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Short Communication

Discovering the ecological secrets of *Alsodes cantillanensis* (Anura: Alsodidae): Dietary knowledge through computerized microtomography

Descubriendo los secretos ecológicos de *Alsodes cantillanensis* (Anura: Alsodidae): Conocimiento de la dieta a través de microtomografía computarizada

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ABSTRACT

Computed microtomography is an important tool for understanding the natural history of species using biological collections. Here we report the first dietary data of *Alsodes cantillanensis* Charrier, Correa, Castro & Méndez, 2015. Using this technique, we found remains of Diplopoda and Coleoptera in the digestive tract of a male from the type locality, providing valuable information on the ecology of the species. These findings may be useful for designing conservation strategies for this endangered species.

Keywords: *Alsodes*, biological collection, computed microtomography, diet, ecology.

RESUMEN

La microtomografía computarizada es una herramienta importante para comprender la historia natural de las especies utilizando colecciones biológicas. Aquí reportamos los primeros datos de dieta de *Alsodes cantillanensis* Charrier, Correa, Castro & Méndez, 2015. Usando esta técnica, encontramos restos de Diplopoda y Coleoptera en el tracto digestivo de un macho de la localidad tipo, proporcionando valiosa información sobre la ecología de la especie. Estos hallazgos pueden ser útiles para diseñar estrategias de conservación para esta especie en peligro de extinción.

Palabras clave: *Alsodes*, colección biológica, dieta, ecología, microtomografía computarizada.

Anurans are one of the groups with the greatest knowledge deficits in ecological aspects such as diet (Solé & Rödder 2010). This is where the biological collections that naturalists have preserved for decades come into play. Currently, many of these collections are being digitized, making them more accessible to researchers in various parts of the world without the need to dissect unique samples (Meineke *et al.* 2019). One of the most powerful techniques for digital specimen analysis is microcomputed tomography (µCT), which allows three-dimensional structures to be reconstructed in high resolution, including external (morphology) and internal (anatomy) features (Mizutani & Suzuki 2012; Keklikoglou *et al.* 2016). Microcomputed tomography has demonstrated its ability to reduce knowledge gaps on biodiversity in several fields of research, such as taxonomy, evolution, ecology, developmental biology, and functional morphology

(Paterson *et al.* 2014; Akkari *et al.* 2015; Blackburn *et al.* 2021).

Anurans exhibit a variety of diets, wich are strongly influencied by microhabitats (Moen 2019; Paluh *et al.* 2020), prey availability, body size, ontogeny, foraging strategies, and even by ancestral-descendant relationship (Pacheco *et al.* 2017; Bayrakcı & Çiçek 2022). For example, some high mountain aquatic species of the Andes, due to their lifestyle, are considered aquatic specialists (e.g., some species of *Telmatobius* Wiegmann, 1834; [Barrionuevo 2016; Araos *et al.* 2022]). On the other hand, species in the forest environment tend to be generalists and/or opportunists (Vidal-García & Keogh 2017; Paluh *et al.* 2020). By understanding aspects of the natural history of species, such as diet, we not only gain information about the energy source, but also about the ecological roles they play within their environments (Vitt & Caldwell 2013).

In Chile, the study of diet and other aspects of natural history in anurans is scarce (Pincheira-Donoso 2002; Díaz-Páez & Ortiz 2003; Gutiérrez *et al.* 2008; Alveal *et al.* 2015; Correa *et al.* 2016; Alveal & Díaz-Páez 2021) For example, the last review on diet was published 15 years ago and few studies have been done since then (Vidal & Labra 2008). One group of which very little is known about their biology and ecology is the genus *Alsodes* Bell, 1843. Species of this genus, as the name implies, are forest dwellers (Lavilla 2020), although some species are aquatic o semiaquatic (i.e., *A. montanus* and *A. pehuenche*). Currently, 19 species are recognized, 18 of them within Chilean territory. However, aspects of its natural history, such as diet and therefore its role in ecosystems, remain mostly unknown. So far, only information has been reported on the diet of *Alsodes coppingeri* (Günther 1881), *A. tumultuosus* Veloso, Iturra-Constant & Galleguillos 1979 and *A. montanus* (Lataste 1902) in adulthood, which mainly consume arthropods of the orders Coleoptera, Diptera, Araneae and the subclass Collembola (Díaz & Valencia 1985; Alveal *et al.* 2015; Alveal & Díaz-Páez 2021)

Here we describe arthropod fragments found in the digestive tract of a specimen of *Alsodes cantillanensis* Charrier, Correa, Castro & Méndez, 2015. *Alsodes cantillanensis* inhabits forest relicts dominated by *Nothofagus macrocarpa* at Quebrada Infiernillo (Metropolitan Region, Chile), its type locality, and at the south of the Altos de Cantillana massif in Cerro Poqui (General Bernardo O'Higgins Region), Chile (Ramírez-Álvarez & Peñaloza 2020). Since *A. cantillanensis* is an endemic species with a limited distribution and anthropogenic threats to its habitat have been identified (Charrier *et al.* 2015; Charrier *et al.* 2017), it is classified as Endangered by the IUCN Red List. Beyond its habitat and threat factors, nothing is known about its diet and other aspects of its natural history. Therefore, the use of new techniques such as µCT allow us to advance in the understanding of its ecology, behavior and role in the ecosystem.

We created a µCT scan of a male specimen of *A. cantillanensis* (MZUC 39909; snout-vent length, SVL = 43.9 mm; Fig. 1A) to study the internal morphology of the specie collected in the Quebrada Infiernillo in September 2015. The scanning was performed at the University of Florida's Nanoscale Research Center on a Phoenix V|tome|X M CT scanner with X-ray tube set to 70 kV, 200 mA, and a detector capture time of 0.200098 seconds, with an average of 3 images/rotation and a voxel resolution of 33.54 µm. Segmentation and visualization were performed using VG Studio Max 3.4 (Volume Graphics, Heidelberg, Germany; https://www.volumegraphics.com).

Through this technique, we were able to identify remains of two arthropods in the digestive tract of *Alsodes cantillanensis*. The largest fragment (approximately 19 mm when stretched and about about 10 mm coiled) was identified as a millipede, due to the presence of two pairs of legs per segment and the specific shape of the paranota, absence of ommatidia, number of rings (20) and general body shape. Specifically, it was identified as a millipede belonging to the order Polydesmida (Fig. 1B). In Chile, two families of this order have native representatives: Dalodesmidae and Paradoxosomatidae (represented in the area by a single introduced species: *Oxidus gracilis* [Koch, 1847]); these families can be distinguished mainly by the gonopods, fused at the base in dalodesmids, and separated in paradoxosomatids (Parra-Gómez 2022). However, other diagnostic characters can be used to determinate the specimen at genus level: *Monenchodesmus* is the only genus present in the country with an acutely shaped posterolateral paraterga, as is present in the specimen (Fig. 1B) (Parra-Gómez 2022). Unfortunately, we cannot determine the species due to the absence of gonopods, as the specimen found inside correspond to a female. Gonopods are a key morphological character only present in males that (mostly) accurately allows to distinguish millipedes at a species-level (Mesibov 2009).

Additionally, we observed smaller remains of another specimen in a more advanced state of degradation which made identification at the generic and/or species level impossible. We identified it as a Coleoptera based on the abdomen (4 mm long and 3 mm wide), in which we were able to count 5 abdominal segments (sternite) (Fig. 1C), so it probably belongs to the Curculionidae family. However, since we could not observe other diagnostic characters, it was not possible to determine with greater precision the taxonomic identity of the specimen, so here it is considered as a Coleoptera item.

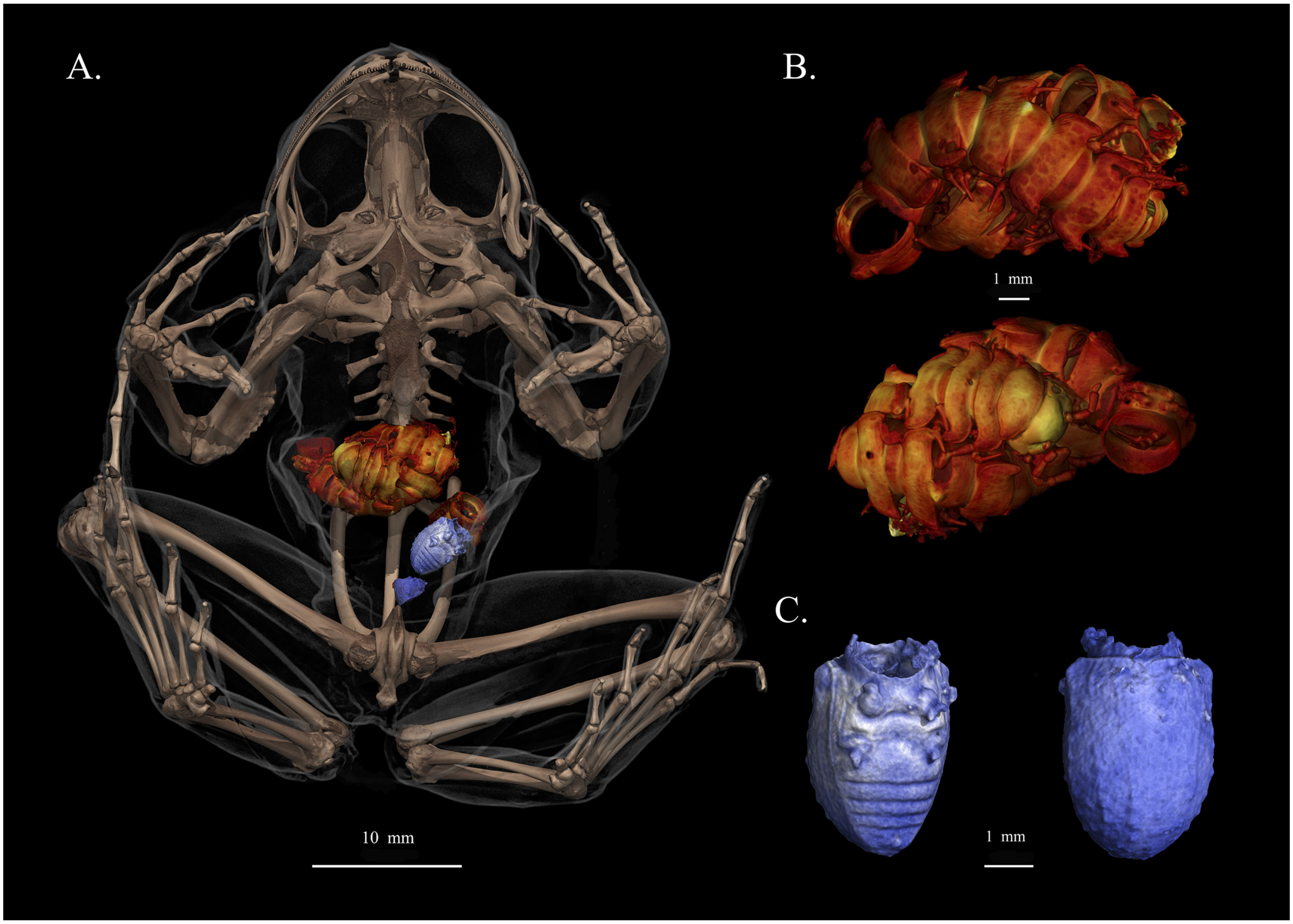

Figure 1: (A) uCT-reconstruction of the skeleton of *Alsodes cantillanensis* (MZUC 39909) in dorsal view, showing remains of Diplopoda (orange color) and Coleoptera (blue color). (B) ventral (upper) and dorsal (lower) views of the remains of the Diplopoda *Monenchodesmus* sp. (C) in blue, exoskeleton of the abdomen of a coleopteran in ventral (left) and dorsal view (right). / (A) Reconstrucción uCT del esqueleto de *Alsodes cantillanensis* (MZUC 39909) en vista dorsal, mostrando restos de Diplopoda (color naranja) y Coleoptera (color azul). (B) vistas ventral (superior) y dorsal (inferior) de los restos del Diplopoda *Monenchodesmus* sp. (C) en azul, exoesqueleto del abdomen de un coleóptero en vista ventral (izquierda) y dorsal (derecha).

This is the first report of diet items of *Alsodes cantillanesis* since its description. Many terrestrial anurans consume arthropods (Filho *et al.* 2021), especially smaller prey such as Formicidae, Diptera, Isoptera, springtails and Coleoptera (Leivas *et al.* 2012; Alveal *et al.* 2015; Alveal & Díaz-Páez 2021; Sant'Anna *et al.* 2022; Mascarenhas *et al.* 2023). The general scarcity of diplopods as prey may be explained by the toxic or repellent secretions that these arthropods use as a defensive mechanism (Ilić *et al.* 2018; Jones *et al.* 2018, 2022; Hassler *et al.* 2020) or relative abundance in the environment. However, relative abundance or others aspect of diplopods in Chilean habitats remains to be elucidated. As most millipedes, the *Monenchodesmus* genus has a nocturnal behaviour and can be typically observed under stones during the day in central Chile (Minelli 2015; Parra-Gómez pers. obs.). The overall size of both *Monenchodesmus* species found in these habitats (*M. chilensis* and *M. michaelseni*) is around 24-25 mm in length, and 2.5 mm wide (Silvestri 1903). There has been no new records about these species since 1957 (Parra-Gómez 2022).

So, studies in dietary ecology are crucial to understanding natural history, population fluctuations and the impact of habitat change on amphibian populations (Ogoanah & Uchedike 2011). Moreover, identifying prey taxa for each species will help to clarify the impact of this group on local invertebrate fauna and determine which prey species are dietary resources (Nakamua & Tominaga 2021; Siliyavong *et al.* 2023). Finally, the use of museum materials plays a crucial role in contributing to our understanding of biodiversity and serves as a cornerstone for conservation initiatives. However, due to the high cost and availability of resources, it is recommended to prioritize the digitization of important biological samples such as type series indivuals or unique material, as well as collections with a high risk of degradation, such as fossil remains, organisms in a deplorable state and rare species from threatened habitats.

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