

TESTING METABARCODING WITH MORPHOLOGICALLY IDENTIFIED SAMPLES RAISES QUESTIONS ABOUT RELIABILITY

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Abstract

Arthropods of 579 species were collected, morphologically identified and grouped into three identical sets. The sets were assessed by two commercial companies using metabarcoding. The results of metabarcoding were compared with those of the morphological identification. Using metabarcoding, only 26% and 56% of morphologically identified species were correctly recognized by Company A in two independent batches, and 35% by Company B. In addition, there were large differences in the recognition rate of individual orders between the two companies and within sets. A large number of species which were identified using metabarcoding were in fact not present in the samples. In our specific study, we found that metabarcoding exhibited restricted reliability, with possible implications of its applicability.

Keywords: metabarcoding reliability, morphological identification, insect monitoring, biodiversity assessment.

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INTRODUCTION

About 50% of all insect species in Thuringia, Germany, are considered threatened according to the Thuringian Red Lists ('Rote Listen Thüringens', TLUBN 2021). Dealing with the

sixth mass extinction (Barnosky et al. 2011) is undoubtedly one of the greatest actual challenges in nature conservation. Following the extensive discussion on the insect biomass decline since 2017 (Hallmann et al. 2017), it became evident that there is a glaring lack of data on insects from many areas when it comes to area-specific proposals for conservation measures and also to regional trend estimates. A part of this data gap could be reduced by the information collected during monitoring programs such as the nationwide insect monitoring in Germany (Züghart et al. 2020). We currently are also in a “taxonomic crisis” (Agnarsson & Kuntner 2007, Löbl et al. 2023). Agnarsson & Kuntner (2007) object that biologists are unable to provide new species descriptions at an appropriate rate or solve taxonomic problems promptly enough. In addition, there is a permanent lack of specialists able to reasonably quickly and safely process insect material for monitoring programs and distribution assessments. On one hand, these problems have been recognised for a long time and become particularly obvious when global distribution assessments are carried out (Wilson 2000). On the other hand, even in countries such as Germany, which benefits from a long tradition of faunistic and taxonomic documentation and research, it is already very difficult to undertake ambitious monitoring programs or to provide specialists able to process and identify sampled insect material satisfactorily (Bundesamt für Naturschutz 2021). This becomes particularly evident in surveys performed in protected habitats or habitats worthy of protection, when many rare and/or difficult-to-identify taxa are to be processed.

For this reason, new methods are being developed, tested and implemented which aim to offer alternatives to the slower morphology-based identification of individuals or at least support it at a large scale so that an assessment becomes feasible in the first place. One example is automatic species recognition using

camera images and artificial intelligence (Yang et al. 2015). However, this method has the disadvantage that only selected, specific groups of insects can be identified with sufficient precision, i.e. down to the species level. Most groups of insects can only be recognized to a high taxonomic rank from habitus images (Wang et al. 2012). Another disadvantage is that there is usually no voucher specimen left after an automatic identification and thus no possibility of verification or further research. Furthermore, retrospective identification is impossible in the case of potential taxonomic changes. These limitations of images-based methods are well known, described (Wang et al. 2012) and can be quickly and transparently demonstrated by specialists in nearly every respective species group.

Metabarcoding promises to remedy the lack of reliable and prompt insect identification down to the species level (Taberlet et al. 2012). In this process, mixed samples of collected insects are assessed by parallel sequencing of DNA fragments, mostly of the 5' end of the CO1 gene region of the mitochondrial DNA (Oliverio et al. 2018), and subsequently evaluated by bioinformatic comparison with DNA databases. In the present paper, these two separate steps are summarized and referred to together as metabarcoding in order to consider this method from the point of view of practical users (often authorities or planning offices), who require a complete and ready-to-use package of species identification.

Metabarcoding can be used for monitoring of biodiversity in agricultural landscapes of Germany (BMEL 2022). Here, a high degree of reliability and verifiability of species identification is particularly important if the method is applied to governmental monitoring programs, as the resulting data will be used for species protection enforcement, elaboration of nature conservation programs or the designation of protected areas based on species evidence.

The verifiability of individuals of a species,

their nomenclature and records, even many decades after the capture or record of an animal, is an indispensable prerequisite for reliably carrying out many taxonomic and species-based studies. While the applicability of metabarcoding in actual taxonomy is already intensively discussed (Zamani et al. 2022), the discussion of its applicability for monitoring programs is not yet as advanced, partly because systematic reviews and long-term experience are still lacking.

The well-established morphological examination of individuals for complementary species diagnosis or as a supplementary tool in taxonomy (Valentini et al. 2009) is difficult and expensive to apply in monitoring programs due to the high effort involved in handling single specimens.

For an assessment of the reliability of the method, comparisons of sets of morphologically identified specimens with subsequent identification using metabarcoding are necessary.

Most studies used samples obtained in aquatic ecosystems. Erdozain et al. (2019) compared morphologically pre-identified specimens with results of metabarcoding assessment and came to a supportive conclusion, as both methods provide similar lists through morphological identification and metabarcoding. However, this study dealt with higher taxonomic units, which have very little or no relevance in monitoring programs for conservation purposes. In contrast, Schenk et al. (2020) report opposing results when comparing different identification methods for aquatic nematode communities. The species identified by the methods were found to have only limited overlap. Cowart et al. (2015) reached a similar conclusion when comparing morphological identification and metabarcoding of seaweed communities. Meredith et al. (2021) compared the performance of morphological identification and metabarcoding of zooplankton communities. Nevertheless, in this instance, only 21 of the 51 species were identified using

both methods. In a similar study, Serrana et al. (2019) found that 35 of the 45 species of macroinvertebrates in a freshwater ecosystem were identified with both morphological methods and metabarcoding.

There are also initial studies of terrestrial species and reviews that urge caution in interpreting metabarcoding-based species data (Liu et al. 2020). Heine et al. (2021) compared fungal species and concluded that metabarcoding is not yet able to be used interchangeably for morphological analysis. Rimmel et al. (2023), do not find significant overlap between the methods employed (~50% overlap between morphological methods and metabarcoding). However, they appear to regard this as a successful outcome. Studies of whole terrestrial communities, especially of insect communities, apart from sampling selected species are still lacking.

Moreover, the majority of studies employ methodologies within a highly controlled setting, frequently utilising the researchers' own laboratories, often with the possibility to repeat the assessment if errors during handling were introduced. As the application of metabarcoding is to be extended to a much wider range of activities than research alone, including large scale monitoring programmes, it will be necessary to engage the services of commercial companies, which may introduce deviations not present if only a single research laboratory is used in a study (such as different handling of samples or different lab protocols) and no possibility to repeat an assessment, as the specimen are usually only sampled a single time.

Metabarcoding usually works in a destructive way. Insect material can hardly be used afterwards for verification of metabarcoding results, nor for further or additional research based on whole specimens. To homogenize the sample, the entire composite material sample is processed into powder. A small portion of powder is consequently used for the assessment itself. However, unless the entire amount of

powder of the composite sample is used, at least a later repetition of the metabarcoding is possible. When examining mixed samples using metabarcoding, it is therefore particularly important that all species or at least all taxa showing divergent DNA barcode in the sample (but only these, without false positive hits) can be reliably detected.

If metabarcoding is to be used on a large scale, for example, to make overarching statements, the comparability and verification of the results is the main basic prerequisite. The results obtained by metabarcoding must depend solely on the sample examined and the state of the corresponding reference database and no other factors. It is imperative that the result is independent of which laboratory or company performed the assessment. In addition, the fact that official orders for metabarcoding are usually placed with different or alternating companies, but the data are subsequently merged, demands unambiguous comparable results. It is also an open question whether companies will persist in the long term and whether questions of methodology and results can be kept transparent and repeatable beyond the existence of a particular company. For this reason, the results of metabarcoding between different companies must be identical, or at least comparable with only very small qualitative differences.

To compare morphological identification and results of metabarcoding, members of the Thüringer Entomologenverband (TEV 2025) compiled three sets of samples, fully identical in species composition and number of individuals of every species, which were first identified morphologically to species level and then by two different companies using metabarcoding. The following questions were raised and should be answered:

1. What is the proportion of reliably identified species in a composite sample? 2. How high is the proportion of incorrectly recognized species in the metabarcoding evaluation? 3. Are there species groups that are better suited for this

method than others? 4. Can metabarcoding in combination with species assignment using reference databases provide a reliable species identification if not whole bodies are present in the sample? 5. Can the results be adequately interpreted by non-taxonomists, less experienced people, or authorities? 6. Are there differences in the results between different companies? And if so, is it possible to identify (without prior assessment) which of the different companies has the better or best quality results?

MATERIAL AND METHODS

Field samples of insects and two arachnid species from Thuringia were collected using the following sampling techniques: manual (hand) collecting, netting, beating, as well as light traps, sieving soil samples and detritus, and suction traps. The specimens were first killed (see below) and preserved in 96% ethanol. Subsequently, in the laboratory, the preserved specimens were morphologically identified by designated group specialists. As only few people were able to identify the specimen, we could not do a double blind identification test or another verification, to check if all identifications were correct. However, all used species were identified to species level, using voucher specimen for confirmation if necessary. Identification was carried out using specific keys for the respective group or genus, including internal morphological characteristics such as genital preparation. Additionally, only renowned entomologists that routinely identify, describe or revise species of the respective group were tasked with identification.

The selection of species for this study was based on two criteria. First, the aim was to have several individuals of each species available, ensuring that at least one individual of the same taxon is present in each sample. Second, species were chosen to include a mix of both common and rare species. The goal was

to imitate a natural assemblage of a naturally rich, mixed sample in terms of species richness (few common, many less abundant species). We intentionally included both very small species such as *Ptenidium pusillum* (Gyllenhal, 1808) (Coleoptera: Ptiliidae) and comparatively large species such as *Cetonia aurata* (Linnaeus, 1761) (Coleoptera: Scarabaeidae) in the samples.

The specimens were mostly transferred directly to 96% un-denatured ethanol in the field and thereby killed. Individuals of ten selected species were killed by freezing. Individuals of 57 species were killed using ethyl acetate and transferred to 96% ethanol shortly thereafter. Diptera specimens caught in traps were kept in saline solution for a maximum of one week, usually less, before being transferred to 96% ethanol. The specimens were removed from ethanol for morphological identification for

several minutes and returned to ethanol after the identification was done. To minimize chances of morphological misidentifications, the samples were identified only by experienced researchers - experts on the regional fauna of Thuringia. Collections of the local fauna were checked for voucher specimen and compared to the identified specimen, to minimize misidentification. At least one specimen of each species is stored preserved in ethanol in the entomological collection of the Museum of Natural History Erfurt to verify the correctness of identification, if necessary.

In each sample, 821 individuals of 579 species of Araneae, Auchenorrhyncha, Coleoptera, Dermaptera, Diptera, Heteroptera, Hymenoptera, Lepidoptera, Neuroptera, Orthoptera, Psocoptera, Raphidioptera, and Trichoptera were identified morphologically (Fig. 1, Appendix 1).

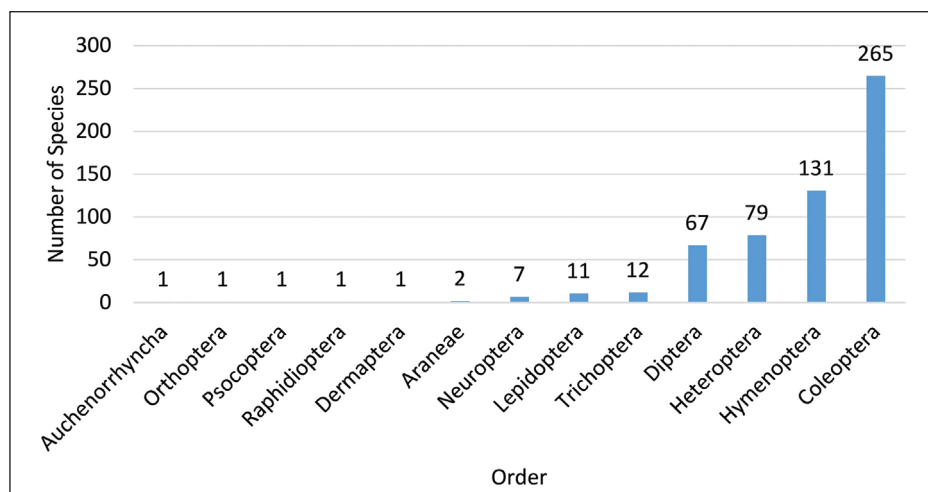


Figure 1. An overview of the composition of mixed samples by major group. See Appendix 1 for a complete list of species.

To address the question of whether only part of an individual is sufficient for identification as well as for practical reasons since there were not enough specimens of uncommon species available, individuals of 106 morphologically

identified species were split into parts and distributed among samples for metabarcoding. For DNA barcoding of arthropods, a leg of a specimen is normally considered enough for genetic assessment (Vaglia 2008). However,

we assumed that a larger minimal amount of tissue was preferable for our study to mitigate a risk of the possible lack or insufficient amount of DNA in the samples. Therefore, when an individual had to be split into three parts for the three samples, portions larger than one leg were detached and used for each sample. For example, for beetles this included thorax, head and pronotum, or abdomen, ensuring that in each case significantly more material than just a single leg is present in each sample.

The individual samples were then collected and merged according to the criteria mentioned above for a total of three sets for three assessments, ensuring that batches identical in terms of specimen numbers and species composition were prepared for further evaluation. These identical batches were then submitted to two commercial companies ('Company A' and 'Company B') for identification using metabarcoding. Both companies are well established, leading companies focused on metabarcoding analysis, routinely managing large samples for universities and government agencies. One company received two identical batches with a time gap of four months. Both batches of this company were handled in an identical way. This was done to evaluate the repeatability of the same experiment performed by the same company and the same lab protocol. Processing was carried out by the companies A and B according to their normal guidelines (Appendix 2). Further evaluation in this paper is based on the result tables provided by the companies to the authors.

For species detection, the companies conducted a comparison with in total three gene databases/methods: BOLD, NCBI Genbank, RDP (Porter & Hajibabaei 2018). Company B indicated a match in species identification in all three gene databases as a confident species identification, whereas Company A listed the species if there was a match in the BOLD database, using a 95% similarity threshold as criterion.

To evaluate whether the incompleteness of

public DNA databases contributed to poor identification of species, we manually cross-checked the species names of the species in our samples against entries in the sequence databases used by the companies to determine whether corresponding reference sequences were theoretically available. In cases where sequences were assigned to species for which DNA data was not released publicly, this process was not possible due to restricted access to these proprietary data.

In our case, after requests, only one of the two companies sent us the actual sequences, potentially allowing for a repeated assessment in the future.

RESULTS

In total, 371 of the 579 species (64%) were correctly detected by at least one of the two companies. Company A, which handled two batches, had an overall detection rate of 56% and 26% per batch, while Company B had a detection rate of 35% (Fig. 2). Depending on the taxonomic group considered, there were significant differences in the detection rate both among taxonomic orders and between companies. E.g., while Company A detected 82% of all Heteroptera species in one batch, Company B only detected 44% of Heteroptera species. The difference is even greater in Lepidoptera (73% versus 4% Company A).

Some taxonomic groups had a better detection rate, while others, such as aforementioned Lepidoptera, had a poor rate of identification. In nearly all cases and for unknown reason, the second batch processed by Company A had lower detection rate than the first batch (56% vs. 26% overall detection), indicating that errors of detection are not only related to use of different protocols, since both batches provided to Company A were handled identically. The source of this substantial variation remains unclear (number of reads per sample, sequence quality metrics).

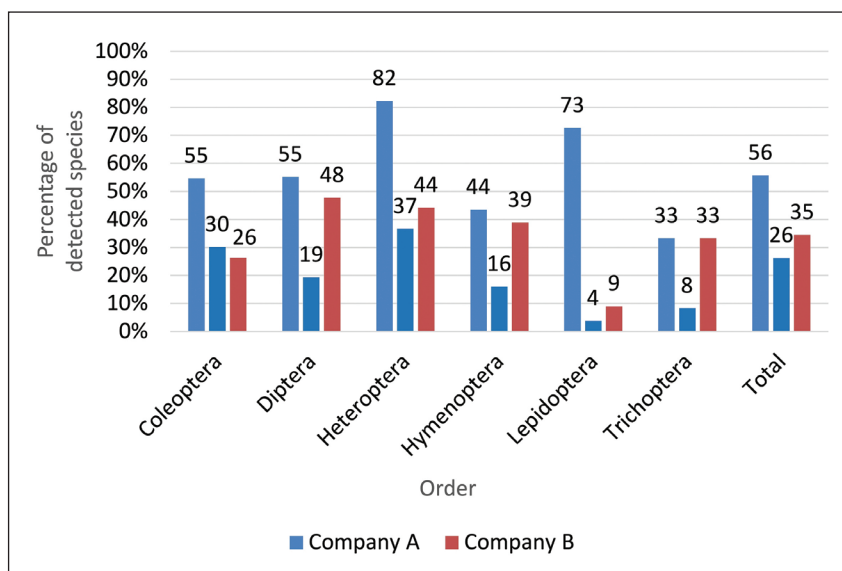


Figure 2. Percentage of detected species per batch, major groups and overall (only taxa with >12 species in one of the resulting tables are represented). For Company A: a species was considered detected if it had $\geq 95\%$ sequence similarity to an entry in the BOLD database. For Company B: the company provided matches for all three reference databases (BOLD, NCBI GenBank, RDP).

To enable comparison with Company A's stricter identification criteria, a species was considered detected in our analysis if it showed $\geq 97\%$ similarity to at least one of the three databases (concerning the criterion whether a species was detected at all), rather than requiring simultaneous matches across all three databases as the company's standard protocol specified.

This more relaxed criterion for Company B allowed us to assess detection rates on a comparable basis with Company A's results in the first place.

In groups that were represented in the batches more abundantly and with higher species number, such as Coleoptera, the differences between the companies have a particularly strong impact on the overall detection rate between companies. Even in these species-rich groups, which have a good faunistic and relatively good sequence data background, species detection rates are poor (Fig. 2).

Despite Company A adopting a less restrictive approach to species identification compared to Company B, only a little over half (56%) of the species were detected in the "best" batch, if we were to follow the guidelines of species detection of the company.

The average detection rate of species represented only by body parts of individuals is approximately 20% lower compared to complete individuals: In total, 111 species were introduced as individual parts into the batches. Of these, 50 species were detected by at least one of the two companies (45%). With at least one complete individual, 468 species were present in the batches, of which 321 species were detected by at least one of the two companies (69%).

In addition to the incomplete recognition of morphologically identified species in the mixed batch, both companies detected a high proportion of additional species that were not present in the sample by complete

or incomplete individuals, or at least not intentionally introduced by our team (Fig. 3). “False positive” identifications constitute a significant portion of the companies’ result tables. At least 20 species detected through metabarcoding have very little chance of being found in Europe. This is due to their geographic ranges being on other continents, their absence from European records, and the fact

that they are not known as invasive species. Additionally, some species have incorrect taxonomic assignments in the lists provided by the companies, such as non-valid or outdated species names. Furthermore, for some of these species, their ecological requirements (e.g., habitat preferences or climatic conditions) are entirely incompatible with the local conditions in Thuringia (e.g. marine species), making their detection highly implausible.

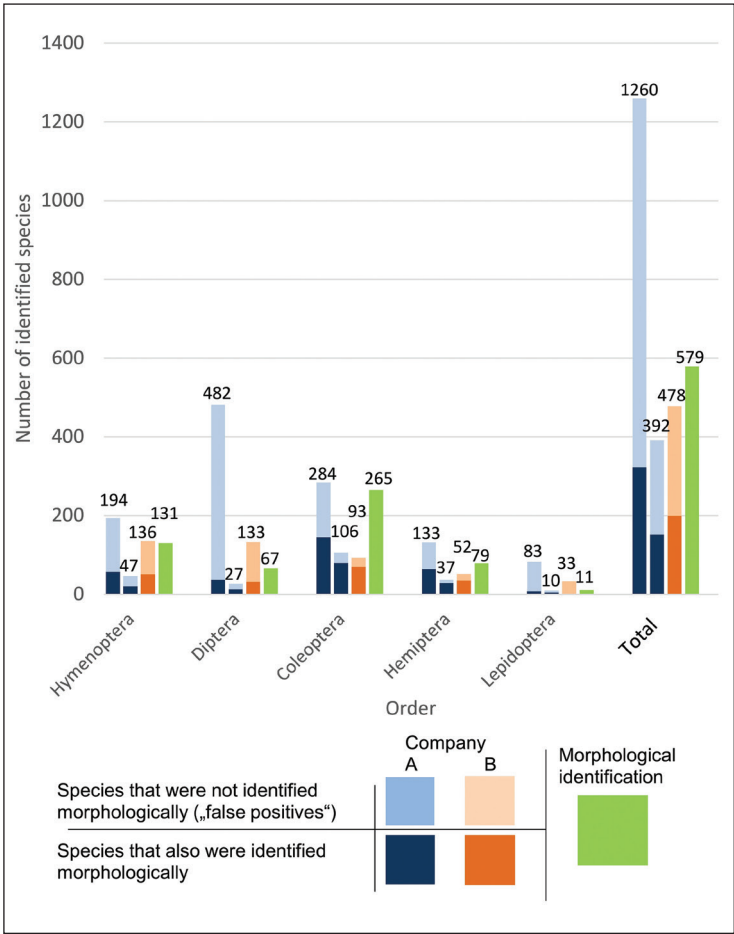


Figure 3. Comparison of identified species by company (metabarcoding) and morphological determination by major groups (depicting taxa with more than 12 species). The number represents the total count of identified species, including suspected false positives.

In total, Company A detected 1260 and 392 species in the two batches, while Company B detected 478 species. The high difference in the number of detected species also results in minimal overlap between the two companies. After comparing the companies' result tables with the results of morphological identification, only 78 of the 579 species were listed in all three result tables, meaning they were recognized correctly by both companies in all the three batches.

Thus, the results of the present study demonstrated that only a small proportion of species were reliably identified using metabarcoding by different companies in a setting, where field sampling and lab work is done by different persons. This kind of setting is a frequent situation in large monitoring programs. However, it should be noted that our number of mixed samples is too small to make a statistically significant statement.

The records of species which have not been reported from Thuringia were verified by the respective species experts and were subjects to plausibility checks. While no claim of completeness is made, several species were detected in the batches whose occurrence in Thuringia is considered highly unlikely. For example, the marine deep-sea polychaete species *Bathyglycinde sibogana* (Augener & Pettibone in Pettibone, 1970) (Goniadidae, Polychaeta) was listed in the result table by Company B. Another species highly unlikely to be found in Thuringia is *Chrysoperla agilis* (Neuroptera: Chrysopidae), which has only been reported from Southern Europe and Switzerland so far. On the other hand, the morphologically identified species *Xyela menelaus* (Hymenoptera: Xyelidae), which has not been previously reported from Thuringia (apart from our mixed batches), was correctly identified by both companies, confirming it as a new species record for the Thuringian fauna. To verify whether the incompleteness of public DNA-databases could be the cause of the inadequate identification rate, the species

presented in our batches were manually checked for their presence in the databases. In total, only seven species present in our batches were not found to be represented in the public databases with at least one reference sequence, indicating a potential good coverage of our species.

DISCUSSION

Overall, the results from both companies as described above raise questions regarding the reliability, replicability, and transparency when applying metabarcoding for species identification, supporting our previously published preliminary results (Förster et al. 2023). While absolute certainty in species identification is not achievable in any case including morphological identification (identification error rate of specialists is usually very low), the available specimens, not just the obtained sequences, can be re-checked any time. Complete individuals were recognized more reliably compared to individuals split into parts. However, it should be noted that the identification rates of partitioned specimen could also be influenced by the overall detectability of each species or chance effects, which might have a bigger influence or larger expression if the sample tissue has a smaller mass. Nevertheless, the use of partitioned specimens for genetic assessments is an established and standardized method for barcoding (Jinbo et al. 2011), so we assume that only a very small portion of the discrepancies can be explained by this factor. Additionally, due to the low number of species that were present in the batches as incomplete individuals, this expected number of unidentified species would be lower than the actual observed number of unidentified species.

To interpret taxonomic issues, such as different genera in the results for the same species and subsequently also in the interpretation of metabarcoding results, expertise, especially in

alpha taxonomy, is still essential for interpreting the results of metabarcoding but also manual identification. In our case, many species were identified or listed as synonyms of the currently accepted taxon or with names that are not used anymore. This important curation can only be performed by taxonomically experienced individuals. Furthermore, subsequent analysis and interpretation of the species assemblage, whether ecological or conservation-related, can still only be conducted by specialists of the relevant group. Thus, the application of metabarcoding requires high expertise in both taxonomy and metabarcoding methodology, limiting its accessibility for use by governmental institutions without specialist evaluation.

It is likely that a small number of species that have not been morphologically identified but were identified through metabarcoding appears correct, for instance, when remnants of other organisms are found in predator stomachs (Paula et al. 2016) or parasitic organisms found in tissues of their hosts. However, in that case the detectability is greatly constrained by decomposition processes in predators' stomachs. This could explain some, but not all, of the additional species, as predators, which were included in our samples, may have prey DNA in their stomachs. The diversity of prey species detectable in predator stomach contents is influenced by several factors, including the predator's diet breadth, prey availability, and digestion rates (Paula et al., 2016; Liu et al., 2020). However, predators often consume only a subset of available prey species, limiting the number of possible additional species. Additionally, digestion processes and DNA degradation can affect prey detection negatively (Kamenova et al., 2018; Sint et al., 2015). Moreover, the dominance of predator DNA in metabarcoding analyses can reduce sequencing depth for prey DNA unless specific measures, such as blocking primers, are applied, which was not the case in our analysis (Paula et al. 2016, Pompanon et al. 2012), thus reducing the

potential impact of prey DNA. In conclusion, we assume that some of the additionally detected species are false positives, while further research regarding stomach content is needed.

In a recent study, Strutzenberger et al. (2023) compared the efficacy of morphological identification of light trap samples with barcoding. Their findings indicate that singleton (thus possibly rare) species are less likely to be detected. In natural communities, most species are rare, with only a few being common. This has implications for the effectiveness of meaningful conservation projects or regulations which often focus on rare species.

Hypothetically, the high false-positive and low actual detection rate of rare species could lead to the incorrect identification of rare species listed in the Red List or protected species and thus even lead to the false creation of protected sites in a real monitoring case.

Although nearly all of our sampled species are present in the databases used, we suspect that the quality of the comparison sequences, especially for rare species, could have a negative impact on the reliability of their identification. In our case for many rare species, there was only a single comparison sequence available. A larger test with a higher number of replicates is needed in order to test this question. From a governmental and monitoring viewpoint, a feasible application of metabarcoding, even with the possibility of false positives, would be for identifying key areas for targeted searches for rare species, where a more thorough assessment might take place later to confirm species. However, it should be noted that there is already a lack of resources and specialists to facilitate targeted actions even with reliable and proven indications of species important to conservation.

Furthermore, flexibility in sample collection during fieldwork is important. A large scale and/or long-term monitoring program should be robust against minor errors. While the method might be able to yield good results

under idealized conditions (Zimmermann et al. 2015), it should also be suitable for various insect groups and be able to handle common problems that usually arise during real-world fieldwork and sample collection. These problems include traps being left exposed for longer than expected, rainwater infiltration and dilution of preservation ethanol or trapping fluid, influence of sunlight/heat, and other issues that have less or no impact on the reliability of morphological identification than potentially on metabarcoding.

The applicability of metabarcoding also depends on the completeness and cleanliness of the gene databases the sequences are compared with. These databases and their completeness can vary from area to area (Ranaweera et al. 2024). Therefore, to identify whether metabarcoding is a suitable method, it is first necessary to assess whether there is sufficient coverage of most taxa in the gene databases for the specific region and taxonomic group (Charrier et al. 2024). In theory, sequencing could be performed at any time, even in the absence of a complete gene database with associated sequences, with the assignment of sequences to taxa taking place at a much later time. However, in the context of conservation-focused monitoring programs, timely data is often necessary to respond promptly to specific issues. In this sense, it is crucial from a practical standpoint to have up-to-date and well-covered species' inventories for the region and group in the databases.

It is also noticeable that some entries in the sequence databases and even in the received result tables are listed with “cf.” [confer, Latin for “compare to”], indicating that the samples used for sequence generation in the underlying database were not confidently identified to the species rank. This could be due to the fact that the identification of the used individuals were not verified before sequencing, or to taxonomic issues that make the identification uncertain in retrospect. In any case, the companies should not have listed these species

in the result tables together with species of high confidence, without clearer labelling (e.g. separate listing), in order to avoid confusion and false results. It's important to note that this issue is not unique to metabarcoding but also exists in morphological identification. The sequence databases are ultimately built on morphological identifications, which are the original source of these “cf.” designations. Taxonomic uncertainties and difficulties in species delimitation persist regardless of the identification method used. This highlights a broader challenge in taxonomy and species identification that affects both traditional morphological approaches and newer molecular methods. The persistence of these issues across different identification techniques underscores the need for continued refinement of taxonomic knowledge and the importance of clear communication about levels of certainty in species identifications, regardless of the identification method employed.

A particularly concerning finding was the substantial variability between two batches processed by Company A using allegedly identical protocols. Detection rates differed by more than a factor of two (56% vs. 26%), with the second batch showing dramatically reduced performance across nearly all taxonomic groups. This variability cannot be attributed to differences in sample composition, as both batches were prepared identically, nor to different laboratory protocols, as the company confirmed using the same procedures. Without access to detailed technical documentation, including sequencing depth metrics, quality thresholds, and bioinformatic parameters, we cannot determine which factors contributed to this dramatic difference. This lack of reproducibility has serious implications for monitoring programs, where consistent methodology is essential for detecting temporal trends or comparing spatial patterns. For regulatory applications requiring defensible data, such variability is unacceptable.

The high variability between Company

A's batches, combined with the absence of raw sequence data from either company, highlights the fundamental lack of transparency and technical documentation in commercial metabarcoding services. Critical methodological details remain ambiguous: DNA extraction procedures, primer sequences, and complete bioinformatic workflows including quality filtering thresholds, error correction methods, and database versions. Standard analytical laboratories establish industry standards through positive and negative controls, replicate analyses, and defined acceptance criteria. Whether such quality control measures are routinely applied in commercial metabarcoding services, and what thresholds determine acceptable performance, remains undisclosed. This opacity is particularly problematic for governmental monitoring programs, where methodological choices must be justified and results must be reproducible.

For metabarcoding to become a reliable tool in biodiversity monitoring, commercial service providers must deliver: 1. complete raw sequence data in standardized formats (FASTQ files), 2. detailed protocols specifying DNA extraction, primers, PCR conditions, and sequencing specifications, 3. complete bioinformatic workflows with quality filtering parameters and clustering thresholds, 4. database versions with timestamps, and 5. quality control metadata. Additionally, the scientific community and regulatory bodies should establish minimum standards and certification procedures for commercial metabarcoding services, specifying required documentation, data formats, quality metrics, and validation procedures. Until commercial services achieve greater transparency and demonstrated reproducibility, metabarcoding should be considered a complementary tool requiring rather than a standalone replacement for morphological identification.

Our study was designed to test metabarcoding from the perspective of typical end-users:

governmental agencies, conservation organizations, or consulting companies, who seek a "plug and play" solution without requiring in-house molecularbiology expertise. From this perspective, several critical challenges emerged.

First, users cannot assess service quality before commissioning expensive analyses. The two companies showed dramatically different detection rates and species numbers, with little overlap. Even repeated analyses by Company A showed unexplained variation, suggesting inconsistent protocols or hidden methodological issues.

Second, results require substantial taxonomic expertise despite delivered species lists. Many species were listed with outdated synonyms, uncertain designations (cf.), or implausible occurrences: marine species in terrestrial samples, requiring extensive faunistic knowledge for validation.

Third, without transparent methodologies, users cannot diagnose whether poor performance results from incomplete databases, suboptimal protocols, inappropriate parameters, or fundamental limitations. This prevents informed decision-making for governmental programs requiring methodological justification. We therefore recommend comprehensive technical documentation, validation data, and pilot studies with morphologically identified reference samples before implementing metabarcoding in operational monitoring. Until commercial services achieve greater transparency and reliability, metabarcoding should be considered a complementary tool requiring expert interpretation.

Currently, more than five million CO1 insect sequences are available worldwide, with Coleoptera, Lepidoptera and Diptera dominating significantly. It is suggested that there are numerous inconsistencies and high error rates in various databases, making manual curation of these sequences urgently necessary (Shen et al. 2013, Magoga et al. 2022). This introduces further uncertainty to the

results, which could be exacerbated by an increased use of species descriptions based on DNA sequences in the future.

Overall, the results provide an initial insight into the reliability of species identification with metabarcoding and complements studies such as Rimmel et al. (2024), which found comparable results between metabarcoding and morphological identification, and Heine et al. (2021) which found the opposite. The statement that a specific species is present in the sample through metabarcoding does not necessarily seem to indicate that the species is actually present in reality. Our findings indicate unfavourable outcomes, regardless of the manner in which a specific company handles the samples. The results from our comparison, with detection rates of less than 60% and many seemingly false positives with large differences even within the same company, demonstrate that this detection method might lead to questionable overall findings and suggest a cautious approach when using this data. However, it is important to acknowledge that this study was based on a small sample size of only three batches. Our results raise important questions about metabarcoding reliability, but future studies with larger sample sizes are needed to draw more definitive conclusions. We recommend conducting similar comparisons with 10 or more replicate batches to increase statistical power and better assess variability between samples and companies.

Existing limitations of the method, such as the still existing inability to identify the abundance of species (detecting only presence/absence) (Effendi et al. 2023, Sickel et al. 2024), also persist and make ecological or functional analyses and applications difficult or even impossible. Whether the sampling itself (by contamination), sample processing, bioinformatic assignment and analysis of sequence data with reference databases, or a combination of all of these, are the cause of these errors remains unclear. However, as demonstrated in our experiment, metabarcoding

can lead to results with an error rate that is not yet low enough for many purposes.

If unverifiable or even false data obtained through metabarcoding in monitoring programmes finds its way into official government databases, this is detrimental to nature and species conservation. The integrity of occurrence databases and the effective use of public funds are jeopardised by such data. Furthermore, conservation programmes cannot and should not be implemented in a targeted and effective manner if there is incorrect knowledge about the distribution and local occurrence of species.

While our results highlight significant limitations of metabarcoding for species identification, the method does offer some potential benefits that warrant further exploration. Metabarcoding can process large numbers of samples more quickly than morphological identification and may detect cryptic species or unexpected taxa. However, the high false positive rate and inconsistent detection of known species we observed indicate that substantial methodological improvements are needed before metabarcoding can be reliably used for biodiversity monitoring. Future research should focus on optimizing protocols, improving reference databases, and developing more robust bioinformatics pipelines to address these issues.

The problem of the shortage of taxonomists within most taxonomic groups (Löbl et al. 2023) of invertebrates and the “high effort” of morphological identifications is currently rarely being addressed in discussions about benefits of using metabarcoding. “Laypersons” do not possess the morphological, taxonomic, or faunistic expertise necessary for a technically correct interpretation of the obtained species lists when either metabarcoding or morphological identification is used. Therefore, even if metabarcoding can overcome the methodological problems, the problem of a lack of taxonomic expertise remains. Rather than viewing metabarcoding as a replacement

for traditional morphological identification, we propose that it may be most effective when used as a complementary tool. For instance, metabarcoding could be used for initial, rapid biodiversity assessments to guide more targeted morphological studies. Additionally, integrating metabarcoding and morphological data could provide a more comprehensive understanding of biodiversity patterns (Rommel et al. 2024). Future research should explore how to optimally combine these approaches to leverage the strengths of each method while mitigating their respective limitations.

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Appendix 1. Species list comparing the morphological identification of species with the identification using metabarcoding by two companies. Column Num: number of individuals in each mixed sample, Columns CA, CA (2) and CB: identified by Company A (CA), Company A (2nd sample) or Company B (CB). Column RSA: Reference sequence available in an accessible gene database. All columns: + indicates yes, - indicates no.

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Phrurolithus festivus</i> (C.L. KOCH, 1835)	0,5	+	-	-	-
<i>Xysticus kochi</i> THORELL, 1872	0,3	+	+	-	-
Auchenorrhyncha					
<i>Asiraca clavicornis</i> (FABRICIUS, 1794)	0,3	+	-	-	-
Coleoptera					
<i>Abraeus perpusillus</i> (MARSHAM, 1802)	1	+	+	-	-
<i>Aderus populneus</i> (CREUTZER, 1796)	1	+	-	-	-
<i>Agriotes pallidulus</i> (ILLIGER, 1807)	3	+	+	+	-
<i>Agriotes pilosellus</i> (SCHÖNHERR, 1817)	1	+	+	+	+
<i>Agriotes sputator</i> (LINNAEUS, 1758)	0,3	+	+	+	-
<i>Agrypnus murinus</i> (LINNAEUS, 1758)	1	+	+	+	-
<i>Amara aenea</i> (DEGEER, 1774)	1	+	+	-	-
<i>Amphicyllis globiformis</i> (SAHLBERG, 1833)	0,3	+	+	-	-
<i>Anaspis frontalis</i> (LINNAEUS, 1758)	2	+	+	+	+
<i>Anaspis maculata</i> (GEOFFROY, 1785)	1	+	-	-	-
<i>Anotylus hamatus</i> (FAIRMAIRE & LABOULBÈNE 1856)	0,3	-	-	-	-
<i>Anotylus inustus</i> (GRAVENHORST, 1806)	1	+	-	-	-
<i>Anotylus nitidulus</i> (GRAVENHORST, 1802)	1	+	-	+	+
<i>Anotylus rugosus</i> (FABRICIUS, 1775)	0,3	+	-	-	-
<i>Anotylus tetracarinatus</i> (BLOCK, 1799)	1	+	-	-	-
<i>Anthaxia nitidula</i> (LINNAEUS, 1758)	1	+	+	+	-
<i>Anthobium atrocephalum</i> (GYLLENHAL, 1827)	1	+	+	+	-
<i>Anthocomus fasciatus</i> (LINNAEUS, 1758)	0,3	+	-	-	-
<i>Anthonomus pomorum</i> (LINNAEUS, 1758)	1	+	-	-	+
<i>Anthonomus rectirostris</i> (LINNAEUS, 1758)	0,3	+	-	-	-
<i>Anthrenus museorum</i> (LINNAEUS, 1761)	0,3	+	-	-	-
<i>Anthrenus scrophulariae</i> (LINNAEUS, 1758)	0,3	+	-	-	-
<i>Anthrenus verbasci</i> (LINNAEUS, 1761)	4	+	+	+	-
<i>Aphthona euphorbiae</i> (SCHRANK, 1781)	0,3	+	-	-	-
<i>Aspidapion radiolus</i> (MARSHAM, 1802)	0,3	+	-	-	-
<i>Aspidapion validum</i> (GERMAR, 1817)	0,3	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Atheta fungi</i> (GRAVENHORST, 1806)	1	+	+	-	-
<i>Atheta gagatina</i> (BAUDI, 1848)	1	+	-	-	-
<i>Atheta inquinula</i> (GRAVENHORST, 1802)	1	+	-	-	-
<i>Atheta marcida</i> (ERICHSON, 1837)	2	+	+	-	-
<i>Atheta orbata</i> (ERICHSON, 1837)	0,3	+	-	-	-
<i>Athous haemorrhoidalis</i> (FABRICIUS, 1801)	1	+	+	+	+
<i>Athous subfuscus</i> (O. F. MÜLLER, 1764)	2	+	+	+	-
<i>Athous vittatus</i> (FABRICIUS, 1792)	1	+	+	+	-
<i>Athous zebei</i> BACH, 1852	1	+	+	+	-
<i>Atomaria linearis</i> STEPHENS, 1830	1	+	-	-	-
<i>Atomaria testacea</i> STEPHENS, 1830	1	+	-	-	-
<i>Atrecus affinis</i> (PAYKULL, 1789)	1	+	-	-	-
<i>Attagenus smirnovi</i> ZHANTIEV, 1973	1	+	-	-	-
<i>Aulonothroscus brevicollis</i> (BONVOULOIR, 1859)	5	+	+	-	+
<i>Axinotarsus marginalis</i> (LAPORTE, 1840)	3	+	+	+	+
<i>Batophila rubi</i> (PAYKULL, 1799)	0,3	+	-	-	-
<i>Bolitophagus reticulatus</i> (LINNAEUS, 1767)	1	+	+	+	-
<i>Bruchela rufipes</i> (A. G. OLIVIER, 1790)	1	+	+	-	-
<i>Bruchela suturalis</i> (FABRICIUS, 1792)	1	+	-	-	-
<i>Bruchidius villosus</i> (FABRICIUS, 1792)	2	+	+	-	-
<i>Byturus ochraceus</i> (SCRIBA, 1790)	4	+	+	-	+
<i>Byturus tomentosus</i> (DEGEER, 1774)	2	+	+	+	+
<i>Callicerus rigidicornis</i> (ERICHSON, 1839)	0,3	+	-	-	-
<i>Calvia quatuordecimpunctata</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Cantharis decipiens</i> BAUDI DI SELVE, 1872	1	+	+	-	+
<i>Cantharis fusca</i> LINNAEUS, 1758	1	+	+	+	-
<i>Cantharis lateralis</i> LINNAEUS, 1758	1	+	+	+	-
<i>Cantharis nigricans</i> (O. F. MÜLLER, 1776)	2	+	+	-	+
<i>Cantharis paradoxa</i> HICKER, 1960	1	+	+	-	+
<i>Cantharis pellucida</i> FABRICIUS, 1792	2	+	+	-	+
<i>Cantharis rustica</i> FALLÉN, 1807	1	+	+	+	+
<i>Cercyon laminatus</i> SHARP, 1873	2	+	+	-	-
<i>Cerylon ferrugineum</i> STEPHENS, 1830	1	+	-	-	-
<i>Cetonia aurata</i> FABRICIUS, 1775	1	+	+	+	-
<i>Ceutorhynchus contractus</i> (MARSHAM, 1802)	2	+	+	-	-
<i>Ceutorhynchus obstrictus</i> (MARSHAM, 1802)	1	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Ceutorhynchus pyrrhorhynchus</i> (MARSHAM, 1802)	6	+	-	-	-
<i>Ceutorhynchus typhae</i> (HERBST, 1795)	1	+	-	-	-
<i>Chaetocnema concinna</i> STEPHENS, 1831	0,3	+	-	-	-
<i>Chaetocnema hortensis</i> (GEOFFROY, 1785)	0,3	+	-	-	-
<i>Charopus flavipes</i> (PAYKULL, 1798)	1	+	-	-	-
<i>Cidnopus pilosus</i> (LESKE, 1785)	1	+	+	+	+
<i>Cionus alauda</i> (HERBST, 1784)	1	+	-	-	-
<i>Cionus hortulans</i> (GEOFFROY, 1785)	1	+	+	-	-
<i>Cionus tuberculosus</i> (SCOPOLI, 1763)	1	+	-	-	-
<i>Cis micans</i> (FABRICIUS, 1792)	0,3	+	-	-	-
<i>Cis castaneus</i> (=nitidus) (HERBST, 1793)	1	+	+	-	-
<i>Coccinella septempunctata</i> LINNAEUS, 1758	1	+	+	-	+
<i>Colenis immunda</i> (STURM, 1807)	0,3	+	-	-	-
<i>Cordylepherus viridis</i> (FABRICIUS, 1787)	4	+	+	+	-
<i>Corticarina similata</i> (GYLLENHAL, 1827)	1	+	-	-	-
<i>Corticara gibbosa</i> (HERBST, 1793)	1	+	+	-	-
<i>Crepidodera aurata</i> (MARSHAM, 1802)	5	+	+	+	+
<i>Crepidodera aurea</i> (GEOFFROY, 1785)	1	+	+	+	-
<i>Crioceris asparagi</i> (LINNAEUS, 1758)	1	+	-	+	-
<i>Cryptocephalus ocellatus</i> DRAPIEZ, 1819	1	+	+	-	-
<i>Cryptophilus propinquus</i> REITTER, 1874	1	+	-	-	-
<i>Crypturgus hispidulus</i> THOMSON, 1870	0,3	+	-	-	-
<i>Cylister lineare</i> (ERICHSON, 1834)	1	+	-	-	-
<i>Cypha longicornis</i> (PAYKULL, 1800)	0,3	+	-	-	-
<i>Dacne bipustulata</i> (THUNBERG, 1781)	1	+	-	-	-
<i>Dacne rufifrons</i> (FABRICIUS, 1775)	1	+	-	-	-
<i>Dalopius marginatus</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Danacaea nigratarsis</i> (KÜSTER, 1850)	2	+	+	-	-
<i>Dasytes aeratus</i> STEPHENS, 1830	1	+	+	-	-
<i>Dasytes plumbeus</i> (O. F. MÜLLER, 1776)	1	+	-	-	-
<i>Dasytes subaeneus</i> SCHÖNHERR, 1817	1	+	+	-	-
<i>Dasytes virens</i> (MARSHAM, 1802)	1	+	-	-	-
<i>Dicronychus cinereus</i> (HERBST, 1784)	1	+	+	+	-
<i>Diplocoelus fagi</i> (CHEVROLAT, 1837)	2	+	+	+	+
<i>Drusilla canaliculata</i> (FABRICIUS, 1787)	0,3	+	-	-	-
<i>Dryophilus pusillus</i> (GYLLENHAL, 1808)	0,3	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Eledona agricola</i> (HERBST, 1783)	1	+	-	-	-
<i>EpiRhynchites auratus</i> (SCOPOLI, 1763)	1	+	-	-	-
<i>Epuraea biguttata</i> (THUNBERG, 1784)	2	+	-	-	+
<i>Epuraea distincta</i> (GRIMMER, 1841)	1	+	+	-	-
<i>Epuraea melanocephala</i> (MARSHAM, 1802)	3	+	+	+	+
<i>Epuraea ocularis</i> FAIRMAIRE, 1849	0,3	+	-	-	-
<i>Euglenes oculatus</i> (PAYKULL, 1798)	2	+	-	-	-
<i>Eusomus ovulum</i> GERMAR, 1824	1	+	+	+	+
<i>Eusphalerum atrum</i> (HEER, 1839)	1	+	+	-	+
<i>Exomias pellucidus</i> (BOHEMAN, 1843)	1	+	+	-	-
<i>Exomias trichopterus</i> (GAUTIER DES COTTES 1863)	1	+	-	-	-
<i>Fabogethes nigrescens</i> (STEPHENS, 1830)	0,3	+	-	-	-
<i>Foucartia ptochioides</i> (BACH, 1856)	1	+	+	-	-
<i>Foucartia squamulata</i> (HERBST, 1795)	1	+	-	-	-
<i>Glaphyra umbellatarum</i> (SCHREBER, 1859)	1	+	+	+	-
<i>Gonioctena olivacea</i> (FORSTER, 1771)	1	+	+	+	-
<i>Grammoptera ruficornis</i> (FABRICIUS, 1781)	1	+	+	+	-
<i>Habrocerus capillaricornis</i> (GRAVENHORST, 1806)	1	+	-	-	-
<i>Harmonia axyridis</i> (PALLAS, 1773)	1	+	+	-	+
<i>Harpalus rufipes</i> (DEGEER, 1774)	2	+	-	+	+
<i>Hemicoelus fulvicornis</i> (STURM, 1837)	1	+	-	-	-
<i>Hermaphysa mercurialis</i> (FABRICIUS, 1792)	1	+	-	-	-
<i>Heterocerus fenestratus</i> (THUNBERG, 1784)	2	+	+	-	+
<i>Hippodamia variegata</i> (GOEZE, 1777)	1	+	+	-	+
<i>Holobus flavicornis</i> (LACORDAIRE, 1835)	2	+	-	-	-
<i>Holotrichapion pisi</i> (FABRICIUS, 1801)	2	+	+	-	-
<i>Homoeusa acuminata</i> (MÄRKEL, 1842)	0,3	+	-	-	-
<i>Hydrobius fuscipes</i> (LINNAEUS, 1758)	0,3	+	-	-	-
<i>Hypebaeus flavipes</i> (FABRICIUS, 1787)	1	+	+	-	-
<i>Ips sexdentatus</i> (BOERNER, 1766)	1	+	+	-	-
<i>Ischnomera cyanea</i> (FABRICIUS, 1792)	0,3	+	+	+	+
<i>Isomira thoracica</i> (FABRICIUS, 1792)	2	+	+	-	+
<i>Latridius hirtus</i> (GYLLENHAL, 1827)	1	+	-	-	-
<i>Leiopus femoratus</i> FAIRMAIRE, 1859	2	+	+	+	+
<i>Leiopus linnei</i> WALL. ET AL. 2009 / <i>nebulosus</i> (L., 1758)	2	+	+	+	+
<i>Lesteva longoelytrata</i> (GOEZE, 1777)	0,3	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Liliocercis lilii</i> (SCOPOLI, 1763)	1	+	+	+	-
<i>Limonijs poneli</i> LESEIGNEUR & MERTLIK, 2007	1	+	+	-	-
<i>Litargus connexus</i> (GEOFFROY, 1785)	3	+	+	+	+
<i>Lithocharis nigriceps</i> KRAATZ, 1859	1	+	-	-	-
<i>Longitarsus anchusae</i> (PAYKULL, 1799)	1	+	-	-	-
<i>Longitarsus echii</i> (KOCH, 1803)	1	+	+	+	-
<i>Longitarsus luridus</i> (SCOPOLI, 1763)	0,3	+	-	-	-
<i>Longitarsus lycopi</i> (FOUDRAS, 1860)	1	+	+	-	+
<i>Longitarsus pratensis</i> (PANZER, 1794)	1	+	-	-	-
<i>Luperus luperus</i> (SULZER, 1776)	2	+	+	+	-
<i>Magdalis phlegmatica</i> (HERBST, 1797)	1	+	-	-	-
<i>Magdalis ruficornis</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Malachius bipustulatus</i> (LINNAEUS, 1758)	5	+	+	+	+
<i>Malthinus flaveolus</i> (HERBST, 1786)	2	+	+	-	-
<i>Malthodes marginatus</i> (LATREILLE, 1806)	1	+	+	+	-
<i>Malthodes pumilus</i> (BRÉBISSE, 1835)	1	+	+	-	-
<i>Malvapion malvae</i> (FABRICIUS, 1775)	1	+	-	-	-
<i>Megasternum immaculatum</i> (STEPHENS, 1829)	1	-	-	-	-
<i>Megatoma undata</i> (LINNAEUS, 1758)	0,3	+	-	-	-
<i>Melanotus brunnipes</i> (GERMAR, 1824)	1	+	+	+	-
<i>Melasis buprestoides</i> (LINNAEUS, 1761)	0,3	+	-	-	+
<i>Metacantharis clypeata</i> (ILLIGER, 1798)	1	+	+	-	-
<i>Microlestes minutulus</i> (GOEZE, 1777)	2	+	-	-	-
<i>Microplontus rugulosus</i> (HERBST, 1795)	1	+	-	-	-
<i>Mniophila muscorum</i> (KOCH, 1803)	1	+	-	-	-
<i>Monotoma brevicollis</i> AUBÉ, 1837	1	+	-	+	-
<i>Mordellistena pumila</i> (GYLLENHAL, 1810)	1	+	-	-	-
<i>Mycetophagus fulvicollis</i> FABRICIUS, 1792)	0,3	+	-	-	-
<i>Mycetophagus quadripustulatus</i> (LINNAEUS, 1761)	2	+	+	+	+
<i>Myrrha octodecimguttata</i> (LINNAEUS, 1758)	0,3	+	-	-	+
<i>Necrobia violacea</i> (LINNAEUS, 1758)	2	+	+	-	-
<i>Nedys quadrimaculatus</i> (LINNAEUS, 1758)	2	+	+	-	-
<i>Nemozoma elongatum</i> (LINNAEUS, 1761)	0,3	+	+	+	-
<i>Neocrepidodera transversa</i> (MARSHAM, 1802)	1	+	-	-	-
<i>Nicrophorus vespillo</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Oedemera virescens</i> (LINNAEUS, 1767)	1	+	+	+	+

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Oiceoptoma thoracicum</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Olibrus aeneus</i> (FABRICIUS, 1792)	2	+	+	+	+
<i>Olibrus corticalis</i> (PANZER, 1797)	0,3	+	+	+	-
<i>Omalium rivulare</i> (PAYKULL, 1798)	2	+	-	-	-
<i>Omophron limbatum</i> (FABRICIUS, 1777)	1	+	+	-	-
<i>Omosita colon</i> (LINNAEUS, 1758)	3	+	-	-	-
<i>Oomorplus concolor</i> (STURM, 1807)	0,3	+	-	-	-
<i>Orthotomicus longicollis</i> (GYLLENHAL, 1827)	2	+	-	-	-
<i>Otiorhynchus lirus</i> SCHÖNHERR, 1834	0,3	+	-	-	-
<i>Otiorhynchus singularis</i> (LINNAEUS, 1767)	1	+	+	-	+
<i>Otiorhynchus subdentatus</i> (GYLLENHAL, 1834)	2	+	-	-	-
<i>Oulema obscura</i> (STEPHENS, 1831)	1	+	+	+	-
<i>Oxyomus sylvestris</i> (SCOPOLI, 1763)	0,3	+	+	+	+
<i>Oxystoma ochropus</i> (GERMAR, 1818)	0,3	+	-	-	-
<i>Oxytelus piceus</i> (LINNAEUS, 1767)	1	+	-	-	+
<i>Oxythyrea funesta</i> (PODA VON NEUHAUS, 1761)	0,3	+	+	-	-
<i>Pachybrachis sinuatus</i> (MULSANT & REY, 1859)	1	+	+	-	-
<i>Paromalus parallelepipedus</i> (HERBST, 1791)	1	+	-	-	-
<i>Pheletes quercus</i> (A. G. OLIVIER, 1790)	1	+	+	+	+
<i>Philonthus quisquiliarius</i> (GYLLENHAL, 1810)	0,3	+	-	-	-
<i>Phyllobius betulinus</i> (BECHSTEIN & SCHARFFENBERG, 1805)	4	+	+	+	-
<i>Phyllobius pomaceus</i> GYLLENHAL, 1834	3	+	+	+	+
<i>Phyllobius roboretanus</i> GREDLER, 1882	8	+	+	+	+
<i>Phyllopertha horticola</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Phyllotreta nemorum</i> (LINNAEUS, 1758)	0,3	+	-	-	-
<i>Phyllotreta nigripes</i> (FABRICIUS, 1775)	1	+	+	-	-
<i>Phyllotreta nodicornis</i> (MARSHAM, 1802)	2	+	+	-	-
<i>Phyllotreta vittula</i> (REDTENBACHER, 1849)	0,3	+	-	-	-
<i>Pityogenes chalcographus</i> (LINNAEUS, 1760)	0,3	+	+	-	-
<i>Placusa pumilio</i> (GRAVENHORST, 1802)	1	+	-	-	-
<i>Placusa tachyporoides</i> (WALTL, 1838)	3	+	-	-	-
<i>Platystomos albinus</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Podagricra fuscicornis</i> (LINNAEUS, 1767)	1	+	+	-	-
<i>Pogonocherus hispidus</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Polydrusus marginatus</i> STEPHENS, 1831	3	+	+	+	+
<i>Polygraphus poligraphus</i> (LINNAEUS, 1758)	5	+	+	+	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Propylaea 14-punctata</i> (LINNAEUS, 1758)	2	+	+	+	+
<i>Prosternon tessellatum</i> (LINNAEUS, 1758)	1	+	+	+	-
<i>Protaetia cuprea METALLICA</i> (HERBST, 1783)	0,3	+	+	+	+
<i>Protapion assimile</i> KIRBY, 1808	1	+	+	-	-
<i>Protapion fulvipes</i> (FOURCROY, 1785)	2	+	+	-	-
<i>Protapion nigritarse</i> (KIRBY, 1808)	1	+	-	-	-
<i>Protapion trifolii</i> (LINNAEUS, 1768)	14	+	+	-	+
<i>Pseudoperapion brevirostre</i> (HERBST, 1797)	3	+	-	-	-
<i>Psyllobora vigintiduopunctata</i> (LINNAEUS, 1758)	0,3	+	+	-	-
<i>Ptenidium pusillum</i> (GYLLENHAL, 1808)	1	+	-	-	-
<i>Pyrochroa serraticornis</i> (SCOPOLI, 1763)	1	+	+	-	+
<i>Pyrrhidium sanguineum</i> (LINNAEUS, 1758)	1	+	+	+	-
<i>Rhagonycha lignosa</i> (O. F. MÜLLER, 1764)	4	+	+	-	+
<i>Rhizophagus bipustulatus</i> (FABRICIUS, 1792)	1	+	+	-	+
<i>Rhopalapion longirostre</i> (OLIVIER, 1807)	3	+	+	-	+
<i>Rhyzobius chrysomeloides</i> (HERBST, 1792)	0,3	+	-	-	-
<i>Rugilus erichsonii</i> (FAUVEL, 1767)	0,3	+	-	-	-
<i>Rugilus rufipes</i> GERMAR, 1836	0,3	+	-	-	-
<i>Salpingus planirostris</i> (FABRICIUS, 1787)	3	+	+	+	+
<i>Scaphisoma agaricinum</i> (LINNAEUS, 1758)	1	+	+	-	-
<i>Scaphisoma boleti</i> (PANZER, 1793)	1	+	-	-	-
<i>Sciaphobus scitulus</i> (GERMAR, 1824)	3	+	+	+	-
<i>Scydmaenus tarsatus</i> Müller & Kunze, 1822	0,3	+	-	-	-
<i>Scymnus ferrugatus</i> (MOLL, 1785)	0,3	+	+	-	+
<i>Sepedophilus testaceus</i> (FABRICIUS, 1792)	2	+	-	-	-
<i>Sericoderus lateralis</i> (GYLLENHAL, 1827)	1	+	-	-	-
<i>Silvanoprus fagi</i> (GUÉRIN-MÉNEVILLE 1844)	1	+	-	+	-
<i>Silvanus bidentatus</i> (FABRICIUS, 1792)	2	+	+	+	+
<i>Sitona lineatus</i> (LINNAEUS, 1758)	0,3	+	+	+	-
<i>Spondylis buprestoides</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Stegobium paniceum</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Stenichnus scutellaris</i> (MÜLLER & KUNZE, 1822)	0,3	+	-	-	-
<i>Stenus impressus</i> GERMAR, 1824	0,3	+	-	-	-
<i>Sulcacis nitidus</i> (=affinis) (FABRICIUS, 1792)	1	+	-	-	-
<i>Symbiotes gibberosus</i> (LUCAS, 1846)	0,3	+	-	-	-
<i>Syntomus foveatus</i> (GEOFFROY, 1785)	1	+	+	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Tachyporus hypnorum</i> (FABRICIUS, 1775)	2	+	+	+	-
<i>Tachyporus nitidulus</i> (FABRICIUS, 1781)	0,3	+	-	-	-
<i>Tachys bistriatus</i> (DUFTSCHMID, 1812)	1	+	-	-	-
<i>Tatianaerhynchites aequatus</i> (LINNAEUS, 1767)	1	+	+	+	-
<i>Thanasimus formicarius</i> (LINNAEUS, 1758)	1	+	+	-	-
<i>Thanatophilus sinuatus</i> (FABRICIUS, 1775)	1	+	+	+	-
<i>Trichodes alvearius</i> (FABRICIUS, 1792)	0,3	+	+	+	+
<i>Trox scaber</i> (LINNAEUS, 1767)	0,3	+	+	+	+
<i>Tychius medicaginis</i> C. N. F. BRISOUT DE BARNEVILLE, 1862	1	+	+	-	-
<i>Tychius meliloti</i> STEPHENS, 1831	1	+	+	-	-
<i>Tychius picirostris</i> (FABRICIUS, 1787)	1	+	+	-	-
<i>Tychius stephensi</i> SCHÖNHERR, 1836	1	+	-	-	-
<i>Tytthaspis sedecimpunctata</i> (LINNAEUS, 1761)	1	+	+	-	+
<i>Uleiota planata</i> (LINNAEUS, 1761)	6	+	+	+	+
<i>Uloma culinaris</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Valgus hemipterus</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Vibidia duodecimguttata</i> (PODA VON NEUHAUS, 1761)	1	+	-	-	-
<i>Vincenzellus ruficollis</i> (PANZER, 1794)	6	+	+	+	+
<i>Xestobium rufovillosum</i> (DEGEER, 1774)	1	+	+	+	+
<i>Xyleborinus saxesenii</i> (RATZEBURG, 1837)	1	+	+	-	-
<i>Xyletinus ater</i> (CREUTZER, 1796)	1	+	-	-	-
<i>Xylocleptes bispinus</i> (DUFTSCHMID, 1825)	3	+	+	+	-
DERMAPTERA					
<i>Labia minor</i> (LINNAEUS, 1758)	6	+	+	-	+
DIPTERA					
<i>(Pseudo-)Lyciella stylata</i> (PAPP, 1978)	0,3	-	-	-	-
<i>Alloborborus pallifrons</i> (FALLEN, 1820)	0,3	+	-	-	-
<i>Bibio johannis</i> (LINNAEUS, 1767)	1	+	-	-	-
<i>Bibio marci</i> (LINNAEUS, 1758)	0,3	+	+	+	+
<i>Bibio pomonae</i> (FABRICIUS, 1775)	1	+	+	+	+
<i>Borborillus uncinatus</i> (DUDA, 1923)	1	+	-	-	-
<i>Brachicoma devia</i> (FALLEN, 1820)	0,3	+	+	+	+
<i>Calliphora vicina</i> ROBINEAU-DESVOIDY, 1830	0,3	+	-	-	+
<i>Chamaepsila nigricornis</i> (MEIGEN, 1826)	5	+	+	-	-
<i>Chloromyia formosa</i> (SCOPOLI, 1763)	0,3	+	+	-	-
<i>Coenosia testacea</i> (ROBINEAU-DESVOIDY, 1830)	1	+	+	-	+

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Coproica ferruginata</i> (STENHAMMAR, 1855)	0,3	+	-	-	-
<i>Coproica vagans</i> (HALIDAY, 1833)	0,3	+	-	-	-
<i>Delia florilega</i> (ZETTERSTEDT, 1845)	0,3	+	-	-	-
<i>Delia platura</i> (MEIGEN, 1826)	10	+	+	+	+
<i>Drosophila hydei</i> STURTEVANT, 1921	0,3	+	+	-	-
<i>Drosophila subobscura</i> COLLIN, 1936	1	+	+	-	+
<i>Eccoptomera pallescens</i> (MEIGEN, 1830)	0,3	+	+	+	-
<i>Empis nuntia</i> MEIGEN, 1838	1	+	+	-	+
<i>Eupachygaster tarsalis</i> (ZETTERSTEDT, 1842)	2	+	-	+	+
<i>Fannia serena</i> (FALLEN, 1825)	0,3	+	-	+	+
<i>Gymnomus caesius</i> (MEIGEN, 1830)	0,3	+	+	-	?
<i>Heleomyza captiosa</i> (GORODKOV, 1962)	0,3	+	-	-	-
<i>Helina evecta</i> (HARRIS, 1780)	0,3	+	+	-	-
<i>Homoneura intersticta</i> (FALLEN, 1820)	2	+	+	-	+
<i>Homoneura thalhammeri</i> PAPP, 1978	0,3	+	-	-	-
<i>Ischiolepta pusilla</i> (FALLEN, 1820)	0,3	+	-	-	-
<i>Leptocera lutosa</i> (STENHAMMAR, 1854)	0,3	+	-	-	-
<i>Leptocera nigra</i> OLIVIER, 1813	0,3	+	-	-	-
<i>Lonchoptera bifurcata</i> (FALLEN, 1810)	1	+	+	-	+
<i>Lonchoptera lutea</i> PANZER, 1809	0,3	+	+	-	+
<i>Lonchoptera tristis</i> MEIGEN, 1824	3	+	+	-	+
<i>Lordiphosa andalusiaca</i> (STROBL, 1906)	10	+	+	-	-
<i>Lordiphosa fenestrarum</i> (FALLEN, 1823)	1	+	-	-	+
<i>Melinda gentilis</i> ROBINEAU-DESVOIDY, 1830	0,3	+	+	-	+
<i>Merodon equestris</i> (FABRICIUS, 1794)	0,3	+	+	+	+
<i>Metopina pileata</i> SCHMITZ, 1936	1	-	-	-	-
<i>Microchrysa polita</i> (LINNAEUS, 1758)	2	+	-	+	+
<i>Musca autumnalis</i> DE GEER, 1776	0,3	+	+	-	-
<i>Nephrotoma appendiculata</i> (PIERRE, 1919)	0,3	+	+	-	+
<i>Oecotha fenestralis</i> (FALLEN, 1820)	0,3	+	-	-	-
<i>Otites lamed</i> (SCHRANK, 1781)	1	+	+	-	+
<i>Pachygaster atra</i> (PANZER, 1798)	2	+	+	-	-
<i>Phaonia subventa</i> (HARRIS, 1780)	0,3	+	+	-	+
<i>Phryno vetula</i> (MEIGEN, 1824)	3	+	+	+	+
<i>Platypalpus agilis</i> (MEIGEN, 1822)	1	+	-	-	+
<i>Platypalpus cothurnatus</i> MACQUART, 1827	1	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Platypalpus stigma</i> (COLLIN, 1926)	0,3	-	-	-	-
<i>Pollenia pediculata</i> MACQUART, 1834	0,3	+	+	-	+
<i>Pteremis fenestralis</i> (FALLEN, 1820)	0,3	+	-	-	-
<i>Sapromyza intonsa</i> LOEW, 1847	1	+	-	-	-
<i>Sarcophaga carnaria</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Sarcophaga incisilobata</i> PANDELLE, 1896	0,3	+	+	-	+
<i>Sarcophaga noverca</i> RONDANI, 1860	0,3	+	+	-	+
<i>Sarcophaga variegata</i> (SCOPOLI, 1763)	0,3	+	+	-	-
<i>Scaptomyza flava</i> (FALLEN, 1823)	1	+	+	-	+
<i>Scaptomyza griseola</i> (ZETTERSTEDT, 1847)	1	+	-	-	-
<i>Scaptomyza pallida</i> (ZETTERSTEDT, 1847)	30	+	-	-	-
<i>Scoliocentra brachypterna</i> (LOEW, 1873)	0,3	+	+	-	-
<i>Spelobia ochripes</i> (MEIGEN, 1830)	1	+	-	-	-
<i>Sylvicola cinctus</i> (FABRICIUS, 1787)	0,3	+	+	+	+
<i>Symphoromyia melaena</i> (MEIGEN, 1820)	0,3	-	-	-	-
<i>Tachina fera</i> (LINNAEUS, 1761)	1	+	+	-	-
<i>Tephrochlamys rufiventris</i> (MEIGEN, 1830)	0,3	+	+	+	-
<i>Themira putris</i> (LINNAEUS, 1758)	0,3	+	-	-	+
<i>Triarthria setipennis</i> (FALLEN, 1810)	0,3	+	+	-	+
<i>Typhamyza bifasciata</i> (WOOD, 1911)	0,3	+	+	-	+
Heteroptera					
<i>Acetropis carinata</i> (HERRICH-SCHAEFFER, 1841)	1	+	+	+	+
<i>Adelphocoris lineolatus</i> (GOEZE, 1778)	1	+	+	-	+
<i>Amblytylus nasutus</i> (KIRSCHBAUM, 1856)	1	+	+	+	-
<i>Anthocoris nemorum</i> (LINNAEUS, 1761)	1	+	+	-	-
<i>Aquarius paludum</i> (FABRICIUS, 1794)	1	+	+	+	+
<i>Aradus depressus</i> (FABRICIUS, 1794)	0,3	+	+	+	+
<i>Atractotomus mali</i> (MEYER-DÜR, 1843)	1	+	+	-	-
<i>Campylomma verbasci</i> (MEYER-DÜR, 1843)	1	+	-	-	-
<i>Capsodes flavomarginatus</i> (DONOVAN, 1798)	1	+	-	-	-
<i>Capsus ater</i> (LINNAEUS, 1758)	1	+	+	+	-
<i>Carpocoris fuscispinus</i> (BOHEMAN, 1851)	1	+	-	-	-
<i>Ceraleptus gracilicornis</i> (HERRICH-SCHAEFFER, 1835)	1	+	+	+	+
<i>Closterotomus fulvomaculatus</i> (DE GEER, 1773)	1	+	+	+	+
<i>Coreus marginatus</i> (LINNAEUS, 1758)	0,3	+	+	+	+
<i>Coriomeris denticulatus</i> (SCOPOLI, 1763)	0,3	+	+	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Corixa punctata</i> (ILLIGER, 1807)	1	+	+	+	+
<i>Cydnus aterrimus</i> (FORSTER, 1771)	1	+	+	-	-
<i>Cymatia coleoptrata</i> (FABRICIUS, 1777)	1	+	+	+	+
<i>Cyphostethus tristriatus</i> (FABRICIUS, 1787)	1	+	+	-	-
<i>Deraeocoris lutescens</i> (SCHILLING, 1837)	1	+	+	+	-
<i>Dicyphus pallidus</i> (HERRICH-SCHAEFFER, 1835)	1	+	-	+	-
<i>Dolycoris baccarum</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Europiella artemisiae</i> (BECKER, 1864)	1	+	+	-	-
<i>Eurycolpus flaveolus</i> (STÅL, 1858)	1	+	+	-	-
<i>Gerris gibbifer</i> SCHUMMEL, 1832	1	+	+	+	+
<i>Gerris thoracicus</i> SCHUMMEL, 1832	1	+	-	+	+
<i>Graphosoma lineatum</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Heterocordylus genistae</i> (SCOPOLI, 1763)	1	+	+	+	-
<i>Heterogaster urticae</i> (FABRICIUS, 1775)	1	+	+	+	+
<i>Heterotoma planicornis</i> (PALLAS, 1772)	1	+	+	-	-
<i>Himacerus apterus</i> (FABRICIUS, 1798)	1	+	+	-	+
<i>Himacerus mirmicoides</i> (O. COSTA, 1834)	1	+	+	-	+
<i>Hydrometra stagnorum</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Ilyocoris cimicoides</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Ischnocoris hemipterus</i> (SCHILLING, 1829)	1	+	+	-	-
<i>Ischnodemus sabuleti</i> (FALLÉN, 1826)	1	+	+	-	-
<i>Kleidocerys resedae</i> (PANZER, 1797)	1	+	+	-	+
<i>Leptopterna dolabrata</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Leptopterna ferrugata</i> (FALLÉN, 1807)	1	+	+	+	+
<i>Liocoris tripustulatus</i> (FABRICIUS, 1781)	1	+	+	-	-
<i>Lygus pratensis</i> (LINNAEUS, 1758)	1	+	-	+	-
<i>Macrolophus pygmaeus</i> (RAMBUR, 1839)	1	+	+	-	-
<i>Macrotylus herrichi</i> (REUTER, 1873)	1	+	+	+	+
<i>Megaloceroea recticornis</i> (GEOFFROY, 1785)	1	+	+	+	-
<i>Micronecta scholtzi</i> (FIEBER, 1860)	1	+	+	-	+
<i>Nabis flavomarginatus</i> SCHOLTZ, 1847	1	+	-	-	-
<i>Notonecta glauca</i> LINNAEUS, 1758	1	+	+	+	+
<i>Oncotylus punctipes</i> REUTER, 1875	1	+	+	+	+
<i>Orius niger</i> (WOLFF, 1811)	1	+	+	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Orthocephalus coriaceus</i> (FABRICIUS, 1777)	1	+	+	+	+
<i>Orthonotus rufifrons</i> (FALLÉN, 1807)	1	+	+	-	+
<i>Orthotylus marginalis</i> REUTER, 1883	1	+	+	-	-
<i>Phytocoris ulmi</i> (LINNAEUS, 1758)	1	+	+	-	-
<i>Phytocoris varipes</i> BOHEMAN, 1852	1	+	+	-	-
<i>Plagiognathus arbustorum</i> (FABRICIUS, 1794)	1	+	+	-	+
<i>Plagiognathus chrysanthemi</i> (WOLFF, 1804)	1	+	+	-	-
<i>Platyplax salviae</i> (SCHILLING, 1829)	1	+	+	+	-
<i>Plea minutissima</i> LEACH, 1817	1	+	-	-	-
<i>Plinthisus brevipennis</i> (LATREILLE, 1807)	1	+	-	-	-
<i>Polymerus nigrita</i> (FALLÉN, 1807)	1	+	-	-	-
<i>Polymerus unifasciatus</i> (FABRICIUS, 1794)	1	+	+	-	+
<i>Psallus albicinctus</i> (KIRSCHBAUM, 1856)	1	+	+	+	-
<i>Psallus lepidus</i> FIEBER, 1858	1	+	+	-	-
<i>Rhabdomiris striatellus</i> (FABRICIUS, 1794)	1	+	+	-	-
<i>Rhyparochromus vulgaris</i> (SCHILLING, 1829)	0,3	+	+	-	+
<i>Scolopostethus affinis</i> (SCHILLING, 1829)	1	+	+	-	-
<i>Scolopostethus thomsoni</i> REUTER, 1875	1	+	-	-	-
<i>Sigara falleni</i> (FIEBER, 1848)	1	+	+	-	-
<i>Sigara iactans</i> JANSSON, 1983	1	+	-	-	-
<i>Sigara lateralis</i> (LEACH, 1817)	1	+	+	-	+
<i>Sigara nigrolineata</i> (FIEBER, 1848)	1	+	+	-	-
<i>Sigara striata</i> (LINNAEUS, 1758)	1	+	+	-	-
<i>Stenodema holsata</i> (FABRICIUS, 1787)	1	+	+	+	+
<i>Stenodema laevigata</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Stenotus binotatus</i> (FABRICIUS, 1794)	1	+	+	-	+
<i>Tritomegas sexmaculatus</i> (RAMBUR, 1839)	1	+	+	-	-
<i>Tropistethus holosericeus</i> (SCHOLTZ, 1846)	1	+	-	-	+
<i>Velia caprai</i> TAMANINI, 1947	1	+	+	-	-
<i>Velia saulii</i> TAMANINI, 1947	1	+	-	-	-
Hymenoptera					
<i>Ammophila sabulosa</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Ancistrocerus nigricornis</i> (CURTIS, 1826)	1	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Andrena bicolor</i> FABRICIUS, 1775	1	+	+	+	+
<i>Andrena cineraria</i> (LINNAEUS, 1758)	3	+	+	-	+
<i>Andrena flavipes</i> PANZER, 1799	3	+	-	+	-
<i>Andrena gravis</i> IMHOFF, 1832	1	+	-	-	-
<i>Andrena haemorrhoa</i> (FABRICIUS, 1781)	3	+	+	+	-
<i>Andrena hattorfiana</i> (FABRICIUS, 1775)	1	+	-	+	-
<i>Andrena helvola</i> (LINNAEUS, 1758)	3	+	+	+	+
<i>Andrena labialis</i> (KIRBY, 1802)	1	+	+	-	+
<i>Andrena minutula</i> (KIRBY, 1802)	3	+	+	+	+
<i>Andrena minutuloides</i> PERKINS, 1914	3	+	+	+	-
<i>Andrena nigroaenea</i> (KIRBY, 1802)	1	+	-	+	-
<i>Andrena nitida</i> (MÜLLER, 1776)	1	+	+	+	+
<i>Andrena nitidiuscula</i> SCHENCK, 1853	1	+	-	-	-
<i>Andrena ovatula</i> (KIRBY, 1802)	1	+	+	-	+
<i>Andrena scotica</i> PERKINS, 1916	1	+	+	-	-
<i>Andrena strophmella</i> STOECKHERT, 1930	1	+	+	+	+
<i>Andrena subopaca</i> NYLANDER, 1848	1	+	-	+	-
<i>Andrena viridescens</i> VIERECK, 1916	1	+	-	-	+
<i>Andrena wilkella</i> (KIRBY, 1802)	1	+	-	-	-
<i>Anthidium byssinum</i> (PANZER, 1798)	1	-	-	-	-
<i>Anthidium punctatum</i> LATREILLE, 1809	1	+	-	-	+
<i>Anthophora aestivalis</i> (PANZER, 1801)	1	+	+	-	+
<i>Anthophora furcata</i> (PANZER, 1798)	1	+	+	-	+
<i>Anthophora quadrimaculata</i> (PANZER, 1798)	1	+	-	-	-
<i>Anthophora retusa</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Apis mellifera</i> LINNAEUS, 1758	3	+	+	-	+
<i>Bombus barbutellus</i> (KIRBY, 1802)	1	+	+	-	+
<i>Bombus hortorum</i> (LINNAEUS, 1761)	1	+	+	-	-
<i>Bombus humilis</i> ILLIGER, 1806	1	+	-	-	+
<i>Bombus lapidarius</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Bombus pascuorum</i> (SCOPOLI, 1763)	1	+	-	-	-
<i>Bombus pratorum</i> (LINNAEUS, 1761)	1	+	-	-	+
<i>Bombus quadricolor</i> (LEPELETIER, 1832)	1	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
<i>Araneae</i>					
<i>Bombus ruderarius</i> (MÜLLER, 1776)	1	+	+	-	-
<i>Bombus subterraneus</i> (LINNAEUS, 1758)	1	+	-	-	+
<i>Bombus sylvarum</i> (LINNAEUS, 1761)	1	+	-	+	+
<i>Cerceris rybyensis</i> (LINNAEUS, 1771)	1	+	+	-	-
<i>Coelioxys conoidea</i> (ILLIGER, 1806)	3	+	+	-	+
<i>Colletes daviesanus</i> SMITH, 1846	1	+	-	-	-
<i>Diodontus luperus</i> SHUCKARD, 1837	1	+	-	-	-
<i>Dolichoderus quadripunctatus</i> (LINNAEUS, 1771)	0,3	+	-	-	-
<i>Dolichurus corniculus</i> (SPINOLA, 1808)	1	+	-	-	-
<i>Ectemnius lituratus</i> (PANZER, 1805)	1	+	-	-	+
<i>Eucera longicornis</i> LINNAEUS, 1758	1	+	-	-	+
<i>Eucera nigrescens</i> PEREZ, 1879	3	+	-	-	-
<i>Halictus maculatus</i> SMITH, 1848	3	+	+	-	+
<i>Halictus rubicundus</i> (CHRIST, 1791)	1	+	-	-	+
<i>Halictus scabiosae</i> (ROSSI, 1790)	3	+	-	-	+
<i>Halictus simplex</i> BLÜTHGEN, 1923	1	+	+	-	+
<i>Halictus tumulorum</i> (LINNAEUS, 1758)	3	+	+	-	+
<i>Hylaeus angustatus</i> (SCHENCK, 1861)	1	+	-	-	-
<i>Hylaeus brevicornis</i> NYLANDER, 1852	1	+	-	-	-
<i>Hylaeus communis</i> NYLANDER, 1852	1	+	-	-	-
<i>Hylaeus dilatatus</i> (KIRBY, 1802)	1	+	-	-	-
<i>Hylaeus gredleri</i> Förster, 1871	1	+	+	+	+
<i>Hylaeus hyalinatus</i> SMITH, 1842	1	+	-	-	-
<i>Hylaeus nigritus</i> (FABRICIUS, 1798)	1	+	-	-	-
<i>Hylaeus signatus</i> (PANZER, 1798)	1	+	-	-	+
<i>Hylaeus styriacus</i> Förster, 1872	1	+	-	+	-
<i>Lasioglossum calceatum</i> (SCOPOLI, 1763)	1	+	-	-	-
<i>Lasioglossum fratellum</i> (PEREZ, 1903)	1	+	-	-	-
<i>Lasioglossum fulvicorne</i> (KIRBY, 1802)	3	+	+	+	-
<i>Lasioglossum glabriusculum</i> (Morawitz, 1853)	1	+	-	-	-
<i>Lasioglossum intermedium</i> (SCHENCK, 1868)	1	+	-	-	-
<i>Lasioglossum interruptum</i> (PANZER, 1798)	1	+	+	-	-
<i>Lasioglossum laevigatum</i> (KIRBY, 1802)	3	+	+	-	-

	Num.	RSA	CA	CA (2)	CB
<i>Araneae</i>					
<i>Lasioglossum laticeps</i> (SCHENCK, 1868)	1	+	+	-	-
<i>Lasioglossum leucopus</i> (KIRBY, 1802)	1	+	-	-	-
<i>Lasioglossum leucozonium</i> (SCHRANK, 1781)	1	+	+	-	+
<i>Lasioglossum malachurum</i> (KIRBY, 1802)	1	+	+	-	+
<i>Lasioglossum minutulum</i> (SCHENCK, 1853)	1	+	+	-	-
<i>Lasioglossum morio</i> (FABRICIUS, 1793)	3	+	+	-	+
<i>Lasioglossum nitidiusculum</i> (KIRBY, 1802)	1	+	+	-	-
<i>Lasioglossum nitidulum</i> (FABRICIUS, 1804)	1	+	+	-	-
<i>Lasioglossum parvulum</i> (SCHENCK, 1853)	1	+	+	-	-
<i>Lasioglossum pauxillum</i> (SCHENCK, 1853)	1	+	+	-	-
<i>Lasioglossum politum</i> (SCHENCK, 1853)	1	+	+	-	-
<i>Lasioglossum pygmaeum</i> (SCHENCK, 1853)	1	+	-	-	-
<i>Lasioglossum quadrinotatum</i> (KIRBY, 1802)	1	+	-	-	-
<i>Lasioglossum rufitarse</i> (ZETTERSTEDT, 1838)	1	+	-	-	-
<i>Lasioglossum tricinctum</i> (SCHENCK, 1874)	1	+	-	-	-
<i>Lasioglossum villosulum</i> (KIRBY, 1802)	1	+	+	-	+
<i>Lestica clypeata</i> (SCHREBER, 1775)	1	+	-	-	-
<i>Macropis europaea</i> Warncke, 1973	1	+	-	-	-
<i>Macropis fulvipes</i> (FABRICIUS, 1804)	1	+	+	+	+
<i>Megachile alpicola</i> ALFKEN, 1924	1	+	+	-	+
<i>Megachile ericetorum</i> LEPELETIER, 1841	1	+	-	-	+
<i>Megachile lapponica</i> THOMSON, 1872	1	+	-	-	-
<i>Megachile maritima</i> (KIRBY, 1802)	3	+	+	-	-
<i>Megachile nigriventris</i> SCHENCK, 1868	1	+	+	-	+
<i>Megachile pilidens</i> ALFKEN, 1924	3	+	+	-	+
<i>Megachile versicolor</i> SMITH, 1844	1	+	+	-	+
<i>Megachile willughbiella</i> (KIRBY, 1812)	1	+	+	-	+
<i>Melitta leporina</i> (PANZER, 1799)	1	+	-	-	-
<i>Myrmecina graminicola</i> (LATREILLE, 1802)	3	+	-	-	-
<i>Nomada bifasciata</i> OLIVIER, 1811	1	+	-	-	-
<i>Nomada castellana</i> DUSMET, 1913	1	+	-	-	-
<i>Nomada fabriciana</i> (LINNAEUS, 1767)	1	+	+	-	-
<i>Nomada flava</i> PANZER, 1798	1	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Nomada flavoguttata</i> (KIRBY, 1802)	1	+	-	-	+
<i>Nomada fucata</i> PANZER, 1798	1	+	-	-	-
<i>Nomada goodeniana</i> (KIRBY, 1802)	1	+	-	-	+
<i>Nomada marshamella</i> (KIRBY, 1802)	1	+	-	-	-
<i>Nomada panzeri</i> LEPELETIER, 1841	1	+	-	-	-
<i>Nomada ruficornis</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Odynerus spinipes</i> (LINNAEUS, 1758)	1	+	+	-	+
<i>Osmia adunca</i> (PANZER, 1798)	1	+	-	-	-
<i>Osmia aurulenta</i> (PANZER, 1799)	1	+	+	+	-
<i>Osmia bicolor</i> (SCHRANK, 1781)	3	+	-	+	-
<i>Osmia cornuta</i> (LATREILLE, 1805)	1	+	+	-	+
<i>Osmia florissomnis</i> (LINNAEUS, 1758)	1	+	-	-	+
<i>Osmia leaiana</i> (KIRBY, 1802)	1	+	-	-	-
<i>Osmia leucomelana</i> (KIRBY, 1802)	1	+	-	-	-
<i>Osmia niveata</i> (FABRICIUS, 1804)	1	+	-	-	-
<i>Osmia rapunculi</i> (LEPELETIER, 1841)	1	+	-	-	-
<i>Oxybelus bipunctatus</i> OLIVIER 1812	1	+	-	-	-
<i>Oxybelus uniglumis</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Philanthus triangulum</i> (FABRICIUS, 1775)	1	+	+	-	+
<i>Polistes dominula</i> (CHRIST 1791)	1	+	+	-	+
<i>Sphecodes ephippius</i> (LINNAEUS, 1767)	1	+	-	-	+
<i>Sphecodes longulus</i> VON HAGENS, 1882	1	+	+	-	-
<i>Sphecodes miniatus</i> VON HAGENS, 1882	1	+	-	-	-
<i>Sphecodes puncticeps</i> THOMSON, 1872	1	+	-	-	-
<i>Sphex funerarius</i> GUSSAKOVSKIJ, 1934	3	+	+	+	+
<i>Spilomena troglodytes</i> (VANDER LINDEN, 1829)	1	+	-	-	-
<i>Tiphia minuta</i> VANDER LINDEN, 1827	1	+	-	-	-
<i>Vespula germanica</i> (FABRICIUS 1793)	3	+	+	+	-
<i>Vespula vulgaris</i> (LINNAEUS, 1758)	1	+	+	-	-
<i>Xyela menelaus</i> BENSON 1960	2	+	+	-	+
Lepidoptera					
<i>Agrotis exclamationis</i> (LINNAEUS, 1758)	1	+	+	+	+
<i>Axylia putris</i> (LINNAEUS, 1761)	1	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Charanyca trigrammica</i> (HUFNAGEL, 1766)	1	+	+	+	-
<i>Eilema lurideola</i> ([ZINCKEN], 1817)	1	+	+	-	-
<i>Hadena rivularis</i> (FABRICIUS, 1775)	1	+	+	-	-
<i>Hoplodrina octogenaria</i> (GOEZE, 1781)	1	+	+	+	-
<i>Ligdia adustata</i> ([DENIS & SCHIFFERMÜLLER], 1775)	1	+	+	-	-
<i>Thyatira batis</i> (LINNAEUS, 1758)	1	+	-	-	-
<i>Trachea atriplicis</i> (LINNAEUS, 1758)	1	+	+	+	-
<i>Xestia c-nigrum</i> (LINNAEUS, 1758)	1	+	+	+	-
<i>Xestia triangulum</i> (HUFNAGEL, 1766)	1	+	-	-	-
Neuroptera					
<i>Chrysopa perla</i> (LINNAEUS, 1758)	0,3	+	+	-	+
<i>Chrysopa phyllochroma</i> WESMAEL, 1841	0,3	+	+	-	+
<i>Chrysoperla carnea</i> (STEPHENS, 1836)	1	+	-	+	-
<i>Coniopteryx borealis</i> TJEDER, 1930	1	+	-	-	+
<i>Coniopteryx haemata</i> McLACHLAN, 1868	0,3	+	-	-	-
<i>Conwentzia pineticola</i> ENDERLEIN, 1905	0,3	+	-	-	+
<i>Parasemidalis fuscipennis</i> (REUTER, 1894)	0,3	+	-	-	-
Orthoptera					
<i>Tetrix tenuicornis</i> (SAHLBERG, 1893)	1	+	+	+	+
Psocoptera					
<i>Loensia fasciata</i> (FABRICIUS, 1787)	0,3	+	+	-	-
Raphidioptera					
<i>Dichrostigma flavipes</i> (STEIN, 1863)	1	+	+	+	+
Trichoptera					
<i>Agraylea sexmaculata</i> CURTIS, 1834	1	+	-	-	+
<i>Drusus annulatus</i> (STEPHENS, 1837)	1	+	+	-	+
<i>Ecnomus tenellus</i> (RAMBUR, 1842)	1	+	-	-	+
<i>Goera pilosa</i> (FABRICIUS, 1775)	1	+	+	+	+
<i>Hydroptila forcipata</i> (EATON, 1873)	1	+	+	-	-
<i>Hydroptila simulans</i> MOSELY, 1920	1	+	-	-	-
<i>Hydroptila sparsa</i> CURTIS, 1834	1	+	-	-	-
<i>Lepidostoma basale</i> (KOLENATI, 1848)	1	+	-	-	-

	Num.	RSA	CA	CA (2)	CB
Araneae					
<i>Psychomyia pusilla</i> (FABRICIUS, 1781)	1	+	-	-	-
<i>Rhyacophila nubila</i> (ZETTERSTEDT, 1840)	1	+	+	-	-
<i>Sericostoma schneideri</i> KOLENATI, 1848	1	+	-	-	-
<i>Silo piceus</i> BRAUER, 1857	1	+	-	-	-

Appendix 2

Methodology Company A (anonymized): Sample preparation and DNA extraction

The mixed samples were divided into two size classes (< 6.5 mm and > 6.5 mm) using analysis sieves, and each fraction was mechanically homogenized separately. From the two ethanol-suspended fractions, 2 ml per fraction were mixed, centrifuged, and dried. The samples were then incubated overnight at 56°C in a solution of 5 ml T1 lysis buffer (Macherey-Nagel, Düren, Germany) and 0.1 ml proteinase K. DNA from the lysates was purified using the NucleoSpin® Tissue Kit (Macherey-Nagel, Düren, Germany).

DNA metabarcoding

PCR and generation of Illumina libraries

A 313-bp fragment of the mitochondrial cytochrome c oxidase gene (COI) was amplified by PCR for each sample using the primers mlCOIintF (LERAY et al. 2013) and jgH-CO2198 (Geller et al., 2013). Each primer was tagged with overhanging Illumina® adapters at the 5' position. The quality of the PCR products was visually checked by gel electrophoresis on a 2% agarose gel stained with GelRed® (Biotium Inc., Fremont, CA, USA). Illumina® libraries were generated using a second PCR to attach dual indices and Illumina® sequencing adapters to the PCR products. The concentration of each PCR product was measured using a fluorometric assay (QuantiFluor®, Promega GmbH, Waldorf, Germany), and the libraries were subsequently mixed in equimolar concentrations. The pooled libraries were sequenced using an Illumina® MiSeq platform (2×250 bp paired-end mode).

Data analysis

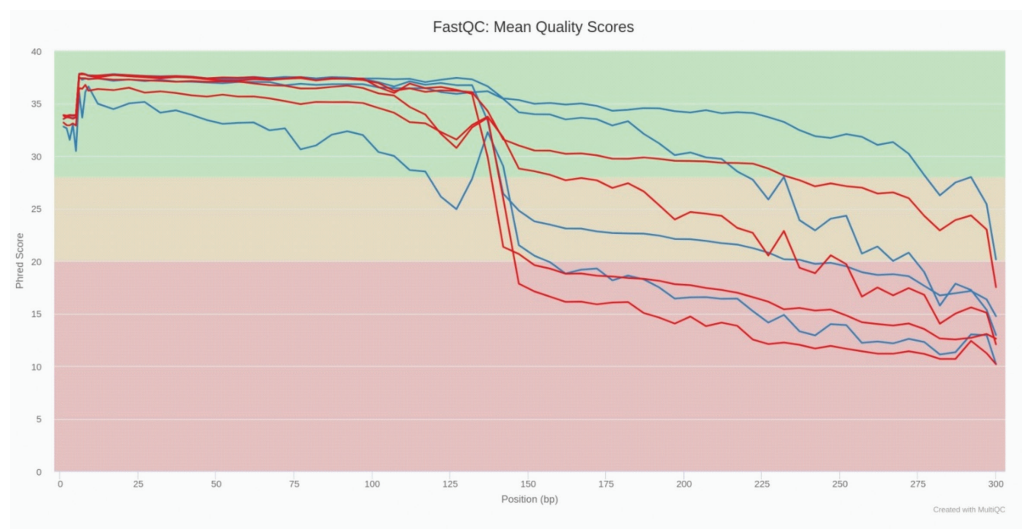
Bioinformatic analysis was performed using the “vsearch” program (Rognes et al., 2016). Paired-end sequences with a minimum overlap of 200 bp were merged, while all remaining unmerged sequences were discarded. Sequences containing at least one unknown base (N) were removed in the filtering step, and identical sequences were merged. Potential chimeric sequences (artifact sequences derived from multiple source sequences) were identified and removed from the sample. The remaining sequences were clustered into Operational Taxonomic Units (OTUs) using a threshold of 3%.

Taxonomic assignment

The OTUs were assigned to taxonomic units by matching them with the available sequences in the BOLD reference database. Only OTUs that were taxonomically assigned with a similarity of over 95% were used (see Table 1). Due to the genetic variability within species, multiple OTUs can be assigned to the same species.

Methodology Company B (anonymized)

An DNA metabarcoding analysis using NGS was performed as shown here. An initial analysis with FastQC Version 0.11.9 revealed that the reads exhibited the following sequence quality scores:



RESULTS

Merging and primer removal (trimming)

The total number of sequences obtained from the sequencer was 146,350 (paired-end) or 292,700 in total. Paired-end merging was performed using “usearch v11.0.667” with parameters “fastq_maxdiffs 99 fastq_pctid 75 -fastq_truncail”. Across the samples, a median of 97.92% of reads were successfully merged (mean 97.94%, minimum 96.94%, maximum 98.98%). Primer removal (trimming) was performed using “cutadapt 3.7 with Python 3.9.12”. Adapters were detected in a median of 99.9% of forward reads and 93.5% of reverse complement reads.

Quality filtering and dereplication

In the next step, we retained sequences above the minimum length of 300 and with a maximum expected error of one. From these, we filtered out duplicate sequences and singletons. Quality filtering was performed using vsearch 2.15.2 with parameters “--fastq_minlen 300 --fastq_maxee 1”. After quality filtering, a total of 63,065 sequences were retained, and 79,301 were discarded. Dereplication was performed, resulting in 45,505 unique sequences from all samples. After dereplication, a total of 3,065 unique non-singleton sequences were obtained.

Clustering and chimera detection

To save computational resources, we performed clustering before removing chimeras. Clustering was done using Swarm 3.1.0 with parameters “-d 13 --usearch-abundance”. Prior to chimera detection, we had a total of 645 OTUs. Chimeras were detected using vsearch 2.15.2 with parameters “--uchime_denovo --nonchimeras”. After de novo chimera detection, we had 523 OTUs, of which 428 showed matches in the databases after OTU table cleanup.