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## **Tardigrades of the canopy: *Doryphoribius elleneddiei* nov. sp. (Eutardigrada, Parachela, Hypsibiidae), a new species from eastern Kansas, U.S.A.**

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A new species of the genus *Doryphoribius* of the phylum Tardigrada is described from eastern Kansas. *Doryphoribius elleneddiei* nov. sp. is a modest tardigrade (200–400 µm) without eyes but with three macroplacoids, polygonal tubercles on the cuticle, sixteen rows of dorso-lateral gibbosities and an additional gibbosity on legs IV. The species was recovered from moss habitat found in the canopy of walnut, cottonwood, and elm trees substrates more than 6 meters (20 feet) above the ground.

*Keywords: Tardigrada, Doryphoribius, gibbosities, canopy, Kansas, new species*

### **INTRODUCTION**

Despite recent success as space travelers (Jonsson et al. 2008) and spectacular SEM images being used in the popular press (Miller 2011; National Geographic 2013) tardigrades remain little known and under studied. Our knowledge of their diversity and distribution is fragmented (McInnis 1994, Kinchin 1994, Miller et al. 2013) and water bears are even less known as a canopy animal (Miller 2004, Miller and Lowman 2012).

The genus, *Doryphoribius* Pilato, 1969 is described with a reinforcing bar under the buccal tube similar to the genus *Macrobotus* and claws similar to the genus *Isohypsibius*. Degma et al. (2009–2014) lists 29 species for the genus but Michalczyk and Kaczmarek (2010) suggest that many species of genus *Isohypsibius* may in fact be *Doryphoribus*.

Michalczyk and Kaczmarek (2010) divided the genus *Doryphoribius* into four “groups” based on the number of placoids (two or three) and the presence or absence of gibbosities. This paper describes a new species in the *Doryphoribus*

*vietnamensis* (Iharos 1969) “group” as modified by Beasley et al. (2006) with three placoids and gibbosities found in Kansas, USA.

### **MATERIALS AND METHODS**

The study area, Douglas County in northeast Kansas, U.S.A., is in the transition zone between the western tall grass prairie and eastern oak-hickory deciduous forest. There are no geographic impediments to the prevailing westerly winds for 500 miles. Vertical transects were conducted by climbing into the canopy with DRT (Double Rope Technique) (Haefke et al. 2013). Samples of tardigrade habitat (moss and lichens) were collected by scraping approximately 50 square centimeters of habitat into a paper lunch bag with a putty knife. In the lab, samples were soaked in bottled spring water for 24 hours (Miller 1997). Three one ml sub-samples were examined with a reflected-light dissecting microscope at 20–30x magnification. Tardigrades were removed with an Irwin Loop (Schram and Davidson 2012) and placed in Polyvinyl Alcohol (PVA) mounting medium on a glass slide. Tardigrades were identified with an Olympus BX60 DIC

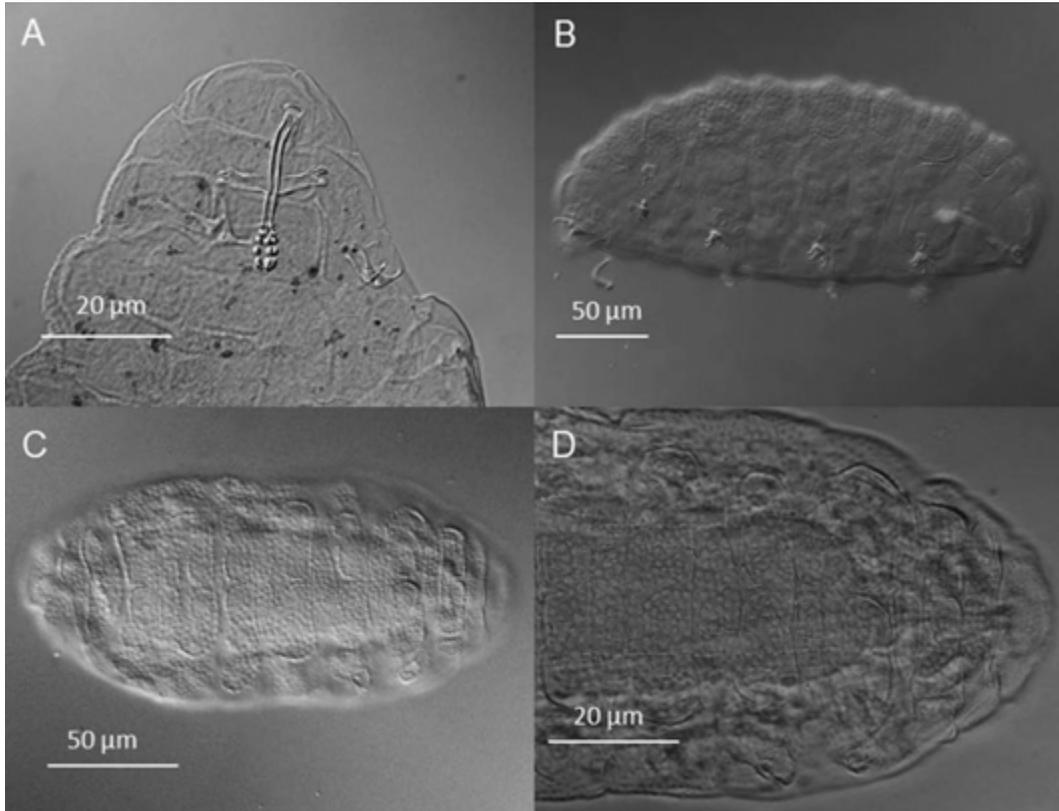


Figure 1. *Doryphoribius elleneddiei* nov. sp. (DIC Images), A. Bucco-pharyngeal apparatus B. Lateral view, C. Dorsal view with gibbosities clearly outlined, D. Dorsal surface.

microscope at 400-1000x magnification. Images and measurements were made with Olympus CellSens Standard 1.6 imaging software.

Tardigrade identifications are based on Ramazzotti and Maucci (1983), Pilato and Binda (2010), and Michalczyk and Kaczmarek (2010). Nomenclature follows Guidetti and Bertolani (2005), Degma and Guidetti (2007), and Degma, Bertolani and Guidetti (2009-2014).

Additional specimens were recovered for scanning electron microscope (SEM) imaging and stored in 70% ETOH. The chemical drying HMDS method described by Shively and Miller (2009) was used in place of the CPD (Critical Point Drying) method. The specimens were placed on an adhesive stud and sputter-coated with gold. SEM imaging was conducted

at the University of Kansas, Microscopy and Analytical Imaging Laboratory using the Carl Zeiss Leo 1550 Field Emission scanning electron microscope.

## RESULTS

Six specimens of the proposed new species were collected in July, 2013, from moss habitat more than 6 meters up the trunks of a black walnut (*Juglans nigra* L.) and an eastern cottonwood tree (*Populus deltoides* W. Bartram ex Humphry Marshall) on the Baker University Wetlands, south of Lawrence, Kansas. The new species was also found in the canopy of an American elm tree (*Ulmus americana* L.) at the University of Kansas Rice Woods near Baldwin City, Kansas.

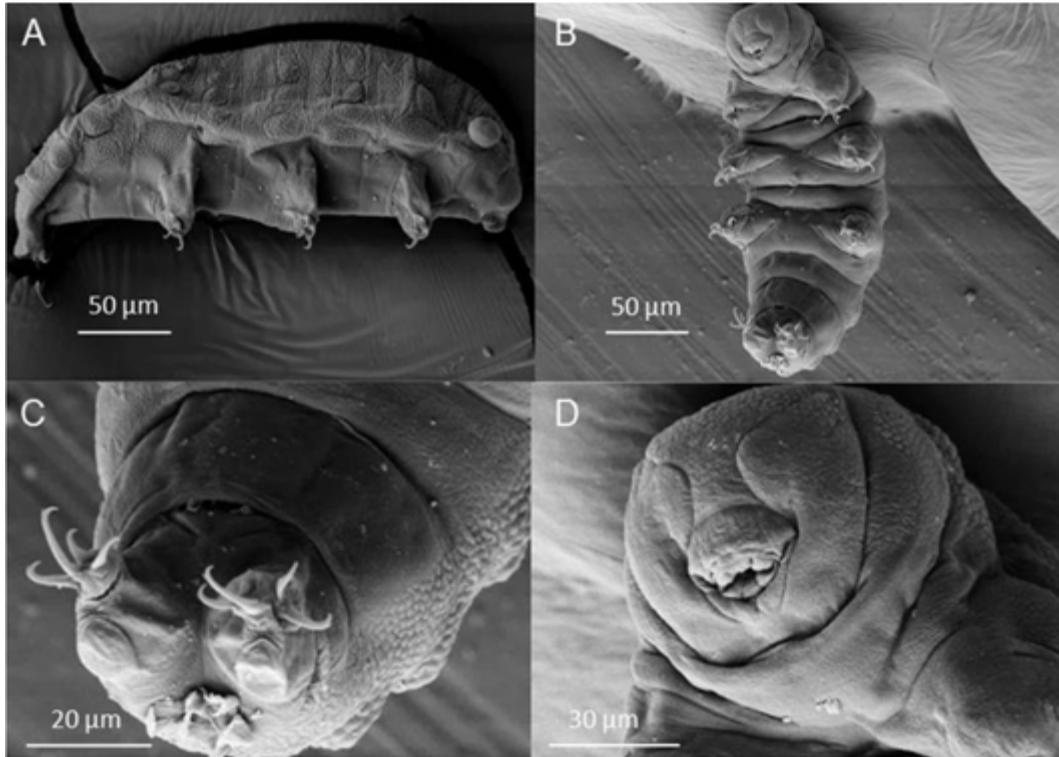


Figure 2. *Doryphoribius elleneddiei* nov. sp. (SEM Images), A. Lateral view showing gibbosities, B. Ventral view, C. Legs IV, showing accessory points on primary branches of claws, gibbosities on leg IV and tubercles on the cuticle, D. Anterior view showing six buccal lamellae.

#### TAXONOMY

Eutardigrada Richters, 1926

Parachela Schuster, Nelson, Grigarick and Christenberry 1980

Isohypsibioidea Marley, McInnes and Sands 2011

Isohypsibiidae Marley, McInnes and Sands 2011

*Doryphoribius* Pilato 1969

*Doryphoribius elleneddiei*, nov. sp.

(Figures 1, 2; Tables 1, 2)

**Diagnosis:** Off-white *Doryphoribius* without eyes but with three macroplacoids, dorsal cuticle with polygonal tubercles, 16 rows of dorso-lateral gibbosities, and gibbosities on legs II, III, and IV.

#### Description of holotype:

Off-white

*Doryphoribius*, length 296.3 μm, eyes absent.

Dorso-lateral cuticle covered with polygonal tubercles (diameter 1-2 μm), not present on legs.

Dorsal tubercles larger than lateral tubercles.

Sixteen rows dorso-lateral gibbosities present:

XVI: 3-4-2-6-2-6-2-6-2-6-2-6-2-3-3+2[L<sub>II-IV</sub>].

Rows V, IX, and XII are aligned with legs I, II,

and III, respectively. Dorsal gibbosities oval,

lateral gibbosities circular. Additional gibbosities

present on anterior side of legs II, III and IV.

Sub-terminal mouth with 6 small pre-buccal lamellae. Buccal tube and pharynx of *Doryphoribius* type, buccal support bar present. Buccal tube length 33.9 μm, internal width 1.4 μm, external width 2.8 μm. Stylet insertion point 22.6 μm. Pharynx with three macroplacoid rows, increasing in length from first to last. Anterior placoid 1.6 μm, middle 2.0 μm, and posterior 3.8 μm. Microplacoid absent.

Table 1. Measurements of morphological features in the holotype and 5 paratypes. Measurements in micrometers ( $\mu\text{m}$ ). Measurements of structures were only taken if their orientations were suitable.

Character	CASIZ Nbr	Holotype 197443	Paratype 1 197444	Paratype 2 197445	Paratype 3 197446	Paratype 4 197447	Paratype 5 197448	Mean
Body Length		296.3	342.1	358.7	315.5	324.2	298.8	322.6
Buccal tube length		33.9	31.9	36.2	35.5	31.9	32.4	33.5
Buccal tube ext. width		2.8	3.6	4.4	3.9	3.4	3.9	3.6
Buccal tube int. width		1.4	2.1	1.8	1.8	1.7	1.6	1.7
Stylet support insertion		22.6	22.2	25.9	24.5	22.2	22.5	23.3
Macroplacoid 1		1.6	1.8	1.9	2.9	1.9	1.7	2.0
Macroplacoid 2		2.0	2.0	2.5	2.1	2.1	2.1	2.1
Macroplacoid 3		3.8	2.8	4.4	3.9	4.2	3.4	3.7
Macroplacoid Row		11.2	9.8	11.2	10.6	10.2	9.4	10.4
Pharynx Length		26.3	30.8	-	30.0	25.0	-	30.0
Pharynx Width		26.2	29.3	-	32.6	23.0	-	32.6
Claw I – external – bc		2.9	5.2	4.2	4.3	2.5	4.9	4.0
Claw I – external – pb		9.3	11.4	9.7	8.3	9.3	9.4	9.6
Claw I – external – sb		7.6	7.1	7.4	7.4	7.0	7.2	7.3
Claw I – internal – bc		3.8	5.7	3.8	3.9	2.6	3.9	3.9
Claw I – internal – pb		6.7	7.9	8.1	10.7	7.3	7.8	8.1
Claw I – internal – sb		6.4	7.3	6.5	8.4	5.3	7.2	6.9
Claw II – external – bc		3.4	-	5.8	3.4	2.5	5.5	4.4
Claw II – external – pb		?	-	12.5	7.4	9.9	10.4	8.9
Claw II – external – sb		7.6	-	9.4	6.5	8.1	8.7	7.6
Claw II – internal – bc		3.3	-	4.3	2.5	2.9	3.4	2.9
Claw II – internal – pb		8.1	-	8.4	10.2	7.7	?	10.2
Claw II – internal – sb		6.2	-	7.5	9.8	6.0	6.2	8.0
Claw III – external – bc		3.3	2.6	4	5.2	3.2	4.9	3.9
Claw III – external – pb		8.5	11.1	11.5	11.9	9.1	11.5	10.6
Claw III – external – sb		8.2	10.6	6.8	8.3	7.9	6.7	8.1
Claw III – internal – bc		3.8	3.8	4.7	5.3	3.1	4.1	4.1
Claw III – internal – pb		9.5	8.9	10.7	8.1	6.6	8.2	8.7
Claw III – internal – sb		7.2	6.5	5.9	8.3	6.1	6.5	6.7
Claw IV – external – bc		2.8	5.0	3.0	-	-	5.0	4.3
Claw IV – external – pb		9.7	13.2	9.1	-	-	10.2	10.8
Claw IV – external – sb		-	9.0	7.4	-	-	5.5	7.3
Claw IV – internal – bc		-	4.6	4.6	4.6	-	4.2	4.5
Claw IV – internal – pb		-	8.7	12.6	9.7	-	8.0	9.8
Claw IV – internal – sb		-	6.6	10.0	7.4	-	4.5	7.1

bc = basal claw, pb = primary branch, sb = secondary branch. - = orientation not suitable.

Claws of the *Isohypsibius* type, internal and external claws different in size. Main branches with two accessory points. Lunules absent. Eggs unknown at this time. Table 1 provides morphometric data for holotype and five paratypes.

**Type depositary:** Holotype CASIZ-197443, collected by Brandon Haefke, 10 July, 2013, from moss habitat 6.2 m (21 feet) up the trunk of a black walnut (*Juglans nigra* L.) on the Baker University Wetlands, 38.91381°N, 95.22765°W. Deposited at the California Academy of Science.

**Paratypes:** CASIZ-197444, collected by Anna Spiers, 10 July, 2013, from moss 8.2 m (27 ft) up the trunk of Eastern Cottonwood tree (*Populus deltoides* W.Bartram ex Humphry Marshall) on the Baker University Wetland. CASIZ-197445, collected by Anna Spiers, 3 July, 2013 from moss 7.6 m (25 ft) up the trunk of American Elm (*Ulmus Americana* L.) at the University Kansas Rice Woods, 38.80900°N, 95.17750°W. Three additional paratypes from same location as holotype and deposited at the California Academy of Science; CCASIZ-197446, CASIZ-197447 and CASIZ-197448.

Table 2. Comparison of key characteristics within *Doryphoribius vietnamensis* "group."

Species	Eyes	Cuticle	Gibbosities	Prebuccal lamella
			Number of rows: number in each row	
<i>D. minimus</i>	No	polygonal tubercles	VII: 2-2-2-2-2-2-2-2	No
<i>D. vietnamensis</i>	Yes	fine granules	IX: 5-4-5-4-5-4-5-2-3	No
<i>D. gibber</i>	Yes	half spherical tubercles	X: 4-4-4-4-4-4-4-2-2-3	No
<i>D. mariae</i>	Yes	Smooth	XII: 2-4-2-4-2-4-2-4-2-4-4-1	No
<i>D. elleneddiei</i> n.s.	No	polygonal tubercles	XVI: 3-4-2-6-2-6-2-6-2-6-2-6-2-3-3+2[L <sub>II-IV</sub> ]	Yes

**Etymology:** The species name *elleneddiei* is a concatenation of the first names of Ellen Zuckerman and Edward Burgess and was selected to honor these two young scientists from Princeton who have demonstrated philanthropic support of world conservation efforts including both forest canopies and tardigrades.

**Differential Diagnosis:** *Doryphoribius elleneddiei* nov. sp. is to be placed into the *D. vietnamensis* group because it exhibits three rows of placoids and cuticular gibbosities. The group includes *Doryphoribius vietnamensis* (Iharos 1969); *Doryphoribius gibber* Beasley and Pilato 1987; *Doryphoribius mariae* Pilato and Binda, 1990 and *Doryphoribius minimus* Bartels, Nelson, Kaczmarek and Michalczyk, 2008. *Dorphyphoribius elleneddiei* nov. sp. differs from all other members of the group by the number and arrangement of gibbosities, the presence of polygonal tubercles on the cuticle, and pre-buccal lamellae (Table 2).

In addition, *D. gibber* has two dorso-lateral teeth not found on *D. elleneddiei* nov. sp. while *D. mariae* has eyes not observed in *D. elleneddiei* nov. sp. and lacks the gibbosity on its legs. Dr. Pilato of Catania, Italy confirmed *D. mariae* also lacks the polygonal tubercles on the cuticle and has eyes (pers. Comm.). Because of these numerous differences, we conclude *Doryphoribius elleneddiei* nov. sp. to be a new species within the genus *Doryphoribius*.

While data are still sparse, it is curious that all specimens of this new species were found in one habitat (moss) on three different substrates (walnut, cottonwood, and elm) and always above 6 meters (20 ft).

#### ACKNOWLEDGMENTS

We wish to thank the anonymous reviewers whose suggestions have improved the manuscript. We express our gratitude to Dr. Pilato for sharing his images and knowledge of *Doryphoribius* to aid our identifications. Likewise, we are indebted to the late Dr. Clark Beasley of McMurry University for access to his collection for the purpose of species comparisons. Our research was funded by the National Science Foundation through DEB: REU #1156550 to Baker University. We would also like to thank the Kansas Biological Survey at the University of Kansas for access to their ecological reserves. Finally, we thank the Microscopy and Analytical Imaging Laboratory at the University of Kansas for instruction and use of their scanning electron microscope. Additional support was provided by the Tree Foundation.

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