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Decoding diets: a novel DNA-based approach for identifying cephalopods from beaks

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Cephalopods play a key role in marine ecosystems but are difficult to study directly; hence, beaks recovered from predator stomachs are critical to further understand their distribution and ecology. Cephalopod species have traditionally been identified from beak morphology, a time-consuming method limited by degree of beak digestion, lack of specialist expertise, and limited coverage in reference collections. Here, we provide the first detailed methodology to identify cephalopod species through DNA analysis of their beaks. By extracting, amplifying and sequencing the cytochrome *c* oxidase I (COI) gene, we successfully annotated DNA sequences to species level in 50.5% of samples and 60% of beaks, obtained from predator stomachs and scientific nets. There was no statistically significant effect of beak section (untanned, tanned, rostrum tip), storage condition, or bleach treatment on assignment success (GLMM; all $p > 0.05$). Although formal statistical comparison was not possible because collection method and storage condition were confounded, success rates appeared to vary by sample origin, with lower success in samples from albatrosses (*Thalassarche* spp.) boluses than either stomachs of toothfish (*Dissostichus* spp.) or scientific nets (27.3%, 59.5% and 55.6%, respectively). Our results show that DNA analysis from cephalopod beaks can enable species-level identification, though expanding genetic databases with sequences from additional taxa and populations is crucial to improve accuracy. This approach enhances the recovery of ecological data from samples that were previously challenging to identify, supporting biodiversity monitoring, food-web research, and assessments of ecosystem change in rapidly shifting oceans.

KEYWORDS
cephalopod beaks, Cytochrome c oxidase I (COI), DNA barcoding, Marine trophic interactions, molecular identification

1 Introduction

Cephalopods play critical roles in marine ecosystems worldwide, acting as both predators and prey across a range of habitats (Clarke, 1996a; Boyle and Rodhouse, 2005; Young et al., 2013; Hoving et al., 2014; Tedesco et al., 2020). As a key link between lower and higher trophic levels, they are pivotal to the structure of many marine ecosystems (Coll et al., 2013; Young et al., 2013; Morato et al., 2016). Despite their importance, our understanding of numerous cephalopod species remains limited due to challenges related to sampling, as research cruises rarely target cephalopods, and only small to medium-sized specimens are caught in pelagic nets (Clarke, 1977; Clarke, 1996a; Xavier et al., 2018; Cherel, 2020; Xavier et al., 2022). To overcome these obstacles, predators are often used as bio-samplers, with analyses of beaks or other tissues found in diet samples allowing researchers to address diverse questions relating to cephalopod biology, ecology and biogeography (Clarke, 1996b; Cherel and Duhamel, 2004; Cherel, 2020; Xavier et al., 2022).

Cephalopod beaks are resistant to digestion, and and accumulate in predators' stomachs with up to several thousand can be found in the stomachs (Clarke, 1962; Clarke, 1980; Cherel, 2020; Xavier et al., 2022). Differences in beak morphology allow species identification by a few global experts with access to appropriate reference collections (Clarke, 1962; Iverson and Pinkas, 1971; Hotta, 1973; Wolff, 1984; Lu and Ickeringill, 2002; Xavier and Cherel, 2021). Correct identification of the species is important as incorrect assignment leads to erroneous conclusions about predator-prey dynamics, trophic ecology and stock assessments (Xavier and Cherel, 2009; Cherel, 2020; Cherel, 2021; Xavier and Cherel, 2021; Xavier et al., 2022). Different tools have been used to overcome the challenges of identifying cephalopods from beaks, including drawings, photographs and videos, summarised in identification guides (Clarke and MBA of the UKNERC (Great B., 1986; Cherel, 2020; Xavier and Cherel, 2021). New geometric morphometric methods used to identify fish otoliths for taxonomic studies and stock assessments (Claude, 2008), have also been applied to cephalopod beaks, such as analyses of Cartesian coordinates of biologically definable points (Bookstein, 1997; Cadrin, 2005), or the entire beak outline (Cadrin, 2000). However, not only are these time-consuming but success greatly depends on beak condition (Fang et al., 2017; Lishchenko and Jones, 2021).

Extracting DNA from cephalopod beaks has proven to be challenging, as beaks have a high chitin and protein content and were assumed to hold insufficient DNA for successful amplification and consequent species identification (Vecchione et al., 2009; Xavier et al., 2016; Xavier et al., 2022). This highlights the need for optimised extraction protocols suited to chitinous materials (Vecchione et al., 2009; Xavier et al., 2022). The translucent section of the beak is often the first to degrade, but this section shows the most recent growth and hence is expected to contain the highest DNA and lowest protein content (Miserez et al., 2008; Tan et al., 2015). Yet, as many beaks are retrieved from predator diet samples (*e.g.*, stomach contents, boluses and scats), having already been subjected to gastric acids as well as mechanical action before collection have already been subjected to gastric acids as well as mechanical processes before collection, removing this translucent section, authors suggest DNA extraction is unlikely to be successful (Vecchione et al., 2009; Xavier et al., 2016).

In this study we analysed beaks that had previously been identified from morphology to test methods for extracting DNA of sufficient quality and quantity to enable identification of Southern Ocean cephalopods to species level. Here, we demonstrate that the majority of cephalopod beaks can yield DNA for species-level identification. This finding offers a potentially scalable tool that would increase our ability to understand the ecosystem role of cephalopods in the changing oceans.

2 Material and methods

Beaks used in this study were extracted from cephalopod specimens caught in scientific nets during research cruises in the Scotia Sea (*e.g.*, using Rectangular Midwater Trawl 25 m^2 (RMT25)) and collected from predator stomach contents and boluses. These predators included i) grey-headed and black-browed albatrosses (*Thalassarche chrysostoma* and *T. melanophris* respectively) breeding at Bird Island - South Georgia and Diego Ramirez Islands - Chile, ii) Patagonian and Antarctic toothfish (*Dissostichus eleginoides* and *D. mawsoni*, respectively) sampled around the South Sandwich Islands and eastern Antarctica, Amundsen Sea and Ross Sea waters, and iii) macaroni penguins (*Eudyptes chrysolophus*) breeding at Bird Island - South Georgia (Supplementary Table 1). All beaks were identified morphologically by an expert prior to the DNA analysis using reference collections and published taxonomic guides (Xavier and Cherel, 2009).

We analysed a total of 103 samples from 35 beaks (3 samples per beak + 1 complete beak) (Figure 1) in this study. Samples were categorized by collection method, *i.e.*, nets (n=27) or predator stomach contents: toothfish (n=37); albatross (n=33); penguin (n=6), preservation method, *i.e.*, ethanol 70% (n=94) or ethanol 99% (n=9), and by cleaning method, *i.e.*, using bleach (n=57) or not (n=46).

In the laboratory, the beaks of whole cephalopods were separated from buccal masses. Both upper and lower beaks were used for DNA extractions. Extra cleaning was performed for 15 beaks, using 1:10 bleach (DanKlorix hygiene cleaner, contains 1-10% J sodium carbonate and 2.5-3% sodium hypochlorite solution; diluted 1:10 with distilled water). Cleaning was done in two steps: first, a swab (FLOQSwabs®) drenched in 1:10 bleach was applied for 2 min, followed by a second swab in MiliQ water for 2 min (swabs were discarded after a single use to ensure no contamination between beaks) (Figure 2). Each beak was sectioned into three different regions – B1 (the tip of the rostrum); B2 (the lateral wall and the hood/tanned section), and B3 (the free corner of the lateral wall and the tip of the wing/untanned section) (Figure 1) - corresponding to three biochemical compositions of the beak, *i.e.*, the ratio between chitin, protein, and water (Miserez et al., 2008). Beak sections were cut into 1-3mm^2 using tweezers and scalpel. Every section was treated as a different sample, therefore, both instruments and the surface area were cleaned before working with each section, using a 1:10 bleach and MilliQ solution, followed by a MilliQ rinse, 70%

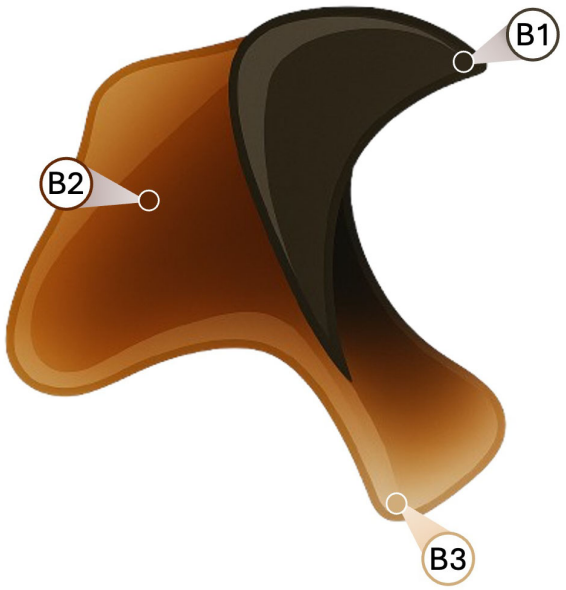

FIGURE 1
Sampled sections of the beak used for DNA analysis: **B1**-rostrum tip (water ~20%; proteins ~70%; chitin ~10%); **B2**-tanned section from the lateral wall (water ~50%; proteins ~25%; chitin ~20%); **B3**-untanned section (water ~70%; proteins ~5%; chitin ~25%) (Miserez et al., 2008).

ethanol, to avoid contamination between samples. One entire beak was also tested, *i.e.*, no sectioning was performed, to also test the protocol under these conditions for replicability.

DNA extraction was performed using the DNeasy Fast DNA Tissue Kit (Qiagen, Germany) according to the manufacturer´s protocol. Using a horizontal vortex, we started by lysing the DNA and homogenising the samples with AVE-Buffer (200 µl), DXL (40 µl), DX reagent (1 µl), Proteinase K (20 µl), and RNase A (4 µl), and incubating at 56°C (two rounds of 5 min vortex and 10 min incubation at 1000 rotations/min and a third round of 5 min vortex and 1 h incubation again at 1000 rotations/min). After incubation, MVL (265 µl) buffer was added and the homogenised mixture was filtered through a silica membrane column that binds the DNA, during centrifugation at 16'100 rcf (relative centrifugal force), for 1 min. The membrane was then washed with two buffers AW1 (500 µl) and AW2 (500 µl), each followed by a 30-s centrifugation step at 16'100 rfc. The DNA was eluted from the membrane using elution buffer ATE pipetted onto the membrane and incubated for 5 min at room temperature. A lower elution volume of ATE buffer (50 µl) was used to account for the potentially low concentrations of DNA recovered after centrifugation for 1 min at 6000 rcf. DNA yield was quantified using NanoDrop One equipment (Thermo Scientific, USA).

The Folmer primers (HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) and LCO1490 (5′-GGTCAACAAAT-CTAAAGATATTGG -3′) (Folmer et al., 1994);) were used to amplify a 658 bp fragment of the cytochrome *c* oxidase I (COI) gene. DNA template was substituted with molecular-grade water in negative controls. PCR reaction volume was 25µl/sample with the following reagent concentrations: Buffer = 1x; MgCl2 = 1.5mM; dNTP = 0.2mM; Primers = 0.5µM; DNA Polymerase = 0.02U/µl. The PCR thermal regime for the primer set was the following: initial denaturation at 94 °C for 3 min; 36 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min followed by a final cycle at 72 °C for 10 min. PCR products were not diluted before sequencing due to the low DNA concentration (0–42 ng/µl), previously measured using the NanoDrop One equipment. PCR products were checked for quality and length using electrophoresis on GelRed-stained, 2% agarose gel. All successfully amplified samples were sent to Eurofins Scientific for Sanger sequencing. The PCR product was sequenced in both directions using both the forward (LCO1490) and reverse (HCO2198) primers.

The chromatographs of all sequenced sequences were visually checked for quality using CodonCode Aligner 8.0.2 (Codon Code Corporation). Both forward and reverse reads were assembled, primer sequences were removed leading to most sequences being 658bp, and reads were compared for uncertain bases and gaps, and screened for the presence of stop codons to exclude the potential of pseudogenes, also using CodonCode Aligner 8.0.2. Sequencing results were compared to reference nucleotide sequences, using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi), from databases such as GenBank (https://www.ncbi.nlm.nih.gov) and the Barcode of Life Datasystems (BOLD) (https://www.boldsystems.org/index.php) for taxonomic assignments. Assignments were checked for overlap in geographic distribution and removed in case of discordance. We applied a similarity threshold of 97%, consistent with widely used COI barcoding

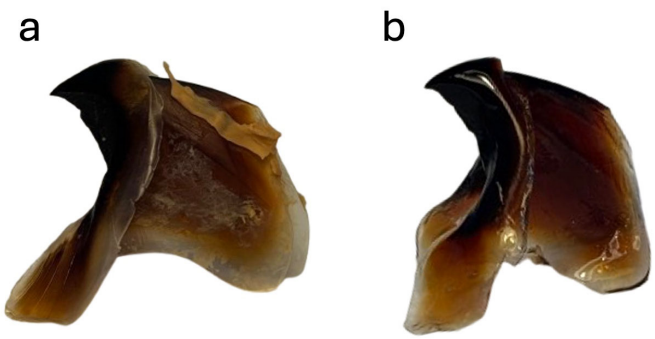

FIGURE 2
Effects of cleaning the beak surface with bleach: a-prior bleach treatment, b-after bleach treatment.

practice (Hebert et al., 2003; Ratnasingham and Hebert, 2007; Dai et al., 2012). This threshold is equivalent to the ≥3% interspecific genetic distance commonly used to discriminate cephalopod species using COI where mean interspecific divergence is typically well above this boundary (Dai et al., 2012). However, we acknowledge that a fixed threshold may not be optimal across all cephalopod taxa, particularly in groups with cryptic diversity or incomplete reference databases (Pappalardo et al., 2025).

To test for the influence of collection type, sample processing and preservation on identification success, a generalized linear mixed model (GLMM) was used in R software 2026.05.1 + 225 (R Core Team 2026). Beak sections, storage condition and bleach treatment variations were compared with beak identity as a random intercept to account for repeated subsamples per beak using *glmer* function from lme4 package (Bates et al., 2015)(distribution: binomial, link function logistic). Prior to model fitting, cross-tabulations were examined to identify complete or quasi-complete separation (Supplementary Table 2). One whole-beak sample (n=1) was excluded due to complete separation in beak section. Collection method could not be included in the final model due to complete separation in penguin-derived samples (n = 6, 100% success) and strong confounding with storage condition, 99% ethanol samples originated almost exclusively from net-caught specimens. These groups are reported descriptively (Bates et al., 2015).

3 Results

A total of 35 beaks were analysed from 10 different cephalopod species, representing nine families, including three octopod and seven oegopsid species from the Southern Ocean (Figure 3). These originated from four different sources: (i) boluses samples from black-browed and grey-headed albatrosses, (ii) stomach samples from macaroni penguins, (iii) stomach samples from Patagonian and Antarctic toothfish, and (iv) beaks extracted from whole cephalopods caught in scientific nets. Thirty-four beaks were divided into three different beak sections, (tanned *vs* non-tanned *vs* rostrum tip) and one was sampled as a whole, resulting in a total of 103 samples (Figure 1; Supplementary Table 1).

DNA extraction was performed for all samples. DNA concentrations in the resulting solutions were too low to be measured accurately, *i.e.*, in most cases were below Nanodrop's measurement threshold of 20 ng/µl. As fluorometric quantification was not performed, DNA concentration values should be interpreted cautiously. Of the DNA samples obtained, 72.8% were successfully amplified using PCR (75 of 103 samples) corresponding to 80.0% of the beaks tested (28 out of 35) (Figure 4; Supplementary Table 1). For 52 samples, corresponding to 21 of the 28 beaks, BLAST searches of the amplified sequences resulted in an assignment to species level that was in agreement with prior morphological identification (Supplementary Table 1).

There was no discrepancy in taxonomic assignment between Gen Bank and BOLD, however sequences for *Slosarczykovia circumantarctica* were only available in the BOLD database. No predator sequences were detected. Some sequences of poor quality assigned to non-cephalopod taxa were removed. Overall, from extraction to successful assignment, the success rate for individual samples was of 50.5% (52 out of 103 samples) and for beaks was 60.0% (21 out of 35 beaks) (Supplementary Table 1). All quality and correctly assigned (i.e. DNA identified the same species as previously identified through morphology) sequences have been submitted to the Barcode of Life Data System (BOLD, project code: LBMTS). Furthermore, 12 additional samples yielded sequence similarity values above 90%, supporting correct family-level assignment, although they did not meet the 97% threshold for species-level identification.

3.1 Effect of sample origin

Due to complete separation in samples from penguin stomachs (n=6, 100% success) and the cofounding effects of storage condition and collection method (Supplementary Table 2), the influence of collection method could not be formally tested in the GLMM. Success rates should therefore be compared with caution. Assignment was possible for 55.6% of samples from beaks extracted

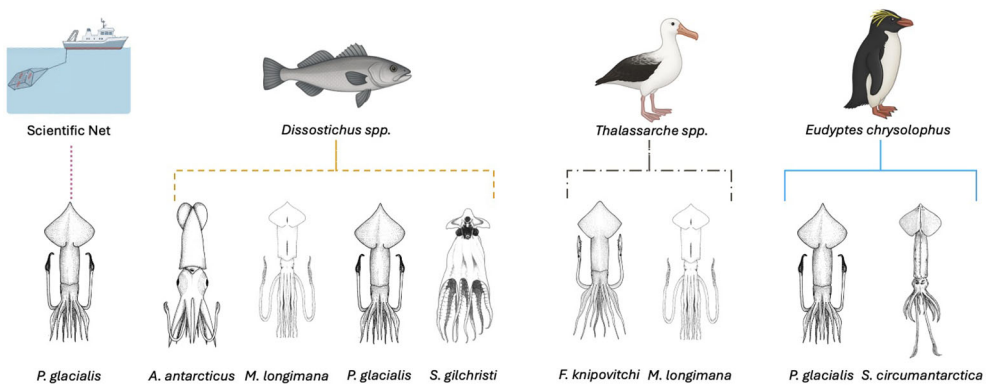

FIGURE 3
Species of cephalopods identified using the different sampling methodologies. Source of cephalopod figures: Reproduced from Jereb, P. & Roper, C.F.E. (eds) (2010) Cephalopods of the World, Vol. 2, FAO Species Catalogue for Fishery Purposes No. 4; and Jereb, P., Roper, C.F.E., Norman, M.D. & Finn, J.K. (eds) (2016) Cephalopods of the World, Vol. 3, FAO Species Catalogue for Fishery Purposes No. 4. © FAO. Licence: CC BY-NC-SA 3.0 IGO.

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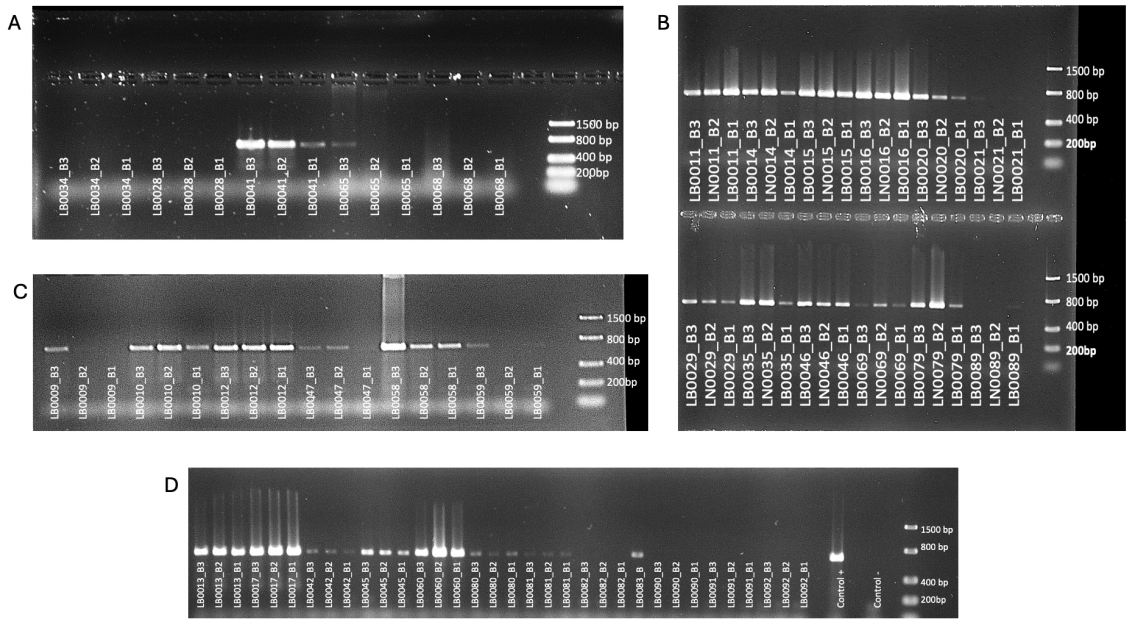

FIGURE 4
Electrophoresis gel showing results from different beak sections: B3-untanned section; B2-tanned section; B1-rostrum tip.

from whole cephalopods caught in scientific nets (15 out of 27), 59.5% from beaks from stomach samples from toothfish (22 out of 37) and 27.3% from stomach samples from albatrosses (9 out of 33). Assignment of beaks was successful for 4 out of the 11 beaks from albatross stomachs, 6 out of the 9 cephalopods caught in nets, 9 out of the 13 beaks from toothfish stomachs and 3 out of the 3 beaks from macaroni penguins (Supplementary Table 1).

3.2 Effect of beak section

Using the protocol described below, we successfully obtained DNA from 52.9% of samples taken from the untanned section (B3, 18 out of 34), 50.0% of samples isolated from the tanned section (B2, 17 out of 34), and 47.1% of samples isolated from the rostrum tip (B1, 16 out of 34). There were no differences in the proportion of samples from different beak sections that were assigned successfully (GLMM: z = −0.452, p = 0.651 for tip; z = 0.451, p = 0.652 for untanned, relative to tanned section) (Table 1).

3.3 Effect of storage condition and bleach treatment

Storage condition and bleach treatment were tested as fixed effects in the GLMM, with beak identity included as a random

TABLE 1 **Final generalized linear mixed model (GLMM) of the effects of storage condition, collection method, beak section and, bleach cleaning on identification success.**

Variable		Sample Size	Estimate	SE	z value	p value
(intercept)			1.428	1.980	0.721	0.471
Storage condition	70% ethanol*	94	–	–	–	-
	99% ethanol	9	-5.450	4.894	-1.113	0.266
Beak Section	Untanned	34	0.413	0.916	0.451	0.652
	Tanned*	34	–	–	–	–
	Rostrum Tip	34	-0.414	0.917	-0.452	0.651
Bleach cleaned	No*	57	–	–	–	-
	Yes	46	-2.381	2.887	-0.825	0.410
Radom Effects						
Beak identity	Variance		26.90	SD = 5.186		

AIC = 106.7; BIC = 122.5; Log-likelihood = −47.4; n = 102 samples, 34 beaks. The model explained 8.8% of the variation through the fixed effects alone ($R^2m = 0.088$) and 90.1% when including the random effect of beak identity ($R^2c = 0.901$). SE: Standard Error; SD: Standard Deviation; "*" indicates reference levels.

intercept (variance = 26.90, SD = 5.19), reflecting substantial variation in success rates among individual beaks. There was no statistically significant effect of either storage condition (estimate = −5.450, SE = 4.894, z = −1.113, p = 0.266) or bleach treatment (estimate = −2.381, SE = 2.887, z = −0.825, p = 0.410) on success rate (Table 1).

4 Discussion

To our knowledge, this study is the first to successfully extract and sequence DNA, correctly identifying to species level individuals using their beaks. We assigned beaks from six cephalopod species (*Alluroteuthis antarcticus*, *Filippovia knipovitchi*, *Moroteuthopsis longimana*, *Psychroteuthis glacialis*, *Slosarczykovia circumantarctica*, *Stauroteuthis gilchristi*), achieving an overall success rate of 50.5% of samples and 60.0% of beaks (Figure 5). An additional 12 samples yielded sequence similarity values sufficient for family-level assignment (>90%). Although these did not meet the threshold for species-level identification, they can still support subsequent morphological classification by taxonomists, particularly given that partial beak sections can be analyzed as shown in this study (Supplementary Table 1). As commonly observed in low-yield extracts, such as those from challenging tissues like cephalopod beaks, most samples were below the limit of quantification of the Nanodrop spectrophotometer. Although DNA quantification in this study relied solely on NanoDrop measurements, future studies should consider using fluorometric assays for DNA quantification, in particular, the Qubit

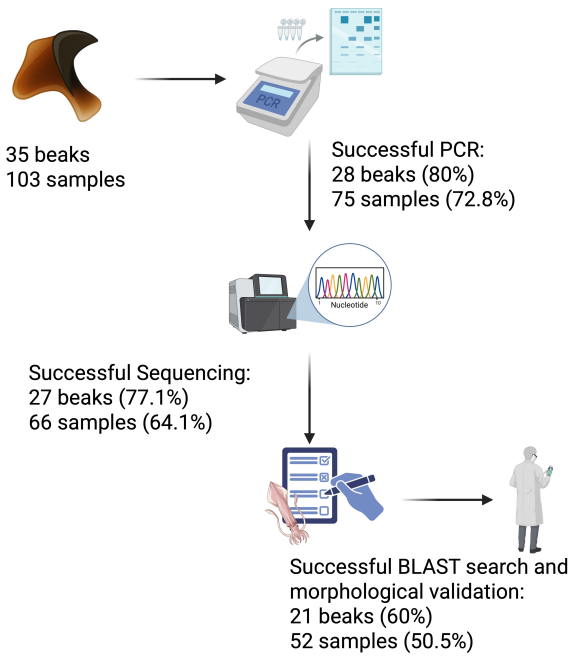

FIGURE 5
Workflow diagram showing sample processing stages and success rates at each step, from DNA extraction through PCR amplification, Sanger sequencing, and taxonomic assignment via BLAST search and morphological validation. Created in BioRender. Bastos, L. (2026) https://BioRender.com/1ewzfb6.

High Sensitivity (HS) assay (Simbolo et al., 2013; Khetan et al., 2019; Billington et al., 2023). This type of methodology has a substantially lower quantification range (0.005–120 ng/μL) (Fisher Scientific T., 2015) than NanoDrop (2 ng/μL) (Fisher Scientific T., 2010) or the Qubit Broad Range (BR) assay (0.2–2000 ng/μL) (Fisher Scientific T., 2022) providing a more accurate estimate of DNA yield from cephalopod beaks. The choice of method may therefore influence whether DNA is considered detectable or sufficient for downstream analyses, potentially preventing the premature exclusion of samples that remain suitable for PCR amplification and sequencing (Simbolo et al., 2013; Khetan et al., 2019; Billington et al., 2023).

Our study provides a molecular alternative that could overcome some limitations related with identification based on beak morphology. DNA analysis of beaks is valuable solution in cases where morphology identification falls short, such as when differentiating between species with similar beak structures or when working with fragmented or isolated beak sections that are otherwise unidentifiable through morphological examination (Xavier and Cherel, 2021). Previous attempts to extract sufficient DNA from beaks in diet samples of predators for species assignment were unsuccessful (Xavier et al., 2016) (Tom Gilbert, personal communication). The use of bleach, and the source of the beak appeared as important factors to successfully extract the DNA (Vecchione et al., 2009; Campos and Gilbert, 2012; Xavier et al., 2016). Our study utilized the DNeasy Fast DNA Tissue Kit (Qiagen, Germany), which was crucial in enabling the extraction of DNA from the chitinous beak structure, a material known for its stiffness (Miserez et al., 2008; Campos and Gilbert, 2012; Xavier et al., 2016). Unlike the more commonly used DNeasy Blood & Tissue Kit, the Fast Tissue Kit includes metal beads within the collection tubes, enabling more efficient mechanical disruption of hard or keratinized materials and thus improving DNA yield. The choice of extraction kit should therefore be considered carefully in future applications of this method. Although this approach proved to be largely effective, for future optimization, an extra enzymatic digestive step could be implemented to disrupt chitin further, using hydrogen peroxide and a chitinase solution, already used for evaluating beak's hormones (Durante et al., 2025).

Cleaning the beaks with bleach was a key step as it removed residual tissue and prevented contamination from non-cephalopod DNA while the beak had been in the predator stomach (Campos and Gilbert, 2012) (Figure 2). Although there was no statistically significant effect of bleach treatment on assignment success (estimate = −2.381, SE = 2.887, z = −0.825, p = 0.410), the tendency towards lower success in bleached samples likely reflects confounding with sample origin and preservation condition rather than a direct effect of bleach on DNA quality (Table 1).In samples obtained from beaks of individuals captured in scientific net, bleach-treated beaks consistently matched reference sequences, indicating that the degradation caused by digestive systems, not the bleach, was the limiting factor (Clarke and MBA of the UKNERC (Great B., 1986; Duffy and Jackson, 1986; Xavier et al., 2016). Therefore, in instances where only the beak is recovered from a predator diet sample, the use of bleach is still advised to reduce the likelihood of contamination. Conversely, in cases where beaks are attached to the buccal mass, there is little need for bleach, as the DNA on the beak surface is likely to originate from the cephalopod itself.

Despite variation in composition of different beak sections that could potentially result in differing DNA concentrations (Miserez et al., 2008; Tan et al., 2015; Xavier et al., 2016), this had no effect on species assignment (Table 1). Analysis of the untanned section of the beak, known for its susceptibility to digestion and hypothesized to contain the highest concentration of genetic material, was no more successful than that of other parts of the beak. These results should however be interpreted cautiously, since despite the GLMM showing no statistically significant differences between sections (Table 1) the dataset was limited to 34 beaks. Nevertheless, this is encouraging for future research as it indicates that there could be no need to select a particular beak section, thereby allowing researchers to apply both DNA assignment and morphological identification without compromising the integrity of the beak, useful when validating possible new species. Moreover, our success in identifying non-sectionized beak fragments (i.e. LB0083B indicates that our method may be applied routinely to both broken beaks often found in diet samples (Clarke and MBA of the UKNERC (Great B., 1986; Duffy and Jackson, 1986) and smaller beaks, where sectioning may be difficult (Xavier and Cherel, 2021).

Success rates appeared to be lower for beaks recovered from albatross stomachs (27.3%) compared to those from toothfish stomachs (59.5%) or scientific nets (55.6%), though this difference could not be formally tested in the GLMM due to confounding of collection method and storage condition (Supplementary Table 1). Albatrosses possess a complex digestive system, including glandular and muscular stomachs which are hyper-acidic, and produce digestive enzymes such as chitinase (Jackson, 1990; Grémillet et al., 2012). Chitinase breaks down the chitin in cephalopod beaks, likely reducing the amount of recoverable DNA (Miserez et al., 2010; Tan et al., 2015; Xavier et al., 2016). Furthermore, beaks from albatrosses are recovered from boluses (pellets), leaving the beak, and consequently the DNA, susceptible to air and weathering (Demay et al., 2013). In contrast, toothfish have a simpler digestive system which appears to preserve the beaks and protect their DNA from degradation and are recovered directly from stomachs (Korovina et al., 2004; Queirós et al., 2022).

Application of DNA analysis to assign beaks to cephalopod species reduces reliance on morphological expertise, providing new opportunities for studying these little-known taxa. Despite our success, there is room for improvement. The DNeasy Fast DNA Tissue Kit has a moderate cost per sample, which should be weighed against the practical advantages of reducing dependence on scarce taxonomic expertise and reference collections. Moreover, genetic databases, such as GenBank and BOLD, need to be updated with sequences from a more comprehensive set of cephalopod species and different genetic markers (e.g., COI, 16S, 18S), and also with sequences from different populations to account for intraspecific variation and potential geographic lineages or species complexes (Wu et al., 2015; Bolstad et al., 2018; Sanchez et al., 2021; Fernández-Álvarez et al., 2022). The integration of molecular methods with traditional morphological approaches will enable more comprehensive studies of cephalopod ecology, biogeography, role in marine food webs, evolutionary relationships and responses to environmental changes.

Data availability statement

The datasets generated for this study can be found in BOLD under project code LBMTS.

Ethics statement

The animal study was approved by British Antarctic Survey (BAS). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

LB: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. CH: Conceptualization, Methodology, Visualization, Funding acquisition, Supervision, Writing – review & editing, Resources. JQ: Formal analysis, Funding acquisition, Visualization, Writing – review & editing. MB: Resources, Writing – review & editing. PH: Resources, Writing – review & editing. RP: Resources, Writing – review & editing. YC: Writing – review & editing. JX: Conceptualization, Funding acquisition, Resources, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2026.1873859/full#supplementary-material

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