Cleaning historical tick specimens using an ultrasonic cleaner

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Received 21st Aug 2019

Accepted: 3rd Dec 2019

Citation: Chitima-Dobler, L., and Dunlop, J. A. 2020. Cleaning historical tick specimens using an ultrasonic cleaner. *Journal of Natural Science Collections.* **7.** pp. 92-97.

Abstract

A method is described for cleaning ticks (Arachnida: Ixodida) preserved for decades in 70% ethanol using an ultrasonic cleaner. The advantages of this approach are that it is relatively inexpensive and does not involve the use of chemical reagents other than ethanol, such as when preparing ticks for scanning electron microscopy or as slide preparations. In a wider context the methods outlined here may be applicable to other relatively robust arthropods preserved in alcohol collections.

Keywords: Arthropoda, Arachnida, Ixodida, cleaning, ultrasonic cleaning, collections

Introduction

Ticks (Arachnida: Ixodida) are an important group of ectoparasites which feed on the blood of vertebrates. About 900 valid species are currently recognized (Guglielmone *et al.*, 2010) and their significance as pathogen vectors in both humans and domestic animals means that there is a considerable body of research relating to their systematics and biology; see e.g. Soneshine & Roe (2013) for an overview. Museum specimens of ticks are a particularly important source of data. Museums can host the type specimens which underlie the identification of species, but even non-type records can provide valuable information on, for example, (historical) distribution patterns, host preferences and morphological variation within a given taxon. However, morphology-based studies of ticks held in museum collections are less effective when the objects are dirty and/or encrusted with detritus, which is often the case with specimens collected in the field. These artefacts can obscure characters necessary for correct identification, or hinder accurate measurements for techniques such as morphometrics. Using the tick collections from the Museum für Naturkunde Berlin as a test case, we demonstrate here a relatively cost- and time-effective method using ultrasonic vibration for cleaning specimens preserved, often for decades, in 70% ethanol.

Methods

Specimens originate from the Museum für Naturkunde Berlin and have repository numbers under the traditional acronym ZMB (for Zoologisches Museum Berlin). This collection hosts ca. 225 valid tick species from throughout the world, with type series of about 160 species (Moritz & Fisher 1981), including historically significant specimens associated with Koch's (1844) groundbreaking study of tick systematics. Note that only about 60% of these type series belong to currently valid species. While some tick specimens in Berlin are pinned and dry or, less

commonly, mounted on slides, most of the collection is preserved in 70% ethanol; as is typical for zoological wet collections. Many of the specimens date back to the mid to late 19th century, i.e. they can be up to 175 years old, with a geographical focus on Europe as well as former German colonies or major international expeditions carried out by German explorers.

For comparative purposes, examples of historical tick specimens in alcohol were photographed using a Keyence VHX-900F microscope (Itasca, Illinois, USA) both before and after the cleaning process (compare left and right panels of Figs. 1–2). Specimens were subjected to ultrasonic vibration at a low intensity for five minutes using a Bioruptor® Sonication System (Diagenode Bioruptor Standard). During this process specimens were still retained within their original vials, which usually contain ca. 2–15 ml of 70% ethanol. This ultrasound treatment can be repeated if necessary. Afterwards, the ticks were placed in a petri dish, still in ethanol, and were manually cleaned of any remaining adhering particles with a small paintbrush; typically sizes 0–2, depending on the sample size. The cleaned ticks were finally placed and dried on a piece of filter paper and manipulated to check from all sides that they were now fully clean. The manual cleaning step can also be repeated if necessary. Once the ticks are in a satisfactory condition they can be returned to the original vials and/or be studied and photographed under the microscope.

Results

The ultrasound method proposed here can return historical tick specimens collected as early as the late 19th century to a near pristine condition. The detritus which adhered to the ticks was successfully removed and high-quality photographs of the clean specimens – including any microstructure and/or setae on their cuticle – are now possible. Figure 1a-d shows part of the type series of *Hyalomma rufipes* CL Koch, 1844 (ZMB 1073) collected pre 1844 from Senegal. Figure 1e–f shows a non-type specimen of *Ixodes bicornis* Neumann, 1906 (ZMB 16777) from Tirrialba in Costa Rica collected in 1913. Figure 2a–b shows a non-type specimen of *Amblyomma pomposum* Dönitz, 1909 (ZMB 15922) from Marromeu in Mozambique collected in 1976. Figure 2g–h shows a non-type specimen of *Rhipicephalus evertsi evertsi* Neumann, 1897 (ZMB 11454) from Mafeking in South Africa; date of collection not recorded.

Discussion

Several methods for cleaning ticks have been proposed in the literature (e.g. Corwin *et al.*, 1979; Dixon *et al.*, 2000), although here the ticks here were specifically being prepared for scanning electron microscopy. The disadvantage of the Corwin et al. (1979) method is the use of a commercial glue, which is not universally available, but was useful for removing dirt particles from the integument of ticks, especially argasids (soft ticks). By contrast, Dixon *et al.*, (2000) proposed a method where they used wax solvent instead of detergents or ethanol. Nevertheless, this method is time consuming and relies on potentially dangerous chemicals like xylene and acetone. These make the techniques more expensive, and introduce additional health risks to the user, making them less appropriate for cleaning and curating large museum or university collections. Larval ticks can also be prepared in Hoyer's medium: a mixture of gum Arabic, chloral hydrate and glycerol (e.g. Stern & Sucena 2000). This is the most satisfactory substance for preparing whole mounts of larval ticks as the setae, the positions of which can be taxonomically important, are seen best when the juvenile specimens are mounted on slides; see also Clifford & Anastos (1960) for details.

Ultrasonic cleaning, often associated with immersion in 5% sodium (or potassium) hydroxide, has also been mentioned in the literature on ticks (e.g. Estrada-Peña *et al.*, 2004; Latif *et al.*, 2012; Barker & Walker 2014), although here the focus was on freshly collected material. In the Latif *et al.*, (2012) study the relatively soft-bodied tick *Nuttalliella namaqua* Bedford, 1931 required careful treatment prior to electron microscopy, namely gradual rehydration and then five 2–3 second bursts in an ultrasonic cleaning bath before the usual critical point drying technique. Barker & Walker (2014) suggested ultrasonic cleaning in a solution of sodium or potassium hydroxide, or if this is not available brushing them with detergent using the stumped bristles of an artist's brush. Although not explicitly stated in these studies, the sodium or potassium hydroxide evidently helps to remove adhering particles.

We demonstrate here that ultrasonic cleaning can also be carried out efficiently on wet samples without the need for additional chemicals beyond the 60–70% ethanol, which would be used for long-term storage anyway. The method is also applicable to historical museum specimens – as opposed to fresh material only – and facilitates the mobilization of high-quality morphological data from older material too. Further advantages of the methods proposed here are that it is relatively quick and, from a curatorial point of view, can be done on specimens still in their original museum vials.

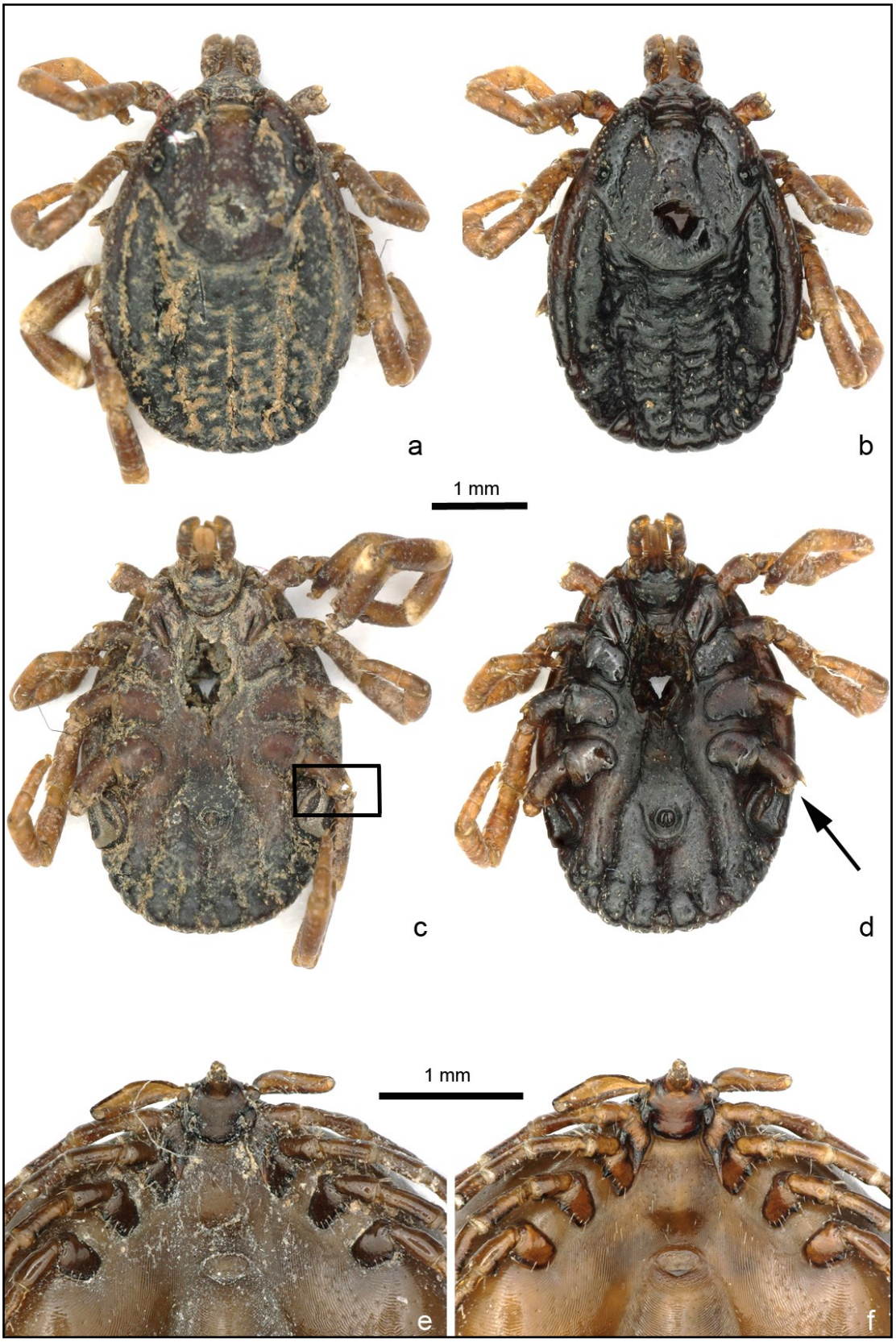

Figure 1. Examples of cleaned ticks. ***a–b.*** Hyalomma rufipes *CL Koch, 1844 (ZMB 1073: from type series) in dorsal view before (a) and after (b) cleaning.* ***c–d****. The same in ventral view before (c) and after (d) cleaning; note that the specimen was originally dried and pinned, and that one leg was weakened (box) and became disarticulated (arrow) during cleaning.* ***e–f.*** Ixodes bicornis *Neumann, 1906 (ZMB 16777), anterior region of engorged individual in ventral view before (e) and after (f) cleaning. Images © Lidia Chitima-Dobler, 2019.*

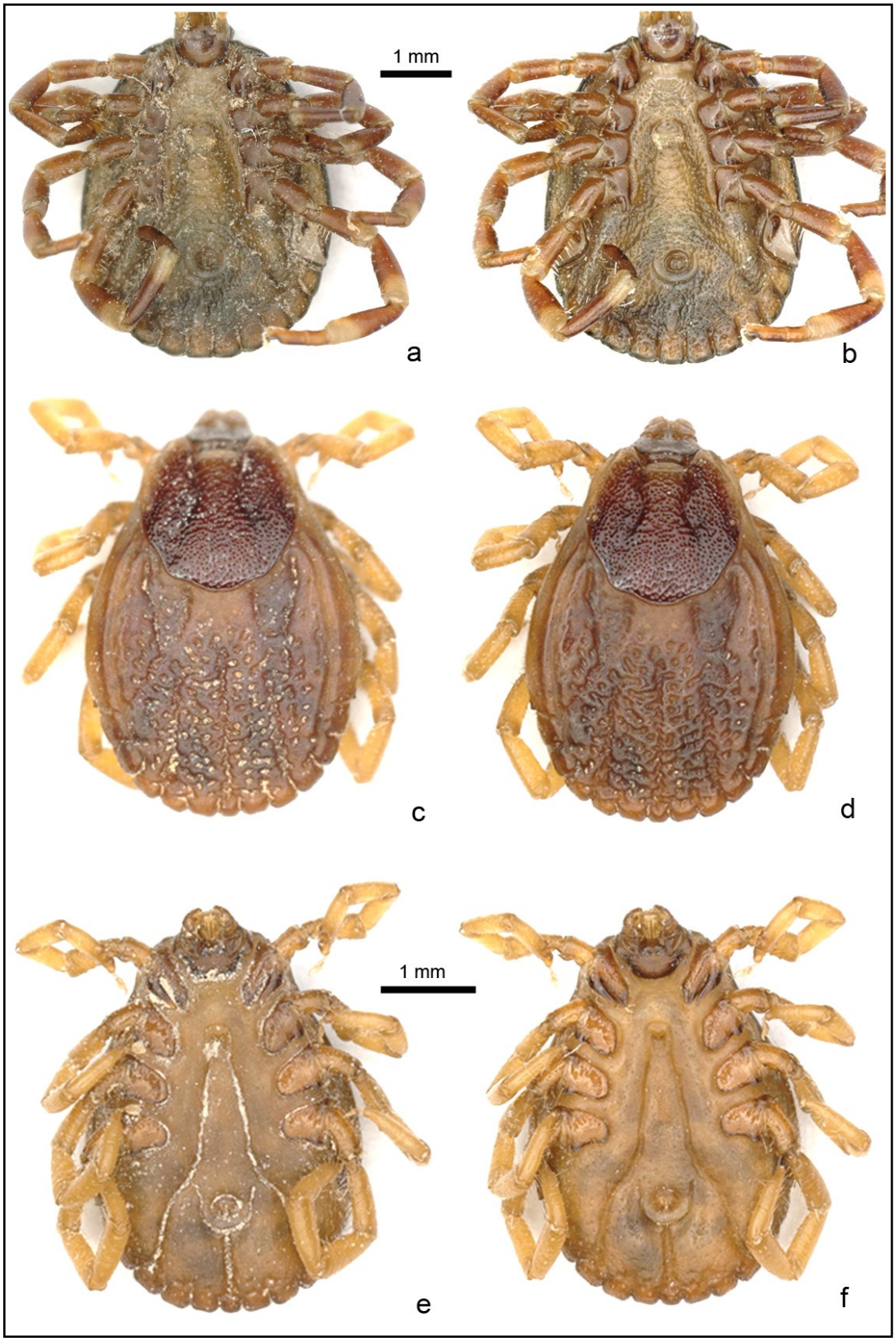

Figure 2. Additional examples of cleaned ticks. ***a–b.*** Amblyomma pomposum *Dönitz, 1909 (ZMB 15922) in ventral view, before (a) and after (b) cleaning.* ***c–d***. Rhipicephalus evertsi evertsi *Neumann, 1897 (ZMB 11454) in dorsal view before (c) and after (d) cleaning.* ***e–f.*** *The same in ventral view before (e) and after (f) cleaning. Images © Lidia Chitima-Dobler, 2019.*

Specimens only have to be removed for the manual cleaning stage with a paintbrush, minimizing risks of them being separated for too long from their original labels and/or being returned to the wrong vial. In other words, they do not necessarily need any new (temporary) labels during the cleaning process. The disadvantages are that the method does need the user to acquire a certain degree of expertise, and patience, to manipulate the ticks during the manual cleaning stage.

We suggest that this ultrasonic method could be applied to clean larger batches of ticks held in natural history collections. Similar methods have also been used to clean spiders and myriapods (Shear & Levi, 1970) and crustaceans (Felgenhauer, 1987). In the latter case ultrasonic cleaning and tumbling in 16% glycerine was used to remove any detritus adhering to the specimen; see also Haug *et al.*, (2011) for its application to gammarid crustaceans prior to imaging by scanning electron microscopy. Several studies have suggested ultrasonic cleaning prior to electron microscopy work on arthropods. Avern (1997) used it, combined with tissue maceration, as a way to clean the internal exoskeleton of arthropods, while Friederich *et al.*, (2014) noted is usefulness for cleaning insects and their (often dirty) mouthparts in particular. One to ten minutes of ultrasonic vibration has also been used to clean the spinnerets of spiders prior to electron microscopy (e.g. Coddington, 1989), and for ten minutes to clean genital preparations of spiders prior to drawing them (e.g. Haddad, 2007). Ticks have a relatively robust and compact body. Harrison (2012) used a similar ultrasonic technique to clean historical specimens of beetles – again typically quite robust arthropods – although it should be added that dry, pinned specimens would have to be rehydrated prior to cleaning. Harrison (2012) also noted that in order to prevent damage to the specimen the ultrasonic equipment should not be too vigorous and we also used a gentle setting here.

Despite this, we should note that in one case (Figures 1b, d) a hind leg did become detached from the body. This happened to the historically oldest specimen we tested: originally a pinned preparation which at some stage was transferred to alcohol. Figure 1c (box) reveals that the weakness in the leg joint was probably already present when the specimen was dry, thus caution may be needed when using these approaches on ticks originating from pinned collections. Essentially, the question is balancing the risks of limb disarticulation against the very obvious improvements (Figure 1d) in the quality of data which can be obtained from the cleaned body. Both Friederich *et al.*, (2014) and Schneeberg *et al.*, (2017) demonstrated that ultrasonic cleaning was not suitable for fragile or delicate insects (especially larvae) and recommended bathing them in potassium hydroxide instead. This alternative method may be appropriate for fragile tick material, and perhaps for other arachnid specimens too.

In a wider context, ultrasonic cleaning has been proposed as a conservation method in various branches of museology; for critical reviews see especially Caldararo (1994; 2005). Fossils can also be cleaned using ultrasonics (reviewed by Pojeta & Balanc, 1989), especially microfossils (Van Bael *et al.*, 2016) or subfossils in sediment cores (Nowak *et al.*, 2008), although here the risks of specimen damage again have to be balanced against the cleaning effect. In another case study, Rull *et al.*, (2016) cautioned that ultrasonics may damage mollusc shells. Still essentially related to natural history (i.e. organic) objects, Barton & Weick (1986) used ultrasonics to clean ethnographic featherwork and Cooke (1989) showed that these approaches were applicable to textile conservation too. Several studies also suggested that inorganic objects (clay tablets, metals) can be cleaned with ultrasonics (e.g. Spier, 1961; Lewis, 1981; Melniciuc Puică, 2005), sometimes in combination with chemical cleaning solutions.

Acknowledgements

We thank Anja Friederichs (Berlin) for curatorial assistance and the reviewers for helpful comments on the typescript and suggestions of additional references.

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