

Fifteen years of elasmobranchs trade unveiled by DNA tools: Lessons for enhanced monitoring and conservation actions

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Declaration of competing interest

The authors declare no competing interests exist.

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3 **Highlights**

- 4 ● Meta-Analysis of DNA-based tools for monitoring trade, focusing on Brazil's
5 elasmobranchs.
- 6 ● Challenges in molecular identification were identified (e.g., limited database
7 resources).
- 8 ● Brazil comprises 203 elasmobranch species, and 64 were molecularly detected in
9 trade.
- 10 ● 83% of species detected are threatened (IUCN), including recently updated
11 assessments.
- 12 ● Ten research gaps were outlined, along with recommended practical solutions for
13 future work.

14 **Abstract**

15 The trade of elasmobranchs (sharks and rays) in Brazil threatens the country's rich
16 endemic biodiversity. The present study explored the use of DNA-based tools to monitor the
17 Brazilian elasmobranch trade, focusing on their role in identifying processed products and
18 supporting conservation efforts. A systematic search of literature was conducted and included
19 35 peer-reviewed papers published between 2008 and 2023. A shift from the development of
20 DNA-based tools to direct trade applications has been observed since 2015. Molecular
21 identification challenges, including costly sequencing and limited resources in national
22 databases, were identified along with proposed solutions, such as protocol optimization and
23 exploration of cost-effective alternatives. Biases in trade analysis papers, particularly the lack
24 of research in the Northeast Region of Brazil, and issues with sample sizes were evident.
25 Species identified using DNA-based tools included the critically endangered Scalloped
26 Hammerhead Shark (*Sphyrna lewini*), which appeared in 46% of the evaluated papers,
27 followed by the Blue Shark (*Prionace glauca*), and several others threatened species, such as
28 the critically endangered and endemic Brazilian Guitarfish (*Pseudobatos horkelii*) and the
29 recently categorized as vulnerable Sharpnose Shark (*Rhizoprionodon porosus*). Other species
30 were reassessed by IUCN, including previously non-threatened species that are now at risk,
31 emphasizing the need for fisheries management, trade monitoring and conservation measures.
32 Our findings highlight the importance of continued genetic monitoring to analyze market
33 trends and adjust legislation, encouraging compliance with frequent inspections to enhance
34 wildlife conservation. We also identified gaps in research and recommended strategies for
35 accurate species identification, broader investigation, and effective management.

36 **Keywords**

37 Brazilian trade; Conservation genetics; DNA-based tools; elasmobranchs (sharks and rays);
38 endemic species; forensics

40 The capture of elasmobranchs (sharks and rays) has been increasing worldwide due to
41 industrial, artisanal, and recreational fisheries catering to diverse markets (Pacoureaux et al.
42 2021). There has been a rising demand for shark fins in Asia and an expanding market for
43 their meat in Europe and South America (Dent and Clarke 2015; Okes and Sant 2019). Brazil
44 has emerged as the largest importer of shark meat and the eleventh-largest country for shark
45 fishing (Barreto et al. 2017; FAO 2020). High demand for shark meat is often attributed to
46 the regulatory practices surrounding finning worldwide, as a substantial growth in the trade
47 has coincided with the global requirement for landings of both fins and the rest of the body
48 (Dent and Clarke 2015; Rangel et al. 2021).

49 Some authors have indicated that the Brazilian market responded to the finning
50 regulation by heightening demand for shark meat in the country so they could continue to
51 obtain fins and export them to the international market, which can be referred to as "fin
52 laundering" (Rangel et al. 2021). Moreover, low meat prices and the umbrella label "cação"
53 (extensively used in Brazil for shark meat) lead to a lack of awareness among consumers that
54 this term corresponds to sharks and some ray species. Consequently, Brazil is currently one
55 of the leading consumers of shark meat globally (Bornatowski et al. 2015; FAO 2020). This
56 is noteworthy, especially considering that fish meat is not the preferred protein source for
57 most of the Brazilian population (Hase Ueta et al. 2023). The consumption pattern varies
58 across macro-regions of the country. The North Region exhibiting the highest consumption
59 compared to others, with 41.7% of its population consuming fish twice or more per week,
60 while the non-coastal Central-West Region has the lowest fish consumption, with only 15.5%
61 of fish consumption twice or more per week (Lopes and Freitas 2023).

62 The global shark trade has undergone significant market shifts recently, with shark meat
63 presenting an increased market value over time, but not reaching the value of fins in market
64 (Niedermüller et al. 2021). Indeed, there is a paradox in the values of shark meat and fins.
65 Even though the overall volume and value of the meat market is higher, the value varies
66 according to the species targeted and regions, and fins always attain higher prices per kg
67 (Niedermüller et al. 2021). The rays trade, although less understood, is also concerning. This
68 is primarily because Brazilian markets consider rays an inexpensive product, and they are
69 caught mainly as bycatch (Dent and Clarke 2015). Nevertheless, Brazil has experienced an
70 increase in ray captures, earning the status of the third-largest exporter of ray meat to South
71 Korea in 2021, the primary consumer of ray meat worldwide (Niedermüller et al. 2021).

72 Elasmobranchs have limited capacity to withstand anthropogenic pressures due to their
73 life history characteristics, including late sexual maturity, low fecundity and slow growth
74 rates (Cortés 2002; Frisk et al. 2005; Stevens et al. 2000). Unreported and unregulated catches
75 have led to substantial extinction risk for elasmobranchs (Mozumder et al. 2023; Sherman et
76 al. 2023a; Worm et al. 2013), making them the primary lineage of marine fish with elevated
77 threats, with over a third of chondrichthyan fish species currently categorized as threatened
78 (Dulvy et al. 2021). The Brazilian Guitarfish (*Pseudobatos horkelii*, Pollom et al. 2020a),
79 Largetooth Sawfish (*Pristis pristis*, Espinoza et al. 2022) and Daggernose Shark
80 (*Isogomphodon oxyrinchus*, Pollom et al. 2020c) are among the Brazilian elasmobranchs
81 facing increased risk of extinction. Decreases in biological diversity of elasmobranchs has
82 led to changes in the whole marine community, including ecosystemic imbalance, shifting in
83 trophic cascades, and decline in seafood catches (Bornatowski et al. 2014; Pimiento and
84 Pyenson 2021; Sherman et al. 2023b).

85 Brazilian legislative measures have been established with the intention of reducing the
86 mislabeling and the commercialization of species categorized as Vulnerable (VU),
87 Endangered (EN), or Critically Endangered (CR) according to the Brazilian Ordinance
88 445/2014 of the Brazilian Ministry of Environment (updated in 2022 and 2023, as ordinances
89 148/2022 and 354/2023). However, mislabeling and the sale of threatened species continue

90 to occur, and monitoring to curb these practices is still an outstanding challenge (Almeron-
91 Souza et al. 2018; Alvarenga et al. 2023; El Bizri et al. 2020; Feitosa et al. 2018; Souza et al.
92 2021; Wosnick et al. 2023). Consumers continue to buy elasmobranch meat inadvertently due
93 the practice of removing heads and fins before selling the processed meat as “cação” filets or
94 steaks (FAO 2020; Rodrigues-Filho et al. 2012). Important diagnostic features of the species
95 are lost in these processes, hampering the morphological identification of the products at
96 landing and commercial sites (Domingues et al. 2021).

97 Molecular techniques have played a fundamental role addressing the illegal commerce
98 of shark and ray products when identification by morphological diagnostic features used for
99 is restricted. These techniques enable the accurate identification of species composition at
100 landings sites, the tracking of finning practices, and the identification of processed meat
101 (Clarke et al. 2006; Domingues et al. 2021). Nevertheless, there is still a need to: a) compile
102 the information available on the use of DNA-based tools to track the Brazilian elasmobranch
103 trade, b) expand the use of molecular techniques to efficiently understand the traded species
104 composition, and c) assist enforcement inspections by using genetic tools and increasing
105 inspection frequency, which can help decelerate the current alarming extinction risk of
106 elasmobranch species.

107 In this context, this study aimed to provide a historical assessment of the contribution
108 of molecular tools in analyzing the Brazilian market of shark and ray products by reviewing
109 all peer-reviewed journals published until June 31st 2023. Our overarching objectives
110 included a comprehensive overview of the research on Brazilian elasmobranch trade and
111 identifying potential areas for further research, which can improve law enforcement, fisheries
112 management, and conservation efforts. To achieve this, five specific goals were established:
113 (i) examine the existing scientific literature in all Brazilian regions, identifying the sampling
114 strategy applied in the context of the papers analyzed and their geographic range, (ii) evaluate
115 the contribution of genetic tools in analyzing the trade of elasmobranchs in Brazil, including
116 an analysis of the most commonly used techniques and the status of genetic databases for
117 Brazilian species, (iii) identify the species composition in Brazilian markets that has been
118 detected with molecular techniques, with a particular focus on the commercialization of
119 threatened and endemic species over time, (iv) assess the extent to which legislation has
120 contributed to monitoring the fishing and trade of elasmobranchs in Brazil, and (v) evaluate
121 mislabeling activities. Furthermore, recommendations are provided to contribute to
122 improving conservation efforts.

123 **2. Material and methods**

124 *Data collection and categorization*

125 We performed our literature search following the protocol from the RepOrting
126 Standards for Systematic Evidence Syntheses (ROSES; Haddaway et al. 2018) to ensure
127 methodological rigor. First, an extensive Boolean search (AND, OR, NOT) on Web of
128 Science and Scopus was carried out to collect peer-reviewed papers that applied molecular
129 tools for the analyses of elasmobranchs catch and trade in Brazil. Our literature collection
130 strategy included keywords, titles, and abstract content related to mislabeling and molecular
131 identification. We included papers that met the following criteria: (i) research carried out in
132 Brazil, (ii) published until June 31st 2023, and (iii) research articles. To consolidate data from
133 both databases and remove potential duplicates, we used the R package Bibliometrix (Aria
134 and Cuccurullo 2017), obtaining a matrix with 67 papers. Second, a Boolean search on
135 Google Scholar was performed, adding the first 50 papers in our matrix (Haddaway et al.
136 2018).

137 To ensure relevancy to our analysis and remove duplicates from the Google Scholar
138 search, we manually inspected the papers matrix for inclusion into our final database. The

139 inspection was performed by three authors, using *strict consensus* criteria, meaning that all
140 the authors that curated the papers agreed to the addition of a paper, resulting in 32 total
141 papers. We computed the inter-rater agreement to infer the consistency and reliability of our
142 assessments by measuring agreement among independent raters evaluating the same set of
143 items (Gisev et al. 2013). Finally, to detect and recover any potentially missed papers, the
144 Snowball Method was applied, which involves a search for papers in the list of citations
145 (forward snowballing) and in their cited references (backward snowballing) (Wohlin 2014),
146 retaining 3 additional papers. For a comprehensive overview of our search process, see Figure
147 1 and Appendix A.

148 To provide a historical assessment on the use of molecular tools to analyze the Brazilian
149 market, we categorized the 35 selected peer-reviewed papers into two groups: "Trade
150 Analysis" (papers focusing mainly on the analysis of elasmobranch trade) and "Methods"
151 (papers that developed DNA-based tools for species identification). These two categories
152 were non-exclusive, which means that there could be papers fitting in both categories and
153 therefore assigned to "Methods/Trade Analysis" (papers combining the development of a
154 DNA-based technique with its applications for trade analysis within the same paper). Initial
155 metadata construction depicts each sample from the respective papers in specific categories
156 to characterize the use of genetics to analyze trade activity in Brazil. For a comprehensive
157 understanding of the metadata and detailed information on the individual categories, see
158 Appendix B. We used the metadata information to conduct a routine exploratory data analysis
159 (data cleaning, summarization, visualization) in R (Luque and Donlan 2019). For a detailed
160 description of the analyzes performed and R packages applied, see Appendix D. We
161 investigated DNA-based tool applications for analyzing the elasmobranch trade in Brazil,
162 assessing papers and samples at national and state levels. In particular, examining the
163 development of genetic tools, the composition of species on trade, threats trends, and
164 mislabeled products.

165 Two additional datasets were generated based on the species composition found to
166 obtain information on their genetic availability, extinction risk status and legislation (Fig. 1).
167 The first dataset focused on genetic information for each of the identified species, specifically
168 the number of sequences available in the National Center for Biotechnology Information
169 (NCBI) GenBank and in the Barcode of Life Data System (BOLD) database. NCBI GenBank
170 sequences were obtained using the *esearch* command in Entrez Direct (EDirect; Kans 2013)
171 while BOLD sequences were accessed through the *bold* package (Chamberlain 2021) in the
172 statistical software R v.2022.02.3 (R Core Team 2021). Both sets of additional data can be
173 found in Appendix C.

174 The second dataset focused on the extinction risk for each species according to the
175 Brazilian Red Book of Threatened Fauna (ICMBio 2018), SALVE System (Portuguese for
176 "Biodiversity Extinction Risk Assessment System", available online at:
177 <https://salve.icmbio.gov.br/>) and the International Union for Conservation of Nature (IUCN)
178 Red List of Threatened Species (hereinafter IUCN Red List, available at:
179 <https://www.iucnRed List.org/>). Whenever available in the online IUCN Red List
180 assessments, additional specific observations on species populations status for the
181 Southwestern Atlantic Ocean (SWA) were also included. In the same dataset, we depicted
182 key legislations for biodiversity conservation and labeling regulations over time.

183 *Data Analysis*

184 In order to achieve the five specific objectives of this work, we divided our analysis into five
185 stages:

186 **2.1 Research Trends**

187 We evaluated research trends using DNA tools to analyze the elasmobranch trade focusing
188 on the main metadata, specifically examining information on publication year, sample size,
189 and market characteristics (Fig. 1).

190 ***2.2 Molecular Species Identification***

191 The analysis centered on how molecular methods were used to identify species composition
192 in the elasmobranch trade using the main metadata, specifically the information on molecular
193 identification (Fig. 1). It also considered the accuracy of the identification (i.e. sample
194 identified at species level). Moreover, we evaluated whether sequences of all species
195 molecularly detected in the papers retrieved were available in the main databases (i.e.
196 GenBank and BOLD System) using the first additional dataset (genetic information
197 metadata).

198 ***2.3 Species Composition in Trade***

199 We assessed all species that were detected in landing sites and in trade using molecular tools,
200 specifically the information on species composition (Fig. 1 - Main metadata). We also
201 evaluated the extinction risk status and assessments updates of each species molecularly
202 detected in the papers retrieved over time based on the main metadata (Fig. 1).

203 ***2.4 Brazilian Legislation***

204 We explored historical trends in Brazilian legislation related to wildlife conservation and fish
205 labeling over time, comparing the legislation metadata with the national extinction risk status
206 only for the species molecularly detected in the papers retrieved (Fig. 1).

207 ***2.5 Mislabeling Activities***

208 We assessed mislabeling activities by comparing the declared market label and the species
209 detected with molecular tools in the main metadata (Fig. 1; Appendix B), checking if the
210 detected species complied with the Brazilian labeling regulation (MAPA 570/2023).

211 For detailed information on statistical and visualization analyses conducted, please see
212 the R Markdown script provided in Appendix D and the GitHub repository available at (link
213 available upon acceptance).

214 **3. Results**

215 ***3.1 Research Trends***

216 A total of 35 peer-reviewed publications (referred here as papers) published between
217 January 2008 and June 2023 were assessed (Fig. 2a). Of these, 24 (69%) were categorized as
218 “Trade Analysis”, eight (23%) as “Methods”, and three (9%) fitting in both categories as
219 “Methods/Trade Analysis” (Fig. 2b). The focus of research has shifted towards trade analysis
220 since 2012, noted by an increase of “Trade Analysis” compared to “Methods” ones (Fig. 2a).

221 Among the 11 “Methods” papers, a variety of DNA-based tools were developed:
222 Multiplex PCR in five papers, PCR-RFLP in four papers, tandem repeats in one paper, and
223 Sanger sequencing in one paper (Table 1; Table S2, in Appendix B). The most frequently
224 employed molecular marker was COI (n = 6), whereas 16S was the least utilized (n = 1).

225 The 27 “Trade Analysis” papers collectively involved 3,784 samples collected for
226 molecular detection (mean = 192; min = 7; max = 747; SD = 194) from 15 Brazilian states,
227 which represent 55% of the total country's federative units, and 88% of those on the coast
228 (Fig. 1c). The sampling sizes and strategies for market screening (e.g., market type, labeling,
229 sample location) varied greatly among the papers (Fig. 1d,e). Out of the total samples, 85%
230 (n = 3,235) were assigned to specific Brazilian states while the remaining samples were
231 labeled “Unidentified Location” due to incomplete information provided in the papers
232 regarding each sample individually (Table S1, in Appendix B). The State of Pará had the
233 highest number of papers and samples (n = 10 and 1,022), followed by São Paulo (n = 8 and
234 998), and Santa Catarina (n = 6 and 194). Notably, there was a scarcity of research from the
235 Northeast Region of Brazil, with only four papers across seven of the nine states in this region
236 and 311 samples collected (Fig. 2c-e).

237 Shark meat was the main target in the Trade Analysis, accounting for 81% of the papers
238 (n = 22) and 1,419 samples, while ray meat was analyzed in 22% of the papers and comprised
239 335 samples (Table S1, in Appendix B). We observed papers that collected specific groups
240 of elasmobranchs such as guitarfishes (Alvarenga et al. 2021 - 75 samples; De Franco et al.
241 2012 - 267 samples), angel sharks (Bunholi et al. 2018 - 85 samples), and whiptail stingrays
242 (Schmidt et al. 2015 - 97 samples). Three papers assessed elasmobranch captured as bycatch
243 (Domingues, 2013 - 317 samples; Ferrette, 2019 - 228 samples, Guimaraes-Costa, 2020 - 73
244 samples), one assessed the fin trade (Ferrette et al. 2019b - 747 samples), and one assessed
245 sawfish rostra (Faria et al. 2013 - 77 samples). In education institutions’ dining services,
246 such as school and universities cafeterias, 23 shark samples were served as a fish protein
247 option, and in commercial restaurants, four out of 14 samples were found to be mislabeled
248 (Alvarenga et al. 2021). A total of nine papers evaluated the mislabeling in elasmobranch
249 trade, including sharks being sold as salmon or croaker (Staffen et al. 2017) (Table 1 and
250 Table S1, in Appendix B).

251 **3.2 Molecular Species Identification**

252 Among the molecular tools applied in “Trade Analysis” papers, PCR-RFLP (n = 1) was
253 the least applied, followed by Multiplex PCR (n = 5). Sanger sequencing (n = 24) was the
254 most applied, and the only technique observed in all papers published since 2018, with the
255 exception of one study in 2019 that used Multiplex PCR (Fig. 3a). The COI was the most
256 applied molecular marker for both shark and ray identification, and the most represented in
257 current databases (Fig. 3b). Among the 26 papers that used COI, the primer set developed by
258 Ward et al. (2005) was applied in 23 papers. Ninety seven percent of the samples (n = 2345)
259 were identified to species level by the authors of the assessed papers using the COI marker,
260 while the remaining 3% of the samples (n = 69) were identified at the family level
261 (Rajiformes, n = 1), genus level (*Dasyatis* sp., n = 39; *Gymnura* sp., n = 5; *Hypanus* sp., n =
262 1; *Mustelus* sp., n = 5; *Narcine* sp.), or as a species complex, in which case it was not possible
263 to assign the sample to a single species (*Carcharhinus obscurus*/*C. galapagensis*, n = 4).
264 Other species complexes were identified through NADH-ubiquinone oxidoreductase chain 2
265 (ND2) (*Squalus brevirostris*/*S. megalops*, n = 1), and 12S/16S (*C. plumbeus*/*C. altimus*, n =
266 4). The region comprising 12S/16S also failed to identify samples at species level
267 (*Rhizoprionodon* sp., n = 35; *Sphyrna* sp., n = 2). Overall, 11 misidentifications were reported
268 (Table S1, in Appendix B).

269 By examining the availability of genetic resources in the public sequence databases, we
270 detected a clear bias toward sharks compared to ray species (Fig. 3b). Furthermore, it is worth
271 noting that public sequence databases still have gaps. For example, the 27 papers assessed
272 here represent 64 species deposited in the NCBI database, but not for all markers (Fig. 3b).
273 Moreover, from the approximately 203 species of sharks and rays known for Brazilian waters
274 (Kotas et al. 2023), only 144 are present in the NCBI database, representing 71% of the

275 overall Brazilian diversity. Gaps in genetic databases are particularly present for species with
276 restricted distribution, such as endemic species of Brazil (Fig. 3c). The availability of
277 complete mitochondrial genomes was higher for species with a wide distribution range,
278 whereas the use of shorter mitochondrial markers showed consistent representation across
279 species with both wide and restricted distributions (Fig. 3c).

280 **3.3 Species Composition in Trade**

281 A total of 73 species (44 genera and 28 families) were reported in all "Trade Analysis"
282 papers (Table S3 in Appendix A). Among these species, 36 shark species belong to 16 genera
283 and 28 rays belong to 20 genera (18 marine and two freshwater). Additionally, we detected
284 nine species of bony fish mislabeled as elasmobranch meat (Fig. 4). Within the identified
285 families, Carcharhinidae was the most abundant, with three genera leading the ranking:
286 *Carcharhinus* (886 samples in 17 papers), *Prionace* (464 samples in 10 papers), and
287 *Rhizoprionodon* (375 samples in 15 papers) (Fig. 4). The top position within paper count was
288 led by the genus *Sphyrna* (345 samples) and *Carcharhinus* (886 samples), both detected in
289 17 papers (Fig. 4). For rays, guitarfishes (genus *Pseudobatos*) were detected in 298 samples
290 across 8 papers, in which 187 samples belonged to the CR *Pseudobatos horkelii* (Fig. S2 in
291 Appendix A), whereas 111 samples belonged to the recently categorized as EN *P. percellens*.
292 The most threatened ray genus, *Pristis*, was also detected in high numbers (113 samples in 3
293 papers), as well as other genera of commercially explored and threatened sharks and rays
294 (Fig. 4).

295 In the trade analyses, we observed threatened species being detected in high (Table 2)
296 and increasing numbers (Fig. S2, in Appendix A). Currently, 83% (n = 54) of the
297 elasmobranch species detected were categorized as threatened (VU, EN or CR) and 12% as
298 Near Threatened (NT) by the IUCN Red List, with only three species categorized as Least
299 Concern (LC) (Table 2). A total of 81% of the valid elasmobranch samples (n = 2,877)
300 detected here belong to threatened categories (Fig. S1, Table S3).

301 The IUCN Red List extinction risk assessments changed over time (Fig. 5, Fig. S2 in
302 Appendix A), with only one species experiencing a decrease in extinction risk (*Rhinoptera*
303 *brasiliensis*, from EN to VU). Contrastingly, we detected 33 cases where the extinction risk
304 has increased, including 17 species not previously considered threatened, and seven species
305 whose extinction risk worsened by more than one category (Fig. 4a). In particular, frequently
306 traded species have become more threatened, such as *Carcharhinus acronotus* (NT to EN),
307 *Rhizoprionodon porosus* (LC to VU), *Sphyrna lewini*, and *S. mokarran* (EN to CR).
308 Additionally, among the 16 species previously categorized as Data Deficient (DD), 14 have
309 had their extinction risk assessed with nine currently considered threatened (Fig. 4b),
310 including two of the most commercially traded species: *R. lalandii* (DD to VU), and *C.*
311 *porosus* (DD to CR). Overall, eight of the species detected are now CR.

312 Among the reviewed papers, only 55% referenced to the Brazilian Red Book, while
313 89% relied only on the IUCN Red List. No paper referenced the SALVE System, since it was
314 made available in 2023. There were discrepancies in species assessment between the
315 extinction risk in the lists analyzed here (Fig. 6), mainly regarding the number of species
316 assessed. All the 64 species found in this study were assessed in the IUCN Red List, while
317 there were Not Evaluated (NE) species in the other two lists: 51% in the Brazilian Red Book,
318 and 8% in the SALVE System. Also, 11% of the species did not have an observation for their
319 IUCN SWA populations. Among the categorized species, we noted some differences in the
320 IUCN Red List assessments of risk of extinction when comparing global and SWA population
321 status. For example, 18% of species present in Brazilian waters were globally assessed as CR,
322 while specific consideration of the SWA populations increased this percentage to 31%.

323 Overall, species assessed at a regional level (Brazil or SWA) tended to have higher
324 extinction risk categories, with 25% of them listed as CR in the Brazilian Red Book and 31%

325 in the SALVE System (Fig. 6a). In contrast, species categorized as EN or CR in the IUCN
326 Red List presented lower risk of extinction categories in the published version of the Brazilian
327 Red Book (e.g., *Carcharhinus perezii* - VU) and SALVE System (e.g., *Pseudobatos*
328 *percellens* and *Zapteryx brevirostris* - VU) (Table S1, in Appendix C).

329 **3.4. Brazilian Legislation**

330 We examined the historical evolution of Brazilian legislation and species extinction risk
331 assessments. First, we analyzed the extinction risk lists (Fig. 6a), including the newly released
332 SALVE System, and the most pertinent regulatory measures based on these assessments (Fig.
333 6b). We identified a discrepancy in the categorization of threatened species (VU, EN, CR)
334 and their protection under Brazilian ordinances. Significant progress was noted from the
335 initial legislation (IN 05/2009), which protected species in Appendices I and II, to subsequent
336 regulations (MMA 445/2014, MMA 148/2022 and MMA 354/2023), which protect VU, EN
337 and CR species. However, the latest regulation (MMA 354/2023) still categorizes a lower
338 percentage (47%) of species as threatened compared to the most recent Brazilian extinction
339 list in the SALVE System (63%). Second, we analyzed labeling regulations, which revealed
340 limited taxonomic classifications and a prevalent use of umbrella labels over time (Table S1,
341 in Appendix C). Only one regulatory measure mandates species-specific labels, and it applies
342 exclusively to one Brazilian state (Paraná) rather than to the entire country.

343 **3.5. Mislabeling**

344 According to the current national fish labeling (MAPA 570/2023), our study identified
345 mislabeling in 237 samples. This included instances where bony fishes were sold as
346 elasmobranchs in 33 samples, with the swordfish *Xiphias gladius* being the most frequently
347 mislabeled species (n = 13). Furthermore, there were 85 cases where rays were sold as sharks
348 and 22 cases where sharks were sold as rays. Specific instances of mislabeling were also
349 recorded, such as sharks, skates, and stingrays being labeled as guitarfishes (n = 65). Also, a
350 common change in labels was noted among angel sharks and guitarfishes, where angel sharks
351 were labeled as guitarfishes (n = 5), and guitarfishes as angel sharks (n = 16). For other
352 mislabeling cases, please refer to Table S2, in Appendix B. Half of the papers analyzing the
353 meat trade included a description on the market label, but only 40% of them aimed to evaluate
354 mislabeling activity (Table 1).

355 **4. Discussion**

356 The present study examined the effectiveness of genetic tools in identifying processed
357 products. Furthermore, it explored their relevance in law enforcement based on market trends
358 detection to inform legislative adjustments as well as continuing genetic monitoring. Since
359 Brazil holds the second-largest number of endemic elasmobranch species globally (IUCN
360 2023), frequent inspections can encourage compliance, thereby improving conservation
361 actions both in Brazil and potentially worldwide. There has been a rise in papers addressing
362 the capture and commercialization of elasmobranchs using genetic tools over the past decade,
363 with a clear temporal trend regarding the type of research ranging from papers developing
364 molecular tools to ones applying them to inspect the trade (Fig. 2). This shift can be attributed
365 to the rapid decrease in supplies prices and an increase in applicability of molecular
366 techniques, including the use of DNA barcoding for elasmobranchs (Ward et al. 2005).

367 **4.1. Current State of the Art**

368 The DNA-based tools applied in Trade Analysis papers experienced a temporal switch,
369 with Sanger sequencing emerging as the predominant technique (n = 24), and the only one

370 applied since 2018, except for a single study in 2019 (Fig. 3b). While Sanger sequencing is
371 highly effective for accurate species identification, its continued high-cost for large sample
372 sizes poses challenges, particularly in resource-limited regions like Latin America, which can
373 restrict frequent market inspections. Several approaches can be pursued to address this,
374 including optimizing DNA sequencing protocols, using high-throughput sequencing (HTS)
375 methods like amplicon sequencing, and exploring modern cost-effective methods, like real-
376 time PCR and closed-tube DNA barcoding (Ballard et al. 2020; Cardenosa et al. 2018;
377 Prasetyo et al. 2023; Yeo et al. 2023).

378 In the economic context of the Global South, it is important to recognize the value of
379 traditional cost-effective techniques, such as PCR-RFLP and Multiplex PCR, which can
380 identify a large set of samples at lower prices (Böhme et al. 2019). Despite Brazil's high
381 elasmobranch biodiversity ($n = 203$, Kotas et al. 2023), it is noteworthy that we found that
382 the 10 species most sold in the Brazilian trade (Fig. S1) contributed to 56% of the trade
383 (sample count). Considering this, focusing on a small subset of the most commonly traded
384 species could be effective for analyzing trade in Brazil. Exceptions to the usual traded species
385 (detected as a negative in cost-effective approaches) could then be analyzed with Sanger
386 sequencing, reducing costs by limiting sequencing to fewer samples. Although these
387 techniques require initial costs for development, some sets that comprise the most traded
388 species in the Brazilian market are already available (Albercrombie et al. 2002; Caballero et
389 al. 2012; Ferrito et al. 2019; Shivji et al. 2002), including those developed nationally (Table
390 S2, in Appendix B). Among the techniques developed in Brazil, only the Multiplex PCR
391 Method developed by De-Franco et al. (2010) was fully implemented in a Trade Analysis
392 paper (De-Franco et al. 2012). Although the others full Multiplex PCR sets were not used,
393 single primers have been used to obtain additional genetic information on shark species
394 (Table S1, in Appendix A). For instance, primers from Pinhal et al. (2012) were used by Davis
395 et al. (2019) to assess population genetics of *Rhizoprionodon* in the Northwest Atlantic
396 Ocean.

397 Fisheries research often relies on opportunistic sampling (Pardo et al. 2016), which can
398 be a result of the constrained availability of research funding in environmental sciences and
399 limited access to the required field and laboratory equipment. This strategy and sample sizes
400 obtained through it can impact forensic analysis. The lack of a statistically calculated
401 sampling campaign may conflate particular aspects of the trade, such as a seasonality, rather
402 than comprehensively covering the entire trade dynamics. This also compromises the
403 accuracy of comparisons across studies (Luque and Donlan 2019; Pardo et al. 2016). Sample
404 sizes varied among the reviewed papers because they were discrete studies, however, it was
405 clear that some regions tended to concentrate more studies and samples than others.

406 A geographical bias was detected along the Brazilian coast, with a high number of
407 research papers conducted in the State of Pará ($n = 11$, samples = 1,022) in the Northern
408 Region of Brazil, followed by São Paulo ($n = 8$, samples = 998) in the Southeast and Santa
409 Catarina ($n = 6$, samples = 194) in the South, while fewer papers were published in the
410 Northeastern Region ($n = 5$, samples = 260) (Fig. 2). This bias is concerning because certain
411 elasmobranch species endemic to the Northeast, such as *Squalus bahiensis* (DD; R Pollom et
412 al. 2020d), *Hypanus marianae* (EN; R Pollom et al. 2020b), and *Squatina varii* (LC; Rincon
413 et al. 2019) may be subject to unacknowledged overexploitation. Overall, bias in biodiversity
414 research can impact or hinder the implementation of conservation efforts, and strategies to
415 avoid it should be implemented (Hickisch et al. 2019). The observed pattern of papers
416 published in the North and South/Southeast regions highlights another issue of national
417 funding distribution, which affects infrastructure and human resources.

418 Another important point is that states with higher tax revenues (e.g., Santa Catarina
419 and São Paulo) usually provide more funding support for research, when compared to states
420 with a lower tax income (e.g., Piauí and Amapá). This could result in different degrees of
421 commitment in monitoring and combating the illegal trade of elasmobranchs and fin exports

422 to foreign markets. Strategic allocation of efforts and resources might reflect in improved
423 inspection; for example, one of the largest seizures of illegal shark fin export in the world
424 took place in São Paulo, with approximately 28.7 tons of illegally obtained fins that would
425 be taken to Asia (Frontini and Mano 2023).

426 Despite having a greater number of endemic and threatened species (IUCN 2023), the
427 number of papers analyzing rays is disproportionately low, considering that the diversity of
428 rays ($n = 104$) is reported as higher than the sharks ($n = 99$) in Brazilian waters (Kotas et al.
429 2023), highlighting a preference for sharks in genetic elasmobranch research (Dudgeon et al.
430 2012; Soares and Petean 2023). Rays are usually captured as bycatch in trawling and gillnet
431 fisheries, and their meat has been undervalued in most Brazilian markets, being sold at low
432 prices, exported, or mislabeled (Dulvy et al. 2014; Ferrette et al. 2019a). Nevertheless, rays
433 have internationally become a valuable fishery resource with well-documented international
434 trade (Sherman et al. 2023a). Currently, Brazil stands as a major exporter of ray meat to South
435 Korea, the largest consumer of ray meat globally (Niedermüller et al. 2021). However, there
436 is a scarcity of peer-reviewed publications evaluating the impacts and products derived from
437 rays captured as bycatch and in industrial fisheries.

438 In general, there is a need to better understand the effects of elasmobranch bycatch and
439 finning, and how these activities drive or are being driven by domestic and international trade.
440 In this sense, molecular tools could be applied, such as the use of environmental DNA
441 (eDNA) to assess bycatch activities using vessel water samples (Albonetti et al. 2023) and
442 regular species identification on landing sites (Ferrette et al. 2019a). Moreover, molecular
443 tools can also be aid in geographical tracking to determine whether Brazilian species are
444 present in international fin markets (Domingues et al. 2021).

445 **4.2. Unraveling Trade with Molecular Tools: Challenges and Future Directions**

446 DNA barcoding, the primary tool for species identification, faces challenges that can
447 affect its accuracy. Incomplete species representation in databases and insufficient curation
448 can lead to misidentification issues, such as the "Rajiformes" case noted here, a label that
449 encompassed 297 species. Additionally, some markers applied to DNA barcoding showed
450 lower discriminatory delimitation for closely related species (Alvarenga et al. 2023). This can
451 impact conservation efforts, especially when differentiating threatened and non-threatened
452 species in the same species-complex (e.g., *Squalus brevirostris* - EN and *S. megalops* - LC).
453 Integrating phylogenetic analysis can enhance identification reliability by considering factors
454 like branch length and unusual relationship patterns (Felsenstein 2004). The most applied
455 molecular marker, COI, has shown limitations in identifying elasmobranchs to species level,
456 possibly due to recent divergences (Marino et al. 2017; Naylor et al. 2012). Alternatively, the
457 fast-evolving ND2 and the mitochondrial encoded 12S gene (Fernandes et al. 2021; Naylor
458 et al. 2012; Valsecchi et al. 2020) have been considered more effective molecular markers for
459 species identification but still remain scarce and underrepresented in current databases (Fig.
460 2b) (Böhme et al. 2019; Shokralla et al. 2012).

461 Furthermore, mitochondrial signal admixture due to historic hybridization occurred
462 between *C. galapagensis* and *C. obscurus* (Corrigan et al. 2017), highlighting the limitations
463 of mitochondrial markers for some species. Broader marker utilization is needed to improve
464 accuracy and expand database entries, including nuclear markers like ITS2, and multiloci
465 approaches that combine slow and fast evolving markers (Domingues et al. 2021). In addition,
466 the adoption of HTS techniques has been very effective in identifying species in food mixtures
467 (DNA metabarcoding) and in detecting bycatch composition via sampling water from trawl
468 fishing nets (eDNA metabarcoding) (Carvalho et al. 2017b; Albonetti et al. 2023; Cermakova
469 et al. 2023). Nonetheless, false positive detections due to failures of primer specificity, eDNA
470 transport in the environment, and database limitations need to be accounted for when applying
471 eDNA for tracking elasmobranch trade (Albonetti et al. 2023). A reduction of such effects

472 could be achieved through the inclusion of a multi-marker approach and occupancy models
473 (Ficetola et al. 2016).

474 A significant challenge in analyzing the fin trade is the level of genetic diversity among
475 global populations. Genetic analysis of shark products derived from worldwide industrial
476 fisheries also shares this challenge. The development of a genetic diversity atlas was
477 considered as essential to trace product origins accurately (Domingues et al. 2021). Thus,
478 incorporating fine-scale information and population-level genetic data in sequence databases
479 can offer significant potential for improving the tracking of wildlife products. This applies
480 not only to the fin market but also to the meat market, allowing for monitoring to trace the
481 origin of both imported and exported products in Brazil. This approach would allow the
482 assessment of local versus international elasmobranch production and provide insights on
483 how the trade impacts Brazilian sharks and rays populations or its effects on the Brazilian
484 trade with foreign populations. Unfortunately, such a comprehensive analysis is currently not
485 feasible with the information available to date. Despite their potential (Albonetti et al. 2023;
486 Mottola et al. 2022) HTS techniques are neglected in elasmobranch detection globally,
487 particularly in Brazil, where no papers have been published yet. Increased research funding
488 for Brazilian research is essential to stay abreast of recent international advancements, and to
489 identify shark and ray presence in processed products [e.g., crab dishes, pet food, and
490 cosmetics (Alvarenga 2020; Cardeñosa 2019)] and in the international trade (Cardeñosa et al.
491 2020)].

492 In reflection of this, establishing a comprehensive national genetic database with
493 accurate molecular markers, complete with both Brazilian species and populations, and with
494 regular curation is crucial. Such an initiative can also serve as a model for other countries in
495 the future, contributing to conservation efforts, trade regulations, and species management.
496 We strongly recommend conducting thorough examinations of other significant
497 elasmobranch trade locations globally to understand and address their specific requirements.

498 ***4.3. Species Composition and Threatened Elasmobranchs in Brazilian Trade***

499 The genetic findings highlight the urgent need for conservation measures to protect Brazil's
500 diverse elasmobranch species, especially those threatened with extinction. Molecular
501 methods detected 64 elasmobranch species traded in Brazil, despite the country's reported
502 diversity of approximately 203 species (Kotas et al. 2023). Around 70% of all Brazilian
503 elasmobranchs have never been molecularly detected as traded, raising questions about
504 factors contributing to it. Such detection pattern could be related to commercial interest,
505 fisheries characteristics, capture challenges, species rarity and non-detections caused by the
506 molecular methods applied. This suggests the need for further research to explore
507 elasmobranch trade composition and implications.

508 Another point we observed was that hammerhead sharks and guitarfishes continue to
509 be sold in alarming numbers, despite their high extinction risk (Alvarenga et al. 2021;
510 Bernardo et al. 2020). Seven out of eight hammerhead sharks and all guitarfishes occurring
511 in Brazil were categorized as threatened (IUCN 2023). This situation emphasizes the
512 significant impact and threat that coastal fisheries represent, especially for endemic [e.g.,
513 guitarfishes and angel sharks (Bunholi et al. 2018)] and highly threatened species [e.g.,
514 sawfishes (Faria et al. 2013)], but also for other threatened and extensively traded
515 elasmobranchs [e.g., Blue Shark (Alvarenga et al. 2021) and Mako Shark (Ferrette et al.
516 2019b)]. Intense fishing pressure on sharpnose sharks (*Rhizoprionodon* spp.) is another
517 conservation concern. Despite their relatively high productivity, high levels of fishing have
518 driven the populations of two sharpnose sharks (*R. porosus* and *R. lalandii*) found in the
519 Brazilian coast to declines, as previously cautioned by Lessa et al. (2006), and hence be
520 recently assessed as VU (Carlson et al. 2021; R. Pollom et al. 2020). The frequent catch of
521 Carcharhinidae species overall is of concern, and the highly traded Blue Shark may face a

522 similar fate as the sharpnose sharks. Other carcharhinid sharks are intensively marketed in
523 Brazil, also experiencing high fishing mortality rates (Bond et al. 2012; Dulvy et al. 2014).

524 The high rates of threatened species found in this study emphasizes the concern for
525 increasing numbers of threatened species. We illustrated a developing trend in extinction risk
526 status, where a growing number of species, especially those frequently traded in Brazil, are
527 becoming increasingly threatened. Overall, 32 out of the 64 species found in this study were
528 reassessed recently in the IUCN Red List, with 18 experiencing an increase in their extinction
529 risk, including seven species receiving multiple uplistings in their assessments. These
530 findings emphasize the detrimental impact of fisheries and trade on elasmobranch populations
531 (Dulvy et al., 2014; Barreto, 2017), adding further pressure to their declines. A single species,
532 *Rhinoptera brasiliensis*, previously categorized as EN showed a slight downlisting, but still
533 remained as VU (Fig. S2). Nonetheless, this positive change is overshadowed by the overall
534 worsening extinction risk status of many other species. The remaining 16 species, previously
535 assessed as DD, were updated and had: 56% assigned to threatened categories (e.g.
536 *Rhizoprionodon lalandii* DD – VU and *Carcharhinus porosus* DD – CR) and 31% assigned
537 to not threatened categories (e.g. *Squalus cubensis* DD – LC, which was one of the least
538 detected species in trade). The remaining 13% corresponded to two Neotropical freshwater
539 stingray species detected in the papers analyzed that remained as DD, emphasizing the need
540 for more research on freshwater elasmobranchs. It is important to note that many DD
541 elasmobranch species have low levels of genetic diversity, which makes them even more
542 vulnerable to stochastic events see (Domingues et al. 2018).

543 Comprehensive conservation strategies are necessary to safeguard and restore genetic
544 diversity among elasmobranch populations in Brazil, mainly given the country's extensive
545 coastline, high levels of endemism, and significant role in conservation efforts, substantially
546 impacting elasmobranch preservation worldwide (Becerril-García et al. 2022). Conservation
547 strategies are crucial for preventing further population declines and genetic erosion in Brazil.
548 The country should prioritize habitat protection, promote sustainable fishing practices,
549 establish marine protected areas, regulate trade, minimize bycatch, and sustainably manage
550 fisheries. Collaboration among researchers, Brazilian government, stakeholders, and
551 international organizations is essential to ensure the success of these conservation efforts.

552 **4.4. Legislative Measures in Brazil: Towards Effective Elasmobranchs Protection?**

553 Fish conservation regulations in Brazil have evolved over the years, reflecting the
554 country's political landscape. Past initiatives, like anti-finning regulations, showcased Brazil's
555 commitment to elasmobranch conservation. The incorporation of the Brazilian Red Book of
556 Threatened Fauna (ICMBio 2018) assessments into legislation have addressed the trade and
557 exploitation of threatened elasmobranchs. Nevertheless, challenges emerged with the
558 substitution or repristination of previous regulations and with the introduction of new
559 regulation actions by ordinances MMA No. 148/2022 and No. 354/2023, raising concerns on
560 the possibility of the interests of fisheries industries being prioritized over conservation
561 strategies. It is expected that the new national database known as SALVE System will
562 contribute to improve conservation initiatives and provide advances, since it contains
563 assessments that are available online, regularly updated, and easily accessible. These
564 assessments hopefully will be considered in future regulations and thereby improve species
565 management and conservation. Even though the recent release of the SALVE System
566 increased the quantity of species assessed and updated previous extinction risk status,
567 discrepancies between the IUCN Red List still occur, with a high rate of species still to be
568 evaluated. We also found differences in the extinction rate between the international and
569 national lists analyzed (Fig. 5a), including in endemic species, which indicates inconsistent
570 categorizations for geographically restricted species. The discrepancies between national and
571 international lists can be attributed to outdated assessments, limited updates in the Brazilian

572 threatened species lists, and factors like data availability, assessment methodologies, political
573 considerations, and regional conservation priorities. To enhance consistency and
574 effectiveness, it is crucial to invest on regular updates, improved integration of data and
575 assessment methodologies, and accurately assess extinction risks and conservation needs. It
576 is important to highlight that while the IUCN Red List is a comprehensive source on the
577 global extinction risk status of animal, fungus and plant species at a global scale, the SALVE
578 System focuses at regional (or national) level by assessing species that are part of the
579 Brazilian biodiversity, being used to delineate legislative measures. Given this, it is important
580 that future studies on trade consider and analyze jointly the current Brazilian regulations and
581 the IUCN Red List assessments when discussing threatened species.

582 Even though the labeling regulations were also updated over time, there have been no
583 significant changes for elasmobranch species, which can still be traded under umbrella-labels
584 nationwide. The lack of species-specific labeling and updated scientific names for sharks and
585 rays in fish labeling regulations poses significant challenges for trade monitoring and
586 conservation efforts (Bornatowski et al. 2013). It reduces commercialization transparency,
587 impeding proper species identification, sustainable fishing practices, and conservation
588 effectiveness. Without accurate identification, tracking and regulating the trade of threatened
589 shark species becomes difficult, potentially hiding and escalating their decline. To address
590 this, the labeling regulations need to be aligned with conservation regulations. This can be
591 achieved by appropriately distinguishing labels for threatened species. Alternatively, by
592 outright prohibiting the trade of "caçãõ", as seen for all sharks in the Bahamas, Palau and the
593 Marshall Islands and for a specific umbrella-term that encompass all elasmobranch species
594 in the Maldives, New Caledonia and the Federated States of Micronesia (Ward-Paige 2017).

595 ***4.5. The Mislabeling Risk: Deceptive Practices Beyond Label Swaps***

596 Generic labeling is also a concerning reality for elasmobranchs in Brazil. Besides the
597 use of umbrella labels at markets and restaurants, we also observed generic labeling of fish
598 in education institution dining services, where elasmobranchs are served without proper
599 species identification, leading unaware customers to consume shark meat (Alvarenga et al.
600 2021). Further investigations are essential to evaluate these cases, as there are also concerns
601 about the presence of shark meat in hospitals, posing risks to both conservation efforts and
602 public health due to high metalloid levels in shark meat (Hauser-Davis et al. 2021; Willmer
603 et al. 2022). Identifying and exposing such activities is crucial to raise awareness, strengthen
604 regulations, and potentially prohibit these practices. Recently, shark meat purchased by the
605 municipality of São Paulo for distribution in schools was recalled due to public pressure,
606 highlighting the effectiveness of raising awareness and taking action against such practices
607 (Bonin 2021).

608 Shark and ray products have also been distributed under mislabeled names such as
609 salmon and croaker (Staffen et al. 2017), while conversely, bony fishes have been frequently
610 sold as elasmobranchs (Almeron-Souza et al. 2018; Calegari et al. 2019; Cruz et al. 2021).
611 Elasmobranchs have been mislabeled not only as bony fish but also as other elasmobranch
612 species (Almeron-Souza et al. 2018; Alvarenga et al. 2021; Bernardo et al. 2020; Bunholi et
613 al. 2018; Palmeira et al. 2013). This deceptive practice not only undermines consumer trust
614 but also poses an especially significant conservation challenge to threatened rays, which are
615 sold at low cost (Niedermüller et al. 2021).

616 We also noted instances of incorrect labeling and incomplete information in the papers
617 analyzed. The misidentifications noted comprised: 1) Failure to differentiate between
618 information related to individual samples, making it impossible to verify the sampling
619 location and label associated with each sample (Table S1, in Appendix B); 2) Variation in the
620 availability of information on sampling date, market sector, price, and origin of capture
621 among the papers (some papers provided comprehensive information on all these aspects,

622 while others had missing or incomplete data) (Table S2, Appendix A); and 3) Non-standard
623 labeling information, often representing only the regional names rather than the regulated
624 name under legislative measures (Table S1, in Appendix B). While documenting diverse
625 regional terminology for traded sharks and rays offers valuable insights into market and
626 cultural distinctions within Brazil, it remains important to also incorporate the label of the
627 marketed product. In cases where such designations are absent, explicit acknowledgment
628 should be indicated in the published papers, as the absence could hinder effective
629 identification of mislabeling patterns. Avoiding such inaccuracies in future papers will be
630 essential to assess the magnitude and dynamics of the Brazilian trade and develop a more
631 precise national-scale analysis of market activity.

632 **5. Current Research Gaps and Required Actions**

633 In conclusion, the present study emphasizes the need to address general research gaps
634 and suggests actions to enhance trade assessments at a national scale (Table 3). By doing so,
635 we can develop effective management and conservation measures to improve shark and ray
636 species protection in Brazil. It is crucial to address these limitations to advance our
637 understanding and take necessary actions to safeguard these vulnerable species.

638 The identification of key governmental gaps that warrant attention is also required, and
639 here we highlight two primary points. First, there is a pressing need for more robust
640 legislation, including protecting threatened species to ensure environmental-based risk status
641 assessment, and proper labeling of products to restrain mislabeling activities. Synchronizing
642 legislative measures for both labeling and conservation is also required to promote best
643 market practices that simultaneously safeguard biodiversity and consumers rights. Second,
644 the need for efficient monitoring mechanisms and frequent inspections can help to restrict the
645 trade of protected species and mitigate mislabeling practices.

646 The Brazilian government is urged to invest in keeping extinction risk assessments
647 updated, enhancing legislative measures and conducting frequent inspections. Additionally,
648 fostering collaborations with research institutions and stakeholders is recommended, such as
649 leveraging on technical expertise and HTS techniques for extensive sampling campaigns and
650 apprehensions utilizing large-scale and cost-effective analysis (e.g., large-scale amplicon
651 sequencing). The proposed actions extend beyond governmental responsibilities, as
652 researchers and citizens are not exempt from contributing to species conservation. It is
653 recommended that these two groups communicate and advocate for new and more effective
654 legislative measures. This can enhance transparency and accuracy in elasmobranch trade and
655 better protect species. A multifaceted approach involving government, researchers, citizens,
656 stakeholders, and international organizations is essential for the comprehensive and effective
657 management of elasmobranch species.

658 **6. Appendix**

659 Additional information can be found in the online version of the article at the publisher's
660 website.

661 Appendix A includes detailed information on data acquisition and supplementary results
662 supporting our findings.

663 Appendix B provides the main metadata built to analyze the data from all peer-reviewed
664 papers collected, alongside a detailed explanation of each category.

665 Appendix C offers additional metadata to support our analysis on the availability of genetic
666 resources for Brazilian species and the evolution of Brazilian legislative measures, as well as
667 national and international conservation lists.

668 Appendix D provides detailed R scripts for the analyses performed in this paper and presented
669 in the main text. The data used with the R scripts are available at GitHub (link available upon
670 acceptance) to ensure reproducibility.

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1090

1092 **Table 1.** Summary of 35 research papers using DNA tools to investigate elasmobranch trade
 1093 in Brazil from 2008 to 2023, including paper categories, sample sizes, the number of
 1094 elasmobranchs and endangered species identified (based on the IUCN Red List at publication
 1095 and current status), dominant species per paper, Brazilian regions studied, type of DNA-based
 1096 tool and molecular marker applied, and if the paper aimed to detect mislabeling activity.

| Authors | Paper category | Sample size | Elasmo branch species | IUCN Listed (paper) | IUCN Listed (current) | Brazilian Regions | Molecular marker | DNA-based tool | Most frequent species | Mislabel ‡ |
|--------------------------------|------------------------|-------------|-----------------------|---------------------|-----------------------|-----------------------------------|------------------|----------------------------------|----------------------------------|------------|
| Pinhal et al. (2008) | Methods | NA | 8 | NA | 7 | All coastal regions | 5S rDNA | Tandem Repeats | NA | NO |
| Mariguela et al. (2009) | Methods | 145 | 3 | NA | 3 | Southeast and South | 16S/COI | PCR-RFLP | <i>Pseudobatos percellens</i> | NO |
| Mendonca et al. (2009) | Methods | 86 | 2 | NA | 2 | Northeast and Southeast | COI | Multiplex PCR | <i>Rhizoprionodon porosus</i> | NO |
| Pinhal et al. (2009) | Methods | 36 | 2 | NA | 2 | Brazilian | 5s rDNA | PCR-RFLP | NA | NO |
| Rodrigues-Filho et al. (2009) | Trade Analysis | 122 | 11 | 1 | 10 | North | 12S-16S | DNA sequencing | <i>Carcharhinus porosus</i> | YES |
| De-Franco et al. (2010) | Methods | 145 | 3 | NA | 3 | Southeast and South | COI | Multiplex PCR | <i>Pseudobatos percellens</i> | NO |
| Mendonca et al. (2010) | Methods | 443 | 25 | NA | 20 | All coastal regions | COI | Multiplex PCR | NA | NO |
| Pinhal et al. (2012) | Methods/Trade Analysis | 62/90* | 7 | NA | 4 | All coastal regions | ITS2 | Multiplex PCR | <i>Rhizoprionodon terranovae</i> | NO |
| De-Franco et al. (2012) | Trade Analysis | 267 | 3 | NA | 3 | Northeast, Southeast and Southern | COI | Multiplex PCR | <i>Pseudobatos horkelii</i> | NO |
| Palmeira et al. (2013) | Trade Analysis | 44 | 8 | NA | 7 | Northern | 16S/CytB | DNA sequencing | <i>Pristis perottepeerti</i> | YES |
| Ribeiro et al. (2012) | Trade Analysis | 41 | 13 | NA | 10 | Southeast | COI | DNA sequencing | <i>Atlantoraja castelnaui</i> | NO |
| Domingues et al. (2013) | Trade Analysis | 317 | 4 | 2 | 4 | Southeast | ITS2/COI | Multiplex PCR | <i>Carcharhinus falciformis</i> | YES |
| Faria et al. (2013) | Trade Analysis | 77 | 1 | 1 | 1 | Northern | CytB | DNA sequencing | NA | NO |
| Falcão et al. (2016) | Methods | 9 | 3 | NA | 3 | Southern | CytB | PCR-RFLP | NA | NO |
| Schmidt et al. (2015) | Methods/Trade Analysis | 97 | 4 | 0 | 2 | Southeast | COI | PCR-RFLP | <i>Dasyatis hypostigma</i> | NO |
| Schmidt et al. (2015) | Trade Analysis | 1 | 1 | 1 | 1 | Southern | COI | DNA sequencing | NA | NO |
| Nachtigall et al. (2017) | Methods/Trade Analysis | 67/51** | 8 | 1 | 7 | Northern | ITS2 | Multiplex PCR | <i>Rhizoprionodon porosus</i> | YES |
| Carvalho et al. (2017a) | Trade Analysis | 8 | 1 | NA | 0 | | COI | DNA sequencing | NA | NO |
| Staffen et al. (2017) | Trade Analysis | 14 | 5 | 2 | 3 | Southern | COI | DNA sequencing | <i>Prionace glauca</i> | YES |
| Almeron-Souza et al. (2018) | Trade Analysis | 63 | 18 | 8 | 14 | Southern | COI | DNA sequencing | <i>Prionace glauca</i> | NO |
| Bunholi et al. (2018) | Trade Analysis | 85 | 3 | 3 | 3 | Southern | COI | DNA sequencing | <i>Squatina guggenheim</i> | YES |
| Feitosa et al. (2018) | Trade Analysis | 427 | 17 | 4 | 15 | Northern | COI | DNA sequencing | <i>Sphyrna mokarran</i> | NO |
| Marques et al. (2020) | Methods | 279 | 10 | 5 | 8 | Southeast | COI/CytB | Morphometrics/DNA sequencing | <i>Gymnura altavela</i> | NO |
| Calegari et al. (2019) | Trade Analysis | 7 | 0 | NA | NA | Southeast | COI | DNA sequencing | NA | NO |
| Ferrette et al. (2019a) | Trade Analysis | 228 | 17 | 5 | 12 | Southeast | COI | DNA sequencing | <i>Dasyatis sp.</i> | NO |
| Ferrette et al. (2019b) | Trade Analysis | 747 | 20 | 9 | 18 | Northern, Northeast and Southeast | COI | Multiplex PCR and DNA sequencing | <i>Prionace glauca</i> | NO |
| Bernardo et al. (2020) | Trade Analysis | 231 | 16 | 7 | 12 | Southern | COI | DNA sequencing | <i>Prionace glauca</i> | NO |
| Camacho-Oliveira et al. (2020) | Trade Analysis | 52 | 4 | 1 | 2 | Southeast | COI | DNA sequencing | <i>Paratrygon ajereba</i> | NO |
| Guimaraes-Costa et al. (2020) | Trade Analysis | 73 | 20 | 4 | 13 | Northern | COI | DNA sequencing | NA | NO |
| Rodrigues et al. (2020) | Trade Analysis | 118 | 9 | 2 | 5 | Northern | COI | DNA sequencing | <i>Hypanus guttatus</i> | NO |
| Alvarenga et al. (2021) | Trade Analysis | 220 | 17 | 13 | 15 | Southeast | COI | DNA sequencing | <i>Prionace glauca</i> | YES |

| | | | | | | | | | | |
|----------------------------|----------------|-----|----|----|----|---------------------|-----|----------------|-------------------------|-----|
| Cruz et al. (2021) | Trade Analysis | 56 | 9 | 6 | 8 | Southern | COI | DNA sequencing | <i>Prionace glauca</i> | YES |
| Martins et al. (2021) | Trade Analysis | 127 | 20 | 12 | 17 | Northern | COI | DNA sequencing | <i>Sphyrna mokarran</i> | YES |
| Merten-Cruz et al. (2021) | Trade Analysis | 57 | 17 | 7 | 13 | All coastal regions | COI | DNA sequencing | <i>Prionace glauca</i> | NO |
| Souza-Araujo et al. (2021) | Trade Analysis | 91 | 13 | 4 | 12 | Northern | COI | DNA sequencing | <i>Mustelus higmani</i> | NO |

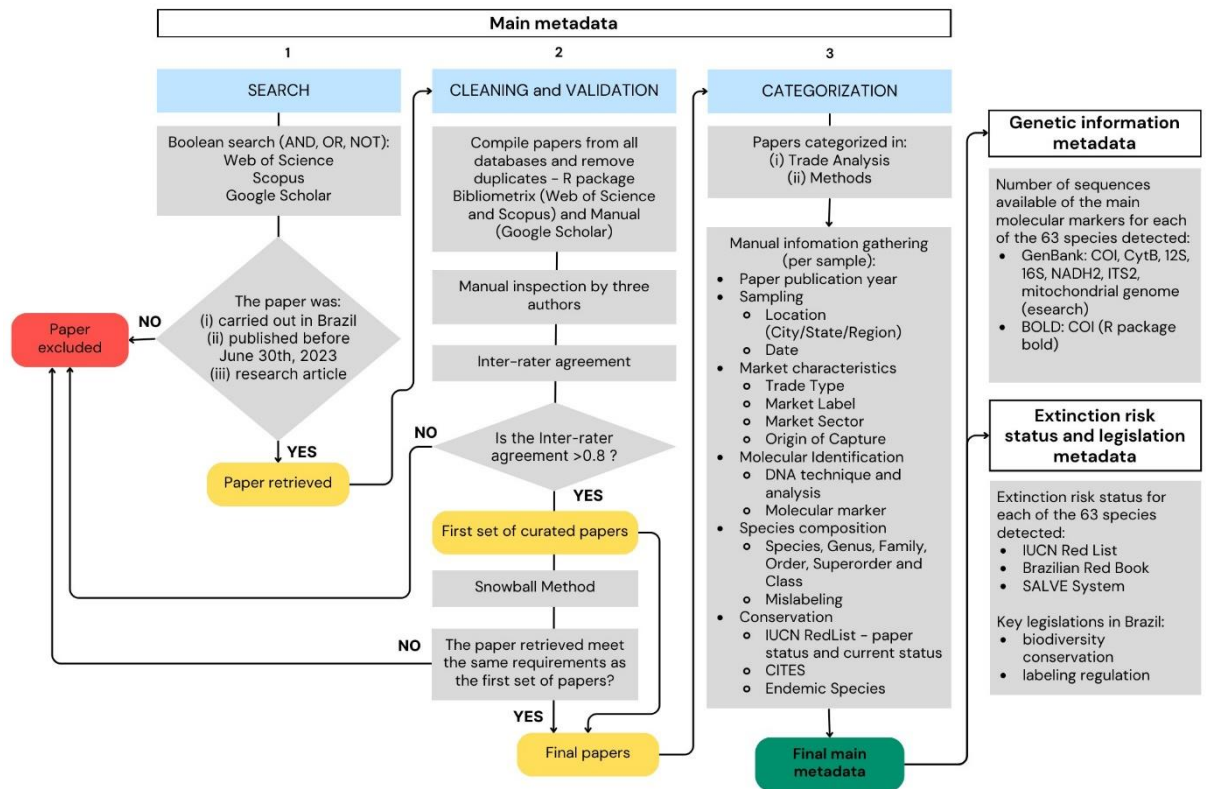
‡ Mislabel = the paper analyzed used the results provided by DNA analysis to infer if the fish sold was mislabeled. * This paper used 166 samples to design the DNA rapid assay, only 62 of which were collected in Brazil. Also, they applied the technique to 90 other samples collected in Brazilian markets. ** This paper used 67 samples to design the DNA rapid assay, 10 of which were newly collected and 57 from other papers. Also, they applied the technique to 51 other samples collected in Brazilian markets.

1098 **Table 2.** Summary of the current risk of extinction status and number of shark and ray species
 1099 found in the papers analyzed. The table includes species categorized CR, EN, VU, NT, LC,
 1100 DD by the International Union for Conservation of Nature (IUCN) Red List of Threatened
 1101 Species, and a detailed list of all species found.

| Status | Number of Species | Species |
|-----------------------|-------------------|--|
| Critically Endangered | 12 | <i>Atlantoraja castelnaui</i> , <i>Carcharhinus porosus</i> , <i>Carcharias taurus</i> , <i>Fontitrygon geijskesi</i> , <i>Galeorhinus galeus</i> , <i>Isogomphodon oxyrhynchus</i> , <i>Pristis pristis</i> , <i>Pseudobatos horkelii</i> , <i>Sphyrna lewini</i> , <i>Sphyrna mokarran</i> , <i>Sphyrna tudes</i> , <i>Squatina occulta</i> |
| Endangered | 20 | <i>Aetobatus narinari</i> , <i>Atlantoraja cyclophora</i> , <i>Carcharhinus acronotus</i> , <i>Carcharhinus obscurus</i> , <i>Carcharhinus perezi</i> , <i>Carcharhinus plumbeus</i> , <i>Carcharhinus signatus</i> , <i>Centrophorus squamosus</i> , <i>Dasyatis hypostigma</i> , <i>Gymnura altavela</i> , <i>Isurus oxyrinchus</i> , <i>Isurus paucus</i> , <i>Mobula thurstoni</i> , <i>Mustelus higmani</i> , <i>Pseudobatos percellens</i> , <i>Sphyrna tiburo</i> , <i>Squalus mitsukurii</i> , <i>Squatina guggenheim</i> , <i>Styracura schmardae</i> , <i>Zapteryx brevirostris</i> |
| Vulnerable | 20 | <i>Alopias superciliosus</i> , <i>Bathytoshia centroura</i> , <i>Benthobatis krefftii</i> , <i>Carcharhinus brachyurus</i> , <i>Carcharhinus brevipinna</i> , <i>Carcharhinus falciformis</i> , <i>Carcharhinus leucas</i> , <i>Carcharhinus limbatus</i> , <i>Carcharodon carcharias</i> , <i>Ginglymostoma cirratum</i> , <i>Hypanus berthallutzae</i> , <i>Hypanus dipterurus</i> , <i>Myliobatis freminvillei</i> , <i>Myliobatis goodei</i> , <i>Rhinoptera bonasus</i> , <i>Rhinoptera brasiliensis</i> , <i>Rhizoprionodon lalandii</i> , <i>Rhizoprionodon porosus</i> , <i>Rioraja agassizii</i> , <i>Sphyrna zygaena</i> |
| Near Threatened | 8 | <i>Carcharhinus altimus</i> , <i>Galeocerdo cuvier</i> , <i>Gymnura micrura</i> , <i>Hypanus americanus</i> , <i>Hypanus guttatus</i> , <i>Mustelus canis</i> , <i>Narcine brasiliensis</i> , <i>Prionace glauca</i> |
| Least Concern | 3 | <i>Carcharhinus galapagensis</i> , <i>Rhizoprionodon terraenovae</i> , <i>Squalus cubensis</i> |
| Data Deficient | 2 | <i>Paratrygon aiereba</i> , <i>Potamotrygon motoro</i> |

Table 3. General research gaps and suggestions of required actions to enhance ability to assess the trade at a national scale.

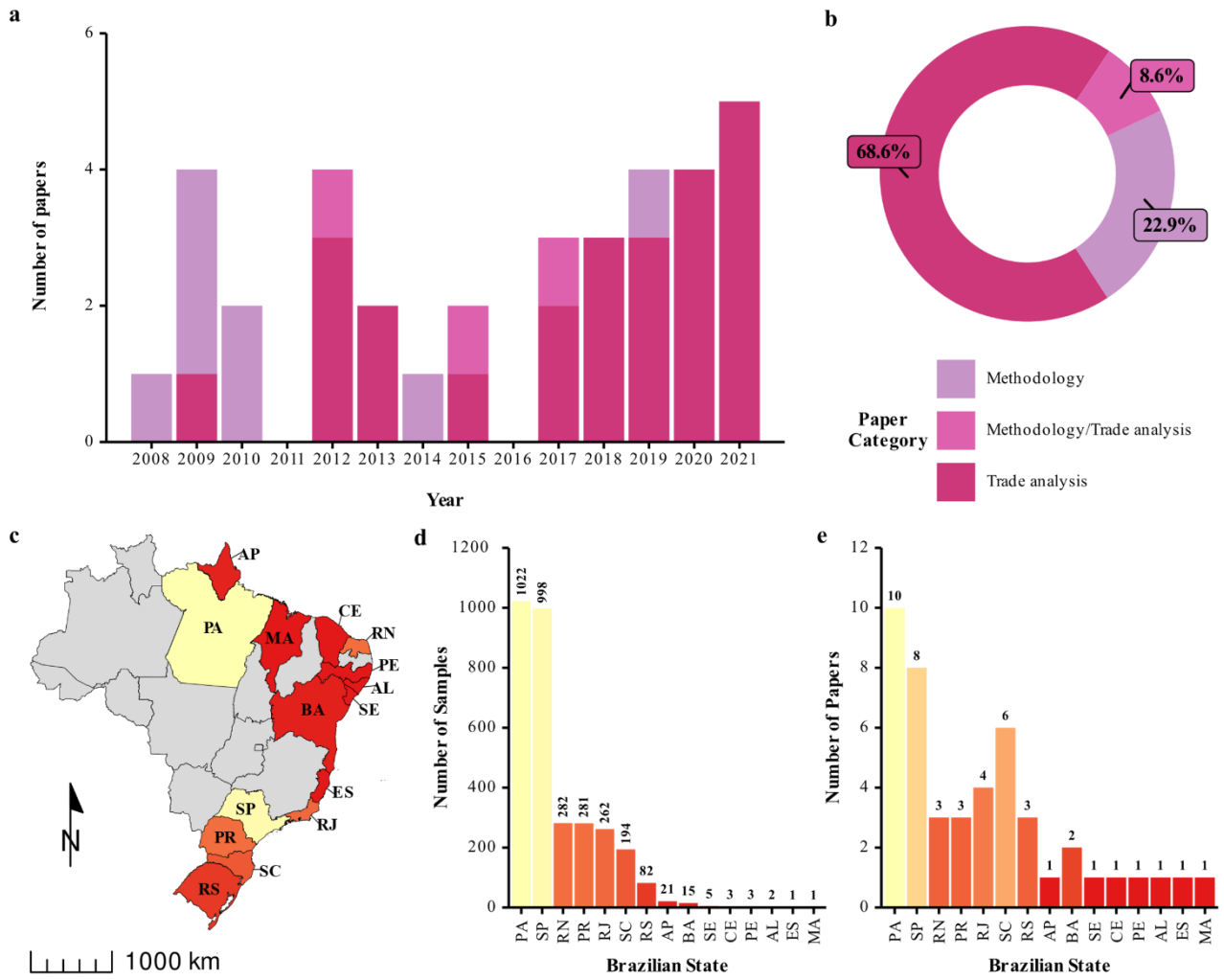
| Goal | Research Gap | Required Action |
|------|--|---|
| i | 1 Lack of a strategic monitoring plan, often relying only on opportunistic sampling, and standardized or minimal documentation (e.g., location, date, price, and market origin). | Formulate a comprehensive and basic sampling protocol, considering regional characteristics and the periodicity of data collection, and establish best practices guidelines to ensure detailed documentation for accurate and comparable data. |
| | 2 Limited number of studies and sampling coverage-on the Northeast Region of Brazil. | Expand research efforts in the Northeast Region of Brazil to address regional disparities. |
| | 3 Concentration of studies primarily focused on analyzing the shark trade. | Expand research to embrace more elasmobranch species in trade (e.g., ray trade, bycatch and finning). |
| | 4 Insufficient application of cost-effective genetic techniques previously developed for elasmobranchs (such as Multiplex PCR and PCR-RFLP) in trade studies. | Promote the utilization of cost-effective DNA-based tools, such as Multiplex PCR and PCR-RFLP, mainly adopting available tools to improve accessibility and frequency of species identification. |
| ii | 5 Underutilization of HTS techniques for species identification in Brazil, with a lack of research using metabarcoding (e.g., detect elasmobranch products in food mixtures), eDNA (e.g., fins dust on markets, water of fishing vessels), large-scale amplicon sequencing (e.g., reduce costs and analyze a higher volume of market samples), among others. | Increase the development and implementation of high-throughput sequencing in cases where it applies (e.g., food mixtures, vessel water analysis, large seizures or sampling campaigns). |
| | 6 Scarcity of DNA sequences from important Brazilian elasmobranch species and populations in genetic databases, particularly ray species | Establish a regularly curated national database with precise species identification and molecular markers, including haplotype differentiation, to effectively support geographical assessments. Foster collaborative initiatives to generate genetic data of Brazilian elasmobranch species, especially rays, and contribute to the expansion of molecular markers in the national genetic database. |
| | 7 Lack of population genetics and phylogeographic studies for elasmobranch species targeting their whole geographic distribution, impeding a proper geographic tracking of both meat and fins trade. | |
| iii | 8 Risk of extinction status (e.g., IUCN, SALVE) is not addressed in certain papers. | Incorporate both IUCN Red List, current Brazilian legislation and national risk of extinction status into papers to evaluate compliance with legislation. |
| iv | 9 Compliance to the regulations to protect threatened species, such as the MMA 354/2023, are not evaluated in most of the papers analysed. | |
| v | 10 Non-standardized or absent labeling notes when sampling, hindering the assessment of mislabeling activity. | Implement standardized labeling notes when sampling elasmobranch products to enable a comprehensive assessment of mislabeling activity and enhance understanding of the dynamics of the shark trade. |



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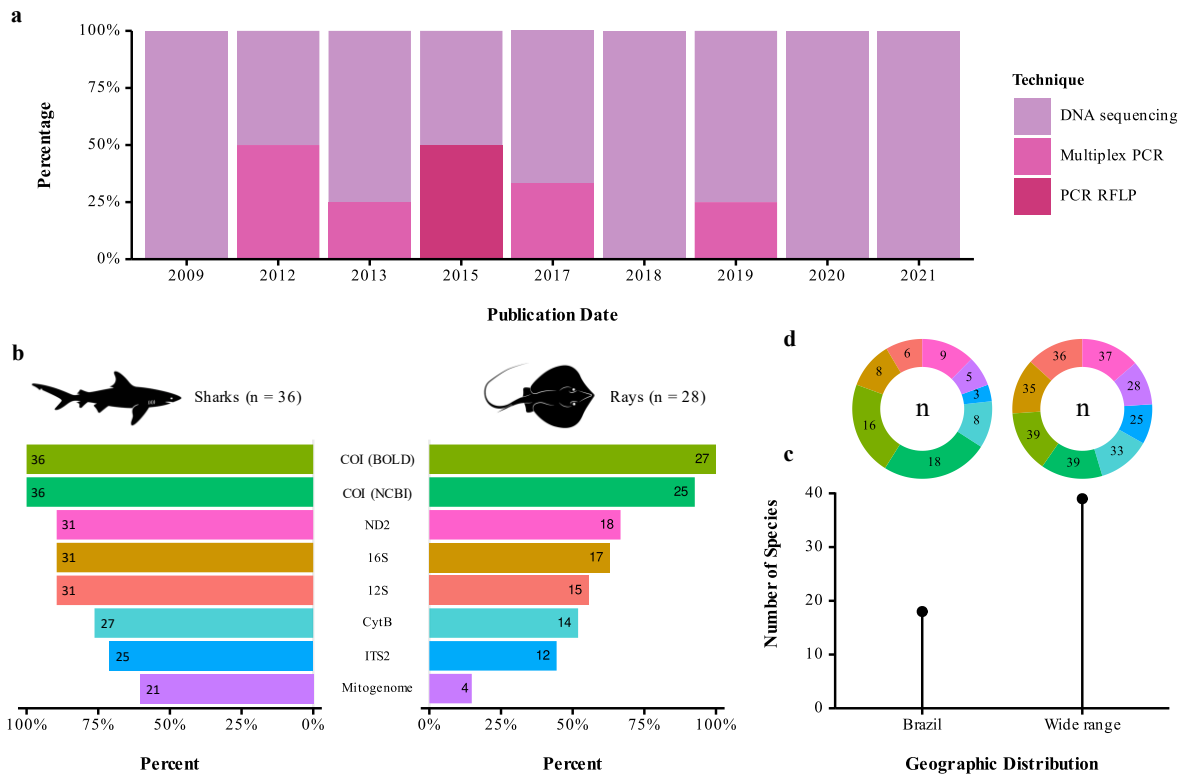
1106 **Fig. 1.** General workflow of the approach used to the assessment of research trends, molecular
 1107 species identification, species composition in trade and the Brazilian legislation consulted.

1108



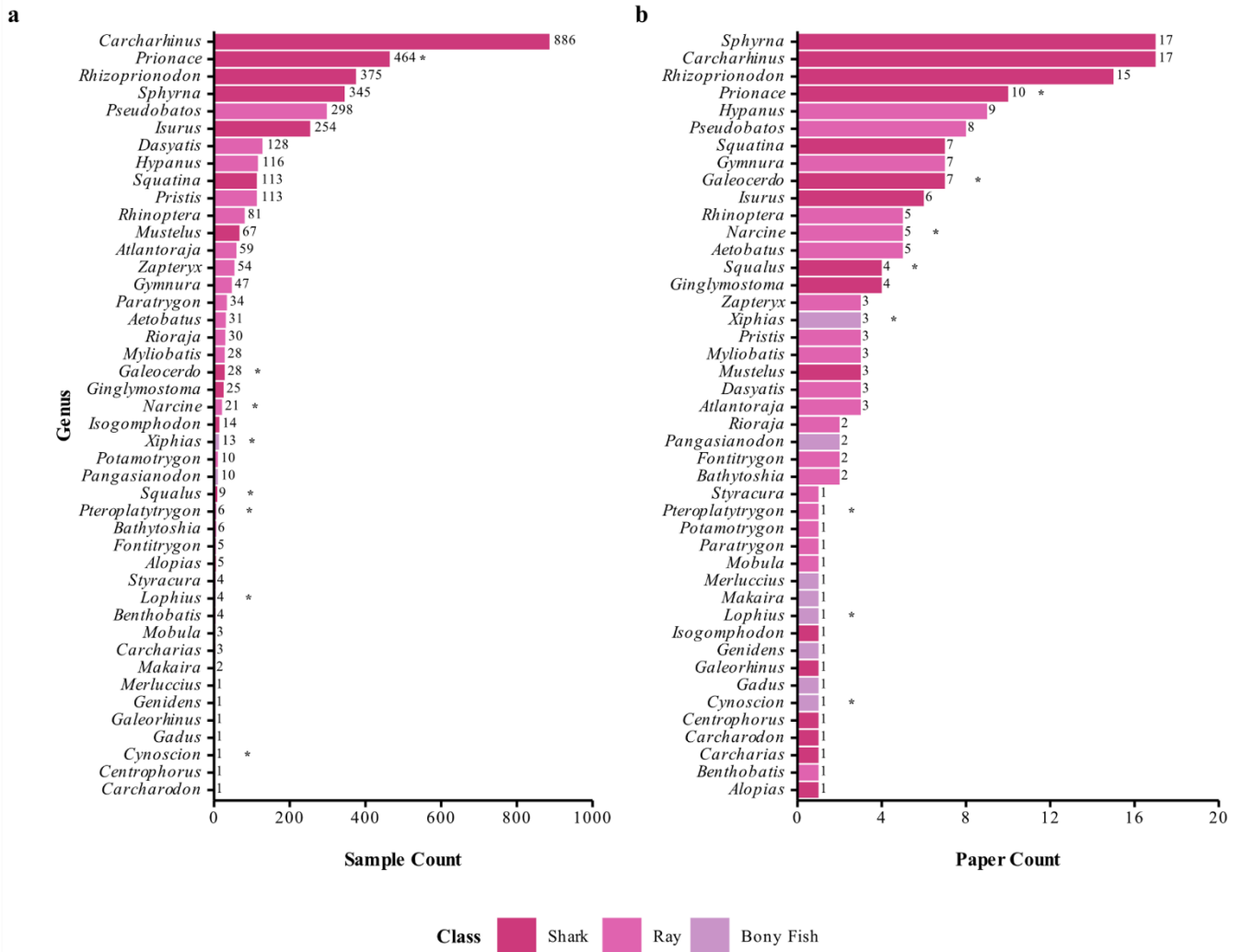
1109

1110 **Fig. 2.** Overview of the papers published on the topic of DNA tools to analyze elasmobranchs
 1111 trade in Brazil between 2008 and 2023, including (A) year of publication, (B) type of research
 1112 in each paper, and number of samples per (C) Brazilian regions, (D) samples and (E) papers.
 1113 Quantity of paper is inversely proportional to the darkness in shade of colors (i.e., in C the
 1114 regions in dark shades are the ones with less papers published, in D the dark shades mean less
 1115 samples available, and in E less papers published), and gray color refers to no paper found in
 1116 the state. The abbreviations refer to Brazilian states, as follows: PA - Pará, AP - Amapá (North
 1117 Region), MA - Maranhão, CE - Ceará, RN - Rio Grande do Norte, PE - Pernambuco, AL -
 1118 Alagoas, SE - Sergipe, BA - Bahia (Northeast Region), ES - Espírito Santo, RJ - Rio de
 1119 Janeiro, SP - São Paulo (Southeast Region), PR - Paraná, SC - Santa Catarina, RS - Rio
 1120 Grande do Sul (South Region).

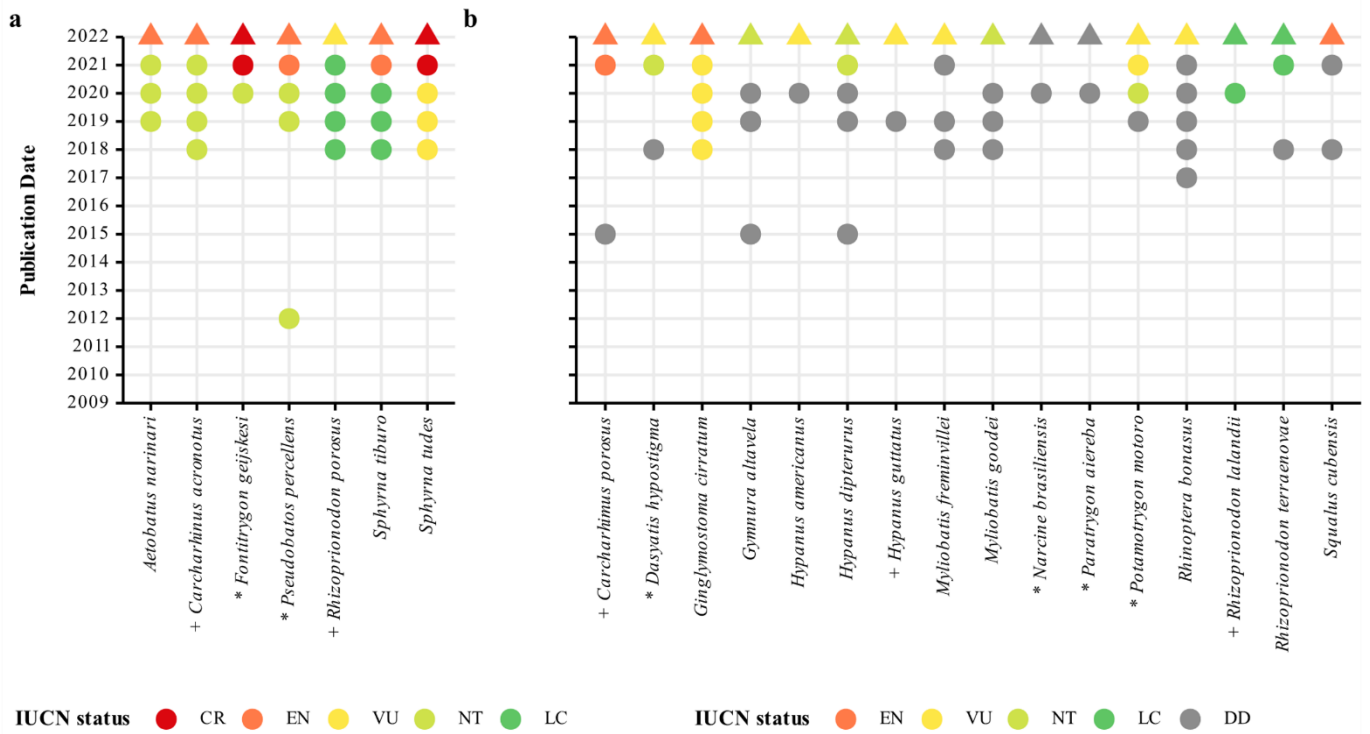


1121

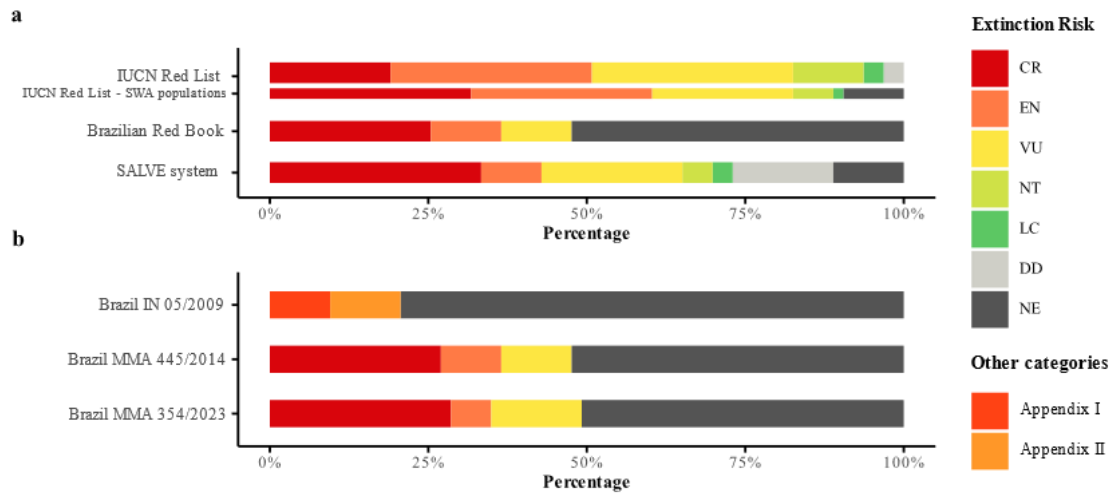
1122 **Fig. 3.** DNA tools in elasmobranch trade analysis. (a) Evolution of the DNA techniques
 1123 applied through time, (b) number of shark and ray species found in all papers depicted
 1124 alongside the animals silhouettes, with the percentage of sequences in GenBank and BOLD
 1125 databases per molecular marker for shark and ray species (absolute numbers depicted inside
 1126 the bar), and (c) number of species with sequences available in both databases for endemic
 1127 species of Brazil versus wide-range species, (d) with the number of species per specific
 1128 molecular marker depicted, using the same color code as in B.



1130 **Fig. 4.** The (a) sample count and (b) paper count of genera found in all papers analyzing the
 1131 elasmobranch trade in Brazil between 2008 and 2023. The class of each genus (shark, ray or
 1132 bony fish) is defined by the color in the caption. The genera that did not contain endangered
 1133 species are marked with *. For a complete list of sample count and paper count of the species
 1134 found in this study, please refer to table S3 in appendix A.



1136 **Fig. 5.** Evolution of extinction risk assessment over time for species found in the present
 1137 study (a) that increased by two categories and (b) that was previously categorized as data
 1138 deficient. The category assigned at the time the analyzed paper was published is represented
 1139 by a circle, while the current category is represented by a triangle. The assessments for all
 1140 elasmobranch species found in the present study can be found in Figure S3.



1141

1142 **Fig. 6.** Categorization of all elasmobranch species identified in the present study according
 1143 to (a) international (IUCN Red List considering global assessment and SWA population
 1144 details) and national assessments of risk of extinction (Brazilian Red Book of Threatened
 1145 Fauna and SALVE System), and (b) the three main Brazilian ordinances. The Brazilian
 1146 Ordinance IN05/2004 was the only one that did not follow the standard extinction risk
 1147 categories (DD, LC, NT, VU, EN, CR), adopting instead two categories: Appendix I
 1148 (Threatened) and Appendix II (Overexploited).