Genetic research to guide conservation and the understanding of freshwater eels of the genus *Anguilla*: A bibliographic analysis

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Abstract

Anguillid eels have facultative catadromous life histories that include long distance oceanic spawning migrations and an extensive leptocephalus larval duration. Less known is the important role genetic research has played in understanding these mysterious fishes of the genus *Anguilla*. A bibliographic literature search was conducted on studies using genetic methodologies to understand *Anguilla* species, and a database of papers from 1973–2025 included 335 publications, increasing over time to reach a maximum of 103 between 2020 and 2025. The DNA literature was dominated by studies on *A. anguilla*, *A. japonica*, and *A. marmorata*, but all species have been included in recent phylogenetic studies. In addition to providing a bibliographic database of genetic papers, this review points to the value of multi-gene or whole genome approaches to clarify relationships within and among anguillid eel species. There is also the need for the development of standardised protocols for environmental DNA (eDNA) research with improved species-specific assays, which can help support the conservation of these remarkable fishes that live in both freshwater and the sea.

Introduction

Eels in the genus *Anguilla* from the monotypic family Anguillidae are unique in various ways, including their semi-global distribution, juvenile growth in freshwater and estuarine habitats, and unusual offshore spawning migration behaviour (Righton et al. 2021). A wide range of interesting aspects of the life histories of eels have been studied over time which has been recently reviewed (Stuart et al. 2024). There are currently 19 recognised *Anguilla* species and sub-species which are distributed in different parts of the world from tropical to temperate

latitudes except in the South Atlantic and eastern Pacific Ocean (see Table 1, and species distribution maps in Watanabe 2003, 2023; Watanabe and Miller 2012; and Stuart et al. 2024). Scientific research on anguillid species composition and life histories has relied heavily on genetic techniques for delineating species and identifying their early life history stages, which can look similar at the larval and glass eel stages. The phylogenetic relationships of anguillids remains of great interest, and most recently environmental DNA (eDNA) has been used to study their distributions, as examined further in this review.

Global interest in anguillids began in the early 1900's with the search for the spawning location of the European eel, *A. anguilla*, which was discovered in the Sargasso Sea of the western North Atlantic, and overlapping with the spawning area of the American eel, *A. rostrata*, (reviewed by Schmidt 1935; Miller et al. 2015). This was followed by the later discovery of the spawning area of the Japanese eel, *A. japonica*, in the western North Pacific (Tsukamoto 1992). These remarkably long distance migrations of adults to offshore spawning locations are followed by return migration to natal freshwater by the oceanic larvae. In the process, the larvae, known as leptocephali, transition to recruitment-stage glass eels, which can happen in freshwater, estuarine, or saline juvenile growth habitats (Arai 2020).

Anguillids are culturally relevant in many societies (reviewed by Tsukamoto and Kuroki 2014), and they are economically important species that have been extensively harvested and cultured, particularly in East Asia (Yuan et al. 2022). Some species of *Anguilla* are part of commercial fisheries, but several of these fisheries have declined as the target species stocks have dwindled (Jacoby et al. 2015). Despite fishery declines and data deficiency for certain species, commercial eel fishing and trading is ongoing globally. Some countries trade tropical glass eel species without conducting prior scientific or management assessments, potentially impeding conservation efforts for species with few to no estimates of population size or

vulnerability (Nijman 2015). Mislabelling or generic labelling of eel species in commercial markets has been reported, allowing suppliers to bypass laws, capture endangered species, and export them to the marketplace (Wallstrom et al. 2020; Stein et al. 2021).

Conservation concerns and research efforts on anguillids have been increasing over the last 20 years because of marked decline in population sizes and recruitment of many species starting in the mid-1970's which cannot be easily explained by any one factor (reviewed by Drouineau et al. 2018). Species of *Anguilla* have come under threat from various human actions including habitat loss, overfishing, diseases, parasites, pollution and changes in environmental conditions associated with climate change (Jacoby et al. 2015; Belpaire et al. 2016; Miller et al. 2016; Drouineau et al. 2018). The reduction in population sizes and recruitment has resulted in a number of anguillid species being categorised as of conservation concern by the International Union for Conservation of Nature (IUCN) (Table 1).

Some aspects of the biology of anguillids have been covered by recent reviews, specifically, general understanding of anguillid biology (Righton et al. 2021), and patterns of studies on anguillid species and life stages (Stuart et al. 2024). Together, these reviews highlight effects of recent global impacts on freshwater eels and provide recommendations to progress the research and management of anguillid species. However, none of these reviews have included much consideration of the significance and increasing use of molecular genetic methods to facilitate the understanding and conservation of anguillids worldwide.

The taxonomy of anguillids was initially based on morphological characteristics, dorsal fin lengths (shortfin or longfin), body colouration (plain or mottled), total number of vertebrae (TV), and patterns of maxillary teeth as identifiers for the tropical mottled species (broad or narrow; Table 1; Ege 1939; reviewed by Watanabe 2003, 2023). The relationships among species identified using morphology was then based on shared features and/or geographic boundaries

separating populations (Ege 1939). This original taxonomy is not supported by more recent studies, including neighbour joining trees based on morphological features and phylogenetic trees from genetic data (Lin et al. 2001, 2005; Watanabe 2003). Furthermore, the four groups of species initially described (Ege 1939) were clearly not monophyletic when analysed using initial molecular markers (Restriction Fragment Length Polymorphism (RFLP) of 16S rRNA; Watanabe 2003), and this lack of monophyly was confirmed in all later phylogenies (e.g., Minegishi et al. 2005; Inoue et al. 2010). Thus, the advent of genetic tools has been invaluable for identifying anguillid species and determining the distribution of their populations.

Beyond morphological similarities among eel species, the spatial scale of population distributions varies markedly among anguillid species, further complicating species identifications. Some species have continental-wide distributions, (e.g., Europe-wide distribution of *A. anguilla*) while others have highly localised populations restricted to narrow geographic scales, such as small island chains (e.g., distribution of *A. luzonensis* is centred around the Philippines) (Table 1). Consequently, the distribution ranges of species frequently overlap, providing further opportunity for genetics to resolve species relationships. For example, early work with genetic methods determined the classification of American and European eels, which spawn in the same area, as separate species (Avise 2003). Additionally, *Anguilla* with small geographic ranges are largely limited to the Indo-west Pacific, such as the species *A. celebesensis*, *A. interioris*, and *A. borneensis*, which all have distributions from Sumatra in the southwest to Papua New Guinea in the northeast (Aoyama et al. 2000; Wibowo et al. 2021; Zan et al. 2022). Perhaps the most renowned example of the value of genetic identification for understanding the distribution and species composition of sympatric eels is *A. luzonensis*, which, because of its morphological similarities with *A. interioris* and *A. celebesensis*, was only recently distinguished using genetic identification (Teng et al. 2009; Watanabe et al. 2013). The species

A. luzonensis has been detected in Mindanao Island, southern islands of Japan, and Taiwan (Han et al. 2016; Shirotori et al. 2016; Kita et al. 2021), but appears to be primarily located on Luzon Island in the Philippines, where its glass eels were possibly misidentified as *A. celebesensis* (e.g., by Arai et al. 2003). Nuclear 18S rRNA (18S rRNA) analyses were used to confirm the first record of *A. reinhardtii* in northern New Zealand (Jellyman et al. 1996). Three tropical anguillid species (*A. marmorata*, *A. megastoma* and *A. obscura*) occur sympatrically across many WSP islands (reviewed by Miller et al. 2024) and while the three species can be morphologically distinguished as juvenile yellow eels (skin pattern, dorsal fin position, dentition; Watanabe 2003, 2023), morphological differences in dorsal fin position and tail pigmentation of glass eels at study sites in Fiji, Tahiti, and Mo'orea were supported by DNA sequence identification (Hewavitharane et al. 2018, 2020; Viana et al. 2025). Similarly, in the southwestern Indian Ocean, morphology has primarily been used for species identifications, but when glass eel specimens cannot be clearly distinguished to species-level, semi-multiplex PCR has been used (Robinet et al. 2008; Gagnaire et al. 2007). These several examples show how delimitation of anguillid species around the world has been made possible due to genetic tools.

Genetic methods are critical to the reliable identification of many anguillid leptocephali, which are highly morphologically similar (Mochioka 2003). Some leptocephali, particularly those found in the Atlantic Ocean, can be distinguished by their different ranges of total myomeres (corresponding to the TV; Avise 2003). Despite this, genetic methods have greatly improved the accuracy of identification of leptocephali in recent surveys of their overlapping spawning grounds in the Sargasso Sea (Fig. 1a), (Hanel et al. 2014). In the Indo-Pacific, the TV of different anguillid species frequently overlap, preventing the use of morphology for reliable identification.

The use of genetic methods to identify the early stages of anguillids have been an essential tool for the identification of anguillid spawning areas. The location of anguillid spawning areas remain cryptic for most species (Miller 2023), hindering conservation and management frameworks. *In situ* collections of genetically identified leptocephali have made it possible to estimate the spawning areas of several species of anguillids (Fig. 1b). For example, genetic methods have been used to validate identification of preleptocephali and eggs of the Japanese eel in order to distinguish these very early stages from *A. marmorata* (Kuroki et al. 2009; Tsukamoto et al. 2011; Aoyama et al. 2014; Takeuchi et al. 2021), which also spawns in the same area (Fig. 1b). For species for which no small larvae have been collected, some spawning areas are not presently known but have been estimated based on the geography of their species ranges and based on the direction of ocean circulation (Miller 2023). Future collection campaigns paired with genetic identification of caught leptocephali will continue to improve conservation of these iconic species.

From an applied perspective, DNA identification techniques have been increasingly used to determine the species identity of eel food products. Glass eels have been transported around the world for use as seedlings in eel aquaculture in East Asia for decades (Shiraishi and Crook 2015). However, in recent years, restrictions were placed on the legal export of European eels for conservation reasons (Jacoby et al. 2015), which has made the use of genetic identification of glass eels or eel products increasingly important (Richards et al. 2020; Ely et al. 2023; Nijman and Stein 2022; Shiraishi et al. 2025).

At the time of the present review, the most actively expanding field of research on anguillids involves the use of eDNA to detect the presence of eel species. This methodology is based on taking water or sediment samples in the natural environment and then extracting total DNA for genetic analysis. It has been used to study the distributions of Japanese (Itakura et al.

2019; Kasai et al. 2021; Ono et al. 2024), European (Weldon et al. 2020; Griffiths et al. 2023) and American eels (Chin et al. 2021; George et al. 2023) in a range of freshwater and marine ecosystems. Laboratory experiments have quantified eDNA release across eel life history stages (Takeuchi et al. 2019a), compared amounts of eDNA and eRNA released by eels (Che-Pelicier et al. 2025), and eDNA analyses of water samples from the Japanese eel spawning area have been used to help identify potential spawning locations (Takeuchi et al. 2019b, 2021).

Analysis of anguillid DNA has also been used to examine a wide range of other aspects of anguillid biology. Hybrids of *A. rostrata* and *A. anguilla* have been detected in Iceland through analyses of 363 amplified fragment length polymorphism (AFLP) loci (Albert et al. 2006) as well as single nucleotide polymorphisms (SNPs) of 18S rRNA gene (Frankowski and Bastrop 2010). Hybrids have also been detected in the WSP where a spawning area appears to be a shared by *A. marmorata* and *A. megastoma* (Schabetsberger et al. 2015). Molecular genetic tools have been used to assess the origin of parasites infecting *Anguilla* species (Wielgoss et al. 2008), examine strains of bacterial and viral diseases (Parchemin et al. 2022), and assess changes in genetic signatures of *Anguilla* specimens in congruence with environmental pollution (Pierron et al. 2014; Laporte et al. 2016). The possible effects of environmental factors, such as temperature, have been evaluated for the genetic composition of anguillid populations that are distributed across a range of latitudes (Gagnaire et al. 2012; Hirase et al. 2022). Molecular tools have been used to assess historical population sizes of *A. anguilla* and *A. rostrata*, suggesting that modern declines in population size are unprecedented and likely related to human actions (Feng et al. 2022).

This current review involved a bibliographic search of published studies that applied molecular genetic methods to investigate the genus *Anguilla.* Here, we briefly assess the content and implications of this wealth of research. A variety of relatively recent books (Aida et al. 2003;

Arai 2016; Tsukamoto et al. 2023), reviews (Aoyama 2009; Drouineau et al. 2018; Righton et al. 2021) and bibliographic analyses (Stuart et al. 2024) have been published about a wide range of subjects, but none have focused directly on the details of how DNA studies have been broadly used to better understand anguillid biology. The types of genetic markers used in these studies are compiled into a database with the goal of providing a resource of information to help guide future research to support the understanding of the ecology and evolution of anguillids and to facilitate their conservation worldwide. All subjects cannot be overviewed in detail here, so more recent studies are emphasised. We consider several subject areas for detailed evaluation such as species identification of anguillids and their products using species-specific assays, anguillid phylogenetics and population structure, and eDNA studies, which are important for future applications and eel conservation.

Methods

There is extensive research using molecular genetic techniques on anguillid eels (Table S1). The review work flow process is outlined in Fig. 2. To identify this body of research, the search terms "eel phylogeny," "eel genetic," "*Anguilla* phylogeny," "*Anguilla* genetic," or "*Anguilla* environmental DNA" were used in "All Fields" on the Web of Science database in May 2025. The Scopus database was also used to conduct the same searches in May 2025 by selecting for the same terms in "Article title," "Abstract," and "Keywords". The same search terms were also used in Google Scholar in May 2025, and the 500 highest ranked manuscripts returned by the search were screened. Several manuscripts were included in multiple databases, and thus duplicates were removed from downstream analyses (Fig. 2). Studies were individually screened to ensure they contained all of the following criteria: (i) the study examined DNA, (ii) it focused on or included species within the genus *Anguilla*, and (iii) specified which gene(s) the study

targeted. Studies that focused on other topics such as chemistry, epigenetics, proteomics, gene expression, or transcriptomics were excluded. Analyses on gut contents were also excluded as these did not look at the DNA of the host *Anguilla* organism itself but focused on the DNA of its food. Reviews were also excluded to focus on publications that directly contributed to novel genetic analyses.

From the selected publications, the authors, year of publication, targeted genes, and species of *Anguilla* were recorded. For this review and database, the most recent name of each species is used. Therefore, *A. nebulosa* is listed as *A. bengalensis*; *A. malgumora* is listed as *A. borneensis*; and *A. huangi* is listed as *A. luzonensis* (Table 1). Many studies that included *A. australis*, *A. bengalensis* and *A. bicolor* did not specify the sub-species, and thus the database compiled from this review only lists *Anguilla* to the species level. The R packages tidyr (Wickham et al. 2023) and dplyr (Wickham et al. 2022) were used to organise data and to count the total in each category (number of publications with each species, number of publications per year, number of times each genetic technique was used). The package ggplot2 (Wickham 2016) was then used to visualise the data (Fig. 3).

The databases used in this review (Web of Science, Scopus, and Google Scholar) are biased to show more publications produced in English. Given that several eel species are native to southeast Asia, it is possible that there are studies not published in English that are missed by this review. Some studies (Gagnaire et al. 2011; Yoon 2015) referred only to using "genomic DNA" or "loci" without specifying the genomic regions examined. Thus, they may have contributed to a more or less commonly studied gene without assignment into those categories. Given that this is a small fraction of the studies presented herein and does not affect the overall pattern of the genetic techniques used, they were retained in our analyses. Other studies may

have used genetic identification of anguillid species as their standard method, but DNA was not in the title, abstract or key words so it was not detected by the search terms.

Results and Discussion

As overviewed above, there has been a wide variety of genetic-related research on anguillids due to their great commercial and cultural importance in many parts of the world (Tsukamoto and Kuroki 2014). With the aim of guiding future research, here we have compiled a bibliography of 335 manuscripts that used some form of genetic analysis to better understand anguillids. From the Web of Science search, "Eel phylogeny" returned 276 results, "eel genetic" returned 865 results, "*Anguilla* phylogeny" returned 122 results, "*Anguilla* genetic" returned 420 results, and "*Anguilla* environmental DNA" returned 226 results. When searching from Scopus, "Eel phylogeny" returned 536 results, "eel genetic" returned 1018 results, "*Anguilla* phylogeny" returned 238 results, "*Anguilla* genetic" returned 490 results, and "*Anguilla* environmental DNA" returned 105 results (Fig. 2).

Mitochondrial *cytb* and 16S rRNA are the two most-commonly used genetic markers for anguillids (Fig. 3), as they are relatively conserved and evolve slowly, and 16S rRNA in particular is considered a suitable marker for taxonomic discrimination of fish species and genera (Meyer 1993). Mitochondrial 16S rRNA is one of the most commonly used markers in studying anguillids and has been used in 62 of the 335 publications (18.5%) in the database of this review (Table S1). The marker COI is also often used for genetic identification of *Anguilla* species, particularly in markets where specimens of unknown species and origin are traded (Stein et al. 2016; Goymer et al. 2023) and is considered a standard "barcode" for identifying organisms (Meyer and Paulay 2005).

The number of genetic publications greatly increased over time, from 23 publications before the year 2000 (1973–1999), 81 during 2000–2009, 128 from 2010–2019, and 103 in just the most recent years 2020–2025 (Table S1). The 103 papers in the most recent five-year period reflect increased interest in tropical species, species identification of eel products, and the expanding use of eDNA for distribution studies. Up to the year 2010, research mostly focused on phylogenetic and DNA species identification studies (Table S1). In genetic studies, *A. anguilla* has received disproportionate research attention (here n = 146 studies); this is followed by studies focused on *A. japonica* (n = 115) and then studies of *A. marmorata* (n = 105). Stuart et al. (2024) found 2535 studies on *A. anguilla*, 1164 studies on *A. japonica*, and 211 studies on *A. marmorata*. From this, it can be seen that genetic studies make up a small proportion of all of the studies on anguillids. The number of genetic studies conducted on the tropical and subtropical *A. marmorata* is third highest (105 genetic studies, 211 overall studies, Stuart et al. 2024), whereas more studies overall were recovered for *A. rostrata* and *A. australis* (76 genetic studies and 597 overall studies on *A. rostrata*; 37 genetic studies and 214 overall studies on *A. australis*, Stuart et al. 2024). The reason why *A. marmorata* has been so highly studied among geneticists could be because it has the largest geographic range of any species of *Anguilla*, from eastern Africa to the eastern Pacific Ocean (Robinet et al. 2008; Escobar-Camacho et al. 2023), the fact that it is abundantly found in its range of distribution (Fahmi et al. 2012; Itakura et al. 2019), and because of its economic value in fisheries and aquaculture (Huang et al. 2016).

Phylogeny of the genus Anguilla

There are 31 intra-genus phylogenetic studies on *Anguilla* species spanning 14 different genetic markers (Table S1). The first concatenated phylogenetic tree of *Anguilla* (excluding the then-undiscovered *A. luzonensis*) was constructed by Aoyama et al. (2001) using a combination

of *cytb* and 16S rRNA genes. This not only provided a visualisation of *Anguilla* genetic relationships as well as hypotheses as to how the genus has evolved but was important in refuting ideas of Ege (1939) on *Anguilla* relationships based on morphology. The same concatenated markers were later used to first distinguish *A. luzonensis* as its own species (Watanabe et al. 2013).

Phylogenetic trees have been constructed using several different methods, including *cytb* (Jamandre et al. 2007; Fahmi et al. 2015), 12S rRNA (Lin et al. 2001), 16S rRNA (Aoyama et al. 2001), Adenosine Triphosphate 6 (ATP6; Jacobsen et al. 2015) genes, as well as whole mitochondrial genomes (Minegishi et al. 2005; Teng et al. 2009; Inoue et al. 2010). Studies have used several substitution models, including general time reversible (GTR; Minegishi et al. 2005, Teng et al. 2009; Inoue et al. 2010), Tamura and Nei (TrN; Lin et al. 2001; Jacobsen et al. 2015), Hasegawa, Kishino, and Yano (HKY; Aoyama et al. 2001; Fahmi et al. 2015) models, and Kimura 2-parameters (Jamandre et al. 2007). Many published phylogenies have incongruences in the relationships between species of *Anguilla*.

Outgroups have been commonly used in previous studies (Bastrop et al. 2000; Aoyama et al. 2001; Lin et al. 2001; Minegishi et al. 2005; Jamandre et al. 2007), although it has been suggested that inclusion of an outgroup could be detrimental to phylogenetic analyses of *Anguilla* due to the "star-like" topology of the genus (Teng et al. 2009). The genetic distances between species of *Anguilla* are low, and the resultant phylogenetic trees of this genus contain multiple short internal branches that can complicate rooting. Additionally, the long-branch attraction effect can further distort the tree if the outgroup clusters on a long branch (Bergsten 2005). Due to the short internal branches within commonly proposed *Anguilla* phylogenies, individual gene trees and mtDNA trees often show conflicting relationships, suggesting that polytomies (dividing into more than three parts) may be a more accurate representation. Many

previous phylogenetic studies identified either *A. borneensis* or *A. mossambica* as the outgroup to all other eels, implying that one of those eels might be most closely related to the common ancestor of all anguillids (Aoyama et al. 2001; Minegishi et al. 2005; Jamandre et al. 2007; Fahmi et al. 2015; Jacobsen et al. 2015).

Resolving phylogenetic relationships within *Anguilla* is a challenge due to the high genetic similarity between species (Minegishi et al. 2009). Relationships among the Indo-Pacific *Anguilla* species vary among studies. The relative placements of *A. japonica* and *A. reinhardtii* in constructed anguillid phylogenies varies based on the gene and substitution model used. Some studies place these two species as sister taxa (Jamandre et al. 2007; Fahmi et al. 2015), whereas others position them variably within or outside the Indo-Pacific clade (Aoyama et al. 2001; Minegishi et al. 2005; Inoue et al. 2010) or depict them as polytomies (Teng et al. 2009; Jacobsen et al. 2015). Phylogenies built using mucosal galectin genes of *Anguilla* (Tsutsui et al. 2015, 2019) have resulted in numerous polytomies. Even analyses of the same mitochondrial *cytb* gene yield conflicting results, as demonstrated by multiple studies (Aoyama and Tsukamoto 1997; Tsukamoto and Aoyama 1998; Bastrop et al. 2000; Aoyama et al. 2001; Lin et al. 2001; Jamandre et al. 2007). The length of *cytb* sequences that are used for these comparisons varies considerably, from just 350 bp (Bastrop et al. 2000) to the entire gene (1140 bp; Lin et al. 2001). Although *cytb* analyses have been found to yield robust phylogenies for anguillids (Fahmi et al. 2015), the discrepancies among these various *cytb* datasets underscore the challenges of interpreting phylogenetic relationships among *Anguilla* species, even when relying on the same genetic marker.

Despite the many inconsistencies among *Anguilla* phylogenies, certain relationships are reliably maintained across gene datasets. *Anguilla anguilla* and *A. rostrata* almost universally appear as sister taxa (Tsukamoto and Aoyama 1998; Aoyama et al. 2001; Minegishi et al. 2005;

Jamandre et al. 2007; Teng et al. 2009; Inoue et al. 2010; Fahmi et al. 2015; Jacobsen et al. 2015) and are considered as most closely related based on mitochondrial genes (Minegishi et al. 2009). Several studies also support a consistently close relationship between *A. obscura* and *A. interiori*s (Minegishi et al. 2005; Inoue et al. 2010; Jacobsen et al. 2015), although introducing *A. luzonensis* sometimes places *A. interioris* as a sister species to *A. luzonensis* (Teng et al. 2009; Fahmi et al. 2015). *Anguilla celebesensis* and *A. megastoma* are frequently identified as sister species (Aoyama et al. 2001; Minegishi et al. 2005; Jamandre et al. 2007; Teng et al. 2009; Inoue et al. 2010; Fahmi et al. 2015; Jacobsen et al. 2015). The identification of the sub-species of *A. bicolor* (*A. bicolor bicolor*, *A. bicolor pacifica*), *A. bengalensis* (*A. bengalensis bengalensis*, *A. bengalensis labiata*), and *A. australis* (*A. australis australis*, *A. australis schmidtii*) appear to all remain stable across most studies. The single exception was likely a misidentified specimen, an outcome emphasising the necessity of reliable genetic identification in *Anguilla* phylogenetic research (Aoyama et al. 2001). Perhaps the strongest phylogenetic analyses of the genus are two studies which both used whole mtDNA genomes of all species of anguillids (15,187 sequences and 13,701 sequences, out of 16,569 total) (Minegishi et al. 2005; Inoue et al. 2010). Analysing genetic relationships among all species of *Anguilla* within all 19 families in the order Anguilliformes suggests that the genus *Anguilla* evolved from a morphologically and ecologically very different type of Anguilliformes, the mesopelagic eels Serrivomeridae (Inoue et al. 2010).

Previous research has demonstrated that analyses of different single gene regions among taxa will result in conflicting relationships at multiple taxonomic levels (Meiklejohn et al. 2014). Additionally, phylogenies built off of partial gene regions have poor confidence and limited agreement with topologies made from full mitochondrial DNA segments (Morón-López et al. 2022). Evolutionary patterns of individual genes are often directly correlated with their function

or chromosomal location, and thus individual genes will accrue complex evolutionary patterns over time (DeSalle and Tessler 2025), and determining which gene is most suitable for studying a particular taxon differs among taxa (He et al. 2023; Main et al. 2024). Among vertebrates, some genes show congruence with the entire mitogenome tree, while trees produced from other genes are highly dissimilar (Main et al. 2024). There are different proposed approaches to resolving this issue, such as the suggestion that mitochondrial studies should be corroborated by nuclear DNA (Shaw 2002; Zink and Barrowclough 2008), or use of multispecies coalescence methods (Kim et al. 2020). For mitochondrial analyses, analyses of entire mitochondrial genomes result in better estimates of species relatedness than single-gene approaches (Meiklejohn et al. 2014).

Overall, it is clear that relatively small methodological differences, such as using shorter sequences or selecting one set of genes over others, can contribute to differences in the outcome of phylogenetic studies of *Anguilla*. To illustrate this, a comparison was made of the relationships among *Anguilla* species for phylogenetic analyses using amino acid sequences with a mitochondrial vertebrate model (mt-Ver; Le et al. 2017) with thirteen genes from mitochondrial genomes, as well as individual trees made from mtDNA protein coding genes ATP6, ATP8, and COI (see Supplementary Materials). The results of this comparison clearly show that the phylogenetic trees comprised of single genes are incongruent with those using larger numbers of sequences (Fig. S1). Notably, there are differences in the outgroup, in which Atlantic eels were recovered as the outgroup to all other anguillids. This is remarkably different from the studies that have found *A. mossambica* or *A. borneensis* as the outgroup to all other anguillid species (Aoyama et al. 2001; Minegishi et al. 2005; Jamandre et al. 2007; Fahmi et al. 2015). Additionally, sub-species could not be resolved as sister taxa when using ATP8 alone, and polytomies were frequently observed despite including *Serrivomer beanii* as an outgroup.

Despite the many incongruences that methods to constructing phylogenies can make, genetic tools have been essential in evaluating relationships among species within the genus *Anguilla*.

Population genetics

Genetic methods have also been used to determine population structure, or lack thereof, of species of *Anguilla*. There are 56 publications on *Anguilla* population genetics and population structure (Table S1). Population genetics methods are especially prevalent in studies of *A. marmorata*, which is unique among *Anguilla* species in that it has multiple spawning locations, genetically different populations, and some populations have a metapopulation structure (Ishikawa et al. 2004; Minegishi et al. 2008; Donovan et al. 2012; Fig. 2b). Panmixia in *A. rostrata* (Ulmo-Diaz et al. 2023), *A. anguilla* (Dannewitz et al. 2005; Palm et al. 2009; Als et al. 2011), and *A. japonica* (Han et al. 2010; Faulks et al. 2022; Zhong et al. 2022) has been indicated via the use of whole genome sequencing for *A. rostrata* and microsatellite loci for *A. anguilla*, as well as analyses of microsatellite loci, *cytb*, mitochondrial D-loop, SNPs, and whole genomes for *A. japonica*. Although other studies have found evidence against panmixia for *A. anguilla* and *A. japonica* (Wirth and Bernatchez 2001; Igarashi et al. 2018), this may have been due to temporal sampling bias, spatial sampling bias, or use of different molecular methods (Dannewitz et al. 2005; Maes et al. 2006). Population genetics not only allow researchers to determine whether each species has one or more breeding populations and thus assist with the management of the distinct populations, but can also point to potential ongoing speciation processes.

Subspecies for three anguillid species have been defined based on geographic ranges and TV, though evidence for genetic differentiation is variable (Watanabe et al. 2008, 2014), generating debate about definitions of populations and subspecies. The genus *Anguilla* contains

16 species, three of which are further divided into subspecies: *A. bengalensis bengalensis*, *A. bengalensis labiata*, *A. australis australis*, *A. australis schmidtii*, *A. bicolor bicolor*, and *A. bicolor pacifica*. Based on analyses of 16S, subspecies show less genetic distance than among species comparisons (Watanabe et al. 2008). These analyses resulted in genetic distances of subspecies pairs ranging from 0.003 between *A. australis australis* and *A. australis schmidtii* to 0.007 between *A. bicolor bicolor* and *A. bicolor pacifica* (Watanabe et al. 2008). The lowest genetic distance between *Anguilla* species is 0.012 between *A. anguilla* and *A. rostrata* (Watanabe et al. 2008). Populations of *A. marmorata* have genetic distances ranging from 0.000 to 0.003 (Watanabe et al. 2008), which is equal to the subspecies of *A. australis*. Morphological characteristics may be insufficient to accurately identify subspecies of *A. bicolor*, but they can be reliably distinguished with a single base pair difference in their 16S gene in correlation with geographic separation (Tanaka et al. 2014). Mitochondrial control region RFLP analyses showed clear division between subspecies of *A. bicolor* and *A. bengalensis*, but *A. australis* subspecies were mixed and had no clustering, suggesting that they should be considered and treated as a single species (Dijkstra and Jellyman 1999; Watanabe et al. 2008), and no significant differences in allozyme frequency between specimens from New Zealand and Australia support the existence of a single species of *A. australis* (Jellyman 1987). However, analyses of whole mitochondrial genomes support clear divergences between all subspecies pairs (Minegishi et al. 2009).

In addition to genetic analyses, these subspecies are also delineated by significant differences in numbers of TV. However, the use of TV to delineate species and subspecies is not without caveats. Embryonic fish developing at lower temperatures have more vertebrae than those at higher temperatures (Brooks and Johnston 1994; Sfakianakis et al. 2011) and heat shock experiments of embryonic fish have induced somite malformations (Connolly and Hall 2008).

Significantly different ranges of TV may be indicative of the water temperature that *Anguilla* eels matured in, and suggest individual spawning areas and reproductive isolation. Different populations of *A. marmorata* have significantly different numbers of vertebrae, though with little genetic differentiation, they are all considered the same species (Watanabe et al. 2025). The issue of differentiating species, sub-species, and populations within *Anguilla* is still being explored, and further genetic analyses such as nuclear genetics are needed in this area.

Certain mitochondrial genes, particularly COI, are standardly used as "barcodes" to identify species and analyse relatedness (Hebert et al. 2003). Mitochondrial DNA sequences often evolve faster than do nuclear DNA sequences, and thus have greater numbers of variable sites, making them useful for species-level and genus-level analyses (Rubinoff and Holland 2005). However, nuclear DNA may be more appropriate to use when analysing population structure, as mitochondrial DNA is only maternally inherited (Rubinoff and Holland 2005). Thus, for every four copies of nuclear DNA passed from one generation to the next, only one copy of mitochondrial DNA is passed on (Ballard and Whitlock 2004). Phylogenies created using mitochondrial DNA are sometimes inconsistent with those created from nuclear DNA; this can be due to differential sorting of genetic lineages that coexisted in ancestral species, differences in gene copy numbers, horizontal gene transfer, and because demographic fluctuations do not impact divergences between lineages of mitochondrial DNA as they do for nuclear DNA (Avise 2012). Issues arise with analyses of nuclear genes as well, such as heterogeneous mutation rates and recombination (Avise 2012). Studies that use mtDNA to assess population structure of species of *Anguilla* (e.g., Huyen and Linh 2020; Arai and Taha 2021; Elmy et al. 2024) are best corroborated with nuclear DNA before making conclusions about genetic structure, connectivity or speciation processes.

Environmental DNA studies

Environmental DNA is defined as the total pool of DNA isolated from environmental samples (Taberlet et al. 2012, 2018), encompassing intra- and extra-cellular traces of genetic material detectable from microbial, meiofaunal, and macrobial organisms in soil, water, and air environments (Pawlowski et al. 2020). Environmental DNA can be more sensitive than traditional survey methods, as it eliminates the need to capture or physically see the target organisms, and it has a higher chance of detecting rare or cryptic species as well as organisms with low densities or biomass in aquatic ecosystems (Jerde et al. 2011; Takahara et al. 2012). Therefore, the application of eDNA is useful for studying anguillids given their often cryptic behaviour, low densities in some locations, and the sympatric ranges of many species.

There are 47 studies using eDNA found in this review (Table S1). Environmental DNA techniques have been used to monitor eel distributions of *A. anguilla* in northern Spain (Burgoa Cardás et al. 2020), Irish lakes (Weldon et al. 2020), and Cyprus (Griffiths et al. 2023). These studies compared field collections of eels to eDNA detections by collecting water samples, filtering the water, and PCR amplification of the captured genetic material. Electrofishing has been traditionally used for sampling *Anguilla*, but it is disruptive to the ecosystem, can damage organisms, can only be conducted in shallow water, and is not possible in saline waters (Vucić et al. 2023). This places further limitations on sampling surveys, which are very labour intensive. Therefore, eDNA mitigates these limitations because one person can do the sampling and water filtration, and samples can be analysed later in the laboratory. Environmental DNA concentration has been used as an estimate of relative abundance of *A. rostrata* in the Bronx River, USA (Chin et al. 2021) and sensitivities of different primer and probe assays targeting different mitochondrial genes (NADH2 and *cytb*; Moyer et al. 2023) for eDNA detections were compared to eel densities and biomass in tributaries to the Hudson River, USA (George et al. 2023). Most

studies using eDNA have targeted *Anguilla* DNA in freshwater systems, however, several studies have also applied eDNA to detect anguillids in in various saltwater habitats (Stoeckle et al. 2017; Knudsen et al. 2019; Takeuchi et al. 2019b, 2022; Vucić et al. 2023; Boyse et al. 2024). Environmental DNA is useful for detecting rare species of *Anguilla* through the use of species-specific primers and probes, which have been developed for some individual anguillid species (Sezaki et al. 2005) for use in semi-multiplex PCR assays (Gagnaire et al. 2007), quantitative PCR (qPCR) assays (Takeuchi et al. 2019c; Moyer et al. 2023) and multiplex droplet digital PCR (ddPCR) assays (Thomson-Laing et al. 2021). There is significant potential to use eDNA to identify the presence of eels during their oceanic life phases, and it has been used to detect a possible spawning event of *A. japonica* (Takeuchi et al. 2022). Spawning sites of some species of freshwater eel are still unconfirmed (Fig. 1b), so the application of eDNA offers the potential to discover these areas.

As with most types of sampling methodologies, there are challenges with using eDNA to study *Anguilla*. Environmental sampling is constrained in vast oceans regions due to the cost of research cruises. Ocean dilution of eDNA affects detection, given the relatively low abundance of anguillids in oceans (Casselman 2003). Environmental DNA becomes degraded with time, reducing ambient eDNA concentrations further, thus eDNA primers often target a short sequence that is usually only about 150 base pairs (bp) long (Cristescu and Hebert 2018). This makes the creation of species-specific primers difficult, as species of *Anguilla* are genetically similar and capturing a section of DNA that can be amplified and is unique to one *Anguilla* species within 150 bp is challenging (Minegishi et al. 2009). A decline in assay specificity can increase the chance of false negatives if non-target substances in the sample interfere with the assay (Wilcox et al. 2013). Having high levels of sensitivity is important, and if the quantity of eDNA in a

sample is below the detection threshold, it can result in a false negative (Wilcox et al. 2013; Che-Pelicier et al. 2025).

To counter these issues, methodological developments with heightened degrees of sensitivity such as ddPCR have been used for studying eDNA of *Anguilla* species (Thomson-Laing et al. 2021). Droplet digital PCR is more sensitive than end-point PCR and qPCR (Wood et al. 2019; Thomson-Laing et al. 2021), as every result is 20,000 individual PCR reactions (Doi et al. 2015). Droplet digital PCR has successfully detected anguillid DNA in environmental samples when DNA was not picked up by metabarcoding (Thomson-Laing et al. 2021).

The only ddPCR studies that have been conducted specifically for anguillids have targeted different mitochondrial genes for each species due to cross-reactivity of single gene primers (Thomson-Laing et al. 2021, 2022). Concentrations of eDNA and environmental RNA (eRNA) shed by *A. dieffenbachii* and *A. australis schmidtii* were quantified using two sets of species-specific primers and probes targeting different genes for each species (Che-Pelicier et al. 2025). Concentrations of shed eDNA and eRNA differed between species, despite each primer and probe set having roughly the same limit of detection (LOD) and limit of quantification (LOQ). Though these thresholds have varying definitions among eDNA publications (Thalinger et al. 2021), here, limit of detection is defined as the minimum DNA dilution at which DNA is detected in two out of three replicates, and limit of quantification is defined as the minimum DNA dilution in which target DNA is quantified in every replicate (Thomson-Laing et al. 2021). Lower LOD and LOQ values indicate that an assay will perform better at lower eDNA concentrations. This can be valuable for finding species of *Anguilla* outside of their currently known ranges, or if they are an introduced species competing with native species (Arai et al. 2017). Differences in eDNA concentrations targeting separate genes has been seen in at least one other study of *Anguilla* species-specific primers targeting NADH2 and *cytb* genes for *A. rostrata*

499 (Moyer et al. 2023; George et al. 2023). The NADH2 marker had slightly lower LOD and LOQ
500 values, indicating it was more efficient than the *cytb* assay. To ensure accurate quantification of
501 *Anguilla* eDNA in future studies, further refinement in genetically distinguishing these species
502 reliably in a short sequence of DNA at low eDNA concentrations is needed.

503 When tested in laboratory conditions, *A. dieffenbachii* and *A. australis schmidtii* released
504 significantly different amounts of eDNA and eRNA (Che-Pelicier et al. 2025). This was the first
505 study of eRNA done on *Anguilla* eels and provides a proxy for how long eDNA and eRNA of
506 these two species is detectable in fresh water. The limitation of this study is that the species-
507 specific assays were designed on different mitochondrial genes (*cytb* for *A. dieffenbachii* and 16S
508 rRNA for *A. australis schmidtii*; Thomson-Laing et al. 2021). This leaves the question as to
509 whether the different amounts of eDNA and eRNA detected were due to species-specific
510 differences, or due to differences in the genes used, highlighting the need for species-specific
511 assays to be designed on the same gene. The development and refinement of these eDNA and
512 eRNA assessment methodologies will allow researchers to detect eNAs of *A. dieffenbachii* and
513 *A. australis schmidtii* in the ocean on the way to their spawning grounds and during their
514 spawning events. This will further enable researchers to develop tracking models of these species
515 in their oceanic migrations and greatly contribute to the overall knowledge of *Anguilla* lifecycles.
516

517 *Genetic tools for fisheries and market product identification*

518 DNA techniques are also important for reliably identifying species of eels in human food
519 and identifying illegal harvesting and trading. There are six publications on using genetic
520 methods to identify *Anguilla* food products to the species level and two publications on detecting
521 illegally trafficked live *Anguilla* animals (Table S1), offering a wide opportunity for using these
522 techniques in future attempts to track and prohibit illegally trafficked *Anguilla* specimens. In

2016, *A. anguilla* glass eels were detected entering the Hong Kong international airport (Stein et al. 2016). This was the first genetic evidence of illegal trading of *A. anguilla* glass eels, and 45% of available eel products at 13 Hong Kong retail outlets were genetically identified as *A. anguilla* by analyses of mitochondrial gene encoding for COI (Richards et al. 2020). European and American eels are increasingly appearing in sushi markets to fill the demand for Japanese eels as the population and availability of *A. japonica* declines (Chang et al. 2021). In American grocery stores and restaurants, American eels (*A. rostrata*) comprised 94% of the samples that were genetically tested, *A. anguilla* comprised 5% and *A. japonica* comprised 0.7% (Ely et al. 2023). In Japan, eel products in supermarkets and other retail stores were identified using 1000 bp fragments of the *cytb* gene were found to be 61% *A. japonica*, 38% *A. rostrata*, and 1.5% *A. anguilla* (Shiraishi et al. 2025).

Despite *A. anguilla*'s status as critically endangered, 13 genetic identification studies on seafood in nine countries matched 59% of recovered mitochondrial COI and *cytb* gene sequences to *A. anguilla* (Nijman and Stein 2022). This exemplifies that this species is still widely traded and consumed, despite laws prohibiting the import and export of endangered species. Tracking illegally harvested anguillids is complex but can greatly benefit by molecular analyses such as loop-mediated isothermal amplification assays, due to their rapid turnaround time and high degree of sensitivity (Soroka et al. 2021). Species-specific primers and probes can be used not only in eDNA studies, but could also help in tracking eel products in marketplaces to assist in preventing future illegal harvesting.

Conclusions

Genetic research techniques have been particularly important for advancing knowledge of anguillids, especially for defining the 19 species and sub-species, and the phylogenetic

relationships among them. The ability to reliably identify species with genetic methods has overcome the constraints of morphological methods and been critical in delineating species ranges and identifying oceanic spawning locations. The recent emergence of eDNA techniques looks set to improve those knowledge outcomes further. Genetic identification is also invaluable for improving understanding of global trading in eel products, including illegal trading of species with legal protections. Genetics have helped to improve the knowledge of the population structure of species which can be used to improve their management. Further refinements of existing techniques such as primer-probe sets on the same gene that can reliably distinguish species, robust population genetic methods utilising a variety of genes, and new analysis methodologies with faster processing times and quicker results, will likely continue to improve the value of genetic techniques and provide better understanding of anguillids. We hope that the review herein compiled will provide a valuable resource for future research on these unique catadromous fishes.

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Author contributions

TCM and XP conceptualised the review in consultation with AJ, OL and AJMS, TCM conducted the bibliographic analysis and wrote the original draft of the manuscript with assistance of MJM,

ECG, AJ, XP. TCM made the figures in consultation with ECG, MJM, OL and XP, ECG worked on phylogenetic tree comparisons, in consultation with TCM, MJM, XP, and all authors reviewed and revised the manuscript.

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Statements and Declarations

The authors have no conflicts of interest to declare.

Data and Availability

Data and code used in this manuscript are uploaded at Dryad.

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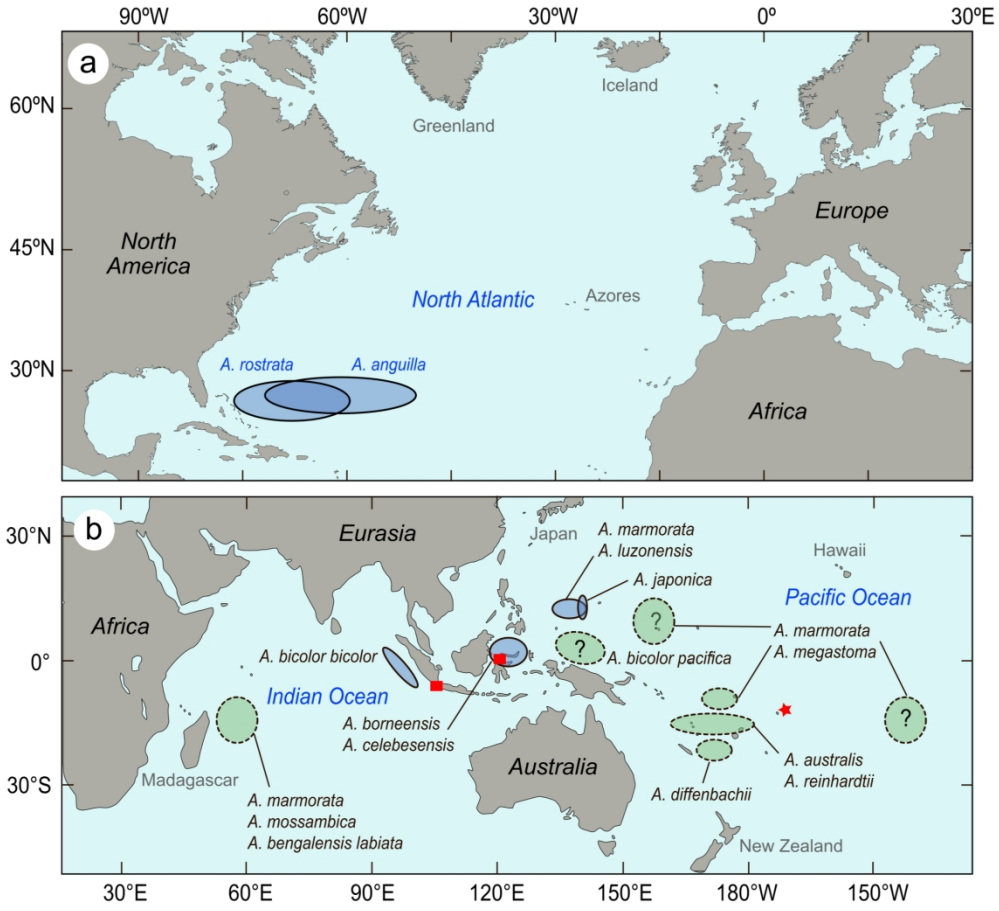
Figure 1. Map of spawning areas of species of *Anguilla*. Map of the north Atlantic Ocean and the overlapping spawning areas of *A. rostrata* and *A. anguilla* in the Sargasso Sea (modified from Watanabe and Miller 2012; Miller et al. 2015) (a). Map of the Indo-Pacific region showing known and estimated anguillid spawning areas for locations of recently spawned *A. interioris* (red squares) and *A. marmorata* (red star) leptocephali (b). Known spawning areas based on egg or larval catches are coloured blue with solid lines (modified from Miller and Tsukamoto 2017, Miller 2023). Estimated spawning areas are coloured green with dashed lines. Areas with question marks have little supporting information other than the geography of species ranges and ocean currents.

Figure 2. Flow chart of the systematic review process of identifying and screening publications from database results in the bibliographic analysis. The number of documents that resulted from the initial searches are listed under "Identification," the number of publications used are listed under "Screening," and the "Removal of duplicates" leads to the number of publications used in the final database (Table S1). Because the first 500 returned results were screened from each Google Scholar search, only the number of publications used that were not found in other database searches is recorded.

Figure 3. Heatmap of ten most commonly used genetic markers and the number of times they have been used for each species of Anguilla in publications included in the bibliographic analysis (see full details in Table S1). COI = cytochrome c oxidase I; SNPs = single nucleotide polymorphisms.

Table 1. Current and previous scientific names, and common names of species of *Anguilla* (Tsukamoto et al. 2020) and their geographic distributions. Categories of Ege (1939) of shortfin (SF), longfin (LF), plain skin (PS), and mottled skin (MS) species with dentition (Dent.) of either broad maxillary bands of teeth (BMX) or narrow bands (NMX), as well as number of total vertebrae (TV) are also listed. Numbers of TV taken from Watanabe 2003; Watanabe et al. 2006, 2008, 2009.

Current Name	Previous Name	Fin, Skin, Dent., TV	Geographic distribution	Common name	IUCN status
A. anguilla		LF, PS, 109-115	Atlantic Ocean	European eel	Critically endangered
A. australis australis		SF, PS, 109-115	Eastern Australia	Australian shortfin eel	Near threatened
A. australis schmidtii		SF, PS, 108-114	New Zealand, South Pacific	New Zealand shortfin eel	Near threatened
A. bengalensis bengalensis	*A. nebulosa nebulosa*	LF, MS, NMX, 106-111	India, Indonesia	Indian Bengal eel	Near threatened
A. bengalensis labiata	*A. nebulosa labiata*	LF, MS, NMX, 110-114	East Africa	African Bengal eel	Near threatened
A. bicolor bicolor		SF, PS, 106-111	East Africa to west Indonesia	Indian bicolor eel	Near threatened
A. bicolor pacifica		SF, PS, 104-109	Southern Japan to east Indonesia	Pacific bicolor eel	Near threatened
A. borneensis	*A. malgumora*	LF, PS, 104-107	Borneo	Borneo eel	Vulnerable
A. celebesensis		LF, MS, BMX, 102-105	Philippines	Celebes eel	Data deficient
A. dieffenbachii		LF, PS, 111-115	New Zealand	New Zealand longfin eel	Endangered
A. interioris		LF, MS, BMX, 101-103	West Sumatra	New Guinea eel	Data deficient
A. japonica		LF, PS. 113-115	East Asia	Japanese eel	Endangered
A. luzonensis	*A. huangi*	LF, MS, BMX, 103-107	Luzon island, Philippines	Luzon eel	Vulnerable
A. marmorata		LF, MS, NMX, 103-110	East Africa through Indo-west Pacific	Indo-Pacific eel	Least concern
A. megastoma		LF, MS, BMX, 107-111	South Pacific islands	Polynesian longfin eel	Data deficient
A. mossambica		LF, PS, 106-108	East Africa	Mozambique eel	Near threatened
A. obscura		SF, PS, 103-105	South Pacific, NE Australia	Polynesian shortfin eel	Data deficient
A. reinhardtii		LF, MS, NMX, 102-104	East Australia, PNG, northern New Zealand	Australian longfin eel	Least concern
A. rostrata		LF, PS, 100-110	Atlantic Ocean	American eel	Endangered



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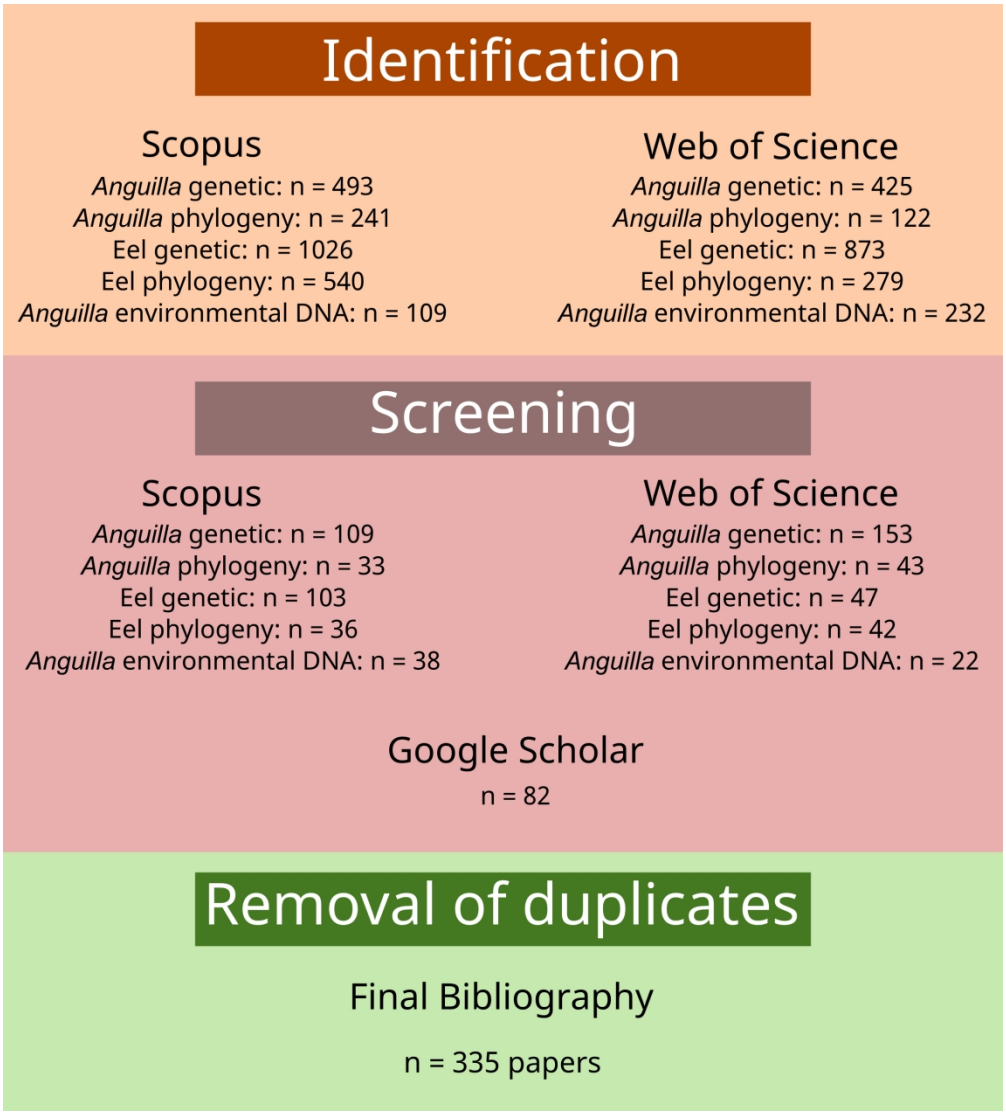

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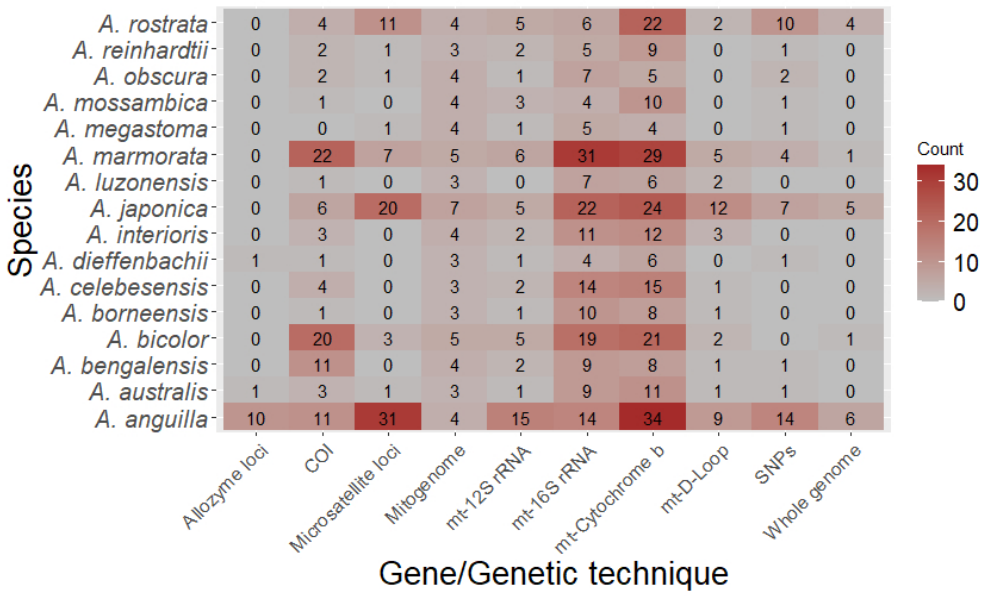

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