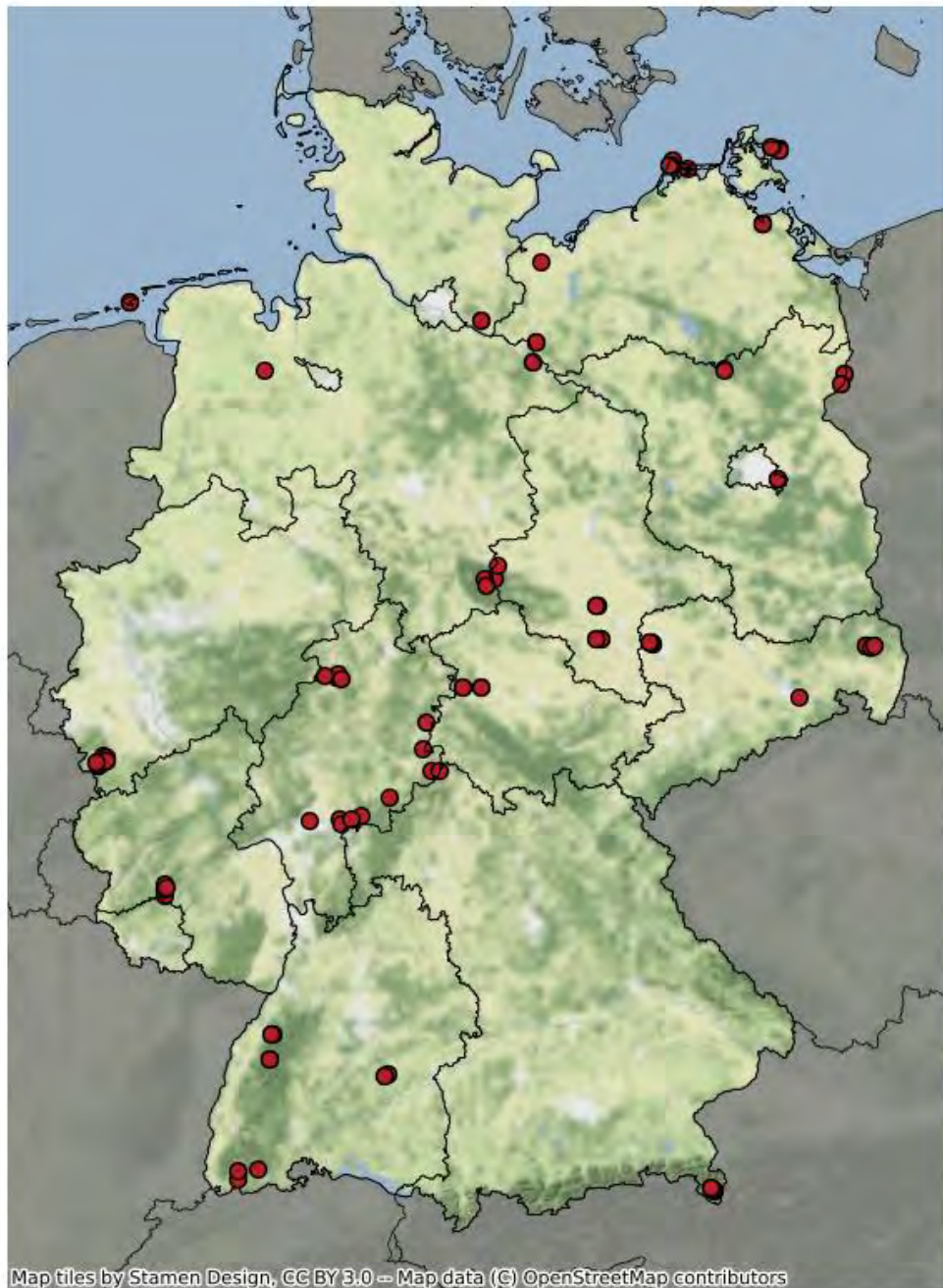


# Bundesweites Insektenmonitoring mittels DNA-Metabarcoding: Übersicht zur Artenvielfalt und deren Plausibilität

LTER-D-Tagung, 06.03.2023

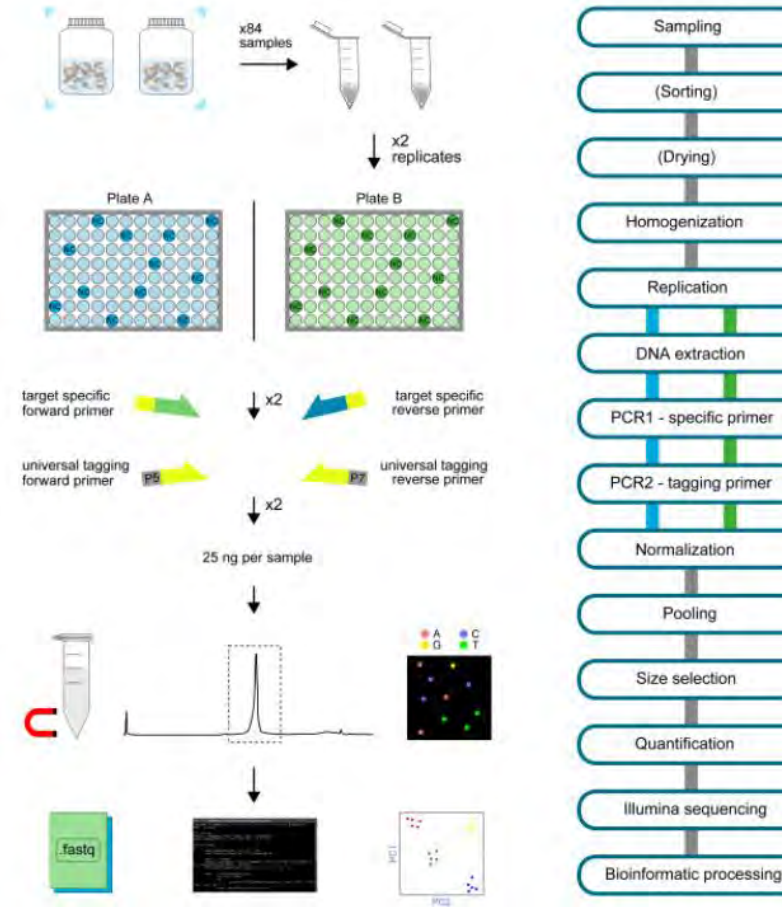
Dominik Buchner, Florian Leese, Peter Haase, Yuanheng Li, Steffen Pauls, Thomas Hörren, UDE/EVK-  
Mitarbeitende, LTER-D-Teams



## Übersicht Auswertung LTER-D Malaisefallenprojekt 2019/2020

- 31 Gebiete
- 130 Fallen
- 1,815 Proben

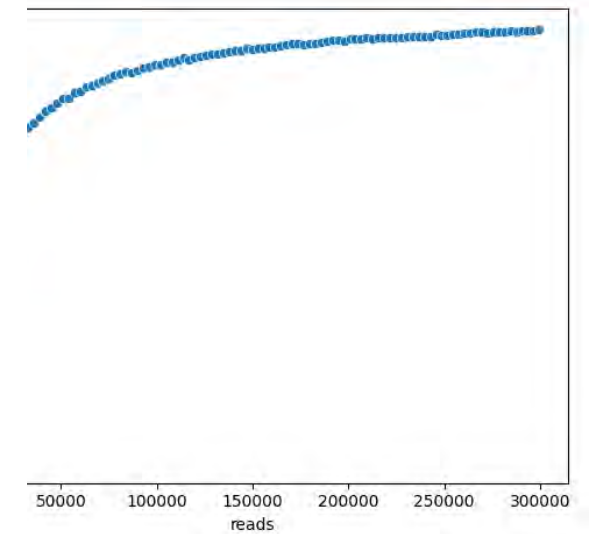
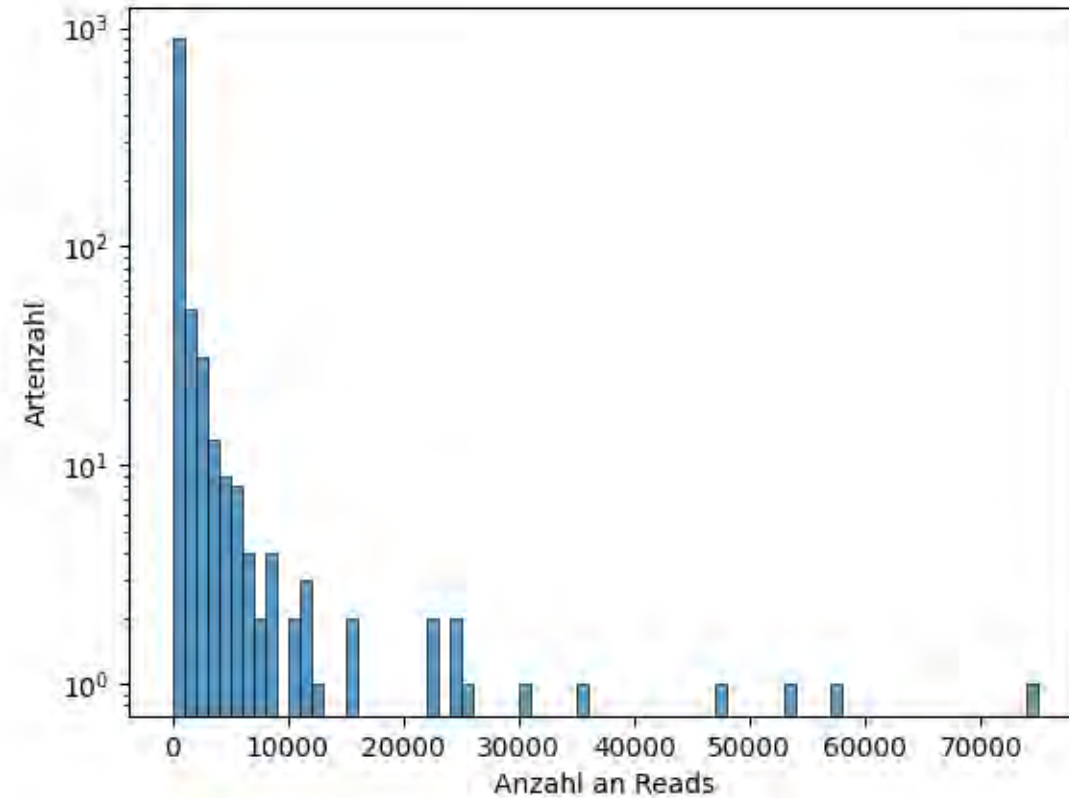
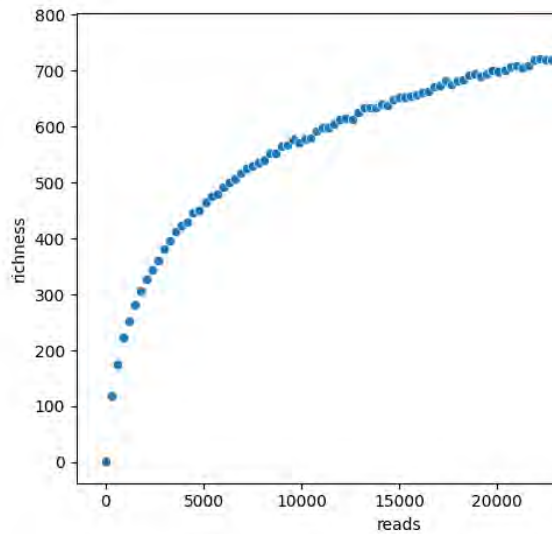
# Laborprozessierung



Buchner et al. 2021

- 2 unabhängige Replikate
- Negativkontrollen
- Dual-Twin Indexing

# Sequenziertiefe



# Bioinformatik und taxonomische Zuordnung

- OTU muss in 2 Replikaten enthalten sein
- Pot. numts werden entfernt
- Taxonomische Zuordnung über BOLD Datenbank

## BOLDigger



downloads 84k

Python program to query .fasta files against the different databases of [www.boldsystems.org](http://www.boldsystems.org)

Buchner et al. 2020

# Auswahl des Top-Hits

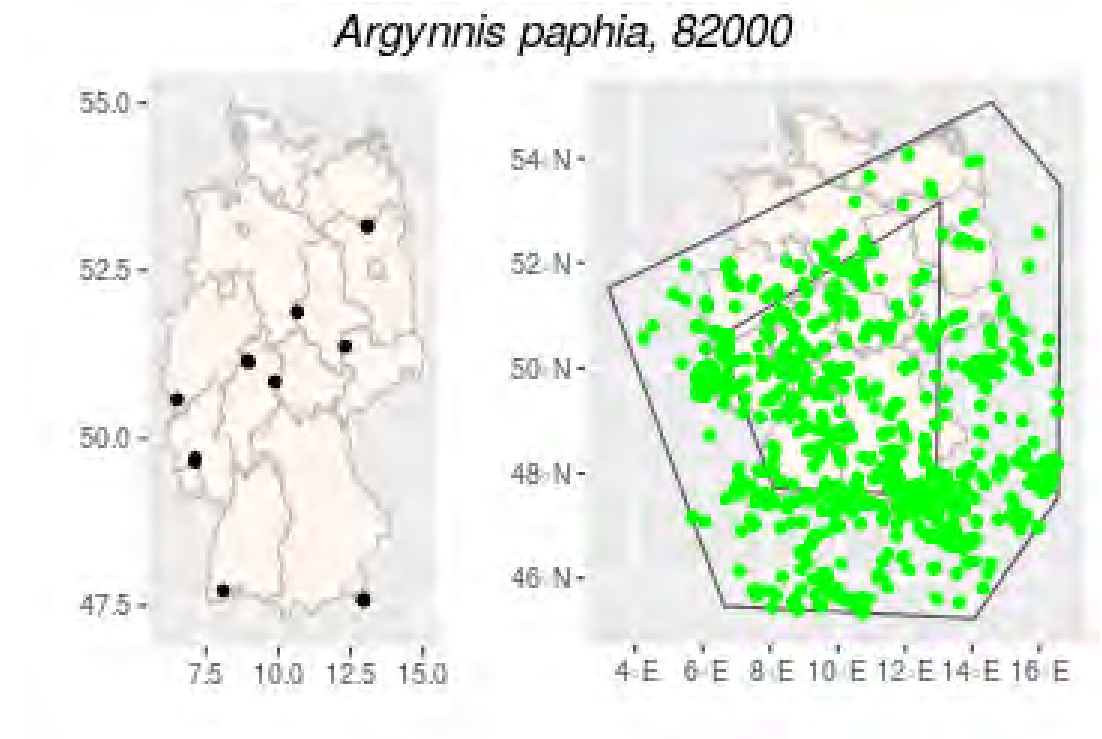
## Top 20 Matches

 Display option: 

| Phylum     | Class   | Order     | Family       | Genus           | Species          | Subspecies | Similarity (%) | Status                      |
|------------|---------|-----------|--------------|-----------------|------------------|------------|----------------|-----------------------------|
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Private                     |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Private                     |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Private                     |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Published <a href="#">↗</a> |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Published <a href="#">↗</a> |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Published <a href="#">↗</a> |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Published <a href="#">↗</a> |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>calcarata</i> |            | 100            | Private                     |
| Arthropoda | Insecta | Hemiptera | Cicadellidae |                 |                  |            | 100            | Private                     |
| Arthropoda | Insecta | Hemiptera | Cicadellidae |                 |                  |            | 100            | Published <a href="#">↗</a> |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>urticae</i>   |            | 100            | Private                     |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>urticae</i>   |            | 100            | Private                     |
| Arthropoda | Insecta | Hemiptera | Cicadellidae | <i>Eupteryx</i> | <i>urticae</i>   |            | 100            | Private                     |

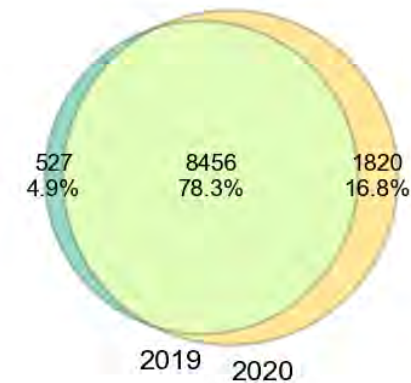
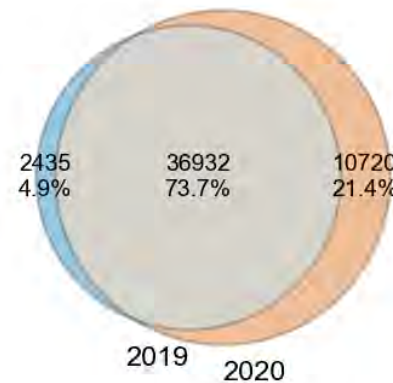
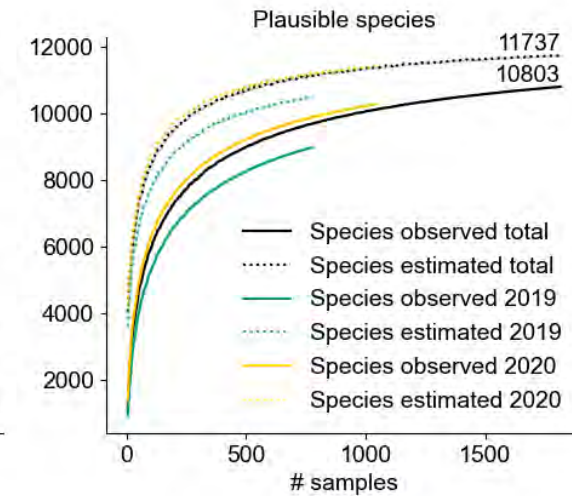
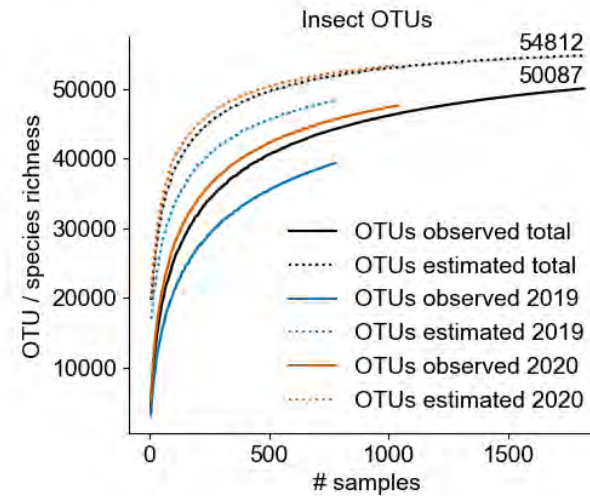
# Ein Versuch der Plausibilisierung

- Insgesamt 11,776 Insekten in 2019 und 2020
- Validiert via GBIF, GBOL und Experteneinschätzung
- 10,803 als plausibel eingeschätzt



# Richness estimation

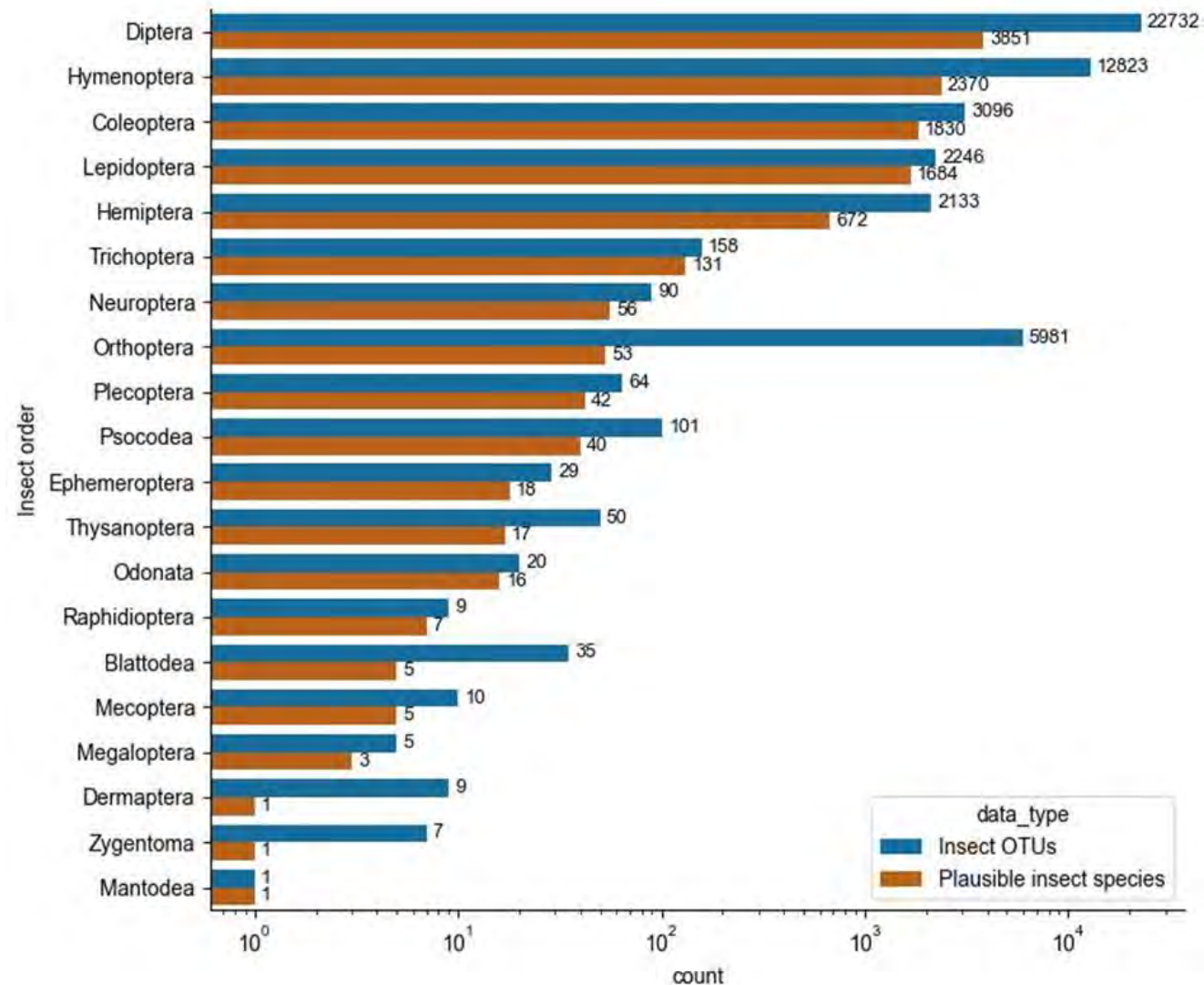
Erwartung:  
~34,000 Insektenarten für  
Deutschland



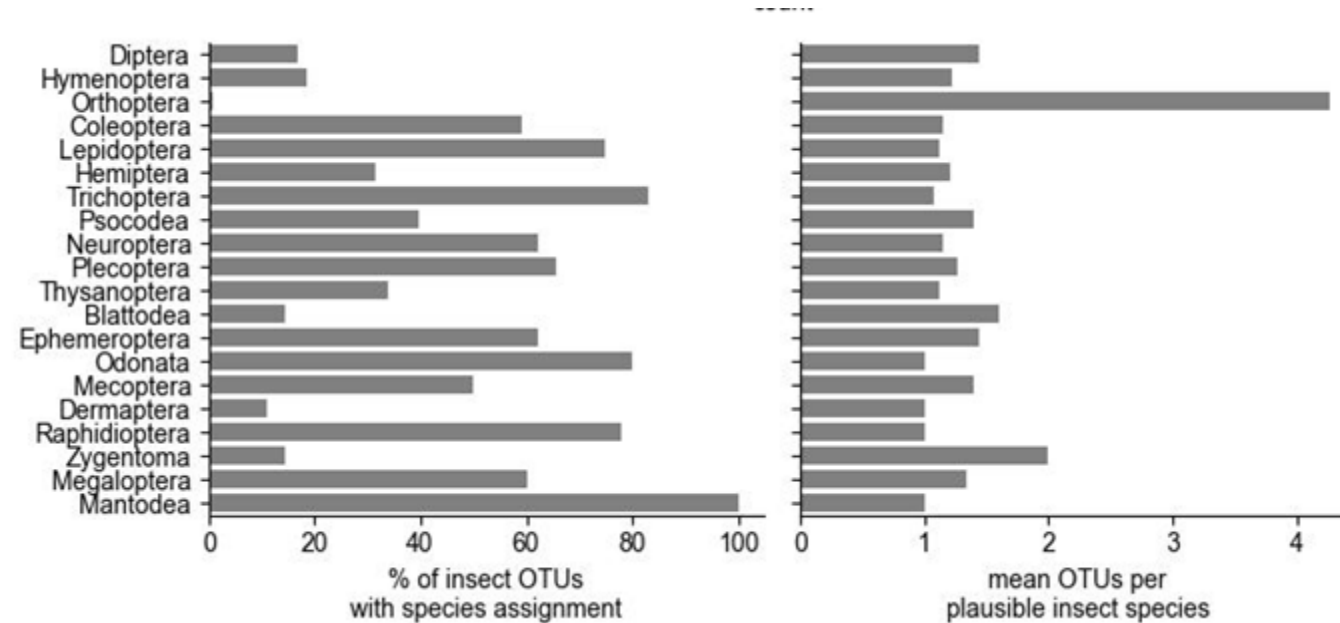
Buchner et al. (in prep)



# Was wir nicht wissen...



# OTUs als gute Abschätzung



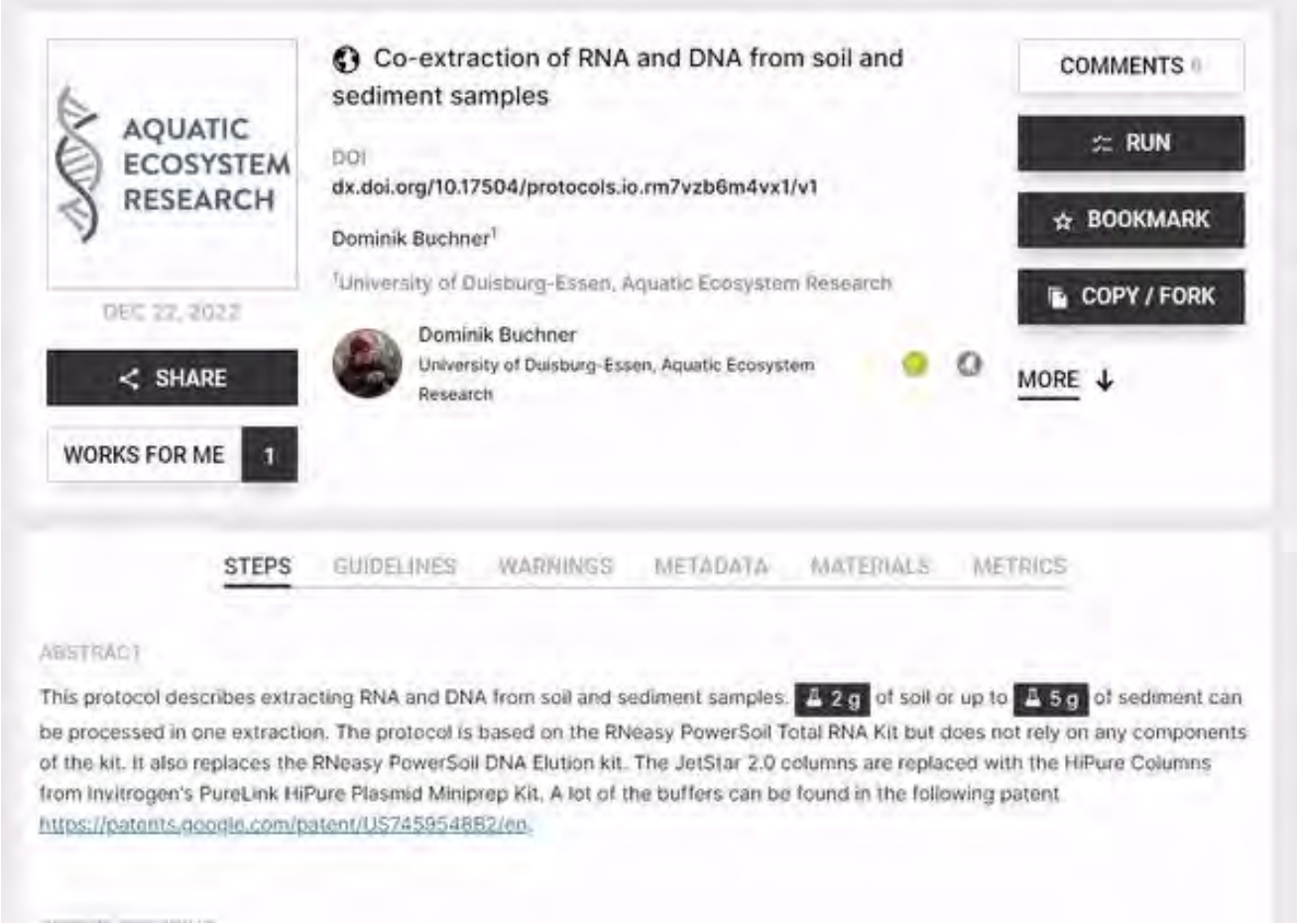
# „Megabarcoding“



# Metabarcoding – Kosten

| Processing step        | Samples (including replicates and negative controls) | Costs per sample [€] | Time per sample [h:min:s] | Total costs [€] | Total time [h:min:s] |
|------------------------|--|----------------------|---------------------------|-----------------|----------------------|
| Size-sorting           | 1,815  | -                    | 0:15:00                   | -               | 453:45:00            |
| Homogenization         | 4,149  | 0.09                 | 0:05:00                   | 379.90          | 345:45:00            |
| Sample lysis           | 4,149  | 0.67                 | 0:00:50                   | 2795.64         | 57:37:30             |
| DNA extraction         | 4,149  | 0.44                 | 0:00:48                   | 1845.55         | 55:00:00             |
| QA DNA extraction      | 4,149  | 0.02                 | 0:00:19                   | 78.48           | 22:00:00             |
| 1 <sup>st</sup> PCR    | 4,149  | 0.72                 | 0:00:10                   | 2994.43         | 11:00:00             |
| PCR cleanup            | 4,149  | 0.21                 | 0:00:38                   | 898.46          | 44:00:00             |
| 2 <sup>nd</sup> PCR    | 4,149  | 0.72                 | 0:00:10                   | 2994.43         | 11:00:00             |
| Normalization          | 4,149  | 0.20                 | 0:00:38                   | 844.47          | 44:00:00             |
| Pooling                | 4,419  | >0.01                | 0:00:02                   | 0.45            | 2:00:00              |
| QA libraries           | 4  | 2.71                 | 0:00:02                   | 10.86           | 2:00:00              |
| Sequencing             | 4 x 2  | 2.61                 | -                         | 11.520          | -                    |
| Bioinformatic analysis | -  | -                    | 0:00:42                   | -               | 48:00:00             |
| <b>Total</b>           | -  | 8.40                 | 24:19                     | 24.362,67       | 1096:07:30           |

# Wie geht es weiter?



The screenshot shows a research protocol page on protocols.io. The title is "Co-extraction of RNA and DNA from soil and sediment samples". The author is Dominik Buchner from the University of Duisburg-Essen, Aquatic Ecosystem Research. The DOI is dx.doi.org/10.17504/protocols.io.rm7vzb6m4vx1/v1. The page includes a "SHARE" button, a "WORKS FOR ME" button with a count of 1, and a "COMMENTS" section with 0 comments. There are also buttons for "RUN", "BOOKMARK", and "COPY / FORK". The abstract section is visible, starting with "This protocol describes extracting RNA and DNA from soil and sediment samples. 2 g of soil or up to 5 g of sediment can be processed in one extraction. The protocol is based on the RNeasy PowerSoil Total RNA Kit but does not rely on any components of the kit. It also replaces the RNeasy PowerSoil DNA Elution kit. The JetStar 2.0 columns are replaced with the HiPure Columns from Invitrogen's PureLink HiPure Plasmid Miniprep Kit. A lot of the buffers can be found in the following patent: <https://patents.google.com/patent/US7459548B2/en>.

**AQUATIC ECOSYSTEM RESEARCH**

DEC 22, 2022

**← SHARE**

**WORKS FOR ME 1**

**Co-extraction of RNA and DNA from soil and sediment samples**

DOI  
dx.doi.org/10.17504/protocols.io.rm7vzb6m4vx1/v1

Dominik Buchner<sup>1</sup>

<sup>1</sup>University of Duisburg-Essen, Aquatic Ecosystem Research

**Dominik Buchner**  
University of Duisburg-Essen, Aquatic Ecosystem Research

**COMMENTS 0**

**RUN**

**☆ BOOKMARK**

**📄 COPY / FORK**

**MORE ↓**

**STEPS** GUIDELINES WARNINGS METADATA MATERIALS METRICS

**ABSTRACT**

This protocol describes extracting RNA and DNA from soil and sediment samples. **2 g** of soil or up to **5 g** of sediment can be processed in one extraction. The protocol is based on the RNeasy PowerSoil Total RNA Kit but does not rely on any components of the kit. It also replaces the RNeasy PowerSoil DNA Elution kit. The JetStar 2.0 columns are replaced with the HiPure Columns from Invitrogen's PureLink HiPure Plasmid Miniprep Kit. A lot of the buffers can be found in the following patent: <https://patents.google.com/patent/US7459548B2/en>.

# Wie geht es weiter?

- Weitere Automation notwendig (Größensortierung / Homogenisierung)
- Langfristige Einlagerung der Proben → Wiederholung mehrerer Jahre möglich
- Was soll mit den Daten passieren?

**Danke für eure Aufmerksamkeit!  
Noch Fragen?**