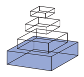
frontiers in
ENVIRONMENTAL SCIENCE

METHODS ARTICLE
published: 29 January 2015
doi: 10.3389/fenvs.2014.00068



A new DNA extraction protocol for screwworm fly *Cochliomyia* species (Diptera: Calliphoridae)

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Modifications to the DNA isolation protocol from *Cochliomyia* spp., are suggested based on the Chelex® 100 reactive. To apply the sterile insect technique (SIT) program it is necessary to study the molecular variations of endemic populations with efficient, fast and low costs techniques. The test samples were collected in the Pichincha province of Ecuador. The isolation protocol had 3 steps: (a) pretreatment (optional), (b) mechanic and chemical lysis, (c) two incubations; then the supernatant were separated by centrifugation. Furthermore, variations in concentrations of magnesium chloride present in the master mix were evaluated. Results showed a high efficiency in isolation with approximately 1.20 h of manipulation (without pretreatment). Additionally, the quality of the amplicon that was visualized on 2% agarose (w/v) showed that the magnesium chloride concentration was influential in the PCR reaction mix.

Keywords: ***Cochliomyia* spp., DNA extraction, magnesium chloride**

INTRODUCTION

Cochliomyia hominivorax (Coquerel) (Diptera: Calliphoridae), is a New World screwworm (NWS) which in its larval stage is an obligate parasite that causes parasitic and zoonotic disease known as traumatic myiasis in warm-blooded animals including humans (Forero et al., 2008; Bárcenas, 2010). The illness is included in the list of multiple species diseases, infections and infestations and their presence is notifiable (OIE, 2013). The NWS is also considered part of Transboundary Diseases in the Americas (GF-TADs, 2007).

NWS is endemic to tropical and subtropical regions of America, where it is widely distributed, mainly in South America and the Caribbean (Wyss and Galvin, 1996; Wall and Shearer, 1997). The annual losses caused by screwworm infestations in the United States, Mexico and Central America were significant; while in some Caribbean islands and South America it remains as a latent problem (Forero et al., 2007; Rodríguez et al., 2011). The programs based on the sterile insect technique eradicated the NWS caused by *C. hominivorax* from the southern United States, Mexico, Central America and some Caribbean islands (Robinson et al., 2009). For the period 2012–2021 the FAO has provided strategies for collecting information about the incidence of populations of screwworm and the impact of NWS on public health, in order to establish the eradication program in South America (FAO, 2011).

In Ecuador, two studies surveyed on animals from tropical areas reported presence of screwworm larvae in 4132 and 830 animals, respectively (Miño et al., 2005; Arteaga et al., 2012). Besides, studies carried out at the International Centre for Zoonosis of the Central University of Ecuador (CIZ-UCE) on adult *Cochliomyia* spp. have shown difficulties in the morphological differentiation of *C. macellaria* and *C. hominivorax* (unpublished data).

Griffiths et al. (2009), Torres and Azeredo-Espin (2009), Robinson et al. (2009) and Lyra et al. (2009) have reported the use of different molecular techniques and several modifications of the DNA isolation protocols for *Cochliomyia* spp. The principal changes were based on the type of sample (legs, abdomen and complete specimen) to be analyzed and focused to phenol-chloroform or commercial kit methods, but the quantification and purity was not reported. The aim of this study was to prove a fast and user-friendly protocol of DNA isolation, that will allow to study the genetic variability and molecular taxonomy of the species in Ecuador. In order to contribute to an efficient biological control method with more genetic information based on a large number of samples in a short period of time (Klassen and Curtis, 2005; McDonagh et al., 2009).

MATERIALS AND METHODS

SAMPLE

The specimens were collected as larvae from animals with myiasis and bovine corpses, which were *in-vitro* cultured in the laboratory until adult stage. All specimens were from the northwest of the Pichincha province. All specimens were labeled and deposited in the bank of specimens of CIZ-UCE; each specimen was preserved dry individually in a tube of 1.5 ml at—20°C for ≈3 months until laboratory process. Details of 9 adults specimens selected