Introduction

To comprehensively assess the biodiversity of a certain location, knowing which species, both native and invasive, exist in that area is essential. However, differentiating between closely related species is often challenging and slips under the radar, possibly causing two slightly different species to be classified as the same type of organism (Deeward, 2011). This leads researchers to either limit their taxonomic scope or to only identify certain species in a higher, less specific, taxonomic category. However, in recent years, a new technology called DNA barcoding has had a high success rate in identifying and differentiating between similar species.

While individually barcoding DNA from different species has been successful, the cutting-edge environmental DNA (eDNA) metabarcoding method promises to rapidly and simultaneously barcode all organisms in a community. Environmental DNA is genetic material that is released into the environment by an organism (Pilliod et al., 2012). Examples of this include shed skin, feces, whole organisms (microscopic), or decaying remains. eDNA represents a major breakthrough in science, since it allows for a cost effective and quick way to identify all species in a community through one sample and protocol. However, while eDNA has appealing features, a major consideration relates to the question of accuracy of specific identification. In aquatic ecosystems, eDNA can be collected through water filtration, DNA isolation, and then amplified for specific genetic markers (COI and 18S) with the polymerase chain reaction (PCR) (Moyer et al., 2014). Then samples are sequenced for metabarcoding using Mi-Seq technologies from Illumina. This ability suggests that eDNA can be used to document community membership within aquatic environments, but the technique has yet to be accepted widely throughout the scientific community.

The major aim of this study was to discover if eDNA provides an accurate representation of an ecosystem's taxonomic profile. By barcoding individual organisms collected from seven ponds where eDNA samples were already sequenced, we tested the accuracy of eDNA results. We hypothesized that the single sample identifications would match the eDNA water samples, thus supporting the notion that eDNA is a reliable source for identifying multiple species within an entire ecosystem.

Objective

The purpose of the experiment was to analyze the accuracy of eDNA testing on water samples from various ponds on Staten Island. In doing so we used DNA barcoding methods to identify arthropods sampled from the ponds and then compared these results to the eDNA arthropod data obtained from the same ponds.

Methods

Our sample size consisted of 50 organisms, which were all collected by a field research team from the College of Staten Island led by our co-mentor Seth Wollney. The seven ponds tested were: Long Pond (Long Pond Park), Sharrotts Pond (Claypit Pond State Park Reserve), B1, B2, and C2 rainwater basins (Freshkills Park), Walker Pond, and Pumphouse Pond (High Rock Park).

The samples were collected during the summer of 2015 using a **timed dip-net sampling method.**

Individual organisms were then stored in 1.5 milliliter cryovials in 95% ethanol and frozen until identification.

We used the **cytocrome-oxidase I** (COI) to isolate the 720 base pair fragments of DNA. The eDNA samples were isolated at the 18S primer location which is a shorter fragment of 150 base pairs.

We performed **Polymerase Chain Reactions (PCRs).**

Once the DNA was isolated, the barcoding samples were sent for sequencing at Genewiz. Editing, BLASTing, and annotation of these sequences was conducted using **DNA Subway.**

We searched eDNA results to check if species identified through single organism barcoding were detected in the metabarcoding procedures. We observed whether the individual organisms identified through extraction were present in the eDNA results at the order, family, genus, and species level.

Results

Out of the 50 samples identified through DNA barcoding, 37 (74%) were from Orders detected by eDNA . As the taxonomic level examined became more specific, the percent similarity between the DNA barcoding results and the eDNA data decreased. The percentages of barcode identifications detected by eDNA were 28% at the family level, 21% at the genus level, and 12.5% at the species level. For ten samples, either one or more taxonomic levels were unavailable or the BLAST provided an identification that was not specific to a certain species or genus. In these situations, the samples were left blank in the comparison chart in Appendix A and not included in calculation of the percentage of sequences present at that specific taxonomic level.

Discussions

Analysis of the eDNA identification showed a low success rate at taxonomic levels below Order, suggesting that the eDNA method was not as accurate and comprehensive as single- species barcoding was for this experiment. For these macro-invertebrates, the results suggest that the 18S primer does not work well in identifying arthropods using eDNA. It is also possible that the 18S primer was too short to react with the denatured DNA. In future studies, other genetic markers should be considered when trying to detect macro-invertebrates from water samples. The low percent similarity between the eDNA results and the single sample DNA barcoding results may also have occurred due to a lack of DNA present in the water samples collected. If the organisms do not shed enough cells into the environment, they will not be detected by the eDNA test. However, it is also possible that DNA was present in the water sample, but eDNA testing is not specific enough to attain barcodes of multiple species from one aquatic environmental sample. Future studies should be conducted in order to test the accuracy of eDNA further.

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Ground Truthing Environmental DNA Arthropod Profiles Through Single Species Barcoding

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Abstract

In order to test the reliability of invertebrate biodiversity assessment using environmental DNA (eDNA), or DNA collected directly from water, we compared these results to single organism DNA barcodes. Environmental DNA may be a more comprehensive, cost effective and less time consuming method of detecting organisms present in an ecosystem. However, it may not be as accurate as the individual extraction method of barcoding. The samples of water used for ground truthing eDNA were collected from ponds on Staten Island during the summers of 2014 and 2015 and sequenced at the 18S primer location. The individual arthropods collected had DNA extracted using Qiagen kits, followed by polymerase chain reactions, and sequencing of the barcodes at the cytochrome oxidase 1 gene. A total of 50 samples were analyzed and compared to the eDNA results to determine similarity between the two methods of identification. Based on this comparison, we found that the eDNA had a low success rate of identification for this set of macro-invertebrates. We concluded that the invertebrates were not detected using eDNA. This was either due to lack of genetic material in the water samples or the inability of the 18S primer to sequence with the denatured DNA.

DNA Barcode Genetic ID	DNA Barcode Genetic ID Order	DNA Barcode Genetic ID Common Name	eDNA Order	eDNA Family	eDNA Genus	eDNA Species
Physella acuta	Gastropoda	Bladder Snail	Present	Present	Present	Present
Chironomidae ablabesmyia	Diptera	Nonbiting Midge	Present	Present	Present	
Atrichopogon fusculus	Diptera	Biting Midge	Present	Absent	Absent	Absent
Caenis	Ephemeroptera	Mayfly	Present	Present	Present	Present
Physella acuta	Lepidoptera	Bladder Snail	Present	Present	Present	Present
Chironomidae	Diptera	Nonbiting Midge	Present	Present		
Culicoides	Diptera	Biting Midge	Present	Absent	Absent	Absent
Chironomidae	Diptera	Nonbiting Midge	Present	Present		
Ischnura verticalis	Odonata	Eastern Forktail	Present	Absent	Absent	Absent
Chironomus	Diptera	Nonbiting Midge	Present	Present	Present	
Limnodrilus hoffmeisteri	Tubificida	Aquatic Worm	Absent	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Chironomidae	DIptera	Nonbiting Midge	Present	Present		
Physa heterostropha (synonym for Physella acuta)	Lepidoptera	Bladder Snail	Present	Present	Present	Present
Lymnaea humilis	Pulmonata	Large Pond Snail	Absent	Absent	Absent	Absent
Rotaria rotatoria	Bdelloidea	Bdelloid rotifer	Absent	Absent	Absent	Absent
Belostoma flumineum	Hemiptera	Giant Water Bug	Present	Absent	Absent	Absent
Rhopalosiphum nymphaeae	Hemiptera	Waterlily Aphid	Present	Absent	Absent	Absent
Hesperocorixa interrupta	Hemiptera	Water Boatman	Present	Absent	Absent	Absent
Enallagma	Odonata	Bluet	Present	Absent	Absent	Absent
Culicoides immaculatus	Diptera	Biting Midge	Present	Absent	Absent	Absent
Caenis diminuta	Ephemeroptera	Mayfly	Present	Present	Present	Absent
Arrenurus	Acarina	Water mite	Absent	Absent	Absent	Absent
Caenis diminuta	Ephemeroptera	Mayfly	Present	Present	Present	Absent
Arrenurus	Acarina	Water mite	Absent	Absent	Absent	Absent
Chironomidae	Diptera	Nonbiting Midge	Present	Present		
Lecane closterocera	Ploima	Worm	Absent	Absent	Absent	Absent
Chaoboridae	Diptera	Phantom Midges	Present	Absent	Absent	Absent
Piona	Trombidiformes	Mite	Absent	Absent	Absent	Absent
Laccophilus	Coleoptera	Water Beetle	Absent	Absent	Absent	Absent
Oecetis inconspicua	Trichoptera	Caddisfly	Absent	Absent	Absent	Absent
Enallagma	Odonata	Bluet	Present	Absent	Absent	Absent
Galerucella nymphaeae	Coleoptera	Water Lily Beetle	Absent	Absent	Absent	Absent
Anopheles melas	Diptera	Mosquito	Present			
Oxyopes sertatus	Acarina	Lynx Spider	Absent	Absent	Absent	Absent
Ahamus yunnanensis	Lepidoptera	Moth	Absent	Absent	Absent	Absent
Physella acuta	Gastropoda	Freshwater Snail	Present	Present	Present	Present
Polistes dominula	Hemiptera	Wasp	Absent	Absent	Absent	Absent
Dysdercus cingulatus	Hemiptera	Red Cotton Bug	Present	Absent	Absent	Absent
Anopheles punctulatus	Diptera	Mosquito	Present			
Anopheles punctulatus	Diptera	Mosquito	Present			
Lucilia cuprina	Diptera	Blowfly	Present	Absent	Absent	Absent
Lucilia sericata	Diptera	Common Green Bottle Fly	Present	Absent	Absent	Absent
Anopheles darlingi	Diptera	Mosquito	Present			

Figure 1. A comparison between the individual DNA barcode BLAST results and the eDNA sample detections.

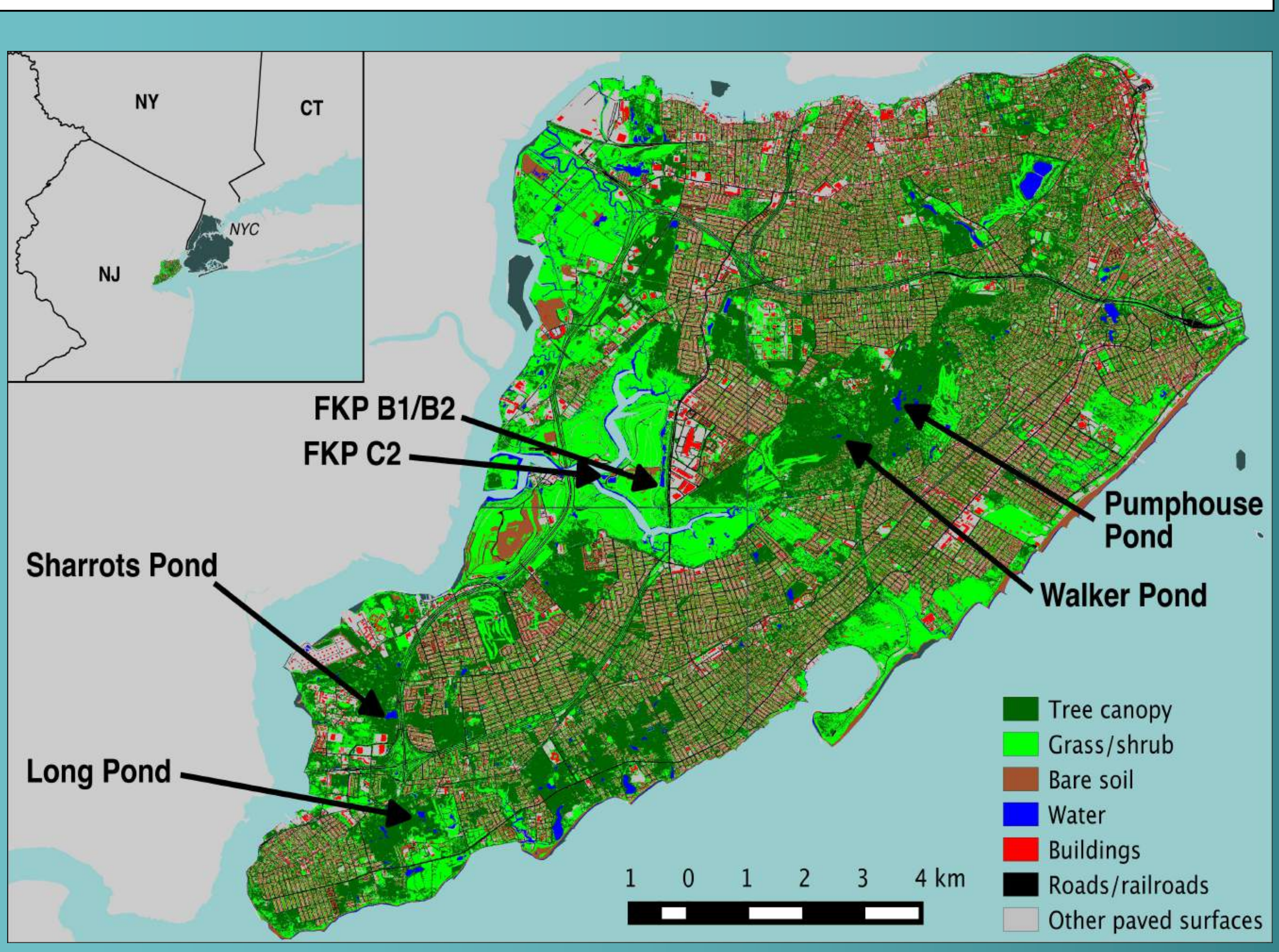

Figure 2. Map of the seven Staten Island ponds used for sampling, courtesy of Anthony Cak, ASRC – CUNY.

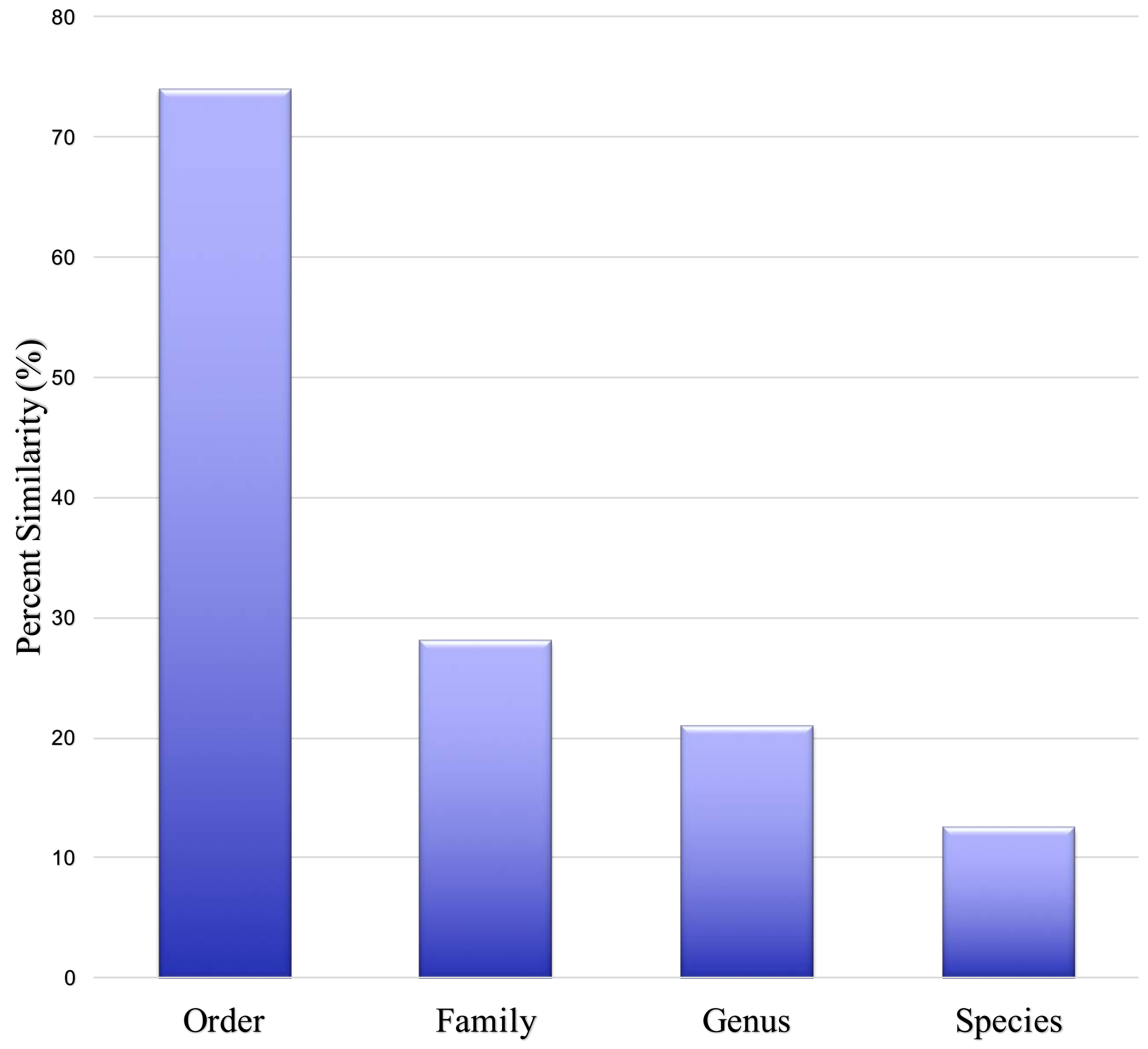
Comparative Analysis of eDNA and Barcodes

Figure 3. A bar graph depicting the percentage of barcodes present in both the eDNA results and the barcode identifications.