

Seasonal Prevalence and Host Specificity of *Lawrencarus* sp.

Mites in Anuran Hosts

HON 4956 004

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## ABSTRACT

Ereynetid mites of the subfamily *Lawrencarinae* are small, free-living, endoparasitic mites found in the nasal passages of anuran hosts. While the physical and physiological attributes of the *Lawrencarena* subfamily have been described, there is little else known about this group of mites. This current study experimentally explores the anuran hosts, specificity of these parasitic mites and establishes the seasonal prevalence, abundance and host specificity of *Lawrencarus* cf. *hylae* mites in anurans over a year-long collection period near Lakeland Highland Scrub in Lakeland, Florida. A total of 378 endoparasitic mites were collected from the nasal cavity of *Rana sphenocephala* (Southern Leopard Frog). Seasonal prevalence, mean abundance, and mean intensity were not significantly different between male and female hosts, and there was no correlation found between host snout-vent length and parasite intensity for male and female hosts. The prevalence of infections in *R. sphenocephala* reached a peak of 90% in the fall, but never dropped below 60% during any season; mean abundance peaked in the spring, and the fewest mites were collected in the winter. The abundance of natural infection in *R. sphenocephala* in comparison to other anuran species studied could be an indication of host species preference. In experimental infections, the prevalence of infection of each anuran host was above 40%, and choice experimental infections indicated a preference of *R. sphenocephala* with a prevalence of infection of 100%. Overall, this study provides an investigation into the host specificity and preference of *Lawrencarus* cf. *hylae* mites, which presents insights into an understudied endoparasitic species.

## INTRODUCTION

Parasites infect almost every species of free-living organisms, yet minimal data for parasite diversity and ecology are available for many species (Marcogliese, 2004). The effect of parasites on their hosts often attract attention once the host species has demonstrated economic or conservation importance. Parasites can have a variety of impacts on their hosts, and these impacts are often realized once the population of a host species begins to decline (Hartigan et al., 2013). These effects can result in energetic demands, alterations of behavior, morphological modifications, fecundity reductions, and a decrease in survival rates (Preston, 2010).

While parasites infect practically every species, there is a limit to which species a parasite is able to infect, since no parasite is universally infective. A fundamental property of parasitic organisms is host specificity, as it reflects the parasite's possible historical association with its hosts (Poulin et al., 2006). Host specificity involves the range and diversity of host species that a parasite is capable of infecting. The two determinants of host specificity are ecological and physiological host specificity. Ecological host specificity consists of the hosts that a parasite would generally encounter in its natural environment. Physiological host specificity involves the hosts that a parasite is physically able to infect because the host and parasite are physiologically compatible. An understanding of ecological and physiological host specificity of a parasitic species would provide a contribution to filling information gaps, where baseline data remains scarce.

The family Ereyenetidae consists of around 200 small, free-living, endoparasitic mite species in the Trombidiformes group of mites (Zhang et al. 20). Endoparasites are parasites that reside in the tissues and organs of their hosts. These mites have a body covered by a soft, finely lined integument, and possess a structure between their propodosoma and hysterosoma (Baker, 1970). The family, Ereyenetidae, contains three subfamilies: Ereyenetinae, Lawrencarinae, and

Speleognathinae. The Ereyenetidae subfamily are free-living predators in mosses, lichens, leaf litter, and under bark, and the Speleognathinae subfamily consists of nasal parasites in birds and mammals (Fain, 1970). Mites of the subfamily Lawrencarinae are found in the nasal passages of anuran hosts but can also be present in the mouth and lungs (Fain, 1962). While the physical and physiological attributes of the Lawrencarena subfamily have been described (Fain, 1961), there is little else known about this group of mites. Ereyenetidae mites are identifiable by the ereyental organ, which is a small pocket located on the dorsal side of the tibia on the organism. The function of the ereyental organ is unknown and is present in all species of Ereyenetidae mites (Fain, 1964). The species of *Lawrencarus* mite used in the current study conforms the most to the species *Lawrencarus hylae* due to their similar morphologies. Some sensillum and hairs did not conform completely to the previous descriptions of *Lawrencarus hylae*, which suggests the mite used in the current study could be a new species of *Lawrencarus* mite or redescription of *Lawrencarus hylae* might be needed.

The morphology and physical attributes of the life cycle stages of a few species of the subfamily Lawrencarinae were previously documented (Fain, 1961); however, information about the host specificity and host-parasite interactions of this elusive group remains scarce. The life cycle of these mites is presumed to be direct based on data gathered from the Speleognathinae family (Baker, 1970), which means there is no need for an intermediate host, and the parasite can move directly from one host to another in favorable conditions (Baker, 1970). The mode of transmission of these mites and the environmental conditions under which transmission occurs also remains unknown. A deeper understanding of host specificity in Lawrencarinae mites could provide knowledge into the extent of the parasitic mites range of host species and geographical area.

Amphibians are one of the most threatened taxa with over 40% of amphibian species in decline, due to the major contributions of fungi, viruses, and parasites. (Marcogliese, 2004). In parasitology, anurans have a history of being study organisms for analyzing ecological and evolutionary mechanisms due to the diversity in the groups' life history strategies and taxonomy. Anurans are model organisms in parasitology because the taxa has been widely studied, are able to be easily manipulated, and mature rapidly. Amphibians are also critical components of both aquatic and terrestrial communities, and a fuller understanding of parasitism in anuran species would provide insight into a taxa that is in rapid decline (Hopkins, 2007). The relationship between anuran hosts and their mites are varied, with some parasitic species being relatively benign, while others are deleterious (Araujo et al., 2003). Although anurans are model organisms to study parasite diversity, no experimental studies on the parasitism of anurans by mites of the lawrencarinae subfamily. As little knowledge has been gathered on the Lawrencarinae subfamily, ecological data would provide a deeper insight into these parasitic mites, and add to a growing understanding of the parasitism of anuran hosts.

Avians host many parasitic mite species, and are among the most-studied species for Ereyneidae mites of the Speleognathinae subfamily. Previous studies of the tissue-feeding Speleognathinae mites in the nasal cavities avian hosts have recognized new species invasions and potential roles of nasal mites in causing avian disease (Hilario-Perez and Dowling, 2018; Knee et al., 2008). These studies on the Speleognathinae mites in avian hosts have shown that species with morphological similarities have the highest abundances of nasal mites (Knee et al., 2008). Previous studies on host specificity on the Speleognathinae subfamily focus on results gathered from field collections and do not explore insights that can be gathered from experimental studies of host specificity on these mites. Modes of transmission of Ereyneidae

nasal mites also remain scarce and further exploration of these mites could provide novel information about the understanding of host-parasite relationships and mite transmission among hosts (Bernardon et al., 2018). Nasal mites are not yet known to cause any significant pathology in their hosts (Knee et al., 2008); however, there remains a lack of information, overall, on this understudied family of parasitic mites. Further investigation into the establishment of the level of host specificity and mean abundance in these mites could provide information into the distribution and diversity of nasal mites in anuran hosts in central Florida.

The current study provides experimental and observational evidence of the mean abundance and host specificity of parasitic mites of the Lawrencarinae subfamily. Our goals were to (1) establish the seasonal prevalence, abundance and host specificity of the *Lawrencarus* mites that conform to *L. hylae* in central Florida and (2) use experimental infections to clarify potential physiological and ecological limitations on host specificity.

## **METHODS**

### **Field Collections and Observations**

To establish seasonal infection parameters of *L. cf. hylae* mites, anurans were collected from October 2010 to August 2011, where *Rana sphenoccephala* (Southern Leopard Frog), *Rana grylio* (Pig Frog), *Acris gryllus* (Southern Cricket Frog), *Bufo terrestris* (Southern Toad), and *Hyla cinerea* (Green Tree Frog) were captured to determine field host specificity of *L. cf. hylae* mites. All anurans were captured near the edges of Lakeland Highlands Road and Broken Arrow Trail Street near Lakeland Highlands Scrub in Lakeland, Florida. These roads followed along a nearby creek and body of water near Lakeland Highland Scrub. During this sampling period, field collections occurred several times during each season to observe the seasonal prevalence of *L. cf. hylae* mites in anuran hosts.

## Experimental Infections

To test host specificity, two different types of experimental infections were conducted in this study. The first experimental infection consisted of 4 anuran species *Rana sphenocephala* (Southern Leopard Frog), *Rana grylio* (Pig Frog), *Bufo terrestris* (Southern Toad), and *Hyla cinerea* (Green Tree Frog) exposed to 10 *L. cf. hylae* in glass dishes. Five individuals of each of the four anuran species were used for the experimental infections. Each anuran species used for the experimental infections were representative of the different habitat types in which the anurans inhabit. These anurans representative of these habitats were used to indicate differences in host specificity in regards to their physiological compatibility. For instance, the Southern Toad represents the terrestrial habitats, the Green Tree Frog represents arboreal habitats, the Southern Leopard Frog represents semi-aquatic habitats, and the Pig Frog represents aquatic habitats. The anuran hosts were reared from tadpoles to ensure they were not infected by nasal mites previously. The mites were sourced from Southern Leopard Frogs. All mites for both experimental infections were acquired by double-pithing the Southern Leopard Frogs. The nasal passages were then opened carefully, and the mites were gently removed with a paintbrush. Double pithing was utilized as opposed to other methods to ensure the mites were not exposed to chemicals before being used in the experimental infections. After the experimental infections, each anuran was euthanized and examined for presence of infection.

The second experimental infection was a choice experimental to simulate transmission of *L. cf. hylae* mites in a natural habitat. For the choice experiment, the same four anuran species as the first experimental infection (*R. sphenocephala*, *R. grylio*, *B. terrestris*, and *H. cinerea*) were exposed to 30 *L. cf. hylae* mites in a critter keeper together for 48 hours. The experiment occurred through seven trials, and the critter keepers contained moist sand, a water dish, and lead

litter. The hosts in the choice experiment were also reared from tadpoles to ensure the anurans had not been infected with nasal mites previously. The mites used in this choice study were sourced from the Southern Leopard Frogs as well as acquired through double pithing. After exposure time, the anurans were euthanized and examined for parasites as well.

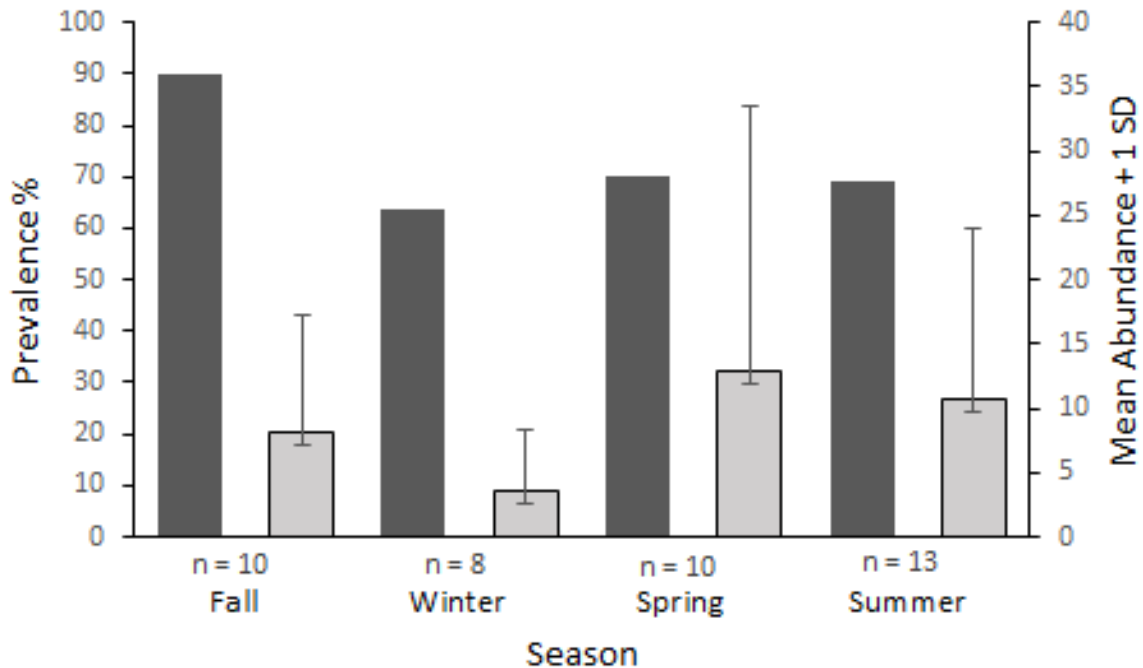
### **Statistical Analysis**

A Z-test for equality of two sample proportions was used to compare the prevalence (P) of infection between male and female Southern Leopard Frogs, and a t-test for two sample means was used to measure both the differences in mean abundance (MA) and mean intensity (MI) between host sexes. A Spearman's rank correlation was used to calculate relationship possibilities between host snout vent length (SVL) and MI; male and female Southern Leopard Frogs were analyzed separately.

## **RESULTS**

### **Field Collections and Observations**





**Figure 1.** Seasonal prevalence and mean abundance of *L. cf. hylae* mites of the collected from the Southern leopard frog (*Rana sphenoccephala*) near Lakeland Highland Scrub in Lakeland, Florida.

Over 12 months, a total of 74 anurans (*R. sphenoccephala*, *R. grylio*, *R. gryllus*, *B. terrestris*, *H. cinerea*) were collected in Lakeland, Fl. A total of 381 endoparasitic *L. cf. hylae* mites (26 larvae, 355 nymphs/adults) were collected from the nasal cavity of 2 anuran species. All mites were found in *R. sphenoccephala* (n = 41) and *R. grylio* (n = 13), whereas *R. gryllus* (n = 7), *B. terrestris* (n = 8), and *H. cinerea* (n = 7) were not infected with mites. A total of 378 mites were found in *R. sphenoccephala* (18 males, 23 females) (P = 43.2%, MA = 5.1 +/- 11.0, MI = 11.9 +/- 14.2). P (female = 69.6%, male = 77.8%, z = 0.50, P = 0.59), MA (female = 9.3 +/- 14.2, male = 9.1 +/- 13.0, t = 0.05, P = 0.96), and MI (female = 13.4 +/- 15.3, male = 11.6 +/- 13.7, t = 0.33, P = 0.74) were not significant between host sexes. There was no significant correlation found for *R. sphenoccephala* between host snout vent length and parasite intensity for males ( $r_s = 0.05$ , df = 8, P = 0.88) or females ( $r_s = 0.07$ , df = 12, P = 0.81). The prevalence of

infections in *R. sphenoccephala* reached a peak of 90% in the fall, but never dropped below 60% during any season. Mean abundance peaked in the spring, and the fewest mites were collected in the winter.

### **Experimental Infections**

The experimental infections were able to establish infections in each anuran species (P = 100%, MI = 5.6 +/- 1.8), *R. grylio* (P = 60%, MI = 3.7 +/- 2.1), *B. terrestris* (P = 40%, MI = 1.5 +/- 0.71), and *H. cinerea* (P = 40%, MI = 2.0 +/- 1.4). The five hosts for the experimental infections included both male and females for each anuran species. The choice experiment infections were not able to establish infections for *H. cinerea*; however, *R. sphenoccephala* (P = 100%, MI = 12.9 +/- 5.0), *R. grylio* (P = 57.1%, MI = 2.0 +/- 0.82), and *B. terrestris* (P = 14.3%) were infected with *L. cf. hylae* mites. Only one mite was discovered in one of the *B. terrestris* hosts, making the mean intensity value and its standard deviation incomputable. During exposures, a Pig Frog ate one of the Green Tree Frogs.

### **DISCUSSION**

This study found the majority of infections from the field and experimental infections occur in the Southern Leopard Frog. The *L. cf. hylae* mites seem to be exclusive parasites of the Southern Leopard Frogs in field collections; however, experimental collections suggest that the mites are physiologically capable of infecting other anurans, at least for short time-periods, if Southern Leopard Frogs are not present. Similarly, Ereynetidae mites of the Speleognathinae subfamily appear to infect host species with morphological similarities (Knee et al., 2008). While the mites in the experimental study had the highest prevalence of infection in the Southern Leopard Frog, the second highest prevalence occurred in the Pig Frog. Pig Frogs are aquatic, while Southern Leopard Frogs are semi-aquatic, which could suggest that due to the similarities

in their natural habitats they could also possess certain morphological similarities that are ideal for the mites. Experimental and field collections results indicated there is both a physiological and ecological host preference in the *L. cf. hylae* mites. The field collections to test ecological host specificity showed that *L. cf. hylae* displays host specificity within the Southern Leopard Frogs as all except 3 mites were collected from Southern Leopard Frogs. The experimental infections suggest there is an physiological host preference for the *L. cf. hylae* mites, and supported our field collection results, which found infections primarily in the Southern Leopard Frogs. The second highest prevalence of infection in the field collections and the experimental infections were in the Pig Frogs, which could suggest that the Pig Frogs are physiologically acceptable hosts, but the mites could rarely come in contact with the Pig Frogs. Since each anuran represented a different general habitat type, the Pig Frogs could rarely be exposed to the mites in comparison to the Southern Leopard Frogs because the Southern Leopard Frogs reside primarily in semi-aquatic habitats, while the Pig Frogs are most commonly found in aquatic habitats. Previous studies and literature regarding the host specificity for *Lawrencarus* spp. remains nonexistent, therefore the current study provides baseline data on the host range in these mites, where information regarding them remains scarce.

This study is the first investigation into the host specificity and seasonal prevalence of a species of *Lawrencarus* mites in central Florida that provides insight into an understudied endoparasitic mite. Also, the mites used in the current study conformed the most to *Lawrencarus hylae*, although some sensillum and hairs did not conform completely. The difference in the previous descriptions and the mites collected in our study could suggest that the mites used in our study is a new species of *Lawrencarus* mite or a redescription of *Lawrencarus hylae* is needed. Interestingly, although a subspecies of these mites, *L. hylae* spp. *Intermedius*, was

originally described from tree frogs in North America (Fain, 1962), the mites in our study did not appear to infect tree frogs often. These differences indicate further that the species of mite in this study is a new *Lawrencarus* species or the redescription of *L. hylae* is needed. There still remains little information on all *Lawrencarus* species of mites including their mode of transmission and life cycle. This current study could provide a start for future studies on *Lawrencarus* mites as data regarding this family of nasal mites remains limited.

## ACKNOWLEDGEMENTS

The research was conducted at Florida Southern College under the Institutional Animal Care and Use Committee protocol #1008, and all the native animals were collected under a Florida Fish and Wildlife scientific collection permit to G.J.L. We thank previous students Mike Politis and Madison Schubert for their assistance in collecting frogs. We also thank Florida Southern College for allowing use of their lab space and resources for the experiments to occur.

## LITERATURE CITED

- Araújo, Adauto, Jansen, Ana Maria, Bouchet, Françoise, Reinhard, Karl, & Ferreira, Luiz Fernando. (2003). Parasitism, the diversity of life, and paleoparasitology. *Memórias do Instituto Oswaldo Cruz*, 98(Suppl. 1), 5-11. <https://doi.org/10.1590/S0074-02762003000900003>
- Baker, R. A. (1970). Studies on the life history of *Riccardoellalimacum* (Schrank) (Acari-Trombidiformes), *Journal of Natural History*, 4:4, 511-519, DOI: 10.1080/00222937000770471
- Bernardon, Fabiana Fedatto, Mascarenhas, Carolina S., Pereira Jr, Joaber, & Müller, Gertrud. (2018). Host-Parasite relationships and co-infection of nasal mites of *Chrysomus ruficapillus* (Passeriformes: Icteridae) in southern Brazil. *Iheringia. Série Zoologia*, 108, e2018025. Epub June 21, 2018. <https://dx.doi.org/10.1590/1678-4766e2018025>
- Fain, A. (1961). Observations sur les acariens de la sous-famille *Lawrencarinae* (Ereynetidae: Trombidiformes); note préliminaire. *Bulletin et Annales de la Société Royale d'Entomologie de Belgique*, 97, 245-255.

Fain, A. (1962). Les acariens parasites nasicoles des batraciens. Revision des Lawrencarinae Fain, 1957 (Ereynetidae: Trombidiformes). *Bulletin Institut Royal des Sciences Naturelles de Belgique*, 38, 1-69.

Fain, A. (1964). Chaetotaxy and specialised sensory organs of the Ereynetidae. *Acarologia*, 6 224-227.

Fain, A. (1970). Nomenclature des poils idiosomaux et description de trois especes nouvelles dans la famille Ereynetidae (Trombidiformes). *Acarologia* 12: 314-325

Fain, A. (1972). Développement postembryonnaire chez les Acariens de la sous-famille Speleognathinae (Ereynetidae: Trombidiformes). *Acarologia*.13: 607-614.

Hartigan, A., Phalen, D. N., & Slapeta, J. (2013). Myxosporean parasites in Australian frogs: Importance, implications and future directions. *International journal for parasitology. Parasites and wildlife*, 2, 62–68. <https://doi.org/10.1016/j.ijppaw.2012.12.002>

Hilario-Perez, A., Dowling, A. (2018). Nasal mites from specimens of the brown-headed cowbird (Icteridae: Molothrus ater) from Texas and Arkansas, U.S.A. *Acarologia*, Volume 58, Issue 2, Pages 296-301, doi: 10.24349/acarologia/20184242

Hopkins, William A. (2007). Amphibians as Models for Studying Environmental Change, *ILAR Journal*, Volume 48, Issue 3, Pages 270–277, <https://doi.org/10.1093/ilar.48.3.270>

Knee, Wayne & Proctor, Heather & Galloway, Terry. (2008). Survey of nasal mites (Rhinonyssidae, Ereynetidae, and Turbinoptidae) associated with birds in Alberta and Manitoba, Canada. *The Canadian Entomologist*. 140. 364-379. 10.4039/N08-017.

Marcogliese, D. (2004). Parasites: Small Players with Crucial Roles in the Ecological Theater. *EcoHealth* 1, 151–164. <https://doi.org/10.1007/s10393-004-0028-3>

Poulin R., Krasnov B.R., Morand S. (2006) Patterns of host specificity in parasites exploiting small mammals. In: Morand S., Krasnov B.R., Poulin R. (eds) *Micromammals and Macroparasites*. Springer, Tokyo. [https://doi.org/10.1007/978-4-431-36025-4\\_13](https://doi.org/10.1007/978-4-431-36025-4_13)

Preston, D. & Johnson, P. (2010) Ecological Consequences of Parasitism. *Nature Education Knowledge* 3(10):47

Zhang, Z., Fan, Q., Pesic, V., Smit, H., Bochkov, A., Khaustov, A., Baker, A., Wohltmann, A., Wen, T., Amrine, J., Beron, P., Lin, J., Gabrys, G., & Husband, R. (2011). Order

Trombidiformes Reuter, 1909. *In*: Zhang, Z.-Q. (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. *Zootaxa*, 3148(1), 129–138.  
doi:<http://dx.doi.org/10.11646/zootaxa.3148.1.24>