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Taxonomic Identification of Two Poorly Known Lantern Shark Species Based on Mitochondrial DNA From Wet-Collection Paratypes

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Etmopteridae (lantern sharks) is the most species-rich family of sharks, comprising more than 50 species. Many species are described from few individuals, and re-collection of specimens is often hindered by the remoteness of their sampling sites. For taxonomic studies, comparative morphological analysis of type specimens housed in natural history collections has been the main source of evidence. In contrast, DNA sequence information has rarely been used. Most lantern shark collection specimens, including the types, were formalin fixed before long-term storage in ethanol solutions. The DNA damage caused by both fixation and preservation of specimens has excluded these specimens from DNA sequence-based phylogenetic analyses so far. However, recent advances in the field of ancient DNA have allowed recovery of wet-collection specimen DNA sequence data. Here we analyse archival mitochondrial DNA sequences, obtained using ancient DNA approaches, of two wet-collection lantern shark paratype specimens, namely *Etmopterus litvinovi* and *E. pycnolepis,* for which the type series represent the only known individuals. Target capture of mitochondrial markers from single-stranded DNA libraries allows for phylogenetic placement of both species. Our results suggest synonymy of *E. benchleyi* with *E. litvinovi* but support the species status of *E. pycnolepis*. This revised taxonomy is helpful for future conservation and management efforts, as our results indicate a larger distribution range of *E. litvinovi*. This study further demonstrates the importance of wet-collection type specimens as genetic resource for taxonomic research.

Keywords: type specimens, *Etmopterus litvinovi*, *Etmopterus pycnolepis*, deep-sea sharks, archival DNA

INTRODUCTION

Shark diversity is poorly represented in the scientific literature. Shark biologists have tended to focus on a few easy-to-access taxa that are assumed to be representative of the groups to which they belong. For example, though there are more than 40 different species of deep-sea lantern sharks (genus *Etmopterus*), nearly a quarter of the 2082 publications devoted to Lantern shark biology (Pollerspöck and Straube, 2021) has focussed on a single species (*Etmopterus spinax*). Thus, most of the diversity of this group remains relatively unexplored (**Figure 1**). To make matters worse, a substantial fraction of lantern shark diversity is known only from formalin preserved type material that was collected prior to the advent of DNA sequencing. Hence, tissue sampling, common practice today for performing DNA sequence-based analysis such as DNA barcoding (Hebert et al., 2003), was not conducted and fixation in formaldehyde and preservation in ethanol causes DNA damage (Gilbert et al., 2007; Hoffman et al., 2015; Hykin et al., 2015; Stiller et al., 2016; McGuire et al., 2018; Hahn et al., 2021). This means that, while we know that the group has diversified extensively (e.g., Straube et al., 2011a; Ebert et al., 2016, 2021; White et al., 2017; Dolganov and Balanov, 2018), it has been hard to decipher how the different species are related to one another and how different ecological pressures have contributed to their diversification. Recently developed tools allow us to obtain DNA sequence data from formalin preserved animals (Gansauge et al., 2017; Hahn et al., 2021; Straube et al., 2021a). In the current contribution we have applied these tools to type material for two species of *Etmopterus* and show how the data collected have implications, not only for understanding their taxonomy and evolution, but also their ranges, which has consequences for their conservation and management. The genus is subdivided into four clades supported by both DNA sequence data and morphological characters (Straube et al., 2010). Morphological characters therefore allow for tentative assignments of species lacking DNA sequence information to one of the four clades. Our first target species, *Etmopterus litvinovi* (Smalleye lantern shark, Parin and Kotlyar, 1990) has been assigned to the *E. spinax* clade (Straube et al., 2010, 2011a) comprising 11 species today (Ebert et al., 2021). The presence and shape of flank markings, dark patterns above the pelvic fins, is a key character allowing for species-to-clade assignments in many *Etmopterus* species. While the character is not present in all species and ontogenetic stages, every species of the *E. lucifer* clade shows distinct flank markings characterised by anterior and posterior branches. Species of the *E. lucifer* clade can further be subdivided into three subclades based on length comparisons of the anterior and posterior flank mark branches (Ebert et al., 2021). The three subclades are the *E. lucifer*, the *E. molleri* and the *E. burgessi* subclades. The *E. lucifer* subclade includes the four species *E. brosei*, *E. lailae*, *E. lucifer* and *E. sculptus*. *E. alphus*, *E. brachyurus*, *E. bullisi*, *E. decacuspidatus*, *E. dislineatus*, *E. molleri,* and *E. samadiae* are the seven species assigned to the *E. molleri* subclade. The *E. burgessi* subclade comprises four species, namely *E. burgessi*, *E. evansi*, *E. marshae*, and *E. pycnolepis* (Ebert et al., 2021). *Etmopterus pycnolepis* (Dense-scale lantern shark, Kotlyar, 1990) is our second target species. Both *E. litvinovi* and *E. pycnolepis* are known from their type specimens only and little is known regarding their biology as they were hitherto sampled only once each in the Salas y Gómez and Nazca submarine ridges in the Southeast Pacific (Kotlyar, 1990; Ebert et al., 2013).

MATERIALS AND METHODS

Etmopterus litvinovi (Smalleye Lantern Shark)

This species is known from 32 type specimens housed in three different museum collections, the Laboratory of Ichthyology at the Zoological Institute of the Russian Academy of Sciences (ZIN), St. Petersburg, Russia (holotype: ZIN 49228; six paratypes: ZIN 49229–32), the Zoological Museum (ZMMU), Biological Faculty, M. V. Lomonosov Moscow State University, Moscow, Russia (21 paratypes ZMMU: P-17989–91; two paratypes P-18222) and the ichthyological collection of the Zoological Museum (ZMH) of the LIB in Hamburg, Germany [paratype ZMH 24994 (ex ISH 6-1989); paratype ZMH 24993 (ex ISH 5-1989)]. We sampled muscle tissue from the paratype specimen ZMH 24994 (**Figure 2A**) at the caudal peduncle using a biopsy needle for minimally invasive sampling. The tissue was preserved in the original preservation fluid of the storage container. The specimen was captured at 25°21′S and 85°8′W at a depth of 720 m on 24.04.1987. It is a juvenile male of 445 mm total length (Thiel et al., 2009; Straube et al., 2011a). Although not explicitly mentioned in the original description, or tested by us, the overall condition of the specimen indicates a fixation in formaldehyde: both body and eyes do not show bleaching of exclusively ethanol preserved samples. Furthermore, the common procedure during research cruises at the time of sampling was a fixation of specimens in 4% formaldehyde and long-term preservation in 70% ethanol.

Laboratory steps and analysis of test-sequencing data of this specimen is described in detail in Straube et al. (2021a). The sample was incubated in a GuSCN-based buffer (Rohland et al., 2004) applying the protocol by Dabney et al. (2013) for DNA purification. A single-stranded DNA library was then constructed, and test-sequencing was performed to check for the ratio of target DNA and contamination. After detection of endogenous DNA in the test-sequencing dataset, target capture for mitochondrial DNA was performed using home-made baits. These were generated from long-range PCR products amplified from the DNA of *Etmopterus* cf. *molleri* tissue housed in the tissue sample collection of the Bavarian State Collection of Zoology (registration number: Ich-P-CH-0264). For the long-range PCR protocol and primers see Straube et al. (2021a). Hybridisation capture was then performed following the protocol of González Fortes and Paijmans (2019), where the single-stranded library is mixed with the denatured bait library after addition of blocking oligos. Hybridisation of target DNA to baits was carried out for 24 h at 65°C. The captured library was then amplified, and the capture procedure and amplification repeated. The resulting double captured library was then sequenced using

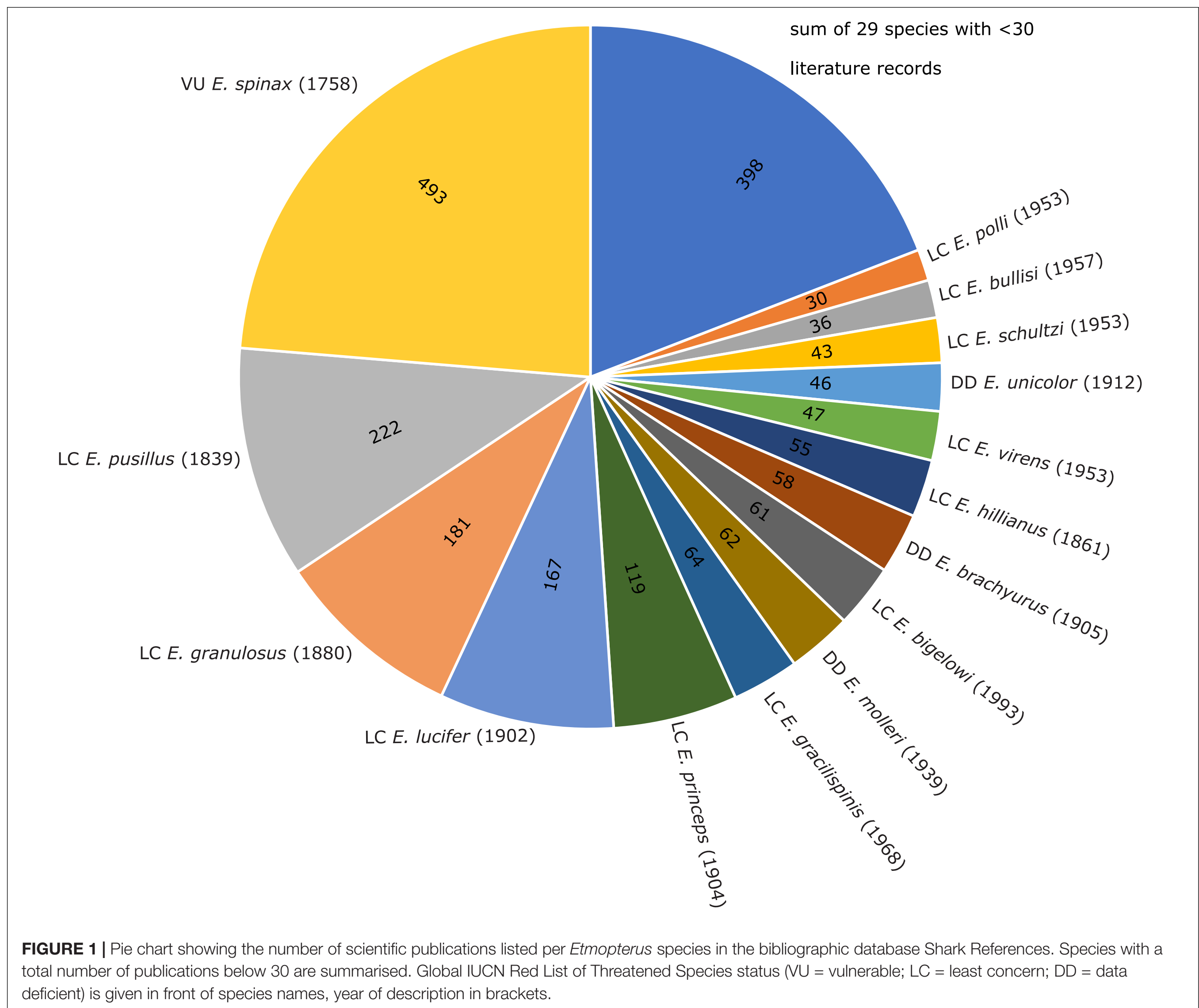

FIGURE 1 | Pie chart showing the number of scientific publications listed per *Etmopterus* species in the bibliographic database Shark References. Species with a total number of publications below 30 are summarised. Global IUCN Red List of Threatened Species status (VU = vulnerable; LC = least concern; DD = data deficient) is given in front of species names, year of description in brackets.

custom sequencing and index 2 read primers (Gansauge and Meyer, 2013; Paijmans et al., 2017) on an Illumina® MiniSeq instrument. We used a mid-output kit in a pool of double indexed samples.

Paired-end raw reads were quality and adapter trimmed with Cutadapt v.1.16 (Martin, 2011) using default settings. The iterative mapping algorithm MitoBim v. 1.9.1 (Hahn et al., 2013) was then used to reconstruct the mitochondrial genome sequence, using default settings and Genbank entry KU892588 (*Etmopterus pusillus*; Chen et al., 2016) as reference for initial baiting. Annotation was performed by aligning the paratype consensus sequence to KU892588 in Geneious® Prime 2021.1 (Biomatters Ltd. Auckland, New Zealand), and checked for internal stop codons. Protein coding genes could not be fully reconstructed. The tRNA-Phe and tRNA-Val transfer RNAs, and the 12S and 16s ribosomal RNAs could be completely reconstructed and were therefore extracted for phylogenetic analysis (2676 bp in total). Reads used in the last iteration of Mitobim were mapped back to the mitochondrial genome consensus sequence as well as to the tRNA and rRNA sequences using BWA aln v.0.7.17 (Li and Durbin, 2009), with default settings, to check if the reads could be unambiguously mapped. Further, BWA was used to align the trimmed and quality filtered reads excluding duplicates to the full mitochondrial genome sequence as well as the tRNA-Phe, the 12S ribosomal RNA, the tRNA-Val and the 16S ribosomal RNA of KU892588 to assess coverage. Obtained sequences were aligned to the sequences of specimens listed in **Supplementary Table 1**, covering nine of the eleven species of the *E. spinax* group (Straube et al., 2010; Ebert et al., 2021). Sequences used to determine the phylogenetic placement of *E. litvinovi* were obtained from the Chondrichthyan Tree of Life (2016) project[1] which are collected from vouchered and validated specimens, as described in White et al. (2018).

[1]https://sharkrays.org

A maximum likelihood tree was computed using RAxML v.8.2.4 (Stamatakis, 2014) under the general time reversible model. Heterogeneity of substitution rates among sites was modelled using a GAMMA distribution. To assess the statistical support for nodes, bootstrapping with 100 replicates was performed and plotted onto the maximum likelihood tree. A haplotype network was reconstructed with POPArt v. 1.7 (Leigh and Bryant, 2015) using the median joining network algorithm (Bandelt et al., 1999) under default settings. The RAxML tree served as a basis for calculating the p-distances between *E. litvinovi* and *E. spinax* clade species analysed herein using the Species Delimitation Plugin 1.4.5 (Masters et al., 2011) in Geneious®.

Etmopterus pycnolepis (Dense-Scale Lantern Shark)

This species is known from six specimens housed in three different museum collections, the ZIN (holotype: ZIN: 49226; two paratypes: ZIN: 49227); the ZMMU (paratype ZMMU: P-17992, paratype ZMMU P-17993) and the ZMH [paratype ZMH: 24995 (ex ISH 4-1989)]. We sampled tissue from the paratype specimen ZMH 24995 (**Figure 2B**) as described previously for the *E. litvinovi* paratype specimen. The specimen was captured at 25°56′ S and 88°33′ W at a depth of 580 m on 30.04.1987. It is an adult male of 426 mm total length (Thiel et al., 2009). As described for the *E. litvinovi* paratype, the overall condition and sampling date of the specimen suggests fixation with formaldehyde.

DNA extraction of the sample involved the same procedure as for *E. litvinovi*. Single stranded library preparation of *E. pycnolepis* DNA followed the protocol described in Gansauge et al. (2017). The *E. pycnolepis* sample underwent different laboratory procedures in comparison to the *E. litvinovi* sample, as the samples were processed with a considerable temporal gap, during which time the standard procedures in the historical laboratory at the University of Potsdam had been updated. Raw test-sequencing reads were analysed as in Straube et al. (2021a). FastQ Screen v0.14.0 (Wingett and Andrews, 2018) was used to check for unique hits to *Etmopterus* references and estimate contamination levels, before proceeding with target capture. After detection of target DNA in the test sequencing dataset, target capture was performed using an Arbor Bioscience myBaits® RNA bait kit. The baits were part of a multi-locus, multi species museum specimen barcoding approach described in Agne et al. (2022). NADH2 bait sequences were derived from representatives of all four *Etmopterus* clades (Straube et al., 2010) deposited in Genbank: *E. lucifer* (JQ518963), *E. gracilispinis* (JQ518960), *E. granulosus* (KF861686) and *E. bigelowi* (JQ518959). The four sequences were initially published in Naylor et al. (2012) and Straube et al. (2015). The single stranded DNA library was captured twice following the protocol described in Huang et al. (2021) using a hybridisation temperature of 65°C for 24 h. Sequencing of the double-captured, indexed library was performed on an Illumina NextSeq 500 System at the University of Potsdam as described in Paijmans et al. (2017). After quality filtering and adapter trimming using Cutadapt v. 2.10 (Martin, 2011) under default settings, reads were processed as described for *E. litvinovi* to reconstruct the NADH2 sequence of the paratype, using the NADH2 sequence of *E. lucifer* (JQ518963; Naylor et al., 2012) as reference.

The NADH2 consensus sequence (1044 bp) of the paratype was subsequently aligned with NADH2 sequences of other *Etmopterus* species with focus on the *E. lucifer* clade (**Supplementary Table 2**). Comparative sequences were obtained from the Chondrichthyan Tree of Life (2016) project (see text footnote 1). For details of NADH2 amplification and sequencing see Naylor et al. (2005, 2012). Forward and reverse sequences were aligned based on chromatograms and edited using Geneious® Pro v. 6.1.7 (Biomatters Ltd. Auckland, New Zealand). The consensus sequences were translated to amino acids and aligned with corresponding NADH2 sequences from representatives of closely related species using the MAFFT (Katoh et al., 2002, 2005) module in Geneious®. The aligned amino acid sequences were translated back in frame to their original nucleotide sequences, to yield a nucleotide alignment 1044 base pairs in length. Analysed samples are listed in **Supplementary Table 2** including 13 of the 15 *E. lucifer* clade species. Phylogenetic inference and species delimitation was performed as described for *E. litvinovi.*

RESULTS

Etmopterus litvinovi (Smalleye Lantern Shark)

A total of 3,734,481 trimmed and quality filtered reads were available after combining test-sequencing and target capture data, including duplicates. MitoBim ran for four iterations. 1,589,598 reads were used for baiting in the final iteration step. The consensus sequence shows 1.26% ambiguities scattered across the mitochondrial genome. Excluding duplicate sequences, 4985 reads map to the consensus sequence resulting from the Mitobim analysis providing an average coverage of 22 reads. The GC content is 40%. The mitochondrial tRNA and rRNA markers used for the phylogenetic analysis showed mapped read lengths mostly larger than 70 base pairs (**Supplementary Figure 1A**) and an average coverage of 58 reads, excluding duplicates (**Supplementary Figure 2A**). They did not show any ambiguous nucleotides.

The maximum likelihood phylogeny of the tRNA and rRNA sequences identifies lineages corresponding to species within the *E. spinax* clade. The relationships in the tree are mostly well-supported with many bootstrap values reaching 100% (**Figure 3A**). The *E. litvinovi* paratype sequence is sister to a sample identified as *E. benchleyi*. This clade also includes a specimen of *E. benchleyi* sampled in the Indian Ocean (GN4952). The clade as a whole is sister to the North Atlantic species *E. princeps* and *E. spinax,* which together form the sister clade to the Southern Hemisphere species *E. viator* (**Figure 3A**). The reconstructed haplotype network detected five haplotypes with 34 segregating sites and 16 parsimony-informative characters. **Figure 3B** shows that the haplotype sequence of the *E. litvinovi*

FIGURE 2 | Paratype images of **(A)** *Etmopterus litvinovi* (ZMH 24994) and **(B)** *Etmopterus pycnolepis* (ZMH 24995). Bars indicate 1 cm.

A

Etmopterus compagnoi GN10443 South Africa South Atlantic
100
Etmopterus unicolor GN7422 Japan Northwest Pacific
Etmopterus sp. B GN7398 New Zealand Southwest Pacific
Etmopterus viator GN7412 New Zealand Southwest Pacific
100
100
Etmopterus benchleyi GN4952 Indian Ocean
100
100
Etmopterus litvinovi paratype ZMH 24994 Southeast Pacific
0
Etmopterus benchleyi GN14570 Costa Rica East Pacific
100
0
Etmopterus spinax GN5162 North Atlantic
100
100
Etmopterus princeps GN1077 North Atlantic
100
Etmopterus princeps GN13688 North Atlantic
100
Etmopterus granulosus GN1447 New Zealand Southwest Pacific
Etmopterus dianthus GN12123 New Caledonia Southwest Pacific
Etmopterus spinax clade
100
Etmopterus gracilispinis clade
100
Etmopterus pusillus clade
100
Etmopterus lucifer clade
100
Oxynotus bruniensis GN1844
0.07

B

Etmopterus princeps GN13688 North Atlantic
Etmopterus benchleyi GN14570 Costa Rica East Pacific
Etmopterus litvinovi paratype ZMH 24994 Southeast Pacific
Etmopterus benchleyi GN4952
Etmopterus princeps GN1077 North Atlantic
Etmopterus spinax GN5162 North Atlantic

FIGURE 3 | (A) Maximum likelihood analysis of *Etmopterus* 12s and 16s rRNA sequences. Numbers at nodes denote bootstrap support values. *Oxynotus bruniensis* was chosen as outgroup. **(B)** Haplotype network of 12s and 16s rRNA sequences including sequences from the *E. litvinovi* paratype, *E. benchleyi* and its sister clade comprising *E. princeps* and *E. spinax*. Crossbars indicate mutational steps. *E. litvinovi* and *E. benchleyi* GN14570 share a haplotype, GN4952 differs in a single mutational step.

paratype is identical to the *E. benchleyi* sample GN14570 and separated by a single mutational step from *E. benchleyi* sample GN4952. The species delimitation analysis shows that the interspecific K2P distance between two valid sister species within the *E. spinax* clade is on average 1.6% (**Supplementary Table 3A**), while the K2P distance between *E. litvinovi* and *E. benchleyi* is substantially smaller (K2P distance = 0.0518%; **Supplementary Table 3A**). Overall, our data does not support the validity of

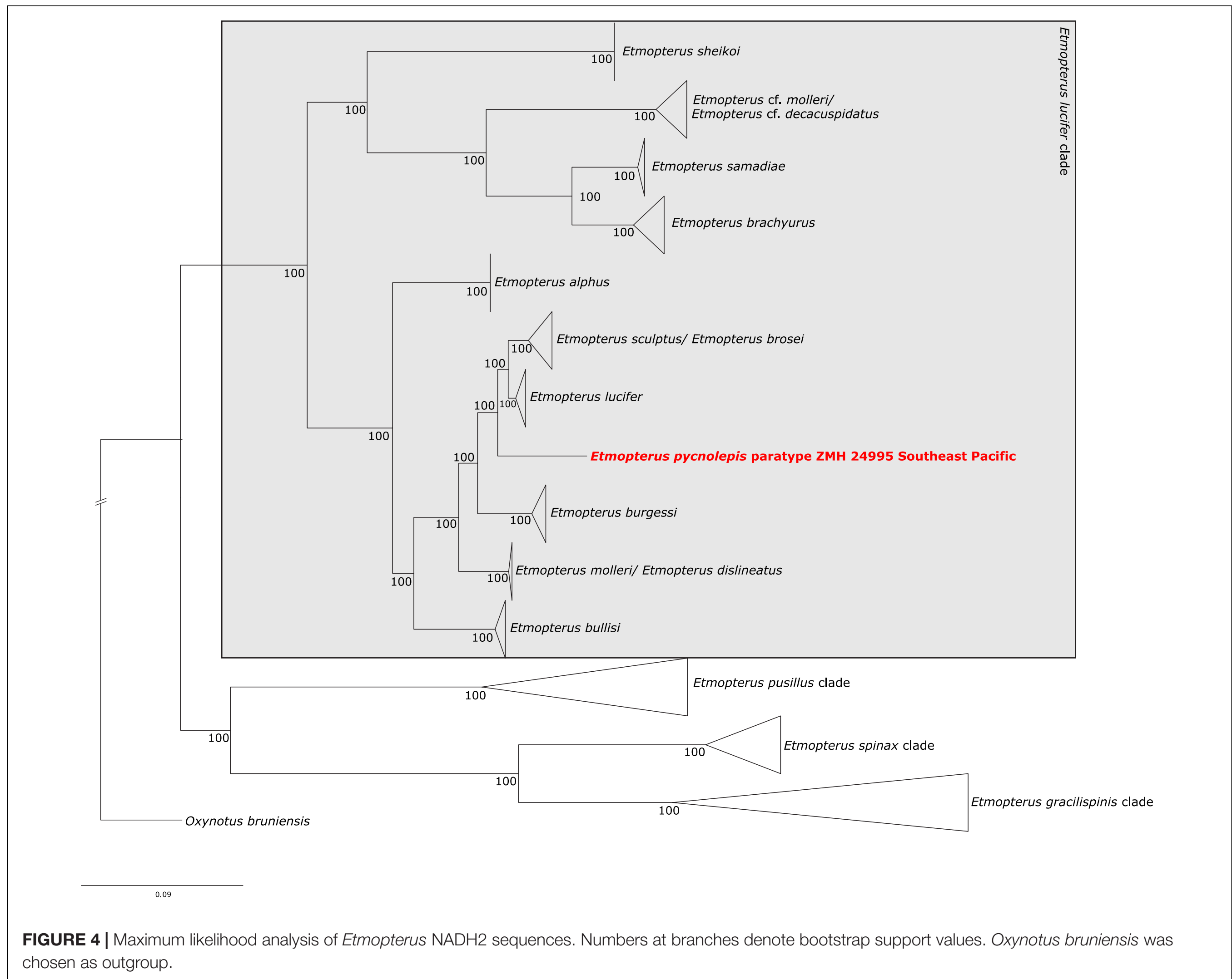

FIGURE 4 | Maximum likelihood analysis of *Etmopterus* NADH2 sequences. Numbers at branches denote bootstrap support values. *Oxynotus bruniensis* was chosen as outgroup.

both species due to the phylogenetic placement of the *E. litvinovi* paratype sequence in the *E. benchleyi* clade and a very small K2P distance between both species.

Etmopterus pycnolepis (Dense-Scale Lantern Shark)

The test-sequencing dataset of 398,752 trimmed reads detected the presence of *Etmopterus* DNA, as indicated by 5.62% unique hits to the *E. spinax* transcriptome used as reference in the FastQscreen analysis. 82.83% of reads were un-assigned to any of the provided references, and contamination with other samples processed simultaneously was not detected. 4,029,200 raw reads were produced by sequencing of the target captured library. Quality filtering and trimming reduced this to 2,680,159 reads. Of these, 139,219 reads mapped to the NADH2 gene of *E. lucifer* (JQ518963). The complete NADH2 sequence was reconstructed after three iterations in Mitobim, using 115,548 reads in the final iteration. The mapped read length distribution is shown in **Supplementary Figure 1B**. The modal fragment length is around 50 base pairs. Mapping those reads back to the reconstructed NADH2 consensus sequence, showed that 277 reads mapped with an average coverage of 13 reads, excluding duplicate sequences (**Supplementary Figure 2B**). The maximum likelihood NADH2 phylogeny shows well-supported clades within the *E. lucifer* group. Several clades do not correspond to species: *E. brosei* clusters with *E. sculptus, E.* cf. *molleri* clusters with *E.* cf. *decacuspidatus* and forms a distinct clade not including *E. molleri. Etmopterus molleri* and *E. dislineatus* do not form two distinct clades. The *E. pycnolepis* paratype specimen is sister to a clade containing the southern hemisphere samples of *E. lucifer* and *E. sculptus* (**Figure 4**). The species delimitation analysis shows that the interspecific K2P distance of the NADH2 gene between sequences of two valid species in the *E. lucifer* clade, excluding clades not corresponding to species, is on average 4% (**Supplementary Table 3B**). Comparing the K2P distance of *E. pycnolepis* to its closest sister taxon, *E. lucifer*, the K2P distance is 3.4%. Our data therefore supports the species status of *E. pycnolepis*.

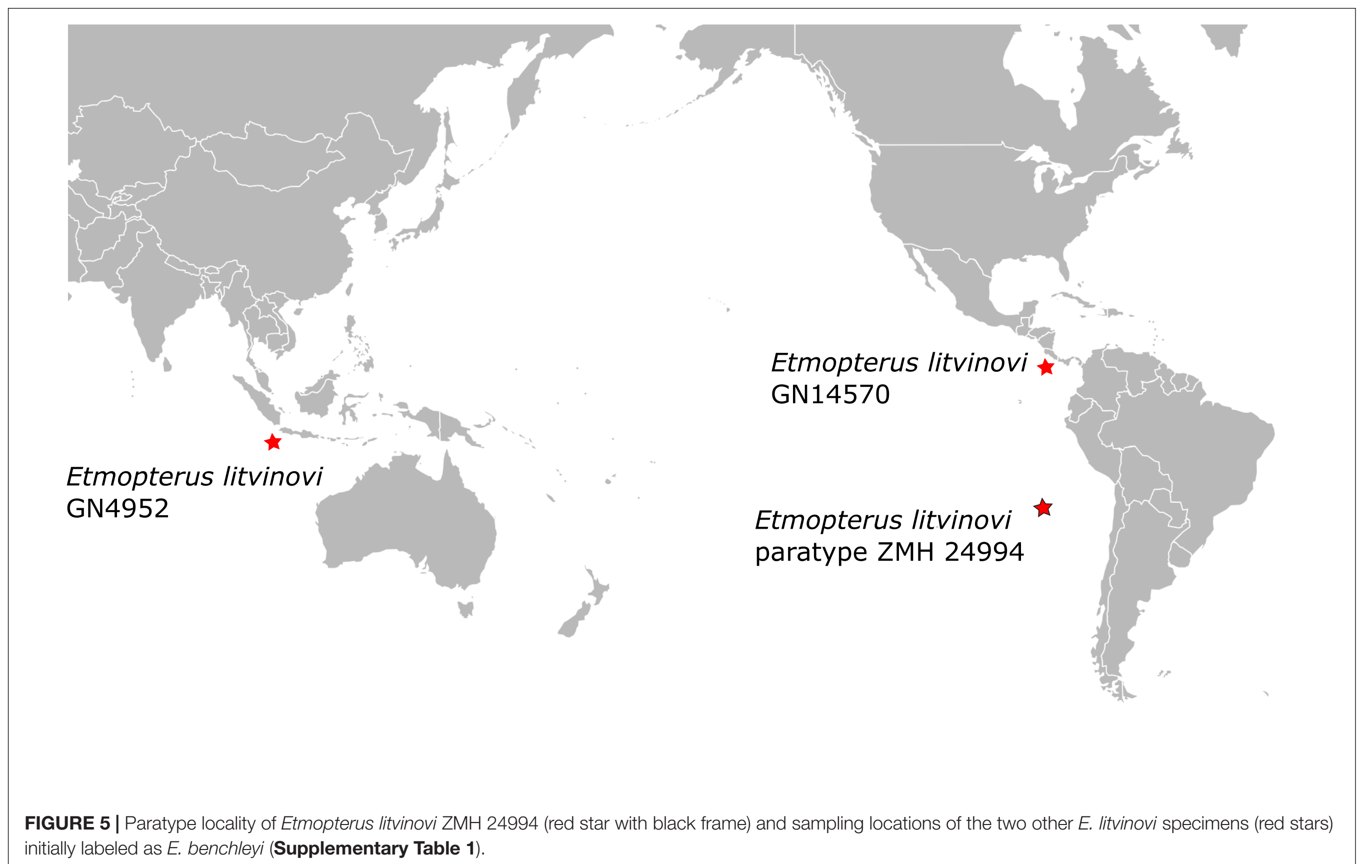

FIGURE 5 | Paratype locality of *Etmopterus litvinovi* ZMH 24994 (red star with black frame) and sampling locations of the two other *E. litvinovi* specimens (red stars) initially labeled as *E. benchleyi* (**Supplementary Table 1**).

DISCUSSION

Mitochondrial DNA Characteristics of the Two Paratype Specimens

The mitochondrial DNA we obtained from both paratype specimens, while fragmented (**Supplementary Figure 1**), was less degraded than other museum samples analysed in previous studies (Straube et al., 2021b). Fixation and preservation cause DNA damage (e.g., Stiller et al., 2016; Hahn et al., 2021; Straube et al., 2021a); however, the mitochondrial DNA of the two paratype samples analysed herein may be less affected due to the comparatively young age of 32 and 34 years, respectively, at the time of extraction. More comparative data is necessary to test if time is correlated with mitochondrial DNA fragmentation levels, and if fragmentation is ongoing under the current storage conditions.

Taxonomic Implications

Etmopterus litvinovi (Smalleye Lantern Shark)

The phylogenetic placement of the paratype sequence aligns with the morphology-based prediction that *E. litvinovi* is a member of the *E. spinax* species clade (Straube et al., 2010). A close relationship of *E. litvinovi* with morphological congeners, including cryptic species, was suggested in Straube et al. (2011a,b). This species complex was recently expanded with several species from which mitochondrial DNA sequence information is, however, available from only two species, *E. benchleyi* (Vásquez et al., 2015) and *E. viator* (Straube et al., 2011a). All analyses, i.e., the phylogenetic reconstruction, the haplotype network and the species delimitation analysis of the *E. litvinovi* paratype specimen suggest that *E. litvinovi* is conspecific with *E. benchleyi*, where *E. benchleyi* forms a junior synonym to *E. litvinovi* (**Figure 3** and **Supplementary Table 3A**). Notably, the sequenced paratype specimen's sampling locality is the Naszca Ridge in the Pacific Ocean, while another *E. litvinovi* haplotype (GN4952) is derived from a specimen sampled in the Indian Ocean (**Figure 5**). This suggests that *E. litvinovi* is widespread and occurs both in the Indian and Pacific oceans. Its overall distribution range may cover an even larger area of Southern Hemisphere oceans, and that its northern and southern distribution limits have yet to be identified. Similarly, wide distribution ranges are also documented for other closely related *Etmopterus* species in the *E. spinax* clade such as *E. granulosus* (Straube et al., 2011a,b, 2015) or *E. viator* (Straube et al., 2011a).

The new data presented herein will be helpful for future assessments of the species in the IUCN Red List of Threatened Species. As of today, the species is evaluated and listed under the "least concern" category justified due to limited fisheries in the area from which the species (i.e., the type material of *E. litvinovi*) was hitherto recorded (Ebert et al., 2020a). Based on our results, a notably larger distribution range should be considered in future evaluations taking different fishing pressure in other regions of occurrence into account. Our results do

not confirm endemic occurrence (Kotlyar, 1990) but support its occurrence in the eastern Pacific as well as the Indian Ocean. The paratype sequence data analysed herein adds important alpha-level taxonomic information on the species, which will ease the collection of data on population size, as well as accurate geographic and depth distribution ranges, in the future. This forms the basis for conservation and management efforts for this poorly known deep-sea shark species.

Etmopterus pycnolepis (Dense-Scale Lantern Shark)

As already indicated by the distinct shape of its flank marking, our analysis further supports the assignment of *E. pycnolepis* as a distinct species (**Supplementary Table 3B**) within the *E. lucifer* clade (**Figure 4**; Straube et al., 2010). Its assignment to the *E. burgessi* subclade (Ebert et al., 2021) is not supported, however. The morphologically defined *E. lucifer* clade subclades described in Ebert et al. (2021) are generally not recovered in our molecular analysis (**Figure 4**). The phylogenetic inference displays to some extent geographic patterns of sampling locations instead. *E. lucifer*, *E. pycnolepis*, *E. brosei* and *E. sculptus* are represented by samples exclusively collected in Southern Hemisphere oceans (**Supplementary Table 2**), while *E. burgessi* samples stem from the Northwest Pacific. *E. brachyurus*, *E.* cf. *molleri*, and *E. samadiae* samples were also collected in the Northwest Pacific. *E. alphus* samples are from the Indian Ocean off Mauritius and *E. bullisi* was sampled in the Northwest Atlantic (**Supplementary Table 2**). Some species seem therefore confined to certain oceanic areas. The three different flank mark shapes characterising the *E. lucifer* clade subclades occur in three different ocean regions in parallel. In our study, *E. brachyurus E. samadiae E.* cf. *molleri*, *E.* cf. *decacuspidatus*, and *E. burgessi* represent the flank mark diversity of all three subclades in the Northwest Pacific; *E. lucifer*, *E. molleri*, *E. dislineatus*, *E. brosei*, *E. alphus*, *E. pycnolepis* and *E. sculptus* in the Indian and South Pacific oceans. In the Atlantic Ocean, only the *E. molleri* subclade type flank marking (Ebert et al., 2021) is represented by a single species (*E. bullisi*); however, *E. bullisi* is the only Atlantic species from the *E. lucifer* clade in general. A denser sampling is necessary to identify detailed species distribution boundaries and clarify indicated synonymies.

The IUCN Red List of Threatened species lists *E. pycnolepis* as least concern under the assumption that the area of origin of the six type specimens representing the species is not exposed to extensive fishing pressure as also in *E. litvinovi*. As mentioned in the evaluation justification, the species may be distributed in Chilean waters as well (Ebert et al., 2020b), which would amount to a large expansion of its distribution area. By providing the first DNA sequences for this species, newly collected samples available for NADH2 sequencing can be correctly assigned to the species and will therefore be useful for documenting its distribution range in the future.

CONCLUSION

Our results demonstrate the importance of archival DNA sequence information from type material for molecular based taxonomy. This is especially true for species which are known from few specimens only, and where re-sampling is hindered by remote sampling localities, as is the case for the two species analysed herein. Our results support the synonymy of *E. benchleyi* with *E. litvinovi,* and consequently suggest a notably larger distribution range than previously known, since the species was assumed to be endemic to the Salas y Gómez and Nazca Submarine Ridges (Kotlyar, 1990). The species status of *E. pycnolepis* is supported by our data, which is now available as reference for future molecular species-level identification of newly collected samples. This will help clarify the distribution of this species. Our results further show that genetic information from collection material can assist in the evaluation of species in a conservation and management context. While it is standard to evaluate morphological characters of wet-collection type material for descriptions of species new to science, the usage of wet-collection specimen DNA sequence information has only recently been established as such (Beermann et al., 2018; Lyra et al., 2020; Rancilhac et al., 2020; Scherz et al., 2020; Straube et al., 2021b) and our work is a further contribution to this.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Figshare (doi: 10.6084/m9.figshare.19446992) and GenBank (accession numbers ON185623-ON185724).

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because no living animals were collected or examined. Tissue samples for DNA sequencing were taken solely from museum specimens and combined with existing data for analysis.

AUTHOR CONTRIBUTIONS

NS, MH, JP, and AB designed the study. SA, MP, and NS conducted laboratory work. GN and LY provided comparative sequences for phylogenetics. SA, NS, LY, and GN analysed the data. RT and SW researched type specimen history, provided and helped sampling the type specimens. NS, SA, and MH wrote the manuscript with contributions from all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.910009/full#supplementary-material

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