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RESEARCH ARTICLE

Relationship of taxonomic error to frequency of observation

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Abstract

Biological nomenclature is the entry point to a wealth of information related to or associated with living entities. When applied accurately and consistently, communication between and among researchers and investigators is enhanced, leading to advancements in understanding and progress in research programs. Based on freshwater benthic macroinvertebrate taxonomic identifications, inter-laboratory comparisons of >900 samples taken from rivers, streams, and lakes across the U.S., including the Great Lakes, provided data on taxon-specific error rates. Using the error rates in combination with frequency of observation (FREQ; as a surrogate for rarity), six uncertainty/frequency classes (UFC) are proposed for approximately 1,000 taxa. The UFC, error rates, FREQ each are potentially useful for additional analyses related to interpreting biological assessment results and/or stressor response relationships, as weighting factors for various aspects of ecological condition or biodiversity analyses and helping set direction for taxonomic research and refining identification tools.

Introduction

[1] discuss biodiversity in terms of not only richness of genotypes, species, and ecosystems, but also evenness of spatial and temporal distribution, functional characteristics, and their interactions. The sheer magnitude of biological species richness is largely unknown, with estimates ranging from 3–100 million [2–7]. For almost 300 years, efforts to organize and understand that diversity have used nomenclature and classification to provide a direct pathway to actual and conceptual catalogues of information about the biota; it is a system that can conceptually and functionally be thought of as a card catalogue in a library. With growing acceptance of the reality of global change and degradation in climate and both small- and large-scale habitat, along with diminishing taxonomic expertise, the task to census and record biota seems ever more daunting and urgent. Increases in computing power, information technology, and molecular techniques are encouraging some optimism in biodiversity research [8–13]. Even with some of these advances, progress in understanding biological diversity is uneven across taxonomic groups representing different segments of the tree of life, the bias mostly reflecting differential research attention and uneven sampling for some taxa in selected geographic areas [5, 14, 15].

Routine biological monitoring and assessment is about gathering representative sample data from defined habitat and using them for quantitative inference of environmental

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Competing interests: Tetra Tech, Inc., the employer of JBS and EWL, allowed some company resources to be applied to some of the data analyses and manuscript preparation. This does not alter our adherence to PLOS ONE policies on sharing data and materials. No individuals, agencies, or private firms have interests in this work relating to employment, consultancy, patents, products in development, or marketed products. conditions [16, 17]. Though such monitoring is not about documenting biodiversity or even absolute richness, the two fields rely on identical basic data as input for indicator calculations, model building, and decision-making, that is, taxonomic identifications. The name of an entity or object, whether individually or as a group or class, associates it with information on observable characteristics, provides answers to questions, and potentially allows new lines of enquiry to be framed and pursued. It is as much a truism of biological taxonomy as it is of basic human language that inconsistency in terminology impedes understanding and progress.

Historical development of biological nomenclature and classification has been considered by anthropologists as a fundamental component of language. Efforts to understand folk taxonomies have been through debating the relative merits of intellectualism vs. utilitarianism [18– 20], approximating the difference between, respectively, basic curiosity and material need. The greater frequency with which an object is observed, there is improved reliability and consistency in its recognition, potentially leading to greater refinement of naming conventions/ nomenclatural structure. In this context, it is important to define what is intended by labelling an object (or a taxon) as rare. From a theoretical perspective, rarity has been defined using niche- or phylogenetic-based concepts of abundance, distribution, rarity, or conservation priority-setting [21–24]. As an operational descriptor, rarity or relative commonness is frequency of encounter or observation.

The first principle and purpose of taxonomic identification and nomenclature is communication, and logically, objects that are more frequently observed will be recognized with increasing speed, reliability, and consistency. Biologist and ecologist perceptions of the relative rarity or commonness of taxa is a combination of life history and encounter frequency. As an example, reliability of botanical nomenclature used by the lay community in Chiapas, Mexico, was evaluated and use of plant names was found to be strongly related to cultural significance [25]. Techniques for communicating about plants with low cultural significance receiving little human attention were imprecise, that is, under-differentiating. Those with moderate cultural significance had a folk taxonomy which came closer to biological taxon definitions; and the extreme, plants with a high cultural significance tended to be over-differentiated. There is a conceptual relationship between cultural significance and familiarity, the latter of which would be enhanced by a high frequency of encounters/observation.

[26] developed a system for distribution classes of benthic macroinvertebrates, based on frequency of occurrence in the Netherlands. Using a combination of species rarity or commonness in their national dataset and direct input from a group of selected taxonomists, they developed a system comprising six different classes (Table 1). One of the driving factors behind their analysis was to have a classification system that would contribute to decision-making relative to conservation of aquatic resources.

Distribution class ¹	Percentage of sites
Very rare	0-0.15
Rare	0.16-0.5
Uncommon	0.6–1.5
Common	1.6–4.0
Very common	4.1–12
Abundant	>12

Table 1. Distribution classes describing relative rarity and commonness of benthic macroinvertebrates in the Netherlands [26].

¹Class definitions are based on frequency of occurrence, calculated as the percentage of sites.

Routine taxonomic quality control (QC) analysis used by the USEPA National Aquatic Resources Surveys (NARS) and several state, regional, and local monitoring programs for benthic macroinvertebrate samples are based on direct inter-laboratory comparisons. Randomly selected samples are identified by independent taxonomists, resulting in quantitative descriptors of data quality, error rates and potential causes, and information used for formulating corrective actions. A secondary use/added benefit of these analyses is that taxon-specific error rates are produced that can be used as direct indicators of taxon uncertainty, as weighting factors during calculation of quantitative indicators, to help guide development of tools for biological monitoring, in general, and taxonomic identification, in particular. The purpose of this paper is to present the process used for deriving the uncertainty values using morphologybased taxonomic identifications, discuss and summarize the results, and provide recommendations for their application and next step analyses.

Methods

Data used in this analysis are from freshwater benthic macroinvertebrate samples, collected from rivers, streams, and lakes across the U.S., including the Great Lakes. All taxonomic identifications were executed in laboratories using necessary sample/specimen preparation techniques, optical equipment, and appropriate technical literature. The level of effort expended by taxonomists for identifications is standardized for individual programs or projects, and is typically genus level, with occasionally more coarse targets for selected taxa. The taxonomic comparison process used for routine QC analysis is described in detail elsewhere [27–29] and involves blind sample reidentification by independent taxonomists in separate laboratories of a randomly selected 10% of each sample lot.

We compiled interlaboratory comparison data for 914 samples from 10 large programs or projects (Table 2) which are conducted at selected local, regional, State, and National scales. Samples used by each of the programs for QC analyses [27, 30] were randomly selected from the full sample load of the program, typically at a rate of approximately 10%. Thus, results reported here can be considered as representative of more than 9,000 samples. There is a total

Entity	Entity Project/Program Name			
Maryland Department of Natural Resources	Maryland Biological Stream Survey (MBSS)	1995-2014	135	
Mississippi Department of Environmental Quality	Mississippi Phased Biological Monitoring	2002-2018	133	
Prince George's County (MD) Department of the Environment	Watershed-Scale Biological Monitoring and Assessment Program	2004-2017	54	
US Environmental Protection Agency/Office of Water (USEPA/OW) (National Survey)	Wadeable Streams Assessment (WSA_2004)	2004	71	
US Army Corps of Engineers-Mobile District	gineers-Mobile District Lake Allatoona/Upper Etowah River Watershed (LAUE) (GA, US)			
USEPA/OW (National Survey)	National Lakes Assessment (NLA_2007)	2007	96	
USEPA/OW (National Survey)	National Rivers and Streams Assessment (NRSA_2008)	2008	134	
EPA/OW (National Survey) National Coastal Condition Assessment (NCCA_2015) (Great Lakes only)		2015	49	
USEPA/OW (National Survey)	PA/OW (National Survey) National Lakes Assessment (NLA_2017) 2017		120	
USEPA/OW (National Survey)	National Rivers and Streams Assessment (NRSA_2018)	2018	103	
1	OTAL		914	

Table 2.	Datasets	compiled	and used	in	this anal	ysis.
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The number of samples generally represents approximately 10% of the entire sample load for each program during the indicated time period.

of 1,003 taxa, primarily at genus level (Fig 1), but also including more coarse levels because the level of effort was limited by defined standard procedures and/or poor specimen condition. Following Genus at 79.9 percent, the most frequently used categories were Family (14.6 percent), and Order and Subfamily (1.9 and 1.6 percent, respectively); other levels represent <1 percent of the dataset. There are occasionally "slash taxa", such as Cricotopus/Orthocladius (Diptera: Chironomidae), and one genus-group taxon, Thienemannimyia genus group which includes the chironomid genera Conchapelopia, Rheopelopia, Helopelopia, Telopelopia, Meropelopia, Hayesomyia, and Thienemannimyia. Truncatelloidea (Mollusca: Gastropoda) is used as a grouping for all Hydrobiidae. Two informal/undefined groupings were used: "Tubificoid Naididae" for those taxa formerly identified as Tubificidae (Oligochaeta: Haplotaxida); and Hydracarina for water mites that could not be taken to genus level.

Two different taxon-specific characteristics are quantified, frequency of observation, or relative rarity, and relative percent difference (RPD). The total number of individuals (count) for a given taxon is the sum across all primary taxonomists (T1), from all samples in all projects. That count is derived in the same manner for the QC taxonomists (T2). Frequency of observation ([FREQ] relative rarity, commonness) for a taxon is the percentage of samples for which a taxon was recorded, calculated as the number of samples in which the taxon was found relative to the total number of samples (n = 914). The number of samples for each taxon is the average between T1 and T2. We plotted numbers of taxa versus numbers of samples using logarithmic scales to illustrate the dominance of taxa observed in a single sample.

The proportional difference between two taxon-specific values is calculated using RPD [31] as an indication of the confidence with which a data user can rely on an identification result. It



Fig 1. Frequency distribution of taxa among hierarchical levels in this dataset.

is calculated as follows:

$$RPD = \left(\frac{|A - B|}{(A + B)/2}\right) * 100$$

where *A* and *B* are the numbers of individuals counted for a taxon by T1 and T2, respectively, and pooled across all samples and projects. Values range from 0, indicating perfect agreement, to 200, or perfect disagreement. A general characteristic of RPD is that low values indicate better consistency of identifications between/among taxonomists, thus conveying greater certainty than high values.

Caution is warranted in using RPD when taxon-specific *counts* are low. If either T1 or T2 recorded ≥ 1 specimen of a taxon, and the other found none (0), RPD would be 200%. Although the number itself (200) would not be informative, it would indicate that one of the taxonomists recognized individuals of a taxon where the other did not. This would be a clue that some morphological key character (and, thus, the taxon) is not being recognized, or incorrect nomenclature is being applied. Other than these cautions, low values of RPD are reliable indicators of consistency. Thus, each taxon is represented by two data values, x = RPD and y = frequency of observation (FREQ) (S1 Appendix), as input for an x:y scatterplot. We used R-script to run a nonlinear regression model relating RPD to FREQ.

Results

The first data visualization was to use a logarithmic plot of numbers of taxa versus numbers of samples (Fig 2). There are 304 taxa that are observed in only 1–2 samples, where the 33 most common taxa are found in anywhere from 200–674 samples. Seventy-five percent (75%) of the taxa were documented in \leq 20 samples. Overall distribution ranged from 200 taxa each being found once (in a single sample), to one taxon, *Polypedilum* (Diptera: Chironomidae: Chironominae: Chironomini), occurring in 674 samples.

Taxon-specific RPD plotted against FREQ (Fig 3) illustrates that most taxa have low taxonomic uncertainty (mostly identified consistently) and are relatively infrequently encountered. The best fit nonlinear regression model is given by the exponential decay equation: RPD = $22.673 + (200.498)^*e^{(-0.192*FREQ)}$, and all model terms were significant at p<0.001 (S1 Table). We delineated six uncertainty/frequency classes (UFC) based on graphic patterns (Figs 4 and 5), resulting in approximately 60% of taxa as being considered rare and identified with a high degree of certainty, that is, low RPD. All taxa are listed with associated numbers of individuals by primary and QC taxonomists, RPD, the number and percentages of samples, and UFC (S1 Appendix). Most taxa fall within UFC3 and 5 (Table 3; Figs 5 and 6), with roughly similar proportions within major taxa (Fig 7). UFC6 should be considered anomalous due to its representation by a small number of taxa (n = 6); otherwise, the mean and median values of RPD and FREQ, respectively, generally decrease and increase from UFC1-5 (Table 4, Fig 8).

We selected several taxa from each UFC (Table 5) to illustrate representative, quantitative outcomes and characteristics. UFC1 is <u>high confidence, common</u>, with representative taxa such as *Pisidium, Stenelmis, Caenis*, and *Hyalella*; overall, taxa in this class are observed in 23–74 percent of samples. Other than *Nais* with an RPD of 20.3, all other taxa in this class have RPD<10. UFC2 is <u>high confidence, moderately common</u>; overall, ranging in frequency of observation from 14–22 percent of samples, these taxa are also identified with low uncertainty (RPD, 0.3–17.6). Example taxa of this class include *Stempellinella, Baetis, Arrenurus*, and *Hemerodromia*. UFC3 groups taxa that are identified with confidence, simultaneous with being relatively rare (low frequency of occurrence) (high confidence, rare). Taxa range from



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Fig 2. Logarithmic scatterplot illustrating that most taxa in this dataset are infrequently observed.
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being observed in only a single sample (0.1 percent of total *n*), such as *Anchycteis*, *Susperatus*, *Marilia*, and *Armiger*, to just under 14 percent, 120–125 samples (*Stenonema*, *Chimarra*, *Limnesia*, *Stictochironomus*). UFC4 groups taxa that are identified with increased uncertainty and are uncommon (Fig 4) (moderate confidence, rare). RPD ranges from 55–82, and taxa represent 0.1 percent of the samples (n = 1) to 5.7 percent (n = 52). Examples of UFC4 taxa include *Halesochila*, *Vacupernius*, and *Macrelmis* from only a single sample to *Cernotina*, *Teloganopsis*, and *Micromenetus* (n = 11, 15, and 52 samples, respectively). UFC5 groups taxa that are simultaneously rare and identified with a high degree of uncertainty (low confidence, rare), with taxa being observed in from 0.1–4.3 percent of samples, and identification uncertainty ranging from 85.7–200 (S1 Appendix). Example UFC5 taxa of lowest observation frequency include *Amphicosmoecus* and *Kogotus* (n = 1 sample) to *Placobdella* and *Sphaerium* in 16 (1.8 percent) and 39 (4.3 percent) samples, respectively. UFC6 taxa are <u>outliers, mixed</u>, not clearly falling in the other classes; there are six in this dataset, three of which are genus level (*Conchapelopia*, *Thienemannimyia*, and *Dero*), and three, family (Polycentropodidae, Libellulidae, and Naididae).

Major taxa are most heavily represented in UFC3 and 5 (Table 6, Fig 6). Chironomidae (n = 104), Trichoptera (n = 72), Coleoptera (n = 68), Ephemeroptera (n = 59), and Plecoptera (n = 47), in descending order, are the top five major taxa in UFC3, while Coleoptera (n = 23),





Chironomidae (n = 21), Annelida (n = 20), Arachnida (n = 19), and Ephemeroptera and Plecoptera (tied, each n = 16) are those for UFC5.

Discussion

Taxa with the highest RPD values, that is, with greater uncertainty, are documented in smaller numbers of sites (Table 7), corresponding with very rare and rare distribution classes of [23], and clearly illustrated by UFC1-2 versus UFC4-5 (Fig 7). In general, the more rare a taxon is, the greater is the uncertainty associated with its identity; and the obverse, increasingly common taxa are better known and identified with elevated confidence. This observation is demonstrated by the near mirror images of error rate (RPD) and rarity (FREQ) for UFC1-5 (Fig 8) and reflects the outcome predicted by [25], i.e., familiarity is borne of repeated encounters. This also speaks, in part, to the collective sense of our limited understanding of biological diversity, and of the most appropriate and effective ways of communicating about that diversity.

Higher level macroinvertebrate taxa in this analysis shown to have *greater identification confidence and consistency* are midges (Insecta: Diptera: Chironomidae), caddisflies (Insecta: Trichoptera), beetles (Insecta: Coleoptera), snails (Mollusca: Gastropoda), and stoneflies



(Insecta: Plecoptera) (Fig 7), as they are mostly made up of finer level taxa within UFC1-3. Conversely, higher level taxa for which identification data seem to be *more problematic* (i.e., greater uncertainty) are bivalves (Mollusca: Bivalvia) and Crustacea (Arthropoda); these groups have a higher percentage of taxa in UFC4-5.

Several potential uses of UFC designations are relevant to informing data analysts and data users on the extent to which confidence can be placed in results. They include being used as taxon-specific weighting factors for calculating biological indicator values, such as indexes of biological integrity (IBI), River Invertebrate Prediction and Classification System (RIVPACS) models, various diversity calculations, species protection, or habitat prioritization. Testing is necessary to determine the effect on indicator values, but a weighted-average index could be formulated to elevate or restrict the importance of a taxon due to the relative potential of identification error. Similar to use of stressor tolerance values in the Hilsenhoff Biotic Index (HBI), UFC numbers could be used as taxon count modifiers. This approach would retain the inherent value and information content of organism identity, and simultaneously help objectively moderate the influence of those taxa on quantitative indicator outcomes.

Taxa demonstrated as having elevated identification uncertainty could be targeted for basic focused research, including morphological re-description, dichotomous identification keys, genetic fingerprinting, or other tools. Commonness values (FREQ) for individual taxa would



Fig 5. Distribution of taxa within uncertainty/frequency categories (UFC1-6). Uncertainty is expressed as relative percent difference (RPD) and relative rarity or commonness as frequency of observation (FREQ). Each point represents a taxon.

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allow users of comprehensive identification manuals (such as, for example, [32]) to evaluate the relative rarity. The need for independent verification of an identification result would be emphasized for those with known elevated error rates (high RPD).

UFC	No. taxa (<i>n</i>)	Percent ¹	Taxonomic certainty	Commonness	
1	30	3.0	High	Common	
2	40	4.0	High	Moderate	
3	606	60.4	High	Rare	
4	79	7.9	Moderate	Rare	
5	242	24.1	Low	Rare	
6	6	0.6	Mixed	Mixed	

Table 3. Identification uncertainty/frequency classes (UFC).

The confidence (certainty) placed in taxonomic identifications is related to both frequency of observation (commonness) and the consistency of identification. ¹Percent is the percentage of taxa relative to the overall dataset.





Another potential use of these results would be in helping target individual taxa for determining causes, beyond lack of familiarity, of higher error rates. A common cause is known to be specimens in poor condition and/or small body size (early life stages, or instars). An outcome of such an investigation might be to specify standard procedures for some taxa, including for sampling, handling, preservation, and identification. An example of this would be a requirement that all larval Chironomidae be slide-mounted for examination under a compound microscope. We do not necessarily advocate this, as slide-mounting is not consistently needed by all laboratories or taxonomists. Rather, we stress that the taxonomist use whatever method is needed to attain the target taxonomic level as defined by program or study goals. The goal in this case is not to require that all taxonomists (or taxonomic technicians) slidemount all chironomid midges; rather, the goal is to acquire genus level data for the taxon. In some cases, slide-mounting might be needed, in others, it would not. Thus, the need for such actions would be determined on a case-by-case, taxon-by-taxon, or even taxonomist-by-taxonomist basis, but the goal of genus level data remains the same.



MAJOR TAXA

Fig 7. Percentages of taxa in "major" benthic macroinvertebrate groups in six uncertainty/frequency classes. 1, high confidence, common; 2, high confidence, moderately common; 3, high confidence, rare; 4, moderate confidence, rare; 5, low confidence, rare; 6, outliers, mixed.

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UFC	RPD								
	Median	Mean	SD^1	Min^1	Max^1				
1	1.3	2.7	4.0	0	20.3				
2	2.3	4.6	5.0	0.3	17.6				
3	7.2	13.0	14.9	0	54.1				
4	66.7	67.2	6.6	54.5	82.4				
5	200	176.4	38.5	85.7	200				
6	90.7	92.4	43.7	45.7	162.2				
	FREQ								
1	31.9	35.4	11.6	22.6	73.7				
2	17.2	17.6	2.6	13.9	22.2				
3	1.2	2.9	3.5	0.1	13.7				
4	0.3	0.9	1.2	0.1	5.7				
5	0.1	0.4	0.6	0.1	4.3				
6	13.3	14.6	8.5	5.9	27.5				

Table 4. Descriptive statistics for relative percent difference (RPD) and frequency of occurrence (FREQ).

¹SD is standard deviation, Min and Max are minimum and maximum. Numbers of taxa (*n*) representing each class are given in <u>Table 3</u>.



Fig 8. Percentile distributions (boxplots) for frequency of taxon occurrence (FREQ) and relative percent difference (RPD) among the uncertainty-frequency classes. FREQ is the percentage of samples for which a taxon was observed; RPD is a measure of uncertainty associated with taxonomic identifications, thus lower values equate to increased confidence. 1, high confidence, common; 2, high confidence, moderately common; 3, high confidence, rare; 4, moderate confidence, rare; 5, low confidence, rare; 6, outliers, mixed.

Our interest is in seeing UFC values used as one tool to enhance biological assessments, whether as direct input to indicator calculations, as information to help formulate additional analytical questions, or to help set or justify interpretive procedures. This analysis was possible

UFC	Class	Order	Family	Genus	T1	T2	RPD	n	Pct.
1	Bivalvia	Veneroida	Pisidiidae	Pisidium	1772	1888	6.3	207	22.6
1	Insecta	Coleoptera	Elmidae	Stenelmis	2507	2522	0.6	248	27.1
1	Insecta	Ephemeroptera	Caenidae	Caenis	16630	16562	0.4	437	47.8
1	Malacostraca	Amphipoda	Hyalellidae	Hyalella	15844	15875	0.2	301	32.9
2	Arachnida	Trombidiformes	Arrenuridae	Arrenurus	1869	1832	2.0	169	18.5
2	Insecta	Diptera	Chironomidae	Micropsectra	2172	2211	1.8	149	16.3
2	Insecta	Ephemeroptera	Baetidae	Baetis	4221	3999	5.4	186	20.4
2	Insecta	Odonata	Coenagrionidae	Enallagma	362	431	17.4	127	13.9
3	Gastropoda	Basommatophora	Planorbidae	Gyraulus	1992	1805	9.8	125	13.7
3	Insecta	Diptera	Chironomidae	Stictochironomus	1438	1406	2.3	125	13.7
3	Insecta	Diptera	Simuliidae	Prosimulium	1363	1363	0.0	95	10.4
3	Insecta	Ephemeroptera	Leptohyphidae	Tricorythodes	3961	4001	1.0	112	12.3
4	Clitellata	Haplotaxida	Naididae	Ripistes	11	6	58.8	6	0.7
4	Crustacea	Isopoda	Asellidae	Asellus	37	19	64.3	4	0.4
4	Gastropoda	Basommatophora	Planorbidae	Micromenetus	592	284	70.3	52	5.7
4	Insecta	Plecoptera	Taeniopterygidae	Oemopteryx	24	11	74.3	6	0.7
5	Annelida	Lumbriculida	Lumbriculidae	Stylodrilus	14	79	139.8	6	0.7
5	Bivalvia	Veneroida	Sphaeriidae	Sphaerium	460	114	120.6	39	4.3
5	Insecta	Diptera	Ceratopogonidae	Mallochohelea	14	93	147.7	18	2.0
5	Insecta	Ephemeroptera	Siphlonuridae	Siphlonurus	9	26	97.1	8	0.9
6	Clitellata	Haplotaxida	Naididae	Dero	2132	3565	50.3	187	20.5
6	Insecta	Diptera	Chironomidae	Conchapelopia	120	1149	162.2	89	9.7
6	Insecta	Odonata	Libellulidae		237	64	115.0	63	6.9
6	Insecta	Trichoptera	Polycentropodidae		179	76	80.8	54	5.9

Table 5. Selected taxa as representative examples of uncertainty/frequency classes (UFC).

The full list of 1,003 taxa is presented in <u>S1 Appendix</u>. T1 and T2 are the summed counts across n samples. RPD is relative percent difference, and Pct. is the percentage of total samples (n = 914) used in this analysis.

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by having access to available output of inter-taxonomist comparisons and demonstrates added benefits of routine QC and operational data management routines.

Table 6. Numbers of taxa by uncertainty/frequency class (UFC).

"Major" taxon		TOTAL					
	1	2	3	4	5	6	
Arachnida	0	3	38	4	19	0	64
Annelida	3	2	41	6	20	2	74
Bivalvia	2	0	9	1	8	0	20
Chironomidae	15	15	104	8	21	2	165
Coleoptera	2	1	68	9	23	0	103
Crustacea	1	2	16	4	11	0	34
Ephemeroptera	1	7	59	9	16	0	92
Gastropoda	1	2	30	5	10	0	48
Plecoptera	0	0	47	6	16	0	69
Trichoptera	3	1	72	7	14	1	98
Total no. taxa	28	33	484	59	158	5	767

Nijboer and Verdo	onschot (2004)		RPD (this study)				
Distribution class	Pct. of sites	n	Median	Mean	SD	Min	Max
Very rare	<0.16	200	200.0	139.2	88.0	0	200
Rare	0.16-0.5	235	40.0	61.9	63.8	0	200
Uncommon	0.6-1.5	181	21.6	44.8	53.8	0	200
Common	1.6-4.0	151	8.5	24.1	34.9	0	180.3
Very common	4.1-12	145	4.9	13.2	23.3	0	162.2
Abundant	>12	91	2.3	7.6	13.8	0	100.7

Table 7. Relating relative percent difference (RPD) to distribution classes.

"n" is the number of taxa that would be categorized as belonging to the [26] distribution classes based on frequency of occurrence in this study.

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Supporting information

S1 Appendix. Uncertainty/frequency dataset, with benthic macroinvertebrate phylogenetic/classification hierarchy. Primary and quality control counts (T1 and T2, respectively) are cumulative across n samples, relative percent difference (RPD), percent of samples, uncertainty/frequency class (UFC), and taxonomic rank. (XLSX)

S1 Table. Nonlinear regression of FREQ against RPD. (XLSX)

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References

- Hooper DU, Chapin FS III, Ewel JJ, Hector A, Inchausti P, Lavorel S, et al. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs. 2005; 75(1):3–35.
- Erwin TL. Tropical forests: their richness in Coleoptera and other arthropod species. Coleopterists Bulletin 1982; 36:74–5.
- May RM. How many species? Philosophical Transactions of the Royal Society of London B. 1990; 330:293–304.
- Hamilton AJ, Basset Y, Benke KK, Grimbacher PS, Miller SE, Novotny V, et al. Quantifying uncertainty in estimation of tropical arthropod species richness. The American Naturalist. 2010; 176(1):90–5. https://doi.org/10.1086/652998 PMID: 20455708
- Titley MA, Snaddon JL, Turner EC. Scientific research on animal biodiversity is systematically biased towards vertebrates and temperate regions. PLoS ONE. 2017; 12(12):e0189577. https://doi.org/10. 1371/journal.pone.0189577 PMID: 29240835
- Lücking R. Three challenges to contemporaneous taxonomy from a lichen-mycological perspective. Megataxa 2020; 001(1):78–103. https://doi.org/10.11646/megataxa.1.1.16.
- Didham RK, Basset Y, Collins CM, Leather SR, Littlewood NA, Menz MHM, et al. Interpreting insect declines: seven challenges and a way forward. Insect Conservation and Diversity. 2020; 13(2):103–14. https://doi.org/10.1111/icad.12408
- Ratnasingham S, Hebert PDN. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes. 2007; 7:355–64. https://doi.org/10.1111/j.1471-8286.2007.01678.x PMID: 18784790
- Hajibabaei M, Shokralla S, Zhou X, Singer GAC, Baird DJ. Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. PLoS ONE. 2011; 6(e17497). https://doi.org/10.1371/journal.pone.0017497 PMID: 21533287
- Parr CS, Guralnick R, Cellinese N, Page RDM. Evolutionary informatics: unifying knowledge about the diversity of life. Trends in Ecology and Evolution. 2012; 27(2):94–103. https://doi.org/10.1016/j.tree. 2011.11.001 PMID: 22154516
- Meier R, Wong W, Srivathsan A, Maosheng F. \$1 DNA barcodes for reconstructing complex phenomes and finding rare species in specimen-rich samples. Cladistics. 2015; 0:1–11.
- Lim NKM, Tay YC, Srivathsan A, Tan JWT, Kwik JTB, Baloğlu B, et al. Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. Royal Society Open Science. 2016; 3(160635). <u>https://doi.org/10.1098/</u> rsos.160635 PMID: 28018653
- Patterson D, Mozzherin D, Shorthouse D, Thessen A. Challenges with using names to link digital biodiversity information. Biodiversity Data Journal. 2016; 4:e8080. <u>https://doi.org/10.3897/BDJ.4.e8080</u>
 PMID: 27346955
- Troudet J, Grandcolas P, Blin A, Vignes-Lebbe R, Legendre F. Taxonomic bias in biodiversity data and societal preferences. Nature (Scientific Reports) 2017; 7:1–14. <u>https://doi.org/10.1038/s41598-017-09084-6 PMID: 28831097</u>
- Blowes SA, Supp SR, Antão LH, Bates A, Bruelheide H, Chase JM, et al. The geography of biodiversity change in marine and terrestrial assemblages. Science. 2019; 366:339–45. https://doi.org/10.1126/ science.aaw1620 PMID: 31624208

- Paulsen SG, Peck DV, Kaufmann PR, Herlihy AT. Meeting the Spirit of the Clean Water Act, Water Quality—Science, Assessments and Policy. In: Summers K, editor. Rivers and Streams: Upgrading Monitoring of the Nation's Freshwater Resources: IntechOpen; 2020.
- Stein ED, Martinez MC, Stiles S, Miller PE, Zakharov EV. Is DNA Barcoding Actually Cheaper and Faster than Traditional Morphological Methods: Results from a Survey of Freshwater Bioassessment Efforts in the United States? PLoS ONE. 2014; 9(4):e95525. <u>https://doi.org/10.1371/journal.pone.</u> 0095525 PMID: 24755838
- Hunn E. The utilitarian factor in folk biological classification. American Anthropologist. 1982; 84(4):830– 47.
- Balée W. BOOK REVIEW: Ethnobiological Classification: Principles of Categorization of Plants and Animals in Traditional Societies. Brent Berlin. Princeton, New Jersey, Princeton University Press, 1992. Journal of Ethnobiology. 1992; 13(1):144–7.
- Brown CH, Anderson EN Jr., Berlin B, Boster JS, Schadeberg TC, Visser LE. The growth of ethnobiological nomenclature (and comments and reply). Current Anthropology. 1986; 27(1):1–19.
- 21. Yu J, Dobson FS. Seven forms of rarity in mammals. Journal of Biogeography. 2001; 27:131-9.
- Kier G, Kreft H, Lee TM, Jetz W, Ibisch PL, Nowicki C, et al. A global assessment of endemism and species richness across island and mainland regions. Proceedings of the National Academy of Sciences. 2009; 106(23):9322–7. https://doi.org/10.1073/pnas.0810306106 PMID: 19470638
- Dopheide A, Makiola A, Orwin KH, Holdaway RJ, Wood JR, Dickie IA. Rarity is a more reliable indicator of land-use impacts on soil invertebrate communities than other diversity metrics. eLife. 2020; 9 (e52787). https://doi.org/10.7554/eLife.52787 PMID: 32423527
- 24. Rosauer DF, Pollock LJ, Linke S, Jetz W. Phylogenetically informed spatial planning is required to conserve the mammalian tree of life. Proceedings of the Royal Society B. 2017; 284:20170627. <u>https://doi.org/10.1098/rspb.2017.0627</u> PMID: 29070718
- Berlin B, Breedlove DE, Raven PH. Folk taxonomies and biological classification. Science. 1966; 154:273–5. https://doi.org/10.1126/science.154.3746.273 PMID: 17810308
- Nijboer RC, Verdonschot PFM. Rare and common macroinvertebrates: definition of distribution classes and their boundaries. Archiv f
 ür Hydrobiologie 2004; 161(1):45–64. https://doi.org/10.1127/0003-9136/ 2004/0161-0045
- Stribling JB, Pavlik KL, Holdsworth SM, Leppo EW. Data quality, performance, and uncertainty in taxonomic identification for biological assessments. Journal of the North American Benthological Society. 2008; 27(4):906–19. https://doi.org/10.1899/07-175.1
- USEPA. National Rivers and Streams Assessment: Laboratory Methods Manual. Washington, DC: U. S. Environmental Protection Agency, Office of Water and Office of Research and Development, 2008 Contract No.: EPA-841-B-07-010.
- USEPA. 2012 National Lakes Assessment. Laboratory Operations Manual. Version 1.1 October 9, 2012. Washington, DC: U.S. Environmental Protection Agency, 2012 Contract No.: EPA-841-B-11-004.
- SFS-TCP. Taxonomic Certification Program: Society for Freshwater Science; 2019 [2019-12-18]. Available from: https://stroudcenter.org/sfstcp/.
- **31.** Keith LH. Environmental sampling and analysis. A practical guide. Chelsea, Michigan: Lewis Publishers; 1991.
- Merritt R, Cummins K, Berg M. An Introduction to the Aquatic Insects of North America. Fifth Edition ed. Dubuque, IA: Kendall Hunt Publishing Co.; 2019. 1498 p.