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Tardigrades of North America: Additions to Montana's Biodiversity Including a New Species, *Platicrista loloensis* nov. sp. (Parachela, Hypsibioidea, Itaquasconinae) †

Chelsea N. Scheirer [1], William R. Miller [2,*] and Jeffrey D. Miller [3]

[1] Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35401, USA
[2] Department of Biology, Baker University, Baldwin City, KS 66006, USA
[3] Biological Research and Education Consultants, Missoula, MT 59801, USA; bioresedcon@gmail.com
* Correspondence: william.miller@bakeru.edu
† urn:lsid:zoobank.org:pub:B8BECA01-877A-4ADD-B836-589CBA15CE1E.

Abstract: A total of 205 tardigrades representing two orders, five families, nine genera and ten species were extracted from a moss sample (104 tardigrades) and a lichen sample (101 tardigrades) collected near Missoula, Montana, in 2016. Three of the species are new to Montana and one is new to science, *Platicrista loloensis* nov. sp., which is distinguished by its smooth cuticle, the presence of internal cuticular bars at the base of the claws of legs II and III and a median cuticular bar between the claws of leg IV.

Keywords: tardigrade; distribution; Montana; new species

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1. Introduction

There are only five reports of the phylum Tardigrada in the state of Montana. Leetham et al. [1,2] twice reported the same three genera (*Macrobiotus*, *Diphascon* and *Hexapodibius*) but did not identify the species. Beasley [3] recorded the first species, when he described *Hypsibius macrocalcaratus* [3] from New Mexico and added Montana as another place he had found the animal. Miller [4] listed 20 species from Montana west of the continental divide. Miller and J.D. Miller [5] extended the known range of eight species into the Bob Marshall Wilderness Complex. Two were additions to Montana's biodiversity list and one was new to science, *Placticrista brunsoni* [5]. This report adds three species to the states' biodiversity list, including one that is new to science.

2. Study Area and Methods

A moss habitat sample was collected from a site on the north side of Lolo Creek (a tributary of the Bitterroot River) near Fort Fizzle south of Missoula, Montana (Figure 1). The moss sample was taken from the center of a plot that measured approximately 30 × 30 cm. The moss was pulled from the gravelly soil substrate and clinging debris was removed using scissors. The moss was put into a plastic bag that contained 510 mL of distilled water and taken to the lab, where it was stored in a refrigerator at approximately 1–2 °C until being processed to extract tardigrades.

A lichen habitat sample was scraped from a lichen-covered fence rail on the north side of an irrigation ditch located on the east side of Rattlesnake Creek (a tributary of the Clark Fork River) north of Missoula (Figure 1). The lichen sample was dry when collected and stored in a paper bag at room temperature (21–22 °C) until processing.

In the laboratory, approximately one-third of each sample was soaked in distilled water. After 24 h, three 2.5 mL aliquots of debris and fluid were extracted from each sample, examined in a black-bottomed, rectangular petri dish using a 25× dissecting microscope illuminated by LED lights to search for tardigrades [6,7]. Tardigrades were removed with

an Irwin loop [8] and placed into a drop of polyvinyl alcohol (PVA) on a glass slide, which was covered with a round glass coverslip. The coverslip was sealed with fingernail polish after five days of drying [6,7].

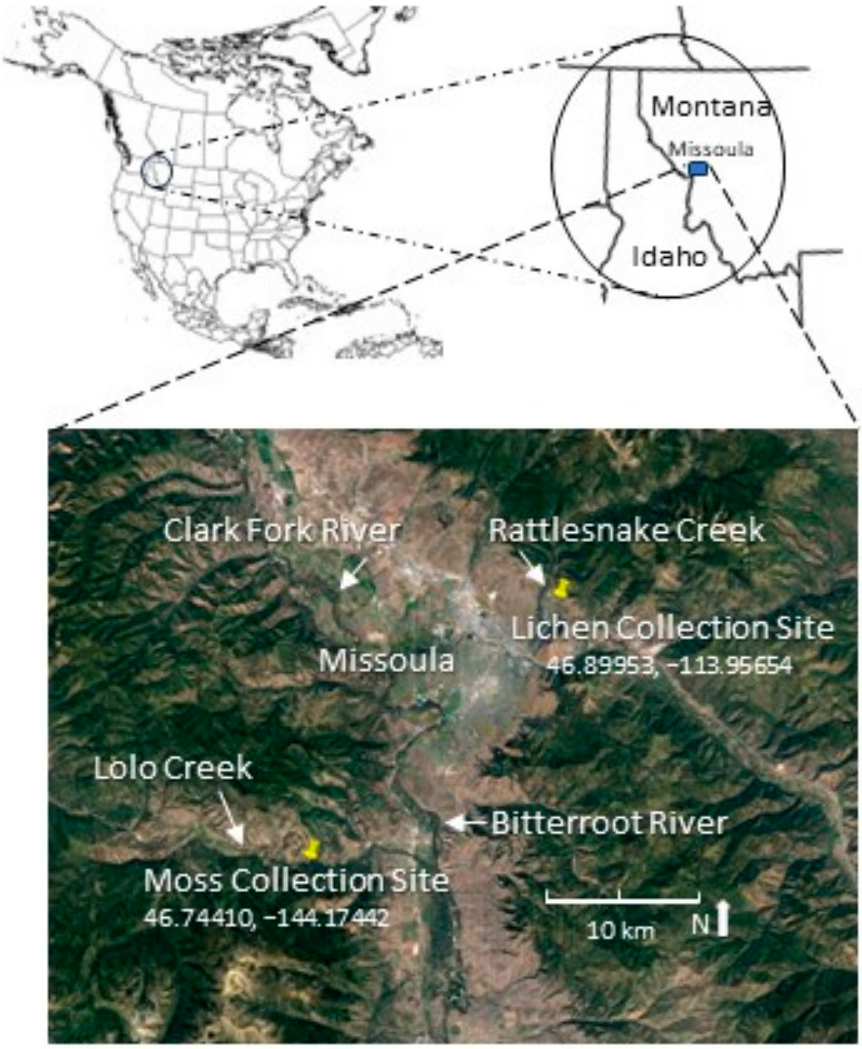

Figure 1. Location of moss and lichen collection sites in western Montana. Based on Natural Earth and Google Earth Pro maps and the images.

Each specimen was examined using an Olympus BX-60 microscope equipped with Nomarski Differential Interference Contrast objectives (DIC). Images were 5.0 MP taken with a Miotic M-500 digital camera and saved in PNG format. The images were cropped to fit the page and only adjusted for brightness and contrast for printability [9].

Measurements were taken µm using AmScope version 64x (2003–2022) software Data were stored and formatted in Excel spreadsheets. A data table was used to characterize species in which N = number of specimens, range = (min–max), mean = average of N specimens, *pt* = character length/buccal tube length, *b* = slope of regression of data, and a* = Y intercept per Bartels et al. [10]. We accept the use of the mean of the *pt* value [11] of the set of measurements used to describe a species and published as the statement of diagnosis for that variable. This, while slightly imperfect, *pt* value allows variable characteristics to be compared within a taxon and among taxa.

Nomenclature was based on the "Actual Checklist of Tardigrades" by Guidetti and Bertolani [12], Degma and Guidetti [13], Degma, et al. [14], and Degma and Guidetti [15]. Identification was based on the work of Ramazzotti and Maucci [16], Pilato and Binda [17], and current literature, including Gąsiorek et al. [18]. Buccal tube measurements followed

the work of Pilato [11]. Abbreviations followed the work of Perry et al. [19,20]. North American distribution and biodiversity were based on the work of McInnes [21], Meyer [22], Kaczmarek et al. [23], and Miller and Perry [24].

3. Results

Both samples were positive for tardigrades. The three aliquots from the moss sample yielded 104 individuals and the three aliquots from the lichen sample yielded 101. The moss sample yielded eight species from three families and five specimens in simplex and five specimens that could not be identified because they were distorted when positioned on the slide. Of those eight species, three were new to Montana and one was new to science (Table 1). The lichen sample yielded four species from four families and three specimens that could not be identified because they were distorted when positioned on the slide (Table 1).

Table 1. Tardigrade species reported from samples collected in 2016 at Lolo Creek and Rattlesnake Creek sites near. Missoula Montana. * = New to Montana. Taxonomy follows Degma and Guidetti [15].

Class Order Superfamily Family Subfamily Genus species	Moss	Lichen
Eutardigrada Richters, 1926		
Apochela Schuster, Nelson, Grigarick and Christenberry, 1980		
Milnesiidae Ramazzotti, 1962		
Milnesium sp.	0	24
Parachela Schuster, Nelson, Grigarick & Christenberry, 1980		
Hypsibioidea Plato. 1969		
Hypsibiidae Pilato, 1969		
Diphasconinae Dastych, 1992		
Diphascon pingue (Marcus, 1936) sensu lato *	1	0
Itaquasconinae Bartoš in Rudescu, 1964		
Adropion scoticum (Murray, 1905)	2	0
Platicrista loloensis nov. sp. *	6	0
Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008		
Ramazzottius oberhaeuseri (Doyère, 1840)	0	64
Isohypsibioidea Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008		
Isohypsibioidae Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008		
Isohypsibius sp.	2	3
Macrobiotoidea Thulin, 1928		
Macrobiotidae Thulin, 1928		
Macrobiotus hufelandi C.A.S. Schultze, 1834 sensu lato	49	0
Mesobiotus harmsworthi (Murray, 1907) sensu lato	15	4
Paramacrobiotus richtersi (Murray, 1911) sensu lato	4	0
Paramacrobiotus tonollii (Ramazzotti, 1956) *	15	0
Identification undetermined	5	3
Total	104	101

The species of tardigrades recovered were not uniformly distributed among the two samples representing two different types of habitats (Table 1). All three members of the family Hypsibiidae were found only in the moss. Three of five Macrobiotidae species were also found only in the moss. *Mesobiotus harmsworthi* sensu lato, known for its intercontinental distribution, was recovered from both the moss and lichen samples. *Milnesium* and *Ramazzottius* were found only in the lichen sample. No pattern of association among the species and their habitats can be determined with only two samples.

4. Taxonomic Treatment of New Species

When a specimen is identified, it is assigned to a genus by confirming the specimen has the diagnostic characteristics for that classification. Then, it is assigned to a species based on the variation in generic characteristics or the addition of a unique specific character(s). Thus, when one or more of a specimen's characters do not correspond with the characters of previously described species and cannot be reasonably explained by variation, then a new species should be created [25].

Phylum	Tardigrada Doyère, 1840
Class	Eutardigrada Richters, 1926
Order	Parachela Schuster, Nelson, Grigarick & Christenberry, 1980
Superfamily	Hypsibioidea Pilato, 1969
Family	Hypsibiiidea Pilato, 1969
Subfamily	Itaquasconinae Rudescu, 1964
Genus	*Platicrista* Pilato, 1987
Species	*Platicrista loloensis* nov. sp.
	Figures 2 and 3, Tables 2 and 3

The number in parenthesis in the description is the measurement of the holotype.

Diagnosis*:** Large, gray ***Platicrista with a smooth cuticle, without eyes before mounting. Smooth, ridged buccal tube, flexible pharyngeal tube, two long, thin macroplacoids in a row, with the second twice as long as the first and no microplacoid or septulum. Claws of *Hypsibius* type, internal cuticular bars at the base of claws on legs II and III, median cuticular bar between claws on legs IV and ragged pseudolunules present on the base of claws IV (Figure 2).

Description*:** Large (615 µm), gray ***Platicrista, without eyes before mounting. Subterminal mouth. Cuticle smooth but may show wrinkles. Pointed, spoon-like furcae away from buccal tube. Buccopharyngeal apparatus with smooth (73.05 µm), ridged buccal tube (36.46 µm) to stylet support attachment point (36.46 µm) and ringed, flexible pharyngeal tube (36.59 µm) posterior to the stylet support attachment point into the oval pharynx and terminating with small distal thickenings (Figure 2A). Buccal tube with an external narrow width (9.24 µm) (Figure 2A) measured at the stylet attachment point (Table 3). Elongated, oval pharynx without apophysis (72.71 µm). Pharyngeal cuticular bars pointing forward in the pharynx anterior to first placoid (Figure 2A, a). Two thin macroplacoids in placoid stack (42.46 µm) with the first measuring 13.29 µm and the second measuring 26.25 µm. Microplacoid and septulum absent. Claws of legs I–III are the *Hypsibius* type (1212), with the largest being the external claws with a stout primary branch, light transmission areas, smooth bases with pseudolunula, and topped with small accessory points (29.39 µm) (Figure 2B–D). Internal claws are smaller but robust with light transmission areas (Figure 2B–F), smooth bases with pseudolunula, and strong accessory points (17.63 µm). No cuticular bars at the base of claws on leg I, with an internal cuticular bar next to the base of internal claws of legs II and III (Figure 2C,D). Claws of leg IV are the *Hypsibius* type (1212), with the posterior claws being the largest (39.59 µm) with a stout primary branch, light transmission areas, topped with small accessory points, and smooth indented bases with thin, flat, serrated pseudolunula. Anterior claws are smaller but more robust (26.95 µm) with stout primary claws, light transmission areas, topped with large accessory points and smooth indented bases with thin, flat, serrated pseudolunula. Median cuticular bar between the anterior and posterior claws of legs IV (Figure 2E,F).

***Holotype*:** Collected from a sample of moss on 28 December 2016 by Chelsea Scheirer on the south side of Lolo Creek near Fort Fizzle, Montana, of the Bitterroot River (46.74410, −114.17442) (Figure 1). Deposited at the University of California Davis, Bohart Museum of Entomology, Davis California (Number S-0025810).

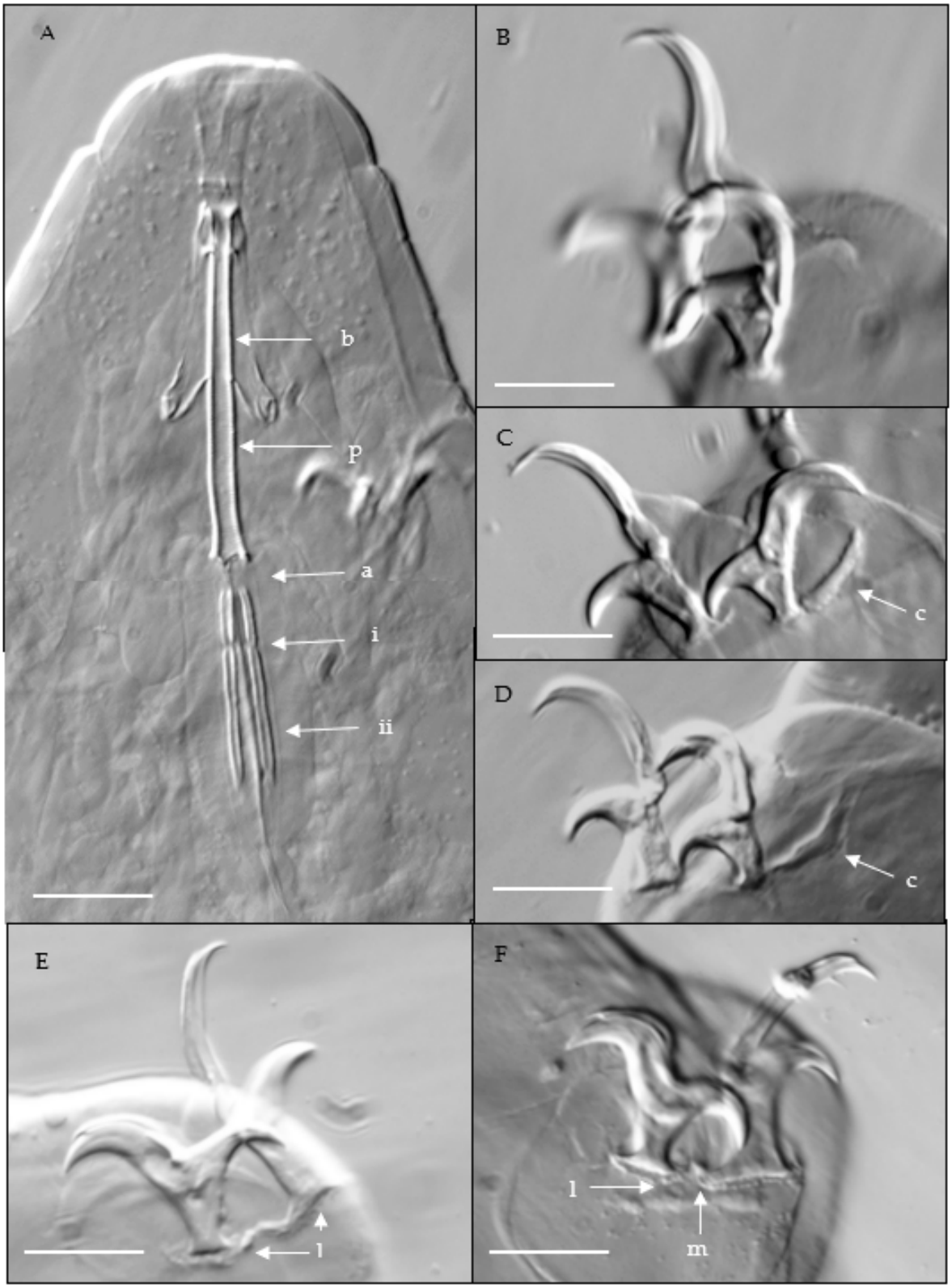

Figure 2. DIC images *Platicrista loloensis* nov. sp. (**A**) Buccal apparatus, a = pharyngeal cuticular bar, b = buccal tube, p = pharyngeal tube, i = first placoid, ii = second placoid (400×), (**B**) claws of leg I; (**C**) claws of leg II, c = internal cuticular bar; (**D**) claws of leg III, c = internal cuticular bar; (**E**) claws of leg IV, l = pseudolunule; (**F**) claws of leg IV, l = pseudolunule, m = median cuticular bar. (**B**–**F**) = 1000×, Scale bar = 10 μm.

Table 2. Morphometric data for *Platicrista loloensis* nov. sp.

		Min	Max	Mean	*Slope (b)*	*Y Intercept (a*)*
Body Length (BL)	6	530.00	750.00	621.33		
pt = BL/BTL (%)	6	1341%	1869%	1666%	0.62	1768%
Bucco-Pharyngeal Apparatus (BPA)	6	70.72	84.84	77.86	0.41	75.51
pt = BPA/BTL (%)	6	194%	237%	210%	0.85	206%
Buccal Tube Length (BTL)	6	29.89	41.26	37.38	0.38	37.38
Buccal Tube Width (BTW)	6	8.59	10.24	9.24	0.25	9.34
pt = BTW/BTL (%)	6	22%	29%	25%	−0.13	26%
Stylet Support Attachment (SSA)	6	29.89	41.26	37.38	0.38	37.98
pt = SSA/BTL (%)	6	100%	100%	100%	0.00	100%
Pharyngeal Tube Length (PTL)	6	36.59	46.32	40.48	0.14	38.63
pt = PTL/BTL (%)	6	94%	137%	110%	0.04	100%
Pharynx Length (PhL)	6	67.32	80.62	74.04	0.37	74.08
pt = PhL/BTL (%)	6	170%	246%	200%	0.28	193%
Placoid Row Length (PRL)	6	41.87	52.35	46.42	0.56	47.60
pt = PRL/BTL (%)	6	106%	146%	125%	−0.20	125%
Placoid 1 Length (P1L)	6	10.90	15.60	13.45	0.75	13.51
pt = P1L/BTL (%)	6	31%	39%	36%	0.37	37%
Placoid 2 Length (P2L)	5	26.25	36.07	31.18	0.51	30.77
pt = P2L/BTL (%)	5	72%	87%	80%	0.42	80%
Exterior Claw I Length (ECI)	5	29.39	33.18	31.36	0.23	31.85
pt = ECI/BTL (%)	5	76%	83%	81%	0.14	83%
Interior Claw I Length (ICI)	6	16.37	24.19	19.98	0.90	20.71
pt = ICI/BTL (%)	6	41%	59%	51%	0.81	54%
Anterior Claw IV Length (ACL-IV)	6	21.65	26.95	25.10	0.41	27.16
pt = ACL-IV/BTL (%)	6	55%	79%	68%	0.00	79%
Posterior Claw IV Length (PCL-IV)	6	32.28	43.97	39.59	0.17	40.01
pt = PCL-IV/BTL (%)	6	94.43%	114.15%	106.18%	−0.23	109.26%

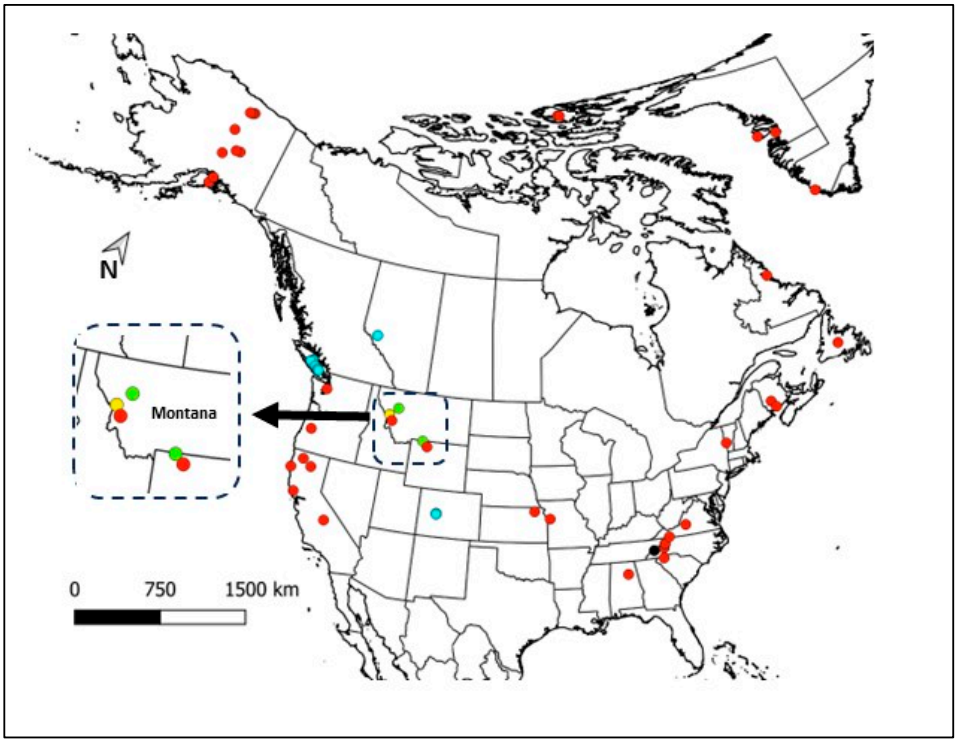

Figure 3. North American records of the genus *Platicista*. Red dots = *Pla. angustata*, light blue dots = *Pla. cheleusis*, green dots = *Plal. brunsoni*, black dot = *Pla. horribilis*, yellow dot = *Pla. loloensis* nov. sp. Locations taken from the work of Kaczmarek et al., 2016 [23].

Table 3. Differential comparison of species of *Platicristia*.

	Platicrista brunsoni	*Platicrista cheleusis*	*Platicrista carpathica*	*Platicrista barneensis*	*Platicrista ramsayi*	*Platicrista loloensis* **nov. sp.**	*Platicrista nivea*	*Platicrista angustata*	*Platicrista horribilis*	*Platicrista aluna*
Specimens used in description	3	14	10	7	3	6	3	28	3	5
Apophyses: Flat	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Furcae: Spoon-like	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Buccal tube: Ridged	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pharyngeal tube: Flexible and striated	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Phaaryngeal apophysis: Absent	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Claws: *Hypsibius* type (2121)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cuticle: Smooth	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes
Cuticle: Low tubercles	Yes	Yes	Yes	No	No	No	No	No	No	No
Cuticle granulated	No	No	Yes	No	No	No	Yes	No	No	No
Pharyngeal tube shorter than buccal tube	Yes	No	No	Yes	Yes	Yes/No	Yes	Yes	Yes/No	Yes
pt SSA	100%	100%	100%	82%	97%	100%	100%	99%	100%	100%
PT/BT ratio	92%	95%	107%	144%	86%	104%	112%	118%	108%	89%
Cuticular bars present	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No
Internal bars on legs I	No	Yes	No	No	Yes	No	No	No	No	No
Median bars on legs I	No	Yes	No	No	No	No	No	No	No	No
Internal bars on legs II–III	Yes	Yes	No	No	Yes	Yes	No	No	No	No
Median bars on legs I–III	No	Yes	No	Yes	No	No	No	No	No	No
Posterior bars on legs IV	No	No	No	Yes	No	No	No	No	No	No
Median bars on legs IV	No	Yes	No	Yes	No	Yes	No	No	No	No
Base of claws on leg IV: Smooth	No	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes
Base of claws on leg IV: Indented	Yes	No	Yes	No	No	Yes	No	No	Yes	No
Pseudolunulae on legs IV	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Pseudolunulae on legs IV Serrated = Ser, smooth = Smo	Ser	No	Smo	No	No	Ser	Smo	Smo	Ser	Smo

***Paratypes*:** Five additional specimens were extracted from the same moss sample. Deposited at the University of California Davis, Bohart Museum of Entomology, Davis California (Numbers S-0025811, S-0025812, S-0025813, S-0025814, S-0025815).

***Etymology*:** The new species is named for the creek that runs beside the collection site. The area has cultural significance for American Indian tribes, specifically the Séliš, QĺIispé, Ksanka and Nimiipuu peoples. The creek and canyon are located within the aboriginal territories of these tribes from time immemorial as a pathway between present day Montana and Idaho. In addition, the Lewis and Clark Expedition used the canyon while travelling west in 1805 and east in 1806.

***Differentiation*:** The new species is similar to the nine other species of the genus *Platicrista* but is different from *Platicrista cheleusis* [26], *Platicrista brunsoni* [5] and *Platicrista carpathica* [18] in Gąsiorek et al. [18] by its smooth vs. tubercated cuticle. *Platicrista loloensis* nov. sp. has internal cuticular bars under the claws that are not found on *Platicrista nivea* [18] in Gąsiorek et al. [18], *Platicrista angustata* [27] or *Platicrista horribilis* [28]. *Platicrista ramsayi* [29] presents internal cuticular bars on legs I, II and III while *Platicrista loloensis* nov. sp. presents internal cuticular bars only on legs II and III. *Platicrista borneensis* [18] in Gąsiorek et al. [18] presents median cuticular bars on legs I-IV, while *Platicrista loloensis* nov. sp. has a median cuticular bar only between the claws on leg IV (Table 3). The new

species has serrated lunules that are similar to *Pla. angustata* and *Pla. horribilis* species with a smooth cuticle. It differs from both by the presence of median cuticular bars in legs IV.

***Discussion*:** The genus *Platicrista* [30] was erected within the subfamily Itaquasconinae Bartoš in Rudescu, [31] to separate the species *Pla. angustata*, originally described by Murray [27] with a flexible bucco-pharyngeal tube with flat ridges for the insertion of stylet muscles and pointed, spoon-like furcae. Pilato [30] included *Diphascon itaquasconoide* [32] and *Diphascon affine* [33] in the new genus. Over the next 16 years, two additional species were described, including *Pla. cheleusis* by Kathman [26] from Vancouver Island and *Pla. horribilis* by Kaczmarek and Michalczyk [28] from Mongolia. Three years later, Marley [29] added *Pla. ramsayi* from Ecuador and followed Dastych's [34] recommendation to declare *Pla. affine* to be a *nomen dubium*. Miller and J.D. Miller [5] found *Pla. brunsoni* in the southern part of the Bob Marshall Wilderness Complex of Montana. Recently, Gąsiorek et al. [18] added three additional species: *Pla. carpathica* from Poland, *Pla. nivea* from Taiwan, and *Pla. borneensis* from Borneo.

The genus *Meplitumen* [35] was separated from *Platicrista* by Lisi et al. [35] based on the presence of annulations on the base of the buccal tube. *Meplitumen aluna* [35] was described as the type species for the genus. Massa et al. [36] transferred *Pla. itaquasconoide* [32] from *Platicrista* to *Meplitumen*. Tumanov and Tsvetkova [37] identified faint annulations on the buccal tube of *Pla. angustata* specimens and by using 18S and 28S rRNA fragments, they moved all the *Meplitumen* species back to the genus *Platicrista* because there were no other significant morphological differences, declaring *Meplitumen* to be a junior synonym to *Platicrista*. Gąsiorek et al. [18] found no differences and formally synonymized *Pla. itaquasconoide* to *Pla. angustata*.

Gąsiorek et al. [18] advanced a re-description of the genus *Platicrista*, (page 27), which contains several diagnostic statements that are not inclusive of some of the species within the genus. The sentence "Cuticle smooth, without pores or undulations. . ." does not include the three species (*Pla. brunsoni*, *Pla. carpathica*, and *Pla. cheleusis*) with notable polyform tubercles. The statement ". . .smooth, ridged buccal tube. . ." does not include the buccal tube annulations of *Pla. aluna* from *Meplitumen*. The sentence "The macroplacoid margins are serrated" should be a species character because some are smooth. The sentence "Pseudolunulae present and well developed" does not apply to all species. Finally, the sentence "Cuticular bars absent." does not apply to four species in the genus with internal and median cuticular bars (*Pla. brunsoni*, *Pla. ramsayi*, *Pla. cheleusis*, *Pla. loloensis* nov. sp.).

Thus, we advance the following amended re-description of the genus *Platicrista* to include all ten species currently assigned to the genus (Table 3).

Amended description of *Platicrista*: Adult body medium-sized to very large, up to 1000 µm, elongated and slightly narrowed anteriorly. Body white to brownish. Head blunt and mouth sub-terminal. Eyes absent. Cuticle without pores, but may be smooth or display irregular wrinkles, fine granulation or shallow polygonal tubercles. Legs very short and plump; legs IV barely distinguishable from the posterior body. Buccopharyngeal apparatus with a wide ridged buccal tube that may be smooth or faintly annulated; flexible, annulated pharyngeal tube; apodeme at the border of the buccal and pharyngeal tube is absent. Stylet supports present and inserted at the caudal position of the buccal tube. Furcae cup shaped with pointed ends. Oral cavity armature absent. Caudal apophyses are wide and show flat ridges. Pharyngeal apophyses absent but the terminal end pharyngeal tube may be slightly enlarged. Pharynx oval and broad, with two long, thin macroplacoids, with the second being about twice as long as the first (1 < 2). Claws of the *Hypsibius* type (1212), large and robust, primary branches of exterior and posterior claws with barely divergent accessory points, slender at their tips. Primary branches of internal and anterior claws are particularly massive with large accessory spines. Pseudolunulae may be present or absent and cuticular bars may be present at the base of the claws in internal or median positions.

The genus seems to have a largely Holarctic distribution with no records of occurrence from Central America [38], or Australasia [39], and only one record from northern Africa [40]. To date, there are three records from South America [41] including *Pla. ramsayi*

from Ecuador [29] near the equator; thus, only the two records from Argentina are significantly below the equator. All three of the new species reported by Gąsiorek et al. [18] are from the equator or above (Borneo, Taiwan, and Poland).

The genus remains infrequently recorded across North America (Canada, Greenland, and USA); there are only 57 records of occurrence for five of the ten species over a period of 120 years. Three of the five species are endemic to North America. Forty-one of those records are *Pla. angustata* from places in Alaska, Kansas, New Brunswick and Greenland, suggesting a continental distribution range (Figure 3), but many records are older and may represent newly described or additional undescribed species. They need verification. *Platicrista cheleusis* has been recorded 11 times from Alaska to Alberta to Vancouver to Colorado, suggesting a northwestern North American range (Figure 3). *Platicrista brunsoni* has two records, including one from western Montana and another from northern Wyoming, suggesting a limited regional range (Figure 3). The remaining record is a single occurrence in Tennessee of *Pla. horribili*, a species known originally from Mongolia. *Platicrista loloensis* nov. sp. has only one record so far (Figure 3).

Platicrista specimens seldom occur in great numbers. Most species have been described based on very few specimens. This new species is no exception, with just six specimens recovered so far and because it has been found at only one location, the presence of a regional population is not yet suggested. The description of five new species in the last four years and the clarification of specific characteristics suggest that the previously reported specimens of *Platicrista* species should be reexamined to have their characteristics confirmed.

5. Comments

There are about 1500 described species of tardigrades worldwide [15]. However, only 320 of these species have been found in North America [22–24]. The records of occurrence of these species are clumped into a few areas because systematic, wide-scale sampling of tardigrade habitats has not been conducted. Generally, collecting occurs through the efforts of a primary researcher and their associates as they address specific research questions or during collecting expeditions (Alaska [42], California [43], Kansas [44], Illinois [45], Tennessee [46,47], New Jersey [48], New Brunswick [49–51], and Greenland [52]).

Recent zoogeographic reviews of the records of occurrence in the literature have provided some understanding of the distribution and diversity of these species [23,24,38–41]. Although there are a few thousand records of occurrence of a tardigrade species being collected at a specific location in North America, there remain great expanses of the continent with no records. There are even fewer data concerning the environments, habitats, and/or relationships to temperature, moisture, or vegetation type. While geopolitical records (states) are important to humans, they mean little to the animals. It would be more useful to consider EPA ecoregions [53] that relate to the conditions of the earth. For example, Missoula is in the 17s Bitterroot-Frenchtown Valley Ecoregion of the level IV EPA.

The diversity of tardigrades in our samples of moss and lichen are typical of habitat samples from around the world (WRM, personal experience). The numbers of one or two species dominate the diversity within the sample, while most species are represented by far fewer specimens. This new species is not an exception. Because it has been found at only one location so far, there is just a single record of occurrence until additional specimens from additional locations are reported.

The addition of three species to the biodiversity list for the state of Montana represents a 10% increase and suggests there are many more species to be found in the geographic boundaries of the state [54].

The Montana biodiversity list for tardigrades has been updated and adjusted for the taxonomic structure and names to reflect the latest guidelines from the "Actual Checklist of Tardigrade Names Version 42" [15] (Table 4).

Table 4. Tardigrade Biodiversity in Montana, U.S.A. in 2024. Taxonomy follows Degma and Guidetti [15].

Class Order Superfamily Family Subfamily Genus species	Leetcham et al. 1980, 1982	Beasley 1988	Miller 2006	Miller and Miller 2021	This Report 2024
Heterotardigrada Marcus, 1927					
Echiniscoidea Richters, 1926					
Ehiniscidae Thulin, 1928					
Acanthechiniscus victor (Ehrenberg, 1853)			X		
Echiniscus arctomys Ehrenberg, 1853			X		
Echiniscus quadrispinosus Richters, 1902			X		
Echiniscus tristosus Cuénot, 1932			X		
Multipseudechiniscus raneyi (Grigarick, Mihelčič and Schuster, 1964)			X	X	
Eutardigrada Richters, 1926					
Apochela Schuster, Nelson, Grigarick and Christenberry, 1980					
Milnesiidae Ramazzotti, 1962					
Milnesium sp.					X
Milnesium tardigradum Doyère, 1840 sensu lato			X		
Parachela Schuster, Nelson, Grigarick and Christenberry, 1980					
Hypsibioidea Plato. 1969					
Hypsibiidae Pilato, 1969					
Diphasconinae Dastych, 1992					
Diphascon alpinum Murray, 1906			X		
Diphascon pingue (Marcus, 1936) sensu lato					X
Diphascon sp.	X				
Hypsibiinae Plato, 1969					
Hypsibius convergens (Urbanowicz, 1925)			X		
Hypsibius macrocalcaratus Beasley, 1988		X			
Itaquasconinae Bartoš, 1962					
Adropion scoticum (Murray, 1905)			X		X
Guidettion arduifrons (Thulin, 1928)			X		
Mesocrista spitzbergensis (Richters, 1903			X		
Platicrista angustata (Murray, 1905)			X		
Placticrista brunsoni Miller and J.D. Miller, 2022				X	
Platicrista loloensis nov. sp.					X

Table 4. *Cont.*

Pilatobiinae Bertolani, Guidetti, Marchioro, Altiero, Rebecchi and Gąsiorek, 2014					
Pilatobius nodulosum (Ramazzotti, 1957)				X	
Pilatobius oculatum (Murray, 1906)			X		
Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008					
Ramazzottius oberhaeuseri (Doyère, 1840)			X		X
Isohypsibioidea Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008					
Hexapodibiidae Cesari, Vechhi, Plamer, Bertolani, Rebecchi and Geodetti, 2016					
Hexapodibus sp.	X				
Isohypsibioidae Sands, McInnes, Marley, Goodall-Copestake, Convey and Linse, 2008					
Isohypsibius prosostomus Thulin, 1928			X		
Isohypsibius sp.				X	X
Macrobiotoidea Thulin, 1928					
Macrobiotidae Thulin, 1928					
Macrobiotus hufelandi C.A.S. Schultze, 1834 sensu lato			X	X	X
Macrobiotus islandicus Richtersi, 1904			X		
Macrobiotus sp.	X				
Mesobiotus harmsworthi (Murray, 1907) sensu lato			X	X	X
Paramacrobiotus areolatus (Murray, 1907) sinsu lato			X		
Paramacrobiotus richtersi (Murray, 1911) sensu lato			X		X
Paramacrobiotus tonollii (Ramazzotti, 1956)					X
Total Species = 27	3	1	20	6	10

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