Amphibia-Reptilia 37 (2016): 437-440

Short Notes

A proposed optimal incision method to obtain gut contents from preserved anurans

Britta Döring[1,*], Sven Mecke[1,*,**], Felix Mader[2], Hinrich Kaiser[3,4]

Abstract. Information on the diet of anuran species based on gut content analyses have been published by numerous researchers, yet the details of the incision method used to open the abdominal cavity of preserved specimens in preparation for such examinations are rarely explained in the presented methods. Our objective is to formally propose an optimal incision into the pleuroperitoneal cavity of liquid-preserved anuran specimens to gain access to and permit easy removal of parts of the digestive tract in preparation for food spectrum analyses. In our experience, this U-shaped cut is easy to perform and teach. It also provides better access to the pleuroperitoneal cavity than a small ventrolateral incision and is less destructive than the classic textbook medial "double T-incision" routinely listed in dissection protocols.

Keywords: anatomy, Anura, food spectrum analysis, gut content analysis, incision, invasive method.

Gut content analysis is an important and efficient tool for determining the diet of amphibians, including anurans. Publications on feeding habits of anurans based on gut content analysis of preserved specimens, however, usually lack information on the type of incision used to open the abdominal cavity, and hence there is no defined consensus on the most appropriate method to use for this purpose (Berry and Bullock, 1962; Zug and Zug, 1979; Vences et al., 1999; Cogălniceanu et al., 2000; Dos Santos et al., 2003; Maneyro et al., 2004; Moseley et al., 2005; Da Silva et al., 2009; Yousaf et al., 2010; Da Silva et al., 2011; Crnobrnja-Isailović et al., 2012; Olson and Beard, 2012; Sugai et al., 2012; Almeria and Nuñeza, 2013; Luría-Manzano and Gutiérrez-Myén, 2014).

During a preliminary study on celiotomies performed on liquid-preserved anuran specimens as part of a broader study (food spectrum analysis of *Duttaphrynus melanostictus*; Döring et al., accepted), we found one method to open the ventral body cavity particularly convincing: a U-shaped cut. This incision technique, that appears most useful when carrying out gut content analyses in preserved anurans, may be well known to some researchers and has already been in use (George Zug, in litt.). It has, however, not been previously described and compared to other incisions in the literature. We describe this U-incision method in the protocol below.

For performance of the U-incision, a rounded, transverse ventral cut at the lowest point in the curve of the U is made at the level of the anterior border of the hind leg insertion into the body wall to penetrate the skin and *musculus rectus abdominis* (fig. 1a). Subsequently, two parallel longitudinal cuts are made, beginning

1 - Department of Animal Evolution and Systematics and Zoological Collection Marburg, Faculty of Biology, Philipps-Universität Marburg, Karl-von-Frisch-Straße 8, 35032 Marburg, Germany
2 - Janusstraße 5, 93051 Regensburg, Germany
3 - Department of Biology, Victor Valley College, 18422 Bear Valley Road, Victorville, California 92395, USA
4 - Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA
*Co-first authors, listed in alphabetical order
**Corresponding author; e-mail: meckes@staff.uni-marburg.de

 DOI:10.1163/15685381-00003061

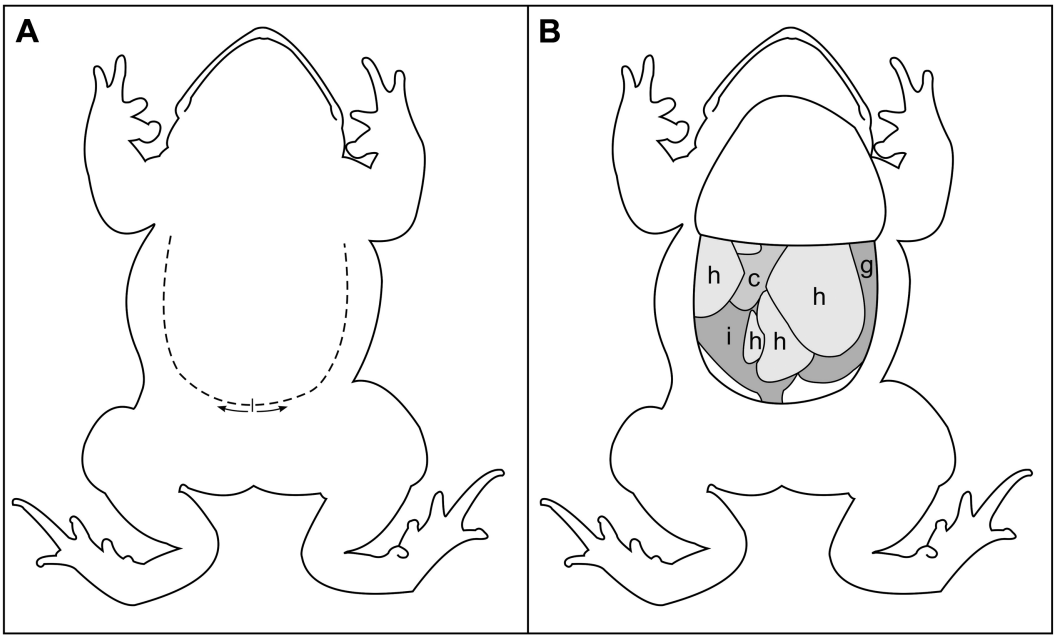

Figure 1. (A) Schematic representation of how to apply the U-incision to a liquid-preserved anuran specimen. (B) View into the body cavity after the single skin and muscle flap is reflected. c = cor (heart), h = hepar (liver), g = gaster (stomach), i = intestinum (intestine).

at the margins of the original incision in direction of the axils, resulting in a U-shaped cut (fig. 1a). After the incision is made, the single flap of skin and muscles is reflected and pinned to the front (in direction of the head) of the respective specimen to allow suitable exposure of the pleuroperitoneal cavity and the inner organs (fig. 1b). Removal of the stomach to analyse its contents does not necessitate the removal of the liver. The *vena abdominalis*, which runs along the inner surface of the abdominal muscles and enters the hepatic portal vein, slightly lifts the liver from its original position when the skin and muscle flap is reflected. This easily allows access to the entire stomach, with a cut necessary at its transition with the oesophagus and at its transition with the duodenum to remove the organ. Furthermore, the larger opening produced by the U-shaped cut also allows access to the lower guts, and thereby easy removal of the intestines is possible by a cut made at the transition of the rectum with the anus. In gravid females, eggs have to be removed prior to the removal of the guts. After completion of food item removal from the guts, the stomach and intestine are repositioned in the pleuroperitoneal cavity, with the stomach held in place by the liver when skin and muscles are flapped back to close the opening. Secure closure of the pleuroperitoneal cavity for the purpose of storage in a collection may be achieved by fixing the skin and muscle flap on each side of the body in vicinity of the hind legs using pins.

For the purpose of a gut content analysis, applying only a small ventrolateral incision does not provide access to all relevant organs. The classic textbook example to open the abdominal cavity in tetrapods is an incision (herein referred to as the double T-incision) along the mid-ventral line (slightly offset from the *linea alba*), beginning at the anterior border of the hind leg insertion into the body wall to a point posterior to the sternum. This mid-ventral cut is extended, using smaller cuts running in a lateral direction (at the level of the limbs), resulting in five separate cuts. This produces two skin flaps that can be reflected laterally and pinned (e.g., Jammes, 1904; Nierstrasz and Hirsch, 1930;

Booloottian and Heyneman, 1969; De Iuliis and Pulerà, 2007; Storch and Welsch, 2009).

The double T-incision, which some researchers have applied in the past to open the abdominal cavity of liquid-preserved anurans, is clearly more destructive than the proposed U-incision, since cutting affects the pectoral girdle and the muscles of the extremities as well as some of the inner organs, if the utmost possible insight into the body's interior is required. By using a double T-incision the resulting skin flaps need to be reflected laterally and pinned to keep the large pleuroperitoneal opening exposed. The U-incision makes specimen handling during examination of the inner body quite simple, since the large opening of the pleuroperitoneal cavity provides general orientation and accessibility to all relevant organs, and the single skin and muscle flap can easily be affixed to a dissection tray using a single pin, or can even simply be held with the fingers.

The level of organ exposure that is produced by the U-incision also provides an excellent view for photography, an important feature given that the morphology of various organs (e.g., liver shape; Hedges, 1989: fig. 12) has been shown to be useful for taxonomic purposes. In studies of eleutherodactylid frogs, Hedges and colleagues (e.g., Hedges, 1989; Hedges et al., 2008) extensively used liver shape as a taxonomic character, and their methodology called for the removal of the entire ventral surface, which would have been unnecessary if using a U-incision.

The U-incision, which is easy to perform and teach, might be a useful method for celiotomies in amphibian groups with relatively elongated body forms (newts and salamanders) as well, but the presence of elongated ribs inside the thorax does not allow an application on lizards. We argue that researchers in their studies should report on the respective incision method used, instead of only stating that an incision was made. This may contribute to the establishment of standardised incision methods in different animal groups.

Acknowledgements. We thank S. Gotte, J. Jacobs and R. Wilson (USNM) for loan of specimens under their care. We sincerely acknowledge M. Kieckbusch, B. Kostron, K. Schuster and H. Worth (Phillips-Universität Marburg, Germany) for providing helpful information and/or suggestions on earlier versions of the manuscript. We thank George Zug (USNM) and an anonymous referee for reviewing the manuscript.

References

Almeria, M.L., Nuñeza, O.M. (2013): Diet of seven anuran species (Amphibia: Anura) in Agusan Marsh, Mindanao, Philippines. Anim. Biol. Anim. Husb. **5**: 116-126.

Berry, P.Y., Bullock, J.A. (1962): The food of the common Malayan toad, *Bufo melanostictus* Schneider. Copeia **1962**: 736-741.

Booloottian, R.A., Heyneman, D. (1969): An Illustrated Laboratory Text in Zoology, 2nd Edition. Holt, Rinehart and Winston, New York, USA.

Cogălniceanu, D., Palmer, M., Ciubuc, C. (2000): Feeding in anuran communities on islands in the Danube floodplain. Amphibia-Reptilia **22**: 1-19.

Crnobrnja-Isailović, J., Ćurčić, S., Stojadinović, D., Tomašević-Kolarov, N., Aleksić, I., Tomanović, Ž. (2012): Diet composition and food preferences in adult common toads (*Bufo bufo*) (Amphibia: Anura: Bufonidae). J. Herpetol. **46**: 562-567.

Da Silva, E.T., Filho, O.P.R., Feio, R.N. (2011): Predation of native anurans by invasive bullfrogs in southeastern Brazil: spatial variation and effect of microhabitat use by prey. S. Am. J. Herpetol. **6**: 1-10.

Da Silva, E.T., Dos Reis, E.P., Feio, R.N., Filho, O.P.R. (2009): Diet of the invasive frog *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae) in Viçosa, Minas Gerais state, Brazil. S. Am. J. Herpetol. **4**: 286-294.

De Iuliis, G., Pulerà, D. (2007): The Dissection of Vertebrates. A Laboratory Manual. Amsterdam. Academic Press, Boston.

Döring, B., Mecke, S., Kieckbusch, M., O'Shea, M., Kaiser, M. (accepted): Food spectrum analysis of the Asian toad, *Duttaphrynus melanostictus* (Schneider, 1799) (Anura: Bufonidae), from Timor Island, Wallacea. J. Nat. Hist.

Dos Santos, J.W.A., Damasceno, R.P., Da Rocha, P.L.B. (2003): Feeding habits of the frog *Pleuroderma diplolistris* (Anura, Leptodactylidae) in quaternary sand dunes of the middle Rio São Francisco, Bahia, Brazil. Phyllomedusa **2**: 83-92.

Hedges, S.B. (1989): Evolution and biogeography of West Indian frogs of the genus *Eleutherodactylus*: slow-evolving loci and the major groups. In: Biogeography of the West Indies: Past, Present, and Future, p. 305-370. Woods, C., Ed., Sandhill Crane Press, Gainesville, USA.

Hedges, S.B., Duellman, W.E., Heinicke, M.P. (2008): New world direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography and conservation. Zootaxa **1737**: 1-182.

Jammes, L. (1904): Zoologie Pratique Basée sur la Dissection des Animaux les Plus Répandus. Masson, Paris, France.

Luría-Manzano, R., Gutiérrez-Myén, G. (2014): Reproduction and diet of *Hyla euphorbiacea* (Anura: Hylidae) in a pine-oak forest of southeastern Puebla, Mexico. Vert. Zool. **64**: 207-213.

Maneyro, R., Naya, D.E., Rosa, I., Canavero, A., Camargo, A. (2004): Diet of the South American frog *Leptodactylus ocellatus* (Anura, Leptodactylidae) in Uruguay. Iheringia, Sér. Zool. **94**: 57-61.

Moseley, K.R., Castleberry, S.B., Hanula, J.L., Ford, W.M. (2005): Diet of southern toads (*Bufo terrestris*) in loblolly pine (*Pinus taeda*) stands subject to coarse woody debris manipulations. Am. Midl. Nat. **153**: 327-337.

Nierstrasz, H.F., Hirsch, G.C. (1930): Anleitung zu Zoologisch-Morphologischen Uebungen. Band II: Wirbeltiere, 2nd Edition. Gustav Fischer, Jena, Germany.

Olson, C.A., Beard, K.H. (2012): Diet of the introduced greenhouse frog in Hawaii. Copeia **2012**: 121-129.

Storch, V., Welsch, U. (2009): Kükenthal. Zoologisches Praktikum, 26th Edition. Springer Spektrum, Heidelberg, Germany.

Sugai, J.L.M.M., Souza Terra, J., Ferreira, V.L. (2012): Diet of *Leptodactylus fuscus* (Amphibia: Anura: Leptodactylidae) in the Pantanal of Miranda river, Brazil. Biota Neotrop. **12**: 99-104.

Vences, M., Glaw, F., Zapp, C. (1999): Stomach content analyses in Malagasy frogs of the genera *Tomopterna*, *Aglyptodactylus*, *Boophis* and *Mantidactylus* (Anura: Ranidae). Herpetozoa **11**: 109-116.

Yousaf, S., Mahmood, T., Rais, M., Qureshi, I.Z. (2010): Population variation and food habits of ranid frogs in the rice-based cropping system in Gujranwala Region, Pakistan. Asian Herpetol. Res. **1**: 123-130.

Zug, G.R., Zug, P.B. (1979): The marine toad, *Bufo marinus*: a natural history resumé of native populations. Smithson. Contrib. Zool. **284**: 1-58.

Submitted: July 21, 2016. Final revision received: August 31, 2016. Accepted: September 7, 2016.
Associate Editor: Uwe Fritz.