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Explaining the worldwide distributions of two highly mobile species: *Cakile edentula* and *C. maritima*

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Abstract

Aim

If we are able to determine the geographic origin of an invasion, as well as its known area of introduction, we can better appreciate the innate environmental tolerance of a species and the strength of selection for adaptation that colonising populations have undergone. It also enables us to maximise the success of searches for effective biological control agents. We determined the number of successful colonisation events that have occurred throughout the world for two *Cakile* species and compared the climates from which they originated, in which they established and then spread.

Location

Worldwide

Taxon

Sea-Rockets (*Cakile* spp.), Brassicaceae

Methods

We used a high throughput sequencing and a genome skimming approach combined with Bayesian Inference phylogenetics to examine variation in entire chloroplast genomes and regions of nuclear ribosomal DNA within native and introduced areas. Databases were used to compare climates between native ranges and introduced regions for multiple clades within each species.

Results

At least seven clades have invaded different regions of the world. In most cases we were able to identify their source regions and climates. The environmental bottlenecks differed in intensity: while some clades colonized into climates that were similar to climates in their native range, other clades had a very broad innate environmental tolerance such that new invasions succeeded beyond the climate range of native populations. We found clear evidence of hybridisation – specifically chloroplast capture - between two species in Australia.

Conclusions

Results here show that these species are sometimes capable of colonizing in climates that are beyond the climate range of native populations. Whether successful colonization beyond native climate niches requires *de novo* adaptation, or whether it represents an innate broad fundamental niche requires further investigation. *Cakile* species provide an excellent opportunity to study replicated climate adaptation trajectories.

KEYWORDS

Chloroplast, climate, invasive plants, origin, phylogenetics.

1 | INTRODUCTION

Colonising genotypes represent only a subset of the variation exhibited across an entire species in its native range (Sotka et al., 2018). Some genotypes within a native range may be “exapted” to the new environments that they encounter during range expansion, since the environments resemble the native environments to some extent, whereas other genotypes may find conditions less suitable. Establishment of a long-distance disperser into an environment that is very different to the source location, however, indicates that founding genotypes have high innate environmental tolerances. The selection pressure that genotypes experience upon colonisation, and thus the subsequent post-colonisation adaptation that will occur, will depend on the magnitude of the difference between native and recipient environments. Evolutionary trajectories of invasive populations that are adapting to new environments will depend on the range of genetic backgrounds on which selection acts, the extent of genetic variation that occurs through processes such as mixing of individuals from different source populations (“admixture”) and hybridisation, and the mating systems of the species (Bock et al., 2015). The relative frequency of colonization events that are either within or beyond the environmental range of a native source population will indicate the likely importance of environmental adaptation to species range expansions.

Although these general principles of invasion ecology are well-understood, it is difficult to determine what processes actually occurred in a specific case. By the time a species invasion is considered sufficiently problematic to warrant study, it may have spread widely, at a continental or even a global scale. Repeated opportunities for multiple introductions from diverse sources, hybridisation, introgression, drift and adaptation in multiple dimensions – spatial, temporal and environmental - make the puzzle complex. Notwithstanding various logical and methodological problems (Colautti et al., 2009), it has become common to use classical common-garden experiments and modern molecular and bioinformatics techniques to uncover plausible signals of mechanisms involved in the invasion of a given region. In addition, repeated introductions into distinct regions around the globe – though not true replicates in a statistical sense - offer contrasting scenarios from which to establish an evidence base.

The first step in a biogeographic study of an invasion is invariably to determine, as near as possible, the source origin for an invasion. This enables appropriate accessions to be included in common-garden comparisons, albeit relying on the implicit assumption that invading phenotypes were once identical, or at least very similar, to those collected today from the region of origin (e.g. Sotka et al., 2018). Successful management of invasive species is also enhanced if searches for biological control agents are made in likely source regions, ensuring compatibility of agent and host genotype. Knowledge of the location of origin also provides information on the source environment. Thus, if we also know the approximate point of entry of an invasion, which we often do from records of deliberate introductions or contemporary herbarium or museum samples, we can estimate environmental parameters along the full pathway of events, from source, to incursion, to the full extent of the (ongoing) invasion. This provides information on the selection pressures (the "environmental filters"), coinciding with the genetic bottlenecks, to which colonising populations will be exposed. Molecular markers are now commonly used forto identify native range sites whose genotypes best match invasive populations, thus facilitating the assessment of these selection pressures.

Two invasive species exhibiting most of the usual complications that we encounter with determining invasion mechanisms are the sea-rockets *Cakile edentula* and *C. maritima* (Brassicaceae). They have a wide geographic native range on either side of the North Atlantic (Labrador to Florida, 22° of latitude and Svalbard to North Africa, 35° of latitude, respectively). They are extremely successful colonisers and have established themselves multiple times, sometimes sequentially, in different regions around the globe (Cousens et al., 2013). *Cakile maritima,* a self-incompatible species, has occupied at least 31° of latitude in western North America (from Baja California to British Columbia), as well as 44° of longitude and 17° of latitude in Australia (from Shark Bay, Western Australia to Brisbane, Queensland), an unknown extent of the eastern coast of South America, as well as the North Island of New Zealand and parts of New Caledonia. The self-compatible *Cakile edentula* colonised 24° of latitude in western North America (from California to Alaska), 38° of longitude and 22° of latitude in Australia (from the western Great Australian Bight to Mackay, Queensland), the length of New Zealand and unknown extent in eastern Russia, Japan and Korea, although in many regions of western North America, Australia and New Zealand it has been displaced by *C. maritima*. The coastal North American *C. edentula* subsp. *edentula* var. *edentula* has appeared in Lake Michigan in historical times, where it is intermixed and perhaps hybridising with var. *lacustris* (Rodman, 1974). Although *C. maritima* has been recorded around ports on the east coast of North America (Riefner, 1982), it has seldom persisted for very long in *C. edentula*'s native range. There is both morphological and molecular evidence that there have been multiple sources of introduction of *C. maritima* as well as hybridisation and introgression with *C. edentula* in Australia (Ohadi et al., 2015). Whether or not this has been the case in other regions remains to be seen.

This paper investigates phytogeography within the native and introduced ranges of both *C. maritima* and *C. edentula*. Specifically, we sought to determine: how many distinct colonisation events there have been in regions throughout the world; whether or not introduced populations are founded from source populations climatically similar to their location of introduction; and the extent to which the climatic range of the species changed after introduction.

2 | MATERIALS AND METHODS

2.1 | Collection locations

Samples were obtained from across the native ranges of *Cakile edentula* (40 samples including subspecies *edentula* (varieties *lacustris* and *edentula*) and *harperi*) and *C. maritima* (44 samples including subspecies *islandica, baltica*, *integrifolia*, *maritima* and *euxina*) and from as many regions as possible where the species are naturalised (Japan (5 samples), Argentina (2 samples), New Caledonia (1 sample), New Zealand (13 samples), Australia (30 samples) and the west coast of North America (5 samples)) (Figure S1, Table S1). Using published distribution maps and a network of collectors, we ensured that all regional subspecies and varieties from the native ranges were included, although subsequent analyses did not include such information explicitly. Samples from introduced regions was uneven, depending on the goodwill of international colleagues and the availability of samples collected for other purposes. In regions where taxa co-occur and may have hybridised (Ohadi et al., 2015), specimens were chosen to be representative of the phenotype of a given taxon. In addition, we collected samples representing most of the *Cakile* variation from the Caribbean and Gulf of Mexico (three subspecies of *C. lanceolata* [*lanceolata*, *alacranensis*, *fusiformis*] and *C. geniculata*), along with samples of the desert species *C. arabica* from Saudi Arabia and *Erucaria hispanica* as an outgroup from the closest related genus within tribe Brassiceae (Willis et al., 2014). Each collector was responsible for compliance with local requirements for permits; no permits were required for the importation of DNA or dried leaf material. Further outgroups from tribe Brassiceae, *Brassica oleracea* and *Sisymbrium irio,* were selected from GenBank® (National Center for Biotechnology Information) (Table S1).

2.2 | DNA extraction, library preparation and sequencing

Genomic DNA was isolated from silica dried leaf tissue using a modified cetyl trimethylammonium bromide (CTAB) protocol (Shepherd & McLay 2011). Library preparation used the protocol outlined by Schuster et al. (2018). Samples were sequenced using Illumina HiSeq2000 and Illumina NextSeq500 sequencing platforms, based at AgriBio (Centre for AgriBioscience, La Trobe University) and the Walter and Eliza Hall Institute in Melbourne, Australia.

2.3 | *De-novo* contig assembly, sequence construction and alignment

Raw sequencing reads were *de-novo* assembled using CLC Genomics Workbench version 12.0.1 (https://www.qiagenbioinformatics.com/**)** with default settings. Resulting contigs were imported into Geneious version 11.1.5 or Geneious Prime v2019.2.1 (http://www.geneious.com, Kearse et al., 2012), along with raw read files. A reference chloroplast genome (*Arabidopsis thaliana* NC_000932.1) and stretch of nuclear ribosomal cistron (*Erucaria hispanica* AY722495) from GenBank were used in the construction of *Cakile* reference sequences. To make a reference upon which all other *Cakile* samples were based, contigs from a single sample were mapped to the *Arabidopsis* and *Erucaria* sequences using custom sensitivity settings; gaps allowed set to a maximum of 20% per read, maximum gap size of 3000 and 2-3 iterations. All raw reads were then mapped back to the consensus sequence and a final consensus sequence extracted using a consensus threshold of 75%, to confirm sequence accuracy. All subsequent *Cakile* sequences were then generated using this same method from the *Cakile* reference sequences. Nuclear ribosomal cistron DNA length varied between samples, from 7,068 to 8,120 base-pairs (bp), with complete coverage of 18S, ITS1, 5.8S, ITS2 and 26S regions and partial coverage of the ETS (external transcribed spacer)/NTS (non-transcribed spacer) regions. The sequence depth of nrDNA samples ranged from 63x to 4,885x, with a median of 678x. Chloroplast genome length varied little between samples, from 153,194 – 153,732 bp. The sequence depth of cpDNA samples ranged from 34x to 2,415x, with a median of 337x. Complete chloroplast genomes were assembled for all samples. cpDNA and nrDNA sequences were aligned separately in Geneious using the MAFFT plug-in (MAFFT version 7.388; Katoh & Standley, 2013) with default settings. Each alignment was checked by eye and adjustments were made manually. Sections of sequence that could not be aligned unambiguously (i.e. due to long stretches of repetitive single base repeats or repetitive indels) were excluded from phylogenetic analyses. One inverted repeat region (IRa) of the cpDNA alignment was excluded from all phylogenetic analyses. Due to poor assembly quality, three cpDNA samples and four nrDNA samples were removed from all analyses.

2.4 | Phylogenetic analyses

Phylogenetic analyses using Bayesian inference (BI) and maximum likelihood (ML) methods were performed using MrBayes version 3.2.7a (Ronquist et al., 2012) and IQ-TREE version 1.6.12 (Nguyen et al., 2015) respectively, on both the cpDNA and nrDNA alignments. Since the results were qualitatively similar, only the BI results will be presented in the main text.

The chloroplast genome alignment was partitioned into four character sets: CDS, rRNA, tRNA, and spacer (SPCR) regions. Models of evolution for each partition were estimated with MrModeltest version 2.4 (Nylander 2004) and selected using the Akaike information criterion (AIC): CDS = GTR+I, rRNA = F81, tRNA = GTR+I, and SPCR = GTR+I+G. The analysis was run using the CIPRES Science Gateway (Miller et al., 2010) for three million generations with unlinked partitions and a tree sampling frequency of 500 generations.

The nrDNA alignment was partitioned into six character sets: SPCR, 18S, ITS1, 5.8S, ITS2, and 26S. The ETS/NTS regions were combined into a single partition (SPCR) due to the uncertainty of region boundaries, partial coverage, and a lack of quality reference sequences for these regions. The selected models were: SPCR = HKY+I+G, 18S = JC, ITS1 = SYM+I, 5.8S = JC, ITS2 = K80+G, 26S = GTR. The BI analysis was run for five million generations with unlinked partitions and a tree sampling frequency of 500 generations.

During both analyses, the average standard deviation of split frequencies reached a value less than 0.01 and the effective sample size reached a value greater than 200. The convergence of MCMC chains was checked in Tracer version 1.7.1 (Rambaut et al., 2018). Twenty-five percent of the trees were discarded as burn-in, a consensus tree was generated, and Bayesian posterior probabilities (PP) estimated for nodes from the consensus tree.

2.5 | Climate analysis

We obtained records for native and introduced locations of *C. edentula* and *C. maritima* from GBIF (Global Biodiversity Information Facility, downloaded 14 June 2020), and removed all non-expert-determined herbarium records and locations distant from the coast for which there was no documentary evidence. Locations of first recorded herbarium samples for a particular part of the world were obtained from Rodman (1974), Cousens & Cousens (2011), Cousens et al. (2013) and from GBIF. For all locations, we accessed eight Bioclim variables (Kriticos et al., 2012) that reflect average (annual mean temperature and precipitation), extreme (mean temperature of the warmest and coldest quarter, precipitation of wettest and driest quarter) and the variability (temperature and precipitation seasonality) of hydrothermal conditions. For clades with sufficient numbers of samples and only a single clade per introduced region, we calculated the Mahalanobis distance between two climate envelope centroids as a metric for climate equivalency, and the proportion of records located outside one reference climate space to describe climate similarity (Mesgaran et al., 2014). We examined differences between centroids of (1) native range GBIF data vs GBIF data from introduced regions attributable to each clade, (2) the sequenced samples from the introduced range vs all native range GBIF records, and (3) the sequenced samples in each clade from the native vs introduced ranges. A permutation test was used to test whether the results were statistically significant. In addition, we verified whether the first introduced record fell within the climate space of the native range. Full details of statistical analyses, data manipulation and software are given in the Supplementary Information.

2.6 | Mapping

All sample locations were presented on a Mollweide map projection using R packages 'ggplot2' (Wickham, 2016), 'maptools' (Bivand & Lewin-Koh, 2020), 'rgdal' (Bivand et al., 2020), 'maps' (Brownrigg et al., 2018), 'sp' (Pebesma & Bivand, 2005) and 'rnaturalearth' (South, 2017).

3 | RESULTS

3.1 | Chloroplast DNA phylogeny

The cpDNA BI tree could not be rooted such that the ingroup was monophyletic, because *Erucaria hispanica* was not placed on a branch shared with the more distant outgroups, *Brassica oleracea* and *Sisymbrium irio*. As a result, the tree was rooted using only *B. oleracea* and *S. irio* as outgroups. Neither *C. edentula* nor *C. maritima* was resolved as monophyletic, but most samples of *C. edentula* were placed in one clade (Group A), and most samples of *C. maritima* in another clade (Group C). *Cakile lanceolata* was also not resolved as monophyletic, with three samples placed in Group B, one in Group A and one in Group C (Figure S2). The ML tree showed very similar results (Figure S3).

Considering the native range *Cakile* samples, the results show some geographic patterns in cpDNA variation. Group B contains most of the collections from the Caribbean and Gulf of Mexico, plus two samples identified by collectors as *C. edentula* from the east coast of Florida (a region in which *C. edentula* subsp. *harperi* and *C. lanceolata* are sympatric). Group C comprises mostly *C. maritima* phenotypes, plus the one sample of *C. arabica* (in clade C11) and *Erucaria hispanica* (in Clade C3). The notable exceptions from the general pattern are a *C. lanceolata* sample from Bermuda (clustered with *C. edentula*, in clade A7) and a *C. lanceolata fusiformis* sample from Puerto Rico clustering with *C. maritima* (clade C12). Interestingly, Group B is more closely related to *C. maritima* than *C. edentula* (see Supplemental Information for further discussion).

Nine clades are evident within group A. Geographically (Figure 1), two clades are clustered in the mid-Atlantic US: clade A5 to the north around New York and New Jersey, and clade A8 further south around Delaware, Maryland and Virginia (though both clades have a geographical outlier further south). Unfortunately, the region south of Cape Hatteras typically associated with *C. edentula* subsp. *harperi* (the Carolinas and Georgia) is poorly represented as is New England. Two clades dominate in the Canadian maritime provinces: clade A9 in the St Lawrence River and on the Atlantic Ocean, with clade A2 in the Gulf of St Lawrence. Of the four samples from the Great Lakes, the most easterly (Lake Erie) is in a clade of its own (clade A1) which is most closely related to Clade A2 in the Gulf of St Lawrence; of the samples from Lake Michigan, one sample clusters with several from the New York region (clade A5), while two samples cluster with two others in the vicinity of the Bay of Fundy (clade A7).

Group C contains 21 clades, of which two are only represented by samples from the invaded range (clades C19, C20). There is a clear geographic separation of clades found only in the eastern Mediterranean (clades C1, C13-C15) and those only in the western Mediterranean (clades C2, C16-C18) (Figure 2). Clade C21 is widespread along the Atlantic coast from the Canary Islands to Denmark). Clade C3 corresponds to the northernmost area of the Atlantic (samples were taxonomically identified as *C. maritima* subsp. *islandica*). Clades C4-C8, which together form a clade, are each represented by just a single sample in the native range and all come from an area of the Atlantic including Northern France, UK and the mouth of the Baltic. The sample from furthest into the Baltic is the only representative of clade C9. Clade C12 is widespread, occurring in the Mediterranean and Atlantic; its most related clades, C10 and C11 are represented by single samples from the Mediterranean.

Regarding the invaded ranges, samples of *C. edentula* from the invaded range come from just two clades, A5 and A9 (Figure 3). Samples from Japan and southern British Columbia were placed in a single clade (A5) along with native range samples predominantly from the New York/New Jersey region. The single Alaskan sample and all New Zealand and Australian samples of the species, plus several *C. maritima* samples, were placed in a clade containing *C. edentula* from the region encircling the Gulf of St Lawrence (A9).

For invasive *C. maritima*, the cpDNA tree also provides good support for the placement of samples in the invaded range. Those from South America are placed in a single clade (C21) containing native-range samples from the Atlantic coast (Figure 4). Those from New Zealand and western North America are placed together in clade C19, but, because this clade sits as part of a polytomy with four other lineages (clades C17, C18, C20, C21), including a mix of native-range and invaded-range samples, the closest relatives of this clade are not resolved. Clades C17-C18 correspond to the extreme western Mediterranean and the Atlantic coast of Europe. Samples of *C. maritima* from Australia are placed in multiple clades, some of which are well-separated in the cpDNA phylogeny. A primarily Western Australian group (clade C8) includes a native-range sample from the Baltic [EW469], while one sample from Western Australia (Clade C5) is sister to a native-range sample from northern France [EW262]. A group comprising samples from eastern Australia and New Caledonia (clade C20) is part of the five-lineage polytomy mentioned previously, so its relationships are not clear. A final group of *C. maritima* samples falls in a clade (A9) that otherwise includes samples of *C. edentula* from Australia, plus native-range samples of *C. edentula* from eastern Canada.

3.2 | Nuclear ribosomal DNA phylogeny

The nrDNA phylogeny was based on substantially fewer sequence data than the cpDNA tree (total alignment length of 8,196 bp with 118 parsimony-informative characters, versus 156,408 bp and 1322) and, accordingly, showed far less resolution and support for relationships (Figures S4, S5). The nrDNA phylogeny, in contrast to that based on cpDNA, showed strong support for the monophyly of *Cakile* and was rooted based on all three outgroup sequences. *Cakile edentula*, *C. maritima* and *C. lanceolata* were not resolved as monophyletic, but there was full support (Posterior Probability, or PP = 1.00) for a clade that included almost all samples of *C. edentula* (except one), and all samples of *C. maritima* were placed as part of a large polytomy (i.e., neither confirming nor refuting the monophyly of that group of samples). In contrast to the cpDNA results, *C. arabica* was distinct from the other taxa.

The nrDNA tree provides little evidence for assessing geographic patterns within either the native or invaded ranges of *C. edentula* and *C. maritima*, but there are some points of potential incongruence within these species between the nrDNA and cpDNA trees. Within *C. maritima*, there are two resolved clades with strong support in the nrDNA tree (clades N1, N2) (Figure S6a). These include five native range samples from the extreme west of the Mediterranean (Clade N1) that are spread across three distinct clades in the cpDNA tree (clades C11, C12, C14), and two invaded range samples (clade N2) from New Caledonia (Fig.S6c) and Vancouver Island, Canada (Figure S6d), that are spread across two distinct clades in the cpDNA tree (clade C19 and C20). Also, the 17 Australian samples of *C. maritima* that were nested among samples of *C. edentula* in clade A9 of the cpDNA tree show no evidence of being nested in *C. edentula* in the nrDNA tree, with full support for the relevant node in the nrDNA tree (pp = 1.00). Within *C. edentula*, there are two resolved clades with strong support in the nrDNA tree (clades N3, N4) (Figure S6b), and includes 19 native range *C. edentula* samples (plus one of *C. geniculata*) that are spread across four distinct clades in the cpDNA tree (clades A2, A4, A9, B1). These *C. edentula* samples are a distinct geographic group encircling the Gulf of St Lawrence (clade N3), plus the three samples from the Great Lakes (clade N4).

3.3 | Climate analysis

Figure 5 shows three *Cakile* invasion events (out of the nine invaded ranges that were associated with a native range clade: Figs 3 & 4) that occurred outside the climate space occupied by the species in its entire native range (*C. edentula* in Japan and Australia; *C. maritima* in New Caledonia). They then spread into a wide range of climates, much of them non-analogous to climates in the native range (Table S2). Climate spaces of the native and invaded ranges of Clade A9 do no overlap at all, while the climate space described by A5 samples was insufficient to for statistical comparision. Both *C. edentula* Clade A9 (Figure 3) and *C. maritima* Clade C8 (Figure 4) originated in temperate regions and have established and spread in Mediterranean regions. In western North America, at least one clade of *C. edentula* (either A5 or A9) established in a Mediterranean climate (near San Francisco) and has then spread northward into colder regions, perhaps as far as Alaska (Figure 5). The first record (representing the presumed area of introduction) and subsequent spread of Clade C21 in South America occurred within a climate window consistent with its source region, although there was considerable uncertainty in the climate of origin. Unfortunately, source and introduced climates of Clades C19 and C20 could not be compared.

4 | DISCUSSION

Distinct cpDNA and nrDNA spatial structure was evident in *Cakile* species despite their high propensity for dispersal. Others have reached a similar conclusion based on morphology (Rodman, 1974), allozymes (Gormally et al., 2011), random amplified polymorphic DNAs (RAPDs), inter-simple sequence repeats (ISSRs) (Clausing et al., 2000) or amplified fragment length polymorphisms (AFLPs) (Kadereit et al. 2005). Sequencing of complete chloroplast genomes enabled us to detect more variation than in previous studies, to determine the origins - and thus source climates - of invasive populations and to confirm the existence of past hybridisation events (Ohadi et al., 2015). By focussing specifically on the climates of origin of the invading genotypes and the climates in the locations of the introductions of those genotypes, we were able to draw more definitive conclusions about responses to invasive range climates than the general species-wide analyses of “niche shifts” by Atwater et al. (2018) and others.

4.1 | Phylogeographic patterns within native ranges

For the European collections, our chloroplast DNA results support a conclusion that the Straits of Gibraltar are an important restriction to gene flow and thus, to the distribution of subspecies of *C. maritima* (Kadereit et al., 2005; Westberg & Kadereit, 2009). Our results also support their observation for a distinction between samples from the eastern and western regions of the

Mediterranean. Within regions, the geographic clustering of our clades is, in general, less distinct than their reports. This is partly because their study contained many more samples separated by shorter geographic distances, especially within the Mediterranean region. Their data were also from AFLP analysis which largely reflects the nuclear genome, which can give a different phylogenetic signal from uni-parentally inherited chloroplast genomes (Rieseberg & Soltis, 1991). Our chloroplast analyses give excellent resolution at a continental scale, clearly distinguishing between the two sides of the Atlantic and between the Caribbean/Gulf of Mexico taxa and the northern *C. edentula*. Moreover, the analysis suggests that North American taxa may have first colonized the Caribbean, since nrDNA indicates that *C. lanceolata* is the basal North American clade (Figure S5), and then extended northward, while recurrent input from European *C. maritima* into the Caribbean (after the expansion of *C. edentula* within North America) appears to have rendered *C. lanceolata* cpDNA more similar to that of *C. maritima* than to *C. edentula*.

4.2 | Sources of invasive populations

We were able to assign sources of all invasive *C. edentula* samples to relatively restricted regions of the eastern seaboard of North America. The northern Atlantic States of the US have long been a centre of trade and the existence of ballast heaps near ports on the east coast is well-documented (Rodman, 1974). There were certainly strong trade links between there and San Francisco, where the *Cakile* invasion of the west coast began, and from there across the Pacific to Japan. The first date for *C. edentula* in Japan is 1982 (Asai, 1982), well after the end of the use of hard ballast by ships, but it is unclear whether or not it had already been present in the western Pacific before then. It is possible that the species is still actively spreading in Asia, perhaps an extension of the invasion that began in California in 1880 (Rodman, 1974) via Alaska. Fruits are dispersed locally along the beach by wind and by seawater; most fruits float and remain viable for no longer than two weeks in seawater under experimental conditions (Heyligers, 2007). A more thorough survey of the west coast of North America is required to determine the spatial distributions of the two chloroplast types that we identified. The east coast Provinces of Canada and Australia and New Zealand had industries in common (e.g. whaling) that could have involved ballast or other materials. The first record of *C. edentula* in Australia was in the 1860s (Rodman, 1986), while it was first found in New Zealand in 1921 (Cousens & Cousens, 2011). Given the similarities in genotypes between Australia and New Zealand, it is possible that the *C. edentula* invasion of New Zealand was a secondary one from Australia.

The published interpretation of the biogeography of *C. edentula* invasion on the Pacific coast of North America (Barbour & Rodman, 1970) is that from its first appearance around 1880 a single colonisation event spread rapidly northwards, reaching Alaska by 1931. Placement of our sample from Alaska suggests at least two introductions on that seaboard. A study of glucosinolate composition (Rodman, 1976) also showed variation within west coast *C. edentula* but was insufficient to determine whether or not there had been multiple introductions. More intensive sampling of this region, along with detailed analysis of historical herbarium specimens, may help to establish the points of entry of the two clades and their subsequent dynamics.

The introduction into South America, at least into Argentina where we sampled, appears to have originated on the Atlantic coast of southern Europe, corresponding to *C. maritima* subsp. *integrifolia*. There appear to have been three introductions into Australia. In Western Australia, most of the samples correspond to the Baltic (*C. m. baltica*), while one matches a sample collected from northern France. Rodman (see Cousens et al., 2013) has previously suggested the Baltic as the origin of *C. maritima* in Western Australia, based on morphology. Glucosinolate composition of two samples from south-eastern Australia indicated origins in western Europe and the western Mediterranean (Rodman, 1974).

The previous interpretation of the invasion dynamics of *C. maritima* in Australia - that a single incursion appeared in Western Australia in the 1890s (Sauer 1988) and spread around the rest of the continent – is a second example of where reliance on species-level databases to determine the trajectories of invasions can be highly misleading. Our results, as well as those of Ohadi et al. (2015) and Cousens et al. (2013), confirm that there was a second invasion that appeared in South Australia by 1918 (Cousens et al., 2013) and that it is this invasion that has proceeded to spread around the rest of the continent. Our results were unable to indicate the source of the second invasion, though the Atlantic coast or the extreme west of the Mediterranean are indicated as possibilities. Ohadi et al. (2015) detected a very low level of nuclear gene flow from the Western Australian introduction into the nearest Eastern populations, but we are unable to say whether this admixture has been in any way responsible for the invasion success of the latter. The population of *C. maritima* in New Caledonia is a similar genotype to Australia, so it could also represent a secondary invasion. *Cakile maritima* has clearly not invaded New Zealand from Australia: that is a separate introduction, from the same region as the invasion of the Pacific coast of North America. But once again, the exact source is unclear but is unlikely to have been from the eastern Mediterranean or the Baltic.

4.3 | Implications for the study of adaptation to climate

Rapidly growing annual species such as *C. edentula* and *C. maritima* can achieve wide latitudinal/climatic ranges by three means. Firstly, genotypes may be already suited to the introduced range if the environmental conditions are similar to those in the native range. This is a plausible explanation for several of the introductions studied here (e.g. *C. maritima* clade C5, C8, and

C21; Figure 5). However, some populations extended their climate range beyond that of the native range after they were introduced (e.g. *C. maritima* C19, C20), and some even colonized locations outside of the range of conditions present in their native range (*C. edentula* clade A5, A9 and *C. maritima* clade C20). In these cases, individual genotypes may have environmental tolerances (fundamental niches) that include conditions beyond those in the native range. When a propagule arrives at a new location, it will only increase in numbers if the genotype can grow and reproduce successfully under those conditions: establishment indicates at least some level of exaptation. Alternatively, they could alter their responses to cues regulating the times during the year at which they emerge or flower; in this way, they avoid the harshest periods (e.g. Stinchcombe et al., 2004) and continue to experience conditions similar to those in their native range. Thirdly, after establishment there may be local adaptation to the new climates so that they are better able to tolerate location-specific growing conditions (Sunday et al., 2019): i.e. evolution of the genotype's niche. Evidence for adaptation has been found in Australian *C. edentula* (Ohadi 2015), in sub-species of *C. edentula* from North America, and in European subspecies of *C. maritima* (Willis (2013).

Evolutionary trajectories in invasive species are likely to depend on the genetic backgrounds of the colonising individuals and the strength of selection pressure, although much more discussion has focussed on the extent to which genetic diversity (genetic bottlenecks) will constrain local adaptation (Bock et al., 2015). Arriving genotypes carry local adaptations from their environment of origin, they arrive in another environment (which may or may not be very different) and may then spread into further environments. The extent of the environmental filters at each of these stages could be crucial to the invader's establishment success and subsequent biogeographic trajectory. Our results show that it is possible to use molecular methods in conjunction with climatic analysis to quantify these environmental shifts. Additional questions then arise for future molecular evolutionary research. Will adaptation be via a different pathway, or of a different form (tolerance vs avoidance), if the selection pressure is greater versus weaker? Are single genes of major effect more likely to be involved than multigenic quantitative traits? If a genotype arrives at one point along an environmental gradient, will it always develop an appropriate phenotype by the same means (so-called "gene re-use")? The broad climatic native ranges, replicated invasions, contrasting environmental filters, and differing breeding systems, would seem to make *Cakile* an ideal system for such investigations.

4.4 | Hybridisation

The success of *C. maritima* as an invasive species is somewhat of a conundrum, as its self-incompatibility would make it prone to mate limitation when undergoing long-distance dispersal and establishment. At least three cases of successful *C. maritima* invasion have followed invasion by the self-compatible *C. edentula* (though it has established on its own in South America and New Caledonia). We have previously published a model showing that hybridisation with a (previously invaded) congener such as *C. edentula* could provide a form of demographic rescue for a self-incompatible incoming species (Mesgaran et al., 2016, 2017). Evidence for hybridisation in Australia has already been confirmed (Ohadi et al., 2015), although it remains unclear whether there is also any adaptive advantage from this hybridisation. In this study, several of our *C. maritima* samples clustered with *C. edentula* based on the chloroplast sequence data, all from locations throughout southern Australia that no longer contain *C. edentula*. All of these samples, however, aligned with *C. maritima* for the nuclear DNA data (Figure S4), indicating that there has been "chloroplast capture" (Rieseberg & Soltis, 1991): at some point in their lineage, these *C. maritima* phenotypes hybridised with a *C. edentula* maternal plant and now retain their chloroplast genomes. These lines are persisting, often at a high percentage, within the *C. maritima* populations long after the invasion front has moved on (Ohadi et al., 2015) and support the predictions of our model.

4.5 | Conclusion

Our results provide information on prehistoric (trans-Atlantic – see S4.6) and recent introductions to new continents in two highly mobile species. Some regions appear to have been colonised from multiple sources and, where the two species have invaded the same regions sequentially, they have clearly hybridised. While both admixture and hybridisation are known in other species to contribute to local adaptation and invasiveness, that remains to be established in *Cakile*. Invasions of *Cakile* have occurred repeatedly into regions that differ in climate to their source regions and from there they have invaded widely, often into further, dissimilar climates. *Cakile* would provide an excellent opportunity for studies of climate adaptation trajectories, with replicate native range and invasive range adaptation along extensive climatic gradients.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All DNA sequences are available in GenBank. All sample details are given in Table S1. TreeBase Accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S26173

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BIOSKETCH

This collaboration emerged from a NIMBioS workshop organised by KD and drew on the resources of various laboratories who are/were previously working on Cakile and who now interact informally.

Author contributions: RDC, SO, AS and KD conceived the ideas. RDC obtained funding and was overall coordinator. JT and MJB supervised DNA library prep work. Early analysis of sequences was by SO (part of her PhD, along with DNA extractions and library prep work) and JT, then RAB; final sequence analyses were by ECS (part of his masters) under detailed supervision of RF and input by MJB. Climate analysis was done by PB. RDC and ECS led the writing team, with input and edits from all other authors.

Figure Legends

FIGURE 1 Native range distributions of samples within groups A and B of the Bayesian Inference chloroplast phylogeny (Figure S2), coloured according to clade. The key above is a stylised version of the phylogeny, with the width of triangles being proportional to the number of samples in both native and introduced regions falling within that clade. Posterior Probability (PP) and Ultra-fast Bootstrap (UFBoot) support values are shown at each node. Samples identified by collectors as *Cakile edentula* (○); *C. lanceolata* or *C. geniculata* (□).

FIGURE 2 Native range distributions of samples within group C of the Bayesian Inference chloroplast phylogeny (Figure S2): *Cakile maritima* samples (△) and *C. arabica* (▽) coloured according to clade, based on chloroplast phylogeny. Unfilled parts of the key represent clades only represented by samples in introduced regions. Posterior Probability (PP) and Ultra-fast Bootstrap (UFBoot) support values are shown at each node.

FIGURE 3 Global distribution of samples from clades within group A that were represented in introduced regions, along with members of those clades in their native range. Unfilled parts of the key represent clades only represented by samples in native regions. Plants displaying *Cakile edentula* phenotype (○); *C. maritima* phenotypes (△). Posterior Probability (PP) and Ultra-fast Bootstrap (UFBoot) support values are shown at each node.

FIGURE 4 Global distribution of samples from clades within group C. Unfilled parts of the key represent clades only represented by samples in native regions. All plants displayed *Cakile maritima* phenotypes (△). Posterior Probability (PP) and Ultra-fast Bootstrap (UFBoot) support values are shown at each node.

FIGURE 5 Principle Components Analysis climate envelopes for those clades recorded in introduced regions: (a) clade A5, (b) clade A9, (c) clade C5, (d) clade C8, (e) clade C19, (f) clade C20, (g) clade 21. Sequenced samples from the native range are shown as open circles; sequenced samples from introduced ranges are closed circles; first records in the introduced region are shown as solid triangles. The pale convex hull encompasses all GBIF (Global Biodiversity Information Facility) records in the native range; the dark convex hull encompasses GBIF records from all introduced regions from which DNA samples of the clade were collected (i.e. the extent of post-introduction spread of that clade). For Clades A5 and A9 (which have many samples introduced to Australia, New Zealand, Japan, or western North America), GBIF data from western North America (but not sampled DNA locations) are omitted since both clades were recorded from that region and it is unclear which records correspond to which clade; *Cakile edentula* subsp. *harperi* samples were excluded from the native range polygons of *C. edentula*, as there are no records of this taxon being introduced anywhere.

Fig. 1.

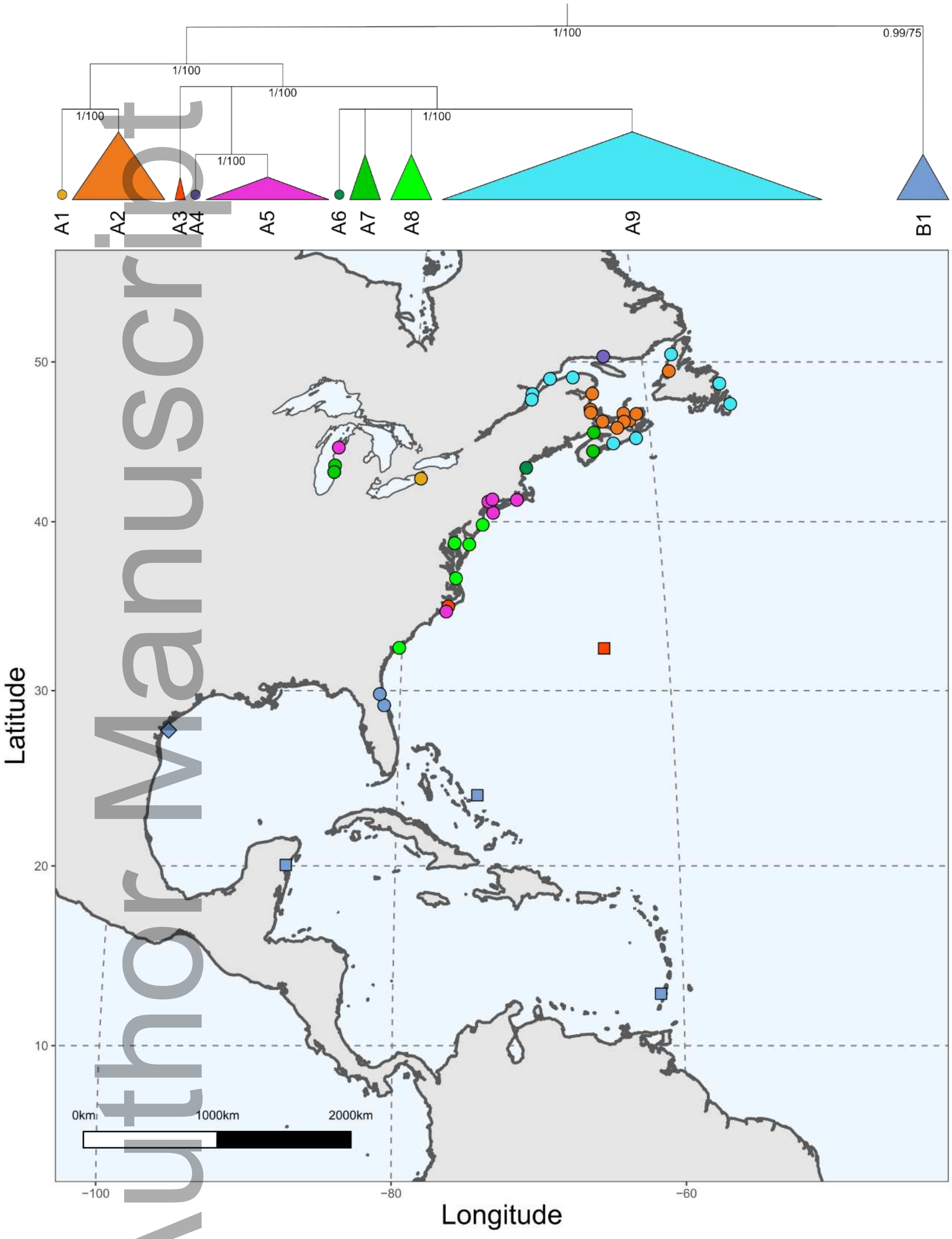
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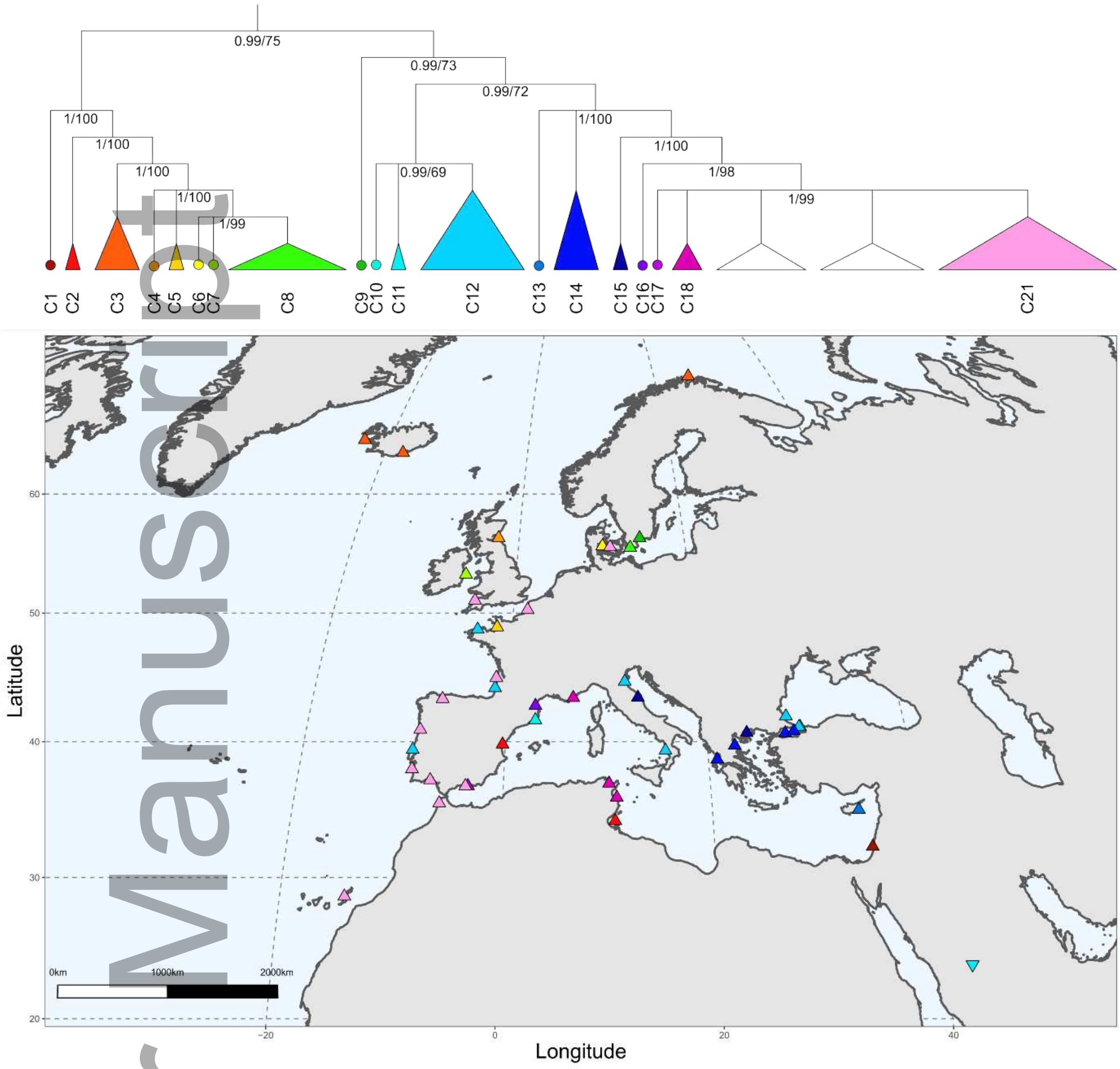
Fig. 2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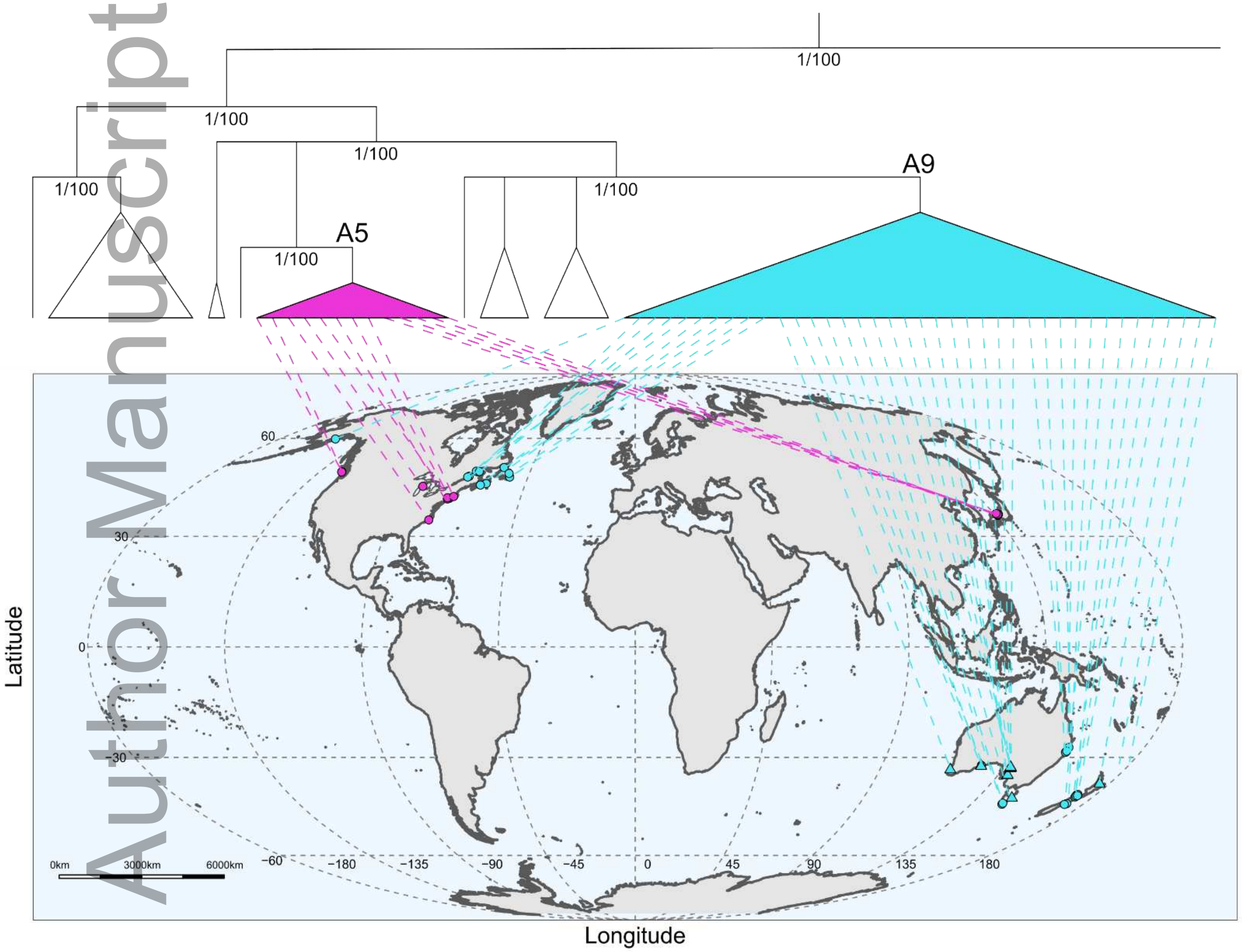
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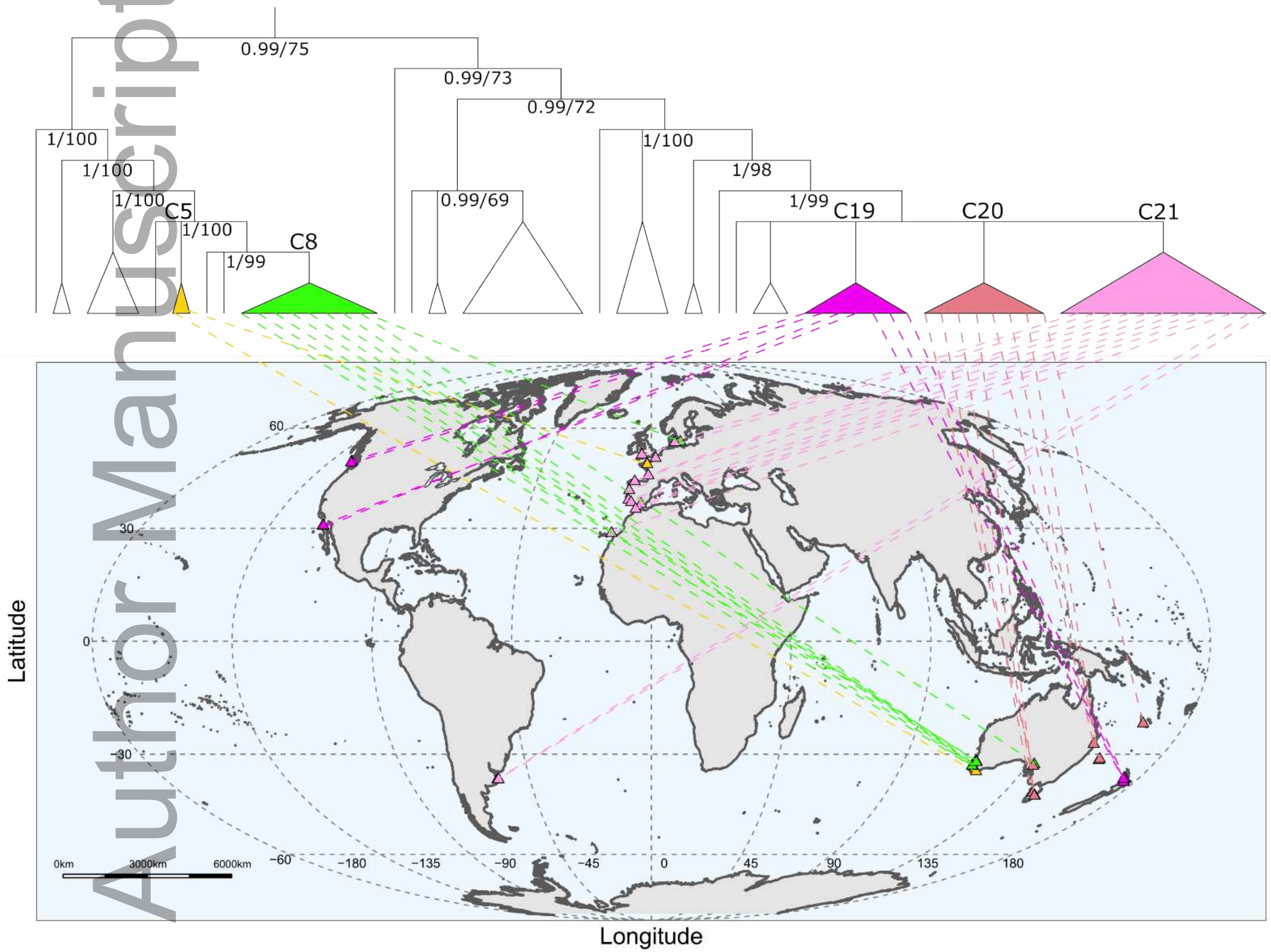
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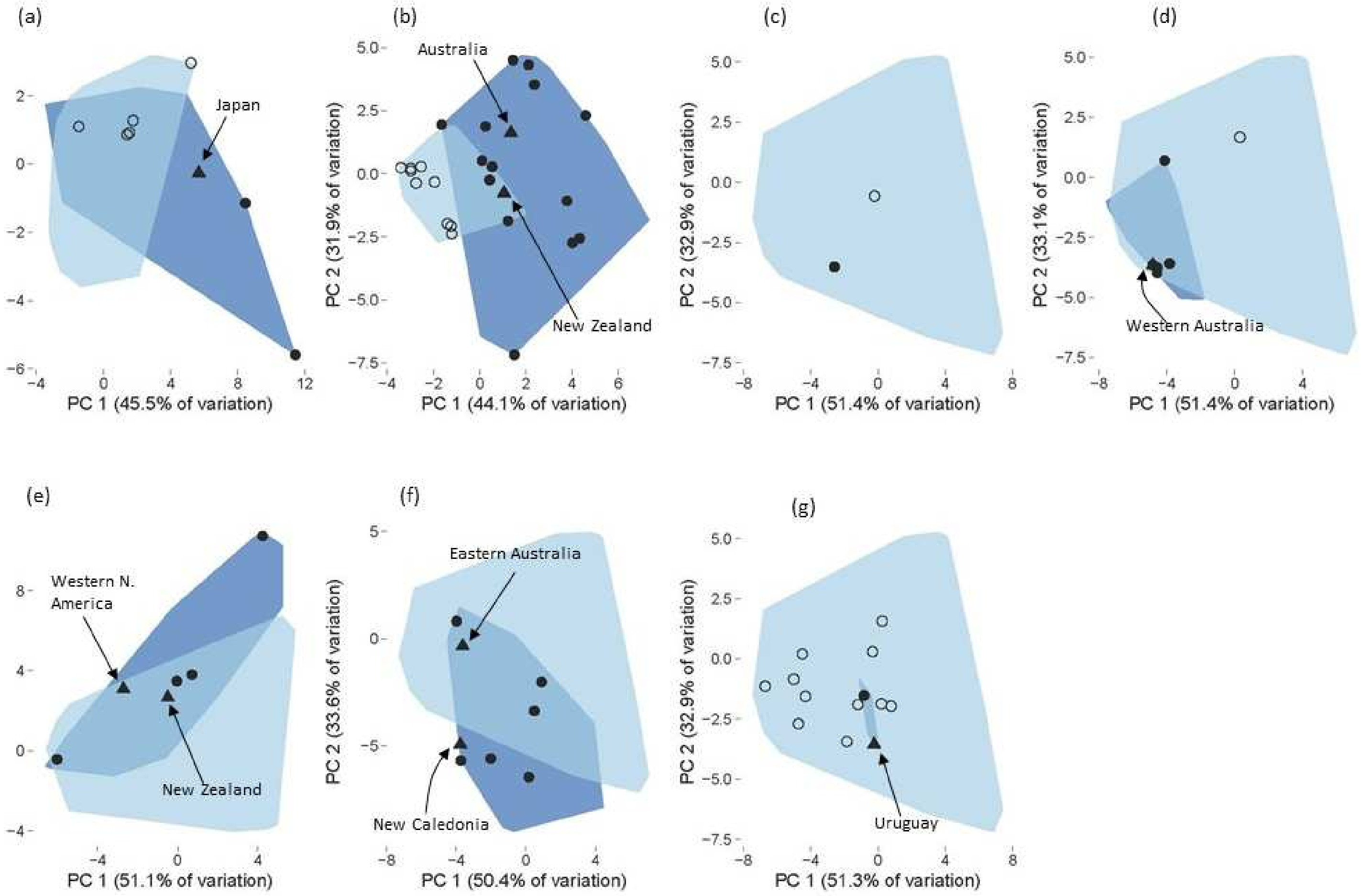
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(d)
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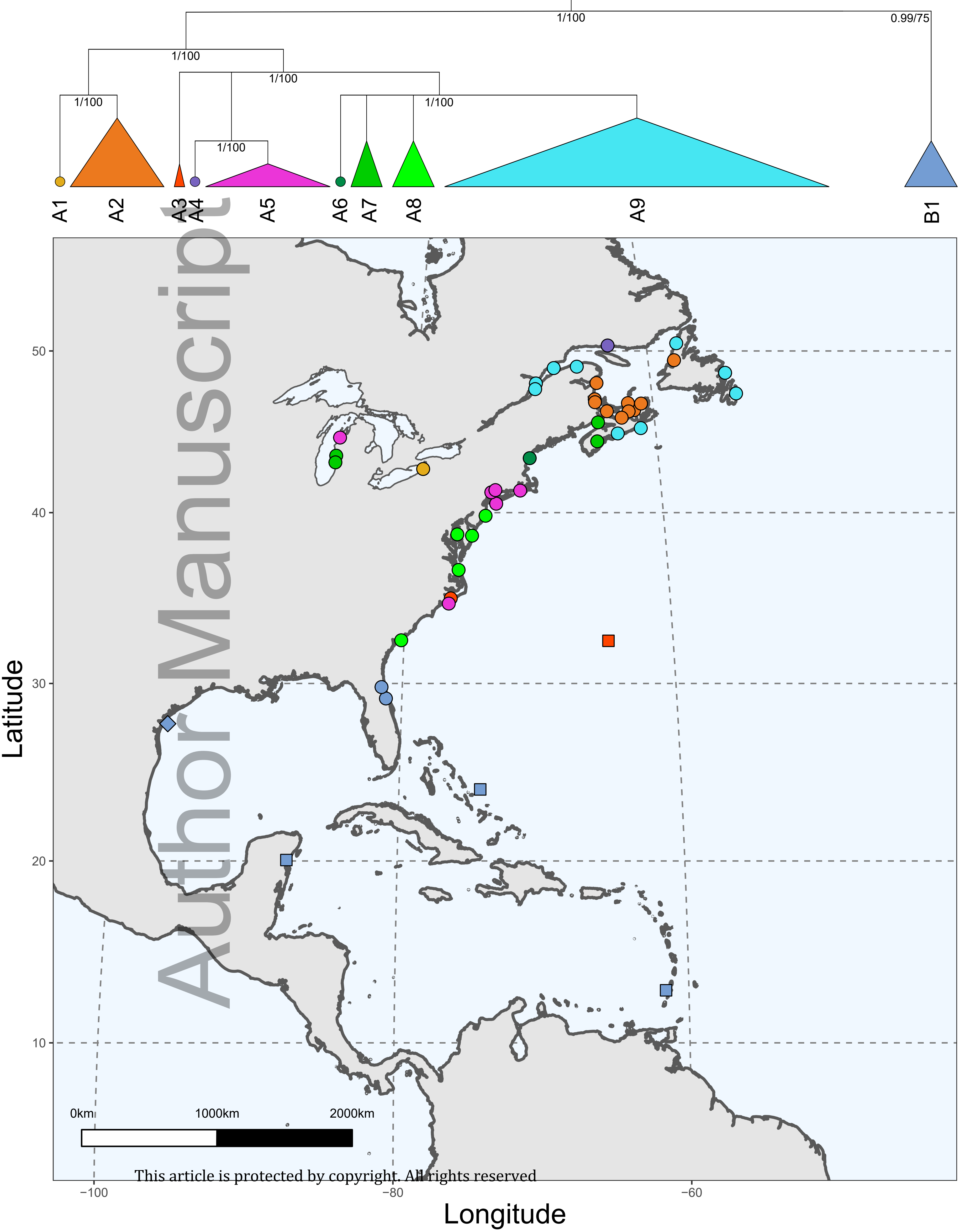
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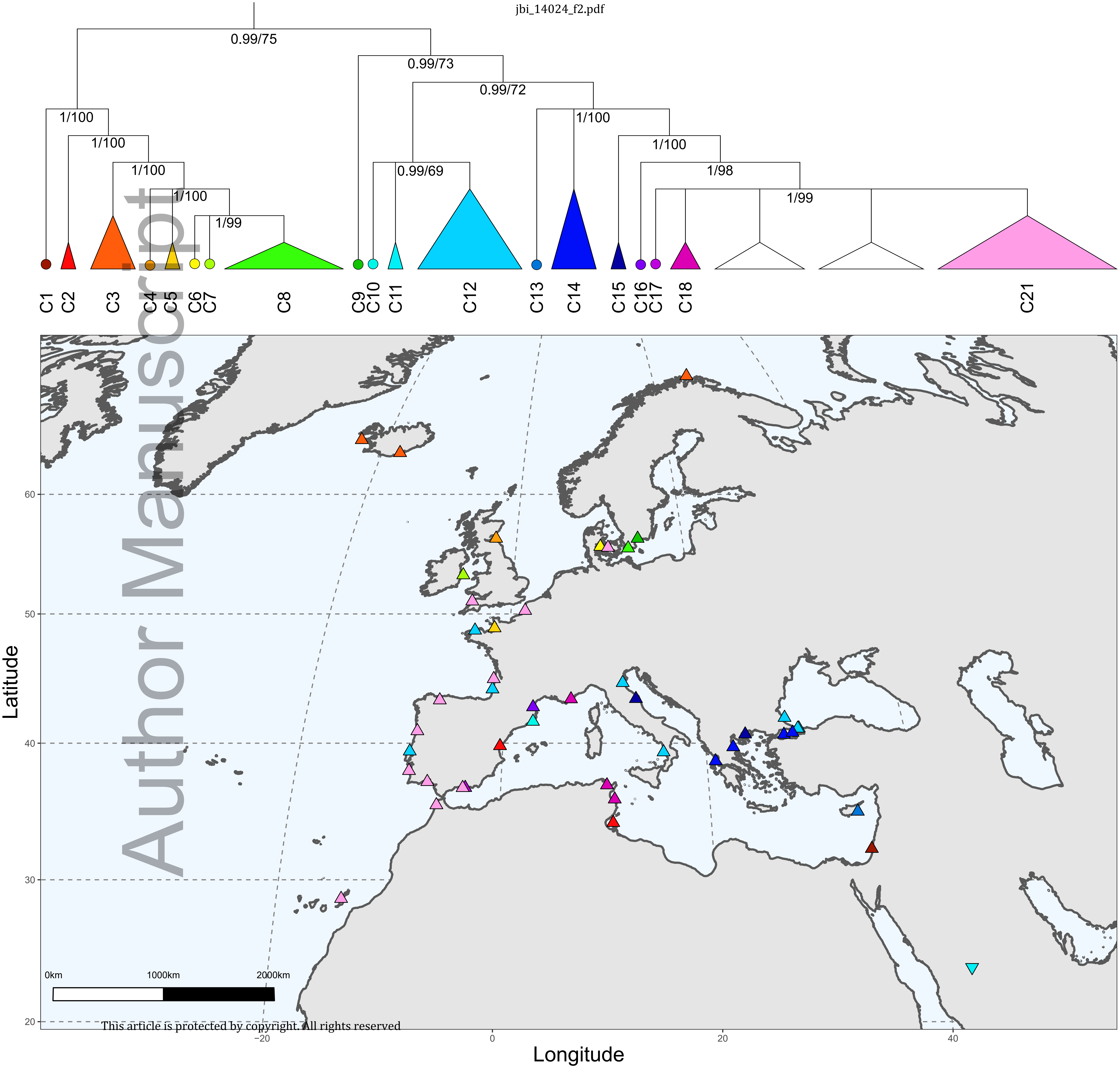
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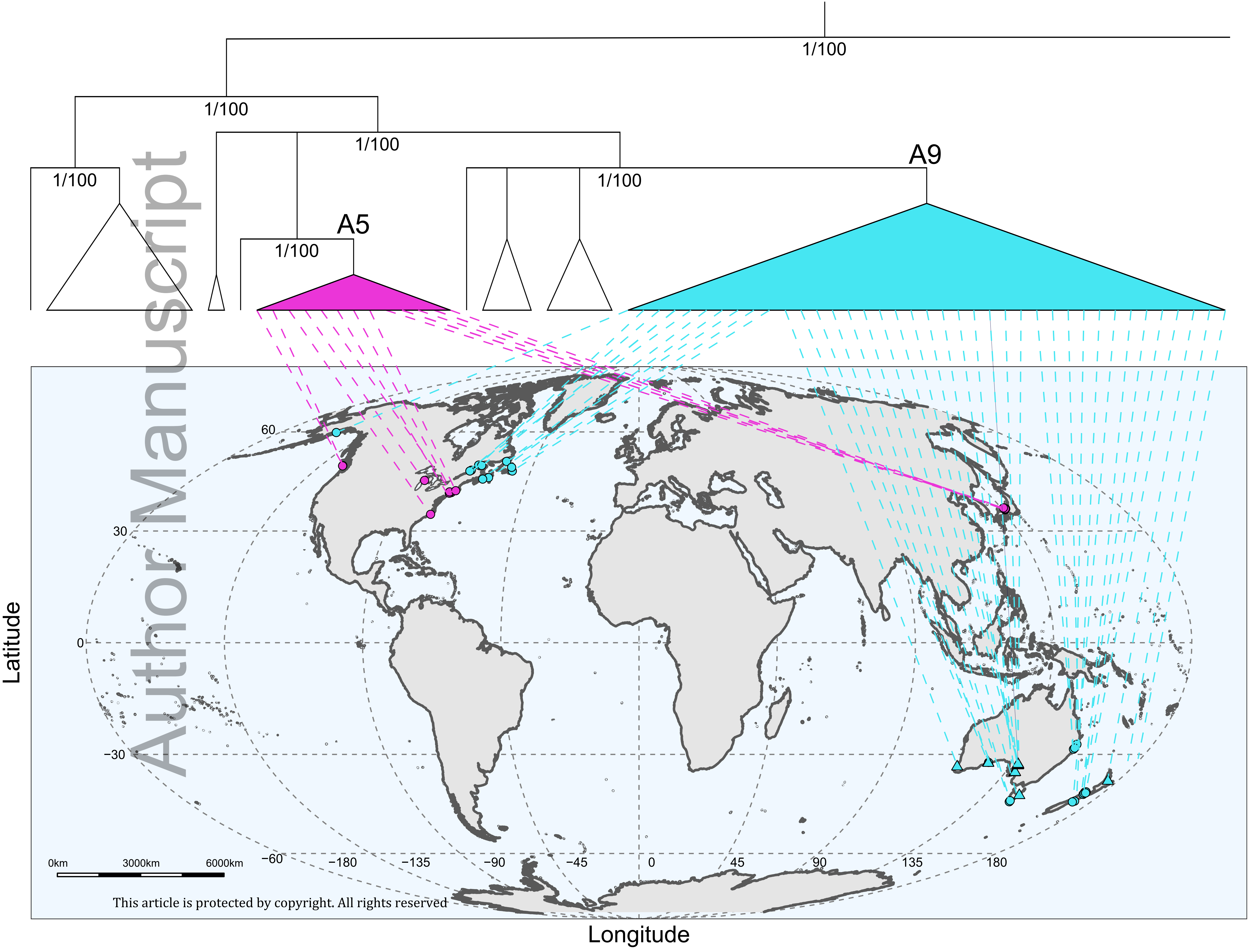
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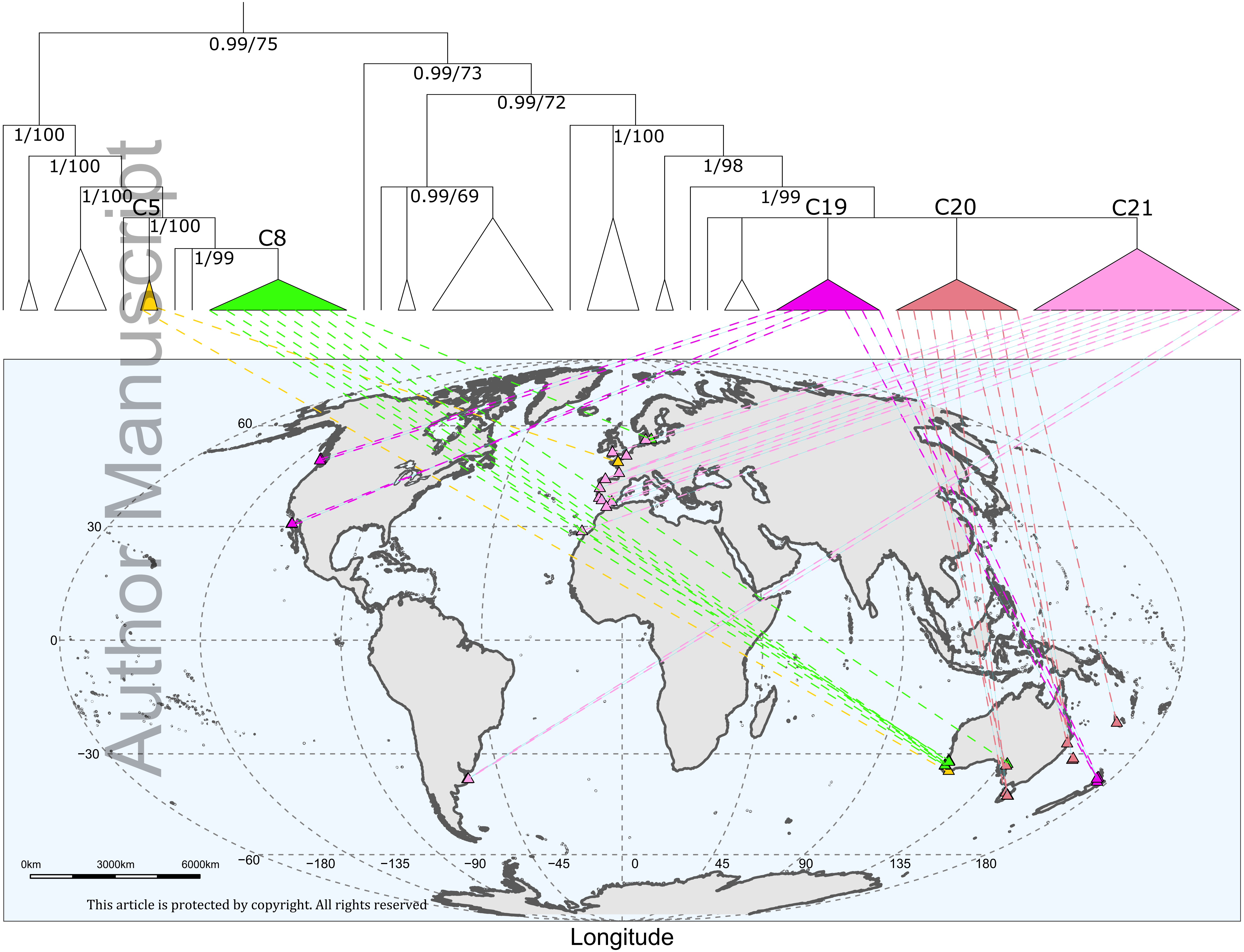
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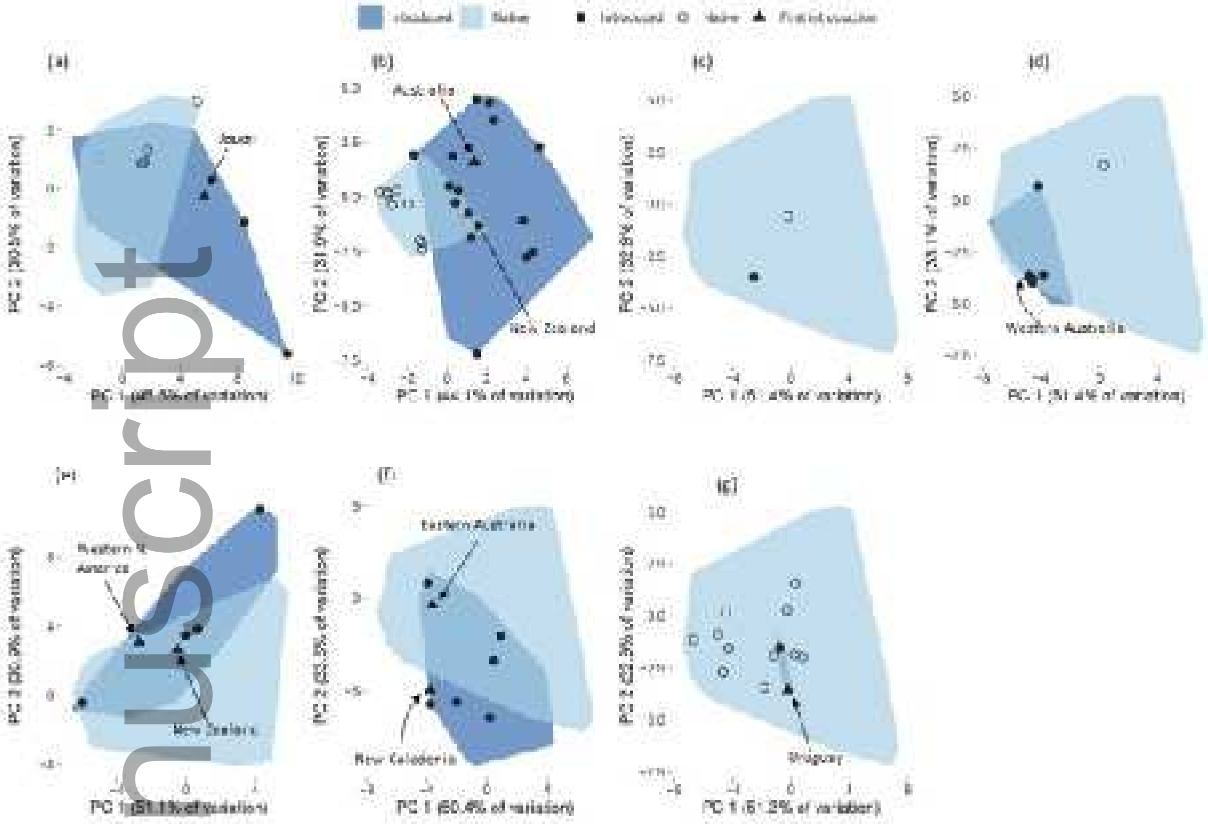

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