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Tropical insect collections and DNA extraction, using *Rhodnius* Stål 1859 (Hemiptera: Heteroptera: Reduviidae: Triatominae)

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Biological collections are central databases for information concerning morphology and diversity in all living groups (Marinoni *et al.* 2005). According to Blaxter (2004), valuable information about taxonomic identification and phylogenetic inference can be provided by DNA sequences. Common methods for DNA extraction normally involve partial destruction of the specimen; this kind of extraction is not attractive for use in the case of historical specimens. The application of that idea in insect collections became possible once Gilbert *et al.* (2007) published a new protocol that allows the extraction of this useful DNA from dry-preserved collection specimens for genetic analyses. However, the authors used specimens from four different collections located only in temperate zones (California Academy of Sciences, Smithsonian Institution's National Museum of Natural History, Carnegie Museum of Natural History, and the National Science Museum, Tokyo).

In the last twenty years, investigations using the sequencing of DNA markers in molecular systematics and taxonomy have grown considerably. The use of molecular markers in triatomines appeared as an informative tool that attempted to establish both systematic and evolutionary questions to help with the investigation of problems concerning taxonomic difficulties, such as the identification of cryptic species (Bargues *et al.* 2002; Ward *et al.* 2009).

Hebert *et al.* (2003) have suggested the use of a DNA barcoding to catalog the world's known animal diversity, thus improving access to biodiversity knowledge for less or non-trained (in taxonomy) scientists.

The purpose of this work was to test the DNA extraction protocol described by Gilbert *et al.* (2007) and the mitochondrial marker cytochrome oxidase subunit I (COI) to identify species of genus *Rhodnius* from the Entomological Collection of the Instituto Oswaldo Cruz, Fiocruz. This collection, located in Rio de Janeiro, is one of the largest collections in Latin America; it comprises five million insects representing most described orders not only from Brazil, but the entire world. This is the first study that has proposed to characterize species from Brazilian entomological collections. The genus *Rhodnius* was chosen because, according to Neiva & Pinto (1923), it can be easily distinguished from other genera and the differentiation between its species is very difficult.

The DNA extraction protocol described by Gilbert *et al.* (2007) was used in 49 dry-preserved insects and the same was applied in two fresh insects (from colonies of Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomineos, Instituto Oswaldo Cruz) to create a positive control. The standard DNA extraction protocol (Qiagen Blood and Tissue kit) for Triatominae was also applied for 10 dry-preserved insects. The extraction products were ran in a 0.8% agarosis gel and then subsequently submitted to PCR using the universal primers LCO-1490/HCO-2198 (Folmer *et al.* 1994) and ShortF/ShortR (Gilbert *et al.* 2007). The amplification and posterior sequencing of the fragment was successful only for the fresh insects (positive control). The unsuccessful sequencing, for us, indicates the advanced degradation state of the genetic material from the collection samples.

The collection used in this work was maintained at an environmental temperature which can range from 18°C to 43°C throughout the year. The humidity is another condition we can not control at this point in our facilities. Together, these factors surely contribute to the rapid and efficient degradation of the DNA material from the insects.

To sum it up, we tested a protocol, described and successful for insects kept in controlled environmental conditions, with samples subjected to the huge climate variation of the tropics. Our results led us to conclude that, in the era of DNA-based studies, biological collections should be held in an extremely controlled environment. Ideally, specimens deposited in collections from now on should have their DNA extracted with a protocol like the one above before voucher numbers are assigned to them.

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