Epizoic cyanobacteria associated with harvestmen (Arachnida: Opiliones) from Tobago, West Indies

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ABSTRACT
On Tobago, there is a species of sclerosomatid harvestman (*Prionostemma* sp.) that serves as a host to a variety of cryptogams. In the living condition, the exterior of the harvestmen is light blue in appearance, which quickly fades to a bluish-green following preservation in ethanol. Observations of specimens with scanning electron microscopy (SEM) revealed the occurrence of several types of epizoic organisms, including prokaryotic cells (several sizes of bacilli and cocci) and at least two species of fungi. Moreover, using PCR with specific primers, we confirmed the presence of at least one species of epizoic cyanobacterium occurring on the tegument of the harvestmen. The tegument of the carapace and abdomen of *Prionostemma* sp. is full of numerous small pits that may facilitate colonisation and survival by epizoic organisms.

Key words Camouflage, Cryptogam, Fungi, Sclerosomatidae, Tegument

INTRODUCTION

Epizoic organisms attach to the external surfaces of animals, living as either commensals or mutualists (reviewed by Machado and Vital 2001). Among terrestrial vertebrates, examples include lichens that dwell on the shells of giant tortoises in the Galapagos Islands (Hendrickson and Weber 1964), bryophytes and algae growing on the heads of lizards in Mexico (Gradstein and Equihua 1995) and algae occurring on the hairs of Neotropical sloths (Thompson 1972). Epizoic organisms have also been observed on terrestrial arthropods, including bryophytes growing upon millipedes in Colombia (Martínez-Torres *et al.* 2011), liverworts and lichens that dwell on mantids in Costa Rica (Lücking *et al.* 2010), and cryptogams and their associated microfaunas (e.g., oribatid mites, rotifers and nematodes) occurring on weevils in New Guinea (Gressitt and Sedlacek 1967; Gressitt *et al.* 1968). Among arachnids, in particular, interactions involving epizoic organisms are known to occur in harvestmen (Order Opiliones), which may carry cyanobacteria, liverworts, and nonpathogenic fungi (Machado *et al.* 2000; Machado and Vital 2001; Proud *et al.* 2012; Townsend *et al.* 2012). Only three species of harvestmen representing two families, Gonyleptidae (suborder Laniatores) and Sclerosomatidae (suborder Eupnoi), have been observed to have relationships with epizoic cyanobacteria or bryophytes (Machado and Vital 2001; Proud *et al.* 2012; Townsend *et al.* 2012).

Machado and Vital (2001) noted variation in the colonisation frequencies of gonyleptid harvestmen by epizoic cryptogams in different types of forests and also noted several similarities shared by arthropod hosts that are inhabited by cryptogams. In both weevils and harvestmen, the surfaces of the exoskeletons feature pits, tubercles and granulations, traits that may facilitate colonisation and survival of the epizoites at early life stages. In addition, arthropod hosts tend to be slow-moving, survive for multiple years and inhabit moist habitats. Machado and Vital (2001) also observed that colonising cryptogams are generally common and widespread taxa, rather than specialists, and also have the ability to colonise ephemeral habitats and grow quickly. Observations of epizoic organisms have generally been made only for harvestmen that occur in moist, tropical environments.

In this study, we used scanning electron microscopy and molecular techniques to investigate further the relationship between epizoic organisms on the carapace and abdomen of an unidentified species of sclerosomatid harvestman from Tobago, West Indies. Specifically, we used SEM to survey the diversity of microorganisms on the external surface of the exoskeleton and molecular techniques to confirm the presence of cyanobacteria among the observed types of cryptogams.

METHODS

Adult sclerosomatid harvestmen, identified as *Prionostemma* sp. 6 by Townsend *et al.* (2012), were collected by hand from the leaf litter and vegetation along the trail connecting the Speyside Overlook to Pigeon Peak from 10-14 August 2010 in Tobago (Townsend *et al.* 2012). The primary habitat in this area (11N 17.845 60W 32.934 at 405 m in elevation) is lower montane rainforest, and *Prionostemma* sp. 6 is one of the most commonly species, with individuals often observed moving across the forest floor at night (Townsend *et al.* 2012). In contrast to their syntopic congeners, the individuals of *Prionostemma* sp. 6 with epizoic cyanobacteria that we observed had a distinct light denim blue colour on their dorsum that faded to bluish-green following preservation in 70% ethanol.

We prepared multiple adults (n = 5) for SEM by dehydrating them in an ethanol ladder and chemically drying them with hexamethyldisilazane. Individuals were mounted on aluminium stubs using carbon adhesive strips, sputter coated with gold for 2 min and examined with a Hitachi S-3400 VP SEM at accelerating voltages of 5-15 kV in the SEM laboratory on the campus of Virginia Wesleyan University. Voucher specimens are deposited in the American Museum of Natural History (AMNH) arachnological collection.

Several techniques were used to remove cyanobacterial cells from the preserved individuals in order to isolate the genomic DNA from the bacteria. Surface films of epizoic cyanobacteria were removed from the harvestmen by applying a piece of tape to their body, and then either scraping or washing the tape into a test tube (following the protocol described by Proud *et al.* 2012). In addition to the scotch tape technique, cyanobacterial cells were also scraped off the harvestmen into a 70% ethanol solution and entire bodies of the harvestmen were added to a 70% ethanol solution and agitated with forceps and scissors to dislodge the bacterial cells. Dislodged cells from these preparations were pelleted in a microcentrifuge tube at 13,000 rpm for 2 min. DNA was isolated using the GE Healthcare Illustra Bacteria Genomic Mini Spin Kit (#28-9042-58), following Protocol 5.3 for purification of genomic DNA from gram-positive bacteria. The cyanobacteria *Oscillatoria* (Carolina Biological Supply) was used as a positive control, as it contains the phycocyanin operon from which the IGS primers can amplify a ~700 base pair PCR product. Both an IGS forward primer (5'-GGCTGCTTGTTTACGCGACA-3') and an IGS reverse primer (5'-CCAGTACCACCAGCAACTAA-3') were used to amplify the IGS with PureTaq RTG PCR beads (GE Healthcare; Proud *et al.* 2012). The PCR settings were: 94^{o} C for 5 min followed by 40 cycles of 94^{o} C for 20 seconds, 60^{o} C for 30 seconds, and 72^{o} C for 1 min. PCR products were analysed by gel electrophoresis.

RESULTS

The tegument of the carapace and abdomen of *Prionostemma* sp. 6 are covered in numerous small pits (Figs. 1, 2A-B). Using SEM, we observed that many of these pits were filled with a mixture of soil particles and other detritus (Fig. 2C). In addition, we observed the occurrence of prokaryotic organisms (Fig. 2C) in many of the pits on the carapace and abdomen. Bacteria included several types of cocci (Fig. 2D) and bacilli (Fig. 2D-F). We also observed the occurrence of larger, eukaryotic cells including leaf-shaped structures (Fig. 2E) that resemble the thalli of Laboulbeniales fungi as well as those that may represent fungal hyphae (Fig. 2F). The results of our analysis of the surface films that we removed from the harvestmen revealed the presence of a distinct band of 700 base pairs, a positive result that confirms the presence of cyanobacteria (Fig. 3).

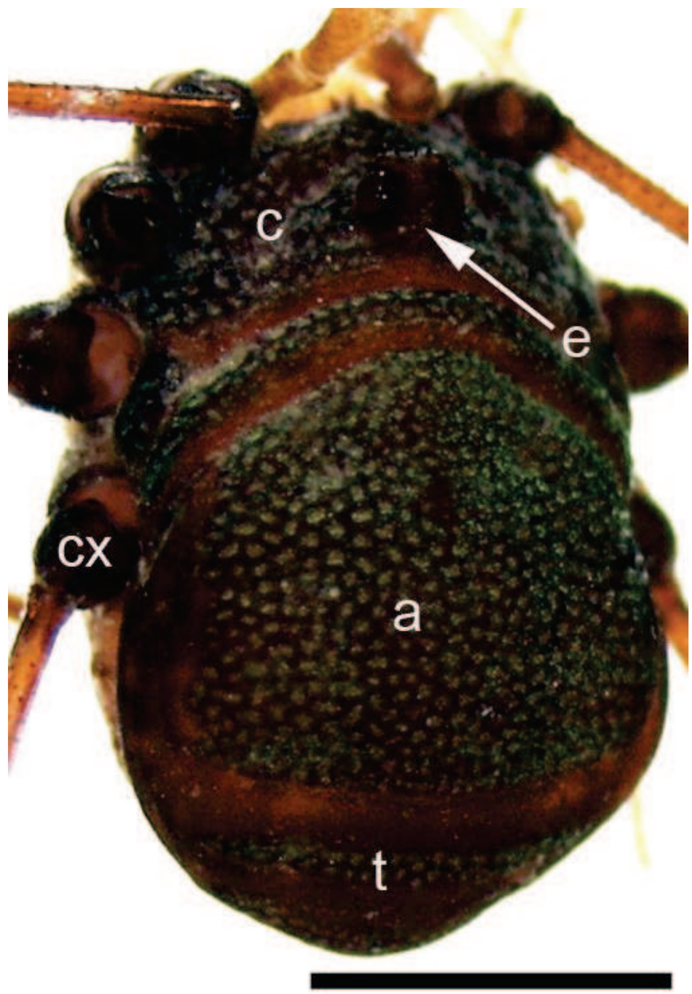

Fig. 1. Photograph of the dorsal habitus of the sclerosomatid harvestman *Prionostemma* sp. 6 with bluish-green cyanobacteria on the dorsal surface. a = abdomen; c = carapace; cx = coxa; e = eye mound (ocularium); t = free tergite. Scale bars = 1.5 mm.

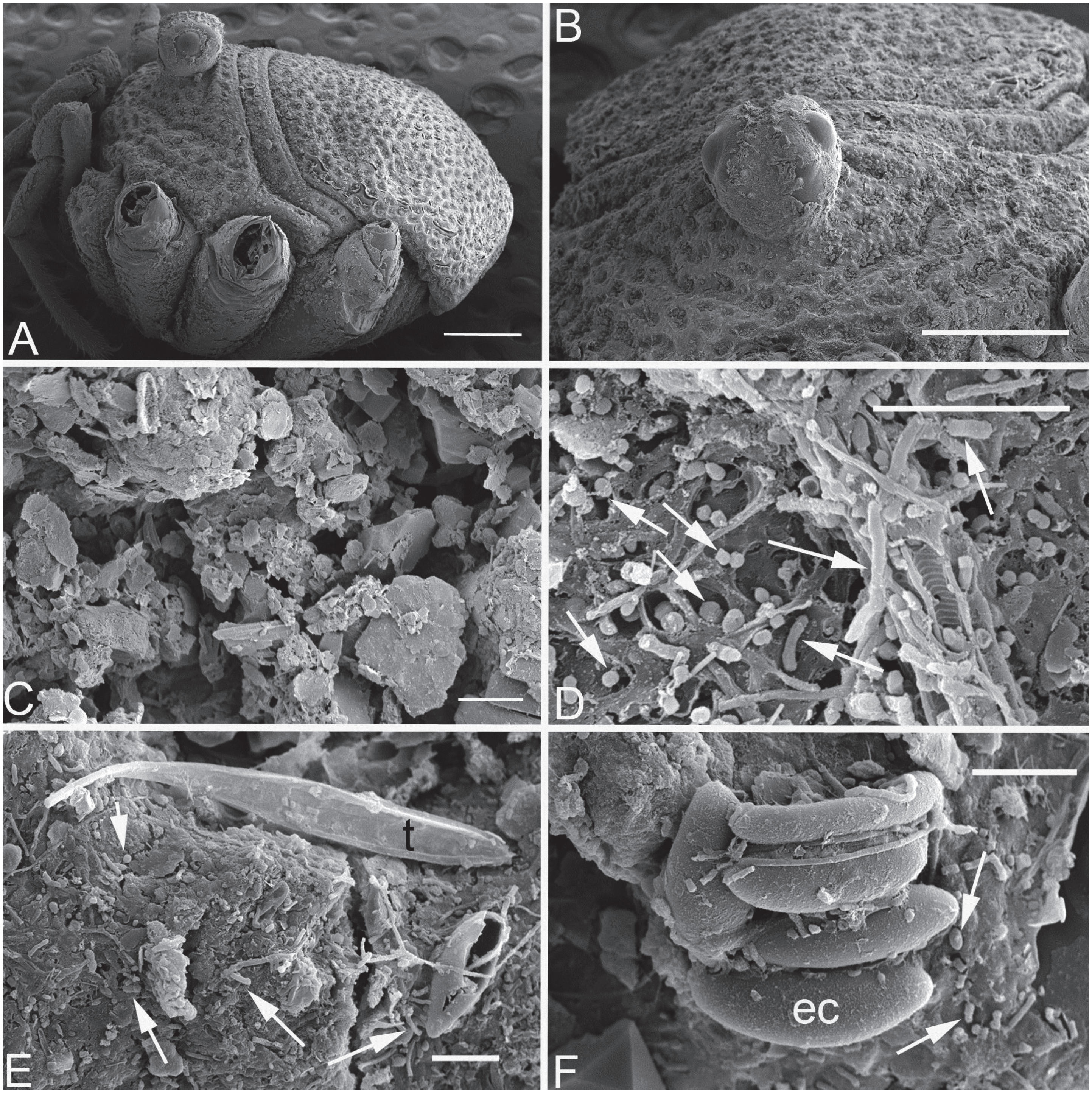

Fig. 2. SEM micrographs showing the mixture of detritus and microorganisms on the tegument of the sclerosomatid harvestman *Prionostemma* sp. 6. Lateral (A) and anterior (B) views of the dorsal scutum revealing the density and distribution of pits on the tegument. C). Detritus from a pit on the posterior region of the body. D) Arrows indicate bacteria (bacilli and cocci) from a pit on the carapace. E) Bacteria (arrows) and a fungal thallus (t) from a pit. F) Large eukaryotic cells (ec) with smaller prokaryotic cells (arrows). Scale bars = 500 μm for A –B and 5 μm for C –F.

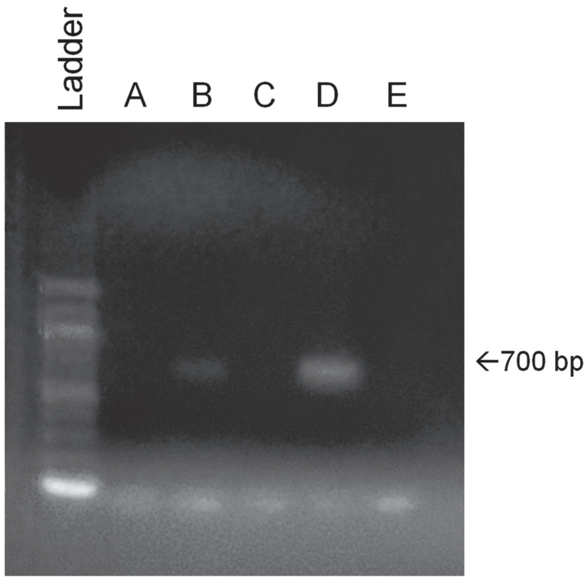

Fig. 3. Gel showing PCR products following amplification of the intergenic sequence of the PC operon within cyanobacteria. DNA from scraping tape applied to the body of an individual of *Prionostemma* sp. 6 (Lane B), and DNA removed from washing the tape applied to the harvestmen body (Lane C) were compared with positive control DNA from *Oscillatoria* (Lane D) and a negative control of no DNA (Lane E). All lanes were compared with the 100 base pair DNA Ladder (New England BioLabs #N3231S). The similar bands on Lane B and Lane D at the 700 base pair region (indicated by arrow) confirm that the cells collected from the tegument of the *Prionostemma* sp. 6 were cyanobacteria.

DISCUSSION

Our confirmation of cyanobacteria living on the dorsal tegument of the carapace and abdomen of adults of the harvestmen *Prionostemma* sp. represents only the third documented case of a epizoic interaction between photosynthetic prokaryotes and harvestmen. Interestingly, all three instances involve host species that live in moist Neotropical environments (Fig. 4). In addition, the species of harvestmen serving as hosts have a tegument dominated by small pits or tubercles, providing suitable habitats for colonisation by epizoic organisms. One striking difference between the cyanobacteria observed by Machado and Vital (2001) and Proud *et al.* (2012) and the cyanobacteria that occurs on the tegument of the harvestman studied here is colour. The specimens that we studied were not green, but rather a distinct blue. We believe that this difference in coloration probably reflects interspecific variation in the pigments used by different species of cyanobacteria. In addition, the epizoic cyanobacteria observed by Proud *et al.* (2012) were generally similar in size to those that we observed on the specimens studied here. However, the epizoites that Proud *et al.* (2012) observed were aggregated in dense patches across most of the dorsal surface of the carapace and abdomen. In contrast, the epizoic cyanobacteria that we observed here occurred generally in a thin film with the most of the small, prokaryotic cells occurring predominantly as dense clusters in the pits on the tegument of the host.

The nature of the epizoic relationship that exists between cryptogams and harvestmen (i.e., commensalism or mutualism) remains to be empirically investigated. The cyanobacteria on the dorsum of the harvestmen are photosynthetic and relatively small. They are probably unable to penetrate the cuticle of the host and, thus, are certainly not parasitic. On the bases of our field observations of harvestmen with cryptogams, the hosts do not appear to be encumbered by the biomass of epizoic organisms that they carry. To the contrary, our observations indicate that individuals with epizoic organisms move normally and are not lethargic.

From the perspective of the epizoic organisms, the body of the host is an unusual microenvironment that supports few, if any, potential competitors for light or other limiting resources. Thus, monotypic colonies of epizoic cyanobacteria can thrive on the tegument of their hosts. However, the benefit, if any, to the host is not as clear. Harvestmen do not appear to be capable of feeding

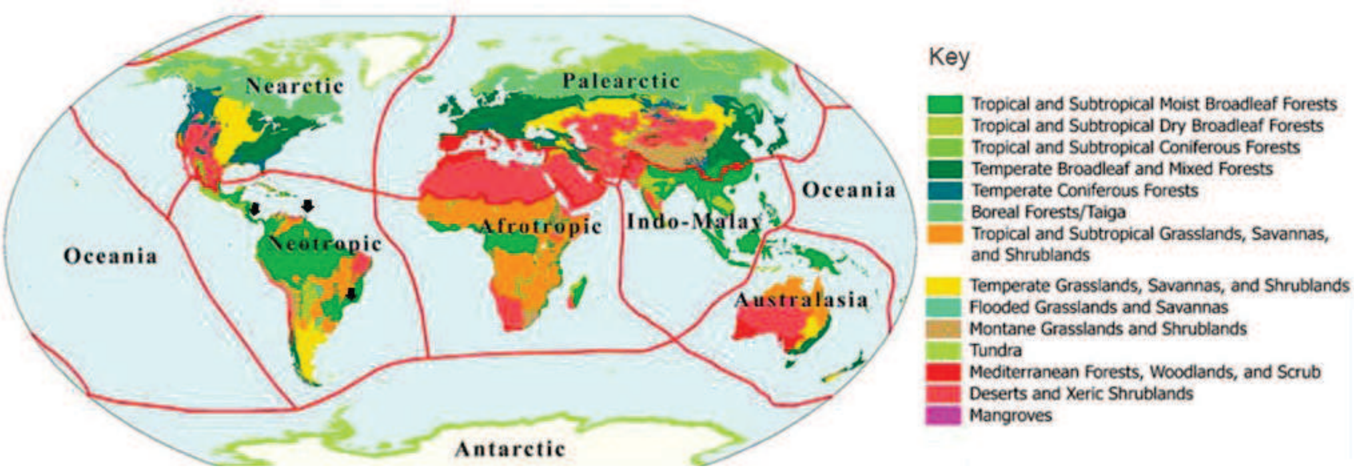

Fig. 4. Arrows indicate the three locations from which harvestmen associated with cyanobacteria have been captured. Map modified from Olson *et al.* 2001.

upon epizoic organisms that live on their dorsum. While harvestmen pass their distal leg segments between the chelicerae in a behaviour known as "grooming", they simply cannot reach the surfaces of the dorsal scutum nor do they have the anatomy necessary (i.e., chelate tarsi) to remove epizoic organisms from the dorsal surfaces of their bodies.

Gressitt *et al.* (1968) hypothesised that the presence of mosses on the dorsal surfaces of weevils in New Guinea conferred crypsis and thus enabled hosts to avoid predators, especially diurnal predators that rely upon visual cues to find their prey. Habitat use and circadian activity patterns of several species of Neotropical *Prionostemma* spp. have been studied (Donaldson and Grether 2007; Grether and Donaldson 2007; Wade *et al.* 2011). In these harvestmen, adults generally are more active in the leaf litter at night, spending the day, occupying perches on tree trunks, buttresses and the exposed surfaces of leaves in the understory. If host species with epizoic cyanobacteria exhibit similar habits, it may be that individuals with cryptogams have better camouflage and are less obvious to visually oriented predators. Unfortunately, the behavioural ecology of *Prionostemma* p. 6 or that of its congeners in Costa Rica has not yet been examined.

ACKNOWLEDGEMENTS

We thank S. Broadbridge (Caribbean Discovery Tours) for helping with the fieldwork and for assistance with ground transportation. We are grateful to R. Shirley, S. Dunn and A. Kaye for assistance with field collections and molecular work and T. Pesacreta and D. Proud (University of Louisiana at Lafayette) for helping with the scanning electron microscopy. We thank G. Machado and an anonymous reviewer for constructive comments on an earlier version of our manuscript. This research was supported by a faculty development grant from the CLA at Mercer University (MKM). We also thank the Forestry Division of the Ministry of Agriculture, Land and Marine Resources of Trinidad and Tobago. All specimens were legally collected and exported under permit number 001414 (to MKM in 2010).

REFERENCES

Donaldson, Z.R. and **Grether, G.F.** 2007. Tradition without social learning: Scent-mark based communal roost formation in a Neotropical harvestman (*Prionostemma* sp.). *Behavioral Ecology and Sociobiology,* 61: 801–809.

Gradstein, S.R. and **Equihua, C.** 1995. An epizoic bryophyte and algae growing on the lizard *Corythophanes cristatus* in Mexican rain forest. *Biotropica*, 27: 265-268.

Gressitt, J.L., Samuelson, G.A. and **Vitt, D.H.** 1968. Moss growing on living Papuan moss-forest weevils. *Nature*, 217: 765-767.

Gressitt, J.L. and **Sedlacek, J.** 1967. Papuan weevil genus *Gymnopholus*: Supplement and further studies in epizoic symbiosis. *Pacific Insects*, 9: 481-500.

Grether, G.F., and **Donaldson, Z.R.** 2007. Communal roost site selection in a Neotropicalharvestman: habitat limitation vs. tradition. *Ethology,* 113: 290–300.

Hendrickson, J.R. and **Weber, W.A.** 1964. Lichens on Galapagos giant tortoises. *Science*, 144: 1463.

Lücking, R., Mata-Lorenzen, J. and **Dauphin L.G.** 2010. Epizoic liverworts, lichens and fungi growing on Costa Rican shield mantis (Mantodea: *Choeradodis*). *Studies on Neotropical Fauna and Environment*, 45: 175-186.

Machado, G., Raimundo, R.L.G. and **Oliveira, P.S.** 2000. Daily activity schedule, gregariousness, and defensive behaviour in the Neotropical harvestman Goniosoma longipes (Arachnida: Opiliones: Gonyleptidae). *Journal of Natural History*, 34: 587-596.

Machado, G., and **Vital. D.M.** 2001. On the occurrence of epizoic algae and liverworts on the harvestmen *Neosadocus aff. variabilis* (Opiliones: Gonyleptidae). *Biotropica,* 33: 535-538.

Martínez-Torres, S.D., Flórez Daza, A.E. and **Linares-Castillo, E.L.** 2011. Meeting between kingdoms: Discovery of a close association between Diplopoda and Bryophyta in a transitional Andean-Pacific forest in Colombia. *International Journal of Myriapodology*, 6: 29-36.

Olson, D.M., Dinerstein, E., Wikramanayake, E.E., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P., and **Kassem, K.R.** 2001. Terrestrial ecoregions of the world: A new map of life on earth: A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity, *BioScience*, 51: 933-938.

Proud, D.N., Wade R.R., Rock, P., Townsend, VR. Jr., and **Jiménez Chavez, D.** 2012. Epizoic cyanobacteria associated with a Neotropical harvestman (Opiliones: Sclerosomatidae) from Costa Rica. *Journal of Arachnology,* 40: 259-261.

Thompson, R.H. 1972. Algae from the hair of the sloth *Bradypus*. *Journal of Phycology*, 8: 8.

Townsend, V.R. Jr., Moore, M.K., Proud, D.N. and **Young, V.A.** 2012. A preliminary survey of the Harvestmen (Arachnida: Opiliones) of Tobago, West Indies. *Living World, Journal of the Field Naturalist Club of Trinidad and Tobago,* 2012: 41-53.

Wade, R.R., Loaiza Phillips, E., Townsend, V.R. Jr. and **Proud, D.N.** 2011. Activity patterns of two species of Neotropical harvestmen (Arachnida, Opiliones) from Costa Rica. *Annals of the Entomological Society of America,* 104: 1360-1366.