



## ***Niku-nuki*: a useful method for anatomical and DNA studies on shell-bearing molluscs**

HIROSHI FUKUDA<sup>1</sup>, TAKUMA HAGA<sup>2</sup> & YUKI TATARA<sup>3</sup>

<sup>1</sup> *Conservation of Aquatic Biodiversity, Faculty of Agriculture, Okayama University, Tsushima-naka 1-1-1, Okayama 700-8530, Japan*

<sup>2</sup> *Department of Biological Science, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

<sup>3</sup> *Department of Biology, Faculty of Science, Toho University, Miyama 2-2-1, Funabashi 274-8510, Japan,*

### **Abstract**

Often only one or a few individuals of rare species are collected. How do we treat them as intact voucher specimens? The shell of the whole individual in formalin or alcohol will corrode or fade. In order to dissect the soft parts, you must crack or dissolve the shell. *Niku-nuki*, a traditional method that has been used by Japanese malacologists overcomes this dilemma. It is also applicable to minute molluscs. The outline is: 1. Prepare boiling hot freshwater, a small beaker, forceps (with fine tips), a small syringe, a petri dish, and a stereomicroscope; 2. When the live animal in the beaker crawls on the bottom, pour boiling hot water over the animal, which is killed immediately. Some seconds later take the specimen out of the hot water, hold it with two fingers of one hand and hold the forceps with another hand; 3. Under the microscope, grab the foot with the forceps and pull carefully to just separate the columellar muscle from the shell; 4. Pull the foot again in a petri dish filled with cold water as under 3. With coiled gastropods, unscrew the specimen by approximately ¼ whorls. If it is difficult to move the soft parts, inject water into the aperture gently with the syringe. Repeat it several times, then you will get an empty shell and the complete soft parts. With this method, we can obtain intact shells and soft parts for multiple purposes such as conchological observation and gross anatomy. DNA can also be extracted from those soft parts because DNA is stable at high temperature. The boiled animal can be dehydrated in alcohol. We can prevent the negative effect of DNase (by heat) and magnesium (by washing in freshwater) on the DNA.

**Key words:** Mollusca, Gastropoda, Bivalvia, Scaphopoda, anatomy, DNA analysis, soft parts, specimen preparation

### **Introduction**

If you are a taxonomist of shelled molluscs, sometimes you should examine a specimen of a very rare species: *e.g.*, endangered species, taxon with a narrow range, material from the locality/habitat, which is very hard to access. When you get only one individual of such a rare species that is extremely important for your study, how do you treat it? If you need to describe the species as new, you must keep the shell intact as the holotype. On the other hand, to determine the systematic position, you may need to dissect the soft parts or extract the DNA from the *same* specimen. In these cases, how is it possible to leave both shell and soft parts as an intact voucher specimen?