



## Biodiversi-TEA—A quick and easy handling method for arthropod trap material in ethanol

TANJA SCHWEIZER<sup>1,3</sup>, DOMINIC WANKE<sup>1,2</sup>, MICHAEL HAAS<sup>1,2</sup> & LARS KROGMANN<sup>1,2</sup>

<sup>1</sup>Department of Entomology, State Museum of Natural History Stuttgart, Rosenstein 1, D-70191 Stuttgart, Germany.

<sup>2</sup>Institute of Zoology, University of Hohenheim, Schloss Hohenheim 1, D-70599 Stuttgart, Germany.

<sup>3</sup>Corresponding author. E-mail: [tanja.schweizer@smns-bw.de](mailto:tanja.schweizer@smns-bw.de)

### Abstract

A quick and easy handling method for ethanol-preserved arthropods is presented, based on tea filter bags and holders. The method is especially suitable for short term storage of specimens resulting from Malaise traps, yellow pan traps and pitfall traps and can be used directly in the field, for subsequent transport and processing of specimens in the laboratory.

**Key words:** Malaise traps, yellow pan traps, pitfall traps, ethanol samples, biodiversity surveys, arthropods, insects, tea filter

### Introduction

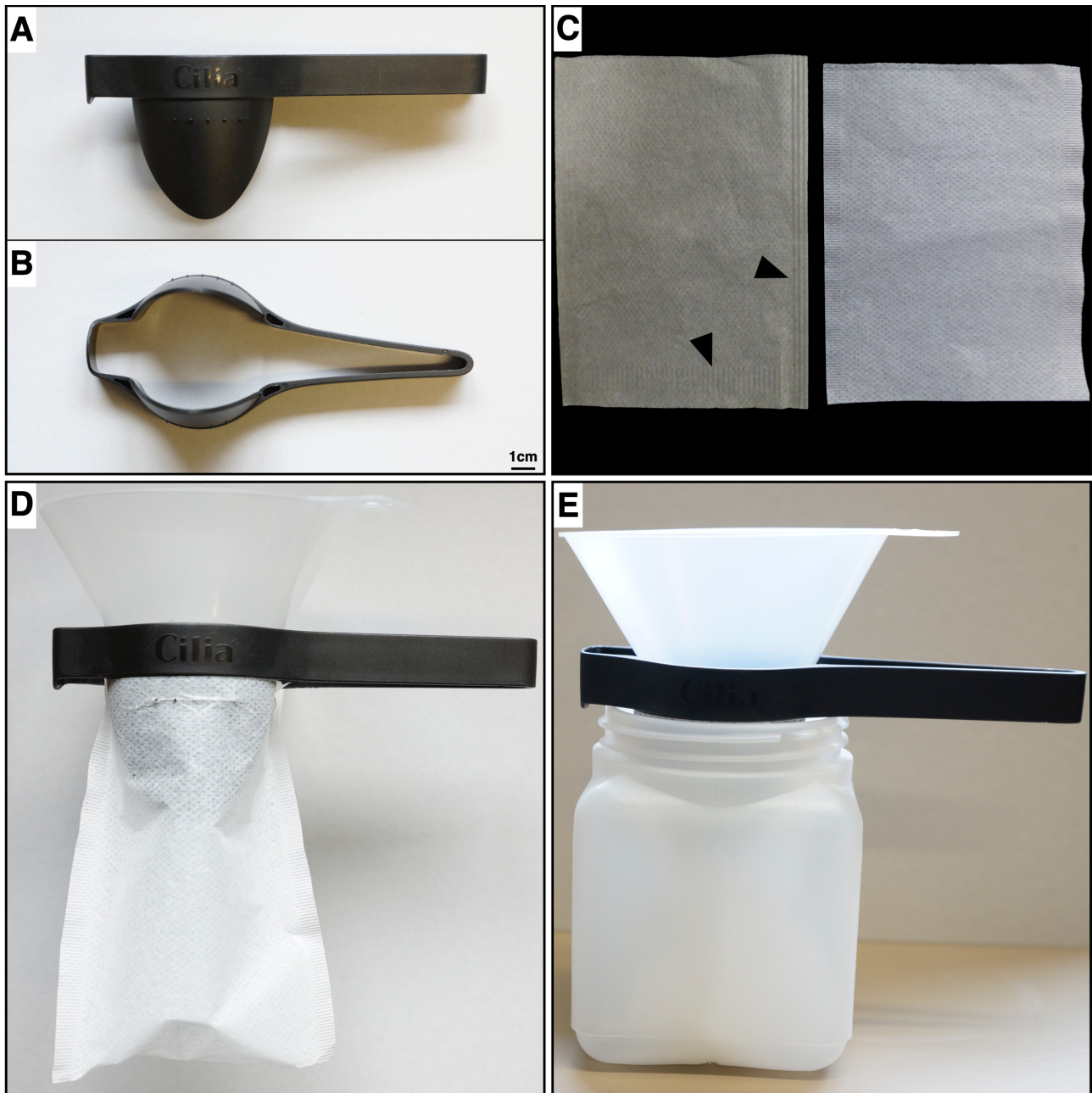
Malaise traps, pitfall traps and yellow pan traps are essential for collecting arthropods for taxonomic and ecological research (Martin 1977; Noyes 1989; Shweta & Rajmohana 2016; Daniel & Ramaraju 2017). Furthermore, global insect decline and the related biodiversity crisis led to a renewed interest in using these traps in a standardized way for biodiversity surveys (e.g., Hallmann *et al.* 2017; Ssymank *et al.* 2018). Trapping and collection methods require large quantities of liquids for preservation and a challenge is to reduce the sample volumes without losing or damaging specimens. Additionally, the transport of ethanol, the most commonly used preservation fluid, is restricted during flights.

Here, we propose Biodiversi-TEA as a quick and easy method for handling and processing of arthropod specimens from traps based on tea filter bags and holders. This method allows safe storage of specimens and easy removal of ethanol or other preservation fluids. The method is especially suitable for short term storage of specimens resulting from Malaise traps, yellow pan traps and pitfall traps and can be used directly in the field, for subsequent transport and processing of specimens in the laboratory.

### Material

Tea filter holder ‘Cilia’ (Melitta), tea filter bags size M ‘Cilia’ (Melitta), funnel with wide opening (Fig. 1). Squeeze bottle, SteriBags sample bags (WHIRL-PAK® bag U.S.A.), plastic boxes (we suggest Lock&Lock food containers, properties: -20°C – +120°C, silicon seal, safety lock, airtight, watertight, stackable, dishwasher-safe, microwaveable, BPA-free, different sizes available: here we used HPL 834).

**Note on the availability of the material.** All material can be easily purchased throughout Europe. Outside of Europe, the necessary tea filters are sold under the Melitta brand but the ‘Cilia’ tea filter holders are often not sold directly. However, through European online shops all products are obtainable and can be sent to any country outside of Europe.



**FIGURE 1.** Material for Biodiversi-TEA. A–B. Tea filter holder in lateral (A) and ventral (B) view. C. Tea filter bags with different properties (on the left with an additional lengthwise running seam, marked with arrows). D. Overview of the assembled tea filter bag, holder and funnel. E. Setup for filtration of trap samples to remove ethanol.

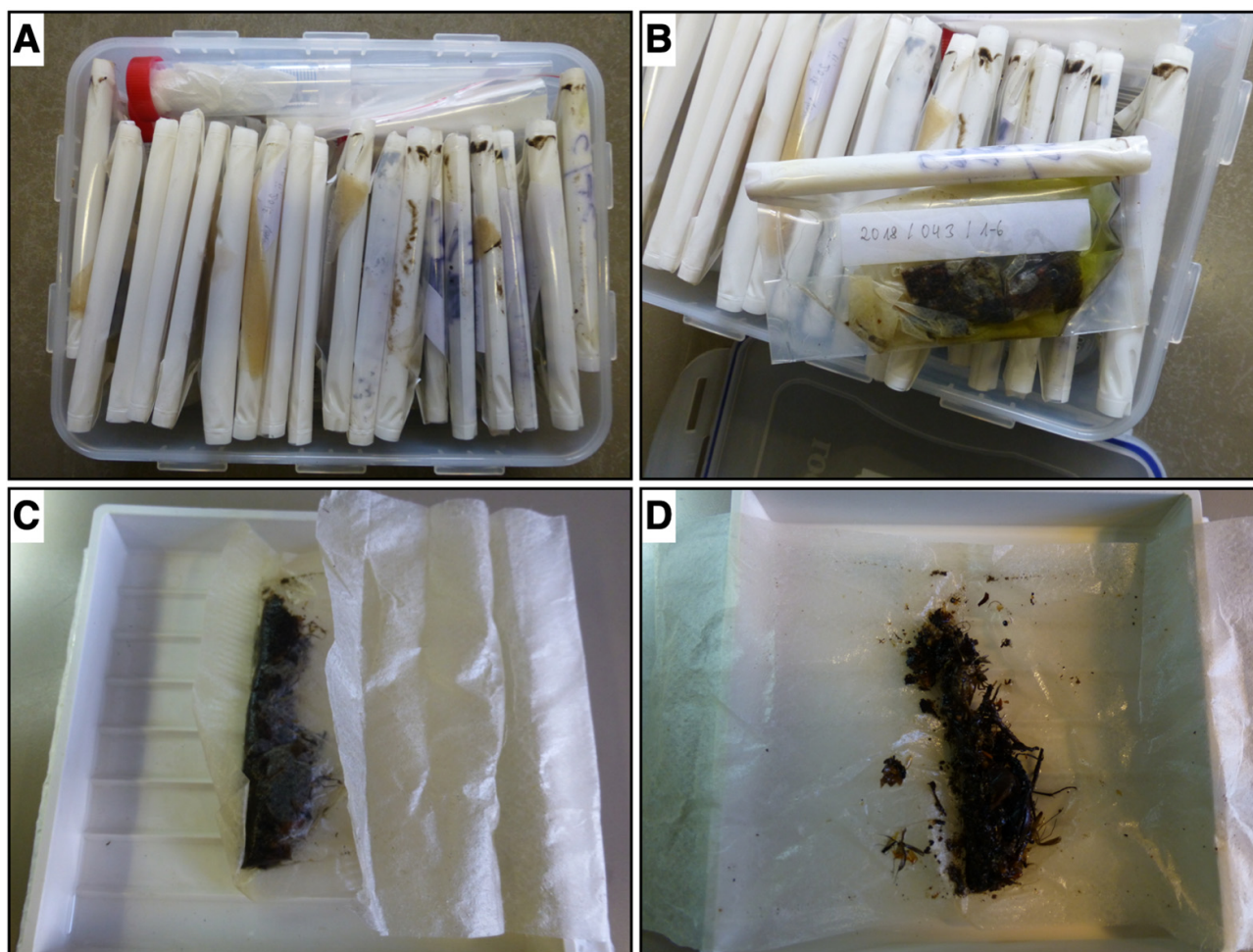
## Methods

**Specimen handling during field trips and for subsequent transport.** For the filtration of ethanol samples during field trips, the entire sample is poured into a tea filter bag fixed to the tea filter holder (Fig. 1). The filter bag is closed by rolling or folding, depending on sample size, and transferred as a whole into a SteriBag for safe storage and transport (Fig. 2). The bag is immediately filled with ethanol to cover the sample, thereby guaranteeing its preservation. A collection label is put inside each SteriBag. The label cannot cause damage to the specimens which are located inside the soft tea filter bag. For safe storage and transport, the bags are densely packed into plastic boxes (Fig. 2).

Before flights, alcohol can be safely removed from each bag. Residual quantities of ethanol will not evaporate in the SteriBag, ensuring a lasting preservation of samples. On field trips where ethanol is a rare commodity, the suggested method can also be used to filter the ethanol from samples even from the smallest impurities such as lepidopteran scales, allowing it to be re-used. Therefore, when emptying Malaise traps the whole sample can be poured through the tea filter and the alcohol can be collected in a bottle (Fig. 1E).

**Processing of specimens.** Many research institutions are faced with the challenge of sorting thousands of arthropods collected in ethanol as part of biodiversity surveys. This is especially difficult for extremely small and abundant arthropods such as Microhymenoptera. Buffington and Gates (2008) suggested a simple method for the mining of tiny arthropods, by dividing the samples into two fractions using the Fractionator. After this process the small fraction, the ‘Black Gold’, has to be poured into a sieve, but subsequent transfer into storage containers is difficult, especially when considering the extremely small size of some parasitoid wasps (down to 0.2 mm in size). Biodiversi-TEA is a useful complement to the Fractionator, as it allows to pour the small fraction directly into a tea filter bag, which can then be placed directly in the storage container. This avoids an additional transfer step, preventing further loss or damage of specimens (Fig. 2C–D).

**Notes on different tea filter bag models.** Tea filter bags of different brands or sizes show different properties, which must also be considered when using this method. For large samples such as Malaise trap samples, tea filter bags with an additional seam and with longitudinal seams at the bottom are recommended (Fig. 1C), as they also facilitate easy opening in the lab (Fig. 2D). Tea filter bags without this additional seam are more difficult to pull apart, but filter the ethanol more rapidly, making them more suitable when the content of a large numbers of traps, e.g., in the case of yellow pan traps, is poured into the filter bags. However, if blocking occurs, filter bags can easily be exchanged and multiple bags can be stored in the same SteriBag.



**FIGURE 2.** Overview of storage, transport and treatment of samples. A. Safely stored samples in plastic box. B. Example of labeled sample from yellow pan traps. C. Sample in tea filter bag placed in sorting tray for further investigation. D. Sample is safely opened by carefully tearing the filter bag apart.

## Discussion

Many possibilities and methods for handling wet samples in the field and laboratory are available (Martin 1977; Krogmann & Holstein 2010). However, their filtration with the aid of sieves or hand nets often represents a challenge due to the amount of liquid needed for removal of specimens which often adhere to the mesh of the nets. This treatment is not only inefficient and time-consuming, but also exposes delicate specimens to unnecessary damage or loss. For transport in the field, samples have often been placed in tubes filled with tissues or cotton wool to prevent damage from movement, but entanglement with this material may again threaten the samples. Biodiversi-TEA enables the safe, easy and quick handling and temporary storage of samples, which can later be separated from the filter bags in the lab. Quantities of ethanol that can be transported during flights are strictly regulated (Pokluda *et al.* 2014). Our method allows one to easily discard ethanol from the samples and the SteriBags can be easily stored in stable plastic boxes to avoid damage (Fig. 2A–B).

The proposed method is particularly suitable for use in surveys based on yellow pan trapping. It functions in the same way as a brine shrimp net, which is for example used by many members of the Hymenoptera community (see Buffington & Gates 2008). However, Biodiversi-TEA is safer and much more efficient for the specimens, as the insect samples do not need to be washed from the inverted net into the final storage jar and can remain within their bag until reaching the lab. This also makes processing of smaller subsets of yellow pan traps much easier, allowing the study of site-specificity and a better assessment of microhabitat attributes.

## Acknowledgements

We thank Sonia Bigalk and Marina Moser (both SMNS) for their help in testing the method in the field. Daniel Whitmore (SMNS) provided helpful comments that greatly improved an earlier version of the manuscript. Steffen Buch (Melitta Group, Minden, Germany) provided helpful information on the global availability of Melitta products. We are thankful to Gary A. P. Gibson (Canadian National Collection of Insects, Arachnids and Nematodes) and Elijah Talamas (Florida State Collection of Arthropods) for constructive comments to the submitted version of this paper.

## References

- Buffington, M.L. & Gates, M.W. (2008) The Fractionator: a simple tool for mining 'Black Gold'. *Skaphion*, 2 (24), 1–4.
- Daniel, J.A. & Ramaraju, K. (2017) A study of three methods of sampling Chalcididae and Pteromalidae in major rice ecosystems of Tamil Nadu. *Journal of Experimental Zoology India*, 20 (2), 1037–1041.
- Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hören, T., Goulson, D. & de Kroon H. (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLOS ONE*, 12 (10), e0185809.  
<https://doi.org/10.1371/journal.pone.0185809>
- Krogmann, L. & Holstein, J. (2010) Chapter 18—Preserving and specimen handling: Insects and other Invertebrates. *In: Eymann, J., Degreef, J., Häuser, C., Monje, J.C., Samyn, Y. & VandenSpiegel, D. (Eds.), Manual on field recording techniques and field protocols for All Taxa Biodiversity Inventories + Monitoring (ATBI+M). AbcTaxa*, 7, 463–481.
- Martin, J.E.H. (1977) *Collecting, preparing and preserving insects, mites, and spiders. The Insects and Arachnids of Canada. Part 1.* Agriculture Canada, Ottawa, 182 pp.
- Noyes, J.S. (1989) A study of five methods of sampling Hymenoptera (Insecta) in a tropical rainforest, with special reference to the Parasitica. *Journal of Natural History*, 23, 285–298.  
<https://doi.org/10.1080/00222938900770181>
- Pokluda, P., Čížek, L., Stříbrná, E., Drag, L., Lukeš, J. & Novotný, V. (2014) A goodbye letter to alcohol: an alternative method for field preservation of arthropod specimens and DNA suitable for mass collecting methods. *European Journal of Entomology*, 111, 175–179.  
<https://doi.org/10.14411/eje.2014.024>
- Shweta, M. & Rajmohana, K. (2016) A comparison of efficiencies of sweep net, yellow pan trap and malaise trap in sampling Platygasteridae (Hymenoptera: Insecta). *Journal of Experimental Zoology India*, 19, 393–396.
- Ssymank, A., Sorg, M., Doczkal, D., Rulik, B., Merkel-Wallner, G. & Vischer-Leopold, M. (2018) Praktische Hinweise und Empfehlungen zur Anwendung von Malaisefallen für Insekten in der Biodiversitätserfassung und im Monitoring. *Series Naturalis*, 1, 1–12.