	*1276–1683*	362	*1412*	338	*161*	330	*1430*	—
Peribuccal papillae length	5	4.2–5.9	*18.2–21.7*	5.1	*20.4*	0.6	*1.5*	4.2	*18.2*	5.2
Buccal tube										
Length	5	23.1–8.0	-	25.5	-	2.3	-	23.1	*100*	25.3
Stylet Support	5	15.5–19.1	*66.6–68.2*	17.2	*67.5*	1.7	*0.7*	15.5	*67*	17.2
Anterior width	5	9.4–11.9	*39.0–42.8*	10.5	*41.3*	1.0	*1.7*	9.39	*40.7*	10.4
Standard width	5	9.2–11.8	*39.2–42.2*	10.3	*40.3*	1.2	*1.3*	9.16	*39.7*	10.2
Posterior width	5	9.3–11.8	*39.9–42.2*	10.4	*40.7*	1.1	*1.0*	9.3	*40.3*	10.4
Standard width/length ratio	5	0.4–0.4	-	40%	-		-	0.39	*1.69*	41%
Posterior/anterior width ratio	5	0.9–1.0	-	99%	-		-	0.99	*4.29*	100%
Claw 1 lengths										
External primary branch	4	11.6–14.4	*50.3–53.7*	12.8	*51.8*	1.4	*1.8*	11.6	*50.3*	12.9
External base + secondary branch	5	8.5–10.3	*35.0–36.6*	9.2	*36.0*	0.8	*0.8*	8.45	*36.6*	9.1
External spur	3	1.4–2.2	*6.2–7.7*	1.7	*7.0*	0.4	*1.0*	1.44	*6.24*	1.9
Internal primary branch	4	11.6–13.0	*48.4–52.2*	12.4	*50.3*	0.7	*1.9*	11.6	*50.3*	12.4
Internal base + secondary branch	5	9.0–11.4	*37.2–40.8*	9.9	*38.9*	1.2	*1.5*	8.97	*38.9*	9.7
Internal spur	2	2.0–2.0	*7.5–8.5*	2.0	*8.0*	0.0	*0.7*	-	-	2.0
Basal thickening width	4	2.1–3.1	*9.0–11.3*	2.7	*10.5*	0.5	*1.0*	2.07	*8.98*	2.7
Claw 2 lengths										
External primary branch	5	11.7–14.0	*49.1–52.0*	12.8	*50.4*	1.3	*1.3*	11.7	*50.7*	12.9
External base + secondary branch	5	8.8–10.7	*36.5–39.9*	9.7	*37.9*	1.0	*1.6*	8.9	*38.6*	9.7
External spur	1	1.8–1.8	*7.8–7.8*	1.8	*7.8*	?	*?*	1.8	*7.81*	?
Internal primary branch	4	12.5 –14.9	*52.3–54.4*	13.3	*53.3*	1.4	*1.0*	12.5	*54.4*	13.0
Internal base + secondary branch	2	8.9–11.5	*41.1–42.9*	9.9	*41.8*	1.1	*1.0*	9.6	*41.5*	10.3
Internal spur	3	1.9–2.3	*7.7–8.3*	2.1	*8.0*	0.2	*0.3*	?	*?*	2.2
Basal thickening width	5	2.3–3.0	*8.1–12.4*	2.4	*9.7*	0.4	*1.9*	2.26	*9.8*	2.4
Claw 3 lengths										
External primary branch	5	11.4–14.2	*49.6–51.2*	12.8	*50.3*	1.3	*0.7*	11.4	*49.6*	12.8
External base + secondary branch	5	9.0–11.1	*38.6–42.0*	10.2	*40.2*	1.0	*1.8*	9.0	*38.9*	10.2
External spur	0	?–?	*?–?*	?	*?*	?	*?*	?	*?*	?
Internal primary branch	4	12.2–13.9	*49.7–53.4*	12.8	*51.4*	1.0	*1.9*	12.3	*53.4*	12.6
Internal base + secondary branch	5	8.3–10.4	*35.9–38.9*	9.5	*37.4*	0.9	*1.7*	8.29	*35.9*	9.6
Internal spur	0	?–?	*?–?*	?	*?*	?	*?*	?	*?*	?
Basal thickening width	5	1.7–2.7	*7.5–9.9*	2.2	*8.6*	0.4	*1.0*	1.74	*7.55*	2.3
Claw 4 lengths										
Anterior primary branch	5	14.0–16.7	*58.3–62.2*	15.4	*60.3*	1.5	*1.7*	14.1	*61*	15.4
Anterior base + secondary branch	5	9.7–11.6	*41.4–43.0*	10.7	*42.1*	0.9	*0.7*	9.7	*41.9*	10.6
Anterior spur	3	1.9–2.2	*7.0–8.2*	2.0	*7.6*	0.2	*0.8*	1.9	*8.15*	2.0
Posterior primary branch	4	14.1–17.6	*58.4–62.8*	16.1	*61.3*	1.9	*2.5*	?	*?*	16.0
Posterior base + secondary branch	5	9.6–11.5	*39.9–43.1*	10.6	*41.8*	0.9	*1.5*	9.57	*41.5*	10.6
Posterior spur	3	1.4–1.7	*6.2–6.2*	1.6	*6.2*	0.2	*0.0*	1.44	*6.24*	1.6
Basal thickening width	5	2.8–3.8	*10.7–14.1*	3.1	*12.3*	0.4	*1.4*	2.82	*12.2*	3.2

A question mark indicates a value that could not be accurately measured. An em-dash indicates a value that is not mathematically applicable.

TABLE 3. Male morphometric data for *Milnesium swansoni* **sp. nov**.

Male Character	N	RANGE		MEAN		SD		a*
		µm	pt	µm	pt	µm	pt	µm
Body length	5	381–448	1165–1493	417	1376	25	133	-
Peribuccal papillae length	3	3.9–4.5	14.1–15.7	4.2	14.7	0.3	0.8	4.2
Buccal Tube								
Length	5	25.8–36.1	-	30.6	-	4.1	-	30.7
Stylet Support	5	17.8–24.2	67.0–71.5	21.1	69.0	2.5	1.9	21.2
Anterior width	5	9.3–12.5	33.3–36.8	10.6	34.7	1.3	1.3	10.7
Standard width	5	9.1–12.4	33.4–36.3	10.6	34.6	1.4	1.2	10.6
Posterior width	5	9.5–12.9	34.8–36.7	10.9	35.7	1.5	0.9	10.9
Standard width/length ratio	5	33%–36%	-	35%	-		-	34%
Posterior/anterior width ratio	5	100%–100%	-	103%	-		-	103%
Claw 1								
External primary branch	4	14.2–17.4	51.5–55.4	15.8	54.1	1.4	2.0	15.8
External base + secondary branch	5	10.1–14.3	38.2–42.4	12.2	39.8	1.5	1.6	12.3
External spur	0	?–?	?–?	?	?	?	?	?
Internal primary branch	5	14.3–18.6	51.2–56.4	16.4	53.8	1.7	2.4	16.5
Internal base + secondary branch	4	10.5–14.2	39.2–41.4	12.7	40.8	1.6	.9	12.8
Internal spur	3	2.5–4.3	9.1–11.8	3.5	10.8	0.9	1.5	3.9
Basal thickening width	4	4.5–4.8	13.0–16.6	4.7	14.8	0.1	1.5	4.7
Claw 2								
External primary branch	4	14.9–19.6	54.1–58.6	16.9	56.2	2.0	2.3	17.1
External base + secondary branch	5	10.7–14.1	39.1–43.3	12.7	41.6	1.5	1.7	12.7
External spur	2	3.4–3.7	10.7–13.4	3.5	12.1	0.2	2.0	3.6
Internal primary branch	5	15.0–19.5	54.1–59.7	17.1	56.2	1.7	2.5	17.3
Internal base + secondary branch	5	10.1–14.0	38.7–42.7	12.3	40.1	1.8	1.7	12.2
Internal spur	2	3.4–3.5	9.7–10.7	3.4	10.2	0.1	0.7	3.5
Basal thickening width	4	3.6–4.3	12.0–13.9	3.8	13.2	0.3	0.8	3.8
Claw 3								
External primary branch	5	15.8–21.3	58.4–62.4	18.5	60.5	2.3	1.8	18.5
External base + secondary branch	5	11.0–13.8	38.2–42.5	12.3	40.5	1.2	1.8	12.4
External spur	2	3.0–3.6	8.4–11.5	3.3	9.9	0.4	2.2	3.0
Internal primary branch	4	14.8–20.2	54.2–57.4	16.9	56.1	2.4	1.4	17.0
Internal base + secondary branch	5	10.1–14.1	36.8–39.9	11.7	38.2	1.6	1.3	11.7
Internal spur	4	2.7–3.6	9.7–12.6	3.2	10.6	0.4	1.3	3.3
Basal thickening width	5	2.7–4.1	9.8–12.9	3.4	11.0	0.6	1.5	3.3
Claw 4								
Anterior primary branch	5	15.9–22.5	61.6–65.5	19.4	63.5	2.6	1.5	19.5
Anterior base + secondary branch	4	11.8–15.5	42.2–45.9	13.7	43.5	1.5	1.6	13.8
Anterior spur	2	1.6–1.6	5.9–6.4	1.6	6.1	0.0	0.3	1.6
Posterior primary branch	5	17.3–23.2	64.3–67.6	20.2	66.2	2.4	1.3	20.3
Posterior base + secondary branch	5	11.0–14.4	39.8–44.7	13.0	42.6	1.4	1.9	13.1
Posterior spur	2	1.0–2.5	3.6–6.8	1.7	5.2	1.0	2.3	2.0
Basal thickening width	5	2.8–5.0	10.2–15.4	4.1	13.3	0.9	2.0	4.4

Paratypes. Four female and five male for a total of nine paratypes deposited at the California Academy of Science, San Francisco, California, U.S.A. Slide Collection numbers: CASIZ-198192, CASIZ-198193, CASIZ-198194, CASIZ-198195, CASIZ-198196, CASIZ-198197, CASIZ-198198, CASIZ-198199, CASIZ-198200 (details in Supplementary Data).

Sexual dimorphism. Species exhibits qualitative sexual dimorphism in the structure of claw I. Males exhibit wide, thick secondary claws lacking sub-branches (Table 3 and Supplementary Data).

A question mark indicates a value that could not be accurately measured. An em-dash indicates a value that is not mathematically applicable.

Eggs. Oval, smooth, deposited in exuvium.

Habitat. The new species was found at all nine of the collection sites indicating wide distribution within the region (Table 1). One-hundred specimens were extracted from lichen habitat and 15 specimens from moss habitat on 38 of the 135 trees climbed, representing 15 of the17 tree species sampled. The new species did not show selection of substrate (tree species) or habitat (moss or lichen).

The new species was collected from ground level up to 20 m with 18, 27, 39, and 31 specimens found at levels 1, 2, 3, and 4 respectively. *Milnesium swansoni* **sp. nov.** was found to occur significantly more often at the three upper levels than was expected for its occurrence to be considered uniform with the base level 1 (X^2= 4.5, 24.5, 9.4 respectively). Thus a preference for upper canopy habitat is suggested.

Differential diagnosis. The new species is in the *tardigradum* group with a smooth cuticle but differs from all species in the group except *M. tetralamellatum* by having only four peribuccal lamellae. It differs from *M. tetralamellatum* by its [3-3]-[3-3] vs [2-3]-[3-2] claw configuration. The new species also differs from *M. tetralamellatum* by not having eyes or claw accessory points, and by having a narrower buccal tube (BTW *pt = 40.2* vs BTW *pt = 49*), and a more posterior stylet support attachment point (SSA *pt = 68* vs SSA *pt =64*). Additionally, it is distinct among the other *Milnesium* with four peribuccal lamellae for its combination of BTW and SSA ranges (Figure 2). The new species is easily distinguished from the other species described from North America with four peribuccal lamellae, *M. lagniappe,* by the smooth cuticle, the claw configuration, the narrower buccal tube and a more anterior attachment point for the stylet support bar (Table 4).

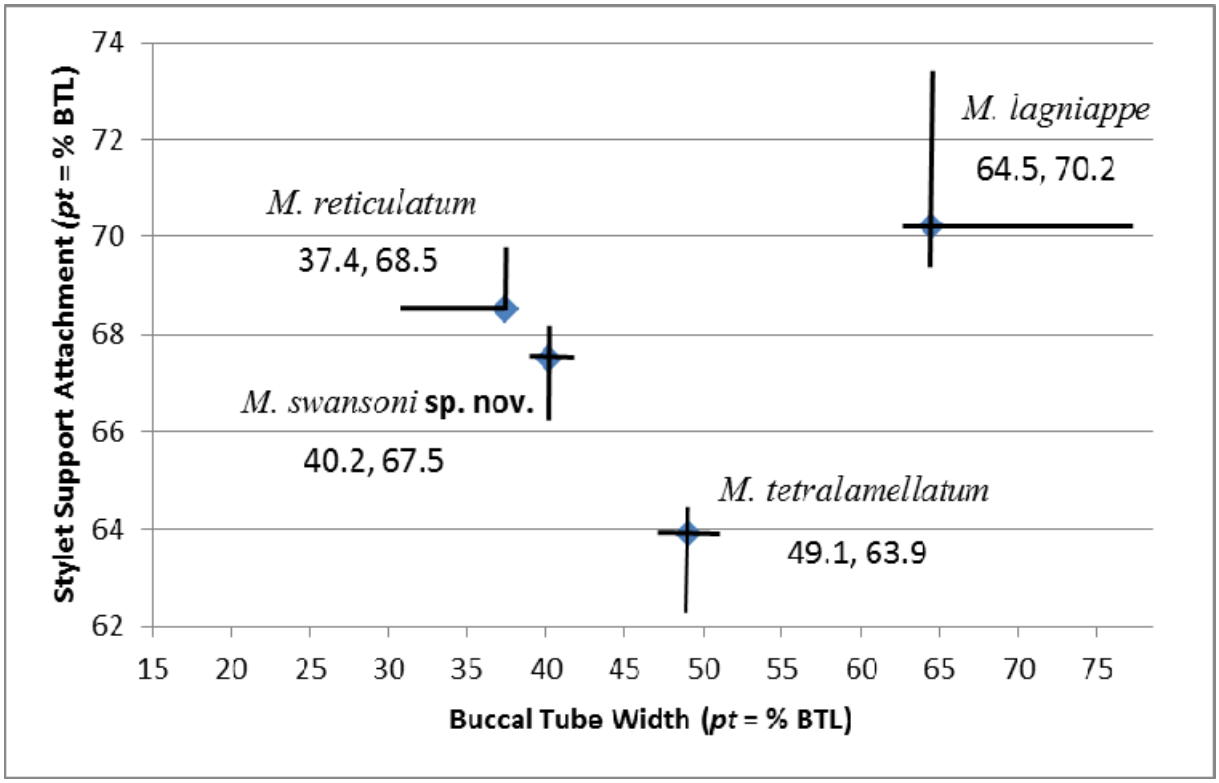

FIGURE 2. Tardigrade species with four peribuccal lamellae group, comparison of *pt* values for Buccal Tube Width (BTW) vs Stylet Support Attachment (SSA) point. *pt* = trait/Buccal Tube Length. Error bars = trait range (BTW/SSA). The numbers in the graph indicate the stylet support attachment and buccal tube width *pt* values of the holotype specimen of each species.

TABLE 4. Comparison of diagnostic characters of *Milnesium* species within the *tetralamellatum* group.

Species	Cuticle surface	Peribuccal lamellae	Eyes	Accessory points	Claw configuration	pt BL	pt BTW	pt SSA
M. reticulatum	gibbosities	4	yes	present	[2-3]-[3-2]	1058	37.4	68.5
M. lagniappe	sculptured	4	no	present	[2-3]-[3-2]	1430	64.5	70.2
M. tetralamellatum	smooth	4	yes	present	[2-3]-[3-2]	1418	49.1	63.9
M. swansoni **sp. nov.**	smooth	4	no	absent	[3-3]-[3-3]	1597	40.2	67.5

Key: *pt* = trait / buccal tube length *100, BL = Body Length, BTW—Buccal Tube Width, SSA = Stylet Support Attachment.

Discussion

Due to the clarification of the nominal species *M. tardigradum* by Michalczyk & Kaczmarek (2012) all specimens identified outside Europe as *M. tardigradum* are suspect and should receive heavy scrutiny given the numerous species additions to the genus in recent years.

TABLE 5. Proposed expansion of species groups within the genus *Milnesium.*

Cuticle	Secondary Claw branching	Peribuccal lamellae Six (6)	Four (4)
M. granulatum	[2-2]-[2-2]	*M. beasleyi*	
(granular cuticle)	[2-2]-[2-2]	*M. katarzynae*	*M. reticulatum*
	[2-3]-[2-2]	*M. berladnicorum*	*M. lagniappe*
	[2-3]-[3-2]	*M. krzysztofi*	
	[3-3]-[3-3]	*M. alabamae*	
	[3-3]-[3-3]	*M. granulatum*	
	[3-3]-[3-3]	*M. beatae*	
	[3-3]-[3-3]	*M. argentinum*	
M. tardigradum	[2-2]-[2-2]	*M. almatyense*	
(smooth cuticle)	[2-2]-[2-2]	*M. kogui*	
	[2-2]-[2-2]	*M. dujiangensis*	
	[2-3]-[2-2]	*M. jacobi*	
	[2-3]-[3-2]	*M. reductum*	*M. tetralamellatum*
	[2-3]-[3-3]	*M. tardigradum* s.s.	
	[2-3]-[3-3]	*M. tardigradum trispinosa*	
	[3-3]-[3-3]	*M. bohleberi*	
	[3-3]-[3-3]	*M. brachyungue*	
	[3-3]-[3-3]	*M. eurystomum*	
	[3-3]-[3-3]	*M. asiaticum*	*M. swansoni* **nov. sp**
	[3-3]-[3-3]	*M. antarcticum*	
	[3-3]-[3-3]	*M. longiungue*	
	[3-3]-[3-3]	*M. zsalakoae*	
	[3-?]-[?-3]	*M. barbadosense*	
	[?-?]-[?-?]	*M. swolenskyi*	

To date, 26 species and subspecies of *Milnesium* have been described (Degma *et al*. 2015). Many have yet to be reported a second time. This paucity of specimens and data should challenge us to mine our existing collections for examples of these newly described species, update their identification, and accurately define the distribution of each species. Such data would expand the biodiversity of many areas, and shed light on *Milnesium* distribution patterns.

To aid in the growing need to separate species of *Milnesium*, we propose to expand the group table for diagnostic characteristics developed by Michalczyk & Kaczmarek (2012) by adding new columns for the pattern of secondary claw branching and peribuccal lamellae number (Table 5).

Miller (2004) speculated that because habitat was available in the canopy, tardigrades should be present. Our new *Milnesium* not only confirms this hypothesis but is the fourth tardigrade species found to be significantly more abundant in the canopy ecosystem of eastern Kansas. Spiers *et al*. (2013) reported 80% of her specimens of *Doryphoribius dawkinsi* Michalczyk & Kaczmarek, 2010 came from the canopy, and Haefke *et al*. (2014) described *Doryphoribius elleneddiei* Haefke, Spiers, Miller & Lowman, 2014 as a new species found exclusively in the canopy. Chappell *et al*. (2015) reports *Doryphoribius gibber* Pilato & Lisi, 2006 as another species with significantly greater numbers higher in the trees. It has been shown that substrate (tree species) influenced the diversity of tardigrades (Mitchel *et al.* 2009) and stratification in the canopy of a single white pine tree has also been demonstrated (Miller *et al.* 2013).

Forests with tree canopies exist all over the world and should be explored for tardigrades. As evidenced by these results from tree canopies in Kansas, the environment of the tardigrade is three dimensional and the upper levels may harbour greater diversity and abundance than we have found for 200 years hunting on the ground, on rocks, and at the base of trees.

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