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Phylogeography of the 'cosmopolitan' orb-weaver *Argiope trifasciata* (Araneae: Araneidae)

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Few spider species show truly cosmopolitan distributions. Among them is the banded garden spider *Argiope trifasciata*, which is reported from six continents across major climatic gradients and geographical boundaries. In orb-weaver spiders, such global distributions might be a result of lively dispersal via ballooning. However, wide distributions might also be artefactual, owing to our limited understanding of species taxonomy. To test the hypothesis that *A. trifasciata* might be a complex of cryptic species with more limited geographical ranges, we investigated the biogeographical structure and evolutionary history of *A. trifasciata* through a combination of time-calibrated phylogenetic analyses (57 terminals and three genes), ancestral range reconstruction and species delimitation methods. Our results strongly suggest that *A. trifasciata* as currently defined is not a single species. Its populations fall into five reciprocally monophyletic clades that are genetically distinct and have evolutionary origins in the Plio-Pleistocene. These clades are confined to East Asia, temperate Australia, Hawaii, the New World and the Old World (Africa and most of the Palaearctic). Our results provide the basis for future investigation of morphological and/or ecological disparity between the populations that are likely to represent species, in addition to examinations of the attributes and dispersal modes of these species.

ADDITIONAL KEYWORDS: biogeography – DNA barcoding – speciation – species delimitation – spiders.

INTRODUCTION

Spiders can be found in almost every terrestrial habitat. Although most of the species diversity (48 643 species; World Spider Catalog, 2020) shows levels of endemicity, some spider species are cosmopolitan, e.g. the woodlouse spider *Dysdera crocata* C. L. Koch, 1838 and the longbodied cellar spider *Pholcus phalangioides* (Fuesslin, 1775), which raises questions about specific ecological attributes or dispersal capacities in these species. At least some of them have been shown to be cosmopolitan owing to anthropogenic dispersal (Schäfer *et al.*, 2001; Duncan *et al.*, 2010). However, other spiders, particularly orb-weavers, achieve long-distance dispersal via aerial travel on silken threads termed ballooning. Other dispersal modes include rafting on tree logs or debris across wide ocean distances (Wheeler, 1916; King, 1962; Heatwole & Levins, 1972; Schiesari *et al.*, 2003) or expanding their ranges over temporary land bridges (Mora *et al.*, 2017). Many spiders are known to be good dispersers, e.g. they are amongst the first invertebrate groups to populate recently formed volcanic islands (Bristowe, 1931; Reimoser & Bristowe, 1934; New & Thornton, 1992).

The banded garden spider, *Argiope trifasciata* (Forsskål, 1775), is an appropriate model species in which to explore the biogeographical imprint of a cosmopolitan distribution and its likely history. Being found in > 50 countries across six continents, this spider might be among the most widespread

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invertebrate species (Fig. 1A). It occurs across an extreme climatic gradient, from the tropics of Central Africa across the Mediterranean area and the Americas to temperate Canada. The morphological identification of *A. trifasciata* as currently defined is straightforward (Levi, 1983). The World Spider Catalog currently lists three subspecies: *Argiope trifasciata trifasciata* (Fig. 1B) described from Egypt, *Argiope trifasciata deserticola* Simon, 1906 described from Sudan and *Argiope trifasciata kauaiensis* Simon, 1900 described from Hawaii (World Spider Catalog, 2020). Its tentative taxonomy and morphological variation are reflected in > 30 junior synonyms available for this species. Recent distributional additions are populations on the Mediterranean islands Malta, Sicily and Sardinia (Di Pompeo, 2011), which may demonstrate recent dispersal events across water.

Given its purported cosmopolitan distribution, surprisingly few biological data exist for *A. trifasciata*, including its modes of dispersal. Studying its aerial dispersal behaviour, Tolbert (1977) concluded that *A. trifasciata* exhibits pre-ballooning behaviour and disperses in large quantities (92% of juveniles) given the right circumstances. Dimassi *et al.* (2017) found high levels of gene flow and little genetic differentiation among Tunisian populations. Ramirez *et al.* (1999) studied the gene flow in *A. trifasciata* among habitat patches on a fragmented landscape and found that dispersal was sufficient to preserve genetic cohesion across a 24 km range (Ramirez & Haakonsen, 1999). Despite these efforts, no comprehensive study has investigated the biogeography and genetic structure of this species across its entire range.

In this study, by investigating the global biogeographical and genetic structure of *A. trifasciata*, in addition to its diversification patterns, we test the single species hypothesis. We perform time-calibrated phylogenetic analyses and species delimitation tests on a dataset of three target genes sequenced for 61 specimens from 14 countries and five continents, representing almost the entire known distribution of the species, including the subspecies from Hawaii. We investigate the evolutionary origins of this species and its genetic variability as a potential cause for morphological variation and genetic differentiation across the known distribution range, through a combination of molecular dating and ancestral range reconstruction analyses. We look for links between the genetic structure of the populations and the geographical variables, aiming to form hypotheses concerning the potential mechanisms that enable adaptation to diverse climates and habitats.

MATERIAL AND METHODS

SAMPLING

The 79 *A. trifasciata* samples were supplied by various researchers and museums (for voucher details, see Supporting Information, Appendix S1) and collected

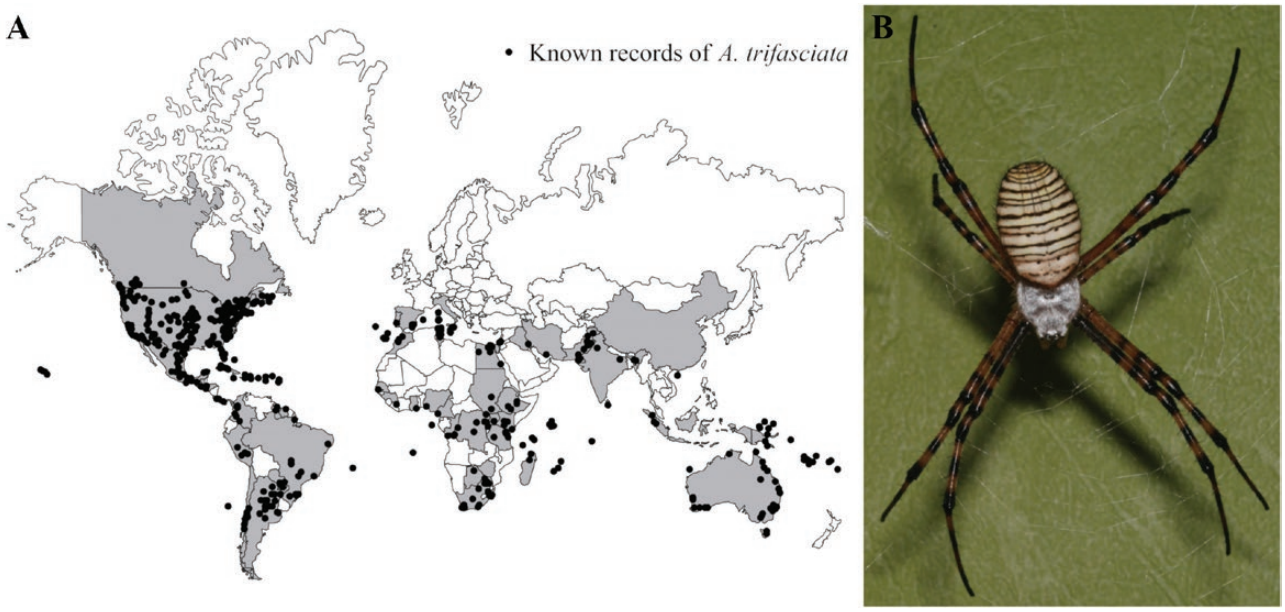

Figure 1. A, distribution map of *Argiope trifasciata*. Localities were obtained from publications listed in the World Spider Catalog (2020), and countries with known occurrences of the species are shaded grey. B, dorsal view of a female *A. trifasciata* (by courtesy of Dr Gabrielle Uhl).

from five continents between 1969 and 2018. All specimens were identified according to morphological key characters by experienced arachnologists before DNA extraction.

Molecular methods

Genomic DNA was extracted using E.Z.N.A. Tissue DNA Kits (Omega Bio-Tek, Norcross, GA, USA) and the standard protocol except for skipping steps 5 and 6, and only 40 µL of Elution Buffer was used to increase DNA yield. Tissue samples included 2–4 mm of whole leg tissue, but more tissue was used if the samples were old or in poor condition. In total, DNA extraction was performed on 79 individuals and protocols adjusted for individual specimens if necessary (for all extraction and polymerase chain reaction protocols, see Supporting Information, Appendix S2). Polymerase chain reactions was performed using standard protocols for three markers: a fragment of the cytochrome *c* oxidase subunit I (*COI*) gene, a fragment of the cytochrome *c* oxidase subunit II (*COII*) gene and the nuclear protein-coding histone 3A (*H3A*) gene. Bi-directional sequencing was done by Macrogen (Amsterdam, The Netherlands).

Sequence editing

The sequences received were checked for contamination by blasting them against comparative sequences on GenBank (Basic Local Alignment Search Tool), annotating the chromatograms by eye and editing ambiguous bases according to the International Union of Pure and Applied Chemistry (IUPAC) codes, and by translating them to check for stop codons. Overall, three of our samples were identified as belonging to different species with BLAST (Altschul *et al.*, 1990), two to *Argiope sector* (Forsskål, 1776) and one sequence to *Argiope blanda* O. Pickard-Cambridge, 1898. Contaminated or otherwise poor sequences were discarded.

Sequence editing and alignment was done using the software package Geneious v.10.2.6 (Biomatters Limited, Auckland, New Zealand) (Kearse *et al.*, 2012) and the integrated MUSCLE algorithm (Edgar, 2004). Four different alignments were created for further analyses: (1) a core alignment of *COI* sequences for molecular dating analyses; (2) an expanded alignment that included additional sequences from GenBank and other online repositories; (3) the complete concatenated dataset that contained the sequences for all three markers; and (4) an alignment of the histone data as the only nuclear gene to contrast against the mitochondrial data. For the expanded *COI* alignment, 30 additional *COI* sequences of *A. trifasciata* from different parts of the world (Supporting Information, Appendix S1) were obtained from NCBI (https://www.ncbi.nlm.nih.gov) and BOLD (Ratnasingham & Hebert, 2007), making up a total of 88 sequences for this species. The choice of outgroup species was according to a molecular phylogenetic analysis for the entire genus *Argiope* (Cheng & Kuntner, 2014) and included eight *Argiope* species and five sequences of specimens of the family Araneidae (Supporting Information, Appendix S1).

Phylogenetic inference

Phylogenetic analyses were performed using both Bayesian inference (BI) and maximum likelihood (ML) approaches. The optimal substitution models for partitions by gene and codon, after partitioning with DAMBE v.7.0.28 (Xia, 2018), were inferred using jModelTest v.2.1.10 (Darriba *et al.*, 2012) based on the corrected Akaike information criterion (AICc) (Hurvich & Tsai, 1993) (see Supporting Information, Appendix S2). Bayesian analysis was performed using MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003;). Four Markov chain Monte Carlo (MCMC) simulations were run for 40 million generations starting from random trees, sampling every 1000 generations, using *Cyclosa conica* (Pallas, 1772) as the ultimate outgroup and discarding the initial 25% of the trees as burn-in. Maximum likelihood analyses were performed using RAxML-HPC2 (Stamatakis, 2014) on XSEDE via the online CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010), using a random starting tree and both GTRgamma and GTRcat models in separate trial runs, a rapid bootstrapping procedure with 1000 iterations and defining *C. conica* as the outgroup. To test for cryptic species, a combination of species delimitation methods and genetic distances was used. Species delimitation for the expanded *COI* dataset was performed using the rather conservative Automatic Barcode Gap Discovery (AGBD; Puillandre *et al.*, 2012) on the web server, using Jukes–Cantor distances and leaving the rest of the options as default, with the relative gap width set to $X = 1.5$. As a second method, a Bayesian implementation of Poisson tree process model (bPTP; (Zhang *et al.*, 2013) was performed on the web server with MCMC generations increased to 500 000, with thinning on 500 because of the high number of taxa. From our experience, this method is highly suitable for taxa with pronounced genetic structuring, as might be expected here (Harms *et al.*, 2018). Genetic distances among and within clades were calculated in the software package Geneious.

Divergence time estimation

To estimate nodal divergence dates, BEAST v.2.5.0 (Bouckaert *et al.*, 2014) was used to analyse the

core and expanded *COI* datasets with two general relaxed molecular clock models (Drummond *et al.*, 2006) for arthropods. Dating was not attempted for the concatenated dataset because there is a lack of suitable fossils, and molecular clocks for the *COII* and *H3A* genes are unavailable. The BEAST input was created by BEAUti v.2.5.0 (Bouckaert *et al.*, 2014), linking site model, clock models and trees and applying HKY (Hasegawa *et al.*, 1985) as the substitution model because GTR overparameterized this analysis and resulted in very low effective sample size (ESS) values. The clock models applied to the *COI* datasets were the general 'Brower' rate of 2.3% pairwise sequence divergence per million years (BEAST input = 0.0115) (Brower, 1994) and the more specific 'Papadopoulou' rate of 3.54% sequence divergence per million years (BEAST input = 0.0177) (Papadopoulou *et al.*, 2010). We constrained the topology according to the results of the BI analyses (Australian samples as the outgroup to all others; see Results) and fixed *C. conica* as the outgroup, but otherwise all analyses ran freely.

A Yule process (Yule, 1925) tree prior was chosen after testing different priors (e.g. a birth–death prior) in trial runs and inferring similar results. The remaining priors were left as default. The analyses were run for 100 million generations, and sampling was every 10 000th generation. TRACER v.1.7.1 (Rambaut *et al.*, 2018) was used to infer ESS values and check for chain convergence. To calculate the final trees, TREEANNOTATOR v.2.5.0 (Bouckaert *et al.*, 2014) was used and 25% of trees were discarded as burn-in. MRBAYES, RAXML and BEAST trees were visualized and edited with FIGTREE v.1.4.3 (Rambaut, 2016).

BIOGEOGRAPHICAL ANALYSES

Reconstruction of ancestral distribution ranges was performed using a Bayesian binary MCMC (BBM) analysis implemented in RASP v.3.2 (Yu *et al.*, 2015). Four different partition schemes were tested to estimate the effects of increasingly complex area partitioning (six to 12 areas) on inference of ancestral distributions. Each sample was assigned to its respective area according to different partitioning schemes (Supporting Information, Appendix S2), from a rough partition into continents to a finer division into more specific geographical regions, to explore the effect of coding strategies on pattern inference. Discrete landmasses, such as Australia and Africa, were initially used for scoring and were subdivided later. All analyses were run using the BEAST trees (10 000 starting trees) derived from the analysis of the expanded *COI* alignment with all outgroups removed. All BBM analyses were run with default settings except that the number of cycles was increased to 100 000.

To test for possible colonization events, a dispersal–vicariance analysis (S-DIVA; Yu *et al.*, 2010) was conducted using the same partitioning schemes and trees as above, and with default settings.

Maps were produced using QGIS v.3.0 (QGIS Development Team, 2018). Figures were edited either with GIMP v.2.10.8 or with Adobe Photoshop CC 2018.

RESULTS

SEQUENCE STATISTICS

Four different datasets were used for phylogenetic analyses (Table 1). The genetic distance between *A. trifasciata* and other *Argiope* species in our phylogeny was ~13% (*Argiope ahngeri* Spassky, 1932, 13%; *Argiope lobata* (Pallas, 1772), 13%), and intraspecies divergences in *A. trifasciata* reached ≤ 8.9% (Supporting Information, Appendix S3).

PHYLOGENETIC INFERENCE

Bayesian inference (Fig. 2) and ML (Supporting Information, Appendix S4) analyses using the complete dataset divided *A. trifasciata* into five distinct genetic lineages with high statistical support (posterior probabilities > 97%, bootstrap values > 85%) except for clade A (posterior probability [PP] = 64%; bootstrap values [BV] = 60%). These clades often corresponded to specific biogeographical regions: clade A, East Asia (two samples, turquoise); clade B, temperate Australia

Table 1. Alignment for phylogenetic analyses, with number of specimens, length and base composition, partitions and proportion of variable sites inferred with MEGAX (Kumar *et al.*, 2018)

Alignment	Number of specimens	Length (bp)	AT content (%)	Partitions	Variable sites (%)
COI, core	70	626	69.2	3	19
COI, expanded	101	626	69.3	3	26.2
Complete dataset, all three genes	70	1502	67	9	14.8
Histone 3A	70	299	49.2	3	4.7

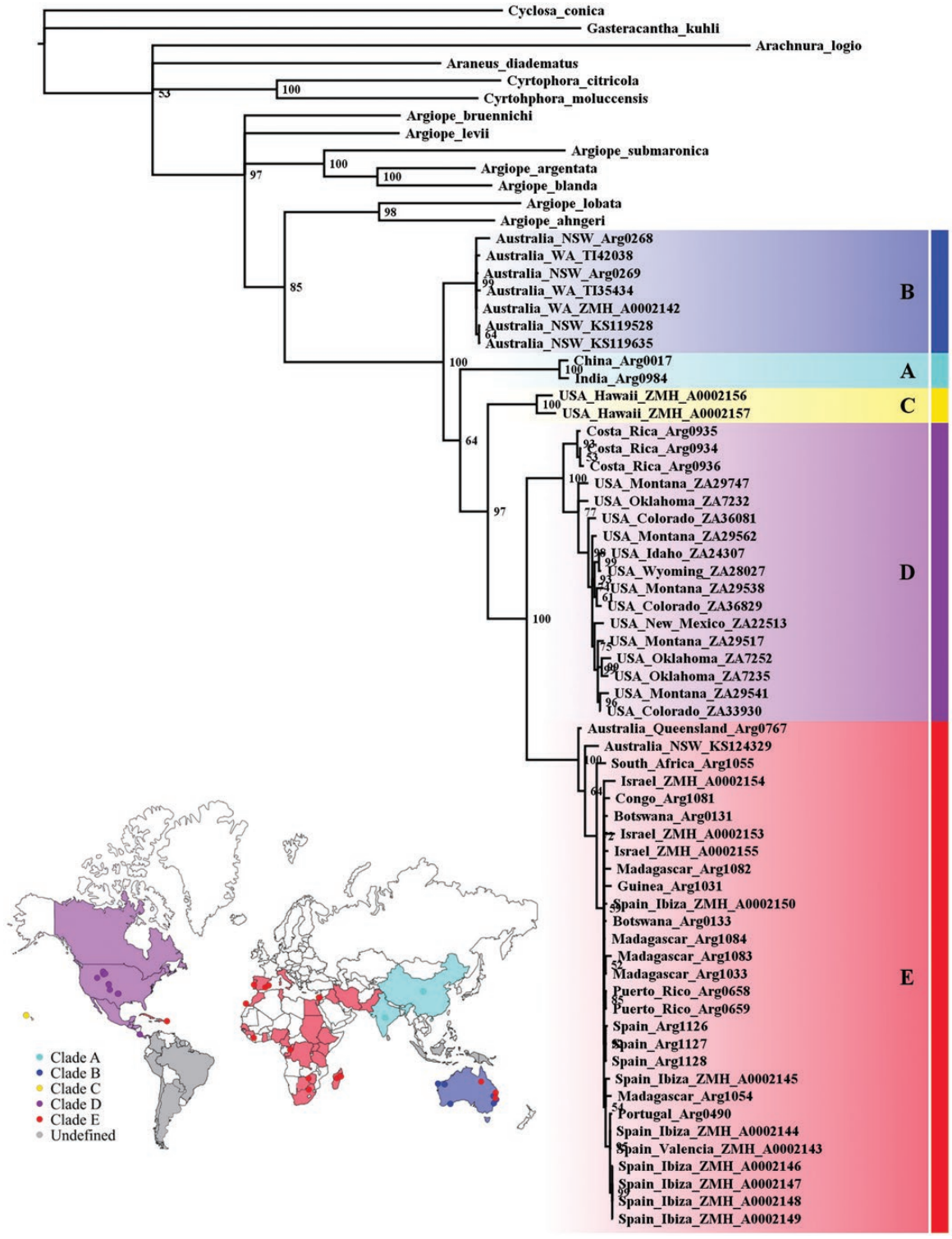

Figure 2. Phylogenetic relationships of *Argiope trifasciata* inferred from a gene- and codon-partitioned MRBAYES analysis of the concatenated matrix with three genes (70 terminals, three genes, nine partitions, 1502 bp). Statistical support values are given for every node as posterior probabilities (as percentages). Countries with known occurrences of *A. trifasciata* were colour coded according to genetic clades inferred in the analyses. Countries shaded in grey have established records of *A. trifasciata* but could not be assigned to an existing genetic clade. Localities of *A. trifasciata* specimens included in the complete dataset are marked with dots.

(seven samples, blue); clade C, Hawaii (two samples, yellow); clade D, New World (17 samples, purple); and clade E, Old World (Africa and the Palaearctic excluding East Asia (29 samples, red).

All analyses of the *COI* datasets (Supporting Information, Appendix S4) recovered the five major clades but differed slightly from those of the concatenated matrix in that the Asian clade split off first (PP = 100%; BV = 72%), followed by the Australian clade (PP = 97%; BV = 28%). Divergences between clades ranged from 3.7% between clade D (34 samples in the expanded *COI* dataset) and clade E (43 samples in expanded the *COI* dataset) to 8.9% between clades A and D in the *COI* gene. Divergences within the clades were usually lower, with ranges from 0 to 5% (Supporting Information, Appendix S3). The BI analysis of the less variable *H3A* data was able to recover three of the five clades (clades A, B and amalgamation) but failed to recover divergence between clades D and E (Supporting Information, Appendix S4).

The Australian fauna comprised two clades, and most terminals from Western Australia and temperate eastern Australia formed one clade, whereas all specimens from Queensland belonged to clade E (Old World). The distinct status of the Hawaiian populations was also highly supported, and both terminals were placed as a long and distinct branch (PP = 97%; BV = 85%). All terminals from the mainland Americas formed a New World clade (PP = 100%; BV = 94%), with all Costa Rican samples (PP = 100%; BV = 99%) and two Floridian specimens in the expanded *COI* dataset as their sister groups. Clade E comprised specimens from Europe, Africa and the Greater Middle East (Israel and Pakistan). Surprisingly, all terminals from the Antilles (Cuba, Dominican Republic and Puerto Rico) nested within clade E (Old World) and not with clade D from the Americas.

The two species delimitation methods discovered between six and nine molecular operational taxonomic units (mOTUs), five of them corresponding to the clades (Fig. 3; Supporting Information, Appendix S3). The conservative Automatic Barcode Gap Discovery (ABGD) split clade D into two mOTUs, and the bPTP split clades C and D further.

Molecular dating analyses

Divergence date estimates using the complete *COI* dataset (101 terminals, three partitions, 626 bp) and two molecular clocks suggested that the split of *A. trifasciata* from its closest relatives, *A. lobata* and *A. ahngeri*, happened in the Late Miocene–Pliocene [Brower rate, mean = 5.13 Mya; 95% highest posterior density (HPD), 6.33–3.90; and Papadopoulou rate, mean = 3.33 Mya; 95% HPD, 4.12–2.43] (Table 2). Diversification of *A. trifasciata* into distinct genetic clades started between the Late Pliocene and Early Pleistocene (Brower rate, mean = 3.59 Mya; 95% HPD, 4.42–2.81; and Papadopoulou rate, mean = 2.33 Mya; 95% HPD, 2.87–1.81) when clade B (Australia) split off. Clade A (East Asia) diverged at the Plio-Pleistocene boundary (Brower rate, mean = 3.27 Mya; 95% HPD, 4.00–2.51; and Papadopoulou rate, mean = 2.12 Mya; 95% HPD, 2.61–1.63), and clade C (Hawaii) emerged slightly after, between 3 and 2 Mya (Brower rate, mean = 2.95; and Papadopoulou rate, mean = 1.91 Mya).The split between the Holarctic clades D (New World) and E (Old World) was comparatively recent and occurred during the Pleistocene (Brower rate, mean = 2.42 Mya; and Papadopoulou rate, mean = 1.57 Mya). We note that divergences within clade D were deep, and the Floridian and Costa Rican populations split off as recently as 2 Mya (Brower rate, mean = 2.01 and 1.45 Mya; and Papadopoulou rate, mean = 1.30 and 0.94 Mya, respectively), and clade E started to diversify only a little later (Brower rate, mean = 1.40 Mya; and Papadopoulou rate, mean = 0.91 Mya).

Biogeographical analyses

The BBM analyses suggested that the origins of diversity in *A. trifasciata* lie in the Southern Hemisphere, and probably in the southern Pacific region, as evidenced by the presence of multiple genetic clades in this region that are also old by comparison. Indeed, the BBM analyses placed the ancestral range of a common progenitor either in Asia or in Australia, with probabilities of 74.3 and 15.6%, respectively (Fig. 4; partition 2, node 175). Reconstruction analyses showed that the species spread into the Northern Hemisphere most probably via dispersal in the Pacific region, as evidenced by the high significance of the Asian/Australian landmasses for nodes 173, 172 and 170 in our phylogeny.

The S-DIVA analysis suggested a complex biogeographical history, with both multiple dispersal and vicariance events shaping the phylogeographical structure. There was strong evidence for several dispersal events between the Middle East, Africa and Europe in all directions and several levels of depth in the phylogeny, in addition to dispersal between African populations and those in north-eastern Australia. The Antilles populations resulted from long-distance dispersal from Africa. However, these results should be treated with caution because our analysis did not include samples from South America.

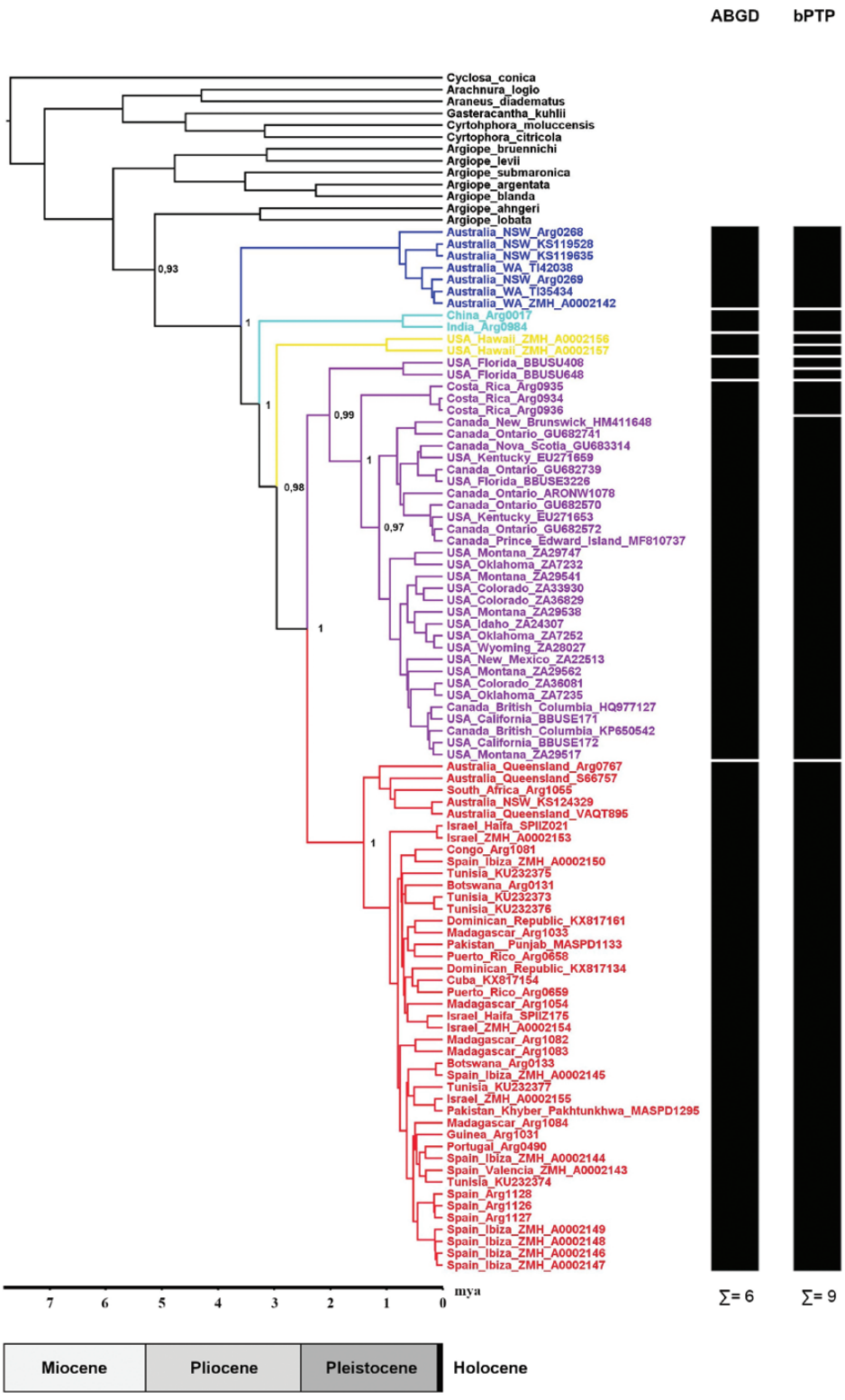

Figure 3. Chronogram for the expanded *COI* dataset (101 terminals, three partitions, 626 bp) inferred using a relaxed log-normal molecular clock (Brower rate: BEAST input = 0.0115) and results of two species delimitation methods. The molecular operational taxonomic units are indicated as black bars. Branch support values are given as posterior probabilities. Abbreviations: ABGD, Automatic Barcode Gap Discovery; bPTP, Bayesian implementation of Poisson tree process.

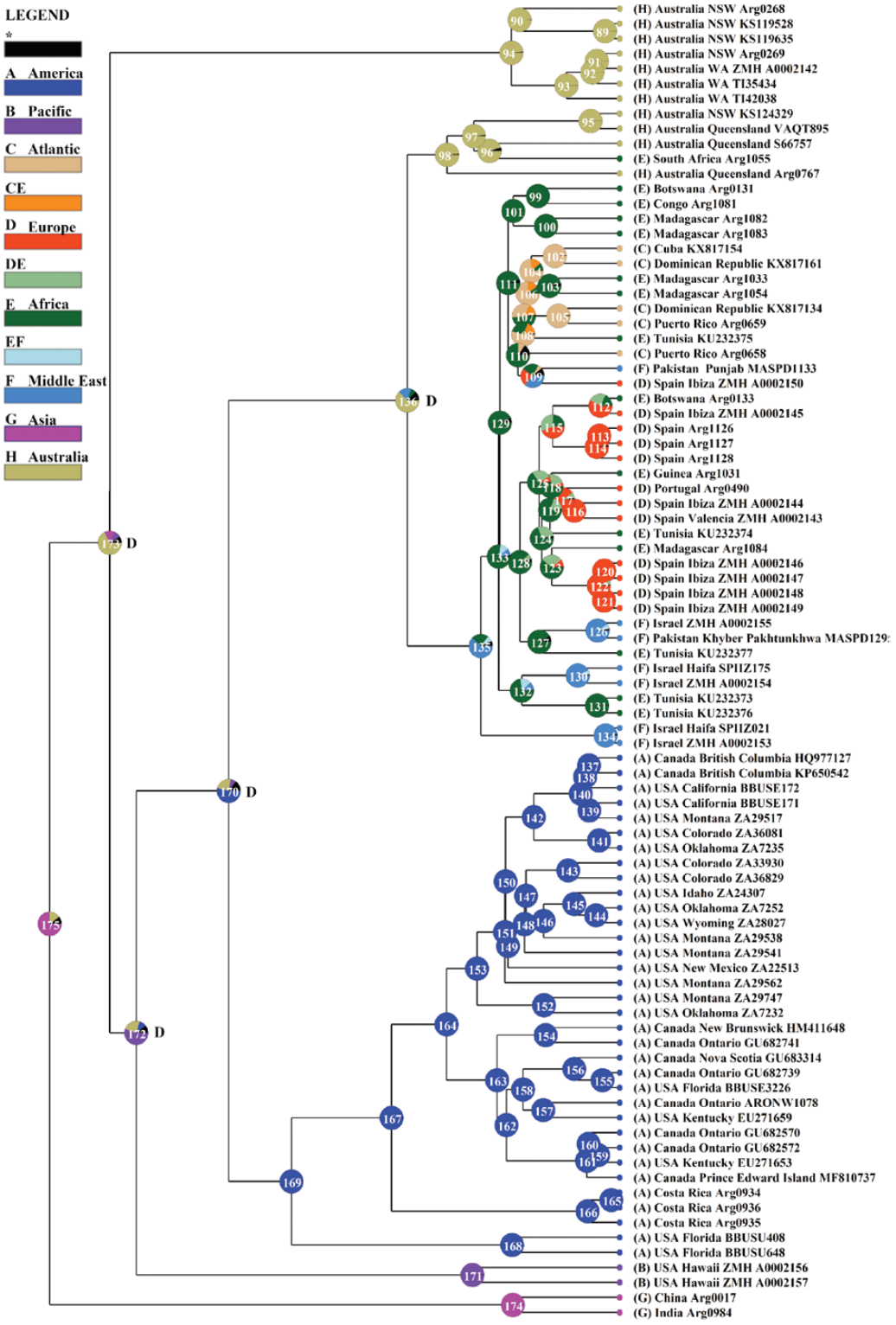

Figure 4. Graphical results of the Bayesian binary Markov chain Monte Carlo (BBM) and statistical dispersal–vicariance (S-DIVA) analyses. Nodal pie charts indicate probabilities of ancestral areas, with colour coding according to regions using partition 2. Black with an asterisk represents other ancestral ranges. The letter D indicates trans-oceanic dispersal events.

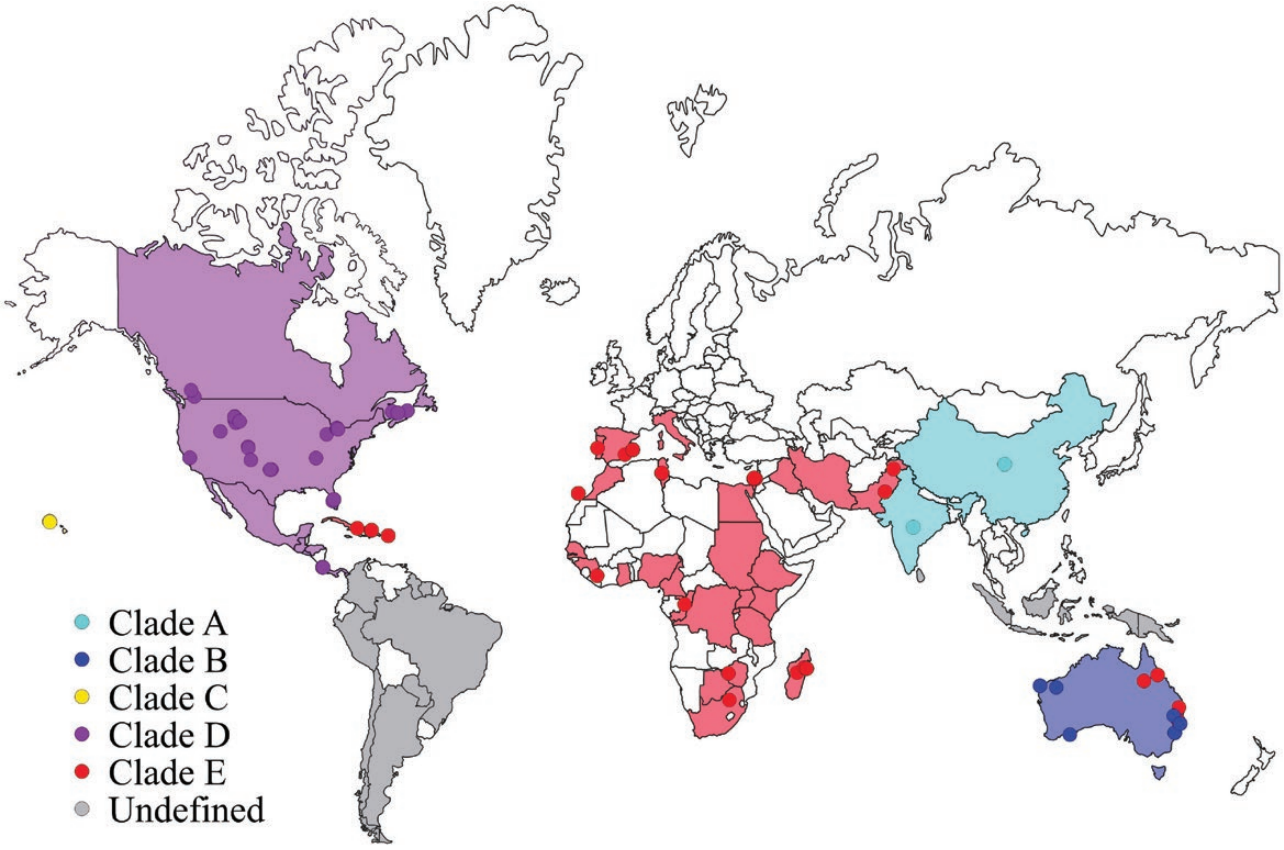

Figure 5. Revised distribution map, coloured according to genetic clades. Localities of *Argiope trifasciata* specimens included in the expanded *COI* dataset are marked with dots.

DISCUSSION

THE BIOGEOGRAPHICAL STRUCTURE OF *A. TRIFASCIATA*

We recovered five reciprocally monophyletic clades within *A. trifasciata* that often show a pronounced degree of genetic structuring and correspond to specific biogeographical areas. The genetic distances between those clades (in some cases > 8%) highlight pronounced patterns of phylogeographical structuring in *A. trifasciata* instead of panmixia and unlimited gene flow between populations (Fig. 5).

Clade A, comprising only the Indian and Chinese specimens, seems to be limited in the west by Greater Middle Eastern countries. It is possible that the Himalayas act as a barrier to dispersal and gene flow, thereby dividing the Palaearctic and the Oriental realm (von Oheimb *et al.*, 2013; Qiong *et al.*, 2017; Golovatch & Martens, 2018). It stands to reason that additional specimens found in Indochina would nest in clade A, although specimens from southern Indonesia and other islands between Asia and Australia should be sequenced to confirm their identity. Clade B is confined to specimens from the Australian continent but includes genetically similar specimens from both western Australian and eastern Australian terminals, despite several thousand kilometres of arid desert between these habitats. Clade C is endemic to Hawaii and currently recognized as the subspecies *A. trifasciata kauaiensis*. Clade D includes all mainland American specimens but shows some interesting patterns. All terminals from Canada and the USA, except two Floridian specimens, are genetically similar, although they were sampled from regions that differ drastically in terms of climate. This clade occurs between New Mexico (south) and British Columbia (north), but there is no obvious biogeographical structure, and genetic divergences (1.5%) are low. Samples from Costa Rica show some genetic divergence from these samples and form an outgroup to clade D. It is possible that these samples represent a more widespread South American lineage, but additional samples from South America would need to be sequenced to test this hypothesis. Clade E has the widest distribution and occurs in the Old World (Africa, Europe and the Greater Middle East),

Table 2. Nodal ages of the different clades for the expanded *COI* dataset inferred using a relaxed log-normal molecular clock, applying both the Brower rate and the Papadopoulou rate

Node	Brower rate (Mya) / Papadopoulou rate (Mya)
Split from *Argiope*	Mean = 5.13; 95% HPD: 6.33–3.90 / Mean = 3.33; 95% HPD: 4.12–2.43
Clade B	Mean = 3.59; 95% HPD: 4.42–2.81 / Mean = 2.33; 95% HPD: 2.87–1.81
Clade A	Mean = 3.27; 95% HPD: 4.00–2.51 / Mean = 2.12; 95% HPD: 2.61–1.63
Clade C	Mean = 2.95; 95% HPD: 3.49–2.22 / Mean = 1.91; 95% HPD: 2.37–1.48
Clade D	Mean = 2.42; 95% HPD: 3.21–1.88 / Mean = 1.57; 95% HPD: 1.96–1.21
Clade E	Mean = 2.42; 95% HPD: 3.21–1.88 / Mean = 1.57; 95% HPD: 1.96–1.21

Abbreviation: HPD, highest posterior density.

spanning a vast terrain of > 8000 km from South Africa to Spain and Pakistan, without significant genetic differences (< 2%). Surprisingly, the specimens from the Caribbean islands, expected to form a clade with the other New World terminals, were placed in this clade with specimens from the Old World and illustrate a capacity for long-distance dispersal. Dating suggests very recent dispersal, which might have been active, via ballooning, or passive (e.g. anthropogenic). Interestingly, clade E also occurs on other islands, such as Madagascar, despite the high degree of faunistic isolation of this island. The lack of genetic divergences between the African mainland specimens and those from Madagascar suggests that that dispersal was recent or that gene flow is continuous.

An interesting pattern is exhibited by the Australian fauna, which is divided into two clades. The specimens from tropical Queensland and one New South Wales sample nested as sister groups in clade E from the Old World and differed significantly from *A. trifasciata* in temperate eastern and western Australia; again, showcasing a capacity for long-distance dispersal. This is not a panmictic population with unlimited gene flow but a genetically diverse and highly structured evolutionary lineage.

The biogeographical and genetic structure in spiders differs vastly across taxa. Intraspecific diversity in spiders can differ from < 1% in some species to > 10% in others (Barrett & Hebert, 2005; Robinson *et al.*, 2009). The highly dispersive *Trichonephila antipodiana* (Walckenaer, 1841) from tropical Asia displays genetic divergences of ≤ 1.5% between populations from the Philippines and Singapore (Su *et al.*, 2011). In the subtropical Asian *Trichonephila clavata* (L. Koch, 1878), specimens from Japan, China and Taiwan diverge by < 1% in the *COI* data (Su *et al.*, 2011). These patterns of small divergences across thousands of kilometres match those of the various *A. trifasciata* clades uncovered here, but not those of the entire species. Anthropogenic dispersal is also not a relevant factor that can account for the deep clade divergences alone. For example, *Latrodectus geometricus* C. L. Koch, 1841 shows average genetic distances of 1.5%, with a maximum of 2.3% between populations from Hawaii, North America, Argentina and South Africa (Garb *et al.*, 2004). On the contrary, *Pholcus phalangioides*, a synanthropic spider, presents maximal intraspecific divergence values of 7.13% in samples from Brazil, Spain and Germany (Robinson *et al.*, 2009), although these spiders differ by a significant amount between populations in different building complexes (Schäfer *et al.*, 2001), and genetic divergences might be only loosely correlated with geographical distance (Astrin *et al.*, 2006).

The biogeographical patterning indicates multiple layers of structure in time and space: deep events in time that established clade structure in the Plio-Pleistocene and more recent ones within clades where anthropogenic dispersal might have been a factor.

Temporal and spatial origins of diversification

The analyses suggest that *A. trifasciata* split from related species around the Miocene–Pliocene boundary. The biogeographical analysis suggests three main diversification events in time. There was one deep diversification event in the Australasian/Southeast Asian region that occurred between the Middle and Late Pliocene and established three genetic clades that are old by comparison (3.6–3.0 Mya) and divided by genetic distances of 4% between clade B (Australia) and clade C (Hawaii) and 7% between clade A (East Asia) and clade B in the *COI* data. A second and more recent event was correlated with the Early Pleistocene of the Northern Hemisphere and associated with genetic splits between the New and Old World populations. The third, most recent event established diversity within the clades defined here and accounts for genetic variation between populations on a common landmass.

A pending question is, how did *A. trifasciata* spread from its origin in the Pacific into the Northern Hemisphere? The reciprocal monophyly of the two Northern Hemisphere clades (clades D and E) and the

timing results and biogeographical analyses suggest that the Northern Hemisphere diversity might originate from a single colonization event, with the most likely scenario being that *A. trifasciata* spread across the Pacific to the Americas first, followed by subsequent dispersal to the Old World. The alternative hypothesis, colonization of the Old World from Asia, is not likely because dispersal events might render the Northern Hemisphere fauna poly- or paraphyletic. Assuming the more likely scenario, *A. trifasciata* could have spread across the entire African continent and dispersed to Madagascar, the Middle East and Europe. It is to be noted that the species was thought to be absent from Europe for a long time (Levi, 1983, 2004) and was first recorded on the European mainland in 1985 (Morano & Ferrández, 1985), which indicates a recent introduction to the continent (Di Pompeo, 2011).

The paraphyletic Australian fauna (one clade tropical and one clade temperate) is a conundrum and best explained by back dispersal to Australia from Asia or Africa > 1 Mya. This colonization is likely to have happened from Africa, owing to the small divergences between samples from both regions. A dispersal event from other parts of the world cannot be refuted completely, because our dataset does not cover all known populations and it is possible that those eastern Australian specimens match haplotypes from regions yet to be sampled.

Diversification within all clades is estimated to have occurred during the Pleistocene. We assume that the genetic patterns within *A. trifasciata* in both the New and the Old World were strongly influenced by glacial–interglacial periods, as suggested previously for the Tunisian populations of *A. trifasciata* and other Mediterranean and American spider species (Ayoub & Riechert, 2004; Planas *et al.*, 2014; Krehenwinkel *et al.*, 2016; Dimassi *et al.*, 2017). Similar divergences might be recovered in the other clades pending more detailed sampling and sequencing.

Mechanisms shaping genetic structure

The biggest factor in shaping the contemporary genetic structure of *A. trifasciata* is likely to be its ability to disperse over long distances. There is strong evidence for dispersal events at different levels in time and various spatial scales. The spreading over the globe in the Plio-Pleistocene pre-dates potential human interference by a long time. *Argiope trifasciata* crossed both the Pacific and the Atlantic at least once on its way eastwards without the help of humans and is likely to have recolonized east Australia with a second immigration event. Crossing the Pacific to Hawaii and America might have been possible by island hopping, as suggested by the occurrence of *A. trifasciata* on several other Pacific islands, such as New Caledonia and Fiji (Koch *et al.*, 1871; Sarasin & Roux, 1914; Levi, 1968, 1983). As discussed previously, the most likely scenario for the colonization of the Old World by *A. trifasciata* is across the Atlantic. The successful trans-oceanic dispersal events across barriers several thousand kilometres wide suggest excellent dispersal capabilities and exceed documented instances of spiders ballooning over hundreds of kilometres (Darwin, 1839; Hardy & Cheng, 1986). Orb-weaver spiders do cross oceanic barriers up to, and probably more than, 4000 km (Turk *et al.*, 2020). Some spiders are even known to withstand encounters with both fresh and marine water and can take off again from the water surface (Hayashi *et al.*, 2015). In addition to 'sailing', sea crossing on large rafts or floating vegetation cannot be ruled out (Harrison *et al.*, 2017). There are several instances of rafting between South America and Africa, across taxonomic groups (Carranza & Arnold, 2003; Vidal *et al.*, 2008), including non-ballooning spider groups (Ceccarelli *et al.*, 2016).

Despite the evidence of multiple trans-oceanic dispersal events for *A. trifasciata*, the existence of phylogenetic clades within this species, corresponding to landmasses, illustrates strong geographical patterning. Thus, although *A. trifasciata* is capable of crossing oceans, trans-oceanic dispersal is not sufficiently frequent to allow for unlimited gene flow and panmixia across the distribution range. The genetic structure of the species suggests the maintenance of gene flow within certain geographical regions but not necessarily across oceans to ensure panmixia across the globe. This supports the existing studies about *A. trifasciata* by Ramirez *et al.* (1999) and Dimassi *et al.* (2017), who found the same on a local scale. Smaller oceanic barriers or connected landmasses seem not to be a limiting factor. Specimens from multiple islands, such as Madagascar, Ibiza or the Canary Islands, are genetically similar to those from the mainland.

Although dating analyses suggest that human-mediated dispersal is not the primary factor in shaping the genetic structure of this species, it might play a role in some cases. It is possible that the occurrence of the species on Madagascar, the European mainland and the Antilles was mediated by humans.. However, it is impossible to distinguish between passive and active dispersal in such recent dispersal events. Certainly, the patterning seen in the Australian specimens clearly pre-dates human history and is a case of active dispersal across wide distances.

Is *A. trifasciata* one species or many?

Argiope trifasciata is a species with a long taxonomic history, which has resulted in > 30 junior synonyms available in the taxonomic record

(World Spider Catalog, 2020). Indeed, *A. trifasciata* is morphologically variable and has been described independently from many parts of the world only to be synonymized into a single species by subsequent authors (Levi, 1983). The species can be viewed as a prime example of taxonomic redundancy, but the genetic data indicate that at least some of these synonymies might have been established in error. Some species concepts take a molecular approach to delineation of putative species, invoking genetic divergences and species delimitation methods, deep divergences in time and phylogenetic clades (DeSalle *et al.*, 2005; Pons *et al.*, 2006), which is especially important in cryptic species (Hamilton *et al.*, 2011).

Application of molecular species concepts and methods to *A. trifasciata* indicates the presence of up to nine mOTUs, which might be classified as separate species, although the more conservative estimate of six mOTUs seems more realistic. These mOTUs have origins in the Plio-Pleistocene and may be young by comparison, but they are clearly defined biogeographically. Given that barcoding thresholds differ from taxon to taxon (Hebert *et al.*, 2003; Barrett & Hebert, 2005; Čandek & Kuntner, 2015), the limits between species are not clearly defined, and divergences of > 8% should not be used to resurrect some of the synonyms or to describe new species in *A. trifasciata*, although they are indicative of distinct evolutionary units. In a previous study, Agnarsson *et al.* (2016) described the new cryptic species *Argiope butchko* LeQuier & Agnarsson, 2016, in a phylogeographical study of the spider *Argiope argentata* (Fabricius, 1775), based on average sequence divergences of 6.3% between American and Cuban specimens; a value that is comparable with the data observed here. It may be interesting in this context that the Hawaiian specimens, which nested in their own monophyletic clade within *A. trifasciata*, were first described by Simon (1900) as *Argiope avara kauaiensis* and later synonymized by Levi with *A. trifasciata* (Levi, 1983), arguing that they represent only a colour morph. Subsequent authors did not agree (Berry & Williams, 2017) and supported the taxonomical standing as a subspecies after evaluation of the male genitalia for the first time.

We refrain from drawing definite taxonomic conclusions from the genetic data presented here because this is a complex case, and other species concepts, such as the biological and morphological ones (Simpson, 1951; Mayr, 1970; De Queiroz, 2007), need to be evaluated for *A. trifasciata*.

Levi (1983, 2004) noted no significant variation between the *A. trifasciata* holotype from Cairo and other *A. trifasciata* specimens but remarked on a slightly different abdomen in Australian specimens. We note, however, that Levi was generally conservative in his species assessments and that several of his synonymized species have been resurrected by subsequent workers. Unfortunately, studies about differences in mating behaviour or ecology in different *A. trifasciata* populations around the world are lacking; therefore, species delineation based on the biological and morphological species concepts is not possible at this stage.

The next steps to verify the existence of putative species should include the detailed examination of morphology, especially the genitalia, mating experiments and ecological studies to reconcile the genetic data with other lines of evidence in the taxonomic process.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. List of specimens included in the analyses with reference to collection localities and list of downloaded sequences from online databases.
Appendix S2. Polymerase chain reaction protocols, primer sequences, substitution models in MrBayes and partitions for biogeographical analyses.
Appendix S3. Genetic distances between clades and results of the species delimitation.
Appendix S4. Phylogenies generated with MrBayes, RAxML and BEAST.