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Assessing global range expansion in a cryptic species complex: insights from the red seaweed genus *Asparagopsis* (Florideophyceae)[1]

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Abstract

The mitochondrial genetic diversity, distribution and invasive potential of multiple cryptic operational taxonomic units (OTUs) of the red invasive seaweed *Asparagopsis* were assessed by studying introduced Mediterranean and Hawaiian populations. Invasive behavior of each *Asparagopsis* OTU was inferred from phylogeographic reconstructions, past historical demographic dynamics, recent range expansion assessments and future distributional predictions obtained from demographic models. Genealogical networks resolved *Asparagopsis* gametophytes and tetrasporophytes into four *A. taxiformis* and one *A. armata* cryptic OTUs. Falkenbergia isolates of *A. taxiformis* L3 were recovered for the first time in the western Mediterranean Sea and represent a new introduction for this area. Neutrality statistics supported past range expansion for *A. taxiformis* L1 and L2 in Hawaii. On the other hand, extreme geographic expansion and an increase in effective population size were found only for *A. taxiformis* L2 in the western Mediterranean Sea. Distribution models predicted shifts of the climatically suitable areas and population expansion for *A. armata* L1 and *A. taxiformis* L1 and L2. Our integrated study confirms a high invasive risk for *A. taxiformis* L1 and L2 in temperate and tropical areas. Despite the differences in predictions among modelling approaches, a number of regions were identified as zones with high invasion risk for *A. taxiformis* L2. Since range shifts are likely climate-driven phenomena, future invasive behavior cannot be excluded for the rest of the lineages.

Keywords: *Asparagopsis*, Cryptic, Distribution Models, Invasion Risk, Hawaiian Archipelago, Mediterranean Sea, Phylogeography.

List of abbreviations

BSP: Bayesian skyline plots
cox: cytochrome oxidase subunit
GBIF: Global Biodiversity Information Facility
OTU: operational taxonomical unit
IGS: intergenic spacer
L: lineage
MGC: University of Málaga herbarium
ML: maximum likelihood
MP: maximum parsimony
MY: million years
SDM: species distribution modelling

Introduction

Human mediated introductions of marine biota represent major drivers of modification of native diversity, ecosystem functioning and associated goods and services (Vitousek et al. 1997). Among the thousands of exotic marine species transported daily via ballast water, aquaculture, aquarium trade and fouling on ship hulls (Ruiz et al. 2000, Hewitt et al. 2007), seaweeds represent a significant component, corresponding to up to 40% of the invasive species found today in the world's oceans (Schaffelke et al. 2006). Many so-called cosmopolitan seaweeds introduced worldwide encompass genetically distinct lineages often characterized by subtle phenotypic differences (Provan et al. 2005, Zanolla et al. 2014), distinct eco-physiological profiles (Zanolla et al. 2015) and variable levels of invasive potential (e.g., the green alga *Caulerpa*; Verlaque et al. 2003, Meusnier et al. 2004, Yeh and Chen 2004). In these cases, attempts to assess species invasiveness, impact and consequences are often arduous or inaccurate since cryptic invasive, introduced or cryptogenic lineages may co-occur with non-invasive native lineages of the same species complex in the same geographical region (Andreakis and Schaffelke 2012).

Pseudocryptic speciation is known in the cosmopolitan red seaweed genus *Asparagopsis* (Bonnemaisoniales, Rhodophyta). *Asparagopsis* comprises two morphospecies, *A. armata* and *A. taxiformis*. The genus is characterized by a triphasic, heteromorphic life history consisting of erect gametophytes (n) that alternate with filamentous free-floating tetrasporophytes (2n) known as the "Falkenbergia" stages, which in turn originate from microscopic carposporophytes (2n) attached to the gametophytes. Both *Asparagopsis* species are considered among the "worst invasive alien species threatening biodiversity in Europe" (EEA 2007) and among the "100 worst invasive seaweeds in the Mediterranean Sea" (Streftaris and Zenetos 2006). Genetic studies have confirmed two cryptic lineages (L1, L2) in the cold-temperate *A. armata* and six (L1 to L6) in the tropical *A. taxiformis* (Andreakis et al. 2004, 2016, Dijoux et al. 2014). Due to their cryptogenic status, the native range of each lineage remains undetermined (Dijoux et al. 2014). However, *A. taxiformis* lineages 2 and 3 are considered introduced and invasive in the Mediterranean Sea and South Africa respectively, and have been recognized as causes of endemic species decline (Andreakis et al. 2004, Altamirano et al. 2008, Bolton et al. 2011). On the other hand, *A. taxiformis* L4 is a presumed recent introduction to the Hawaiian Islands and is found sympatrically with L1, with unknown consequences for the local coastal community (Sherwood 2008). In this manuscript, for sake of clarity, we refer to *A. armata* L1 and L2 and *A. taxiformis* L1-L6 as operational taxonomic units (OTUs). In light of recent findings however, we propose that their taxonomic status requires revision (Andreakis et al. 2007, 2015, Dijoux et al. 2014, Zanolla et al. 2014, 2015).

The combination of molecular phylogeographic inference, ecophysiological studies and SDM represents a powerful tool for the detection of introduced species and the identification of their source populations (Booth et al. 2007, Zanolla and Andreakis 2016) but also for predicting species and population expansion and assessing the impact and invasive potential of invaders at multiple stages in the invasion process (Peterson 2003, Bolton et al. 2011, Marcelino and Verbruggen 2015). In particular, the combination of species distribution modelling (SDM) and molecular phylogeography represents a high resolution tool for distinguishing between cryptogenic endemisms and cryptic introductions. For instance, in cases of cryptic species complexes, the occurrence of individuals in locations not predicted by the models may indicate a novel cryptic OTU, which can represent either an endemic species or a cryptic introduction that requires further attention for management and conservation.

The power of integrating multiple lines of evidence in invasion biology relies primarily on accurate delineation of the transported or introduced taxonomic units. Solid taxonomic unit delineation is an absolute necessity in SDM because the species hypothetical distribution range is predicted on the basis of environmental affinities between locations where a species occurs and locations where the same species has never been reported (Austin 2002). Species distribution modelling is routinely used to identify suitable areas for invasive species on the basis of species-level accurate georeferenced data (species presence or absence). The erroneous inclusion of multiple genetically and geographically distinct cryptic lineages in the model as a single biological species is expected to lead to erroneous results for the model itself as well as in a posteriori actions such as decision making for management planning and conservation (Rissler et al. 2006, Rosauer et al. 2015).

We argue that multiple lines of evidence (i.e., haplotype analysis, historical demography and SDM) are necessary to assess lineage-specific invasive risk, predict their future distribution and establish sustainable management and conservation plans (Ferrier and Drielsma 2010). Our study focuses on two areas of high maritime traffic (the Hawaiian Islands and Mediterranean Sea) where combinations of *Asparagopsis* OTUs co-occur. Following cryptic OTU identification via mitochondrial DNA sequencing, we employed two different approaches to examine past and future expansion patterns for each of the lineages detected in this study. Firstly, historical demographic processes were analyzed through phylogeographic reconstructions, Bayesian Skyline Plots and neutrality tests, inferred from globally collected molecular data (Andreakis et al. 2007, Sherwood 2008). Secondly, future distribution patterns of invasive *A. taxiformis* and *A. armata* OTUs were evaluated by means of SDM applied under present and future climate projections. Our results are further discussed in light of field observations, eco-physiological profiles of *Asparagopsis* lineages

(Zanolla et al. 2015), their life history traits and dispersal vectors between locations where *Asparagopsis* OTUs are considered invasive.

Material and methods

Specimen collection and identification

A total of 115 *Asparagopsis* gametophytes and 56 tetrasporophytes were collected in the Mediterranean Sea and the Hawaiian Islands by snorkelling (0 to 2 m depth). Morphological identification was possible only for *A. armata* gametophytes, due to the presence of typical harpoon-like lateral branches (Harvey 1849). All gametophytes and tetrasporophytes were genetically analyzed for species and mitochondrial lineage identification. Vouchers were deposited in the MGC (see Table S1 in the Supporting Information for specimen details and sequence accession numbers).

Molecular analysis, phylogenetic and genealogical network reconstruction

DNA extraction and sequencing of the mitochondrial *cox*2–3 IGS were performed as described previously (Zuccarello et al. 1999). Newly produced sequences were compared with data mined from NCBI to create seven sequence alignments: one global *Asparagopsis* alignment, five alignments corresponding to each of the *Asparagopsis* OTUs sampled, and one global *A. taxiformis* alignment. For species and OTU delineation, exploratory MP phylogenies were computed in PAUP* for Windows version v4.0 (Swofford 2002). Model-corrected (Posada and Crandall 1998) ML phylogenies were also inferred in PAUP*. Bootstrap support (Felsenstein 1985) was calculated using 1,000 replicates with the same model parameters used in the tree inferences, but with identical sequences represented only once. Finally, genealogical networks were computed using the Median Joining algorithm in Network v4.5.1.6 (http://www.fluxus-technology.com; Bandelt et al. 1999; see Appendix S1 in the Supporting Information for more details).

Genetic differentiation and past demographic history

Genetic differentiation within and between *Asparagopsis* OTUs was calculated as F_{st} in the DnaSP software (Librado and Rozas 2009) using Tamura-Nei distances. Past demographic fluctuations for each *Asparagopsis* OTU were examined as follows: firstly, assuming neutrality of the marker used, deviations from migration-drift equilibrium were assessed in DnaSP using Fu's F_S (Fu 1997) and the R_2 statistics (Ramos-Onsins and Rozas 2002). Secondly, mismatch distributions (i.e., the frequency distribution of the pairwise differences between haplotypes) were evaluated to detect recent population expansion, indicated as unimodal distributions under a demographic expansion model and 1,000 bootstraps or as bimodal distributions as an outcome of expansion if admixture occurred between two divergent cryptogenic lineages (Rogers and Harpending, 1992). Bayesian skyline plots (BSPs) were computed in BEAST v1.7.5 for past population dynamics estimates (Drummond et al. 2005, Drummond and Rambaut 2007). A time scale to the population size estimates was assigned by fixing the per lineage evolutionary rates at 0.28% per MY (Andreakis et al. 2007). Credibility intervals for the BSPs were visualized in Tracer v1.4 (http://beast.bio.ed.ac.uk/Tracer; see Appendix S1 for details and rational of analyses).

Distribution modelling

An inductive approach was used to infer macroecological affinities for each OTU from their known distribution range. For *A. taxiformis*, we only used sequences assigned to a specific lineage (e.g., those listed in Table S1). Species distribution models were run under the hypothesis that environmental characteristics of known occurrences are useful to identify suitable new settlement habitats (Muñoz and Real 2006) and predict colonization routes for each OTU. Predictor variables were obtained from AquaMaps (Kaschner et al. 2010) at half-degree spatial resolution and were chosen on the basis of potential predictive power and availability for future climate change scenarios. We used mean primary productivity, mean annual sea surface temperature, sea bottom temperature, mean surface salinity and bottom salinity, which were assumed to be at least correlated with more proximal causal factors.

Distribution modelling was performed with R 2.15.2 (R Core Team 2005) using the R script supplied in Appendix S2 in the Supporting Information. For each OTU, preliminary modelling analyses examined the correlations between variables within occurrence areas, as well as their response curves and contributions to the models. To avoid problems related to collinearity between variables, whenever two variables were highly correlated within a model (> 0.8), the one with the lowest contribution was excluded and the model was re-run. Variables were mapped with the *raster* package (Hijmans et al. 2013) in R and overlaid on the occurrence records for which geographical coordinates were available (see Table S1). For *A. armata*, 1132 geo-referenced records from GBIF (http://www.gbif.org) were added to our occurrence database, to enable more robust models by increasing the amount of information on the environmental conditions under which the species can live. While GBIF data still suffer from survey bias like any biodiversity dataset (Barbosa et al. 2013), adding these data strengthened our dataset and diminished the regional bias in our sampled data. The modelled area included coastal worldwide marine waters (within the same distances to land as the observed presences). Distribution models were built with the *dismo* R package (Hijmans et al. 2012) using three modelling algorithms: Bioclim, Domain and Maxent. Bioclim and Domain use presence data; for Maxent, which also requires pseudo-absences, 10,000 points were randomly generated within the study area, following published recommendations (Barbet-Massin et al. 2012).

Models were projected to predict the potential worldwide distribution of each OTU under the present climate scenario. Potential ranges are linear features along coastlines, since these seaweeds typically inhabit a relatively narrow band along the shore within a particular depth or tidal range. Maxent models were also extrapolated to predict suitable areas under the environmental values forecasted for the year 2050 (Kaschner et al. 2010). Bioclim and Domain were not extrapolated, as they are known to perform poorly under climate change scenarios (Hijmans and Graham 2006).

Results

Identification of Asparagopsis *OTUs and phylogenetic inference*

All specimens were successfully sequenced (see Table S1 for specimen collection information and accession numbers). Maximum parsimony (MP) phylogenies showed variable levels of phylogenetic signal in each alignment (Table 1) and enabled the identification of seven *A. armata* and 50 *A. taxiformis* tetrasporophytes (see Table S2 in the Supporting Information). Five reciprocally monophyletic *Asparagopsis* OTUs were recovered (see Table S2 and Fig. S1 in the Supporting Information for specimen assignment to OTUs). Most western Mediterranean isolates belonged to *A. taxiformis* L2, 10 to *A. armata* L1 and five to *A. taxiformis* L3, which represents a first record from the western Mediterranean (Alboran Sea). In the Hawaiian Islands, most isolates belonged to *A. taxiformis* L2, followed by L1 and L4. An updated distribution map of Mediterranean and Hawaiian *Asparagopsis* is shown in Figure 1.

Genealogical networks

Genealogical network reconstruction revealed highly frequent *Asparagopsis* haplotypes and multiple singletons in each of the OTUs analyzed. Low frequency haplotypes found at the same sampling location were also recorded (Fig. 2, a-e). Eighteen haplotypes were recovered within *A. armata* L1 (Fig. 2a) with the most frequent one representing eight specimens collected from the Alboran Sea, South Africa and Australia. A less frequent haplotype was found representing four specimens collected from the Strait of Gibraltar. Eighteen haplotypes were recovered within *A. taxiformis* L1 with the most frequent haplotype including 10 specimens collected from the Hawaiian Islands, Panama and French Polynesia (Fig. 2b). Multiple haplotypes were found on Oahu shores (Hawaii, USA) differing in all cases by one substitution. Lineage 2 of *A. taxiformis* was composed of 45 distinct haplotypes (Fig. 2c). The most frequent haplotype included 99 specimens from the Hawaiian Islands, South Africa, Greece, the Chafarinas Islands (Spain) and the majority of the Andalusian specimens. Nine specimens were found in the next most frequent haplotype, all collected from the Hawaiian Islands. All specimens from Almeria (Spain) shared the same haplotype of L3 previously found in Tenerife and Lebanon (Fig. 2d). Specimens from South Africa and Brazil differed by two substitution steps from the most common L3 haplotype. Finally, *A. taxiformis* L4 contained a frequent haplotype shared by 11 specimens and four less frequent haplotypes (Fig. 2e).

Lineage genetic differentiation and past demographic history

Variable levels of genetic divergence among OTUs and low levels of intra-clade genetic variation were observed, reflecting distinct genealogical chronicles for each OTU (Fig. 3, a and b; Table S3 in the Supporting Information). The results are herein inferred from the sequence sampling size available for each *Asparagopsis* lineage. We acknowledge, however, that the elevated number of *A. taxiformis* L2 sequences may have affected the calculations for that lineage. Pairwise F_{st} comparisons confirmed high genetic differentiation among *Asparagopsis* OTUs ($p<0.005$; Fig. 3c). Fu's F_s neutrality tests showed significant departure from expectation, suggesting substantial population expansion for *A. taxiformis* L2 and L4 (Table 1). Sudden population expansion was additionally supported for L2 by the R_2 statistic, while population expansion for L4 was marginally rejected (Table 1). In *A. armata* L1 and *A. taxiformis* L2, mismatch distributions showed no significant difference from a population expansion scenario (Fig. S2 in the Supporting Information). BSPs showed stable past population sizes for *A. armata* L1 and *A. taxiformis* L3 and L4, yet a sudden increase in population sizes for *A. taxiformis* L1 and the full *Asparagopsis* dataset (Figs. 2b, S1). *Asparagopsis taxiformis* L2 in particular showed a decrease in effective population size prior to the last expansion step (Fig. 2c).

Predictive occurrence models - Asparagopsis armata *L1*

Mean surface salinity was highly correlated with mean bottom salinity where *A. armata* occurs. However, given its low contribution to the models, bottom salinity was excluded from the calculations. Under present climatic conditions, Maxent predicted high suitability values for *A. armata* L1 in the British Isles, the Atlantic coast of the Iberian Peninsula, the Alboran Sea and north-western Africa. South Africa, South Oceania, and Central-west South America were identified as areas of intermediate suitability (Fig. 4, a and b). Bioclim (Fig. S3a in the Supporting Information) delivered a more restricted prediction compared to Maxent while an intermediate prediction was given by Domain (Fig. S3b). The predicted suitable areas of this OTU on the Atlantic and the Mediterranean coasts varied among models, with Maxent suggesting the widest distribution on the European Atlantic (Fig. 4b; habitat suitability up to 60º N). Using Maxent, north-western Africa was found suitable for *A. armata* L1, suggesting a distribution limit of 16º N (Fig.4b). On the Mediterranean coasts, Domain projected a wider distribution, especially in its central part (Fig. S3b), while Maxent suggested the Alboran Sea to be highly suitable for *A. armata* compared to the central part of the basin (Fig. 4b). Maxent and Domain predictions were congruent for this taxon's distribution in South Africa and the south-eastern coast of Australia (Figs. 4a, S3, a and b). Furthermore, both models suggested the south-eastern coasts of South America as suitable areas for this OTU, in particular the coasts of Brazil, Uruguay and Argentina. Finally, only Domain predicted intermediate suitability for *A. armata* in the Japan Sea and the north-eastern coast of the United States of America (Fig. S3b). A future distribution of *A. armata* L1 projected for the year 2050 is shown in Figure S4 in the Supporting Information. Importantly, a regression of the species' distribution is expected on the Atlantic coasts of Europe, together with a shift of suitable areas in the Mediterranean Sea, from Alboran coasts to the central Mediterranean basin. Finally, changes at the margins of the lineages' distribution are predicted by the models in other areas (Fig. S4a).

Predictive occurrence models - Asparagopsis taxiformis *lineages*

None of the variables were highly correlated where *A. taxiformis* L1 occurs and the models therefore included all five predictors under current climatic conditions. Numerous Pacific islands, including the Hawaiian Archipelago, small islands in the Atlantic and the Indian Ocean, such as Mauritius and Réunion, and some regions of the Black Sea were predicted as potential L1 occurrence areas (Fig. 4, c and d). Domain delivered the widest distribution prediction for L1, covering the tropical waters of all oceans with the Pacific exhibiting the highest suitability (Fig. S3d). These models have not predicted L1 in latitudes greater than 45º N. The Mediterranean Sea showed marginal suitability for this lineage as indicated by all models. Comparisons between current Maxent predictions and future projections in the year 2050 suggested a significant expansion in suitable areas for L1, although within the same latitudes of current suitability (Fig. S4b).

In the case of *A. taxiformis* L2, sea bottom salinity and temperature were excluded due to their high correlation with mean annual sea surface temperature. The latter variable contributed the most to the models under current climate conditions. Multiple potential occurrence areas were identified, including multiple Mediterranean locations, the Adriatic Sea, Macaronesia, north-western and southern Africa, Australia and New Zealand, Hawaii, Melanesian and Polynesian Islands, Japan, and South and North America (Fig. 4, e and f). Maxent and Domain provided similar predictions (Fig. S3, e and f). Bioclim was slightly more conservative, predicting more restricted potential ranges (Fig. S3e). L2 was not predicted to be distributed in cold temperate regions. Under the future climatic scenarios, L2 is expected to lose environmental suitability in the western Mediterranean, the northwestern coast of Africa, Australia and Macaronesia (Fig. S4c). Some suitable areas, however, remain roughly similar to the present, and others such as the Caribbean, the eastern coast of South America and South-West Africa, become more suitable for L2 (Fig. S4c). Finally, significant models could not be obtained for *A. taxiformis* L3 and L4, given the low number and limited environmental variation of the available geo-referenced records.

Discussion

We report historical demographic expansion events for *A. taxiformis* L1 and L2 and *A. armata* L1 which are congruent with the present day lineages' continuous expansion profiles suggested by SDM. Projected distribution range scenarios for the OTUs meet lineage-specific ecophysiological and photosynthetic traits estimated for *A. taxiformis* L1 and L2 and *A. armata* L1 (Zanolla et al. 2015) confirming that any physiological adaptation underlying rapid range expansion can be crucial for a successful invader (Sakai et al. 2001). In this case, the combination of multiple lines of evidence was effective in accurately testing invasion risk useful for the development of surveillance plans against highly invasive *Asparagopsis* OTUs. Due to intense maritime traffic, the Mediterranean basin and the Hawaiian archipelago may represent important sources of invasive populations of *Asparagopsis* worldwide and this is one reason why we focused on *Asparagopsis* lineages in these areas. Further, these regions have experienced increased sea surface temperatures and decreased precipitation since the last glacial maximum (Giambelluca et al. 2008, Coma et al. 2009, Xie et al. 2010, Hoerling et al. 2012). Extensive niche shifting phenomena have been observed for many taxa, which have promoted dramatic biotic changes at the community level (Giambelluca et al. 2008, Xie et al. 2010, Jezkova et al. 2011, Hoerling et al. 2012). Among the environmental variables explored in our models, temperature was the only one associated with the geographical distribution of *A. taxiformis* OTUs. Fluctuations in local temperature can strongly affect growth, survival and reproduction in marine seaweeds, therefore altering the species' optimal distribution range (Breeman 1988, Smith et al. 2007). In species with heteromorphic life cycles, temperature changes may differentially disturb each life history stage, with unpredictable effects on local ecosystem functioning, depending on the morpho-functional group to which the affected life history stage corresponds. In seaweeds with heteromorphic life strategies, temperature plays a pivotal role in species dispersal since each stage may contribute differently to the invasive process. Temperature variation is therefore expected to be critical in invasive *Asparagopsis* OTUs as their tetrasporophytes exhibit distinct thermal ranges; they presumably contribute the most to the species' long distance dispersal potential (Zanolla et al. 2015). However, distribution models only consider the general effect of temperature upon a specific species, diluting the possible differences among distinct life stages.

Overall, the range expansions for *A. taxiformis* L1 and L2 in the Hawaiian Islands and L2 and L3 in the Mediterranean Sea are supported by low genetic diversity and the absence of private haplotypes within recently colonized areas. These patterns meet the expectations for sudden population expansion and are consistent with previous findings (Sherwood 2008).

The two *Asparagopsis* macroscopic life stages (gametophytes, tetrasporophytes) play different roles in the course of the invasion as they occupy distinct functional groups: tetrasporophytes are likely responsible for remote dispersal and gametophytes for population maintenance and short distance dispersal (Zanolla et al. submitted). This hypothesis is supported by higher survival rates in ballast waters associated with higher thermal photosynthetic performance of the tetrasporophytes compared to gametophytes (M. Zanolla pers. obs.; Mata et al. 2006, Zanolla et al. 2009).

Three *A. taxiformis* OTUs occur sympatrically in the Hawaiian archipelago: L1 abounds in the Main Hawaiian Islands; L2 dominates the Northwestern Hawaiian Islands; L4 is apparently present only along a localized region of the south shore of Oahu (Andreakis et al. 2007, Sherwood 2008). Compared to these previous studies, this study revealed extended distribution ranges for L1 and L2 on Oahu and multiple new haplotypes for all *A. taxiformis* OTUs.

Asparagopsis armata *L1*

Asparagopsis armata has been reported from the Mediterranean Sea since the first half of the 20th century (Bonin and Hawkes 1987, Drew 1950). Recently identified as a species complex consisting of two mitochondrial lineages (Dijoux et al. 2014), this taxon showed a documented negative impact on the local marine communities of south-western Spain including the Strait of Gibraltar (Flores-Moya and Conde Poyales 1992).

In the present study, invasion risk was limited for *A. armata* L1, on the basis of range shift dynamics predicted by SDM and distinct differences in photosynthetic plasticity among lineages (Zanolla et al. 2015).

Following the introduction of *A. taxiformis* L2, populations were initially reported in sympatry with *A. armata*, with the former occupying deeper sites (Altamirano et al. 2008). Subsequently, *A. taxiformis* L2 populations have partially or totally replaced those populations of the originally dominant *A. armata* at many sites, independent of depth (Altamirano et al. 2008; J. De la Rosa pers. obs.). This competitive relationship established between the two *Asparagopsis* OTUs likely denotes a physiological response to recent global warming (Schaffelke and Hewitt 2007, Rilov and Crooks 2009, Mainka and Howard 2010). In this sense, the greater photosynthetic plasticity of *A. taxiformis* L2 compared to *A. armata*, indicates adaptive benefits that likely support its invasive success (Zanolla et al. 2015).

Temperature optima for survival and reproduction may have also compromised the cold-temperate *A. armata* and favoured the tropical-temperate *A. taxiformis* L2. This hypothesis is further supported by Maxent predictive modelling which delivered an important decrease in suitability for *A. armata* in the Alboran Sea, which agrees with the observed species decline in this region.

Asparagopsis taxiformis *L1*

Three *A. taxiformis* OTUs occur sympatrically in the Hawaiian archipelago: L1 abounds in the Main Hawaiian Islands, L2 dominates the Northwestern Hawaiian Islands, and L4 is apparently present only along a localized region of the south shore of Oahu (Andreakis et al. 2007, Sherwood 2008). Compared to these previous studies, this study revealed extended distribution ranges for L1 and L2 on Oahu and multiple new haplotypes for all *A. taxiformis* OTUs. Fifteen L1 haplotypes were found around Oahu, corresponding to a 68% increase in haplotype diversity for L1.

In the year 2050 a significant expansion is expected for this lineage in the tropics, and it is predicted to be almost nonexistent in latitudes higher than 45º N.

It is worthwhile mentioning that *A. taxiformis* L1 gametophytes were often observed on live coral colonies in Lanikai beach in Ohau (Hawaii), with possibly negative consequences to the corals of the reef (M. Zanolla pers. obs.).

Asparagopsis taxiformis *L2*

Asparagopsis taxiformis L2 dominates the central Mediterranean, and the lineage was recently found in the eastern Mediterranean where its behavior is often invasive (Tsiamis et al. 2010). In the western Mediterranean, undetermined lineages of *A. taxiformis* have been previously reported from the archipelago of the Chafarinas Islands since 1999 (Altamirano 1999). The taxon has been since found in the south-eastern coast of Spain, the north coast of Morocco and the Strait of Gibraltar (Altamirano et al. 2008, Dijoux et al. 2014). Along the Andalusian coastline, *A. taxiformis* L2 gametophytes persist and abound along a depth range of 1-15 m throughout the year (Zanolla et al. 2017a). The presence of L2 in these habitats may compromise both diversity and species richness of native communities, including protected maërl assemblages (Zanolla et al. submitted). These populations are well established, presenting both sexual reproduction and vegetative propagation, with the subsequent production of dispersal units (i.e., tetrasporophytes; Zanolla et al. 2017a,b). The native status of L2 in Hawaii (Sherwood 2008) is supported by the result of this study given its high level of haplotype variation. Only 11 haplotypes of L2, including the most frequent central Mediterranean haplotype, were found at multiple Spanish locations, compared to the higher haplotype diversity reported previously from the central Mediterranean Sea (Andreakis et al. 2004, 2007). These findings, supported by our field observations, suggest that the western Mediterranean L2 populations under expansion are of central Mediterranean origin. Hawaiian L2 haplotypes have been recovered in few Spanish sampling sites and central Mediterranean haplotypes were found in Scottburgh (South Africa) (Bolton et al. 2011), suggesting remarkable levels of survival potential and confirming the feasibility of *A. taxiformis* L2 remote dispersal (South Africa-Mediterranean-Hawaii; e.g., via ballast water; Flagella et al. 2007). The haplotype composition found in Spanish populations suggests an *A. taxiformis* L2 range expansion westwards following introduction of immigrants from central Mediterranean sources. Successful persistence and survival of select genetic variants have also been reported for introduced populations of *Codium fragile* subsp. *fragile* (Provan et al. 2005, 2008). This hypothesis is further supported by the environmental continuum between central and western Mediterranean and the connection roots within the basin that may facilitate L2 dispersal towards environmentally suitable areas for colonization.

An invasion meltdown hypothesis may additionally explain the introduction of *A. taxiformis* L2 to the Andalusian coasts assuming that communities under stress from an invasive species (i.e., *A. armata*) are more susceptible to additional invaders (i.e., *A. taxiformis* L2; Simberloff and Von Holle 1999). Under this caveat, it is noticeable that in Almerian localities where the two *Asparagopsis* OTUs co-occur, the subtidal communities are additionally affected by the invasive *Caulerpa cylindracea* (Altamirano et al. 2014). Despite the lack of impact assessments for these invasive OTUs from Almeria, our overall impression during this work was one of biotic homogenization and species richness decline (M. Altamirano, J. De la Rosa and M. Zanolla pers. obs.).

Invasion risk is herein inferred to be high for *Asparagopsis taxiformis* L2 on the basis of range shift dynamics predicted by SDM and superior performance of vegetative propagation capability for *A. taxiformis* L2 associated with constant presence of fertile individuals of *A. taxiformis* L2 throughout the year, indicative of sexual propagation (Zanolla et al. 2015, 2017a, b).

Asparagopsis taxiformis L3

Falkenbergia isolates of L3 are reported for the first time in this study from the Alboran Sea (western Mediterranean, Almeria, Southern Spain). Populations of *A. taxiformis* L3 are known from Lebanon (Andreakis et al. 2007) and South Africa where the lineage is considered invasive (Bolton et al. 2011). Atlantic and Lebanese isolates and those from the Alboran Sea shared identical haplotypes while L3 haplotype diversity was the lowest. Since L3 was not found in intermediate locations among Lebanon, Alboran Sea and the Canary Islands, its source population cannot be assessed. However, L3 is here considered as a new introduction in this part of the Mediterranean Sea that remained hidden in previous studies conducted in the area mostly because of insufficient sampling effort (Andreakis et al. 2004, 2007, 2009, Dijoux et al. 2014). Due to its characteristic photosynthetic plasticity, L3 is likely to expand in the Mediterranean Sea (Zanolla et al. 2015). At present, given the lack of systematic biodiversity surveys in the Mediterranean Sea, we are uncertain of the lineage's distribution range in that region. In addition, the presence of only L3 tetrasporophytes does not exclude the presence of L3 gametophytes, which may have been missed because of unsuccessful tetrasporogenesis due to unfavorable environmental conditions at the sampling site (Breeman 1988, Ni Chualain et al. 2005). Whether L3 is capable of completing its full life cycle and establishing populations along the Andalusian coasts still remains uncertain. However, given its invasive character (Bolton et al. 2011) the lineages' local survival potential requires further investigation, which is critical to prediction modelling and establishing early-stage detection programs.

Asparagopsis taxiformis L4

Only three new L4 haplotypes were detected in the Hawaiian Islands, further supporting the lineage's recent introduction to the Islands (Sherwood 2008). The presence of identical haplotypes of L4 in the Hawaiian Islands and in Costa Rica suggests that both populations may be acting as donors of propagules for L4.

Modelling seaweed invasions

The use of mechanistic models to predict species distribution can often be a challenging process since it is very sensitive to the input data (e.g., see Araujo and Guisan 2006, Marcelino and Verbruggen 2015). SDM is a correlative approach that describes patterns rather than explaining the mechanisms of species distribution. Since our input data are strictly environmental and related to present presence/absence of the lineages, we acknowledge that the output of the models may be affected. In addition, despite the *A. taxiformis* records included in this study were all genotyped at the lineage level, several records of *A. armata* were reported before the cryptic speciation discovered recently (Dijoux et al. 2014) and they could not be associated with any of the *A. armata* cryptic lineages.

The distribution models obtained suggest that *A. armata* L1 and *A. taxiformis* L1 and L2 have considerable potential for surviving upon introduction worldwide. This is further supported by the broad-scale tolerance inferred from the values of environmental variables associated with their known distribution range. These values likely reflect a wide environmental tolerance of *Asparagopsis*, which in the case of the invasive OTUs, is expected to increase with time. Also, genetic variation within species is known to enhance species survival under unfavorable conditions or escalate species fitness following introduction (Knop and Reusser 2012).

Assuming that the future environmental projections (Kaschner et al. 2010) are generally fulfilled, the invasive OTUs, in particular *A. taxiformis* L1, may represent future high profile invaders along the suitable coasts including their currently occupied habitats.

It is worth mentioning that some model predictions suggest changes in OTU invasiveness in certain regions over time. For example, Maxent models suggested a shift in invasive potential between *A. taxiformis* L2 and *A. armata* L1 in the central Mediterranean by 2050, probably due to the predicted regional decrease in thermal suitability for the former OTU (Zanolla et al. 2015).

Conclusions

Demographic statistics and behavioral changes such as sudden range expansion suggest *A. taxiformis* L2 to be the most invasive OTU within the genus. Our work illustrates that long-term programs for monitoring marine aliens are necessary in areas particularly vulnerable to biological invasions (e.g., Mediterranean Sea and the Hawaiian Islands), and in the case of *Asparagopsis*, in the high invasion-risk areas predicted by the models (Figs. 4, S3, S4). Early-stage detection protocols in place can be facilitated through the use of recently proposed morphological characters useful for inexpensive and rapid identification of invasive *Asparagopsis* OTUs (Zanolla et al. 2014).

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Table 1. Sequence and alignment statistics. *h*, number of distinct haplotypes; *p*, parsimony informative sites given the alignment; -g1, estimate of the phylogenetic signal given the alignment; Fu's F_s and R_2, population expansion and departure from neutrality statistics; in bold, statistically significant values (p<0.002). Model of sequence evolution given the alignment; α, gamma shape parameter; I: proportion of invariable sites.

OTU	length	# sequences	h	p	*-g1*	Fu's Fs	R_2	Model	α	I
full database	365	263	101	91	-0.85	-0.7^{ns}	0.102(0.825)	HKY	0.568	n/a

A. taxiformis	338	334	71	38	-0.71	-0.8^{ns}	0.063(0.331)	HKY	0.258	n/a
***A. armata* L1**	364	29	18	3	-2.70	-4.8^{ns}	0.104(0.197)	F81	0.635	0.9242
***A. taxiformis* L1**	268	38	9	6	-1.48	-2.3^{ns}	0.102(0.427)	F81	n/a	0.9658
***A. taxiformis* L2**	338	166	46	12	-2.33	**-10.1**	**0.031(0.055)**	F81	n/a	n/a
***A. taxiformis* L3**	338	13	7	3	-0.69	-0.8^{ns}	0.224 (0.815)	HKY	n/a	0.9941
***A. taxiformis* L4**	324	16	3	n/a	n/a	**-0.4**	0.165 (0.654)	F81	n/a	n/a

Figure captions

Figure 1. Updated distribution occurrence map of the Mediterranean Sea (a, b) and the Hawaiian Islands (c) for each of the *Asparagopsis* OTUs based on genetically delineated specimens (Andreakis et al. 2004, 2007, Sherwood, 2008, Dijoux et al. 2014). *A. armata* L1 (▲); *A. taxiformis* L1 (X); *A. taxiformis* L2 (●); *A. taxiformis* L3 (■); *A. taxiformis* L4 (♦).

Figure 2. Genealogical network reconstruction based on the *cox*2–3 spacer through Bayesian Skyline Plots (BSPs) of *Asparagopsis* OTUs and updated haplotype distribution based on genetically delineated specimens. a) *A. armata* L1; b) *A. taxiformis* L1; c) *A. taxiformis* L2; d) *A. taxiformis* L3; e) *A. taxiformis* L4. Circles denote distinct haplotypes; circle sizes are proportional to haplotype frequencies. Haplotype numbers and specimen details are given in Table S1. Bars across lines connecting haplotypes denote substitution steps. Time on BSPs is given in millions of years (MY).

Figure 3. In a) TN-corrected genetic distances within lineages; h= number of haplotypes encountered within each lineage; b) TN-corrected inter-species and lineage distance comparisons, and c) F_{st} values denoting lineage-specific genetic differentiation; d) Haplotype diversity expressed as number of haplotypes recovered over the total number of samples analysed in former studies (dark gray) compared to this paper (light gray). AA: *A. armata* L1; AT: *A. taxiformis*; L: Lineage.

Figure 4. Potential distribution of *A. armata* L1 (a, b), and *A. taxiformis* L1 (c, d) and L2 (e, f) as predicted by Maxent models. Panels b, d and f represent detailed potential distribution patterns of the aforementioned *Asparagopsis* lineages in the Mediterranean Sea. Predictions given by additional models are provided in the Supporting Information. Color scale next to each map in the figure indicates environmental suitability for occurrence.

Supplementary files

Appendix S1. Details and rational of analysis methods chosen.

Appendix S2. R functions used for modelling.

Supplementary Figure 1. Midpoint-rooted maximum likelihood phylogenetic reconstruction in a) and Bayesian Skyline Plot (BSP) in b) of the genus *Asparagopsis* based on *cox*2–3 spacer sequences. AA, *Asparagopsis armata*; AT, *Asparagopsis taxiformis*; F, Falkenbergia stage; GR, Greece; ITA, Italy; HI, Hawaiian Islands. Specimen details are given in Table S1. Time in the BSP is given in millions of years before present.

Supplementary Figure 2. Mismatch distributions calculated for lineages of the genus *Asparagopsis*. The thin line represents the expected mismatch distribution of a stationary population. The dotted line represents the observed mismatch from segregating sites of the aligned sequences of the *cox*2-3 mitochondrial marker. AT, *A. taxiformis*; L, lineage.

Supplementary Figure 3. Potential distributions of *Asparagopsis armata* L1, *A. taxiformis* L1 and L2 as predicted by Bioclim (a, c, e) and Domain (b, d, f) models respectively under current environmental conditions. Color scale indicates environmental suitability for occurrence.

Supplementary Figure 4. Hypothesised potential distribution of *Asparagopsis armata* L1 (a), *A. taxiformis* lineages L1 (b) and L2 (c) in the year 2050, as predicted by Maxent models applied to projected AquaMaps environmental data. Color scale indicates environmental suitability for occurrence.

Supplementary Table 1. List of specimens used in this study.

Supplementary Table 2. Number of specimens and haplotypes collected from each geographical region.

Supplementary Table 3. Tamura-Nei corrected genetic distances amongst *Asparagopsis* species and lineages**.**

Table 1. Sequence and alignment statistics. h, number of distinct haplotypes; p, parsimony informative sites given the alignment; -g1, estimate of the phylogenetic signal given the alignment; Fu's F_s and R_2, population expansion and departure from neutrality statistics; in bold, statistically significant values ($p<0.002$). Model of sequence evolution given the alignment; α, gamma shape parameter; I: proportion of invariable sites.

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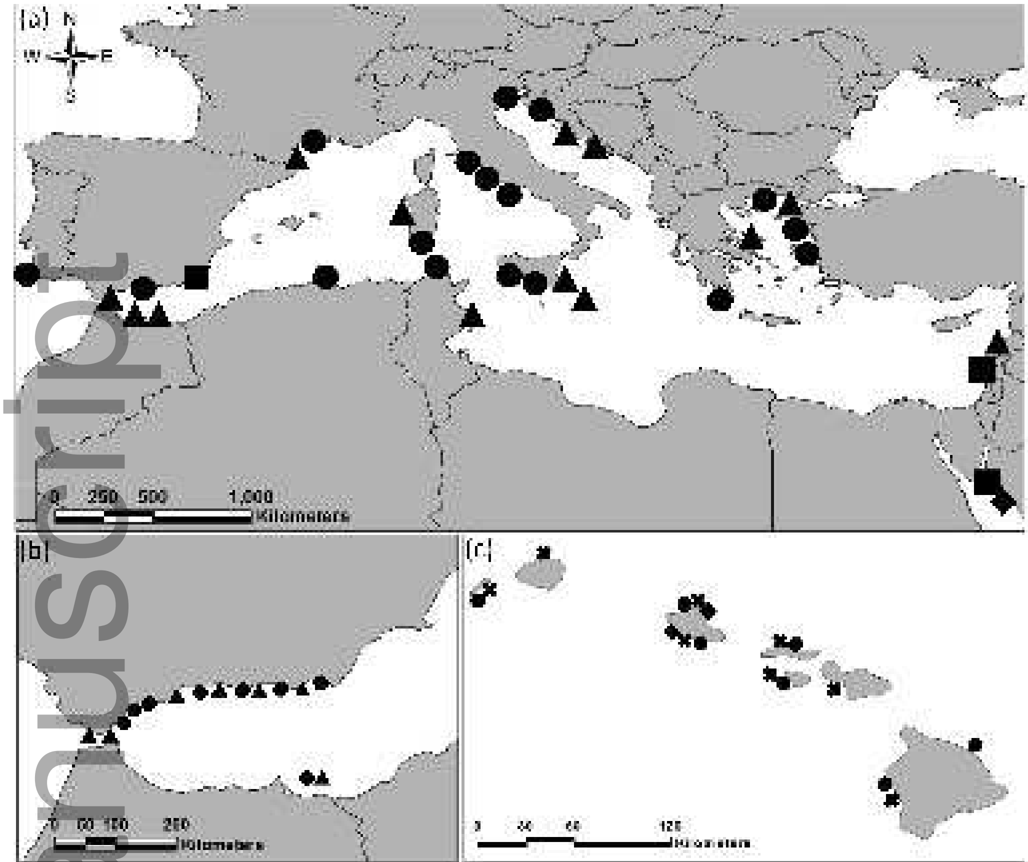
A. taxiformis L3	338	13	7	3	-0.69	-0.8^{ns}	0.224 (0.815)	HKY	n/a	0.9941
A. taxiformis L4	324	16	3	n/a	n/a	**-0.4**	0.165 (0.654)	F81	n/a	n/a



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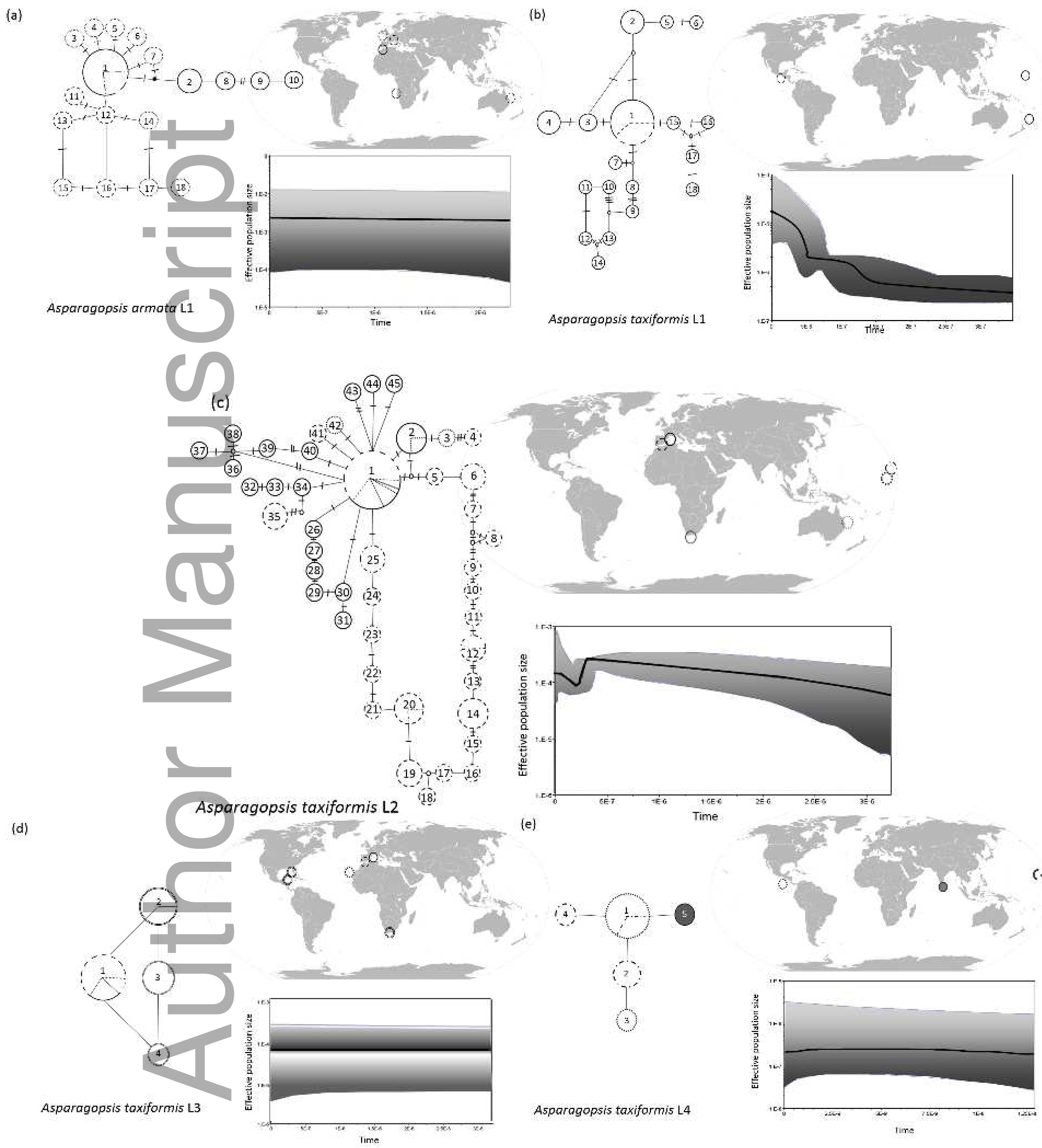

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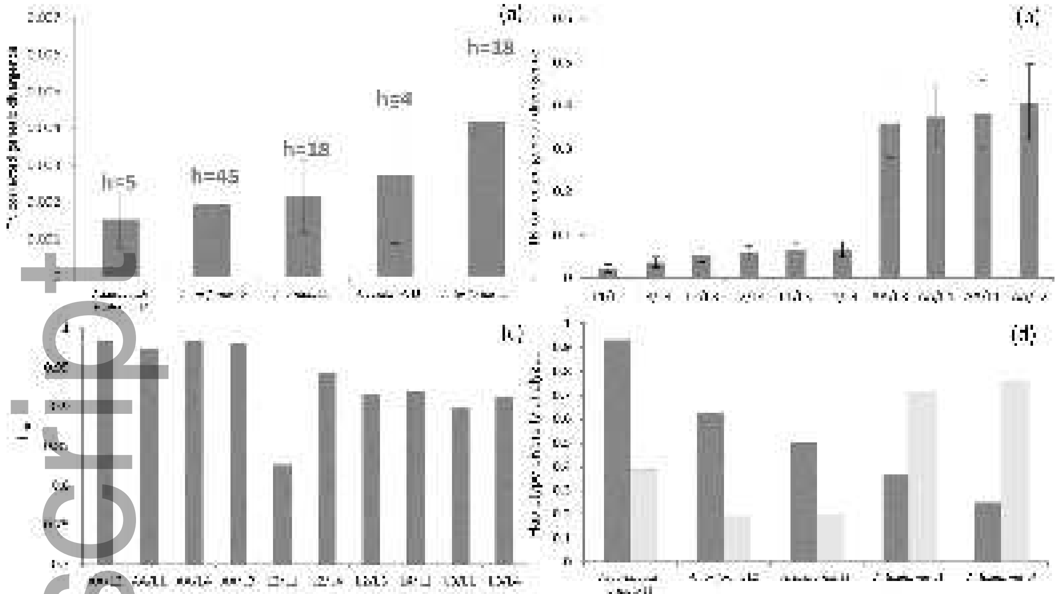

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