Molecular Phylogenetics and Evolution 148 (2020) 106817

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Recent diversification of *Chrysoritis* butterflies in the South African Cape (Lepidoptera: Lycaenidae)

Gerard Talavera[a,b,*], Zofia A. Kaliszewska[b,c], Alan Heath[b,d], Naomi E. Pierce[b,*]

[a] Institut de Biologia Evolutiva (CSIC-UPF), Passeig Marítim de la Barceloneta 37, 08003 Barcelona, Catalonia, Spain
[b] Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, United States
[c] Department of Biology, University of Washington, Seattle, WA 98195, United States
[d] Iziko South African Museum, Cape Town, South Africa

ARTICLE INFO

Keywords:
Butterflies
Chrysoritis
Fynbos
Phylogeny
Radiation
Speciation
Taxonomy

ABSTRACT

Although best known for its extraordinary radiations of endemic plant species, the South African fynbos is home to a great diversity of phytophagous insects, including butterflies in the genus *Chrysoritis* (Lepidoptera: Lycaenidae). These butterflies are remarkably uniform morphologically; nevertheless, they comprise 43 currently accepted species and 68 currently valid taxonomic names. While many species have highly restricted, dot-like distributions, others are widespread. Here, we investigate the phylogenetic and biogeographic history underlying their diversification by analyzing molecular markers from 406 representatives of all described species throughout their respective ranges. We recover monophyletic clades for both *C. chrysaor* and *C. thysbe* species-groups, and identify a set of lineages that fall between them. The estimated age of divergence for the genus is 32 Mya, and we document significantly rapid diversification of the *thysbe* species-group in the Pleistocene (~2 Mya). Using ancestral geographic range reconstruction, we show that West Fynbos is the most likely region of origin for the radiation of the *thysbe* species-group. The colonization of this region occurred 9 Mya and appears to have been followed by a long period of relative stasis before a recent increase in diversification. Thus, the *thysbe* radiation does not appear to have resulted from the colonization of new biogeographic areas. Rather, the impact of species interactions (with ants and plants), the appearance of key innovations, and/or the opening of new ecological niche space in the region might explain the sudden burst of speciation that occurred in this group 2 Mya. The biogeographic model suggests two different diversification processes with few historical cross-colonisations, one in eastern South Africa for the *C. chrysaor* group and the other in western South Africa for the remaining taxa. Distributional range assessments and ecological niche models for each species show important niche overlap, and in a few cases, complete overlap. However, these shared traits are not explained by phylogenetic history. *Chrysoritis* taxa frequently fly in sympatry and gene tree reticulation appears to be widespread at the species level, suggesting that several episodes of range shifts might have led to secondary sympatries, allowing limited gene flow that challenges species delimitation efforts. In addition, the unusually high diversification rate for the *thysbe* clade of 1.35 [0.91–1.81] lineages per million years also suggests the possibility of taxonomic oversplitting. The phylogeny presented here provides a framework for a taxonomic revision of the genus. We highlight cases of potential synonymy both in allopatry and sympatry, and stress the importance of dedicated studies to assess potential pre- and post-zygotic barriers giving rise to species delimitations of the *thysbe* group.

1. Introduction

The South African Cape Belt forms a unique eco-region in the narrow, southernmost tip of Africa. Also known as fynbos, it is one of the most endemic-rich regions in the world (Cowling and Richardson, 1995; Allsopp et al., 2016). One of these endemic lineages is the lycaenid genus *Chrysoritis,* which comprises 43 currently accepted species distributed in biomes ranging from semi-desert to closed canopy deciduous forest, from coastal to montane, and from areas of winter rainfall to those of summer rainfall (Terblanche and van Hamburg, 2004). The genus' centre of diversity is in the Western Cape Province (Terblanche and van Hamburg, 2004), a region that includes the Cape

* Corresponding authors at: Institut de Biologia Evolutiva (CSIC-UPF), Passeig Marítim de la Barceloneta 37, 08003 Barcelona, Catalonia, Spain (G. Talavera).
E-mail addresses: gerard.talavera@csic.es (G. Talavera), npierce@oeb.harvard.edu (N.E. Pierce).

https://doi.org/10.1016/j.ympev.2020.106817
Received 4 August 2019; Received in revised form 30 March 2020; Accepted 1 April 2020
Available online 11 April 2020
1055-7903/

Fold Mountains, a winter rainfall area, unlike other South African provinces. Due to the high degree of endemicity and limited distributions of many taxa, these butterflies are of conservation concern, with at least ten of the species and subspecies classified as critically endangered or vulnerable (Mecenero et al., 2013).

The large number of species found in such a relatively small geographic area is unusual among butterflies. A few other examples of butterflies in Southern Africa include *Aloeides, Thestor, and Lepidochrysops* (Cottrell, 1978; Heath and Pringle, 2004), or *Erebia* in the Palearctic mountains (Peña et al 2015, Schmitt et al., 2016); although species packing may be more common in plants: for example the Southern African semi-desert ice plants (Klak et al., 2004) and *Erica* (Pirie et al., 2016) rival this type of diversity. Geographic distributions in *Chrysoritis* are also intriguing, and while some species such as *C. adonis*, *C. lyndseyae*, *C. rileyi*, *C. daphne* or *C. blencathrae* have restricted, dot-like distributions (known from fewer than 3 sites), others such as *C. pan* and *C. chrysaor* are widespread. In addition, multiple *Chrysoritis* species are often found flying in sympatry. For example, in an area a little more than a hectare in size near Hondeklip Bay on the West Coast, as many as seven *Chrysoritis* species occur together, four of which belong to a clade known as the *thysbe* species-group. The species' apparent reproductive isolation suggests that speciation might sometimes occur in sympatry or that range expansion after allopatric divergence leads to secondary sympatry.

The genus comprises 68 currently valid taxonomic names and it has a complex taxonomic history. Species descriptions in the past have been based almost entirely on differences in wing markings, and minor regional or population variation in wing markings have prompted descriptions of new "species", especially in the *thysbe* species-group (Heath 1997, 2001; Rand et al 2000; Heath and Pringle 2007). As a result, 43 species names are currently considered valid, 28 of which belong to the *thysbe* species-group. *Chrysoritis* genitalia are fairly uniform and are nearly invariant among members of the *thysbe* species-group, making it difficult to use traditional methods to delimit species (Heath, 1997). Host-plant associations have also been analysed in an attempt to describe *Chrysoritis* diversification. For example, 19 plant genera from 13 families have been recorded as host-plants of species in the genus, with as many as nine host-plant species recorded for a single species, *C. thysbe* (Supplementary Table S5). However, it seems unlikely that host-plant shifts play a major role in speciation (Heath et al., 2008; Terblanche and van Hamburg, 2004).

Like most Lycaenidae, *Chrysoritis* larvae are myrmecophilous: the immature stages associate with ants (Heath and Claassens, 2003). The larvae of almost all *Chrysoritis* species associate with ants in the genus *Crematogaster*, with just two taxa associating with species of *Myrmicaria*. Although *Chrysoritis* species are mostly herbivorous, one species, *C. dicksoni* (Gabriel, 1946), is aphytophagous. Larvae of this species live in ant nests and are fed by ants that regurgitate to them via trophallaxis (Heath, 2014).

Thus, the potential drivers of *Chrysoritis* diversification are not clear. Most debates concerning *Chrysoritis* diversity and evolution have been made in the absence of a phylogenetic framework, although an early molecular phylogeny using a limited number of taxa (19) and a single mitochondrial marker (COI) inferred relationships for part of the group (Rand et al. 2000). Here we expand the dataset to include nearly all *Chrysoritis* species and subspecies and four molecular markers (3332 bp). We investigate the timescale of diversification and explore aspects of the group's biogeographic history, including colonization of new habitats that might have led to diversification in such a limited area.

2. Methods

2.1. Taxon sampling and molecular data

We included 406 wild-caught *Chrysoritis* specimens and representatives of four outgroups for this study. Sampling was designed to include all described *Chrysoritis* species and as many subspecies as possible according to the taxonomic assignments of Heath (1997, 2001, 2011) and Heath and Pringle (2007). Identifications were based on original descriptions and examination of type specimens. Specimens of *Pseudaletis clymenus* (Druce), *Cigaritis lohita* (Horsfield), *Cigaritis syama* (Horsfield) and *Crudaria capensis* (van Son) were selected as outgroups based on a recent higher level phylogeny of the Aphnaeinae (Boyle et al., 2015). Ingroup specimens were chosen to maximize coverage of the full geographic ranges of each taxon. Thus, several specimens from multiple populations were included for most species and subspecies. Only one specimen was collected for the few taxa that are known to be geographically restricted and/or of conservation concern. Samples (bodies in ethanol and wings in glassine envelopes) are deposited in the DNA and Tissues Collection of the Museum of Comparative Zoology (Harvard University, Cambridge, MA, USA). Identification codes and collection localities are listed in the Supplementary Table S1.

We extracted genomic DNA from legs or abdomens using a DNeasy Tissue Kit (Qiagen, Inc.). Mitochondrial *cytochrome c oxidase I* (1220 bp, COI) was sequenced from all 410 samples. From a subset of 96 specimens, we also sequenced three nuclear loci: *elongation factor 1α* (1039 bp, EF1α), *carbamoylphosphate synthase* (745 bp, CAD) and *histone 3* (328 bp, H3). This subsampling includes at least one specimen for each species/subspecies. PCR and sequencing conditions followed those of Kaliszewska et al. (2015). The sequences were submitted to GenBank (accession numbers in Supplementary Table S1).

2.2. Phylogenetic inference, divergence times and diversification rates

A data matrix was assembled for each marker by editing and aligning individual loci with Geneious 6.1.5 (Biomatters, Auckland, New Zealand; http://www.geneious.com/). Two datasets were then assembled: a large 410-specimen dataset including only COI; and a reduced 96-specimen dataset with full coverage of markers. For the 410-specimen dataset, Bayesian inference using BEAST 2.3 (Bouckaert et al., 2014) was employed to estimate evolutionary relationships and divergence times simultaneously. Best-fitting DNA substitution models were selected according to the Akaike Information Criterion (AIC) in jModeltest 2.1.4 (Guindon and Gascuel, 2003; Darriba et al., 2012). The GTR + I + G model was selected for COI, EF1α and CAD, and the GTR model was used for H3. The gamma distribution was estimated automatically from the data using six rate categories. A constant size coalescent approach and the uncorrelated relaxed clock (Drummond et al., 2006) were set as priors. We used a molecular rate calibration to date the phylogeny in the absence of fossil calibration points available for this genus or recent fossil calibrated butterfly phylogenies with adequate sampling to permit adopting secondary calibrations from them. A normally distributed substitution prior was set between 0.0075 and 0.0015 (within the 95% confidence interval), corresponding to substitution rates widely used for invertebrates: a slower 1.5% uncorrected pairwise distance per million years (Quek et al., 2004) and a faster 2.3% (Brower 1994). Two independent MCMC chains were run for 50 million generations each, sampling values every 5000 steps. A conservative burn-in of 500,000 generations was applied after checking for convergence in Tracer v1.5 (Rambaut and Drummond 2007). Maximum likelihood (ML) inference was carried out using PhyML 3.0 (Guindon et al, 2010), with a GTR + I + G substitution model as chosen by Smart Model Selection (SMS) (Lefort et al. 2017), a NNIs tree topology search, and 100 bootstrap replicates. A coalescent multilocus approach in *BEAST 2.3 was used to reconstruct a species tree (consisting of the 43 currently accepted *Chrysoritis* species) from the 96-specimen dataset, using a Yule Process tree prior, and a linear piecewise demographic model. Other settings were kept as a default in *BEAST. The resulting species tree was used to investigate diversification-rate heterogeneity using the software BAMM 2.2 (Rabosky et al., 2013; 2014a). Four MCMC chains were run for 10 million generations, and convergence

was tested using the CODA package in R (Plummer et al., 2006) with a burn-in of 10%. The R package BAMMTOOLS 2.2 (Rabosky et al., 2014b) was used to estimate the number of rate shifts and rate shift configurations, and to generate a phylorate plot for average net diversification rate. Net diversification rates were estimated for *Chrysoritis* and *thysbe* crown ages using Magallon and Sanderson's (2000) method with the function "bd.ms" in the R package geiger.

2.3. Distributional ranges and sympatry data

We compiled distribution information for each of the 43 *Chrysoritis* species from the Red List and Atlas (Mecenero et al., 2013), Heath (2011), and have expanded the dataset using our own observations. To translate our observed occurrences into latitude and longitude coordinates, we built a grid of 52 × 68 cells (3536 cells) for southern Africa corresponding to the distribution maps in Mecenero et al. (2013). Presence points for subspecific taxa were merged at the species level. Coordinates at centroids were extracted for the cells labelled as present, using the "*xyFromCell*" function in the R package raster (Hijmans, 2019). Resulting data points ranged from one cell per species (*C. daphne*) to 218 cells (*C. chrysaor*).

2.4. Niche modelling and niche/range overlap

We used all 19 BIOCLIM variables for climatic niche modelling (Hijmans et al, 2005). We adopt the term "climatic niche" because we consider only macroclimatic variables, but these exclude important components of an insect's niche, such a hostplant range. To avoid model over-parameterization, we reduced the number of variables by discarding correlated variables that shared a Pearson correlation coefficient of 0.7 or higher. Subsequently, eight ecological variables were retained: annual mean temperature (BIO1), isothermality (BIO3), temperature annual range (BIO7), mean temperature of the wettest quarter (BIO8), mean temperature of driest quarter (BIO9), annual precipitation (BIO12), precipitation seasonality (BIO15) and precipitation of coldest quarter (BIO19). Species with fewer than five occurrence points were not modelled. Climatic niches for each species were modelled from presence-only data with MaxEnt (v. 3.3.3: Phillips et al., 2006). Model performance was evaluated by cross-validation using the area under the receiving operating characteristic curve (AUC).

We estimated climatic niche overlap between all species pairs based on the predicted probability surfaces using the function "*niche.overlap*" in the R package phyloclim (Heibl, 2012) according to Warren's I statistic (Warren et al., 2008). Geographic distributions were estimated by means of convex Hull areas (minimum convex polygons) using the R package dismo (Hijmans, 2012). For most species, geographic distributions were better estimated using a single convex Hull area, but in a few cases, more than one convex Hull area was necessary. We then compared climatic niche overlap with degrees of geographic overlap. Intersecting polygons between areas of species distributions and of overlapping regions were estimated for all pairs of species. The proportion of overlap between species was obtained by dividing the area of the intersect polygon by the area of the species with the smallest distribution. Consequently, two species were scored as having an overlap of 1 if the species range of one was completely enclosed in that of the other (Joly et al., 2014). R packages phyloclim (Heibl, 2012), PBS mapping (Schnute et al., 2015), maps (Becker et al., 2018) and maptools (Bivand and Lewin-Koh, 2013) were used for these calculations, and the "*heatmap*" function in R was used to visualize these data.

2.5. Biogeography

Ancestral geographic ranges were estimated to reconstruct the most likely dispersal history of *Chrysoritis* lineages within their restricted distribution in Southern Africa. The likelihoods of different dispersal scenarios were assessed in the R package BioGeoBEARS 0.2.1 (Matzke 2013). The distribution range of *Chrysoritis* was divided into 6 regions: West Coast, West Plateau, West Fynbos, East Fynbos, East Plateau and East Coast (Supplementary Table S2). The inferred species tree was used as a reference topology, and geographic regions for each tip were assigned according to the presence grid previously assembled. A dispersal–extinction–cladogenesis (DEC) model (Ree and Smith, 2008), a maximum-likelihood version of the dispersal–vicariance model (DIVALIKE) (Ronquist, 1997) and a Bayesian biogeographical inference (BAYAREALIKE) (Landis et al., 2013) were tested. A dispersal multiplier matrix was coded, with lower probabilities of dispersal between non-adjacent areas (Supplementary Table S3). Likelihood ratio tests and AIC scores were used to select the best-performing models (Supplementary Table S4).

3. Results

3.1. Phylogenetics and diversification

Both BI (Bayesian Inference) and ML (Maximum Likelihood) methods recover *Chrysoritis* as monophyletic with two well-supported clades (*thysbe* and *chrysaor* species-groups) and a grade of lineages sister to the *thysbe* clade (*chrysantas, pyroeis, felthami, zeuxo,* and *zonarius*) (Fig. 1, Supplementary Fig. S1). Phylogenetic relationships within the *chrysaor* clade are generally concordant between methods. *Chrysoritis chrysaor* and C. *midas* are recovered as polyphyletic, and *C. natalensis* is nested within that clade (Supplementary Fig. S1). Different methods infer different phylogenetic relationships within the *thysbe* clade, and several taxonomic units are not supported or are paraphyletic (Supplementary Fig. S1). The species tree inference shows high levels of uncertainty within the *thysbe* clade (Fig. 1). Thus, most taxonomic designations within the *thysbe* clade are not supported by our genetic data, contrasting with differentiation in wing marking and behavior. All phylogenetic approaches consistently recover the taxon *chrysantas* as the sister lineage of the *thysbe* species-group. A *zonarius-zeuxo* clade is recovered, but each is mutually paraphyletic.

The estimated age of divergence of *Chrysoritis* is 32.17 Mya [22.25–41.22] Mya. The first split occurred 17.28 Mya [12.33–22.56] between the *chrysaor* clade and the rest. We document a very recent time of diversification for the *thysbe* clade: 2.77 Mya [1.96–3.61] Mya according to the full BI tree, and 1.4 Mya [1.00–1.98] according to species tree inference. This represents an exceptional burst of diversification, with a rate of 1.35 [0.91–1.81] lineages per million years compared to only 0.18 lineages per million years overall for the genus. BAMM detects a significant rate shift for the *thysbe* species-group (Fig. 1), which preceded a period of no diversification between approximately 2 and 6 Mya, according to a lineage through time plot (Fig. 2).

3.2. Biogeographic patterns

The distribution data indicates that the *chrysaor* group is predominantly distributed in eastern South Africa, with the exceptions of the widely distributed *C. chrysaor* and its phylogenetically nested species *C. midas*, and the basal *C. dicksoni* that is restricted to a few spots in West Fynbos (Fig. 3) (Supplementary Fig. S2). Conversely, taxa in the *thysbe* species-group are mostly distributed in West Fynbos and the western coast of South Africa. *Chrysantas* lineages are also distributed in the Cape Fold Belt and the West Coast, and no species reaches eastern South Africa, thus not overlapping with the *chrysaor* group (other than the previously mentioned exceptions).

When reconstructing ancestral geographic ranges along the tree, the DIVALIKE model was the best among all models tested with BioGeoBEARS (LnL, −121.30; AIC) (Supplementary Table S4). Among the six potential ancestral regions, the model inferred an early geographic split with strong support. Dispersal between east and west rarely occurred after this early split, and the majority of geographic

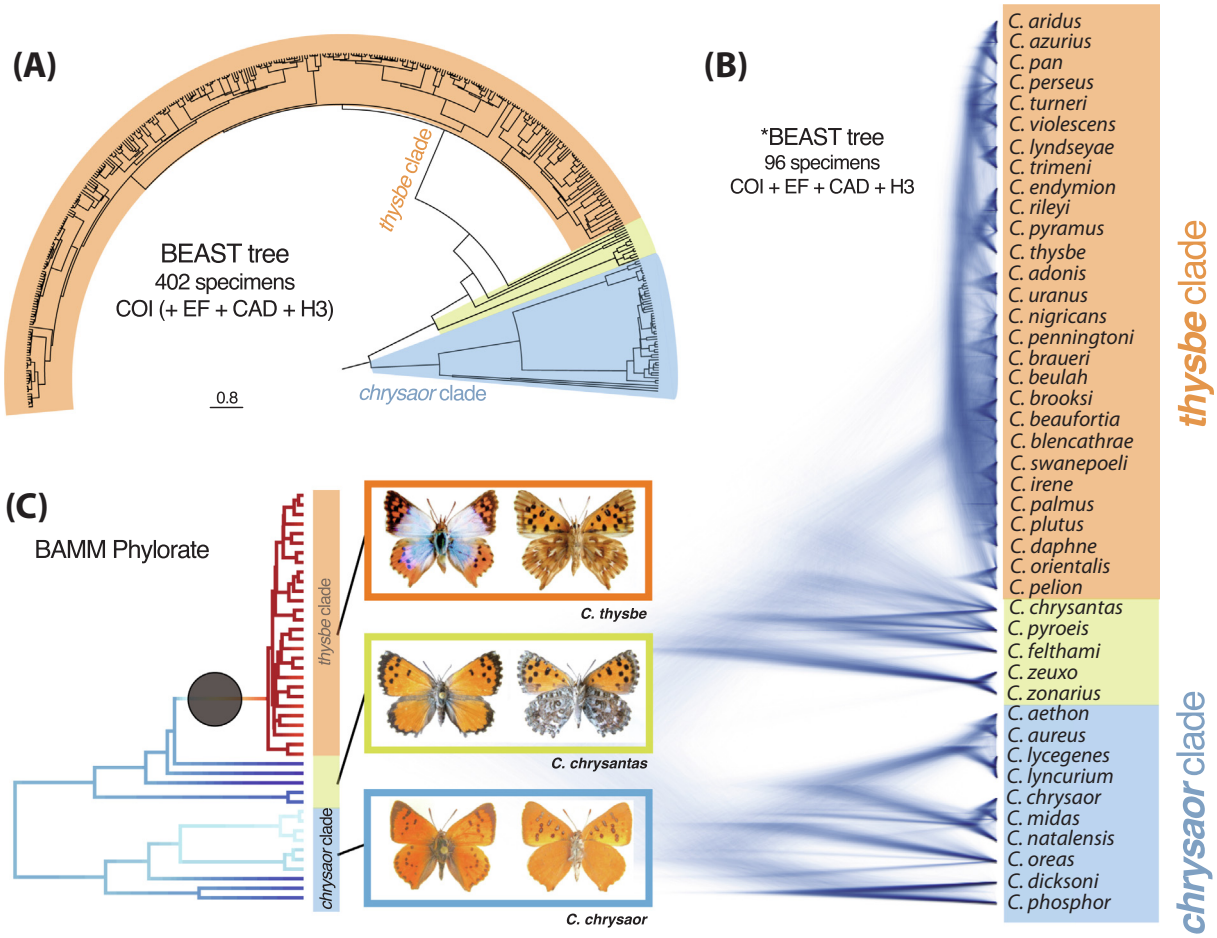

Fig. 1. Phylogenetic overview of *Chrysoritis*. A) Bayesian phylogenetic tree for the 410-specimen dataset. B) *BEAST species tree inference based on the 96-specimen dataset. Tree is plotted with DensiTree (Bouckaert, 2010) displaying all trees of the MCMC chain after a 10% burn-in. C) BAMM phylorate plot showing average net diversification rates along the *Chrysoritis* species tree. Warmer colours denote faster diversification rates (in lineages per Mya) and the circle denotes a significant increase in diversification rate.

shifts occurred within the eastern or the western regions (Fig. 2). Thus, *Chrysoritis* in western and eastern South Africa diversified independently.

The model describes various gains of new geographic areas along the phylogeny, which are generally clustered into three periods: (1) Five gains between 12 and 6 Mya, (2) 18 gains earlier than 8 Mya and (3) five gains during the last 2 Mya. Two of these timeframes coincide with an increase in the accumulation of lineages, but during the period between 3 and 5.5 Mya, where geographic gains are likely to be much higher, phylogenetic splits are not present (Fig. 2). This suggests a scenario of multiple colonization events but no speciation.

4. Discussion

4.1. A diversification burst for the thysbe species-group in West Fynbos

Based on the ancestral geographic range reconstruction, West Fynbos appears to be the most likely region of origin for the radiation of the *thysbe* species-group (Fig. 2). However, the colonization of West Fynbos from the West Coast in the *Chrysoritis* western lineage occurred 9 Mya, and no diversification occurred until ~2 Mya. Such a long time without diversification in West Fynbos is interesting, and we can conclude that it is unlikely that the *thysbe* radiation occurred due to the colonization of a new biogeographic space. On the contrary, the appearance of key innovations in the lineage or the opening of new niche space in the region might explain this sudden burst at ~2 Mya.

Previous *Chrysoritis* colonisations of West Fynbos did not bring about important diversification events. For example, although the ancestors of *C. chrysantas* and *C. pyroeis* appeared soon after West Fynbos colonization *ca.* 9 Mya, neither of these lineages further diversified. *Chrysoritis felthami, C. zeuxo and C. zonarius* also did not diversify, although their West Fynbos colonization occurred more recently. Only three taxa from the *Chrysoritis* Eastern Lineage colonized West Fynbos: the *chrysaor/midas* species-pair expanded to all regions; and an early dispersal event (*ca.* 11.5 Mya) gave rise to the West Fynbos endemic *C. dicksoni*. There is no evidence of major geologic or macroclimatic events in West Fynbos ca. 2 Mya. The main geologic events of the region are older than 2 Mya, and the region has been stable since then (Cowling et al., 2009).

Two main uplift events occurred in southern Africa in the early Miocene (about 22 Mya) and in the Pliocene (about 5 Mya), causing a profound impact on the landscape of the fynbos. It is possible that the latter tectonic event triggered numerous cross-colonization events between regions. On the other hand, it might have promoted fragmentation in *thysbe* populations in the fynbos. The southern Cape Belt includes a complex suite of environments, diverse topography, many endemic plants, and microhabitats that have promoted diversification in many other insect groups (Procheş and Cowling, 2006). The mountains, cliffs, and valleys of the Cape Fold Mountains have surely played a role in isolating populations of *Chrysoritis*. These butterflies do not seem to be good dispersers, and usually remain in restricted populations. This may be partly due to selection favouring low vagility and thus increased fidelity to ant species that obligately tend their myrmecophilous larvae. The taxa *C. blencathrae, C. adonis, C rileyi* and *C.*

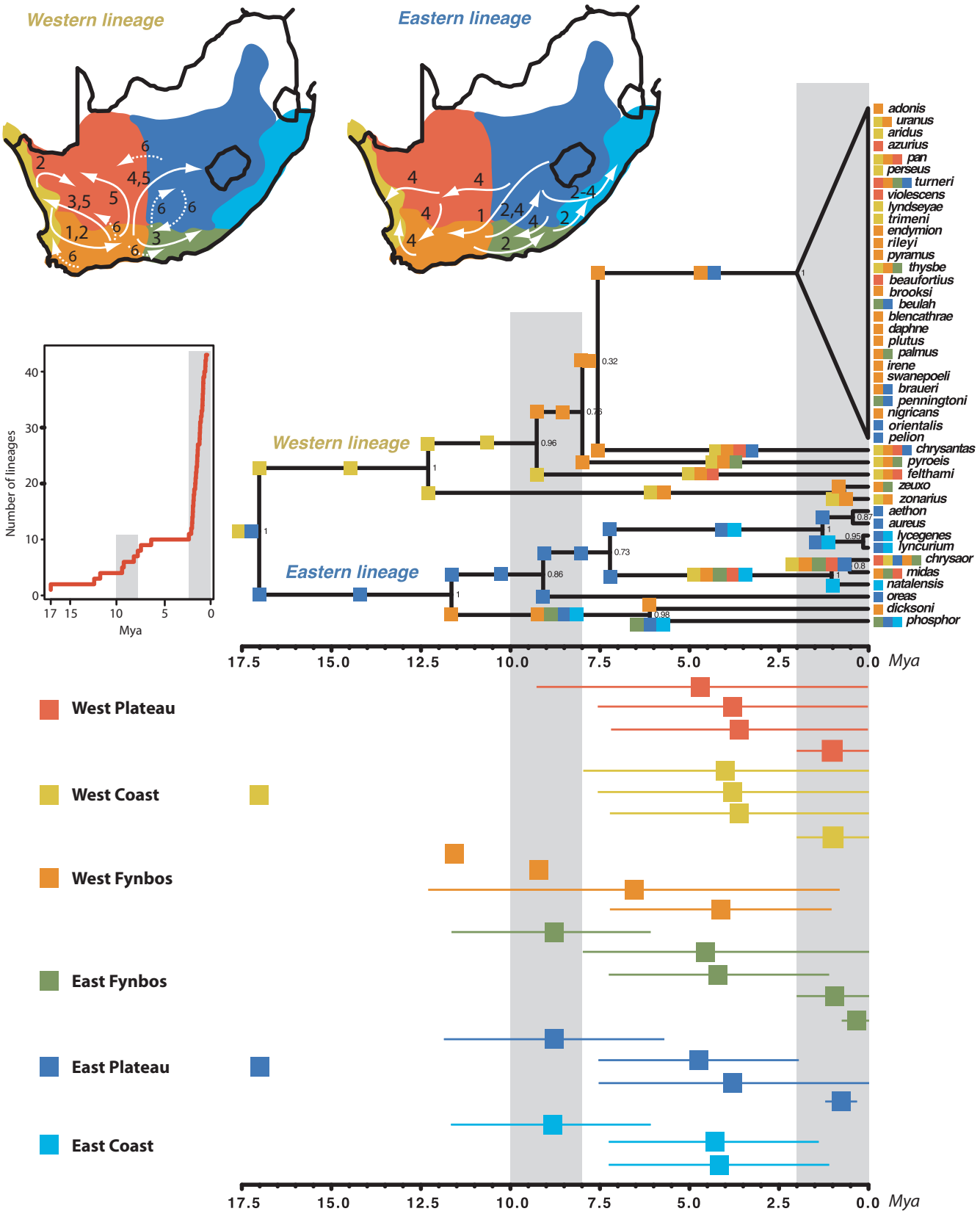

Fig. 2. Ancestral range reconstruction of *Chrysoritis*. The entire geographic range of the genus was divided into 6 regions indicated by different colours. The chain of biogeographic gains is shown in a temporal scale below and on the species tree of the group. Each square, placed at the mid point of the branch, indicates colonization of a new region. Lines through the squares represent the length of the branches where the event could have happened and denote temporal uncertainty. Three main period intervals concentrate most gain events, two coinciding with increases in the accumulation of lineages (highlighted in grey) and a period in between with lower phylogenetic splits but where geographic gains are likely to be higher. Numbers in arrows represent approximate steps, and dotted arrows denote uncertain steps within the *thysbe* group.

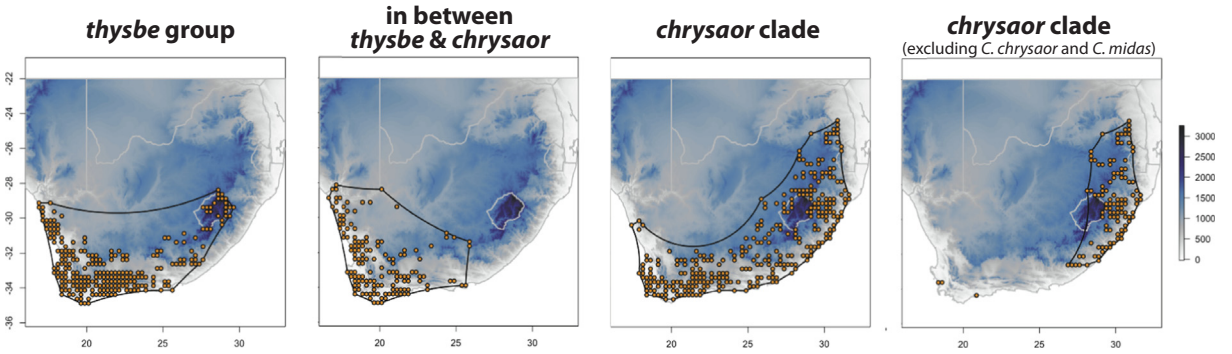

Fig. 3. Distributions of main *Chrysoritis* lineages: the *thysbe* species-group, the *chrysaor* clade (both with and without the widespread taxon *C. chrysaor*), and a set of lineages between them (*chrysantas, pyroeis, felthami, zeuxo,* and *zonarius)*. The axes indicate longitude and latitude, and the intensity of blue coloration denotes altitude in meters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

daphne are good examples of species with such narrow geographic distributions, and illustrate how neighbouring valleys can harbour different *Chrysoritis* species with no apparent distributional overlap. In addition to the role of biogeography in diversification (mostly via allopatry), there may be traits or key innovations that facilitate ecological divergence (mainly in sympatry). European butterflies that are more specialized, smaller, and more restricted in their distributions tend to have higher rates of genetic differentiation (Mackintosh et al., 2019; Dapporto et al., 2019), a pattern that could apply to *Chrysoritis* butterflies as well.

Myrmecophyly is an ancestral characteristic of *Chrysoritis* butterflies (Rand et al. 2000). Larval ant association has proved to be a key factor in promoting ecological niche shifts in other lycaenid butterflies (Pierce et al., 2002; Schär et al., 2018). Southern Africa is a hotspot for myrmecophilous lycaenids such as *Aloeides*, *Thestor,* and *Lepidochrysops*, all of which have undergone significant radiations. Ants in the genus *Crematogaster* (with two exceptions in the genus *Myrmicaria*) are the main ant species interacting with *Chrysoritis*. At least three *Crematogaster* species have been reported to associate with larvae of *Chrysoritis* species (Rand et al., 2000; Heath et al., 2008). However, cryptic, unrecognized *Crematogaster* species are likely, and the true specificity and diversity of these ant-butterfly associations is thus poorly known. *Crematogaster* is one of the largest ant genera globally (approximately 470 species), and it is also a complex taxonomic group (Blaimer, 2012). Species of *Crematogaster* are common and widespread in South Africa, and *Chrysoritis* populations likely encountered ant colonies frequently during their evolutionary history. It was once thought that *Chrysoritis* species might be generalists of *Crematogaster* species (Rand et al., 2000). Ant-association shifts do not appear to be correlated with speciation in *Chrysoritis*. However, some sympatric *Chrysoritis* taxa have specialized associations with different ants, and this could function as a barrier to gene flow. For example, in the Leipoldville-Lambert's Bay area, two subspecies, *C. pan pan* and *C. pan lysander,* seem to associate exclusively with *Crematogaster liengmei* and *C. peringueyi*, respectively, yet these two *C. pan* subspecies occur sympatrically (Heath, pers. obs.). Detailed studies of specific butterfly-ant interactions are needed to test the hypothesis that association with different ant species has promoted divergence between these two subspecies.

Host-plant shifts have been suggested to explain speciation in other butterfly taxa (*e.g.,* Fordyce, 2010; Hernández-Roldán et al., 2016), and it seems plausible that diversification of lycaenids in the fynbos might be correlated with shifts in host plant use. However, taxa in the *thysbe* group are often polyphagous, and many species have been recorded feeding on multiple plant families (Supplementary Table S5).

Another distinctive trait that makes the *thysbe* clade unique is the presence of brilliant iridescence in their wings. Only one species outside the *thysbe* clade, *C. pyroeis*, shares this trait, which suggests an independent gain. Wing iridescence has been attributed to mate recognition and sexual selection in many butterflies (Lukhtanov et al., 2005; Stavenga et al., 2010; Douglas et al., 2007), although further research characterizing the wavelengths and/or potential polarization of this trait is required.

The generation of *ca*. 25 species in about 2 Mya represents an exceptional burst of diversification not only in Lepidoptera, but in animals more generally (Coyne and Orr, 2004). A net diversification rate of 1.35 [0.91–1.81] lineages per million years is comparable only to a few butterfly groups, and is similar to the highest known rate of 1.78 in the subgenus *Agrodiaetus* (Polymmatina, Lycaenidae), which has produced ca. 120 species in only 2.3 Mya (Kandul et al., 2007, Talavera et al., 2013). The evolutionary mechanisms driving such fast radiation in *Agrodiaetus* relate to chromosome evolution and have been well characterized (Kandul et al., 2007, Vershinina and Lukhtanov 2017). Further investigation of potential evolutionary drivers leading to speciation in *Chrysoritis* would be fruitful.

4.2. Geographic/climatic niche overlap and evolution in sympatry

Overlapping distributions and environmental niches among species raise the question whether speciation with gene flow might be a common process in *Chrysoritis* (Fig. 4, Supplementary Fig. S2). This idea is reinforced by field observations of different taxa flying in sympatry in numerous localities (Fig. 5). Many localities harbouring species in sympatry include the four most widespread: *pan*, *turneri*, *thysbe* and *chrysaor* (Supplementary Table S6). Of 38 localities with two or more sympatric *Chrysoritis* species, 29 include one of these four widespread species, and 20 include two or more additional non-widespread species.

Some of these localities have closely related, sympatric species, and these would be good candidates for studies of reproductive viability and assessment of species limits. Different behaviours that could promote mate discrimination have been observed in some of these closely related, sympatric species. For example, Heath and Claassens (2000) document hilltopping, where males differentially patrol hilltops or ridges, or select gullies or other landmarks that may facilitate mate-searching by virgin females.

Localities with many sympatric species are concentrated in Western South Africa, maps with overlapping known and modelled species distributions also show this pattern, particularly in the West Fynbos (Fig. 5). Species from the eastern lineage (the *chrysaor* clade) rarely co-occur with congeners other than *C. chrysaor.*

Interestingly, species pairs outside the *thysbe* clade showing low genetic diversity, such as *zeuxo/zonarius*, *aethon/aureus* or *lycegenes/lyncurium*, do not overlap geographically. These examples highlight cases of recent divergence in allopatry where assessment of species limits is challenging. The triplet *chrysaor/natalensis/midas* are not recovered as reciprocally monophyletic. These species are distributed parapatrically but are not syntopic and show a certain degree of

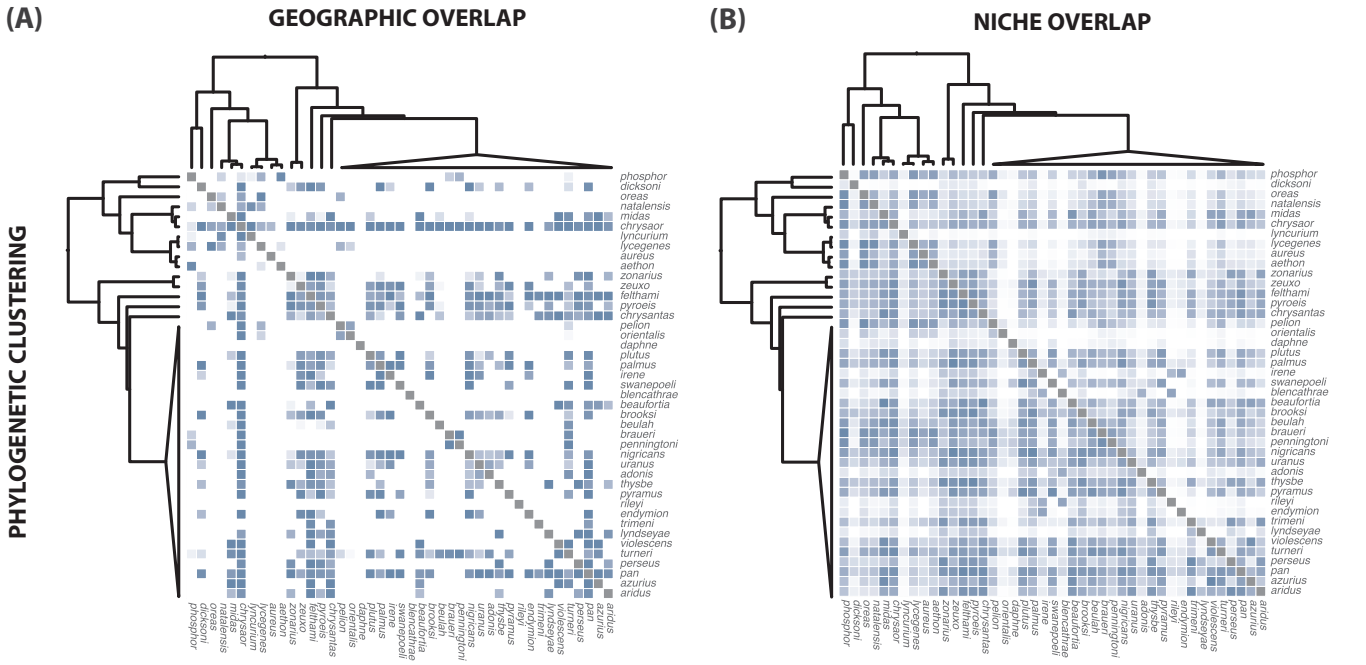

Fig. 4. Heatmaps of the percentage of A) geographic overlap and B) climatic niches overlap between *Chrysotitis* species. Species are sorted according to the *BEAST species species tree (phylogenetic clustering). Geographical overlap (or range area shared between each species pair) was calculated by means of convex Hull areas (minimum convex polygons) using R package dismo (Hijmans et al, 2012). Niche range per species was calculated based on the best climatic niche fit models estimated from 8 ecological variables and modelled with MaxEnt. Niche overlap was predicted by calculating the probability surfaces using the function "*niche.overlap*" in the R package phyloclim (Heibl, 2012). White indicates no overlap, dark blue indicates total overlap. Climatic niche overlap between most species is remarkable. Geographic overlap seems to mainly involve widespread species. In both cases, there seems to be no tendency to overlap by phylogenetic proximity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

climatic niche differentiation (Fig. 4). Since species pairs within *thysbe* are not resolved, no accurate assessments can be made regarding evolution in sympatry or allopatry, unless a scenario of widespread gene flow is accepted for the species-group as a whole. Several localities contain species from the *thysbe* group in sympatry, with up to four at Hondeklip Bay. It is worth noting that certain species with extremely restricted distributions, such as *adonis*, *irene*, *endymion*, *brooksi* or *lyndseyae* occur in sympatry with close relatives (Fig. 5, Supplementary Fig. S2). The apparent reproductive isolation in these sympatric localities might be explained by historical distribution shifts leading to secondary sympatry. Given their low genetic divergence, these secondary encounters might have happened after some degree of differentiation in isolation, long enough to fix a few characters but not long enough to generate complete reproductive barriers (Pigot and Tobias, 2012, 2015). Alleged microclimatic fluctuations that happened during the Pleistocene in South Africa (Cottrell, 1978) might have promoted processes of this sort, and might explain the reticulated phylogenies within the *thysbe* group better than ecological specialization leading to speciation in sympatry, which would result in monophyletic taxa. Populations may well have shifted their distributions depending on recent palaeoclimatological changes, particularly in a relatively small region as such as the Western Fynbos where most *Chrysoritis* diversity is concentrated and thus where sympatric encounters are more likely. Given this scenario of partial sorting but reproductive compatibility, the delimitation of species is particularly challenging.

4.3. Chrysoritis taxonomy

Chrysoritis has been the centre of a passionate but largely unpublished debate (Williams, 2004). This debate has taken place from the species level to deeper taxonomic levels. In a review of the subfamily, Heath (1997) subsumed and synonymised the genera *Poecilmitis* Butler, 1899; *Bowkeria* Quickelberge, 1972; and *Oxychaeta* Tite and Dickson, 1973 within *Chrysoritis* Butler, based primarily on the relative uniformity of the male genitalia. Such synonymies are reasonable in the current phylogenetic context, since genera would otherwise become paraphyletic. It is worth noting though, that the age of the genus *Chrysoritis* (32 Mya) is among the oldest in the Aphnaeinae (Boyle et al., 2015) and quite old compared with typical ages of genera in the Lycaenidae. For example, Polyommatinae includes genera as young as 4 Mya (Talavera et al., 2013). The two biogeographically differentiated lineages in East and West South Africa, each undergoing different rates of diversification, raises the question of splitting *Chrysoritis* into two genera, one for the *chrysaor* clade, and one for the rest (including the *thysbe* species-group). However, the relatively uniform genitalia throughout the genus (Heath, 1997) together with the unique coloration within the subfamily mitigate against such action. Other higher-level clusters typically used in *Chrysoritis* are the informal categories "species-groups" (Heath, 1997; Williams, 2018). The concept of species-groups blends the species and the subgenus categories, and is traditionally employed to explain unusual species diversity with uncertain characters. We emphasize that the use of 'species-groups' in *Chrysoritis* (which implicitly attempts to identify synapomorphic traits) is largely unnecessary, especially given the phylogenetic evidence presented here, with the exception of the *thysbe* species-group, where it is still useful because taxa in this clade are so clearly unresolved. For all other taxa, reference to clades should replace reference to species-groups.

Because *Chrysoritis* genitalia hardly vary, species level taxonomy is based primarily on differences in wing patterns. Population variation has further injected subjectivity into species delimitation in this group, and encouraged a tendency toward oversplitting (Heath, 1997). To rectify this situation, Heath (2001) synonymized 17 species, based on morphological similarities; thereby reducing the number of *Chrysoritis* species from 59 to 42 but causing much consternation (Williams 2004). Terblanche and van Hamburg (2004) noted a lack of sufficient supporting evidence for these synonymies, although they did not address the lack of adequate justification for species designation in the first place. Heath and Pringle (2007) inferred the need for further

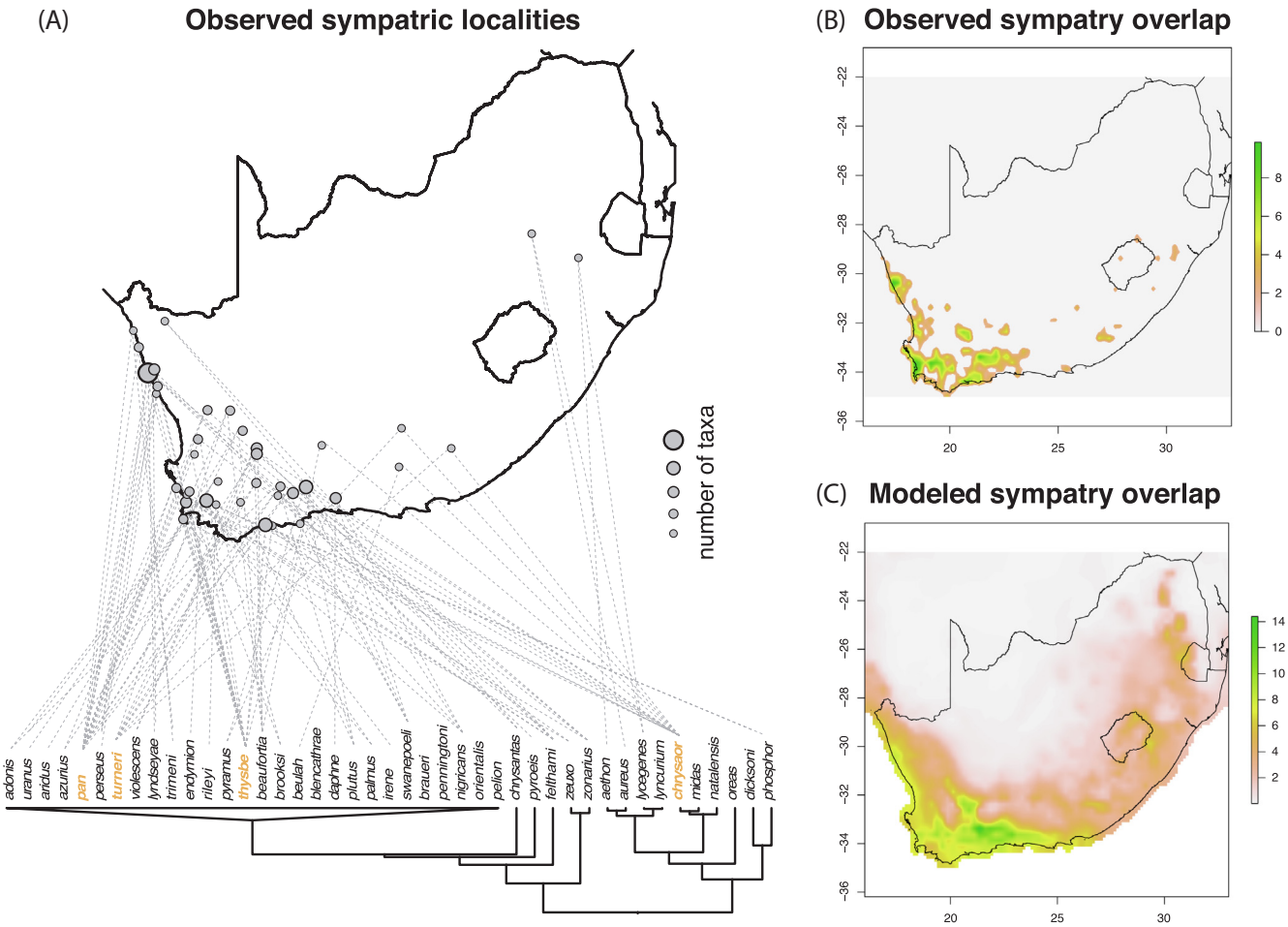

Fig. 5. A) Localities with sympatric *Chrysoritis* species their phylogenetic affinities. Taxa in orange are those most widely distributed and show higher number of shared localities. B) Patterns of distributional overlap based on observed distributions and C) based on modelled distributions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

synonymies, but chose not to make changes at the species level until molecular evidence was available.

The phylogenetic evidence presented in this paper suggests that *Chrysoritis* is oversplit. The fact that the diversification rate of the *thysbe* species-group without synonymization approximates that of *Agrodiaetus*, which has unusual chromosomal instability and has diversified faster than almost any other insect, further suggests that oversplitting is likely. By strictly applying a phylogenetic systematics criteria, only monophyletic species should be retained, and this would suggest that the entire species-group should simply be designated as *thysbe*. However, this approach fails to recognise significant morphological and ecological variation in this group. It is possible that deeper genomic assessments could unravel evolutionary traces of differentiation beyond the resolution power of the four molecular markers used here, although the extremely young age (~2 Mya) has afforded little time for lineage sorting to occur. A detailed taxonomic revision will need to integrate molecular evidence in the light of known morphology and ecology, as well as distribution and climatic niche overlap patterns. Additional natural history information that would complement molecular data would be helpful. Potential pre- and post-zygotic barriers for species in sympatry should be studied on a *per case* basis, and further applying this knowledge to those several cases of allopatric species, for which species limits are more complex to address. Species with dot-like distributions including *adonis, lyndseyae, endymion, rileyi, blencathrae, daphne, irene* probably need stronger justification to sustain their specific status, particularly in the light of low genetic divergence (Vila et al., 2010). These cases contrast with the ancient, biogeographically relictual species *C. dicksoni*. Allopatric species-pairs (or closely related species) with low genetic divergence, such as *aridus/azurius, lyndseyae/trimeni*, *endymion/rileyi*, *aethon/aureus* or *lycegenes/lyncurium*, also need to be re-examined, partly considering their relative genetic divergences in relation to other well characterized species pairs.

Synonymizing species within *thysbe* should therefore be considered, unless additional, strong alternative evidence can be obtained that could explain the observed phylogenetic reticulation. On a practical level, the singularity of these species is important and should be recognized in conservation policies and this could be achieved by considering the currently designated *thysbe* taxa as subspecific categories. This would enable all *thysbe* taxa to be designated by phenotype (based on wing shape, extent of iridescence, ant association or male patrolling preference, for example) in a manner that would allow them to count as discrete and identifiable taxonomic units for conservation.

5. Conclusions

Chrysoritis butterflies have diversified in fynbos as found in a variety of other groups of insects and plants. Our analyses show a set of relatively old lineages, but the largest group of species arose in an explosive radiation that is too recent for lineage sorting to have taken place. The molecular loci we used are not monophyletic for most of these species, and taxonomic designations are thus highly contingent on the species concept used and other hard-to-get information, such as reproductive compatibility. This explosive radiation in the *thysbe* species-group seems to not be due to geography. Instead, ecology seems to be

important, but future work is needed to ascertain what the precise factors are, which may be related to specialized habits of the myrmecophilous larvae.

CRediT authorship contribution statement

Gerard Talavera: Conceptualization, Methodology, Investigation, Writing - original draft, Visualization. **Zofia A. Kaliszewska:** Conceptualization, Investigation, Resources, Data curation, Writing - review & editing. **Alan Heath:** Conceptualization, Investigation, Resources, Writing - review & editing. **Naomi E. Pierce:** Conceptualization, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgements

We thank Steve Woodhall, Harald Selb, Simon Joubert, Alf Curle, and Ernest Pringle for contributing samples. We thank David J. Lohman and two anonymous reviewers for helpful comments. GT was supported by the MINECO programme Juan de la Cierva Incorporación (IJCI-2016-29083), the Marie Curie Actions FP7-People-2013 IOF (project 622716), "La Caixa" Foundation (LCF/BQ/PR19/11700004), and the National Geographic Society (grant #WW1-300R-18). ZAK was supported by an NSF Graduate Research Fellowship. Collecting expeditions were made possible by grants from the Putnam Expeditionary Fund of the Museum of Comparative Zoology (MCZ, Harvard University) to ZAK, and the research was supported by NSF DEB-0447244 and DEB-1541560 to NEP.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2020.106817.

References

Allsopp, N., Colville, J.F., Verboom, G.A. (Eds.), 2016. Fynbos: Ecology, Evolution and Conservation of a Megadiverse Region. Oxford University Press, pp. 414.
Becker, A., Wilks, A.R., Brownrigg, R., Minka, T.P., Deckmyn, A., 2018. maps: Draw Geographical Maps. R package version 3 (3).
Williams, M.C., 2018. Afrotropical Butterflies and Skippers: a digital encyclopaedia. Lepidopterists' Society of Africa. http://www.lepsocafrica.org/?p=publications&s=atb.
Bivand, R., Lewin-Koh, N., 2013. maptools: tools for reading and handling spatial objects. R package version 0.8-25. Available at: http://CRAN.R-project.org/package=maptools.
Blaimer, B.B., 2012. Acrobat ants go global – Origin, evolution and systematics of the genus *Crematogaster* (Hymenoptera: Formicidae). Mol. Phylogenet. Evol. 65, 421–436.
Bouckaert, R.R., 2010. DensiTree: making sense of sets of phylogenetic trees. Bioinformatics 26, 1372–1373.
Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10, e1003537.
Boyle, J.H., Kaliszewska, Z.A., Espeland, M., Suderman, T.R., Fleming, J., Heath, A., Pierce, N.E., 2015. Phylogeny of the Aphnaeinae: myrmecophilous African butterflies with carnivorous and herbivorous life histories. Syst. Entomol. 40, 169–182.
Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. PNAS 91, 6491–6495.
Cottrell, C.B., 1978. Aspects of the biogeography of southern African butterflies: revealed by an investigation of the nature of the Cape butterfly fauna. Supplement to Zambezia (J. Univ. Rhodesia) i-viii, 1–100.
Cowling, R.M., Richardson, D., 1995. Fynbos: South Africa's Unique Floral Kingdom. Fernwood Press, Vlaeberg, pp. 156.
Cowling, R.M., Procheş, Ş., Partridge, T.C., 2009. Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. Mol. Phylogenet. Evol. 51, 64–74.
Coyne, J.A., Orr, A.H., 2004. Speciation. Sinauer, Sunderland, MA.
Dapporto, L., Cini, A., Voda, R., Dinca, V., Wiemers, M., Menchetti, M., Magini, G., Talavera, G., Shreeve, T., Bonelli, S., Casacci, L.P., Balletto, E., Scalercio, S., Vila, R., 2019. Integrating three comprehensive data sets shows that mitochondrial DNA variation is linked to species traits and paleogeographic events in European butterflies. Mol. Ecol. Resour. 19, 1623–1636.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Meth. 9, 772.
Douglas, J.M., Cronin, T.W., Chiou, T.H., Dominy, N.J., 2007. Light habitats and the role of polarized iridescence in the sensory ecology of neotropical nymphalid butterflies (Lepidoptera: Nymphalidae). J. Exp. Biol. 210, 788–799.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLOS Biol. 4, e88.
Fordyce, J.A., 2010. Host shifts and evolutionary radiations of butterflies. Proc. Royal Soc. B Biolog. Sci. 277, 3735–3743.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
Gabriel, A.G., 1946. A new species of *Phasis* (Lepid. Lycaenidae) from South Africa. The Entomologist 80, 60–61.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.
Heath, A., 1997. A review of African genera of the tribe Aphnaeini (Lepidoptera: Lycaenidae). Metamorphosis 8 (Suppl. 2), 1–60.
Heath, A., 2014. Uncovering secrets of the "cuckoo" butterfly species *Chrysoritis dicksoni* (Gabriel, 1947), a social parasite of *Crematogaster* ants: A summary to the end of the 20th century with current conclusions. Metamorphosis 25, 5–10.
Heath, A., Claassens, A.J.M., 2000. New observations of ant associations and life history adaptations (Lepidoptera: Lycaenidae) in South Africa. Metamorphosis 11, 3–19.
Heath, A., 2001. New synonymies and taxonomic notes on the genus *Chrysoritis* Butler (Lepidoptera: Lycaenidae). Metamorphosis 12, 85–98.
Heath, A., Pringle, E.L., 2004. A review of the southern African genus *Thestor* Hübner (Lepidoptera: Lycaenidae: Miletinae). Metamorphosis 15, 91–151.
Heath, A., Pringle, E.L., 2007. Biological observations and a taxonomic review based on morphological characters in the myrmecophilous genus *Chrysoritis* Butler. (Lepidoptera: Lycaenidae: Aphnaeini). Metamorphosis 18(1) + CD.
Heath, A., Claassens, A.J.M., 2003. Ant-association among Southern African Lycaenidae. Journal of the Lepidopterists' Society 57 (1), 1–16.
Heath, A., McLeod, L., Kaliszewska, Z.A., Fisher, C.W.S., Cornwall, M., 2008. Field notes including a summary of trophic and ant-associations for the butterfly genera *Chrysoritis* Butler, *Aloeides* Butler and *Thestor* Hubner (Lepidoptera: Lycaenidae) from South Africa. Metamorphosis 19, 127–148.
Heath, A., 2011. Taxonomic issues between Chrysoritis lyndseyae (S.F. Henning) stat. rev. and C. thysbe bamptoni (Dickson) (Lepidoptera: Lycaenidae: Aphnaeini). Metamorphosis 22: 97–101 + 4 pl.
Heibl, C. 2012. phyloclim: Integrating phylogenetics and climatic niche modelling. URL: http://cran.r-project.org/web/packages/phyloclim/.
Hernández-Roldán, J.L., Dapporto, L., Dinca, V., Vicente, J.C., Hornett, E.A., Šichová, J., Lukhtanov, V., Talavera, G., Vila, R., 2016. Integrative analyses unveil speciation linked to host plant shift in *Spialia* butterflies. Mol. Ecol. 25, 4267–4284.
Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965–1978.
Hijmans, R.J., 2012. Cross-validation of species distribution models: removing spatial sorting bias and calibration with a null model. Ecology 93, 679–688.
Hijmans, R.J. 2019. raster: Geographic analysis and modeling with raster data. R package version 3.0-7. URL: http://CRAN.R-project.org/package=raster.
Joly, S., Heenan, P.B., Lockhart, P.J., 2014. Species radiation by niche shifts in New Zealand's rockcresses (Pachycladon, Brassicaceae). Syst. Biol. 63, 192–202.
Kandul, N.P., Lukhtanov, V.A., Pierce, N.E., 2007. Karyotypic diversity and speciation in *Agrodiaetus* butterflies. Evolution 61, 546–559.
Kaliszewska, Z.A., Lohman, D.J., Sommer, K., Adelson, G., Rand, D.B., Mathew, J., Talavera, G., Pierce, N.E., 2015. When caterpillars attack: biogeography and life history evolution of the Miletinae (Lepidoptera, Lycaenidae). Evolution 69, 571–588.
Klak, C., Reeves, G., Hedderson, T., 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. Nature 427, 63.
Mackintosh, A., Laetsch, D.R., Hayward, A., Charlesworth, B., Waterfall, M., Vila, R., Lohse, K., 2019. The determinants of genetic diversity in butterflies. Nat. Commun. 10, 3466.
Magallon, S., Sanderson, M.J., 2000. Absolute diversification rates in angiosperm clades. Evolution 55, 1762–1780.
Matzke, N., 2013. BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R scripts, CRAN: The Comprehensive R Archive Network. cran.r-project. Retrieved from org/web/packages/BioGeoBEARS/index.html.
Mecenero, S., Ball, J.B., Edge, D.A., Hamer, M.L., Henning, G.A., Krüger, M., Pringle, E.L., Terblanche, R.F., Williams, M.C. (Eds)., 2013. Conservation assessment of butterflies of South Africa, Lesotho and Swaziland: Red List and Atlas. pp. 13 –33. Saftronics (Pty) Ltd., Johannesburg & Animal Demography Unit, Cape Town. 676 pp.
Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. Syst. Biol. 62, 789.
Lukhtanov, V.A., Kandul, N.P., Plotkin, J.B., Dantchenko, A.V., Haig, D., Pierce, N.E., 2005. Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiaetus* butterflies. Nature 436, 385–389.
Lefort, L., Longueville, J.-E., Gascuel, O., 2017. SMS: Smart model selection in PhyML. Mol. Biol. Evolut msx149.
Peña, C., Witthauer, H., Klečková, I., Fric, Z., Wahlberg, N., 2015. Adaptive radiations in butterflies: evolutionary history of the genus Erebia (Nymphalidae: Satyrinae). Biol. J. Linn. Soc. 116, 449–467.
Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231–259.
Pierce, N.E., Braby, M.F., Heath, A., Lohman, J., Mathew, J., Rand, D.B., Travassos, M.A., 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu. Rev. Entomol. 47, 733–771.

Pigot, A.L., Tobias, J.A., 2012. Species interactions constrain geographic range expansion over evolutionary time. Ecol. Lett. 16, 330–338.
Pigot, A.L., Tobias, J.A., 2015. Dispersal and the transition to sympatry in vertebrates. Proc. Royal Soc. B 282, 1799.
Pirie, M.D., Oliver, E.G.H., de Kuppler, A.M., Gehrke, B., Le Maitre, N.C., Kandziora, M., Bellstedt, D.U., 2016. The biodiversity hotspot as evolutionary hot-bed: spectacular radiation of Erica in the Cape Floristic Region. BMC Evol. Biol. 16, 190.
Plummer, M., Best, N., Cowles, K., Vines, K., 2006. CODA: convergence diagnosis and output analysis for MCMC. R news 6 (1), 7–11.
Procheş, S., Cowling, R.M., 2006. Insect diversity in Cape fynbos and neighbouring South African vegetation. Glob. Ecol. Biogeogr. 15, 445–451.
Quek, S.P., Davies, S.J., Itino, T., Pierce, N.E., 2004. Codiversification in an ant-plant mutualism: Stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution 58, 554–570.
Rabosky, D.L., Santini, F., Eastman, J., Smith, S.A., Sid- Lauskas, B., Chang, J., Alfaro, M.E., 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nature Commun. 4, 1958.
Rabosky, D.L., Donnellan, S.C., Grundler, M., Lovette, I.J., 2014a. Analysis and visualization of complex macroevolutionary dynamics: an example from Australian scincid lizards. Syst. Biol. 63, 610–627.
Rabosky, D.L., Grundler, M., Anderson, C., Title, P., Shi, J.J., Brown, J.W., Huang, H., Larson, J.G., 2014b. BAMM-tools: An R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods Ecol. Evol. 5, 701–707.
Rambaut, A., Drummond, A.J., 2007. Tracer version 1.5. Available at: http://beast.bio.ed.ac.uk/Tracer.
Rand, D.B., Heath, A., Suderman, T., Pierce, N.E., 2000. Phylogeny and life history evolution of the genus *Chrysoritis* within the Aphnaeini (Lepidoptera: Lycaenidae), inferred from mitochondrial *cytochrome oxidase I* sequences. Mol. Phylogenet. Evol. 17, 85–96.
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.
Ronquist, F., 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.
Schär, S., Eastwood, R., Arnaldi, K.G., Talavera, G., Kaliszewska, A., Boyle, J.H., Espeland, M., Nash, N.R., Vila, R., Pierce, N.E., 2018. Ecological specialisation is widely associated with genetic structure in the ant-associated butterfly family Lycaenidae. Proc. Royal Soc. B 12, 285.
Schmitt, T., Louy, D., Zimmermann, E., Habel, J.C., 2016. Species radiation in the Alps: multiple range shifts caused diversification in Ringlet butterflies in the European high mountains. Org. Divers. Evol. 16, 791–808.
Schnute, J.T., Boers, N.M., Haigh, R., and Couture-Beil, A., 2015. PBSmapping 2.69: user's guide revised from Canadian Technical Report of Fisheries and Aquatic Sciences 2549: vi + 43 p.
Stavenga, D.G., Giraldo, M.A., Leertouwer, H.L., 2010. Butterfly wing colors: Glass scales of *Graphium sarpedon* cause polarized iridescence and enhance blue/ green pigment coloration of the wing membrane. J. Experim. Biol. 213, 1731–1739.
Talavera, G., Lukthanov, V.A., Pierce, N.E., Vila, R., 2013. Establishing criteria for higher level taxonomic classification using molecular data: The systematics of *Polyommatus* blue butterflies (Lepidoptera, Lycaenidae). Cladistics 29, 166–192.
Terblanche, R.F., van Hamburg, H., 2004. The taxonomy, biogeography and conservation of the myrmecophilous *Chrysoritis* butterflies (Lepidoptera: Lycaenidae) in South Africa. AGRIS 46, 65–81.
Vershinina, A.O., Lukhtanov, V.A., 2017. Evolutionary mechanisms of runaway chromosome number change in *Agrodiaetus* butterflies. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-08525-6.
Vila, R., Lukhtanov, V.A., Talavera, G., Gil-T, F., Pierce, N.E., 2010. How common are dot-like distribution ranges? Taxonomical oversplitting in Western European *Agrodiaetus* (Lepidoptera, Lycaenidae) revealed by chromosomal and molecular markers. Biolog. J. Linnean Soc. 101, 130–154.
Warren, D.L., Glor, R.E., Turelli, M., 2008. Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. Evolution 62, 2868–2883.
Williams, M.C., 2004. Editorial. Metamorphosis 15, 90.