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A NEW METHOD OF PREPARATION TO IDENTIFY ARTHROPODS FROM STOMACH CONTENTS OF BIRDS

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Abstract.—A micropaleontology method modified for the study of fragments of arthropods in stomach contents of birds is described. Fragments are sorted then mounted with gum tragacanth onto numbered grids of micropaleontological slides. Specimens on these slides are easily compared to museum reference collections of arthropods for identification. Reference collections of fragments with data fully computerized are proposed as vouchers for resultant analyses thereby aiding additional dietary investigations by subsequent workers. This method can be adapted readily for similar studies in non-avian vertebrates.

UN NUEVO MÉTODO PARA IDENTIFICAR ARTRÓPODOS EN CONTENIDOS ESTOMACALES DE AVES

Sinopsis.—Se describe un método usado en micropaleontología el cual ha sido modificado para el estudio de fragmentos de artrópodos en contenidos estomacales de aves. Una vez que los fragmentos han sido escogidos usando un microscopio binocular, las partes identificables son montadas con goma tragacanto en láminas micropaleontológicas con cuadrícula numerada. Los especímenes de estas láminas pueden ser comparados usando colecciones de referencia de artrópodos. Se propone la formación de colecciones de láminas con fragmentos de artrópodos montados, como referencia del análisis resultante y ayuda para futuras investigaciones. Este método puede ser considerado para estudios similares en otros vertebrados.

Analysis of diet from fragments recovered from stomach contents, fecal samples, forced regurgitation, flushing, ligatures and pellets can be a critical aspect in studies of foraging behavior, resource use, community organization, energetics, ecomorphology and predation (Rosenberg and Cooper 1990). However a major deficiency lies in the near absence of lists, sketches or photographs for identification of food fragments. Only two papers address this part of the work: Tatner (1983) offers a list with drawings of various prey fragments, and Ralph et al. (1985) provides a list of commonly found fragments of a variety of arthropod taxa, accompanied by photographs of the parts. Regardless, these kinds of studies are increasing and it is necessary to begin organizing the methodology for such investigations. Usually, when stomach contents are used for a particular study, data on diets are collected, but fragments are returned to a vial and stored remixed in bulk. Furthermore, the methods employed for sorting and identification of fragments in most cases go unpublished. Hours of work are lost because voucher specimens of fragments are not readily available to other scientists, especially those who might test the hypotheses of the initial investigation.

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For more than three decades, paleontologists have used microslide cards (microslide) for studies of various taxa of small fossils, e.g., Coleoptera (Coope 1986). They mount the fragments with gum tragacanth (a water-soluble glue) in numbered grids on the microslide for identification and study (A. C. Ashworth and S. A. Elias, pers. comm.). In an effort to find a reliable and practical technique to identify fragments in stomach contents, I adapted these paleontological methods to my studies of the Wedge-billed Woodcreeper (*Glyphorhynchus spirurus*).

These methods can be applied readily to qualitative and quantitative investigations of diets in other groups of vertebrates.

As this technique proved to be successful in my studies, I describe it here. I also describe a database consisting of a searchable inventory of all fragments and point to its importance in these kinds of study.

MATERIALS AND METHODS

Storing samples.—Collected samples in the field should be stored in glass vials in 70–80% ethanol with the catalog number of the collector, until microslide preparations are made.

Sorting samples.—To sort and identify fragments of Coleoptera to ordinal level, I use a stereo microscope, a petri dish with alcohol and forceps. The fragments to be mounted are moved from the petri dish to absorbent filter paper. Unmounted arthropod fragments (non-target taxa) and seeds from vials are retained in the whole stomach sample well-marked with the museum or collector's catalog number. Also, in some cases, when there are some doubts about identification at ordinal level, all fragments in question should be mounted, then later, if determined not to be of the Order under study, removal is easy and they may be restored to the vial with other non-target orders.

Mounting fragments.—Microslides (Fig. 1) are available in various sizes from Brandt and Lawson Printing, Inc., Houston, Texas. I recommend the use of 28 ply for study of arthropods in the diets of small and medium-size birds. Each microslide is composed of a multi-ply paper slide with 60 numbered squares, an aluminum slide holder and a glass microscope slide (Fig. 1). The fragments can be mounted each in a square of the multi-ply slide with a water-soluble glue that is prepared in a vial with one part ethanol, two parts water and one part gum tragacanth powder. The gum is first laid with a fine stick over the squares and the fragment is mounted after. It is possible to put glue on four or five squares, then the fragments are placed on the glue.

Orientation of the fragments depends on the part of the arthropod recovered and type of arthropod. Very large fragments may require more than one square of the slide and, if so, the lowest square number is used as a database reference location. All fragments must be mounted to reveal maximum characters depending on the group. For beetles, dorsal surfaces should show for heads, prothoraces, and elytra. Orientation should be a "north/south" axis on the square. The water-soluble glue allows easy subsequent reorientation. All fragments of Coleoptera, including the small

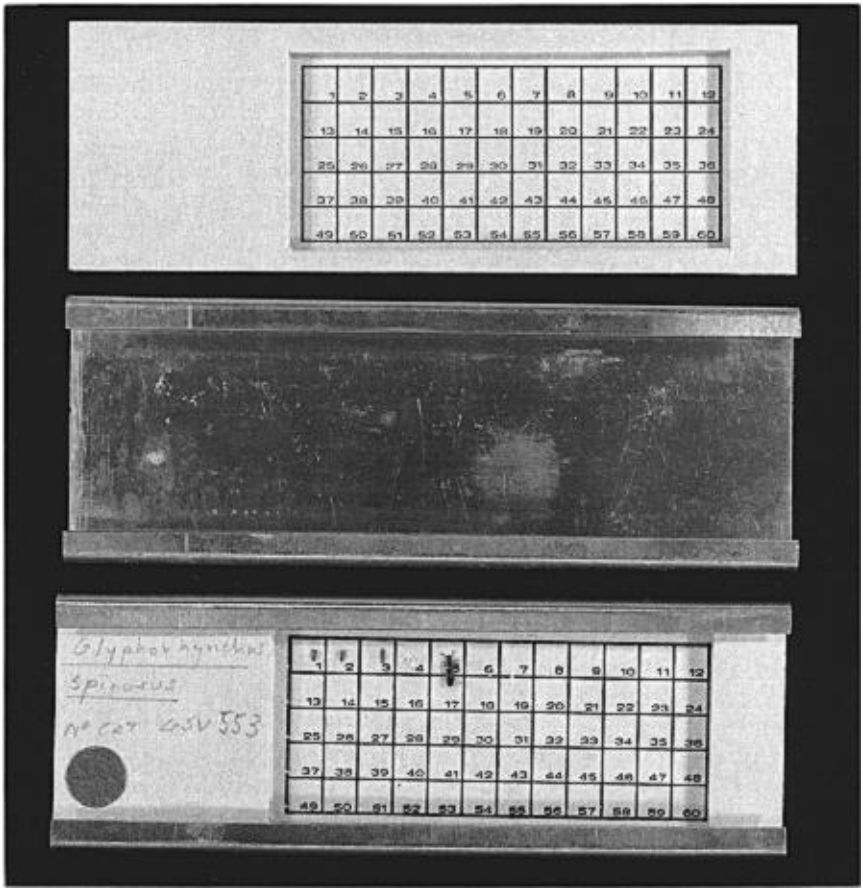

FIGURE 1. Micropaleontological slide card, slide holder and mounted fragments of Coleoptera.

ones, must be mounted for study. Diversity of beetles at all sites is maximum among orders of insects, thus failure to determine each fragment from a sample may exclude a species used as a prey item.

Upon identification, it is possible with forceps to move conspecific fragments into a series after softening the glue with a drop of water. This softening method also facilitates arrangements of the fragments for drawing or photography. It is not necessary, however, to reorder the pieces, as long as the database inventory allows rapid location of a desired fragment. The microslide label block should indicate the species of bird and the specimen catalog number. The latter will be helpful to readily locate the record for adding data considered useful for the study and for collection inventory.

After the glue is dry, the multi-ply holder is secured in the aluminum slide holder and covered with the glass.

Identifying fragments.—Each fragment may have essential characteristics for identification of genera or species, e.g., form of the humerus, size of the elytral punctures, presence of pubescence, or color. All of these may be determined from even a small fragment. Legs of Coleoptera are important because the first pair often differ in structure or vestiture from others pairs and offer additional characteristics. If all leg fragments are from the same beetle, it is still necessary to mount all of them on the slide to determine if more than one individual was eaten. Two elytra together can give an estimate of the beetle's volume and even an impression of form of what to look for in the reference collections.

The identification of arthropods is greatly facilitated by a large museum reference collection of the order of arthropods under study. Even more useful is a reference collection of fragmented parts taken from intact specimens collected in the same area in which the birds were collected and also mounted on microslides for making comparisons.

Fragments identified are recorded on a 7.6 × 12.7 cm card at appropriate taxon levels (family, genus, and/or species). The record contains the number of individuals on the microslide, the name of the parts, and the characteristics of the fragment, e.g., color, texture, size.

A database can be created using available computer software (e.g., dBase for DOS machines and Excel for Macintosh computers). With this, it is possible to maintain a searchable inventory of all fragments and their identifications for each bird species (Fig. 2). Report design can be a powerful tool for support of research projects, as well as collection management, and should be a part of all fragment collections from the start.

Slide-mounted fragment measurements, a major analytical feature of the database, are made only when the piece is complete (using an ocular micrometer), for example the length of the elytron or width of the head. These measurements are taken for additional help in identification and for making regression analyses, etc.

Storing the microslide.—Upon completion of the study, the microslides may be stored in a reference collection ordered by the family/genus of bird (or arthropod order), with the bird specimen's catalog number.

DISCUSSION

There are numerous advantages to this method of data collection. 1) The fragments are easy to manipulate without risk of damage or loss from the sample. 2) A reference collection of identified fragments is produced that serves as a voucher, and as a resource for future studies. 3) It permits identification of fragments stored in a database that can serve supplemental kinds of studies. 4) It is time-saving, because specimens are prepared only once and each successive investigation adds information directly to the database, thus speeding the analysis process. 5) This method can also be applied to other vertebrates and items in their diet.

RECORD No.	BIRD DATA					ORDER	QUAD No.	COLEOPTERA DATA							FRAGMENT DESCRIPTION			
	FAMILY	GENUS	SPECIES	CATALOG No.	LOCALITY			FAMILY	TRIBE	GENUS	SPECIES	REFER. MUSEUM	WHOLE SIZE LENGTH	WHOLE SIZE WIDTH	PART	COLOR	PATTERN Y/N	VESTITURE
1/1	DEND	Glyphorynchus	spirurus	GSV 546	PAK	COL	1	TRGS		Tenebroides		TBPT 84	4.40	1.75	ELYT (F)	LBRWN	N	G
1/1	DEND	Glyphorynchus	spirurus	GSV 546	PAK	COL	2	TRGS		Tenebroides		TBPT 84	4.40	1.75	ELYT	LBRWN	N	G
2/1	DEND	Glyphorynchus	spirurus	GSV 553	PAK	COL	1	COLY		8u' 973		SAM 90	1.85	.80	ELYT	DBRWN	N	P
2/1	DEND	Glyphorynchus	spirurus	GSV 553	PAK	COL	2	COLY		8u' 973		SAM 90	1.85	.80	ELYT	DBRWN	N	P
2/1	DEND	Glyphorynchus	spirurus	GSV 553	PAK	COL	3	SILV		Nausibius		TBPT 83	3.75	.95	ELYT (F)	DBRWN	N	P
2/1	DEND	Glyphorynchus	spirurus	GSV 553	PAK	HYM	4											
2/1	DEND	Glyphorynchus	spirurus	GSV 553	PAK	COL	5	SILV		Nausibius		TBPT 83	3.75	.95	WHOLE	DBRWN	N	P

FIGURE 2. Example of partial database for the study of Arthropoda fragments in stomach contents of birds. RECORD No: The first number indicates the microslide number, the second, the number of microslides in the total ordinal sample.

The disadvantages are the cost of the microslides, which is approximately $0.40 US each, and the necessity to purchase or build storage cabinets for rapid access to individual collections (although they may be kept in boxes until the collection grows). Like other collections, these slides require curatorial work and protection from humidity, fungus or other plagues. It is necessary to keep the collection in a dry place.

Studies of fragments have become significantly easier and faster since adopting the described technique. I investigated 105 stomach contents from *G. spirurus,* and mounted 78 slides with Coleoptera fragments (74% of the total samples of this species of bird) in 120 h. A less experienced worker would take possibly 80 h more. I then spent 200 h identifying 66 species of Coleoptera (76% of the specimens on the microslides). This part also depends on the experience and knowledge of the investigator.

If these methods are adopted by others, and museums begin accumulating microslide-prepared materials, special consideration will be needed regarding access, data storage, specimen loans, and housing for the specimens.

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