UV-Induced Fluorescence of Two Puerto Rican Endemic Arthropods: The Harvestman, *Yunquenus portoricanus* and the Millipede, *Spirobolellus richmondi* [1]

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Abstract: Two endemic arthropods, the harvestman, *Yunquenus portoricanus*, and the millipede, *Spirobolellus richmondi*, from a humid montane forest in Puerto Rico, are first reported to fluoresce when they are illuminated with ultraviolet light (360 nm). Both species are relatively small, nocturnal dwellers and have a dark exoskeleton, three attributes that make them almost imperceptible during night surveys using white lights. The use of UV illumination enhances the detection of these animals during field surveys.

Key Words: Biofluorescence, biophosphorescense, bioluminescense, ultraviolet illumination, *Yunquenus portoricanus*, Opiliones, *Spirobolellus richmondi*, Diplopoda, Spirobolida, Carite Forest, Puerto Rico

Introduction

Light production is ubiquitous in life. In the presence of some sources of electromagnetic radiation, many organisms glow either by fluorescence or by phosphorescence. Fluorescence is the absorption of light (e.g., ultraviolet) and its reemission in another, usually longer, wavelength (e.g., blue). When the source of light is eliminated, the fluorescence ceases (Wannas et al. 2019). Different wavelengths have been found to elicit distinct fluorescent patterns useful for quality imaging (Haug et al. 2011). On the other hand, phosphorescence is the emission of light, usually green, pink, red orange or purple, during the time the object is being illuminated and for a time after the source of illumination has been removed (Wannas et al. 2019). In contrast to both fluorescence and phosphorescence, bioluminescence is the cellular production of light by organisms without an external source of electromagnetic illumination. Bioluminescence, as in the light that fireflies and numerous other organisms produce, is a biochemical reaction involving an enzyme, generically known as "luciferase", and a substrate, broadly known as "luciferin", with minimal production of heat (Harvey 1952, Shimomura 2011). Bioluminescence is being used in many practical biological applications (Rich and Douillet 2009, Kim 2016).

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Fluorescent organisms have a wide array of chemicals that make them glow in the dark in the presence of electromagnetic radiation. Those compounds include flavins, reduced NADH and NADPH, lipofuscins, reticulin fibers, collagen, elastin, chitin, pterins, among many others that are integrated in their integument or other tissues (Lawrence 1954, Main 2013, Jeng 2019, Wannas et al. 2019). Biofluorescence has been documented in scorpions, in which the use of UV illumination is a common practice to detect these generally nocturnal organisms, spiders (Andrew et al. 2007), millipedes (Sierwald et al. 2019), including fossil polyxenid millipedes [*Phryssonotus burmiticus* (Cockerell, 1917), Su et al. 2019], among many other vertebrates and invertebrates.

In arachnids, Andrews et al. (2007) reported fluorescence in 41 genera of spiders, an attribute that has been recently extended to opilionids or harvestmen (Rubin et al. 2017), such as *Yunquenus portoricanus* Šilhavý, 1973 (Agoristenidae), the latter herein also reported for the first time as fluorescent.

In millipedes, numerous extant species out of the over 12,000 described are known to fluoresce. Some examples of extant species include, *Motyxia sequoiae* (Loomis and Davenport, 1951) (Kuse et al. 2001, as *Luminodesmus sequoiae*), *Parafontaria laminata* (Attems, 1909) (Kuse et al. 2014), and several species of *Pseudopolydesmus* (Sierwald et al. 2009), all placed in the Order Polydesmida. Herein, we report *Spirobolellus richmondi* (Chamberlin, 1922; Diplopoda: Spirobolida: Spirobolellidae), a millipede endemic to montane habitats in Puerto Rico (Galanes and Thomlinson 2011)[4], such as the Carite State Forest, as fluorescent for the first time.

Methods

Site Description. The Carite Forest (Cayey, Puerto Rico: 18°3'58.86" N, 66° 7'55.77" W; elevation ~680 m above sea level) ecosystem is characterized by having high rainfall (annual mean = 181.6 mm), cool temperatures (average minima and maxima = 17.5ºC and 26.6ºC, respectively; Colón-Torres 2009). Carite is a subtropical wet montane forest remarkable for its lush vegetation. Epiphytic ferns, bromeliads, and orchids are common, coexisting with more than 150 species of trees that forms a dark and closed canopy at about 20 meters (Miller and Lugo 2009). This floral tropical jungle provides a continuous income of litterfall, which becomes available for saprophytic organisms, like millipedes.

Equipment and Procedures. Four nocturnal (8:00 – 11:00 pm) surveys of invertebrates were conducted on September 9-12, 2019 along a 200 m long forest patch in the Carite State Forest. A Police Security Elite 1,800-Lumen Ultra Bright Flashlight and an HK 100 LED 360 nm ultraviolet (UV) Blacklight Flashlight, were employed to detect the presence of soil and tree trunk dwellers. The UV lamp eased author ENLN spot the glow of fluorescent and phosphorescent

[4] In Puerto Rico, millipedes are known by several common names, including “gongolí”, “gongolo”, “gongolón”, “gungulén”, “gongola”, “gongolimbo”, “milipiés”, and “milpatas”, depending on the size of the millipede and the region of the island where it is seen.

animals. Both lamps were pointed to all possible directions within the sampling area (e.g., soil, rocks, tree trunks and leaves, snags). Night sampling detected only two types of animals exhibiting fluorescence: a pair of harvestmen or daddy longlegs (Arachnida: Opiliones) and hundreds of millipedes (Diplopoda). The fluorescing invertebrates were photographed and digitally video recorded *in vivo* with and without UV light using a SONY Alpha 6500 with a SONY 19-135 mm Macro lens.

Organisms' preservation and identification. Given only two opilionids were found to glow, they were imaged and released. The arachnid was identified comparing photographs and anatomical attributes following (Šilhavý 1973) and Pinto-Da Rocha and Ryotara-Hara (2009). Some diplopods were collected and preserved in 70% ethanol and identified following Vélez (1965, as *Microspirobolus richmondi*). Updated millipede nomenclature followed Hoffman (1999) as well as Sierwald and Spelda (2019). Voucher specimens of adult *S. richmondi* (four males and four females) have been deposited in the Museum of Entomology and Tropical Biodiversity of the University of Puerto Rico, Mayagüez Campus. The MEBT is located at the Estación Experimental Agrícola, Jardín Botánico Sur in San Juan, Puerto Rico.

Results and Discussion

Two arthropods endemic of Puerto Rico were found to fluoresce in the sampling area during the four nights' survey: the harvestman, *Yunquenus portoricanus* (Silhavý, 1973; Agoristenidae), and the millipede *Spirobolellus richmondi*. (Chamberlin, 1922; Diplopoda: Spirobolida). As far as we know, this is the first report of biofluorescence for these species. The harvestman is naturally dark brown and glowed blue green (Figure 1), a finding very similar reported for spiders (Andrew et al. 2007) and other Opiliones (Rubin et al. 2017). The following link shows the glow of *Yunquenus portoricanus*: https://youtu.be/mHV5DN1Ux58.

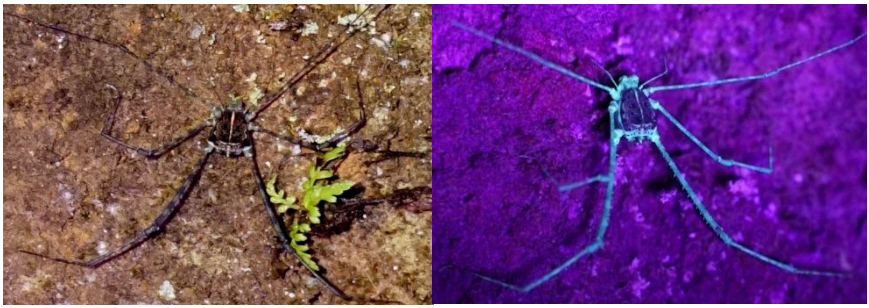

Figure 1. The harvestman *Yunquenus portoricanus* (Opiliones) showing its natural dark (left) and its UV induced fluorescent color (right).

Thirteen species of harvestmen, most of them endemic, have been reported for Puerto Rico (Alegre-Barroso and de Armas 2012, Kury 2003). Harvestmen are omnivorous and their diet includes small, soft skinned arthropods and other invertebrates, as well as carrion, plants and fungi (Pinto-Da Rocha et al. 2007). The observed harvestmen *Yunquenus portoricanus* is a solitary arachnid that dwells in the humid ground of the Carite State Forest. Its body is ~0.50 cm long, while the length of the posterior and spinier pair of legs if sixfold that. According to Šilhavý (1973) and Pinto-Da Rocha and Ryotara-Hara (2009), *Y. portoricanus* most prominent characteristic is its relatively spiny opisthosoma, which includes two notorious spines (Figure 2). Besides its taxonomy, there is no ecological or natural history information about this species.

Figure 2. The harvestman *Yunquenus portoricanus* (Opiliones) prominent spiny opisthosoma. Dorsal (left panel). Posterior (right panel). Photos taken by author ENL.

Millipedes are well characterized in Puerto Rico, where about 50 species and subspecies have been described, exhibiting a high degree of endemism, with 39 described endemic species and subspecies (Vélez 1965, Santiago-Blay and Vélez 1985, Galanes and Thomlinson 2011). Hundreds of *Spirobolellus richmondi* were found actively foraging on the soil, within the litterfall, and on tree trunks, particularly on the West Indian tree fern, *Cyathea arborea* (L.) Sm. (Cyatheaceae), less than 2 m above the ground. They were easily detected by their blue-glowing (Figure 3). Although their relative abundance, they were generally found as singletons or as mating pairs, not aggregated. This species of millipede is endemic to Puerto Rico (Vélez 1965, Galanes and Thomlinson 2011, see also Torres and González 2005).

Figure 3. Glowing *Spirobolellus richmondi* dwelling over litterfall.

According to Vélez (1965), adult *Spirobolellus richmondi* are dark brown, medium sized (approximately 20-35 mm long) millipedes with a strongly hardened integument, and over 30 acarinated diplosegments. *Spirobolellus richmondi* often have two mid-dorsal lines consisting of pale brown spots. The antennae and legs are pale yellow to nearly white and the eyes have about 15 ocelli. The collum is narrowed laterally and does not ventrally surpass the level of the following diplosegments. The openings of the repugnatorial glands, or ozopores, when present, are located in the posterior half, or metazonite, of the diplosegments (Figure 4). The anal tergite generally does not surpass the paraproct. The posterior margin of the epiproct is concave and posterior margin of the hypoproct is angular (Figure 4). Coleopods as in Figure 4.

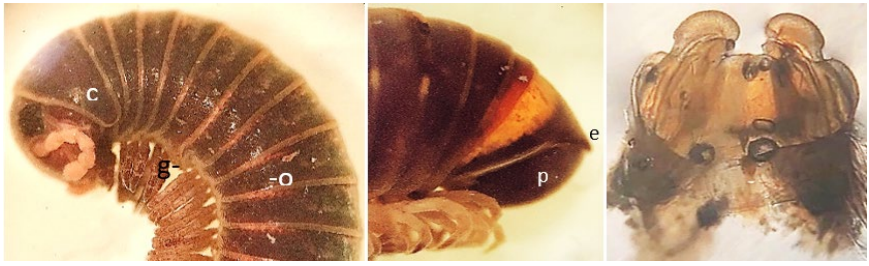

Figure 4. Male *S. richmondi*. Lateral view of head, collum (c), and first ten segments (left panel, including apex of coleopods, g). Lateral view of posterior segments, including epiproct (e) and paraproct (p, central panel). Anterior views of coleopods (right panel).

Spirobolellus richmondi tends to occupy montane regions characterized by an elevation not exceeding 850 meters above sea level, generally eight kilometers or more from the coast, moderately high annual precipitation (140-265 mm) and cooler temperatures 23-24°C. Given its coloration and the typical dark foraging habitat, the species is almost imperceptible to the unaided eyes, but effortlessly detectable with ultraviolet light sources. During the day, the species assumes a cryptic behavior resting in tree crevices, under rocks or snags, or within the litterfall. A video clips showing *S. richmondi* glowing in the forest can be accessed at https://youtu.be/cuENV3v61qA.

The use of UV light seems to provide advantages for population studies. UV light enhanced the detection of *Yunquenus portoricanus* and *Spirobolellus richmondi* and the unidentified opilionid, two dark nocturnal dwellers. Figures 5 and 6 depict two scenarios where the use of UV light allowed the detection of hidden or camouflaged individuals of *S. richmondi*. These organisms are relatively small, mobile, dark-bodied, and nocturnal dwellers, four attributes which make them almost imperceptible with white light. Although these results are preliminary, there is no doubt the application of UV light improved the detection of the above fluorescent organisms in dark and humid challenging environments, a finding very similar to that reported by Rice et al. (2015).

Figure 5. Comparison of a pair of *Spirobolellus richmondi* millipedes photographed with incident white light (upper photo) and with UV light (lower photo).

Figure 6. Simple demonstration of field counting enhancement of millipedes when using white light (above) versus UV light (below).

Future studies need to validate wavelength, distance from subjects, climatic conditions, and type of habitat among other factors to assess the potential application of UV light for nocturnal animal surveys. The evolutionary significance of fluorescence in millipedes as well as for other invertebrates, remains elusive.

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