# **RESEARCH ARTICLE**



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# Using eDNA for mammal inventories still needs naturalist expertise, a meta-analysis

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#### Abstract

DNA from the environment (eDNA) has been increasingly used as a new tool to conduct biodiversity assessment. Because of its noninvasive and less time-consuming nature, many studies of recent years solely rely on this information to establish a species inventory. eDNA metabarcoding has been shown to be an efficient method in aquatic ecosystems, especially for fish. However, detection efficiency is not clear for mammals. Using the existing literature, we conducted a meta-analysis to investigate if eDNA metabarcoding allows greater detection success compared to conventional surveys (such as field surveys, camera traps, etc.). Although only 28 articles were retrieved, showing the lack of comparative studies, still representing more than 900 taxa detected, we found that detection success was method dependent, but most importantly varies on the taxonomy of the targeted taxa. eDNA metabarcoding performed poorly for bats compared to the traditional mist nests. However, strong detection overlaps were found between conventional surveys and eDNA for large-bodied mammals such as ungulates, primates, and carnivores. Overall, we argue that using both molecular and field approaches can complement each other and can maximize the most accurate biodiversity assessment and there is much room for metabarcoding optimization to reach their full potential compared to traditional surveys.

#### **KEYWORDS**

assessment, biodiversity, eDNA, environmental DNA, iDNA, mammals, meta-analysis, survey

TAXONOMY CLASSIFICATION Biodiversity ecology, Demography, Ecological genetics, Population ecology, Zoology

#### | INTRODUCTION 1

Mammals are one of the taxa undergoing drastic declines through fast-paced anthropogenic changes in the landscape, many of them being secluded in protected areas, with extreme conservation status (Benítez-López et al., 2019; Hill et al., 2020; Pacifici et al., 2020). Hence, we strongly need rapid, cost-effective, noninvasive biodiversity assessments to monitor the variation of animal communities across changing environments. To conduct such assessments, especially for mammals, conventional methods such as trapping through cages, nets, or camera, as well as field surveys, are conducted (Table 1). Those methods provide direct observations and identifications, as well as information on the studied population (reproductive status, sex, potential disease status of each individual).

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TABLE 1 Rapid description of common traditional methods for mammalian surveys.

Method	Description
Camera traps	Disposition of a set of camera over multiple locations that record according to movement, day and night. Species identification by a research team based on morphological and behavioral elements from footage.
Field surveys and transects	Visual observation on the field location on a grid and transect unit. Considering the living characteristics of terrestrial species, direct observation and traces (caves, excrement, footprints, etc.) were checked and investigated while moving. Usually conducted during the day (unlikely to detect nocturnal animals).
Field signs	Footprints and scats at sampling locations (or along transects) collected on each occasion (daily).
Areal counts	Helicopter-based, with trained observers and a data recorder.
Mist netting	Mist nets positioned across potential flight paths of bats along a transects. Set up from duck and were monitored continuously until midnight.
Cage traps	Grid composed of transects with trap stations of two cages with strict intervals. Cage traps baited and checked daily.
Pitfall traps	Approx. 100m pitfall line of plastic buckets, spaced 10m apart. Drift fences, consisting of a continuous barrier running the total length of each line, made of strips of hardware clear polyethylene clipped to vertical stakes hammered into the ground.

Such surveys have been deployed and improved over multiple decades to capture a maximum of individuals, with specificities according to each targeted taxon.

However, environmental DNA-eDNA-has emerged as a new tool to conduct biodiversity assessment over the last two decades. In this study, we will only focus on eDNA metabarcoding coined by Taberlet et al. (2012) as high-throughput multi-species detection using DNA extracted from environmental samples based on PCR and NGS technologies, intentionally excluding single-species specific detection assay via qPCR, for example, that do not fall into broad mammalian inventory assessments. On a broader scale, eDNA metabarcoding is being used to characterize past and present biodiversity patterns (Zinger et al., 2019), to understand trophic interactions and diet preferences (Galan et al., 2018) and to monitor ecosystem health and dynamics (Evrard et al., 2019). In the case of vertebrates, many different substrates have been used to establish species inventories and can be grouped into three classes. First, the eDNA group, using substrates from the animal's environment such as water (seawater, freshwater, Ficetola et al., 2008; Foote et al., 2012), soil (Andersen et al., 2012; Taberlet et al., 2012), sediments (McDonald et al., 2023; Ryan et al., 2022), and air through filters (Garrett et al., 2023; Lynggaard et al., 2022). Another type of substrate can be considered eDNA traps, as they allow DNA concentration due to their intrinsic properties, such as feces (Van Der Heyde et al., 2021; Walker et al., 2019, hair (Croose et al., 2023; Lee et al., 2016), saliva bait (Nichols et al., 2015; Piaggio et al., 2019); saltlicks (Ishige et al., 2017), vegetation (Allen et al., 2023; Van Der Heyde et al., 2021) and even spider webs (Gregorič et al., 2022). Finally, a third group of DNA originates from invertebrates-iDNA-that are ectoparasites of the targeted taxa and blood/fecal meals are used as DNA sources (Calvignac-Spencer et al., 2013). Among them are leeches (Hanya et al., 2019; Schnell et al., 2012), flies (Fernandes et al., 2023; Schubert et al., 2015), mosquitoes (Danabalan et al., 2023; Massey et al., 2022), and dung beetles (Drinkwater et al., 2021).

While at first used as a complementary method to conventional surveys, eDNA metabarcoding has become a main stand-alone technique to estimate species richness on a defined location, because this method presents multiple benefits compared to conventional surveys (Thomsen & Willersley, 2015; Ruppert et al., 2019). Briefly, sampling collection needs less manpower, taxonomic expertise, and time (linked to costs) to be implemented compared to traditional methods. Collection standardization is also possible over multiple habitat types due to the limited constraint for sample collection. eDNA metabarcoding also implies being less dependent on weather conditions or seasons for substrate access compared to traditional methods that are more dependent on logistic issues, and is completely noninvasive towards the targeted species, as it can also provide high sensitivity, especially for species with cryptic lifestyles. However, due to the PCR-based nature of eDNA metabarcoding, this method still represents a number of pitfalls and challenges (Beng & Corlett, 2020; Coissac et al., 2012; Taberlet et al., 2012; Thomsen & Willerslev, 2015). The main disadvantages are lack of laboratory access by field scientists in many countries, DNA degradation, persistence and inhibition due to its origin, state, transport and fate in the environment (Barnes & Turner, 2015); PCR and sequencing errors, as well as human-induced contamination from the field and the wet lab; the accuracy of identification being highly biased by differing quality of reference databases, and finally the lack of information on specimens (age, living or dead, hybrid, sex).

To date, it has been shown that eDNA from water is particularly efficient for aquatic ecosystems, especially amphibians, fishes and freshwater mussels (Carvalho et al., 2021; Keck et al., 2022; Svenningsen et al., 2022) due to the release of DNA through mucus secretion or free gamete and larvaes and their circulation in freshwater (Barnes & Turner, 2015). When it comes to mammals, few studies to date looked at the global detection success for eDNA metabarcoding compared to conventional methods for species richness assessment. Our goal was thus to explore the literature for comparison between eDNA metabarcoding

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and traditional survey methods and to distinguish which methods allow more taxa detection depending on method types and more particularly according to which terrestrial mammalian taxa. Because the presence of shed DNA is based on physiology, behavior, and ecology of the targeted species (Seeber & Epp, 2022), we expected that eDNA would be less effective for the detection of small mammals and Chiroptera compared to cage trapping and mist nets. We argue that the amount of DNA shed by those taxa would be in lesser amount compared to large ungulates and carnivores. Although bat DNA captured through air filters seems a promising strategy, its utilization is still at the infancy level and studies did not meet our selection criteria (Garrett et al., 2023; Johnson et al., 2023). Moreover, we hypothesized that eDNA from soil was less efficient than originating from freshwater due to the patchy distribution of mammals on land (Seeber & Epp, 2022). To verify that, we retrieved articles where a direct comparison in the same location was done between eDNA metabarcoding and conventional methods, going up to the species level, for mammals, and conducted a meta-analysis based on detection ratios and overlaps between methods.

# 2 | METHODS

# 2.1 | Data extraction

A literature search was conducted from 2005 to 2023 using Google Scholar, Pudmed, Scopus, Science Direct databases as well as in depth search on Environmental DNA and Metabarcoding and Metagenomics journals. Search terms included "(metabarcod\* OR "Environmental DNA" OR edna OR "ingested DNA" OR idna) AND (vertebrate OR mammal) AND (traditional OR conventional OR survey OR inventory)". Papers (n = 5168) were manually assessed for relevance based on the abstract to ascertain whether the study met the criteria listed in Figure 1. The following data were extracted from each accepted article: article metadata, sampling information (geographical information, taxa detected, sampling size) and methodological details about methods (length of survey, markers, technology). When extracting data for species richness, data were retrieved as followed when a study compared two methods: the number of species only detected by eDNA, the number of species only detected by a traditional method, and the number of species detected by both methods. The values were separated by taxonomical order, although identification ranged from family to species level individually. In the same way, if traditional survey methods only targeted a reduced set of taxa (bats for mist netting, traps for small mammals), no comparison was included with eDNA barcoding for other taxa when multiple methods were involved within a publication. Additionally, information about the taxa's habitat uses (arboreal, terrestrial, subterranean, aquatic, aerial) and taxonomy were verified and documented using Wilson and Mittermeier (2009).

# 2.2 | Meta-analysis of data

Following Keck et al. (2022) and Carvahlo et al. (2021), we used the log-ratio risk (LRR) as the log of (ai/n1i)/(ci/n2i), where ai and ci are the number of taxa not detected by eDNA/iDNA or conventional methods, respectively; and n1i and n2i the sample size of each of these two groups (Viechtbauer, 2010). The LRR value is above one when traditional methods detected more taxa than DNA barcoding, below one when DNA barcoding detected more taxa than traditional methods and equals one when both methods are equal. To study the proportion of overlap (PO) between methods, proportion of taxa in common for all sites between eDNA and conventional methods was estimated as a ratio of taxa detected overlapping to the total number of taxa for each publication with double arcsine transformation.

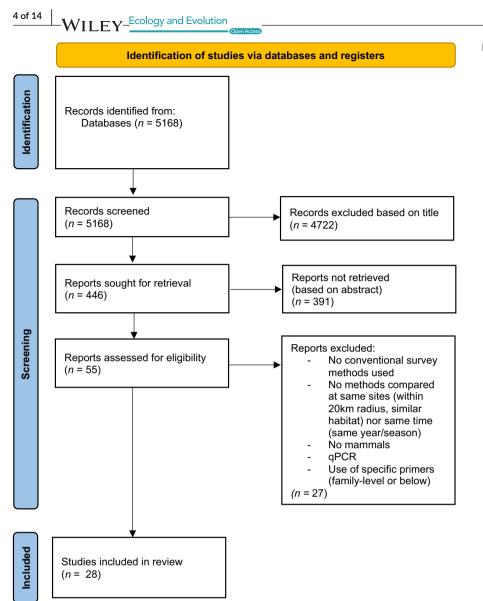
Data were grouped for the statistical analysis based on each moderator investigated and by publication. Therefore, if a study evaluated multiple methods, a single study could yield multiple groups to be included in the meta-analysis. All data analysis was conducted in R v.4.0.3 (R Core Team, 2018). An overall test for heterogeneity was conducted to inspect publication bias, as well as a funnel and Egger's test for funnel asymmetry. The Cochran's chi-squared test (Q-test), tau<sup>2</sup>, and Higgin's index ( $I^2$ ) were used to measure heterogeneities in the overall dataset and for group analyses.  $I^2$  estimates greater than 75% were considered high heterogeneities (Wang, 2018).

Random effects model estimations with restricted maximum likelihood method were used to explain the heterogeneity in effect sizes in different moderators using the *metafor* package (V3.0-2, Viechtbauer, 2010). The following moderators were inspected separately: taxa-related moderator (taxonomy, habitat use) and survey method-related moderators (sample type for eDNA, type of conventional method, reference database choice, and number of barcodes). Within study variation was assumed to be different between moderators. Each moderator was evaluated separately in univariate models, while the interaction between animal taxonomy and type of methods were being tested simultaneously in meta-regression models.

#### 3 | RESULTS

#### 3.1 | Literature search

A total of 5168 articles were identified within all databases from January 2005 to September 2023 (Figure 1). Articles were retrieved from 2022, 2021, 2020, 2019, 2018, 2017, and 2016, with a maximum of five articles for 2020. A total of 14 mammalian orders composed of 979 taxa were detected overall within the 28 articles retrieved (Appendix S1). The most detected orders (23% of the dataset) were carnivores (n=229/979), followed by rodents (21%, n=202), Artiodactyla (14%, n=138), bats (12%, n=119), marsupials



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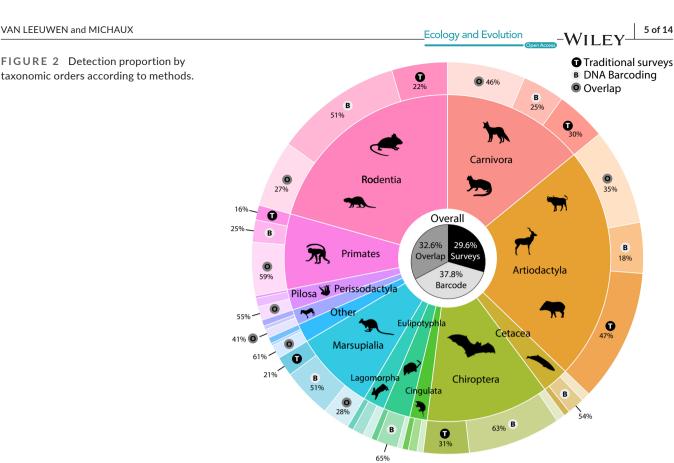
FIGURE 1 PRISMA flow diagram.

(8%, n=78), and primates (7%, n=73, Figure 2). A majority of the animals were sampled in South America (39%), followed by East Asia (14%), Africa (14%), Europe (13%), and North America (12%). In the same way, 53% of mammals detected were reported to be terrestrial, 13% arboreal, 12% aerial, and 11% semi-arboreal while semiaquatic, marine and subterranean represented less than 10% in the total dataset. Most of the dataset compared traditional surveys with eDNA coming from water samples (50.4%) and iDNA from flies, leeches, and mosquitoes (35.3%), while few involved water sediments (7%) soil (5%) and tree hollow samples (2.3%). 68% of the dataset included comparison between eDNA/iDNA against camera traps, but other conventional methods such as mist netting (5%), field surveys and transects (18%), cage trapping (3.5%), as well as pitfall traps, areal counts and trawl surveys for marine species (<3%) were also investigated. Studies used the genetic markers 12S, 16S, CO1 and cytochrome b for eDNA/iDNA barcoding, 70% of taxa investigated involved a single genetic marker, two markers for 20%

and three for 6% of the dataset, all through an Illumina sequencing platform.

# 3.2 | Overall heterogeneity assessment and publication bias

Heterogeneities in effect sizes were identified and quantified in the entire dataset for both LRR and overlap proportion (OP). The overall estimate in LRR was -0.1026 (95% CI=-0.228; 0.023) while OP overall estimate was 31.23% (95% CI=24-38%). Moderate to high overall heterogeneities were observed (LRR: Tau<sup>2</sup>=0.058; SE=0.029;  $I^2$ =59.14%; OP: Tau<sup>2</sup>=0. 0.029; SE=0.011;  $I^2$ =79.9%), with significant variation in LRR and OP between studies (LRR: Q=70.29, p<.0001; OP: Q=117, p<.0001). In the analysis of publication bias, nonsignificant asymmetries of the funnel plot were noted for LRR and OP among all mammals'



**FIGURE 3** Funnel plots for overall models of LRR and OP.

(b) (a) C 0 0.06 Standard Error 0.14 Standard Error 0.28 0.11 0.17 0.559 0.2 -1.5 -0.5 0.5 -1 0 1 0.13 0.52 0.72 0.89 0.02 0.31 Log Risk Ratio Proportion

detection (Figure 3; LRR: Egger's test: z = -1.0447, p = .2962; OP: t = 0.2243, p = .8243).

# 3.3 | Moderator analysis

### 3.3.1 | LRR – Taxa only detected by each method

When considering taxonomy of animals detected at the order level, moderator differences for estimates variation in LRR were statistically significant (Q=36, p=.0003) with moderate but significant heterogeneity ( $l^2$ =48.1%, Q=158, p<.0001). Higher LRR was observed when comparing traditional methods compared to eDNA for carnivore detection, meaning more detection through traditional methods (Table 2A). Lower LRR is also observed for aerial animals compared to others (Table 2A, Figure 4a). The type of sample for

DNA barcoding had no significant impact on LRR variation within the dataset ( $l^2$ =85.6%, Q=1.87, p=.74). According to the model, type of traditional method influenced LRR, with greater detection using eDNA compared to transects and trawl surveys (Q=15.4, p=.017, Table 2A, Figure 4b). The use of different reference databases for metabarcoding had nonsignificant impact on LRR estimates (Q=3.87, p=.276), while lower LRR estimates are observed when studies used only one barcode despite great within group heterogeneity (Q=10.3, p=.006).

# 3.3.2 | PO between methods

Variation in the estimates for PO between the two detection methods was significant according to taxonomy (Q=120, p<.0001) with moderate heterogeneity ( $l^2=56.2\%$ ). Higher OPs between

# TABLE 2 Moderator analysis results.

Interpretain </th <th>Moderator</th> <th>Subgroup</th> <th>k</th> <th>RR</th> <th>95% Cl</th> <th>Tau<sup>2</sup></th> <th>Tau</th> <th>Q</th> <th>l<sup>2</sup> (%)</th> <th>p-Value random model</th>	Moderator	Subgroup	k	RR	95% Cl	Tau <sup>2</sup>	Tau	Q	l <sup>2</sup> (%)	p-Value random model
Image: space of the stand state of the s	(A) Log risk ratio									
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Primates         11         0.9717         0.8476; 1.160         <0.0010         0.0012         1.2.2         1.8.2         0.902           Rodentia         21         0.7030         0.4803; 1.0289         0.3841         0.6213         6.425         6.49         0.679           Arboreal         13         0.7330         10.7213         0.121         0.423         6.433         6.423         6.433         6.423         6.433         6.423         6.433         6.423         6.433         6.433         6.433         6.433         6.433         6.433         6.433         6.433         6.433         6.433         6.433         6.443         6.443         6.443         6.443         6.443         6.443         6.443         6.443         6.443         6.443         6.44		Perissodactyla	10	0.9957	[0.6701; 1.4794]	0	0	4.98	0.0	.9808
Radentia210.70300.4803; 1.0290.38610.62136.4256.890.677Habitat useArboreal130.2730.283; 1.74000.1510.7972.7446.4.4471Habitat useArboreal120.8310.550; 1.25100.15100.15822.7436.4.4471Semi-aquatic120.8365; 1.25030.02500.15122.1326.15.7132.7452Semi-aquatic120.4200.02500.02500.15122.02.7132.7452Marine70.4720.47410.01000.1008.442.9.1189Harine70.6720.2517, 3.3020.40100.0108.442.9.1282Marine130.2910.2517, 3.3020.4540.4724.34.753.402Sample type for DNA barcolinInvertebrates80.2519.12710.1260.4385.701.42.929Mater130.2990.25190.4260.4385.708.14.142.919Sample type for DNA decInvertebrates120.57810.1260.4385.708.14.919Mater140.7590.25190.1260.4385.708.14.919Mater150.75990.20100.6260.78711.691.69.921Mater140.7590.63770.1610.7871.69.7221.69<		Pilosa	5	0.7827	[0.4732; 1.2947]	<0.0001	0.0016	4.53	11.6	.2479
Habitat use         Other         10         1.2739         (0.928, 1.748)         0         0         4.97         0.0         1.175           Habitat use         Arboreal         13         0.8317         (0.530; 1.2510)         0.1581         0.3976         27.54         56.4         .3447           Semi-aquatic         12         0.8398         (0.5170; 1.4300)         0.1554         0.3942         1.732         36.5         .5268           Semi-aboreal         22         0.0412         (0.7487; 1.2329)         0.2149         0.4638         160.27         7.9         .7103           Terrestrial         13         0.2416         (0.7487; 1.2329)         0.2100         0.4015         6.07         1.2         .3288           Marine         7         0.6720         (0.3936; 1.1474)         0.0100         0.0015         6.07         1.2         .3288           Semi-subterranean         7         1.267         (0.3936; 1.1474)         0.0101         0.0105         6.07         1.2         .3288           Marine Ware         13         0.2671         (0.3581         1.4757         0.4388         5.87         8.14         .919           Inverterbartes         8         9.9908		Primates	11	0.9917	[0.8476; 1.1603]	<0.0001	0.0012	12.22	18.2	.9082
Habitat use         Arboreal         13         0.8317         0.5330, 1.2510         0.1514         0.3976         27.54         5.64         .3447           Semi-aquatic         12         0.859         10.5170; 1.4300         0.1554         0.3942         17.32         3.65         .5268           Semi-arboreal         22         1.034         10.8565; 1.2502         0.0250         0.1582         2.81         7.9         .7103           Marine         7         0.672         1.3267         0.03936; 1.1474         0.0100         0.001         6.07         1.2         .3928           Semi-subterranean         7         1.326         10.5959; 3.14371         0.0100         0.001         6.07         1.2         .3928           Aerial         13         0.2916         10.241; 0.6851         1.201         1.0191         43.63         7.8         .7051           Sample type         Invertobrates         8         0.908         10.5189; 1.10731         0.1264         0.438         5.81         .1328         .1267           Mater         501         0.741; 1.2488         0.1565         0.4148         1.919         .4025         .4029           Log debris         1.0         0.6395		Rodentia	21	0.7030	[0.4803; 1.0289]	0.3861	0.6213	64.25	68.9	.0679
Semi-aquatic120.8590.5170; 1.43000.15540.39421.7326.565.268Semi-arboreal221.0340.8565; 1.25020.02500.15822.2817.97.103Terrestrial230.4210.7487; 1.23390.21490.463510.6277.937.452Marine70.6720.3936; 1.14740.01000.1008.442.8.109Arrial130.2910.21470.001110014.337.051Ontomon51.2611.0217; 6.30200.45450.6746.433.76.0101Sample type for DNA barcodingInvertebrates80.9081.0519; 1.10830.26670.51659.145.75.1022Sample type for DNA barcodingCarear traps110.7510.12410.53610.7521.2.692.1.9507Sample type for DNA barcodingCarear traps110.5091.02170.53610.78771.9.888.7.9.1402Sample type for DNA barcodingCarear traps110.5591.05160.4180.78771.9.888.7.9.1402Sample type for DNA barcodingCarear traps110.5971.04250.53610.1490.78771.9.888.7.9.2.140Sample type for DNA barcodingCarear traps10.5261.0412; 4949010.42160.14121.9.91.4.9.2.149		Other	10	1.2739	[0.9283; 1.7480]	0	0	4.97	0.0	.1175
Semi-arboreal         22         1.0348         0.8555, 1.2502         0.0250         0.1582         2.81         7.9         .7133           Terrestrial         23         0.642         0.7487, 1.2339         0.2149         0.4635         106.27         7.9.3         .7452           Marine         7         0.720         0.3936, 1.1474         0.0000         0.005         6.07         1.2         .9292           Semi-subterranean         7         1.367         10.2517, 6.3802         0.4010         0.005         6.07         1.2         .9292           Invertebrates         8         0.9206         10.2517, 6.3802         0.4545         0.4388         58.70         88.1         .9192           Sample type for DNA barcoding         Invertebrates         8         0.9806         10.5197, 1.0831         0.2667         0.1585         8.14         8.57         .4021           Sample type for DNA barcoding         Nater/Sediment         15         0.7584         [0.341; 9.4990         0.2626         0.7897         1.398         8.57         .5294           Sample type for DNA barcoding         Quetr/Sediment         1         0.5597         0.5161         -         0.00         .0205         .5294         .5294	Habitat use	Arboreal	13	0.8317	[0.5530; 1.2510]	0.1581	0.3976	27.54	56.4	.3447
Internetial         23         0.9421         0.7487; 1.2339         0.2149         0.4635         106.27         7,3         .7452           Marine         7         0.6720         0.3936; 1.1474         0.0100         0.1000         8.44         28.9         .1189           Semi-subterranean         7         1.3676         [0.5950; 3.1437]         <0.0011		Semi-aquatic	12	0.8598	[0.5170; 1.4300]	0.1554	0.3942	17.32	36.5	.5268
Marine70.67200.3936; 1.147i0.01000.10008.442.801.367Semi-subterranea71.3670.5950; 3.1437<.0001		Semi-arboreal	22	1.0348	[0.8565; 1.2502]	0.0250	0.1582	22.81	7.9	.7103
Semi-subterranean         7         1.3676         (0.595): 3.1437         <0.0011         0.0015         6.07         1.2         .3928           Aerial         13         0.2916         (0.1241; 0.6851)         1.201         1.1091         43.63         72.5         .0084           Sample type for DNA barcoding         Invertebrates         8         0.9088         (0.6519; 1.4757)         0.1926         0.4388         58.70         88.1         .9139           Mater         15         0.7584         (0.5189; 1.1083)         0.2667         0.5165         98.14         85.7         .1402           Sail         0.312         0.9590         (0.0010; .906.7766)         0.5361         0.7322         12.69         92.1         .9507           Traditional method compared         Water/Sediment         3         1.1288         (0.1341; 9.4990)         0.52361         0.7897         1.3.98         85.7         .8294           Traditional method compared         Field surveys & transects         10         0.570         [0.4025; 0.8878]         0.1400         0.3377         25.20         6.3         .0209           Traditional method compared         Field surveys & transects         1         0.6326         [0.4087         0.916         .1<472		Terrestrial	23	0.9612	[0.7487; 1.2339]	0.2149	0.4635	106.27	79.3	.7452
Aerial130.29160.1241; 0.6811.2011.10914.3.6372.5.0084Sample type for DNA barcodingInvertebrates80.56170.51520.45450.67426.4337.8.7051Sample type for DNA barcodingInvertebrates80.8080.6519; 1.47570.19260.438858.7088.19139Gater130.7540.5189; 1.10830.26670.516598.1485.7.402Soil20.5900.0010; 906.776610.53610.732212.6992.1.507Traditional method compared10.750110.341; 949900.62360.789713.9885.7.294Traditional method compared10.750210.341; 949900.62360.789713.9885.7.294Traditional method compared10.750210.341; 949900.62360.789713.98.852.294Traditional method compared10.75710.4925; 0.88780.140210.41210.00.0007.0007.0007.0007.0007.000.4723.4723Traditional method compared20.58960.0003; 1.6447: 1380.0374.6814.725.4345Traditional method compared10.43970.2254; 0.8491.0375.0341.788.873.4616Traditional method compared10.43970.5540; 1.47170.6045.62.4<		Marine	7	0.6720	[0.3936; 1.1474]	0.0100	0.1000	8.44	28.9	.1189
Unknown         5         1.2671         0.2517; 6.3802         0.4545         0.6742         6.43         37.8         7051           Sample type for DNA barcoding         Invertebrates         8         0.9808         [0.519; 1.4757]         0.1926         0.4388         58.70         88.1         9139           Soil         2         0.7584         [0.519; 1.1083]         0.2667         0.5165         98.14         85.7         1402           Soil         2         0.7590         [0.0010; 906.7766]         0.5361         0.7322         12.69         92.1         9507           Traditional         Camera traps         21         0.7500         [0.5350; 1.0514]         -         -         0.00         -         0.951           Traditional method compared         Fiel surveys         1         0.7500         [0.5350; 1.0514]         -         -         0.00         -         0.951           Traditional method compared         Fiel surveys         1         0.7500         [0.0035; 0.7815]         -         -         0.000         -         .4723           Traditional method compared         Fiel surveys         1         0.5256         [0.0003; 1.6467.2800]         -         -         0.000         -		Semi-subterranean	7	1.3676	[0.5950; 3.1437]	<0.0001	0.0015	6.07	1.2	.3928
Sample type for DNA barcoding         Invertebrates         8         0.9808         [0.6519; 1.4757]         0.1926         0.4388         58.70         88.1         .9139           Mater         15         0.7584         [0.5189; 1.1083]         0.2667         0.5165         98.14         85.7         .1402           Soil         2         0.9590         [0.0010; .906.7766]         0.5361         0.7322         12.69         92.1         .9507           Traditional method compared         Water/Sediment         3         1.1288         [0.1341; 9.4990]         0.6236         0.7897         13.98         85.7         .8294           Traditional method compared         Ing debris         1         0.7502         [0.4025; 0.8878]         0.1412         120.15         83.4         .8829           Traditional method compared         Field surveys & transects         10         0.5978         [0.4025; 0.8878]         0.1410         0.3377         25.20         64.3         .0209           Traditional method compared         Field surveys & transects         1         0.5675         [0.4005; 0.7815]         -         -         0.00         -         .4723           Gage trapping         2         2.0856         [0.0003; 16469.2182]         0.97341 <td></td> <td>Aerial</td> <td>13</td> <td>0.2916</td> <td>[0.1241; 0.6851]</td> <td>1.2301</td> <td>1.1091</td> <td>43.63</td> <td>72.5</td> <td>.0084</td>		Aerial	13	0.2916	[0.1241; 0.6851]	1.2301	1.1091	43.63	72.5	.0084
for DNA barcodingWater150.7584(0.5189; 1.1083)0.26670.516598.148.57.1402Soil20.9590(0.001); 906.7766)0.53610.732212.6921.09507Water/Sediment31.1288(0.1341; 9.4990)0.62360.789713.988.578294Log debris10.7500(0.5350; 1.0514)0.000951Camera traps210.9832(0.7741; 1.2488)0.19550.442212.0158.34.8829Field surveys & transects100.5778[0.4025; 0.8878]0.11400.337725.206.43.0209Traditional method compared100.565[0.0035; 0.7815]0.000421Areal counts10.6876[0.0000; 1.64780.79884.03.0209.0423Mist netting20.589[0.0003; 1.64792182]0.97864.05.7450.7453Reference databaseCustom70.2355(0.5003; 1.64692182]0.9384.7898.39.4616Mather Lange10.4375[0.254; 0.8491]0.00.1438Reference databaseCustom70.9383[0.554; 1.7459]0.30870.55563.7308.39.5254Mather Lange10.4395[0.601; 1.3170]0.16510.40636.2.178.2.3.5254Mather Lange1 <td></td> <td>Unknown</td> <td>5</td> <td>1.2671</td> <td>[0.2517; 6.3802]</td> <td>0.4545</td> <td>0.6742</td> <td>6.43</td> <td>37.8</td> <td>.7051</td>		Unknown	5	1.2671	[0.2517; 6.3802]	0.4545	0.6742	6.43	37.8	.7051
barcoding barcoding         Water         13         0.7364         (0.3167, 11063)         0.2367         0.3103         36.14         6.3.7         1.442           Soil         2         0.9590         [0.0010; 906.7766]         0.5361         0.7322         12.69         92.1         .9507           Water/Sediment         3         1.1288         [0.1341; 94990]         0.6236         0.7897         13.98         85.7         .8294           Log debris         1         0.7500         [0.5350; 1.0514]         -         -         0.00         -         .0951           Traditional method compared         Camera traps         21         0.9832         [0.7741; 1.2488]         0.1955         0.4422         120.15         83.4         .8829           Tradi surveys & transects         1         0.578         [0.4025; 0.8878]         0.1140         0.3377         25.20         64.3         .0209           Tradi surveys & transects         1         0.578         [0.4025; 0.8878]         0.1140         0.3377         25.20         64.3         .0209           Tradi surveys & transects         1         0.556         [0.4148; 1.1395]         -         -         0.000         -         .4723           Cage tr	Sample type	Invertebrates	8	0.9808	[0.6519; 1.4757]	0.1926	0.4388	58.70	88.1	.9139
Soil         2         0.9590         [0.0010; 906.7766]         0.5361         0.7322         12.69         92.1         9507           Water/Sediment         3         1.1288         [0.1341; 9.4990]         0.6236         0.7897         13.98         85.7         .8294           Log debris         1         0.7500         [0.5350; 1.0514]         -         -         0.00         -         .0951           Traditional method compared         Camera traps         21         0.9832         [0.7741; 1.2488]         0.1955         0.4422         120.15         83.4         .8829           Traditional compared         Field surveys & transects         10         0.576         [0.4025; 0.8878]         0.1140         0.3377         25.20         64.3         .0209           Traditional compared         Field surveys & transects         1         0.556         [0.0005; 0.7815]         -         -         0.00         -         .4723           Mist netting         2         0.5898         [0.0000; 1.1370]         0.7738         0.9868         4.47         7.6         .7435           Cage trapping         2         2.0856         [0.0003; 1.1469.2182]         0.3977         0.556         37.30         83.9         .4516		Water	15	0.7584	[0.5189; 1.1083]	0.2667	0.5165	98.14	85.7	.1402
Log debris         1         0.7500         [0.5350; 1.0514]         -         -         0.00         -         0.951           Traditional method compared         Camera traps         21         0.9832         [0.7741; 1.2488]         0.1955         0.4422         120.15         83.4         .8829           Field surveys & transects         10         0.5978         [0.4025; 0.8878]         0.1140         0.3377         25.20         64.3         .0209           Trawi surveys         1         0.6526         [0.0035; 0.7815]         -         -         0.00         -         .4723           Areal counts         1         0.6875         [0.4148; 1.1395]         -         -         0.00         -         .4723           Mist netting         2         0.8598         [0.0000; .11647.3804]         0.9738         0.9341         7.88         87.3         .4616           Cage trapping         2         2.0856         [0.2254; 0.8491]         -         -         0.00         -         .1438           Reference choice         Quistom         7         0.9355         0.540; 1.7459         0.3087         0.556         3.730         83.9         .2554           Matabase choice         Genbank	barcounig	Soil	2	0.9590	-	0.5361	0.7322	12.69	92.1	.9507
Traditional method compared         Camera traps         21         0.9832         [0.7741; 1.2488]         0.1955         0.4422         120.15         83.4         .8829           Field surveys & compared         Field surveys & transects         10         0.5978         [0.4025; 0.8878]         0.1140         0.3377         25.20         64.3         .0209           Trawl surveys         1         0.0526         [0.0035; 0.7815]         -         -         0.00         -         .0421           Areal counts         1         0.6875         [0.4148; 1.1395]         -         -         0.00         -         .4723           Mist netting         2         0.5878         [0.0000; .11647.3804]         0.9738         0.9868         4.47         7.7.6         .7435           Cage trapping         2         2.0856         [0.0003; .16469.2182]         0.8725         0.9341         7.88         87.3         .4616           Reference choice         Qustom         7         0.8355         [0.6010; 1.3170]         0.3087         0.5556         37.30         83.9         .9458           Reference choice         Gustom         7         0.8334         [0.4332; 1.3359]         0.2899         0.5384         68.17         87.3		Water/Sediment	3	1.1288	[0.1341; 9.4990]	0.6236	0.7897	13.98	85.7	.8294
method compared         Field surveys & transects         10         0.5978         [0.4025; 0.8878]         0.1140         0.3377         25.20         64.3         .0209           Trawl surveys         1         0.0526         [0.0035; 0.7815]         -         -         0.00         -         .0421           Areal counts         1         0.6875         [0.4148; 1.1395]         -         -         0.00         -         .4723           Mist netting         2         0.5898         [0.0000; .11647.3804]         0.9738         0.9868         4.47         .7.6         .7435           Cage trapping         2         0.8898         [0.0003; .16469.2182]         0.8725         0.9341         7.88         8.7.3         .4616           Pitfall traps         1         0.4375         [0.2254; 0.8491]         -         -         0.000         -         .1438           Reference         Custom         7         0.9835         [0.5540; 1.7459]         0.3087         0.5556         37.30         83.9         .9458           Genbank         12         0.8937         [0.6010; 1.3170]         0.1651         0.4063         62.26         82.3         .5254           Kobic         EMBL         8		Log debris	1	0.7500	[0.5350; 1.0514]	-	-	0.00	-	.0951
compared compared compared framework         reference database choice         reference database choice         1         0.0526         [0.4025; 0.8878]         0.1140         0.3377         23.20         84.3         0.029           Mist netting         1         0.0526         [0.0035; 0.7815]         -         -         0.00         -         .0421           Areal counts         1         0.6875         [0.4148; 1.1395]         -         -         0.00         -         .4723           Mist netting         2         0.5898         [0.0000;         0.9738         0.9868         4.47         77.6         .7435           Cage trapping         2         2.0856         [0.0003;         0.8725         0.9341         7.88         87.3         .4616           Cage trapping         2         2.0856         [0.2254; 0.8491]         -         -         0.00         -         .1438           Reference         Custom         7         0.9835         [0.5540; 1.7459]         0.3087         0.5556         37.30         83.9         .9458           Mist Dell         0.8037         [0.6010; 1.3170]         0.1651         0.4063         62.26         82.3         .5254           Mumber of borcodes used		Camera traps	21	0.9832	[0.7741; 1.2488]	0.1955	0.4422	120.15	83.4	.8829
Areal counts       1       0.6875       [0.4148; 1.1395]       -       -       0.00       -       .4723         Mist netting       2       0.5898       [0.0000; 11647.3804]       0.9738       0.9868       4.47       77.6       .7435         Cage trapping       2       2.0856       [0.0003; 16469.2182]       0.8725       0.9341       7.88       87.3       .4616         Pitfall traps       1       0.4375       [0.2254; 0.8491]       -       -       0.00       -       .1438         Reference       Custom       7       0.9835       [0.5540; 1.7459]       0.3087       0.5556       37.30       83.9       .9458         BolD       7       0.9835       [0.6010; 1.3170]       0.1651       0.4063       62.26       82.3       .5254         BolD       1       0.5517       [0.3552; 0.8569]       -       -       0.00       -       .0081         Number of barcodes used       1       0.5517       [0.5582; 1.0235]       0.2372       0.4871       148.64       .60707         Seconds       2       8       0.9841       [0.4837; 2.0024]       0.2505       0.5005       28.51       .75.5       .9591			10	0.5978	[0.4025; 0.8878]	0.1140	0.3377	25.20	64.3	.0209
Mist netting       2       0.5898       [0.0000; 11647.3804]       0.9738       0.9868       4.47       77.6       .7435         Cage trapping       2       2.0856       [0.0003; 16469.2182]       0.8725       0.9341       7.88       87.3       .4616         Pitfall traps       1       0.4375       [0.2254; 0.8491]       -       -       0.00       -       .1438         Reference database choice       Custom       7       0.8897       [0.6010; 1.3170]       0.1651       0.4063       62.26       82.3       .5254         BOLD       1       0.5517       [0.3552; 0.8569]       -       -       0.00       -       .0081         Number of barcodes used       1       0.5517       [0.552; 0.8569]       -       -       0.00       -       .0081		Trawl surveys	1	0.0526	[0.0035; 0.7815]	-	-	0.00	-	.0421
InfedT.3804]       Cage trapping       2       2.0856       [0.0003; 16469.2182]       0.8725       0.9341       7.88       87.3       .4616         Pitfall traps       1       0.4375       [0.2254; 0.8491]       -       -       0.00       -       .1438         Reference choice       Custom       7       0.9835       [0.5540; 1.7459]       0.3087       0.5556       37.30       83.9       .9458         BolDn       12       0.8034       [0.6010; 1.3170]       0.1651       0.4063       62.26       82.3       .5254         BolDn       12       0.8034       [0.4832; 1.3359]       0.2899       0.5384       68.17       89.7       .3426         Number of barcodes used       1       0.5517       [0.3552; 0.8569]       -       -       0.000       -       .0081		Areal counts	1	0.6875	[0.4148; 1.1395]	-	-	0.00	-	.4723
Pitfall traps       1       0.4375       [0.2254; 0.8491]       -       -       0.00       -       .1438         Reference database choice       Custom       7       0.9835       [0.5540; 1.7459]       0.3087       0.5556       37.30       83.9       .9458         Reference database choice       Genbank       12       0.8897       [0.6010; 1.3170]       0.1651       0.4063       62.26       82.3       .5254         BOLD       8       0.8034       [0.4832; 1.3359]       0.2899       0.5384       68.17       89.7       .3426         Number of barcodes used       14       0.5517       [0.3552; 0.8569]       -       -       0.000       -       .0081		Mist netting	2	0.5898	- /	0.9738	0.9868	4.47	77.6	.7435
Reference database choice         Custom         7         0.9835         [0.5540; 1.7459]         0.3087         0.5556         37.30         83.9         .9458           Genbank         12         0.8897         [0.6010; 1.3170]         0.1651         0.4063         62.26         82.3         .5254           EMBL         8         0.8034         [0.4832; 1.3359]         0.2899         0.5384         68.17         89.7         .3426           BOLD         1         0.5517         [0.3552; 0.8569]         -         -         0.000         -         .0081           Number of barcodes used         1         18         0.7825         [0.5982; 1.0235]         0.2372         0.4871         148.64         88.6         .0707		Cage trapping	2	2.0856	. ,	0.8725	0.9341	7.88	87.3	.4616
database choice       Genbank       12       0.8897       [0.6010; 1.3170]       0.1651       0.4063       62.26       82.3       .5254         EMBL       8       0.8034       [0.4832; 1.3359]       0.2899       0.5384       68.17       89.7       .3426         BOLD       1       0.5517       [0.3552; 0.8569]       -       -       0.000       -       .0081         Number of barcodes used       1       18       0.7825       [0.5982; 1.0235]       0.2372       0.4871       148.64       88.6       .0707		Pitfall traps	1	0.4375	[0.2254; 0.8491]	-	-	0.00	-	.1438
choice       Genbank       12       0.8897       [0.8010; 1.3170]       0.1851       0.4063       62.26       82.3       .5254         EMBL       8       0.8034       [0.4832; 1.3359]       0.2899       0.5384       68.17       89.7       .3426         BOLD       1       0.5517       [0.3552; 0.8569]       -       -       0.000       -       .0081         Number of       1       18       0.7825       [0.5982; 1.0235]       0.2372       0.4871       148.64       88.6       .0707         barcodes       2       8       0.9841       [0.4837; 2.0024]       0.2505       0.5005       28.51       75.5       .959	Reference	Custom	7	0.9835	[0.5540; 1.7459]	0.3087	0.5556	37.30	83.9	.9458
EMBL       8       0.8034       [0.4832; 1.3359]       0.2899       0.5384       68.17       89.7       .3426         BOLD       1       0.5517       [0.3552; 0.8569]       -       -       0.00       -       .0081         Number of       1       18       0.7825       [0.5982; 1.0235]       0.2372       0.4871       148.64       88.6       .0707         barcodes       2       8       0.9841       [0.4837; 2.0024]       0.2505       0.5005       28.51       75.5       .959		Genbank	12	0.8897	[0.6010; 1.3170]	0.1651	0.4063	62.26	82.3	.5254
Number of         1         18         0.7825         [0.5982; 1.0235]         0.2372         0.4871         148.64         88.6         .0707           barcodes         2         8         0.9841         [0.4837; 2.0024]         0.2505         0.5005         28.51         75.5         .959           used         3         0.9841         [0.4837; 2.0024]         0.2505         0.5005         28.51         75.5         .959	choice	EMBL	8	0.8034	[0.4832; 1.3359]	0.2899	0.5384	68.17	89.7	.3426
barcodes 2 8 0.9841 [0.4837; 2.0024] 0.2505 0.5005 28.51 75.5 .959 used		BOLD	1	0.5517	[0.3552; 0.8569]	-	-	0.00	-	.0081
used 2 6 0.7641 [0.4657; 2.0024] 0.2505 0.5005 26.51 75.5 .759	Number of	1	18	0.7825	[0.5982; 1.0235]	0.2372	0.4871	148.64	88.6	.0707
used 3 2 1.2245 [0.5693; 2.6341] 0 0 0.49 0.0 .1841		2	8	0.9841	[0.4837; 2.0024]	0.2505	0.5005	28.51	75.5	.959
	used	3	2	1.2245	[0.5693; 2.6341]	0	0	0.49	0.0	.1841

# TABLE 2 (Continued)

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Moderator	Subgroup	k	OP	95% CI	Tau <sup>2</sup>	Tau	Q	I <sup>2</sup> (%)	p-Value random mode	
(B) Overlapping p	proportion									
Таха	Artiodactyla	23	0.4923	[0.2874; 0.6981]	0.0807	0.2841	61.29	64.1	<.0001	
	Carnivora	26	0.3127	[0.2225; 0.4087]	0.0115	0.1071	34.82	28.2	.0645	
	Cetacea	5	0.1533	[0.0000; 0.6613]	0.0740	0.2721	10.97	63.5	.0626	
	Cingulata	5	0.2428	[0.0219; 0.5471]	0	0	2.05	0.0	.2261	
	Chiroptera	13	0.0000	[0.0000; 0.0107]	0.0071	0.0844	8.12	0.0	<.0001	
	Eulipotyphla	10	0.0634	[0.0000; 0.2921]	<0.0001	0.0013	8.52	0.0	.0092	
	Lagomorpha	13	0.2593	[0.0091; 0.6201]	0.0494	0.2222	16.04	25.2	.2684	
	Marsupialia	7	0.2067	[0.0012; 0.5427]	0.0593	0.2436	20.91	71.3	.0262	
	Pilosa	5	0.5641	[0.1760; 0.9198]	<0.0001	0.0001	4.28	6.5	.6116	
	Perissodactyla	10	0.4889	[0.0562; 0.9305]	0.0737	0.2714	13.87	35.1	.9791	
	Primates	11	0.5149	[0.2414; 0.7849]	0.0673	0.2594	27.04	63.0	.6060	
	Rodentia	21	0.2510	[0.1561; 0.3560]	0.0147	0.1214	32.30	38.1	.0162	
	Other	10	0.6573	[0.3024; 0.9493]	0	0	7.99	0.0	.3601	
Habitat use	Arboreal	13	0.2920	[0.1233; 0.4858]	0.0358	0.1892	28.79	58.3	.4064	
	Semi-aquatic	12	0.3895	[0.1110; 0.7003]	0.0412	0.2030	17.80	38.2	.0606	
	Semi-arboreal	22	0.4493	[0.2649; 0.6389]	0.0282	0.1678	33.61	37.5	.1332	
	Terrestrial	23	0.3582	[0.2682; 0.4528]	0.0283	0.1681	72.33	69.6	.4795	
	Marine	7	0.1435	[0.0000; 0.4804]	0.0546	0.2336	13.35	55.1	.4850	
	Semi-subterranean	7	0.1127	[0.0000; 0.5385]	0	0	4.50	0.0	.0748	
	Aerial	13	0.0000	[0.0000; 0.0107]	0.0071	0.0844	8.12	0.0	.0115	
	Unknown	5	0.0231	[0.0000; 0.2478]	0	0	1.50	0.0	.0218	
Sample type	Invertebrates	8	0.3460	[0.1635; 0.5534]	0.0501	0.2238	53.60	86.9	.4547	
for DNA	Water	15	0.2755	[0.1682; 0.3954]	0.0260	0.1613	49.92	72.0	.5737	
barcoding	Soil	2	0.4197	[0.3580; 0.4825]	0	0	0.00	0.0	<.0001	
	Water/Sediment	3	0.2834	[0.0198; 0.6629]	0.0109	0.1046	3.56	43.8	.9882	
	Log debris	1	0.5217	[0.3151; 0.7248]	-	-	0.00	-	.3214	
Traditional	Camera traps	20	0.3545	[0.2686; 0.4449]	0.0251	0.1583	68.94	72.4	.0224	
method compared	Field surveys & transects	10	0.2641	[0.1322; 0.4166]	0.0154	0.1239	16.75	46.3	.5660	
	Trawl surveys	1	0.0000	[0.0000; 0.1827]	-	-	0.00	-	.3346	
	Areal counts	1	0.1739	[0.0415; 0.3600]	-	-	0.00	-	.9792	
	Mist netting	2	0.1587	[0.0426; 0.3146]	0	0	0.02	0.0	.5923	
	Cage trapping	2	0.0764	[0.0000; 1.0000]	0.0340	0.1844	3.58	72.1	.9792	
	Pitfall traps	1	0.0455	[0.0000; 0.1849]	-	-	0.00	-	.4709	
Reference	Custom	7	0.4168	[0.2171; 0.6303]	0.0387	0.1968	28.77	79.1	.0232	
database	Genbank	12	0.2848	[0.1611; 0.4245]	0.0222	0.1489	30.31	63.7	.0805	
choice	EMBL	8	0.3188	[0.2104; 0.4375]	0.0144	0.1200	27.36	74.4	.0634	
	BOLD	1	0.0465	[0.0008; 0.1351]	-	-	0.00	-	.1897	
Number of	1	18	0.2871	[0.2127; 0.3671]	0.0176	0.1327	59.79	71.6	.1042	
barcodes	2	8	0.2602	[0.0785; 0.4903]	0.0499	0.2233	26.14	73.2	.8765	
used	3	2	0.6259	[0.0000; 1.0000]	0.0326	0.1805	4.73	78.9	.2539	

*Note*: Significant interactions (<.05) are bold in table 2A. *p*-value under p = .05 are bold in table 2B.

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methods were observed for Artiodactyla (49%), while Chiroptera and Eulipotyphyla (<10%) were significantly less detected by both methods (Table 2B, Figure 5a). Null overlapping proportions are observed when animals are aerial, compared to other habitat type (0%, Table 2B). The type of barcoding sample also significantly influenced estimates PO (Q=10.34, p=.035), with highly variable overlapping proportion when soil is used as DNA sample (42%, 95% CI=35-48). In the same way, type of traditional methods also influenced overlapping proportion of taxa detected (Q=30.5, p<.0001) with increasing proportion when researchers used camera traps. Greater overlapping detection proportions are also significantly found if a custom database is used for molecular taxonomical identification (Q=21.8, p<.0001, Table 2B), while the number of used barcodes did not significantly correlate with OP estimates (Q=5.2, p=.076).

# 3.4 | Meta-regression analysis

### 3.4.1 | LRR – taxa only detected by methods

In the multivariate analysis, 58.7% of the total heterogeneity in the dataset was accounted for (F=2.0156, p < .0001,  $l^2$ =7%). The interactions of both types of molecular and traditional detection methods as well as taxonomy of the species impacted LRR (Table 3). Strikingly, using mist nests to detect chiropters allowed significantly more taxa than eDNA (Figure 5b), as well as camera traps for carnivores and marsupials. Otherwise, eDNA extracted from water samples allows an increased rodents, marsupials and cetaceans detection compared to surveys and camera traps (Table 3; Figure 5c).

#### 3.4.2 | PO between methods

A meta-regression model for the OP of taxa detection accounted for 67.9% of the total variation within the dataset and moderator differences in OP were statistically significant (F=2.0998, p<.0001;  $l^2$ =13.4%). Greater overlapping proportion between methods was in majority directed towards large taxa such as Primates, Perissodactyla, Artiodactyla, and Carnivores (Table 4).

# 4 | DISCUSSION

An important consideration in interpreting our results is that data reviewed in this analysis are not a random sampling. We recognize that each study would have its own questions, motivations, and limitations for comparing survey methods which introduce inherent biases of these results. Moreover, the fact that only 28 articles matched the inclusion criteria shows the lack of replication for this type of comparative study in mammals, compared to other taxa present in aquatic ecosystems (Carvalho et al., 2021; Keck et al., 2022). This does, however, provide us the opportunity to identify these biases and report trends in the literature which will be crucial to evaluate which techniques allow greater species detection.

Overall, this study shows that detection success is taxonomy dependent, whether it concerns conventional or DNA-based methods. As stated by Beng and Corlett (2020), in order to enhance the detection probability, collecting biological samples should be done where the target is most likely to be detected, based on data ecology. So, it is very unlikely that one method fits all mammals, due to their extensive variation in size, physiology, behavior and ecology. In this way, it is not surprising that we found that traditional survey methods targeting specific taxa outperformed metabarcoding, especially mist nets for Chiroptera.

Despite the results obtained through our analysis, we need to recognize that data extracted from the included articles are highly biased towards camera trapping as a conventional method. Hence, we need more replication studies with other survey tools to confirm our results. However, it is logical that camera traps are a useful tool to survey carnivores due to their size and behavior (Seeber & Epp, 2022). However, because such methods are taxa targeted and that have been used and perfected for decades, it is important to acknowledge the promising future of DNA metabarcoding and that sampling methods still need standardization (Thomsen & Willerslev, 2015). All articles used the same barcodes (COI, 16S, and 12S) with almost the same primers. They also used next-generation Illumina sequencing and most of them used the same public reference databases (NCBI, EMBL, BOLD). However, custom databases yielded greater overlapping proportions of taxa detected, showing the importance of knowledge and reference barcodes of the area of interest. Moreover, it is critical to notice that sampling collection varied between studies in the number of samples, space, and time, greatly influencing taxa detection. This sampling variation is observed in our results through high between studies heterogeneity values  $(I^2)$  in pairwise moderator analysis. This shows that despite unaccounted indicator variation for moderators, we do find differences between moderators overall.

The use of iDNA for mammal inventory also seemed promising due to the facility to trap invertebrates and the DNA quality coming from blood as a substrate. However, our analysis showed that this method still needs improvements. As stated by Calvignac-Spencer et al. (2013), the host preferences of the blood-feeding invertebrates are still poorly understood, as blood meals might not reflect local host availability in density, space, and time. Overall, this method needs further studies to investigate host range variation, as it would be expected to be taxa-specific and no one invertebrate fits all.

eDNA, however, had strong detection overlaps with conventional methods for specific taxa such as ungulates and primates. This evidences that it can be an adequate method for specific uses. All surrounding over 50% of detection overlap between methods, one however does not outperform the other, but rather complements each other. Given the practicality of eDNA sampling, it is

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(a)	Habitat use	Traditional surve Events Total E		IA barcod Total	ing Risk Ratio	RR	95%–CI
	Arboreal Random effects model Heterogeneity: 1 <sup>2</sup> = 56% , t		106	132		0.83	[0.55; 1.25]
	Semi–aquatic Random effects model Heterogeneity: 1 <sup>2</sup> = 36% , t	23 38 <sup>2</sup> = 0.1554 , <i>p</i> = .10	30	38	₩	0.86	[0.52; 1.43]
	$\begin{array}{l} \mbox{Semi-arboreal} \\ \mbox{Random effects model} \\ \mbox{Heterogeneity:} \ \ \mbox{I}^2 = 8\% \ , \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $	76 107 = 0.0250 , p = .35	76	107		1.03	[0.86; 1.25]
	<b>Terrestrial</b> Random effects model Heterogeneity: 1 <sup>2</sup> = 79% , τ		352	523		0.96	[0.75; 1.23]
	Marine Random effects model Heterogeneity: 1 <sup>2</sup> = 29% , τ		32	38	-#	0.67	[0.39; 1.15]
	Semi-subterranean Random effects model Heterogeneity: $I^2 = 1\%$ , $\tau^2$	7 10 = < 0.0001 , p = .41	5	10		1.37	[0.59; 3.14]
	<b>Aerial *</b> Random effects model Heterogeneity: 1 <sup>2</sup> = 72% , τ		82	119	-0-	0.29	[0.12; 0.69]
	Undocumented Random effects model	7 12	б	12		1.27	[0.25; 6.38]
	Heterogeneity: $I^2 = 38\%$ , $\tau^2$	$p^2 = 0.4545$ , $p = .17$		1.000			
	Heterogeneity: $1^2 = 38\%$ , $\tau$ Random effects model Heterogeneity: $1^2 = 60\%$ , $\tau$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences	609 979 <sup>2</sup> = 0.1665 , <i>p</i> < .01	689 ( <i>p</i> = .04	979		00	[0.79; 1.05]
(b)	Random effects model Heterogeneity: $I^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18)	609 979 <sup>2</sup> = 0.1665 , <i>p</i> < .01	( <i>p</i> = .04 /s DN	979 <sup>4)</sup> A barcodi	Favor eDNA Favor Trac	00	ods
(b)	Random effects model Heterogeneity: $1^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences	609 979 $^{2} = 0.1665$ , $p < .01$ s: $\chi_{7}^{2} = 14.45$ , df = 7 Traditional survey Events Total E 449 658	( <i>p</i> = .04 /s DN	979 <sup>4)</sup> A barcodi	Favor eDNA Favor Trac	기 00 Jitional meth RR	ods 95%–C
(b)	Random effects model Heterogeneity: $I^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences Traditional methods Camera traps Random effects model	$609   979$ $= 0.1665, p < .01$ s: $\chi_7^2 = 14.45, df = 7$ Traditional survey Events Total E $449   658$ $= 0.0409, p = .09$ $80   181$	(p = .04 vs DN vents	979 <sup>4)</sup> A barcodi Total	Favor eDNA Favor Trac	기 00 Jitional meth RR	ods 95%–C [0.96; 1.13]
(b)	Random effects model Heterogeneity: $ ^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences Traditional methods Camera traps Random effects model Heterogeneity: $ ^2 = 11\%$ , $\tau^2$ Field surveys & transects * Random effects model	$609   979$ $= 0.1665, p < .01$ s: $\chi_7^2 = 14.45, df = 7$ Traditional survey Events Total E $449   658$ $= 0.0409, p = .09$ $80   181$	(p = .04 vs DN vents 454	979 4) A barcodi Total 658	Favor eDNA Favor Trac	□ 00 ditional meth RR 1.04	ods 95%–C [0.96; 1.13] [0.69; 0.93]
(b)	Random effects model Heterogeneity: $1^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences Traditional methods Camera traps Random effects model Heterogeneity: $1^2 = 11\%$ , $\tau^2$ Field surveys & transects * Random effects model Heterogeneity: $1^2 = 2\%$ , $\tau^2 = 10\%$	$609   979 \\ = 0.1665  , p < .01 \\ s:   \chi_7^2 = 14.45  , df = 7 \\ Traditional survey \\ Events   Total  E \\                                   $	(p = .04 vs DN vents 454	979 4) A barcodi Total 658	Favor eDNA Favor Trac	☐ 00 ditional meth RR 1.04 0.80 0.01	ods 95%-C [0.96; 1.13] [0.69; 0.93] [0.00; 0.78]
(b)	Random effects model Heterogeneity: $ ^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences Traditional methods Camera traps Random effects model Heterogeneity: $ ^2 = 11\%$ , $\tau^2$ Field surveys & transects * Random effects model Heterogeneity: $ ^2 = 2\%$ , $\tau^2 =$ Trawl surveys * Areal counts Random effects model	$609   979$ $x_7^2 = 0.1665, p < .01$ s: $\chi_7^2 = 14.45, df = 7$ Traditional survey Events Total E $449   658$ $= 0.0409, p = .09$ $80   181$ $= 0.0409, p = .44$ $11   23$ $= 0.0409, p = .34$ $19   35$	(p = .04 vs DN vents 454 157	979 4) A barcodi Total 658 181	Favor eDNA Favor Trac	00 Jitional meth RR 1.04 0.80 0.01 0.91	ods 95%-C [0.96; 1.13] [0.69; 0.93] [0.00; 0.78] [0.41; 1.99]
(b)	Random effects model Heterogeneity: $1^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences Traditional methods Camera traps Random effects model Heterogeneity: $1^2 = 11\%$ , $\tau^2$ Field surveys & transects * Random effects model Heterogeneity: $1^2 = 2\%$ , $\tau^2 =$ Trawl surveys * Areal counts Random effects model Heterogeneity: $1^2 = 12\%$ , $\tau^2$	$609   979$ $= 0.1665, p < .01$ s: $\chi_7^2 = 14.45, df = 7$ Traditional survey Events Total E $449   658$ $= 0.0409, p = .09$ $80   181$ $= 0.0409, p = .44$ $11   23$ $= 0.0409, p = .34$ $19   35$ $= 0.0409, p = .47$ $43   51$	(p = .04 vents 454 157	979 4) A barcodi Total 658 181 23	Favor eDNA Favor Trac	7 00 ditional meth RR 1.04 0.80 0.01 0.91	ods 95%-C [0.96; 1.13] [0.69; 0.93] [0.00; 0.78] [0.41; 1.99]
(b)	Random effects model Heterogeneity: $ ^2 = 60\%$ , $\tau^2$ $t_{101} = -1.34$ (p = 0.18) Test for subgroup differences Traditional methods Camera traps Random effects model Heterogeneity: $ ^2 = 11\%$ , $\tau^2$ Field surveys & transects * Random effects model Heterogeneity: $ ^2 = 2\%$ , $\tau^2 =$ Trawl surveys * Areal counts Random effects model Heterogeneity: $ ^2 = 12\%$ , $\tau^2$ Cage trapping Random effects model Heterogeneity: $ ^2 = 0\%$ , $\tau^2 =$ Mist nets Random effects model	$609   979$ $= 0.1665, p < .01$ s: $\chi_7^2 = 14.45, df = 7$ Traditional survey Events Total E $449   658$ $= 0.0409, p = .09$ $80   181$ $= 0.0409, p = .44$ $11   23$ $= 0.0409, p = .44$ $19   35$ $= 0.0409, p = .47$ $43   51$ $= 0.0409, p < .01$ $7   22$	(p = .04 vents 454 157 16 22	979 4) A barcodi Total 658 181 23 35	Favor eDNA Favor Trac	00 Jitional meth RR 1.04 0.80 0.01 0.91 0.91 2.21	ods 95%–C [0.96; 1.13] [0.69; 0.93] [0.00; 0.78] [0.41; 1.99] [0.60; 1.38]

FIGURE 4 Forest plot for LRR moderator analysis for (a) animal habitat use and (b) type of traditional survey method.

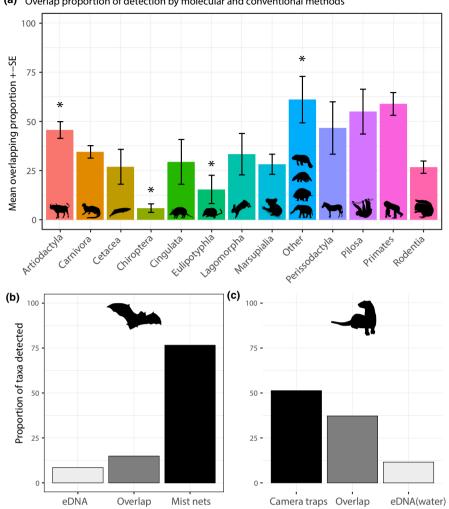


FIGURE 5 (a) Mean overlapping proportion of species detected by both methods ( $\pm$ SE) according to taxonomic order across all studies. \* Represents significant variation in the effect sizes for each taxon according to moderator analysis. (b) Variation in the proportion of species detected according to detection methods for chiropters and (c) carnivores. Overlap stands for the proportion of taxa detected by both methods.

TABLE 3 Log risk ratio meta-regression analysis results for significant interactions.

Log risk ratio			Estimate	SE	T-value	df	p-Value	CI lower bound	Cl upper bound
Intercept			-0.0885	0.2457	-0.3603	242	0.7189	-0.5726	0.3955
Barcode sample type	Traditional method	Таха							
Water	Camera traps	Carnivora	0.5047	0.2706	1.8655	242	0.0633	-0.0282	1.0377
Water	Trawl surveys	Cetacea	-2.8559	1.3032	-2.1914	242	0.0294	-5.4230	-0.2888
iDNA	Camera traps	Chiroptera	-1.8886	0.6568	-2.8753	242	0.0044	-3.1824	-0.5947
idna	Field surveys and transects	Chiroptera	-1.5209	0.8682	-1.7519	242	0.0811	-3.2310	0.1892
idna	Mist nets	Chiroptera	1.1872	0.6615	1.7946	242	0.0740	-0.1159	2.4902
Soil	Camera traps	Chiroptera	-1.6828	0.8573	-1.9628	242	0.0508	-3.3716	0.0060
Water	Camera traps	Chiroptera	-1.8008	0.7254	-2.4827	242	0.0137	-3.2297	-0.3720
Water	Field surveys and transects	Chiroptera	-1.7224	0.7015	-2.4552	242	0.0148	-3.1043	-0.3405
Water	Mist nets	Chiroptera	1.5022	0.3953	3.8000	242	0.0002	0.7235	2.2809
Soil	Camera traps	Marsupialia	0.6313	0.3630	1.7394	242	0.0832	-0.0836	1.3463
Water	Field surveys and transects	Marsupialia	-1.5787	0.6358	-2.4829	242	0.0137	-2.8312	-0.3262
Water	Field surveys and transects	Rodentia	-0.7105	0.3407	-2.0853	242	0.0381	-1.3817	-0.0393

Note: Significant interactions (<.05) are in bold. Estimates in blue translate a higher log-ratio risk, corresponding to more detection from traditional survey methods compared to eDNA, while estimates in pink translate more detection from eDNA compared to traditionals methods.

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(a) Overlap proportion of detection by molecular and conventional methods

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	Cl upper bound	0.5734		1.5416	1.5416	0.8181	0.7936	0.9750	0.7366	0.7048	1.7561	1.2401	1.2821	0.9319	0.9004	1.5416	1.7561	0.9518	1.7561	1.1342	1.5336	1.5416	1.1249	1.0776	1.3300	1.2733	0.9557	1.1167	0.6839	0.6521	
	CI lower bound	-0.0216		0.2628	0.2628	0.1407	0.0199	0.1077	0.0469	0.0637	0.0483	0.0817	0.2002	0.0871	0.0308	0.2628	0.0483	0.1662	0.0483	0.2578	0.3752	0.2628	0.1384	0.1228	0.3951	0.2868	0.2859	0.1959	0.0237	0.0040	
	<i>p</i> -Value	0.0689		0.0059	0.0059	0.0057	0.0394	0.0146	0.0262	0.0190	0.0385	0.0255	0.0074	0.0183	0.0359	0.0059	0.0385	0.0055	0.0385	0.0020	0.0013	0.0059	0.0123	0.0140	0.0003	0.0021	0.0003	0.0054	0.0358	0.0473	
	df	242		242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	242	
	t-Value	1.8269		2.7796	2.7796	2.7881	2.0711	2.4589	2.2375	2.3614	2.0813	2.2478	2.6989	2.3760	2.1094	2.7796	2.0813	2.8030	2.0813	3.1288	3.2461	2.7796	2.5226	2.4765	3.6345	3.1153	3.6511	2.8079	2.1111	1.9939	
	SE	0.1510		0.3246	0.3246	0.1719	0.1964	0.2201	0.1751	0.1627	0.4335	0.2940	0.2746	0.2144	0.2207	0.3246	0.4335	0.1994	0.4335	0.2225	0.2940	0.3246	0.2504	0.2424	0.2373	0.2504	0.1700	0.2337	0.1676	0.1645	
	Estimate	0.2759		0.9022	0.9022	0.4794	0.4067	0.5413	0.3917	0.3843	0.9022	0.6609	0.7411	0.5095	0.4656	0.9022	0.9022	0.5590	0.9022	0.6960	0.9544	0.9022	0.6317	0.6002	0.8625	0.7801	0.6208	0.6563	0.3538	0.3280	
וור ווורבו פררוחווא.			Таха	Artiodactyla	Artiodactyla	Artiodactyla	Artiodactyla	Carnivora	Carnivora	Carnivora	Eulipotyphla	Eulipotyphla	Lagomorpha	Marsupialia	Marsupialia	Other	Other	Other	Perissodactyla	Perissodactyla	Pilosa	Pilosa	Pilosa	Pilosa	Primates	Primates	Primates	Rodentia	Rodentia	Rodentia	
			Traditional method	Camera traps	Camera traps	Camera traps	Field surveys and transects	Camera traps	Field surveys and transects	Camera traps	Cage trapping	Camera traps	Camera traps	Camera traps	Field surveys and transects	Camera traps	Field surveys and transects	Camera traps	Field surveys and transects	Camera traps	Field surveys and transects	Field surveys and transects	Camera traps	Field surveys and transects	Camera traps	Camera traps					
	Overlap proportion	Intercept	Barcode sample type	Soil	Water	iDNA	Water	Soil	Water	Water	iDNA	Water	Soil	Soil	Water	Water	Soil	iDNA	Water	Water	iDNA	Water	Water	iDNA	Water	iDNA	iDNA	iDNA	iDNA	Water	

TABLE 4 OP Meta-regression analysis results for significant interactions.

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also necessary to acknowledge that conventional methods also face challenges, especially because they are time-consuming and require a lot of manpower. Most of all, they rely on taxonomic expertise which is becoming scarce (Hopkins & Freckleton, 2002; Wägele et al., 2011). However, we argue that even if taxonomists are not needed for eDNA metabarcoding sampling, they still have a crucial role, as well as their natural collections, to provide accurate identification based on barcode sequences coming from reference holotypes (De Santana et al., 2021; Paknia et al., 2015). On the way around, low detection overlaps highlight the need to use both types of methods to increase the overall success of mammalian inventories, and this is particularly the case for bats (Figure 2). The use of air filter to gather bat DNA is promising and could greatly complement mist netting (Garrett et al., 2023; Johnson et al., 2023). In the same way, the ease of sampling and high DNA yield from leaf swabs seems a promising technique for eDNA capture (Lynggaard et al., 2023), which predicts a bright future for eDNA biodiversity assessments.

Here, we want to argue that our results show that eDNA metabarcoding still needs conventional methods for cross-validation and to reduce the chance for not detecting a species. Sampling strategy for biodiversity assessment should not be overlooked and thought based on the environment's locality, as well as the targeted taxa. Moreover, using both molecular and traditional tools can be combined to expand detection success. First, in a way that one can increase the chances to find mammalian DNA by increasing the diversity of substrates (iDNA, water, soil, vegetation, feces...) that makes sense in light of the targeted ecology's DNA (Barnes & Turner, 2015). Second, if a particular taxon is targeted, a combination of both DNA and trapping can maximize detection. For example, airDNA has been shown to be a promising way of detecting bat species (Garrett et al., 2023), which is completely noninvasive, and coupled with mist net surveys, to cross-validate metabarcoding results. The "tangible proofs" coming from direct observations of specimens is still important because it is not possible to ignore the presence of eDNA in the absence of living target and the absence of eDNA in the presence of the living target without actual field surveys (Beng & Corlett, 2020). Another example of method combination for carnivores can be to use DNA traps to obtain hair and saliva from baits, as well as camera trapping. As taxonomic expertise and field experience are crucial to apprehend the challenges that one can face when sampling a location for animal detection, a greatly beneficial partnership between taxonomists, naturalists and molecular biologists has to be developed. This would also help in the implementation and enrichment of local/international reference databases, that can lack in under-sampled locations or environments.

We conclude that when it comes to biodiversity assessment, especially for mammals, there is no one size fits all, and it is up to biologists to find the appropriate threshold between molecular tools and conventional survey methods to maximize detection success. However, eDNA still being at its infancy compared to traditional survey methods, this study shows that it is a promising and powerful tool. As already discussed by Carvhalo et al. (2022), there is urgency to conduct more comparison studies between methods, as well as a need to better understand the ecology of the DNA targeted. This will allow us to further tune methods for the most accurate biodiversity assessment.

### AUTHOR CONTRIBUTIONS

**Pauline Van Leeuwen:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); software (equal); visualization (equal); writing – original draft (equal). **Johan Michaux:** Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); writing – review and editing (equal).

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#### CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

# DATA AVAILABILITY STATEMENT

Data and R script are available from the Figshare Repository: https:// figshare.com/articles/dataset/Meta-analysis\_eDNA\_vs\_convention al\_surveys/23284298.

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#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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