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

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Milnesium burgessi nov. sp. is a new species of tardigrade described from the canopy of eastern Kansas, USA. This unique tardigrade of class Eutardigrada, order Apochela and family Milnesiidae is characterized by its smooth cuticle, six peribuccal lamellae, long primary branch of the claws with accessory points, a claw configuration of [3-3]-[3-3] and a buccal tube that is significantly shorter than other species.

Introduction

The tardigrade genus *Milnesium* Doyèr, 1840 was described for the species *Milnesium tardigradum* Doyère 1840, based on the sloping body, six peribuccal papilla and lamellae, a wide buccal tube, a lack of placoids and separated double claws. For 150 years this large, easily recognized, limno terrestrial genus of water bears was considered monotypic (Ramazzotti and Maucci 1983) and cosmopolitan (McInnes 1997).

Only two varieties were proposed during that time, both from South America and based on cuticular variation. *Milnesium tardigradum tripinosa* (Rahm 1931) was described from four specimens with three posterior spines and *Milnesium tardigradum granulatum* Ramazzotti 1962 was described for specimens with a fine granulation on the cuticle. Ramazzotti and Maucci (1983) did not find sufficient evidence to elevate either to a species level.

Since 1990, 36 new species have been described (Degma et al. 2017) based on differences in cuticular granulation, the number of peribuccal lamellae, the size, and shape of the claws, and variation in width of the buccal tube and stylet support attachment point relative to the length of the buccal tube. Recently, Michaelczyk et al. (2012) re-described the nominal species, *Milnesium tardigradum* from Germany, near but not from the original type location in France. They brought the description to modern taxonomic standards by defining a suite of both diagnostic and measured characteristics necessary to differentiate the species within the genus. They elevated *Milnesium tardigradum granulatum* to species level as *Milnesium granulatum* Ramazzotti 1962 and divided the genus into two groups, one with smooth cuticles (*tardigradum*) and the other with a reticular or granulated dorso-lateral cuticle (*granulatum*) and provided a detailed key to the described species.

Young et al. (2015) further divided the genus by the number of peribuccal lamellae (4 or 6) and the number of points or sub-branches found on the secondary branch of each claw on each leg (Table 4). Since then 4 species have been added to the group and this report adds another species to the smooth cuticle group (*tardigradum*) with six peribuccal lamellae and a [3-3]-[3-3] secondary branch pattern, its habitat is the canopy of eastern Kansas, USA.

Materials and Methods

Eight sites in north eastern Kansas were surveyed. The sites range through four biome classes and three georegions. The three sites were in the pre-Illinoian Glaciated Region (Aber 1991), an area covered with up to 150 meters of ice just 600,000 bp (Dort 2006). Four sites, were in the Osage Cuestas and one was in the Flint Hills georegion (Aber 1991).

Multiple species of trees were climbed using the double-rope climbing technique (Haefke 2013, Kilgore et al. 2008) and samples of moss and lichen were collected from eight forests in Eastern Kansas. Collection was stratified at 3-meter increments. In the laboratory, the samples were soaked with bottled water in small plastic cups for 24 hours (Miller 1997). Three 1 ml sub-samples were examined for tardigrades using reflected-light and a dissecting microscope at 25x magnification. Tardigrades were caught using an Irwin loop (Schram and Davidson 2012). Specimens were mounted on slides in PVA, sealed with fingernail polish and examined with an Olympus BX60 DIC (differential interference contrast) microscope equipped with a 12 megapixel camera. Digital images were stored in tiff format and have not been enhanced except to adjust contrast and brightness to achieve a printable image (McInnes 2001).

Measurements

Olympus cellSense Standard® ver. 1.6 software was used for morphometric measurements (Table 1). Measurements are in micrometers (μm) and *pt* ratios are the ratio of a structure to the length of the buccal tube (Pilato 1981), expressed as a percentage. Only fully elongated tardigrades were measured as Tumanov (2006). The table of morphometric data follows the Apochela ver. 1.1 template from the Tardigrada Register (Michalczyk and Kaczmarek 2013). The list includes the number of points on each secondary branch of the claws and the anterior and posterior widths of the buccal tube as described by Michalczyk et al. (2012). The measurement of the basal thickening for each claw was added by Young et al. (2015). The inclusion of allometric factors (a*) and (b) (Bartels et al. 2011) in Table 1 follows the pattern used by Bartels and Nelson (2014) to remove the effects of body size in morphometric analysis.

A hypothesis of uniformity or mean distribution was assumed between locations, substrates, and habitats. The generally accepted short-distance wind-distribution concept for tardigrades (Ramazzotti and Maucci 1983) predicts that all tardigrades have equal opportunity of reaching a new location. Both were evaluated with chi-square where a calculated value greater than the critical value of 3.84 was interpreted to be significantly different from a random result (P value of less than 0.05 for one degree of freedom).

For 200 years tardigrades have been extracted from habitats (moss or lichen) collected at or near ground level on the trunks of trees. Thus, ecological relationships in the canopy were tested statistically by using chi-square to compare the observations at upper levels against an expectation of uniformity with the observations found at the base level. 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, Bertolani and Guidetti (2009-2017).

Results

During the summer of 2014, a biodiversity survey was conducted to demonstrate that tardigrades inhabit the canopy. More than 8,000 tardigrades were extracted from 800+ samples of two habitats (moss or lichens) collected at four levels in 135 substrate trees of 17 species climbed at eight locations. One hundred fifty-seven specimens of a *Milnesium* did not match a published description and are described here as a new species.

Taxonomy

Eighteen fully extended specimens were used, measurements are those of the holotype. The range of the population is presented in Table 1. Measurements are in micrometers (µm).

Eutardigrada Richters 1926
Apochela Schuster, Nelson, Grigarick and Christenberry 1980
Milnesiidae Ramazzotti 1962
Milnesium Doyère 1840
Milnesium burgessi nov. sp.
(Figure 1, Tables 1- 4)

Table 1. Measurements and *pt* values of selected morphological structures of 18 selected specimens from populations of *Milnesium burgessi.* nov. sp. (N = number of specimens measured; Range = largest and smallest measured; Stdev = Standard Deviation).

Character	N	Range				Mean		Stdev		Alomentry	
		um		*pt*		um	*pt*	um	*pt*	a*	b
Body Length	18	1030	343	*2409*	*2024*	734.28	*2203*	133.26	*0.92*		
*pt*BL	18	2409%	2024%			2203%		0.92	*0.16*	2255%	0.06
Peribuccal papillae length	16	11.76	5.64	*34.6*	*0.0*	8.96	*24.2*	1.56	*0.10*	8.94	0.59
Lateral papilla Length	15	9.37	5.03	*29.7*	*0.0*	7.39	*19.3*	1.08	*0.09*	7.58	0.47
Buccal Tube											
Buccal Tube Length	18	48.30	16.95			33.33		6.21		32.59	0.94
Stylet Support Attachment Pt	18	29.27	11.90	*74.0*	*60.6*	22.21	*66.8*	4.02	*0.04*	21.98	0.87
*pt*SSA	18	74.0%	60.6%			67.0%		0.04	*0.01*	67.2%	-0.07
Anterior BTW	18	29.77	9.53	*75.5*	*56.2*	22.14	*66.4*	4.13	*0.05*	22.86	1.03
Standard Buccal Tube Width	18	28.38	9.34	*68.5*	*52.9*	20.82	*62.4*	4.02		21.31	0.98
ptBTW	18	68.5%	52.9%			62.4%		0.05	*0.00*	65.4%	0.04
Posterior BTW	18	27.57	9.20	*70.1*	*54.3*	20.77	*62.4*	3.80	*0.05*	21.54	0.97
Post/Anterior Width Ratio	18	100.00	85.19			93.98		3.74		94.39	0.05
Leg I											
I-External Primary Branch	18	25.94	11.65	*79.5*	*52.2*	20.73	*62.7*	3.75	*0.08*	20.94	0.77
I-External Base + Sec Branch	18	19.41	7.64	*55.2*	*38.8*	14.94	*45.2*	2.43	*0.04*	15.43	0.80
I-External Spur	18	6.66	2.45	*21.9*	*7.1*	4.89	*14.7*	1.35	*0.03*	4.86	0.85
I-Internal Primary Branch	18	25.09	12.82	*75.6*	*47.9*	19.77	*60.0*	3.23	*0.07*	19.43	0.62
I-Internal base + Sec Branch	18	18.75	8.00	*53.7*	*36.7*	14.59	*44.2*	2.40	*0.05*	14.97	0.74
I-Internal Spur	18	22.75	4.16	*134.2*	*11.3*	7.11	*24.5*	4.70	*0.28*	4.42	-1.05
Leg II											
II-External Primary Branch	17	29.58	11.44	*78.3*	*0.0*	21.94	*62.2*	4.04	*0.17*	21.94	0.85
II-External Base + Sec Branch	18	21.66	6.91	*51.5*	*40.8*	15.16	*45.4*	3.08	*0.03*	14.99	1.05
II-External Spur	18	7.21	2.71	*22.3*	*11.3*	5.40	*16.3*	1.24	*0.03*	5.66	0.79
II-Internal Primary Branch	17	24.49	11.21	*76.6*	*0.0*	20.97	*59.6*	3.67	*0.16*	21.72	0.81
II-Internal base + Sec Branch	18	18.88	6.76	*52.3*	*35.2*	14.20	*42.8*	2.58	*0.05*	14.76	0.88
II-Internal Spur	18	8.31	2.49	*22.7*	*9.7*	5.61	*16.9*	1.53	*0.04*	5.65	0.94
Leg III											
III-External Primary Branch	17	29.01	11.94	*76.1*	*0.0*	22.18	*62.9*	4.05	*0.17*	22.17	0.85
III-External Base + Sec Branch	18	19.60	8.55	*58.2*	*40.4*	15.92	*48.2*	2.48	*0.05*	16.47	0.75
III-External Spur	18	7.29	2.51	*22.6*	*9.6*	5.27	*15.9*	1.44	*0.04*	5.48	0.86
III-Internal Primary Branch	17	26.67	12.61	*74.4*	*0.0*	21.64	*61.7*	3.28	*0.16*	22.02	0.70
III-Internal base + Sec Branch	18	17.63	6.82	*51.3*	*36.5*	14.82	*44.7*	2.51	*0.04*	15.73	0.88
III-Internal Spur	18	8.44	2.70	*27.3*	*8.0*	5.82	*17.8*	1.67	*0.05*	6.58	0.55
Leg IV											
IV-Anterior Primary Branch	18	34.15	14.31	*89.6*	*70.7*	27.39	*82.7*	4.36	*0.07*	28.18	0.80
IV-Anterior Base + Sec Branch	18	20.53	8.21	*61.6*	*41.1*	16.43	*49.6*	2.91	*0.06*	17.14	0.81
IV-Anterior Spur	17	14.51	2.14	*46.9*	*0.0*	6.80	*19.3*	2.91	*0.10*	6.97	1.08
IV-Posterior Primary Branch	18	33.51	13.88	*96.2*	*66.6*	27.92	*84.3*	4.49	*0.08*	29.26	0.83
IV-Posterior Base + Sec Branch	18	23.60	8.22	*60.5*	*44.5*	17.51	*52.6*	3.30	*0.04*	17.72	0.96
IV-Posterior Spur	17	9.64	3.20	*23.8*	*0.0*	5.91	*16.8*	1.64	*0.06*	5.82	0.73
IV-Base Width	18	7.34	3.00	*23.7*	*10.2*	5.42	*0.2*	1.05	*0.04*	6.08	0.49

Table 2. Collection Data for Paratype specimens. * = Type specimen.

CASIZ	Slide #	Date	Place	Latitude	Longitude	Substrate	Habitat	Height
224121	R042564*	6-20-14	Baker Wetlands	38.91378	-95.22961	Hackberry	Lichen	>10 m
224122	R040284	6-11-14	Baker Prairie	38.76711	-95.12850	Red Oak	Lichen	>07 m
224123	R042363	6-18-14	Baker Woods	38.81060	-95.17389	Hickory	Lichen	>10 m
224124	R043807	6-24-14	Baker Campus	38.77771	-95.18786	Hackberry	Lichen	>10 m
224125	R044240	7-02-14	KU Field Station	39.04424	-95.20440	White Ash	Lichen	>10 m
224126	R044487	6-27-14	Baker Wetlands	38.91528	-95.23338	E. Cottonwood	Lichen	>13 m
224127	R047285	7-08-14	Konza LTER/NEON	39.10428	-96.59519	American Elm	Lichen	>13 m
224128	R048042	7-11-14	KU-Rice Woods	38.80996	-95.17830	Hickory	Moss	>10 m

Diagnosis: Yellowish *Milnesium* without eyes; with smooth cuticle, very short and wide buccal tube length , with six peribuccal lamellae. Main branch of claw long, with very small accessory points. Short cuticular bars on legs IV.

Description of Holotype: Body colorless to yellowish (transparent), eyes may be absent, cuticle smooth. Six peribuccal papillae, two lateral papillae, and six peribuccal lamellae. Body length 862 µm, buccal tube length 40.4 µm, *pt* BL = 2133%. Standard buccal tube width 25.92 µm, *pt* BTW = 64.1%. Stylet supports attachment point 28.6 µm, *pt* SSA = 70.9%. Pharyngeal bulb elongated, pear shaped, without placoids or septula. Claws of *Milnesium* type, main branch long with very small accessory points, separated from secondary branch. Secondary branch with primary, secondary, and basal spurs [3-3]-[3-3]. Length of external primary branch of claw I 25.8 µm, *pt* = 63.8%, and length of posterior primary branch of claw IV 33.5 µm, pt = 82.9%. Length of base plus secondary branch of external claw I 17.0 µm, *pt*=42.1%; length of base plus secondary branch of posterior claw IV 21.5 µm, *pt*=53.2%. Base width of secondary branch of posterior claw IV 6.1 µm. Long cuticular bars just below claws on first three pairs of legs, and short cuticular bars at the base of each leg IV. Males present with enlarged, simple, secondary branch of claws I. Eggs deposited in shed cuticle. (Table 1)

Collections: Samples collected June 1- July 31, 2014 within 160 kilometers of the Baker University Campus in Baldwin City, Kansas, U.S.A.

Etymology: Named ***Milnesium burgessi*** nov. sp. to honor a young, budding adventurer, philanthropist and tardigrade supporter, Jim Burgess, son of co-author Lowman.

Holotype: Collected from a sample of lichen growing at level 3 (6-9 meters from the ground) on the trunk of a Hackberry (*Celtis occidentalis*) tree on June 20, 2014 at the Baker Wetlands, Lawrence, Kansas, U.S.A. by Kaylyn Hobelman. Deposited at the California Academy of Science, San Francisco, California, U.S.A. Slide Collection number: CASIZ-224121.

Paratypes: Seven paratypes are also deposited at the California Academy of Science, San Francisco, California, U.S.A. All paratypes are from Kansas, U.S.A. CASIZ-224122 to CASIZ-224128 (Table 2).

Differential Diagnosis: *Milnesium burgessi* nov. sp. is a member of the smooth cuticle group (Table 3) with a smooth cuticle, six peribuccal lamellae and a sub group with a secondary claw branch code of [3-3]-[3-3]. It is distinctively different from the other twelve members of the group by having a significantly shorter buccal tube compared to its body length (*pt* BL > 2000%; Table 3; Figure 1). It also differs by the presence of short cutiular bars at the base of legs IV (Figure 1E). There

Figure 1. (RIGHT) *Milnesium burgessi* nov. sp. A. Habitus, buccal tube length (BTL), body length (BL), *pt*=BL/BTL> 2000%, B. Mouth, C. Mouth, D. Stomach content with tardigrade mouth parts (a) and claws (b) and ascomycete spores (c), E. Claws of leg IV showing short cuticular bars (arrows) and [3-3]-[3-3] secondary claws.

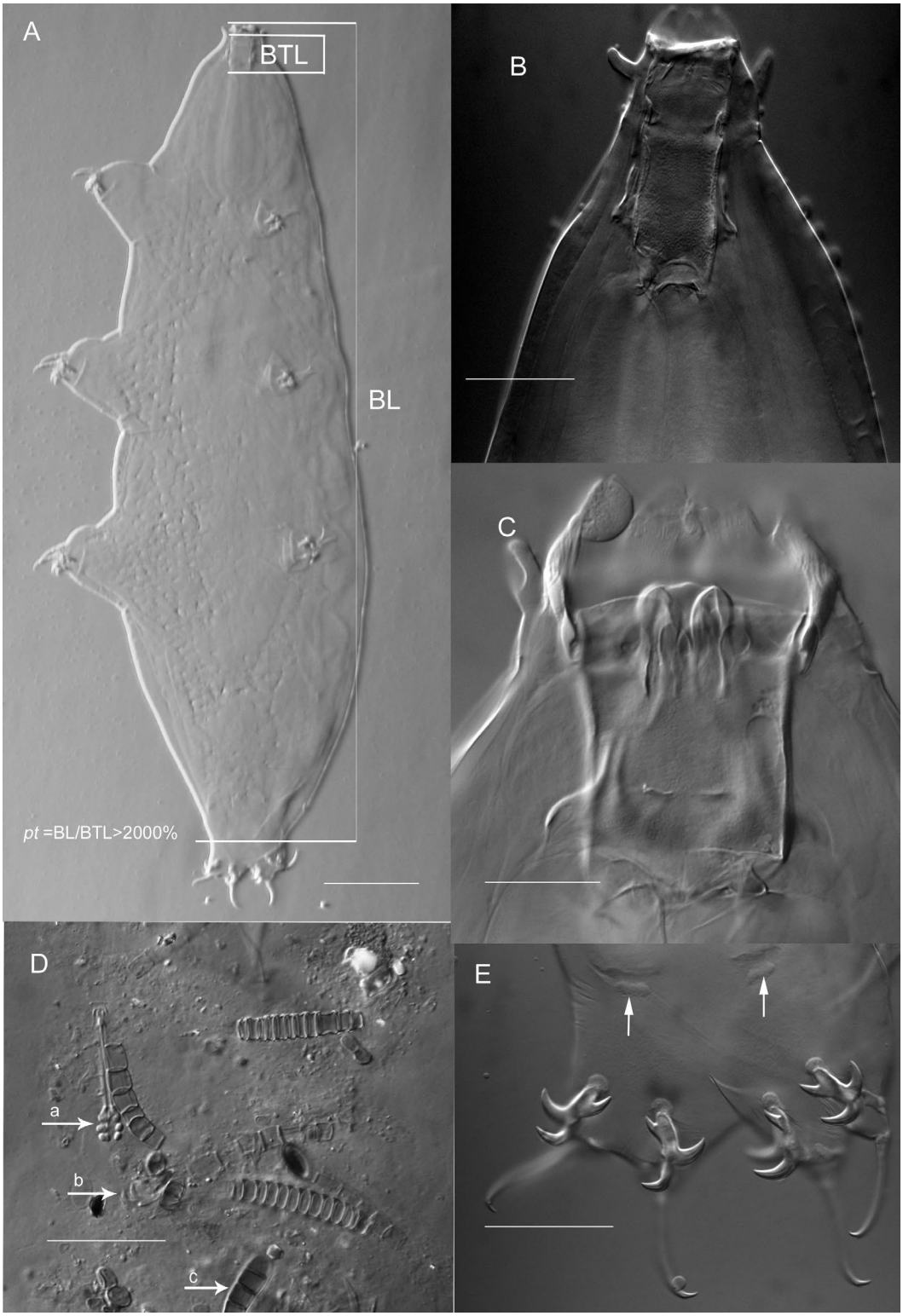

A
BTL
BL
pt =BL/BTL>2000%
B
C
D
a
b
c
E

Table 3. Comparison of relationship of measured characters within the *Milnesium* smooth cuticle and [3-3]-[3-3] secondary claw arrangement and mean *pt* values.

Species	AccPt	Eyes	*pt* BL	*pt* BTW	*pt* SSA
M. alpigenum	Present	Present	1573%	31.8%	65.7%
M. antarcticum	Present	Present	1120%	39.2%	71.5%
M. asiaticum	Present	Present	1437%	35.2%	65.3%
M. bohleberi	Present	Present	1321%	59.5%	67.9%
M. brachyungue	Present	Present	1148%	37.0%	69.4%
M. burgessi n sp.	Present	Absent	2203%	62.4%	67.2%
M. eurystomum	Present	Present	1128%	63.3%	60.2%
M. longiungue	Absent	Present	1630%	43.6%	64.1%
M. minutum	Present	Present	1101%	41.0%	64.0%
M. shilohae	Present	Present	1533%	51.3%	76.2%
M. sandrae	Present	Present	1497%	44.9%	58.0%
M. tumanovi	Present	Present	1525%	55.1%	52.3%
M. zsalakoae	Absent	Present	1511%	39.4%	69.7%

is no way to compare the short cuticular bars on legs IV as they are only mentioned in the comparative description of *Milnesium beatae* by Tibbs et al. (2016), a species in the pseudopore cuticle group.

Ecology: The new species was found at all eight collection sites, which spanned more than 160 kilometers from the Konza LTER near Manhattan, Kansas on the west to the Baker Prairie near Baldwin City on the east. Based on the hypothesis of equal distribution (distribution at random among different sites), half of the sites show significant variation from the mean number of specimens expected to be found at each site. The sites range through four biome classes and three georegions. The three sites in the pre-Illinoian Glaciated Region (Aber 1991), an area covered with ice just 600,000 bp (Dort 2006), yielded significantly more specimens than expected ($P<0.05$). Two additional sites, one from the Osage Cuestas and one from the Flint Hills georegion (Aber 1991), yielded significantly fewer specimens than expected ($P<0.05$). The remaining three sites exhibited specimens as expected. The new species adds to the biodiversity of Kansas and the region (Lehmann et al. 2007, Meyer 2013).

Milnesium burgessi nov. sp occurred in both moss and lichen habitats as expected on the basis of random distribution among the habitat of the region. It was found on 14 of the 17 species of substrate trees. The hypothesis of equal occurrence was also true on 12 substrates but the new species occurred on Shag Bark Hickory and Red Oak substrates more frequently than expected ($P<0.05$), suggesting different suitability. *Milnesium burgessi* nov. sp was collected at all four levels in the trees. The number of individuals found at each of the three upper levels compared to the base level was significantly greater ($P<0.05$) than expected by chance (Table 4), suggesting an advantage to living higher in the canopy for this species.

Several specimens were observed to have the mastex of rotifers, several types of ascosporous, and mouthparts of both *Minibiotus* and

Table 4. *Milnesium burgessi* nov. sp. by level in canopy.

Level	Height - meters	# Tardigrades
4	9 – 12 m	50*
3	6 – 9 m	60*
2	3 – 6 m	35*
1	0 – 3 m	12
Total		157

* = $X^2 > 3.85$, 1 df.

Table 5. Comparison of diagnostic characteristics and mean *pt* values of measured characters within the genus *Milnesium*. P = Present, A = Absent.

	Diagnostic Characteristics					Measured Characters		
Milnesium species	Cuticle Group	Peribuccal lamellae	Claw Spurs	Acc pts	Eyes	*Pt* BL	*Pt* BTW	*Pt* SSA
M. lagniappe		4	[2-3]-[3-2]	P	A	2199%	69.0%	71.8%
M. variefidum			[2-2]-[2-2]	P	P	1288%	26.2%	70.85%
M. berladnicorum			[2-3]-[2-2]	P	P	1619%	33.5%	69.2%
M. beasleyi	Pseudopores	6	[2-3]-[3-2]	P	-	1513%	36.9%	64.2%
M. argentinum			[3-3]-[3-3]	P	P	1093%	27.4%	71.7%
M. beatae			[3-3]-[3-3]	P	P	1401%	61.9%	65.1%
M. domensis			[3-3]-[3-3]	P	-	1730%	44.1%	66.0%
M. reticulatum		4	[2-2]-[2-2]	P	P	1058%	33.9%	69.2%
M. katarzynae			[2-2]-[2-2]	P	A	969%	21.7%	78.3%
M. krzysztofi	Reticulated	6	[2-3]-[3-2]	P	A	1363%	35.1%	65.5%
M. alabama			[3-3]-[3-3]	A	A	1082%	36.5%	63.7%
M. granualtum			[3-3]-[3-3]	P	P	1244%	39.0%	66.5%
M. tetralamellatum		4	[2-3]-[2-3]	P	P	1419%	49.1%	63.9%
M. swansoni			[3-3]-[3-3]	A	A	1430%	34.6%	69.0%
M. kogui			[2-2]-[2-2]	P	P	1094%	34.1%	71.1%
M. reductum			[2-3]-[2-2]	A	P	1539%	38.1%	67.2%
M. almatyense			[2-3]-[2-2]	P	A	1789%	39.6%	67.3%
M. barbadosense			[2-3]-[2-3]	P	A	1188%	39.2%	72.4%
M. tardigradum SS			[2-3]-[3-2]	P	P	1314%	38.4%	65.4%
M. vorax			[2-3]-[3-2]	P	P	1644%	59.9%	62.1%
M. jacobi			[2-3]-[3-3]	P	P	1473%	57.0%	87.7%
M. alpigenum			[3-3]-[3-3]	P	P	1573%	31.8%	65.7%
M. antarcticum	Smooth	6	[3-3]-[3-3]	P	P	1120%	39.2%	71.5%
M. asiaticum			[3-3]-[3-3]	P	P	1437%	35.2%	65.3%
M. bohleberi			[3-3]-[3-3]	P	P	1321%	59.5%	67.9%
M. brachyungue			[3-3]-[3-3]	P	P	1148%	37.0%	69.4%
M. burgessi n. sp.			[3-3]-[3-3]	P	A	2203%	62.4%	67.2%
M. eurystomum			[3-3]-[3-3]	P	P	1128%	63.3%	60.2%
M. longiungue			[3-3]-[3-3]	A	P	1630%	43.6%	64.1%
M. minutum			[3-3]-[3-3]	P	P	1101%	41.0%	64.0%
M. shilohae			[3-3]-[3-3]	P	P	1533%	51.3%	76.2%
M. sandrae			[3-3]-[3-3]	P	P	1497%	44.9%	58.0%
M. tumanovi			[3-3]-[3-3]	P	P	1525%	55.1%	52.3%
M. zsalakoae			[3-3]-[3-3]	A	P	1511%	39.4%	69.7%
M. quadrifidum			[4-4]-[4-4]	-	-	-	-	-

Ramazzottus species of tardigrades in their guts (Figure 1D). Our observations are in line with Roszkowska et al. (2015) who suggested the selection and size of the food was related to the dimensions of the buccal apparatus.

Discussion

Tardigrades have not been thought of as canopy animals in the past. While there is literature suggesting they would be found above ground

level in the trees, the research is not extensive (Kimmel and Meglitsch 1969, Voegtlin 1982, Counts et al. 2001, Miller 2004, Kilgore et al. 2008, Mitchell et al. 2009). However, Miller et al. (2013) and Chang et al. (2015) reported greater tardigrade diversity and density at greater heights in trees in Kansas, thus opening the question of tardigrades' distributional patterns in the canopy.

Milnesium burgessi nov. sp. is the fifth species documented by this project to be more common in canopies in Kansas than at ground level (*Doryphoribius dawkinsi* Michalczyk and Kaczmarek, 2010 by Spiers et al. (2013); *Doryphoribius elleneddiei* Haefke, Spiers, Miller and Lowman, 2014 by Haefke et al. (2014); *Doryphoribius gibber* Pilato and Lisi, 2006 by a Chappell et al. (2015); and *Milnesium swansoni* Young, Chappell, Miller and Lowman (2016) by Young et al. (2016)). We believe there will be many more species to be documented and discovered as the canopies of the world continue to be explored.

Due to the proliferation of species of *Milnesium*, and to aid in the identification, we support the grouping concept of diagnostic characteristics introduced as a table by Michalczyk and Kaczmarek (2012) and amended by Young et al. (2015). We updated and expanded the table (Table 5) to include the species described since 2012, modified the first column to the name of the cuticular appearance (reticulated, pseudopore and smooth). From the literature we have added two columns for the descriptive characters of the presence of eyes and accessory spikes on the primary branches of the claws and the three measured characters of body length (BL), buccal tube width (BTW), and stylet support attachment (SSA) expressed as the mean *pt* value for each.

To date, 36 species of *Milnesium* have been described (Degma et al. 2017). Most have yet to be reported a second time and many are based on very few specimens. Michalczyk et al. (2012) challenged the identification of every *Milnesium tardigradum* reported outside of Europe. The paucity of specimens, locations, and ecology of the new species should challenge us to mine our existing collections for examples of these newly described species that may have in the past been lumped as *M. tardigradum*. Only when such data become available can we start to form a true picture of the global distribution of the genus and it is likely that the biodiversity of many areas of the globe will be changed.

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