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CHEMICAL COMPOSITION OF *Physalaemus* FOAM NESTS BY GC-MS: A NEGLECTED BUT RELEVANT DIMENSION?

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The Amphibia class contains 8,911 documented species, distributed into three orders: Gymnophiona, Caudata, and Anura. Anurans represent the most diverse order (7,858 species). and their secretions contain specialized metabolites such as peptides, biogenic amines, and alkaloids contributing to several aspects of their biology. Despite their significance, the role of these compounds in reproductive context are little understood. The construction of foam nests by few lineages like those in the family Leptodactylidae stands out, as this reproductive mode provides key functions to embryos including protection against predators, gas exchange regulation, and embryonic dehydration. However, the chemical composition and function of the metabolites present in foam nests have been only partially described. Thus, this study aims to provide a comprehensive characterization of nonpolar metabolites from the foam nests of *Physalaemus* spp. from the Leptodactylidae family. To this end, lyophilized samples of foam nests of four species (i.e., *P. cristinae*, *P. olfersii*, *P. obtectus*, and *P. cuvieri*) were extracted, concentrated, resuspended, and analyze using Gas Chromatograph coupled to a quadrupole Mass Spectrometer (GC-MS). The data generated was analyzed using MZmine 4.7 and Metaboanalyst, and also through manual annotations. Our findings identified that Fatty Acid Methyl Esters (FAME) constitute the most abundant chemical class, with the highest concentration (51.72%) in *P. obtectus*. Free fatty acids are also high concentrations, with considerable variation between species. Strikingly, steroids were also recovered at high levels, suggesting they play crucial functions, likely related to the nest structure. Minor metabolites, such as aldehydes, hydrocarbons, vitamins, ketones, alcohols, and terpenoids, occurred in lower proportions but were consistently found in the nests. Overall, this study represents a unique contribution for understanding the contributions of non-polar metabolites in the structural and functional composition of amphibians' foam nests, possibly mediating embryo protection and foam stability. These findings reinforce the importance of further studies on the ecological and evolutionary role of nonpolar metabolites in anuran foam nests.

Keywords: *Leptodactylidae; Physalaemus; GC-MS.*