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Landscape genetics indicate recently increased habitat fragmentation in African forest-associated chafers

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Abstract

Today, indigenous forests cover less than 0.6% of South Africa's land surface and are highly fragmented. Most forest relicts are very small and typically occur in fire-protected gorges along the eastern Great Escarpment. Yet, they hold a unique and valuable fauna with high endemism and ancient phylogenetic lineages, fostered by long-term climatic stability and complex microclimates. Despite numerous studies on southern African vegetation cover, the current state of knowledge about the natural extension of indigenous forests is rather fragmentary. We use an integrated approach of population-level phylogeography and climatic niche modeling of forest-associated chafer species to assess connectivity and extent of forest habitats since the last glacial maximum. Current and past species distribution models ascertained potential fluctuations of forest distribution and supported a much wider potential current extension of forests based on climatic data. Considerable genetic admixture of mitochondrial and nuclear DNA among many populations and an increase in mean population mutation rate in Extended Bayesian Skyline Plots of all species indicated more extended or better connected forests in the recent past (<5 kya). Genetic isolation of certain populations, as revealed by population differentiation statistics (G'_{ST}), as well as landscape connectivity statistics and habitat succession scenarios suggests considerable loss of habitat connectivity. As major anthropogenic influence is likely, conservational actions need to be considered.

Keywords: beetles, niche modeling, phylogeography, *Pleophylla*, Scarabaeidae

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Introduction

Forests cover about one-quarter of earth's surface (Bartholomé & Belward, 2005) but harbor more than half of terrestrial biodiversity (Millennium Ecosystem Assessment, 2005a). They provide important ecosystem services on a global and a regional scale, including climate regulation, carbon storage, and erosion control (Foley *et al.*, 2005; Millennium Ecosystem Assessment, 2005b; Newbold *et al.*, 2015). Although there is evidence that South Africa's land surface was covered by extended forests in the past (Deacon *et al.*, 1983), it is today predominantly covered by open habitats like grassland, Fynbos, Karoo, and savannah biomes (Mucina & Rutherford, 2006; Huntley *et al.*, 2016). Less than 0.6% of the area of South Africa is covered by indigenous forests (Low & Rebelo, 1996; Mucina & Rutherford, 2006) which are predominantly found along the Great Escarpment as well as the South and South-Eastern coasts. The vast majority of the forests is highly fragmented with 78.5% of the recorded forest patches being smaller than 1 km² (estimate based on data of Mucina & Rutherford, 2006). Diversity and patchiness of the forests imply their relictual character (Deacon *et al.*, 1983; Mucina & Rutherford, 2006). Although past climatic fluctuations in southern Africa repeatedly caused forests to expand and retreat again (Geldenhuys, 1997; Dupont *et al.*, 2011; Huntley *et al.*, 2016), anthropogenic influence is major cause for an extreme forest retreat within the last centuries (Fourcade, 1889; Bews, 1913, 1920; King, 1941; Acocks, 1953; Bond *et al.*, 2003). Records of first explorers (e.g., Vasco da Gama 15th century; Ravenstein, 1898) might be interpreted in a way that coastal indigenous forests were widely expanded. Also field surveys, paleoenvironmental evidence, and climatic niche modeling proposed that areas in south-eastern South Africa had and have the potential to be widely covered by forest (Acocks, 1953; Eeley *et al.*, 1999; Bond *et al.*, 2003; Chase & Meadows, 2007; Quick *et al.*, 2011).

Grasslands are thought to have expanded at the expense of forests in many parts of the world because they withstand conditions that limit the establishment or survival of woody species (Bond, 2008; Edwards *et al.*, 2010). Besides grazing of large herbivores and xeric climatic conditions, fires are one of the most important natural drivers of vegetation structure in southern Africa (Phillips, 1930; Little *et al.*, 2013), but man-made fire regimes with more frequent burning of

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smaller areas have replaced natural ones (Deacon, 1983a; Archibald *et al*., 2013) and sometimes even threat fire-adapted ecosystems (Reside *et al*., 2012). There is evidence that man used fire for vegetation management for at least 100 ky (Deacon, 1983a; Deacon & Deacon, 1999) and cleared large portions of forest particularly at the east coast of southern Africa (Fourcade, 1889; Bews, 1913, 1920; King, 1941; Acocks, 1953; Castley & Kerley, 1996). When Vasco da Gama sailed along the southern African coast in 1498, burning fires along the coast probably led him to name places 'Ponta das Queimadas' (Gulf of forest fires, St. Francis Bay) or 'Terra dos Fumos' (Land of smoke, around todays Maputo) (Ravenstein, 1898; Bews, 1913). Today, native forest-ecosystems are invaded by fire-adapted alien plants (Brooks *et al*., 2004) and agricultural areas are retained by fires. Currently, South African conservation management controls alien invaders' expansion by burning of uprising woodland to preserve fire-adapted Fynbos and grassland biomes (van Wilgen, 2009; van Wilgen *et al*., 2012), which also inhibits restoration of indigenous forests (Luger & Moll, 1993). The crucial question in this context is, whether or not and to which extent indigenous forest is the potential natural vegetation (vs. grassland). Besides that, indigenous forests are also threatened by their exploitation to satisfy needs for building material, fuel wood, food, and medicine (Mucina & Rutherford, 2006).

Most forest plants and associated insects disappear quite abruptly with forest clearance. However, depending on land use intensity and abiotic factors like shading, forest soils retain their original properties for several years (Balesdent *et al*., 1988; Lemenih *et al*., 2005) and thus stay suitable for most of its soil fauna. One such element of soil fauna is *Pleophylla*, a genus of soil dwelling scarab chafers (Coleoptera: Scarabaeidae) which occurs predominantly in the isolated forest patches throughout South Africa. It expands with a few species in the Afromontane forests along the Eastern Arc up north to Uganda and D.R. Congo (Eberle *et al*., 2016a), a pattern that is also observed in other forest-associated species (e.g., Huber, 2003). It is one of the oldest lineages of the highly diverse tribe of Sericini (Ahrens, 2006; Eberle *et al*., 2016b) that originated ca. 79 Mya and showed a burst of speciation since the Miocene (Eberle *et al*., 2016c). Most available records of *Pleophylla* are located in or in close vicinity to forest remnants; therefore, the genus is suspected to be forest associated. However, their polyphagous feeding habits makes them quite independent from specific forest plant species: the fully winged adults of *Pleophylla* feed, as most Sericini, polyphagously on leaves of a variety of angiosperms including many allochthonous ones, while their larvae develop in the upper soil strata, feeding on organic matter and plant roots. Therefore, these beetles can still exist when primary indigenous forest plants have gone and thus may serve as a proxy for past or potential forest distribution in South Africa.

In the present study, we address the question on the natural extent of South African forests by combining population genetics, spatial distribution, and climatic niche modeling (Fig. 1a) of populations of nine species of *Pleophylla* to assess present and past connectivity of forest habitats. We investigated the degree of genetic isolation of populations since the last glacial period, using fast evolving mitochondrial and nuclear loci. Good genetic admixture is expected if forests are currently or were recently more extended or at least well connected through migration corridors. The opposite (i.e., poor admixture and extreme endemism) is expected for poorly connected, long-term isolated and little extended forests. The demographic histories of the species were examined to detect population size alterations that might be related to habitat expansion or fragmentation. Current and past distribution models of the species were used to infer potential fluctuations of distribution ranges which might be linked to forest distribution and to further explore the hypothesis of historically more extended forest. Genetic population structure inference was backed by landscape connectivity analyses based on species distribution models (SDM) inferred from climatic data. Three different scenarios were evaluated by using the unrestricted SDM (F0), by additional consideration of forest patches (forest-accounting SDM; F1) and by excluding potentially suitable soils for *Pleophylla* larvae outside forest occurrences (forest-restricted SDM; F2) (Fig. 1). Knowledge about the connectivity among *Pleophylla* and other forest dwelling species' populations (Fig. 1a, F1) appears to be crucial to identify areas for high-priority conservation of forest faunas. Based on the combined evidence from *Pleophylla*, we discuss the potential distribution of forests and the anthropogenic influence on forest habitats in South Africa.

Materials and methods

Sampling and assessment of forest association

The distribution data of *Pleophylla* included 319 specimens of which DNA data were available (Tables S1 and S2) and 828 dry specimens from 12 different museum collections (Eberle *et al*., 2016a). Most specimens were collected with light traps at 172 unique localities. They were identified by examining the dissected male genitalia, females partly through match of species-specific DNA markers (Eberle *et al*., 2016a,c). Collection locations of museum specimens without GPS data were localized using the GeoNames geographical database

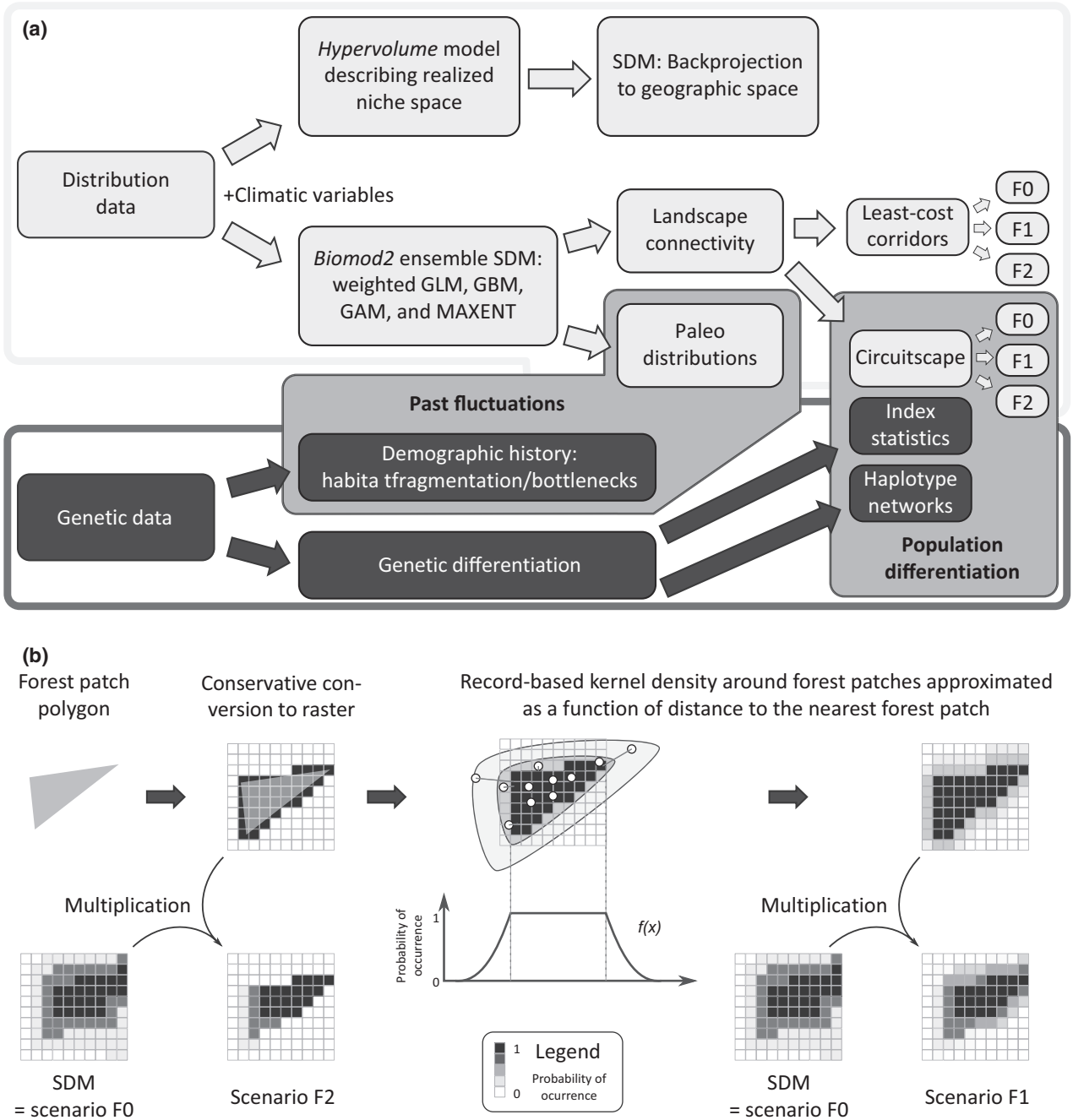

Fig. 1 Integration of climatic niche modeling and molecular analyses. (a) Flow chart illustrating batched analysis steps in two lines of evidence (dark gray: genetic and light gray: niche modeling). Medium gray background boxes highlight complementary results. (b) Diagrammed steps to SDMs based on actual forest distribution (scenarios F1 and F2). Please refer to the main text for detailed explanation.

(www.geonames.org) and Google Maps (www.google.com/maps). The forest-association hypothesis of *Pleophylla* was assessed using the distribution data of all southern African Sericini species (20 000 specimens comprising ca. 400 species; Eberle *et al*., 2016a). Collection sites, where no species of *Pleophylla* were recorded, are highly informative in this context, because all Sericini are collected with the same method during the same season (predominantly with light traps). Therefore, these localities can be likely considered absence data on a rough scale. The forest association of *Pleophylla* is a fundamental assumption of this study. Its reliability was evaluated by measuring the distance of the sampling points from forest patch polygons of Mucina & Rutherford (2006) in QGIS (v. 2.10, www.qgis.org, NNjoin plugin). Eight records of *Pleophylla*

specimens with spuriously large distances to forest (>20 km) were checked on satellite images (CNES/Astrium, Feb. and Apr. 2016; DigitalGlobe®, Sep. 2014; accessed via Google Earth) and not considered in this analysis, because very small forest patches were found nearby that were not digitized by Mucina & Rutherford (2006). An analysis of variance (ANOVA) of all specimens of *Pleophylla*, all other southern African Sericini, and 1000 randomly distributed points in South Africa was performed in R (R Core Team, 2015), followed by pairwise *t*-tests; *P*-values were adjusted by the Holm method (Holm, 1979).

Analyses of genetic variation

Specimen collection, preservation, and DNA extraction followed Ahrens & Vogler (2008). A total of 347 specimens were used for molecular analyses. Qiagen® Multiplex PCR Kits were used to amplify the 3′ end of cytochrome oxidase subunit 1 (*cox1*) with the primer pairs stevPat and stevJerry (Timmermans *et al.*, 2010). Primer pairs ITS1F and ITS1R (Hillis & Dixon, 1991; Vogler & Desalle, 1994) were used for the amplification of the internal transcribed spacer *ITS-1*. Sequencing was done by Macrogen (Seoul, South Korea). Sequences were aligned per marker using MAFFT (v7.017; Katoh *et al.*, 2002) and subsequently checked by eye in Geneious® 7.1.8. All DNA voucher specimens are deposited in the collections of the Zoological Research Museum A. Koenig, Bonn (ZFMK). GenBank Accessions are listed in Table S1.

The genetic variation of South African *Pleophylla* species was investigated on the basis of haplotype networks in *pegas* (v. 0.8-2; Paradis, 2010; R Core Team, 2015). Distances between haplotypes were calculated under pairwise deletion of missing data. The networks were colored according to the sampling localities to visualize genetic differentiation in geographical context. Spatial isolation might indicate limited gene flow since the last glacial due to range fragmentation (Templeton *et al.*, 2001). Therefore, we calculated the standardized measure of genetic differentiation G'_{ST} (Hedrick, 2005) between the sampling localities of each species separately for *cox1* (826 bp length) and ITS1 (853 bp length) using *diveRsity* (v. 1.9.73; Keenan *et al.*, 2013).

Genetic bottlenecks may occur by strong reduction or fragmentation of a species range and leave traces in the DNA of populations. Indications for such reductions in *Pleophylla*, most likely linked to the loss of habitat (i.e., forests), were inferred by the reconstruction of the species' demographic histories using Extended Bayesian Skyline Plots (EBSP; Heled & Drummond, 2008) of sufficiently sampled species (i.e., with a sample size >10 specimens). The analyses were conducted with BEAST (v. 1.8.1; Drummond *et al.*, 2012) on both markers. The substitution models, clocks, and trees were unlinked for *cox1* and ITS1 partitions. Optimal substitution models were inferred with PartitionFinder (Lanfear *et al.*, 2012, 2014) (Table S3). Because low levels of rate variation are expected in intraspecific data sets, a strict molecular clock was used for each partition (Brown & Yang, 2011). The analyses were time calibrated by setting the pairwise divergence rate of *cox1* to 3.54% My^{-1} (Papadopoulou *et al.*, 2010). The rate of ITS1 was estimated relative to that rate under a uniform prior. The analyses were all run twice for 60 million generations and subsequently combined with *burntrees* (v. 0.2.2; Nylander, 2014) after removing a burnin of 10%. Stationarity of repeated runs at similar values and convergence was assessed with Tracer (v.1.6.0; Rambaut *et al.*, 2014) before conducting the demographic analyses on the combined output.

Species distribution modeling

Environmental predictors for species distribution models (SDMs) were compiled from a set of 19 bioclimatic variables (http://www.worldclim.org/bioclim) at a spatial resolution of 30 arc seconds available from WorldClim (Hijmans *et al.*, 2005). To restrict the overall environmental background, a BIOCLIM model (Busby, 1991; Booth *et al.*, 2014) based on all sampling points of *Pleophylla* was calculated, and original variables were clipped to it (Table S6). This initial step was necessary to reduce computation time without omitting potentially suitable areas, as hypervolume models (see below) are per definition nested within an overall BIOCLIM model. Following Blonder *et al.* (2014), a spatial principle component analysis was performed based on the clipped background to create an orthogonal niche space, only retaining components with Eigenvalues >1. This step is crucial as the hypervolume analyses require an orthogonal parameter space to avoid pseudo-replication.

Niches were quantified following Hutchinson's original niche concept of n-dimensional hypervolumes (Hutchinson, 1957) enclosing all environmental conditions which allow infinite existence of populations. Recently Blonder *et al.* (2014), Blonder (2015) provided the R package *hypervolume* (Blonder, 2015; R Core Team, 2015) allowing for the first time to compute even high-dimensional hypervolumes that are based on multidimensional kernel density estimators to derive a density distribution of species records in PCA space. This density distribution is used to compute the total volume of the species' realized niche space and allows geometric operations of multiple hypervolumes including intersection, unique proportions etc. (Blonder *et al.*, 2014). These hypervolumes were projected back in geographical space indicating those geographical areas that provide suitable conditions for populations of the species. However, with increasing dimensionality, the required minimum number of species records increases with this method exponentially. Therefore, it was possible to include in this analysis only nine of the 13 species with a minimum of five unique sampling locations. For comparisons between hypervolumes, the Sørensen Index was calculated as a measure of niche overlap (Sørensen, 1948) based on shared and unique proportions of two hypervolumes following Blonder *et al.* (2014).

Evidence for past distribution and landscape connectivity of *Pleophylla* species came from *biomod2* ensemble SDMs (Thuiller, 2003; Thuiller *et al.*, 2013) which were modeled using a subset of the 19 previously mentioned bioclimatic variables: to remove possible negative effects of spatial autocorrelation, intercorrelation structure among the variables throughout the study area was assessed by computing

pairwise squared Spearman's rank correlation coefficients. In cases, where r^2 exceeded 0.75, only the putatively biologically most important variables were chosen. Using this strategy the following variables were retained: mean diurnal range (BIO2), temperature annual range (BIO7), mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter (BIO11), annual precipitation (BIO12), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18).

Modeling techniques employed in *biomod2* ensembles were the Generalized Linear Model (GLM), the Generalized Boosting Model (GBM), the Generalized Additive Model (GAM), and Maximum Entropy (MAXENT) (Thuiller, 2003; Thuiller *et al*., 2013). As environmental background two different sets of each 10 000 pseudo-absence records were created within a circular buffer of 200 km enclosing the respective species records, but not closer than 100 km. We preferred to use pseudo-absences here as it is generally difficult to proof the absence of a *Pleophylla* species at a given site, especially given the varying degrees of sampling effort and focus taxa in the data set of Sericini species. All models were repeated five times for each set of pseudo-absences randomly splitting the species records in 80% used for model training and 20% used for model evaluation resulting in 40 single SDMs per species (2 × pseudo-absences × 4 algorithms × 5 repetitions). As the evaluation measures, we computed the area under the receiver operating characteristic curve (ROC; Swets, 1988), Cohen's Kappa and the True Skills Statistic (TSS) (Allouche *et al*., 2006). For the calculation of the final ensemble model, the best fitting models (with ROC >0.7) were proportionally weighted according to their fit, as recommended in the *biomod2* manual (Thuiller *et al*., 2016). When projecting, areas requiring extrapolation beyond the environmental training range of the SDMs were discarded. Species with less than 24 spatially unique records were excluded from the *biomod2* approach (retaining *P. fasciatipennis*, *P. ferruginea*, *P. navicularis*, *P. nelshoogteensis*, and *P. pilosa*).

To evaluate past habitat expansion of *Pleophylla*, potential distributions of *Pleophylla* species in the Last Glacial Maximum (LGM, 21 kya) and the Holocene Altithermal (HA, 6 kya) were inferred. The species' weighted ensemble models were used to assess past potential distributions based on 11 different global circulation models of the Paleoclimate Modelling Intercomparison Project (PMIP) 3 (Braconnot *et al*., 2011, 2012): bcc-csm1-1, CCSM4, CNRM-CM5, COSMOS-ASO, CSIRO-Mk3-6-0, FGOALS-g2, GISS-E2-R, IPSL-CM5A-LR, MIROC-ESM, MPI-ESM-P, and MRI-CGCM3 (Table S4). Original monthly outputs of the global circulation models run with r1i1p1 initial conditions were downscaled to a resolution of 2.5 arc min (approximately 4 km in the study area) using the delta method proposed by Peterson & Nyári (2008). Subsequently, the respective BIOCLIM variables were computed using the relevant functions of the *dismo* package for cran R (Hijmans *et al*., 2015).

Landscape connectivity analyses

Two methods for modeling the potential connectivity of the sampled *Pleophylla* populations and for identifying important dispersal corridors and pinch points were applied: *Circuitscape* (Shah & McRae, 2008) and *Least Cost Corridors* (LCCs, Adriaensen *et al*., 2003; Verbeylen *et al*., 2003). *Circuitscape* adapts concepts from electric circuit theory because many parallels exist between organism movement and electric current flow (McRae, 2006; Shah & McRae, 2008). It is able to assess the amount of gene flow in complex landscapes and seems particularly suited to evaluate the isolation of populations as species movement (i.e., current) over long distances and high resistances is allowed to end by death of the moving individual (analog to groundings). In contrast to *LCCs* models, it can incorporate the effects of wider habitat swaths and of independent, parallel pathways connecting samples (McRae, 2006). We used as current sources in the resistance landscape all sampling localities of a species which was modeled with *biomod2* (landscape resistance model F0). Additionally, two derivatives of F0 were used which were informed by the actual distribution of indigenous forests (Fig. 1b). These derivatives were employed to model the distribution of *Pleophylla*, not only considering climatic factors but also the actual forest occurrences. The first forest-accounting derivate (F1) models the current potential distribution, while the second (F2) describes a scenario without potentially suitable soils for *Pleophylla* larvae outside forest occurrences (forest-restricted). Therefore, the vector format forest patch polygons of Mucina & Rutherford (2006) were transformed into a binary raster layer of forest patches in QGIS (v. 2.10), by assigning all pixels the value 1 (i.e., forest present) which fully or partially overlapped the polygons (Fig. 1b). This approach artificially enlarges the forest patches (i.e., the potential habitat of *Pleophylla*) slightly, leading to a more conservative approach of habitat fragmentation inference than the alternative approach of selecting only fully overlapped pixels would. To consider also the occurrence of *Pleophylla* individuals in yet humic soils outside forest patches (forest-accounting scenario F1), its probability of occurrence outside forests was approximated as a function of distance to the nearest forest patch based on a kernel density of all available sampling points (Fig. 1b). For scenario F1, gradients of decreasing occurrence probability around forest patches (with values ranging from 1 to 0) were added to the above-mentioned raster layer prior to multiplying with the *biomod2* ensemble SDMs. The forest-restricted scenario F2 was produced by multiplying the raster layer without gradients of decreasing occurrence probability with the *biomod2* ensemble SDMs.

Least Cost Corridors and *Paths* (LCPs) were inferred with *SDMtoolbox* (v. 1.1c; Brown, 2014) in ArcGIS® 10.2.2. Due to computational limitations, occurrence data of each species were spatially rarified with *SDMtoolbox*, i.e., records with high spatial autocorrelation were removed (Brown, 2014). *Biomod2* SDMs and its derivatives were resampled to 50% of the original resolution. LCCs were calculated in a pairwise manner between sampling sites. The results were visualized with the *raster*-package in R (version 2.4-15; Hijmans, 2015; R Core Team, 2015) and in QGIS.

An overview of the entire pipeline of species distribution modeling and landscape connectivity analyses is shown in Fig. 1.

Additionally, landscape connectivity metrics based on forest patch characteristics were calculated with *FRAGSTATS* (McGarigal *et al.*, 2012) employing the binary raster layer of indigenous forest patches (see above). Besides the number of forest patches and forest patch density, the edge-to-edge Euclidean distances between all nearest neighboring patches (McGarigal & Marks, 1995), the connectance index (CI, McGarigal *et al.*, 2012), and the degree of landscape division (LDI, Jaeger, 2000) were calculated. The CI gives the percentage of pairwise patch-comparisons that are expected to be connected under a given threshold. The threshold was set to the maximum distance of all *Pleophylla* records to the nearest neighboring forest patches which also includes occurrences in forest plantations. Additionally, the CI was calculated for 1 and 5 km thresholds. The LDI is interpreted as the probability that two randomly chosen pixels in the landscape are not situated in the same patch. The eight cell neighborhood rule was applied for all calculations.

Species richness was estimated using the TOMBIO-PLUGIN (v. 2.5.0; http://www.tombio.uk/qgisplugin) for QGIS, by counting the number of species per 100 km grid cell in all available records of *Pleophylla*.

Fire frequencies in South Africa were inferred using the MODIS Burned Area Product (Collection 5.1, MCD45; Roy *et al.*, 2002, 2005, 2008) which covers 13 years from 2001 to 2013. It counts no more than one burning event for a given pixel per month if a fire was detected. We summarized the data in a raster layer giving the number of burning events in 13 years using the *raster*-package in R (Hijmans, 2015). As fires are largely of anthropogenic origin, these data were not used for modeling purposes.

Results

Assessment of forest association

Pleophylla species were shown to be strictly forest-associated, although differences were found in the occurrence of *Pleophylla* species in the different forest subtypes (Fig. S24). The mean distance for randomly chosen points, sample sites of other Sericini, and *Pleophylla* species to the nearest forest patch were 161.5, 58.5, and 3.5 km, respectively (Fig. 2). The ANOVA of distances to nearby forest patches found highly significant differences ($P < 0.001$) among the examined groups and in pairwise *t*-tests ($P < 0.001$ in all pairwise comparisons).

Present and past distribution models

Dimension reduction of the *BIOCLIM* model retained three principal components for the n-dimensional hypervolume approach so that distributions of species with more than five spatially independent records could be considered (Table S5). The climatic elements most driving divergence in *Pleophylla*

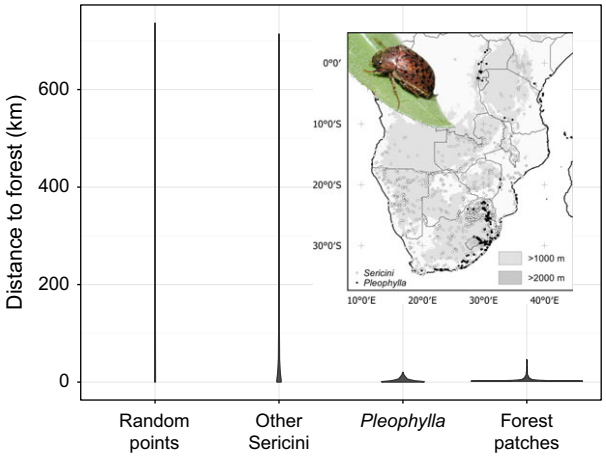

Fig. 2 Dependence of *Pleophylla* species on forest habitat. Equal area violin plots illustrating the distance of all available *Pleophylla* locality data to the closest forest edge compared to other Sericini occurrences, randomly distributed points in South Africa, and nearest neighbor distances of all indigenous forest patches. The widths of the violins depict the probability of occurrence density at a given distance. The inset shows the geographical distribution of all specimens under study including absence records (Eberle *et al.*, 2016a) and *P. fasciatipennis* on a leaf. [Colour figure can be viewed at wileyonlinelibrary.com]

bioclimatic records were found along principal components (PCs) one and two: annual precipitation and precipitation in warmest and wettest (BIO 12, 13, 16, and 18) vs. precipitation in coldest and driest periods (BIO 14, 17, and 19) as well as mean and extreme temperatures (BIO 1, 5, 6, 8, 9, 10, and 11) vs. annual and diurnal temperature ranges (BIO 2 and 7) (Fig. S1, Table S6).

Test statistics in terms of ROC, TSS and Cohen's Kappa indicate an overall good discrimination ability of both hypervolume and *biomod2* ensembles (Tables S5 and S8).

Highest niche similarity was found among species that predominantly occurred in KwaZulu-Natal (*P. fasciatipennis*, *P. ferruginea*, *P. navicularis*, and *P. pilosa*) and among northern South African species (*P. harrisoni*, *P. pseudopilosa*, and *P. warnockae*) (Fig. S2, Table S7). Climatic niches of *P. nelshoogteensis* and *Pleophylla silvatica* slightly overlapped with the latter but were more similar to the southern species. The divergence of hypervolumes of northern and southern South African *Pleophylla* species was mainly driven by PC2 which was dominated by variables of precipitation. Although the estimated species distribution models distinctly differed in their extent, they were all restricted to the southern, south-eastern, and eastern parts of South Africa, enclosed by the Great Escarpment and the coastline (Fig. 3). The models predicted by far larger areas to

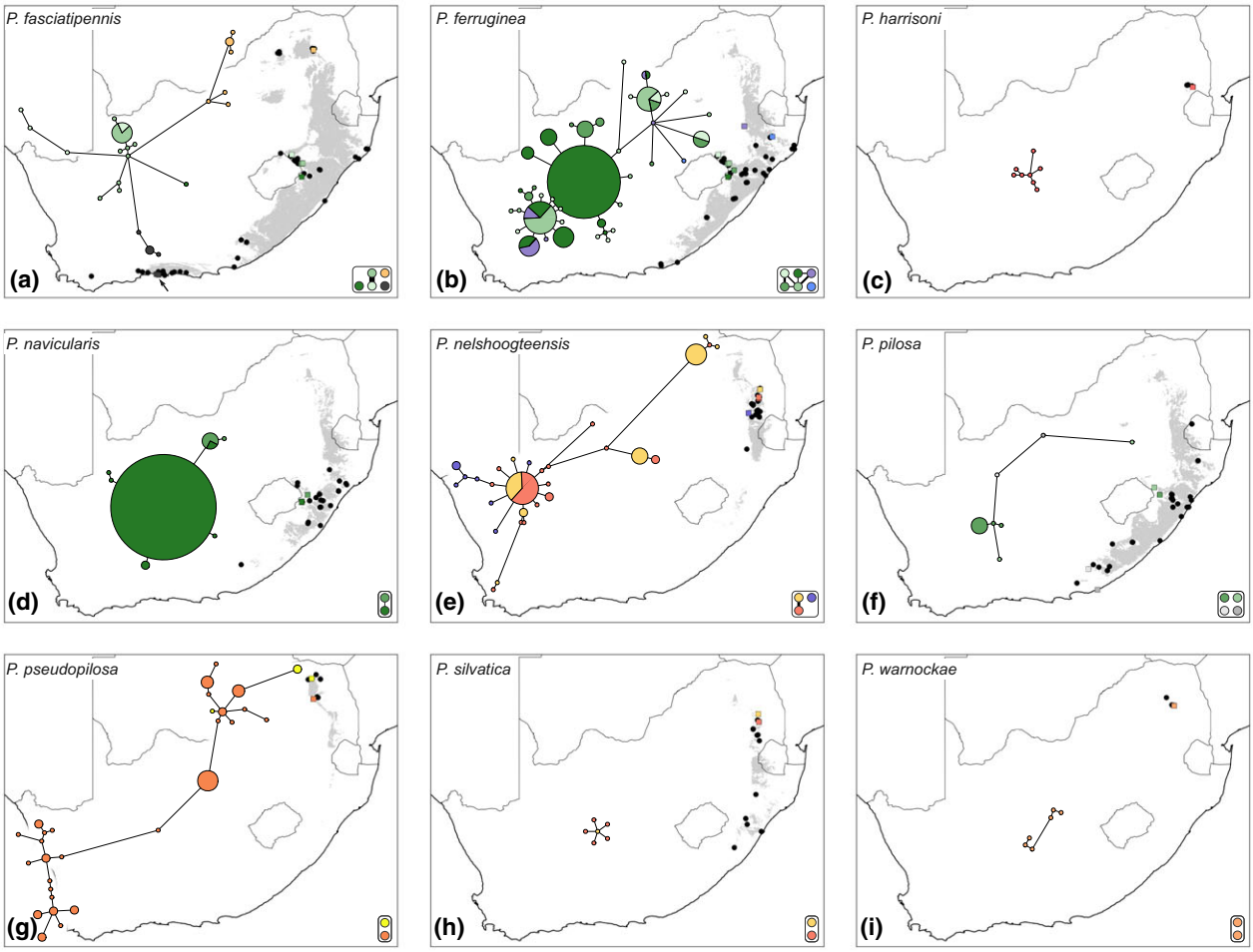

Fig. 3 Mitochondrial genetic structure (*cox1*) and population differentiation of nine *Pleophylla* species and their modeled potential distributions (SDM). Pie chart sizes in haplotype networks correspond to the number of haplotypes, colors indicate geographical origin of haplotypes like coded on the respective maps, and branch lengths indicate the amount of mutational change. Non-DNA sampling sites that were used for SDM are shown as black dots. Back-projected SDM from n-dimensional hypervolumes are shaded in gray. Where applicable, boxes at the bottom-right corner show genetic admixture among sampling sites measured by G'_{ST} values with thick, thin, or missing lines between color-coded localities, respectively, depicting high or low genetic exchange or isolation. (a) *P. fasciatipennis*, (b) *P. ferruginea*, (c) *P. harrisoni*, (d) *P. navicularis*, (e) *P. nelshoogteensis*, (f) *P. pilosa*, (g) *P. pseudopilosa*, (h) *P. silvatica*, (i) *P. warnockae*. [Colour figure can be viewed at wileyonlinelibrary.com]

be climatically suitable for *Pleophylla* than are currently covered by forests.

Compared to the hypervolume models, potential distributions under current climate conditions as suggested by the *biomod2* ensembles were slightly larger for *P. ferruginea*, *P. nelshoogteensis*, and *P. pilosa*, in particular in the Eastern and Western Cape provinces (Figs S3, S7, S11, S15, and S19). The predicted potential distribution (Fig. S3) was smaller in the *biomod2* ensemble for *P. fasciatipennis*, showing a more fragmented pattern than the hypervolume SDMs (Fig. 3a). According to the *biomod2* ensembles, climatic niches of all species were mainly defined by precipitation variables (Table S8), followed by annual and mean diurnal temperature ranges, being congruent to the results from the hypervolume approach.

Paleo-distribution models vastly differed in all species for the different PMIP3 global circulation models, mostly caused by differing reconstructions of precipitation among the alternative climate models (PMIP3 synthesis maps, pmip3.lsce.ipsl.fr; accessed March 20, 2016) (Varela *et al.*, 2015). For instance, the distribution of *P. fasciatipennis* in the LGM (Fig. S5) in southern Africa ranged from few small and less suited patches along the eastern coast and in the Soutpansberg area of northern South

Africa (model IPSL-CM5A-LR) to an extensive and well-suited area similar today's range (model MIROC-ESM).

Genetic differentiation and demographic history

Generally, good admixture of haplotypes was found for molecular data with some exceptions as outlined in detail below (Figs 3 and 4). An exceptional pattern was observed in *P. fasciatipennis* which showed remarkable concordance in geographical and mitochondrial genetic differentiation between populations in Limpopo, KwaZulu-Natal, and Western Cape (Fig. 3a). It is the only species of the genus that is distributed from the Cape to the north of the country at Soutpansberg (Fig. 3).

Despite apparent admixture, which was evident from haplotype networks, population differentiation statistics found high genetic differentiation among many sampling sites (Figs 3 and 4, Table S9). Particularly southern populations were strongly isolated (Figs 3a, f and 4f). Further isolated populations were inferred at northern KwaZulu-Natal (Ngome forest; Figs 3b and 4b; blue symbols), and in northern South Africa (Soutpansberg; Figs 3g and 4g; yellow symbols). Good admixture was found among sampling sites at the Drakensberge (Figs 3a, b, d and 4b, d; green squares) and among sites in northern Mpumalanga (Figs 3e and 4e, reddish, yellow, and purple squares). Shared haplotypes over long distances between the Drakensberge and southern Mpumalanga were found for *P. ferruginea* (Figs 3b and 4b; green and purple squares; *cox1*: G'_{ST} = 0.78 and 0.87, ITS1: G'_{ST} = 0.62–0.89; Table S9).

Haplotype network analyses revealed repeated reciprocal exchange of related haplotypes between two or more sampling sites (Figs 3 and 4), which was not detected by differentiation statistics but likewise indicated gene flow, although potentially more ancient than evident from shared haplotypes. A local differentiation of haplotypes, indicating limited gene flow, was found in *P. ferruginea*, *P. nelshoogteensis*, and *P. pseudopilosa* (Fig. 3). These populations appeared to be completely differentiated according to G'_{ST} due to the lack of shared haplotypes. ITS1 sequences of *P. silvatica* and *P. warnockae* all belong to the same haplotype.

We had sufficient data to infer the demographic history (EBSP) of six species (*P. fasciatipennis*, *P. ferruginea*, *P. navicularis*, *P. nelshoogteensis*, *P. pilosa*, and *P. pseudopilosa*). A recent increase in mean population mutation rate over the last 5–10 ky was a basic pattern observed in all species (Fig. S23). This implied an increase in effective population size (N_e) because the mutation rate was constant over time. There were no fluctuations prior to the LGM (21 kya) indicating a loss of demographic signal in the utilized markers.

Landscape connectivity

Patch-based landscape measures indicated a highly fragmented distribution of indigenous forests in South Africa. A forest patch density of 0.054 patches per 100 ha was calculated with a mean nearest neighbor distance between patches of 4.17 km (median: 2.42 km). For comparison, the mean distance of *Pleophylla* records to the nearest forest patch was 3.47 km (median 1.94 km) (Fig. 2). The connectance index for

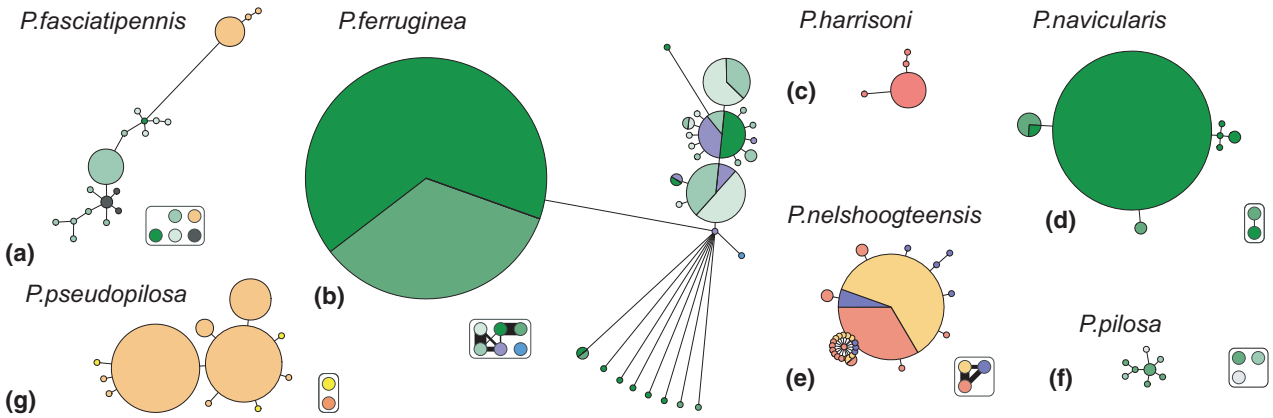

Fig. 4 Nuclear genetic structure (ITS1) and population differentiation of seven *Pleophylla* species. Pie chart sizes in haplotype networks correspond to the number of haplotypes and are the same as in Fig. 3. Colors indicate geographical origin of haplotypes like coded on the respective maps in Fig. 3, and branch lengths indicate the amount of mutational change. Where applicable, boxes at the bottom-right of the networks show genetic admixture among sampling sites measured by G'_{ST} values with thick, thin, or missing lines between color-coded localities respectively depicting high or low genetic exchange or isolation. (a) *P. fasciatipennis*, (b) *P. ferruginea*, (c) *P. harrisoni*, (d) *P. navicularis*, (e) *P. nelshoogteensis*, (f) *P. pilosa*, (g) *P. pseudopilosa*. [Colour figure can be viewed at wileyonlinelibrary.com]

the 1, 5, and 20 km migration capability thresholds were 0.00%, 0.21%, and 0.97%, respectively. That is, assuming that specimens of *Pleophylla* may disperse up to 20 km outside indigenous forest patches (based on present records; Eberle *et al.*, 2016a), only less than 1% of the pairwise forest patch evaluations resulted connected for *Pleophylla*. Likewise, the Landscape Division Index indicated strong fragmentation of indigenous forest patches. The probability that two randomly chosen points were not in the same patch was 98.2%.

Circuitscape models allow migration to end between sampling points due to high resistances or too long distances and are therefore supposed to reflect genetic admixture. In case of *Pleophylla*, they were strongly affected by the reduction of potential distributions to actual forest patches (Figs S6, S10, S14, S18, S22). In all species, potential migration routes through wider habitat swaths were narrowed to corridors in the resistance landscape informed by forest-accounting scenario (F1). The migration intensity was distinctly increased in these corridors. Migration was restricted to within forest patches in the forest-restricted scenario (F2), completely isolating nearly all sampling localities from each other. Areas with the highest potential loss of connectivity (from F0/F1 to F2) were found on the slopes of the Drakensberg east of the border to Lesotho and in Central KwaZulu-Natal (*P. fasciatipennis*, *P. ferruginea*, and *P. navicularis*), along the cost between Port Elisabeth and Durban (*P. pilosa*) and north of Swaziland between Mbombela to the Motlatse River Canyon (*P. nelshoogteensis*) (Figs S6, S10, S14, S18, S22). In concordance with the genetic differentiation, populations of *P. fasciatipennis* in Limpopo, KwaZulu-Natal and Eastern Cape were not connected in any model (Figs 3a and S6). The connection between the Drakensberg mountains and southern Mpumalanga (purple square), which was inferred by the haplotype networks and the genetic differentiation index (G'_{ST}) for *P. ferruginea* (Figs 3b and 4b), was observed along the Great Escarpment (Fig. S10, F1). Localities of intermediate *cox1*-haplotypes of *P. nelshoogteensis* and *P. pilosa* (Fig. 3e, f) were also connected in the circuitscape models (scenario F1; Figs S18 and S22, arrows). The populations of *P. fasciatipennis* that appeared isolated from DNA data were also disconnected in the circuitscape analyses (Figs 3a and S6).

Least cost corridor/LCP models always spanned even long distances between sampling points (Figs S6, S10, S14, S18, S22). Restrictions of potential migration routes by forest-informed *biomod2* derivatives F1 and F2 altered the results only marginally. As LCCs/LCPs also connected localities that were inferred to be isolated by circuitscape and molecular methods, they marked connecting areas between those populations that had the highest density of indigenous forest patches and good climatic suitability (Fig. 5).

Discussion

Forest association of Pleophylla species

Our distance-to-forest analysis of sampling plots confirmed *Pleophylla* as strictly forest associated (Fig. 2). Due to its polyphagous feeding style of larvae and adults, *Pleophylla* is not restricted to specific plant species or forest subtypes (Fig. S24) and may therefore serve as a proxy for potential forest distribution in South Africa. The proven link between forests and *Pleophylla* established the basis for our use of *Pleophylla* species as a proxy for indigenous forests' distribution in a landscape genetics context, an approach that is for the first time applied to South African forest remains. Records outside forest patches might be reconcilable by the ability of light traps to attract insects over certain distances. However, although short-distance dispersal out of forests at least in some species may rarely occur, the mean and maximum collection distance from forests of 3.5 and 20 km, is better explained by suitable replacement habitats that may often exist in sufficient number in the vicinity of current forest patches (Fig. 2). Some records used for our analyses date back more than 20–30 years, in which the extension and quality of forest habitats might have undergone significant changes due to human land management (e.g., controlled burning, expansion of industrial forestry, etc.) and thus discrepancies between specimen records and recently digitized forest patches might have become even stronger. Further support for *Pleophylla* being a forest related species is found in the high influence of precipitation variables in all species' models (Figs S1 and S2), because precipitation is a crucial factor for forest development (Sankaran *et al.*, 2005). The exclusive occurrence in forests might be attributable to the beetle's dependence on humic forest soils for larval development. Depending on the climatic conditions, these soils remain suitable for considerable time after deforestation because its degradation takes several years (Lemenih *et al.*, 2005).

The distribution models likely improved by past and recent records outside current forest patches, which reveal suitable areas that would have been disregarded by other approaches. The beetles' ability to fly prevents extreme genetic structuring between only little separated forest patches which might lead to overly strong conclusions of a general connectivity breakdown.

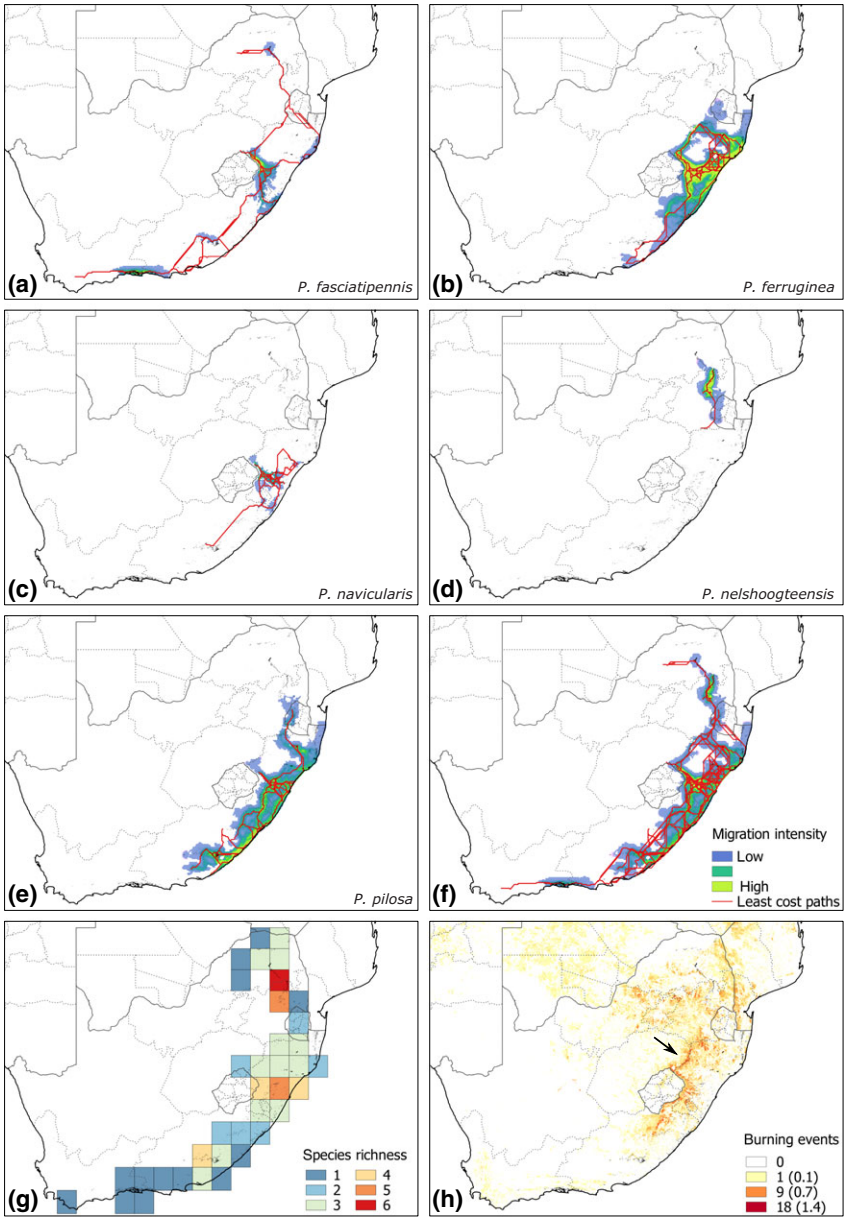

Fig. 5 Connectivity among sampling sites for five species of *Pleophylla*, fire frequency, and species richness in the investigated area. (a–f) Migration intensity that was inferred with circuitscape based on the forest-accounting species distribution models (F1) (light green: high migration density, blue: low migration density) and the least cost paths (LCPs, red lines) among available sampling sites are overlaid. LCPs roughly illustrate areas that connect current occurrences of *Pleophylla* most parsimoniously. (f) Cumulative migration intensity of the above five species and all LCPs. These areas are climatically best suited for afforestation and intensified conservation of existing forest patches. (g) Regional species richness is depicted as number of species occurring per 100 km grid cell based on all available *Pleophylla* records. (h) Distribution of fire frequency (legend: summarized number of months with burning events over 13 years; numbers in brackets are averaged burning events per year). The arrow points to areas with high burning frequency along the Great Escarpment which coincide with LCPs and high migration intensity. [Colour figure can be viewed at wileyonlinelibrary.com]

Past development of Pleophylla species ranges

Miocene. The Miocene is known for a general cooling and aridification (Zachos *et al.*, 2001) and for the onset of intensified diversification in *Pleophylla* (Eberle *et al.*, 2016c) and other forest-associated organisms (Measey & Tolley, 2011; Mlambo *et al.*, 2011; Menegon *et al.*, 2014; Eberle *et al.*, 2016b). This was argued to be attributable to fragmentation and isolation of previously widespread species in forest remains (Maley, 1996).

Furthermore, the interaction of general long-term climatic stability since the Miocene and complex microclimates was suggested to be the driver of the exceptional plant diversity in the Cape region of southern Africa (Schnitzler *et al.*, 2011) which might also apply for faunal elements. Highly regional forest endemics, like they are for instance found in many flightless and little vagile dung beetles (Davis *et al.*, 2001; Medina & Scholtz, 2005; Deschodt & Scholtz, 2008; Mlambo *et al.*, 2011), rose chafers (Šípek & Malec, 2016), or long-horned grasshoppers (Naskrecki *et al.*, 2008; Samways *et al.*, 2012), might derive from such past subdivisions. However, in most cases, these are very rare species which are reported from only a tiny fraction of existing forest patches. Being much more narrowly adapted to forest habitats (shadow, host plants, mammal dung), it seems unlikely that they could persist in only a few tiny and interrupted areas of a few hectares since the Miocene. Also in *Pleophylla* we encounter several highly endemic species (Eberle *et al.*, 2016a) which are scattered over numerous isolated forest patches whose distances exceed by far the dispersal capacity of the species, indicating that forests were until the recent past more extended than currently observed.

Pleistocene. Pleophylla's population demography showed, at least for the species for which we had sufficient data, no fluctuations prior to the LGM, indicating a loss of demographic signal in the utilized molecular markers (Fig. S23). However, late Pleistocene regional paleoenvironmental data for eastern South Africa revealed complex climatic mosaics and frequent changes in vegetation cover with periodically more extended forest patches all over South Africa (Scott, 1999; Parkington *et al.*, 2000; Finch & Hill, 2008; Chevalier & Chase, 2015; Quick *et al.*, 2016), which is also confirmed by climatic models (Huntley *et al.*, 2016). The lack of demographic signal in the EBSP (Fig. S23) that was observed for all investigated *Pleophylla* species may indicate a strong bottleneck during the LGM in result of habitat reduction (e.g., Heled & Drummond, 2008) that erased earlier signals in the utilized markers (Ho & Shapiro, 2011). The EBSP therefore supported a strong decline of populations due to the diminution of forests during dry periods around the LGM and only slow recovery of populations. This is largely concordant with paleoenvironmental evidence which supposes a general cooling and drying for South Africa (Scott, 1989; Partridge *et al.*, 1999; Finch & Hill, 2008) except for the western part (current winter rainfall zone) that appears to have been moister than today (Chase & Meadows, 2007). Contemporary evidence for moister conditions inland (Free State province and Drakensberg escarpment) (Scott, 1989; Norström *et al.*, 2014) strengthen the impression of a highly complex mosaic of climatic conditions.

Concordant with the paleontological record, the SDMs based on the climatic models IPSL-CM5A-LR, CCSM4, and CNRM-CM5 (Table S4) inferred highly reduced distributions and drier conditions in south-eastern South Africa for all investigated *Pleophylla* species during the LGM (Figs S5, S9, S13, S17, S21). The highly contrasting and contradicting inferences among PMIP3 models (Braconnot *et al.*, 2011) for South Africa (e.g., Fig. S5) may reflect instable climatic conditions and frequent fluctuations that are also evident from paleoenvironmental records throughout the country. Recently, long-term precipitation trends in northern South Africa were explained by sea-surface and continental temperature trends, while a main influence of the Southern Hemisphere westerlies was deduced for central South African precipitation cycles (Chevalier & Chase, 2015), placing South Africa in a transition zone of multiple climatic influences. A dynamic mosaic of microclimates, providing numerous refugial areas over long time time-spans and sustaining ancient phylogenetic diversity like in the case of *Pleophylla*, seems thus likely in the light of fossil records and modeling approaches.

Our data renders potential glacial refugia for *Pleophylla* in coastal areas of today's KwaZulu-Natal and northern South Africa most likely. The best fitting LGM-models (IPSL-CM5A-LR, the CCSM4, and the CNRM-CM5) all showed a shift of species' distributions toward the south-eastern coast (Figs S5, S9, S13, S17, S21), which was also proposed for eastern forests (Scott, 1989; Finch & Hill, 2008). More northern occurring species, such as *P. nelshoogteensis* and *P. fasciatipennis*, appeared to have persisted in northern refugia (Figs S5 and S17). Such potential long-term refugia (Ibrahim *et al.*, 1996; Nistelberger *et al.*, 2014) of *Pleophylla* in the northern parts of the Great Escarpment were also supported by the high haplotype diversity that was recovered for the northern species in this study (Figs 3e, g and 4e, g) as well as the occurrence of locally endemic species (e.g., *P. warnockae*, *P. pseudopilosa*, *P. ruthae*; Eberle *et al.*, 2016a).

Holocene. The late increase in some species' population size after the HA (<6 kya; Fig. S23) might indicate forest expansions from refuges at the eastern coastal areas and southern KwaZulu-Natal, where a more humid climate and increased forest cover were documented (Eeley *et al.*, 1999; Neumann *et al.*, 2010, 2014; Chevalier & Chase, 2015). Otherwise, drier conditions in the HA (Jolly *et al.*, 1998) likely prevented earlier expansions. It is also to consider that time calibration of population-level analyses based on mutation rates inferred from

interspecific analyses – although being common practice – can be problematic (Ho *et al*., 2005; Ho & Larson, 2006; Grant, 2015). Mutation rates can be an order of magnitude higher than substitution rates that are observed among species (Hoareau, 2016). The time estimates from the EBSP might therefore be biased toward older ages, i.e., the observed increase in population size in all species (Fig. S23) might have occurred even more recently. This fits further palynological evidence that suggest rather recently (>3 kya) more widespread forests and a steady decrease from this time on (Finch & Hill, 2008; Neumann *et al*., 2008, 2010; Finch *et al*., 2009). Increasing pollen of neophytes (e.g., *Zea mays* and *Pinus*) and Poaceae in concert with a drastic decline of *Podocarpus* and other trees ca. 700 years ago marks the appearance of the first iron age settlers at the coast of KwaZulu-Natal and in today's northern Limpopo province (Scott, 1987; Neumann *et al*., 2010).

Current population connectivity

For landscape connectivity modeling of current *Pleophylla* populations, we used three resistance layers circumscribing various probabilities for specimen dispersal and occurrence (F0, F1, and F2; Fig. 1b). The models were completed by population genetic inferences which showed good concordance with circuitscape landscape connectivity models based on forest-accounting scenario F1, i.e., circuitscape inferred no or low dispersal between genetically isolated populations (e.g., Figs 3a, 4a and S6). Complete isolation of sampling sites that was often found by G_{ST}, G'_{ST}, and D_{Jost} statistics (Table S9), which all depend on shared haplotypes, is likely to be caused by limited sampling in some cases. However, a re-evaluation using increased specimen sampling and thorough inference of panmictic populations, which was impossible in the framework of the present study, may reveal more genetic mixture than is currently evident. Despite this potential underestimation, considerable genetic mixture was evident. Therefore, the hypothesis that South African forests have been highly fragmented to isolated patches over long time spans is not supported by our data. This argumentation holds despite a possible alternative explanation for the observed haplotype network pattern for *P. fasciatipennis*: a long distance dispersal from Kwazulu Natal (green) to Limpopo (orange) might have occurred in the past (the yellow haplotypes already diverged and diversified; Figs 3a and 4a). However, the climatic niche models which show a broad suited connection (Figs 3a and S3) as well as the hypothesis of a northern glacial refuge in Limpopo render a relictual population in the past more likely. For *P. ferruginea*, *P. pilosa*, and *P. pseudopilosa*, two or more of the naturally rare long distance dispersal events would have been necessary to explain the observed patterns, rendering this scenario unlikely as well. Given the high mutation rates (as discussed above) in the employed markers, considerable genetic exchange should thus be assumed at least over the last 5 ky. However, in the present study, we found high geographical distances between forest patches that were already slightly larger than the maximum distance that *Pleophylla* species were supposed to migrate between forest patches. Strong fragmentation and low connectance of forest patches (fragstats analyses, Fig. 2) supported this reasoning. The current data cannot exclude that gene flow among many populations already ceased during the last centuries by anthropogenic influence. The observed genetic mixture of the markers used in this study bears the signature of the recent past (<5 ky) when possibly most populations were still better connected. Comparing the mean record distances of *Pleophylla* specimens from forests with distances among forest patches (3.5 vs. 4.4 km; see also Fig. S2) revealed that the maximum tolerated migration distance between forest patches is reached in many cases.

For all species that were suitable for landscape connectivity modeling, sampling localities were found that were completely isolated from neighboring populations under current conditions (forest-accounting SDMs; F1; Figs S6, S10, S14, S18, S22). The models inferred potential connections for some of them under optimal conditions (i.e., unrestricted SDMs; F0) so that they might be re-connected to larger populations (Figs 5 and S10). Those regions are very likely to be disconnected from other populations by anthropogenic influence. For the reconnection of such isolated populations, burning and intensive forestry in those regions should be reconsidered and re-establishment of indigenous forests in suited areas should be promoted. Well-suited areas for afforestation and high-priority conservation of existing forest patches might be found along the LCPs that were inferred in this study based on the forest-accounting scenario F1 (Fig. 5). As they trace the most parsimonious path between larger populations that has the highest forest patch density and the highest climatic suitability, best chances for the reconnection of isolated populations are given there, although being partly under strong impact of man-made fire management (Fig. 5h). Restricting specimen movements to forest patches (forest-restricted SDMs; F2) resulted in a drastic deterioration of predictions of population connectivity (Figs S6, S10, S14, S18, S22).

Implications for conservation management and future research

Our results improve the understanding of the forests' natural extension, contributing to potential solutions to the long lasting conservational dilemma whether fire-adapted grasslands and fynbos or forest should be fostered in specific areas. Reliance on two lines of evidence from independent sources of data strengthened the results, which were further backed up by reflections on past development of forest cover in South Africa. Conclusions are closely linked with the conservational importance of South Africa as a cradle of evolution (Pickford, 2004; Eberle *et al.*, 2016b), promoting the persistence of a rich and valuable (phylogenetic) diversity (Sechrest *et al.*, 2002; Schnitzler *et al.*, 2011; Huntley *et al.*, 2016). Frequent natural climate fluctuations that led to repeated forest retreats and expansions in southern Africa (Deacon, 1983b; Eeley *et al.*, 1999) might have acted as speciation pump (Terborgh, 1992; Voelker *et al.*, 2010; McDonald & Daniels, 2012) producing a unique and diverse flora and fauna. Besides *Pleophylla*, other highly diverse insect groups like canthonine dung beetles (Canthonini), which bear many flightless taxa, exclusively occur in South African forest remains (Davis *et al.*, 2001; Medina & Scholtz, 2005; Deschodt & Scholtz, 2008; Mlambo *et al.*, 2011). Likewise, considerable diversity of flightless species is found in grassland biomes (e.g., Pope, 1960; Naskrecki *et al.*, 2008), indicating a certain stability of both biomes in the region. This supports the fossil- and modeling-based idea of a long-term mosaic of sufficiently connected grassland and forests that was able to persist in refugia provided by a variety of geological features and different climatic influences during glacial periods. However, with intensified human land use, urban development, and fire management (Fig. 5h), the ability of forests to track environmental change is seriously limited (Eeley *et al.*, 1999) which might result in precarious habitat loss, particularly in times of global climate change. Although more detailed field observations of the specimens' migration between forest patches are necessary for a more detailed insight of today's patterns, a breakdown of many current gene-flow corridors is likely (forest-restricted SDMs, F2), since intensive land use and fires degrade soil organic matter within few decades (Mills & Fey, 2003), with serious consequences for forest fauna relics. With further degradation of soils, stepping stone populations that currently connect populations between indigenous forest remains will disappear and further cease gene flow. Such potential stepping stone populations are frequently found in vicinity of forest plantations, along certain river valleys or suburban sites where soils stay suitable for many years. However, most plantations are usually burned after logging to remove decaying wood which inevitably impoverishes the soil fauna. It was also shown for other species like the red colobus monkey (*Procolobus gordonorum*) that burning and urban development strongly diminishes gene flow among populations in forest patches (Ruiz-Lopez *et al.*, 2016). It is therefore important to protect forest remains and to ensure connectivity among them by reforestation of suited connecting areas. Our results support previous findings that current climatic conditions support a much wider forest extent in South Africa (Eeley *et al.*, 1999). The inference of extremely recent drops of population sizes, like they may have occurred by anthropogenic deforestation over the last few hundred years, has to be one major aspect of future studies. Molecular studies employing extremely fast evolving genetic loci like microsatellites and a careful calibration of the molecular clock (Ho & Larson, 2006) could provide improved evidence. Shotgun sequencing or restriction site-associated DNA analysis (Davey *et al.*, 2011; Hohenlohe *et al.*, 2011) may deliver large datasets for more analyses at finer time scales. In this context, it will be fruitful to take into consideration other factors like grazing of large herbivores and the putative influence of wild-fires, also in the light of the risk of invasive plants that endanger ancient grasslands (Bond, 2016). This future research should also include other alternative taxa, in particular less mobile model groups like wingless insects that do not have passive dispersal.

Conservation area connectivity is one major issue in the light of global climate change; however, its understanding requires the recognition of current and past patterns. Our results can be used as a step toward the identification of concrete areas where re-establishment and protection of existing indigenous forest could be more effective for connecting forest species populations. It can be seen as a primer to identify areas or problematic regions where additional research at the local scale needs to be conducted (Fig. 5) to apply adequate conservation management (e.g., reforestation vs. burning) and thus to ensure the protection of ancient and evolutionary distinct species.

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Conflict of interest

The authors have no conflict of interest to declare.

Data accessibility

DNA sequences: A detailed listing of GenBank accessions is available in Table S1. Sampling locations are uploaded as Table S2. Species distribution models and results from landscape connectivity analyses for use in Geographic Information Systems are available at Zenodo (doi: 10.5281/zenodo.58181).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Results from spatial principle component analysis for hypervolume models performed on the clipped climatic background.
Figure S2. N-dimensional hypervolumes of *Pleophylla* species.
Figure S3. *Biomod2* ensemble distribution models of *P. fasciatipennis* for current, Holocene Altithermal (HA, 6 kya), and Last Glacial Maximum (LGM, 21 kya) conditions.
Figure S4. Distribution models of *P. fasciatipennis* for Holocene Altithermal (HA, 6 kya) conditions inferred by single PMIP3 experiments.
Figure S5. Distribution models of *P. fasciatipennis* for Last Glacial Maximum (LGM, 21 kya) conditions inferred by single PMIP3 experiments.
Figure S6. Circuitscape and Least Cost Corridor landscape connectivity models of *P. fasciatipennis* based on three *biomod2* distribution models: (F0) the unrestricted ensemble, (F1) the ensemble restricted by actual forest patches including a probability of occurrence gradient zone, and (F2) the ensemble strictly restricted to forest patches.
Figure S7. *Biomod2* ensemble distribution models of *P. ferruginea* for current, Holocene Altithermal (HA, 6 kya), and Last Glacial Maximum (LGM, 21 kya) conditions.
Figure S8. Distribution models of *P. ferruginea* for Holocene Altithermal (HA, 6 kya) conditions inferred by single PMIP3 experiments.
Figure S9. Distribution models of *P. ferruginea* for Last Glacial Maximum (LGM, 21 kya) conditions inferred by single PMIP3 experiments.
Figure S10. Circuitscape and Least Cost Corridor landscape connectivity models of *P. ferruginea* based on three *biomod2* distribution models: (F0) the unrestricted ensemble, (F1) the ensemble restricted by actual forest patches including a probability of occurrence gradient zone, and (F2) the ensemble strictly restricted to forest patches.
Figure S11. *Biomod2* ensemble distribution models of *P. navicularis* for current, Holocene Altithermal (HA, 6 kya), and Last Glacial Maximum (LGM, 21 kya) conditions.
Figure S12. Distribution models of *P. navicularis* for Holocene Altithermal (HA, 6 kya) conditions inferred by single PMIP3 experiments.
Figure S13. Distribution models of *P. navicularis* for Last Glacial Maximum (LGM, 21 kya) conditions inferred by single PMIP3 experiments.
Figure S14. Circuitscape and Least Cost Corridor landscape connectivity models of *P. navicularis* based on three *biomod2* distribution models: (F0) the unrestricted ensemble, (F1) the ensemble restricted by actual forest patches including a probability of occurrence gradient zone, and (F2) the ensemble strictly restricted to forest patches.
Figure S15. *Biomod2* ensemble distribution models of *P. nelshoogteensis* for current, Holocene Altithermal (HA, 6 kya), and Last Glacial Maximum (LGM, 21 kya) conditions.
Figure S16. Distribution models of *P. nelshoogteensis* for Holocene Altithermal (HA, 6 kya) conditions inferred by single PMIP3 experiments.
Figure S17. Distribution models of *P. nelshoogteensis* for Last Glacial Maximum (LGM, 21 kya) conditions inferred by single PMIP3 experiments.
Figure S18. Circuitscape and Least Cost Corridor landscape connectivity models of *P. nelshoogteensis* based on three *biomod2* distribution models: (F0) the unrestricted ensemble, (F1) the ensemble restricted by actual forest patches including a probability of occurrence gradient zone, and (F2) the ensemble strictly restricted to forest patches.
Figure S19. *Biomod2* ensemble distribution models of *P. pilosa* for current, Holocene Altithermal (HA, 6 kya), and Last Glacial Maximum (LGM, 21 kya) conditions.
Figure S20. Distribution models of *P. pilosa* for Holocene Altithermal (HA, 6 kya) conditions inferred by single PMIP3 experiments.
Figure S21. Distribution models of *P. pilosa* for Last Glacial Maximum (LGM, 21 kya) conditions inferred by single PMIP3 experiments.
Figure S22. Circuitscape and Least Cost Corridor landscape connectivity models of *P. pilosa* based on three *biomod2* distribution models: (F0) the unrestricted ensemble, (F1) the ensemble restricted by actual forest patches including a probability of occurrence gradient zone, and (F2) the ensemble strictly restricted to forest patches.
Figure S23. Extended Bayesian Skyline plots of demographic histories.
Figure S24. Percentage occurrence of *Pleophylla* species in forest subtypes defined by Mucina & Rutherford (2006).
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Table S3. Substitution models of nucleotide evolution that were used for Extended Bayesian Skyline inference.
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Table S7. Hypervolume overlap statistics.
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Table S9. Comprehensive compilation of population connectivity statistics.