



## **Note on Photographing Embryos and Hatchlings of Leiobunum sp. Harvestmen (Arachnida: Opiliones: Sclerosomatidae: Leiobuninae)**

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Source: Florida Entomologist, 96(2) : 679-681

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.096.0246>

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NOTE ON PHOTOGRAPHING EMBRYOS AND HATCHLINGS OF  
*LEIOBUNUM* SP. HARVESTMEN (ARACHNIDA: OPILIONES:  
SCLEROSOMATIDAE: LEIOBUNINAE)

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The arachnid harvestmen (“daddy long-legs”) of Genus *Leiobunum* (Koch 1839; 1848) (Opiliones: Sclerosomatidae) are nearly ubiquitous animals within various natural and urban mesic habitats of the Holarctic ecozone (Giribet & Kury 2007), including the species-rich Leiobuninae assemblages of the eastern and central United States and Canada (Ingianni et al. 2011). To date, the embryology and ontogeny of the harvestmen have received less attention than those of the other arachnids, but this is changing for the better (Gnaspini 2007; Gnaspini & Lerche 2010). Here we describe our attempt to create a laboratory breeding colony of *Leiobunum* sp. harvestmen, and also our observations on eggs, embryonic development, and hatchling morphology.

On 6 Oct 2011, we received a plastic container holding 18 live *Leiobunum* sp. adults collected from a back yard in Gastonia, North Carolina (N 35° 15' 44" W 81° 11' 14"; 240 m asl). We identified the harvestmen as members of Genus *Leiobunum* using Pinto-da-Rocha & Giribet (2007). W. A. Shear (Biology Dept., Hampden-Sydney College, Hampden-Sydney, Virginia, USA personal communication) examined one of our adult females (dried, then preserved in ethanol); he confirmed Genus *Leiobunum*, but could not identify the species. The adults were housed in 2 plastic terraria (volume = 13.6 liters each) with soil, leaf litter, and a moistened sponge. The adults were maintained at ambient lab temperature (approximately 24 °C) and fed a diet of dead ants, bits of squashed crickets, and

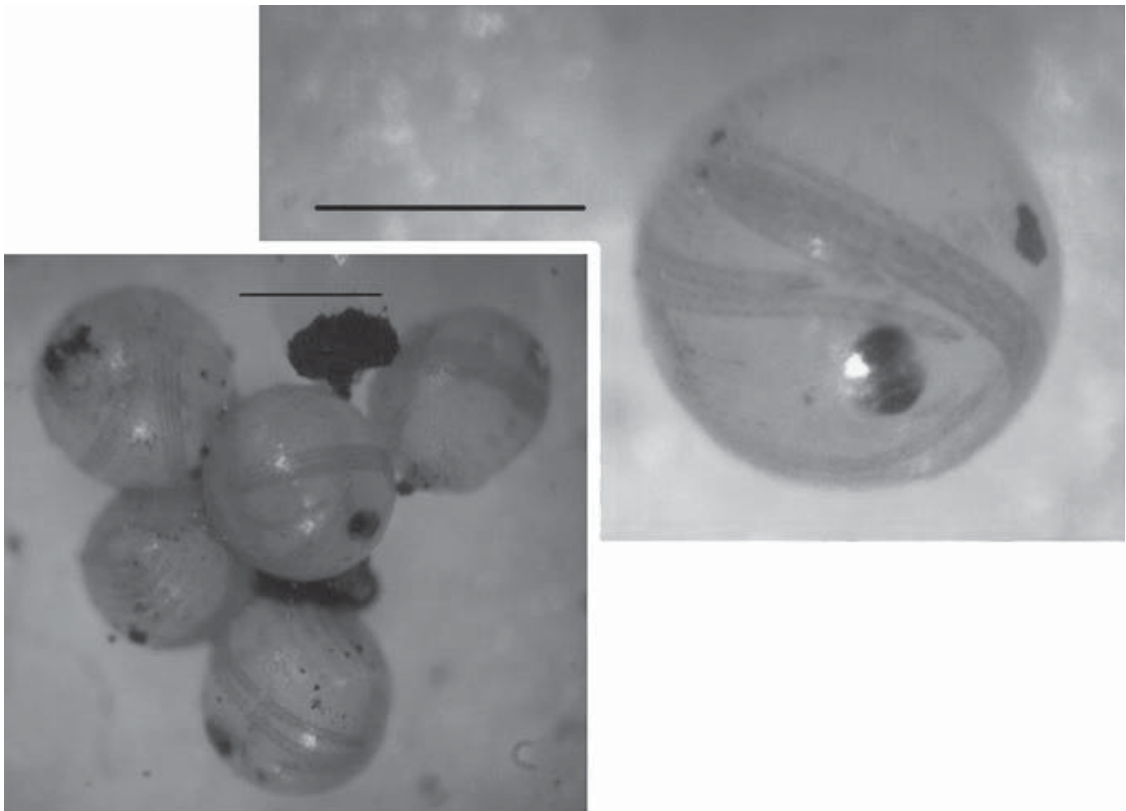


Fig. 1. *Leiobunum* sp. Egg and egg clutch (scale-bar = 1.00 mm).

small bits of apple. We observed some *Leiobunum* sp. courtship and mating, and on 18 Nov 2011, we found one small clutch of 25 whitish eggs, each approximately 1.4 mm in diam. Most of our caged *Leiobunum* sp. adults survived for several weeks; 4 adults survived a total of 44 days, but none lived beyond 15 Dec 2011.

The eggs were washed in 1X PBS (phosphate buffered saline) to remove soil debris; this solution was prepared by dilution of concentrated 10X Dulbecco's PBS (MP Biochemicals, LLC; Solon, Ohio). The eggs were carefully placed onto Whatman filter paper suspended over a petri dish. PBS was rinsed over the eggs drop-wise using a plastic transfer pipette until most of the soil debris had washed away from the surface of the eggs. The Whatman filter paper was placed into a small plastic container with the lid perforated with pinholes for aeration. Eggs were checked visually every other day for changes, and the filter paper was kept moistened with tap water. Hatchlings ( $n = 5$ ) were maintained in the same container with the washed egg clusters.

Photographs of eggs and hatchlings were taken using an Optronics 2.0 20 MPX digital microscope camera attached to an Olympus SZH10 research stereo microscope. The images were processed using Rincon Image Analysis Software. To determine scale on the digital photographs, a digital image of a metric ruler was acquired using the same microscope, magnification setting, and camera used when taking images of the specimens. We opened the digital image of the metric ruler in Microsoft *Paint*, and used the pixel counter in

*Paint* to determine the number of pixels per mm length on the ruler image. Three px/mm measurements were taken at different spots on the ruler image, then averaged together to determine the px/mm for that particular magnification setting on that particular microscope using that particular camera. Scale bars were then drawn using Microsoft *Paint*, according to pixel length, then converted to mm for description in the figure caption.

Fig. 1 shows an egg and a small clutch of eggs photographed on 2 Dec 2011. The most prominent features of an embryo include the two large eyes and the long legs wrapped within the egg. Fig. 2 shows a hatchling also photographed on 2 Dec 2011 standing in front of an egg. These photos were taken roughly 6 weeks after the eggs first appeared.

None of our field-captured adults survived beyond mid-Dec 2011, and it is likely that the last to expire were very old animals; Edgar (1971) noted that temperate-latitude *Leiobunum* spp. adults rarely survive beyond several months. Gnaspini (2007) and Gnaspini & Lerche (2010) published excellent photos of embryonic development for several lineages of tropical harvestmen, but to our knowledge, we have captured perhaps the only photo image of a *Leiobunum* embryo and hatchling together.

This project was supported by the NIH-NIGMS MARC: T34 GM008021 and the NIH-NIGMS MBRS RISE: R25 GM059244 awards to Barry University. We are grateful to Dr. William Shear (Hampden-Sydney College, Hampden-Sydney, Virginia, USA) for his advice and encouragement.

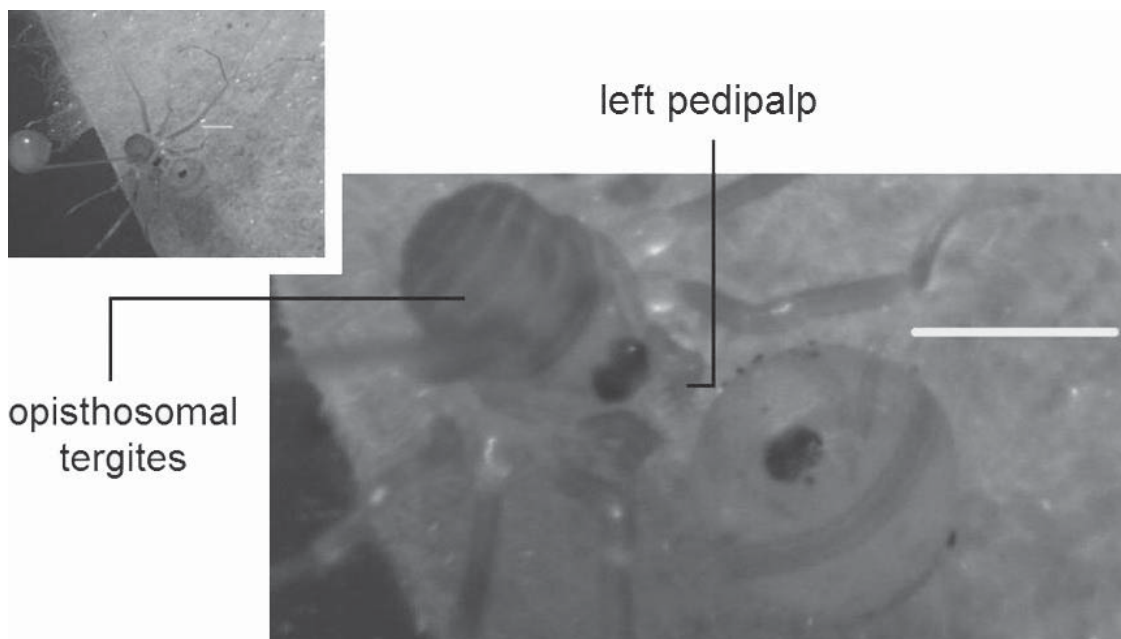


Fig. 2. *Leiobunum* sp. hatchling and egg (scale-bar = 1.00 mm).

## SUMMARY

We reared a lab culture of *Leiobunum* sp. harvestmen adults in 2011, and captured several unique photo images of developing eggs and a hatchling. These images are perhaps the first to be recorded for this genus.

## RESUMEN

Se criaron adultos opiliones del género *Leiobunum* sp. en el laboratorio en el 2011, y se capturó varias fotos con imágenes únicas de los huevos desarrollándose y un individuo recién nacido. Estas imágenes son quizás las primeras en ser tomadas para este género.

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