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Population Genomics of Adaptive Radiations: Exceptionally High Levels of Genetic Diversity and Recombination in an Endemic Spider From the Canary Islands

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ABSTRACT

The spider genus *Dysdera* has undergone a remarkable diversification in the oceanic archipelago of the Canary Islands, with ~60 endemic species having originated during the 20 million years since the origin of the archipelago. This evolutionary radiation has been accompanied by substantial dietary shifts, often characterised by phenotypic modifications encompassing morphological, metabolic and behavioural changes. Hence, these endemic spiders represent an excellent model for understanding the evolutionary drivers and to pinpoint the genomic determinants underlying adaptive radiations. Recently, we achieved the first chromosome-level genome assembly of one of the endemic species, *D. silvatica*, providing a high-quality reference sequence for evolutionary genomics studies. Here, we conducted a low coverage-based resequencing study of a natural population of *D. silvatica* from La Gomera island. Taking advantage of the new high-quality genome, we characterised genome-wide levels of nucleotide polymorphism, divergence and linkage disequilibrium, and inferred the demographic history of this population. We also performed comprehensive genome-wide scans for recent positive selection. Our findings uncovered exceptionally high levels of nucleotide diversity and recombination in this geographically restricted endemic species, indicative of large historical effective population sizes. We also identified several candidate genomic regions that are potentially under positive selection, highlighting relevant biological processes, such as vision and nitrogen extraction as potential adaptation targets. These processes may ultimately drive species diversification in this genus. This pioneering study of spiders that are endemic to an oceanic archipelago lays the groundwork for broader population genomics analyses aimed at understanding the genetic mechanisms driving adaptive radiation in island ecosystems.

1 | Introduction

Understanding how species evolve and adapt to their environments stands as a central issue in evolutionary biology (Austin and Arnold 2001; Ravinet et al. 2017). Yet, although this knowledge is essential for biodiversity management and conservation, particularly in the face of climate change (Mergeay and Santamaria 2012), the evolutionary mechanisms and the key

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genomic targets that drive species diversification remain elusive. Certainly, evolutionary inference in rapidly evolving traits is more feasible in organisms with shorter generation times (Coyne and Orr 2009). Island radiations especially those occurring in oceanic archipelagos offer experimental conditions for studying diversification in animals and plants. The small size and clear boundaries of oceanic islands, jointly with their simplified ecosystems that are repeated in the same or different oceans (resulting in multiple independent replicates of evolution), make islands test tubes for evolutionary studies (Hodges and Derieg 2009; Schluter 2000). For this reason, the oceanic archipelagos have long been recognised as natural laboratories to study short-term evolution (Fernández-Mazuecos et al. 2020), having received much attention over time (Carson and Kaneshiro 1976; Emerson 2002; Gillespie 2004; Grant and Grant 2008; Juan et al. 2000; Machado et al. 2017).

Despite important recent efforts, the relative contributions of adaptive and nonadaptive forces in species diversification are still a matter of debate (Choi et al. 2020, 2021; Muschick, Indermaur, and Salzburger 2012; Rundell and Price 2009; Rundle and Nosil 2005; Simões et al. 2016). The Canary Islands, a volcanic archipelago of eight islands with significant climate variability (Fernández-Palacios 2011) and high endemism, are an interesting model system to study the main drivers of adaptive radiation. In these islands, endemism is particularly important among arthropods, with up to 40% of the species being unique to the archipelago (Martín et al. 2010). The spider genus *Dysdera* Latreille, 1804 (Araneae: Dysderidae) ranks among the lineages with the highest number of endemic species in the Canaries, providing one of the most spectacular examples of island radiations among spiders worldwide (Arnedo, Oromí, and Ribera 2001; Arnedo et al. 2007; Macías-Hernández et al. 2016; Řezáč et al. 2021; Bellvert et al. 2023). Indeed, about 20% of the ~300 species described in this Western Palearctic genus (World Spider Catalog 2024) are endemic to this archipelago. *Dysdera* spiders are nocturnal ground-dwelling hunters recognised as one of the few examples of prey specialisation among spiders (Pekár, Líznarová, and Řezáč 2016). The species of this genus evolved different degrees of stenophagy, being some of the species facultatively or even obligately specialised to feed on woodlice. This prey is generally avoided by generalist spiders mainly due to their defensive strategies, which make them particularly difficult to capture (e.g., clinging, rolling and fast running behaviours or distasteful chemical secretions) or digest (e.g., high concentrations of heavy metals in the exoskeleton; Pekár, Líznarová, and Řezáč 2016). Many *Dysdera* spiders have developed morphological adaptations, behavioural strategies and metabolic mechanisms to successfully capture and digest these isopods. Remarkably, this eco-phenotypic diversity has been observed in both mainland and oceanic islands (Hopkin and Martin 1985; Řezáč, Pekár, and Lubin 2008; Řezáč and Pekár 2007; Toft and Macías-Hernández 2017; Bellvert et al. 2023). However, most of the species in Canary Islands evolved from a single generalist common ancestor in a very short evolutionary period (Adrián-Serrano et al. 2021; Bellvert et al. 2023). At least nine cheliceral morphotypes clearly related to the preference for consuming isopods have evolved independently several times in this archipelago, with co-occurring endemic species tending to diverge in size and mouthpart morphology (Řezáč et al. 2021). These observations provide strong arguments for considering the diversification of *Dysdera* in the Canary Islands as a repeated case of adaptive divergence driven by dietary preferences (independent of what occurred on the mainland), which provides a unique system for studying the genomic basis of adaptation and predictability in evolution.

In a first attempt to determine the molecular basis of such repeated adaptations, we performed a comparative transcriptomic analysis of species with different levels of stenophagy from two evolutionarily independent shifts towards feeding on woodlice (Vizueta et al. 2019). This study uncovered several candidate genes likely associated with dietary diversification within this genus in the archipelago. Specifically, we identified genes involved in heavy metal detoxification and homeostasis, as well as in the metabolism of crucial nutrients and venom toxins. Recently, we reported a chromosome-scale genome assembly of *D. silvatica* (Escuer et al. 2022), a species with low levels of dietary specialisation that inhabits the three more western Canary Islands (La Gomera, La Palma and El Hierro; Macías-Hernández et al. 2016). The genome size of *D. silvatica* is estimated to be 1.7 Gb based on flow cytometry (1.4 Gb in the published assembly), organised into seven holocentric chromosomes (Schrader 1935).

A central question in island adaptive radiations is how the bottleneck associated with the colonisation event and the geographic limitation imposed by small island size, influences the adaptive potential of endemic species. Island endemics are expected to have low effective population sizes, not only because of the founder effect during colonisation, but also because they are persistently confined to very small areas and are frequently subject to stochastic events and environmental disturbances. In this scenario, geographic isolation and genetic drift would dominate over natural selection as the main diversification forces. However, the occurrence of adaptive radiations, such as that observed in the genus *Dysdera* in Canary Islands, appears to be a common feature of these islands (Cerca et al. 2023). Furthermore, many studies on this subject have found no differences in the levels of variability or in the effectiveness of selection between mainland and island species (James, Lanfear, and Eyre-Walker 2016; Yang et al. 2024). We hypothesize that this is the case for the genus *Dysdera*, where rapid adaptive radiation associated with dietary shifts would have been occurred in the presence of sufficient genetic variation and selection efficiency. In this context, we expect to find meaningful levels of variability in the surveyed population of this genus, as well as signals of selection efficiency in the form of recent footprints of positive selection across the genome of *D. silvatica*.

To test for these expectations, we used the high-quality reference assembly of *D. silvatica* and resequencing data from this species to conduct the first population genomics study in an endemic *Dysdera* species. We have characterised and quantified the levels and patterns of natural genomic variation in a natural population of *D. silvatica* and assessed the impact of recent selection on genome-wide patterns of polymorphism and divergence across its genome. We have analysed genome-wide patterns and levels of nucleotide variation and linkage disequilibrium and estimated per-site recombination rate in 12 genomes of *D. silvatica* sampled at a single locality in La Gomera. We found very high levels of nucleotide diversity and

recombination in the genome of this species, as well as the footprint of recent positive selection. These results support environmental stability, with high selection efficacy associated to large long-term effective population size, as the most likely scenario underlying the diversification of the genus *Dysdera* in the Canary Islands.

2 | Material and Methods

2.1 | Sampling, DNA Extraction and Whole-Genome Sequencing

We collected 12 individuals of *D. silvatica* (two males and 10 females) from the same locality in La Gomera Island (Teselinde; Ermita de Santa Clara, Vallehermoso; Table S1). Additionally, we collected one male of *D. bandamae* from Gran Canaria (Llanos de la Pez; Tejada; Figure 1; Table S1) to be used as the outgroup for evolutionary inference. The DNA was extracted using the Gentra Puregene Cell kit (Qiagen) and sequenced on the NovaSeq 6000 (*D. silvatica* samples) and HiSeqX (the *D. bandamae* individual) platforms in Macrogen Inc. (Seoul, Korea).

Due to the large genome size of *D. silvatica* (about 1.7 Gb estimated from flow cytometry; Sánchez-Herrero et al. 2019), we opted for a low-coverage whole-genome resequencing strategy to generate the *D. silvatica* intraspecific data. We obtained an average raw sequencing depth for each *D. silvatica* individual of 6.7× (Table S1), and 31.8× for *D. bandamae* (Table S1). We also obtained the genome sequence of one female of *D. silvatica* at medium coverage (41.2×).

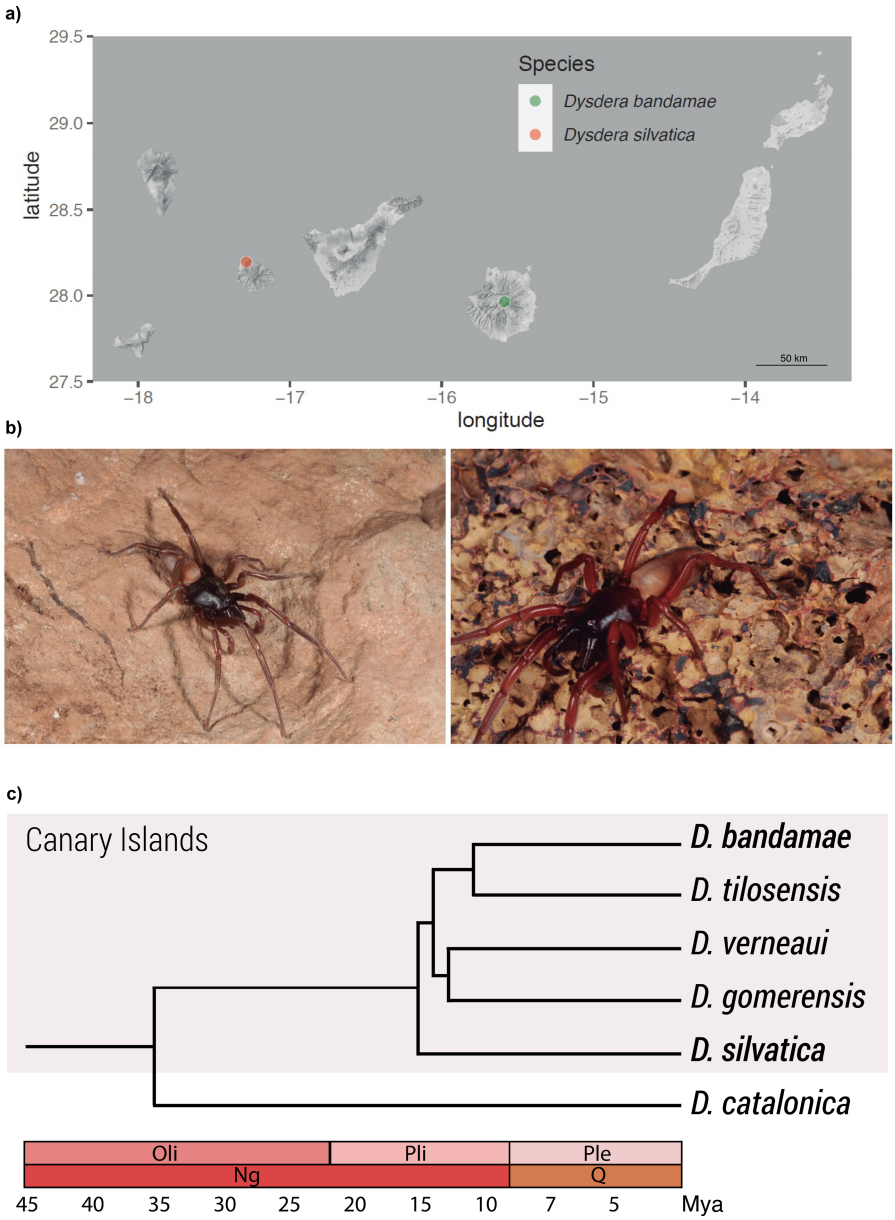

FIGURE 1 | Sampling localities, images and phylogenetic context of the *Dysdera* species surveyed in this study. (a) Map of Canary Islands showing the geographical localisation of the individuals sampled in this study. (b) Images of the two studied *Dysdera* species; left, *D. silvatica*; right, *D. bandamae*. (c) Phylogenetic relationships and divergence times among four Canary Island *Dysdera* species and one continental member of this genus; based on Vizueta et al. (2019) and Crespo et al. (2021).

2.2 | Read Quality Assessment, Trimming and Mapping

We used FASTQC v0.11.9 (Andrews 2010) to assess the quality of raw reads and Trimmomatic v0.39 (Bolger, Lohse, and Usadel 2014) for the trimming process. We filtered out adapters, all reads shorter than 50 bp, or those with bad quality scores (< 15) across sliding windows of 4 bp length. We also removed leading and trailing bases with low quality scores (< 3) and all missing bases.

Filtered reads were mapped independently for each individual of both species, against the reference genome of *D. silvatica* (Escuer et al. 2022) using bwa mem v0.7.16 (Li and Durbin 2009) with short split hits labelled as secondary alignments (–M). Then, we used Samtools v1.11 (Danecek et al. 2021) to filter out these secondary alignments. We removed duplicates from the resulting BAM files and added read groups labels, and the alignments were indexed and sorted using Picard Tools v2.26.10 (Broad Institute 2016). Finally, we estimated the average and median read depth for each scaffold using Samtools v1.11 and Bedtools v2.29.1 (Quinlan and Hall 2010), along with Qualimap v2.2.1 (Okonechnikov, Conesa, and García-Alcalde 2016). The average net coverage per site of the *D. silvatica* sample is 44.3×, with the average net coverage per individual ranging from 3.3× to 4.3× (Table S2). Since *D. silvatica* males are X0, the average sequencing depth in the X chromosome is about half that of autosomes (Table S2). We conducted all analyses separately for the autosomes ($n = 24$) and the X chromosomes ($n = 20$). We excluded the two males from the X-chromosome analysis because ANGSD (Korneliussen, Albrechtsen, and Nielsen 2014) cannot handle haploid data, as it calculates diploid genotype likelihoods (GLs). The average net coverage of the female sequenced at medium coverage is 33.1×.

2.3 | Genome-Wide Nucleotide Polymorphism and Divergence

We utilised ANGSD along with related software (e. g. ngsLD; Fox et al. 2019), ngsDist (Vieira et al. 2016) and ngsTools (Fumagalli et al. 2014), which consider genotype uncertainty for the downstream population genomic analysis of the low-coverage samples.

To obtain a consensus sequence of *D. bandamae* in FASTA format we use the `-doFasta` option of ANGSD. Before estimating GLs, we applied specific quality filters in ANGSD to remove non-informative positions. We filtered out positions with very low or very high net coverage across samples (`-setMinDepth 3, -setMaxDepth 92`), corresponding to the 2.5th and 97.5th percentiles of the per-site net coverage distribution across all individuals. In addition, we only retained positions with high base calling quality, properly paired and high mapping quality reads (`–minQ 20, –only_proper_pairs and -minMapQ 20`). We adjusted the mapQ parameter for excessive mismatches (`-C 50`) and the qscores around indels (`–baq 1`), and discarded not primary, failure and duplicate reads (–remove_bads), as well as reads that did not map uniquely (`-uniqueOnly`). We set the *D. silvatica* genome as a reference genome (`–ref`) and estimated the GLs for each individual using the GATK algorithm (`–GL 2`). To mitigate potential bias introduced by repetitive elements, which might affect the mapping results, we conducted additional analyses with different coverage filters (`-setMinDepth 20, -setMaxDepth 72`), encompassing the 66.6% of the net coverage distribution, and excluding masked regions of the genome identified by RepeatMasker.

We analysed the levels and patterns of nucleotide variation using the unfolded site allele frequency (SAF) likelihood obtained from the *realSFS* program within ANGSD and utilising the sequence of *D. bandamae* as the ancestral reference (`–doMajorMinor 5, –anc`). We estimated the SAF based on individual GL assuming HWE (`-doSaf 1`). From the estimated SAF, and using the *thetaStat* program within ANGSD, we calculated several summary statistics and neutrality tests, including the number of polymorphic sites (S), nucleotide diversity (π; Nei 1987), Watterson estimator of theta (θ_w; Watterson 1975), Tajima's D (Tajima 1989), Fu and Li's D and F (Fu and Li 1993), Fay and Wu's H (Fay and Wu 2000) and Zeng's E (Zeng et al. 2006). We computed these summary statistics and neutrality tests for both the entire chromosome and in a sliding-window approach (in non-overlapping windows of 1 and 50 kb).

We used ngsDist v.1.0.10 (Vieira et al. 2016) to compute the nucleotide divergence between *D. silvatica* and *D. bandamae* based on the estimated GLs. For that, we first generated a GLs file in BEAGLE format using the BAM files of the 13 individuals (12 *D. silvatica* and one *D. bandama*e for the autosomes; 10 *D. silvatica* and one *D. bandamae* for the X chromosome). Using these GLs, we calculated the evolutionary distance between the two species as the average pairwise JC69 corrected distances between each individual of *D. silvatica* and *D. bandamae* (`––evol_model 2`; Jukes and Cantor 1969). We computed nucleotide divergence for the entire chromosome and in non-overlapping windows of 1, and 50 kb.

We used Picard (Broad Institute 2016) to incorporate read groups to the BAM file of the individual sequenced at medium coverage and called their variants with GATK HaplotypeCaller (Van der Auwera and O'Connor 2020). We selected SNP variants with total depth between 10 and 60, phred-scaled quality score > 30, normalised variant quality > 2, StrandOddsRatio < 3 and FisherStrand < 60 (both measuring phred-scaled probability of strand bias), and Mapping quality > 40, to generate the VCF of this individual using GATK VariantFiltration tool.

2.4 | Population Structure and Demography

We conducted a principal component analysis (PCA) to explore population structure in our low-coverage sample. We used the ngsCovar software from ngsTools v3 (Fumagalli et al. 2014). This analysis was based on the estimated GL and was performed separately for autosomes and the X chromosome. We applied the same filters and options used to estimate GL for variation analyses but generating a binary file with GL (`-doGeno 32`), assuming that we know major and minor alleles (`–doMaf 1`), excluding triallelic positions (`-SkipTrialletic 1`) and filtering out those SNPs with p-values > 1×10^{-6} (`–snp_pval`).

We used Stairway Plot2 (Liu and Fu 2020) and the maximum likelihood estimate of the unfolded SFS to infer the recent demographic history of the *D. silvatica* population. The analysis was conducted separately for autosomes and the X chromosome. We set the generation time to 1.5 years (Cooke 1965) and the neutral mutation rate per-site and per-generation (μ) to 6.07×10^{-9}. This mutation rate was obtained from the nucleotide divergence estimated between *D. silvatica* and *D. bandamae* ($K=0.120$; Table 1), and the estimated divergence time for these species of 14.8 mya (Crespo et al. 2021).

2.5 | Linkage Disequilibrium and Recombination Rate

We estimated the site pairwise linkage disequilibrium (LD) using the ngsLD v1.1.1 software (Fox et al. 2019), taking genotype uncertainty into account. Initially, we generated a file with GL in BEAGLE format (`—doGlf 2`) with ANGSD by applying the same filters and options as described above. Then, LD was estimated as the average r^2 between genotypes (i.e., the *ZnS* statistic; Kelly 1997) in non-overlapping windows of 1.5 and 50 kb. To reduce the number of pairwise comparisons, we randomly sampled 0.1% of the sites to estimate the decay of LD with distance.

We used the composite-likelihood approach implemented in pyrho v0.1.6 (Spence and Song 2019) to estimate fine-scale per-base per-generation recombination rate (*r*) in *D. silvatica* based on LD patterns. For each chromosome, we run pyrho under two scenarios: (i) a constant size demographic model, using the size estimated for the present in the demographic analysis, and (ii) the best-fit demographic scenario from this analysis. We used a multi-variant call format (VCF) file with SNPs called in ANGSD (`-doGeno 1, —doMajorMinor 5, -doPost 1, —skipTriallelic 1, —snp_pval 1e-6` and `-dobcf`) as the input for pyrho. We masked repetitive regions in the reference before the variant calling. The specific parameters set in pyrho can be found in Table S3. We also used the sequentially Markovian coalescent model implemented in iSMC (Barroso, Puzović, and Dutheil 2019) and the VCF of the individual of *D. silvatica* sequenced at medium coverage, to infer the population scale recombination rate (Rhro) in this species.

2.6 | Genome Scans for Selection

We used the RAiSD v2.9 software (Alachiotis and Pavlidis 2018) to search for the characteristic hallmarks of positive selection within the *D. silvatica* genome. This method relies on a composite statistic (μ-*R*), calculated through a SNP-driven, sliding-window algorithm (using the default window length of 50 SNPs), integrating evidence indicative of selective sweeps, such as μ-*var* (reduction in nucleotide variation), μ-*sfs* (shifts to low and high-frequency derived variants) and μ-*ld* (elevated levels of LD around the selective site). RAiSD uses the VCF file with called genotypes (see above) as the input. Subsequently, we utilised the script *vcfutils.pl* from Samtools v1.11 (Danecek et al. 2021) to convert the resulting BCF file into VCF format. We used the -X the option to exclude repetitive regions from the analysis.

We used a three-step approach to identify outlier genomic regions within the empirical distribution of μ-*R*. First, we retained windows exhibiting the top 0.1% values of the μ-*R* statistic. Second, we examined the retained windows for consecutive regions showing μ-*R* values above 0.01% of the genome-wide empirical distribution. Among these, we selected those with a minimum of two consecutive windows with μ-*R* values above 0.001%. We then used structural annotations within the reference genome to identify genes or other functional elements located within these outlier regions.

We also applied the McDonald and Kreitman test (MKT) (McDonald and Kreitman 1991) to coding sequences to detect the footprint of positive and negative selection in the genome of *D. silvatica*. We first obtained a VCF file containing the genotypes of the 12 individuals of *D. silvatica* and the individual of *D. bandamae* using ANGSD. We applied the same filters and options as those used for estimating GL and for preparing the VCF input for pyrho. We used snpEff v.5.1d (Cingolani et al. 2012) to predict the functional effects of genomic variants (including synonymous and non-synonymous mutations) across the VCF containing data from 13 individuals. The annotated VCF file was used to run the MKT on all functionally annotated protein-coding genes by using a modification of Tomas Blankers' script (available at https://github.com/thomasblankers/popgen/blob/master/MKTtest). We filtered out sites with a minor allele frequency <0.1 (i.e., singletons).

2.7 | Mitochondrial Assembly and Phylogenetic Analyses

We assembled the mitochondrial genomes of *D. silvatica* (one per individual) and *D. bandamae* using NOVOplasty v4.3 (Dierckxsens, Mardulyn, and Smits 2017) and the raw reads of these species. For the assembly, we applied a Genome Range of 14,000–20,000 bp and a K-mer size of 33. Subsequently, we generated a multiple sequence alignment of the genomes (13 individuals) using MAFFT (Katoh and Standley 2016) with the following options `--maxiterate 1000, —-globalpair` and the *G-INS-i* algorithm. We then built a phylogenetic tree with IQ-TREE2 v1.6.12 (Minh et al. 2020) with parameters `-m MFP -B 1000`, and visualised and edited the tree with iTOL web interface (Letunic and Bork 2021).

2.8 | GO Enrichment

We used InterProScan v.5.57–90.0 (Jones et al. 2014) to extract the GO terms associated with the genes annotated in the reference genome v.2.3 of *D. silvatica* (Escuer et al. 2022). For the GO enrichment analysis, we used the R packages GSEABase v.1.60.0 (Geistlinger et al. 2021; Morgan, Falcon, and Gentleman 2023), GOstats v.2.64.0 (Falcon and Gentleman 2007) and org.Dm.eg.db v.3.16.0 (Carlson 2019) with the GO associated to the protein-coding genes located in the outlier regions in the RAiSD analysis (Alachiotis and Pavlidis 2018). We used the R package *q*-value v.2.30.0 (Storey, Bass, and Dabney 2022) to transform the obtained *p*-values into *q*-values. The GOstats analysis was run under the conditional option. We considered as significantly overrepresented those GO terms with an associated *p*-value <0.01 or with *q*-values <0.10. We used the R packages clusterProfiler v.4.8.1 (Wu et al. 2021) and AnnotationForge v.1.42.0 (Carlson and Pagès 2023) to transform the obtained results

TABLE 1 | Summary statistics of population genomic parameters.

	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	ChrX[a]	Total
n	12	12	12	12	12	12	10	
L	177,130,251	176,685,884	174,193,557	129,208,650	125,942,283	80,935,077	317,833,791	1,181,929,493
Polymorphism								
Sites	129,374,671	129,641,630	127,754,355	94,246,118	92,862,055	58,761,896	219,789,797	852,430,522
% analysed sites	73.04%	73.37%	73.34%	72.94%	73.73%	72.60%	69.15%	72.12%
S	9,971,265	10,155,556	10,511,148	7,598,590	7,385,301	4,896,494	7,718,288	58,236,642
π	0.0147	0.0145	0.0152	0.0148	0.0148	0.0151	0.0080	
Divergence								
Sites	124,507,188	125,033,364	122,943,662	91,492,164	89,021,276	56,912,792	261,430,051	871,340,497
K	0.119	0.119	0.118	0.121	0.119	0.120	0.121	0.120
Linkage disequilibrium								
Average *ZnS*[b]	0.095	0.095	0.095	0.094	0.095	0.095	0.116	

Note: n, sample size; L, chromosome length in bp; Sites, number of analysed sites (after excluding filtering positions) in bp; *S*, number of segregating sites; *K*, nucleotide divergence.
[a]Using information of only the 10 females.
[b]Calculated in windows of 50 kb.

from GOstats into an enrichResult object and plotted the results employing the R packages enrichplot v.1.20.0 (Yu 2023), europepmc v.0.4.1 (Ferguson et al. 2021) and ggplot2 v.3.4.2 (Wickham 2016). The library enrichplot was used to reduce the complexity of the enriched GO terms for better interpretability of the results. The number of clusters (nCluster option) was set to the default value of 5.

3 | Results

High levels of nucleotide polymorphism and recombination in *D. silvatica*.

After excluding positions that did not meet strict quality filters, we analysed 632.64 Mbp and 219.80 Mbp in autosomes and the X chromosome, respectively, which correspond approximately 72% of the total sequenced genomic positions (Table 1). These positions contained 50,518,353 autosomal and 7,718,288 X chromosome SNPs. Levels of nucleotide diversity are high in *D. silvatica* autosomes ($\pi = 0.015$; Table 1; Tables S4 and S5) and evenly distributed across the genome (Table 1; Figure 2a). As expected, the X chromosome is less variable, owing its smaller effective size ($\pi = 0.008$; Table 1; Figure 2a). Tajima's *D* values are consistently negative across the genome (Table 2; Tables S4 and S5). Overall, autosomes tend to have more negative Tajima's *D* values than the X chromosome (Table 2; Tables S4 and S5). Nucleotide divergence is also homogeneously distributed across chromosomes, with an average *K* value of 0.120 (Table 1; Figure 2b).

Linkage disequilibrium, estimated as *ZnS*, exhibits a rapid decay with distance (Table 1; Figure 2d, Table S6), suggesting high levels of genetic recombination in the genome of this species. As expected, *ZnS* values are consistently higher in the X chromosome compared to the autosomes (Table 1; Figure 2d).

In fact, the estimates of local per-base per-generation recombination rate (r) are unusually high in this species, regardless of the underlying demographic model used for the inference (see below) or the chromosome analysed, with most genomic windows displaying values of r between 10^{-7} and 10^{-6} (Table S3). These estimates are generally higher in autosomes than in the X chromosome (Tables S3). The levels of intraspecific variation, but not the divergence estimates, differ between the X chromosome and the autosomes of *D. silvatica*, likely reflecting the expected smaller effective population size of the sex chromosome. Even so, the X/autosomes π ratio (0.53) is lower than theoretically expected (0.75) and even far from the median value estimated for Arthropods (~1; Leffler et al. 2012). We found a significant yet modest correlation between r and π, with the X chromosome showing higher correlation coefficients compared to the autosomes (Figures S2 and S3).

3.1 | Large Historical Effective Population Sizes and no Evidence of Population Structure

We inferred two major demographic events in the recent history of *D. silvatica* populations that are supported by bootstrap analysis (Figure 3). The older event reflects a population bottleneck that occurred around 600 kya, which resulted in a five-fold reduction in population size. Following the complete recovery, the effective size of the population remained stable until around 20 kya, when an abrupt reduction dropped the population size to N_e of ~25×10^3 individuals. Autosomes and the X chromosomes show concordant demographic histories (data not shown).

On the other hand, no evidence of population structure was found in the sample (Figure 4; Figure S1). While PCA results would suggest some degree of differentiation between certain *D. silvatica* individuals with respect to PC2 (Figure 4), this

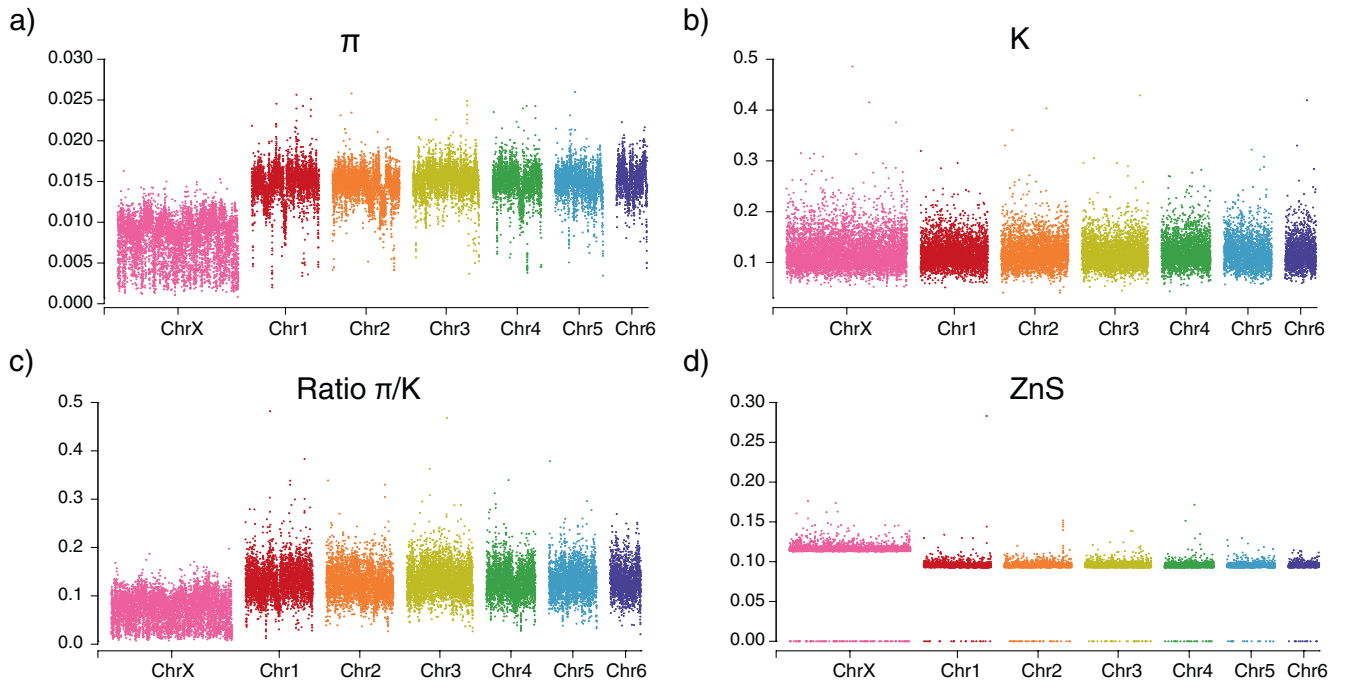

FIGURE 2 | Genome-wide distribution of summary statistics of genome variation. (a) Nucleotide diversity (π). (b) Nucleotide divergence per site (K); (c) Ratio π/K; (d) Linkage disequilibrium (estimated as ZnS). Each point depicts the value calculated in a window of 50 kb.

TABLE 2 | Results of the neutrality tests.

	Chr1	Chr2	Chr3	Chr4	Chr5	Chr6	ChrX[a]
Tajima's *D*	−1.170	−1.251	−1.269	−1.273	−1.248	−1.304	−0.781
Fu and Li's *F*	−1.592	−1.771	−1.786	−1.805	−1.757	−1.854	−0.997
Fu and Li's *D*	−1.358	−1.540	−1.550	−1.572	−1.524	−1.616	−0.820
Fay and Wu's *H*	0.206	0.197	0.210	0.205	0.205	0.206	0.147
Zeng's *E*	−0.401	−0.417	−0.428	−0.426	−0.420	−0.435	−0.286

[a]Using information of only the 10 females.

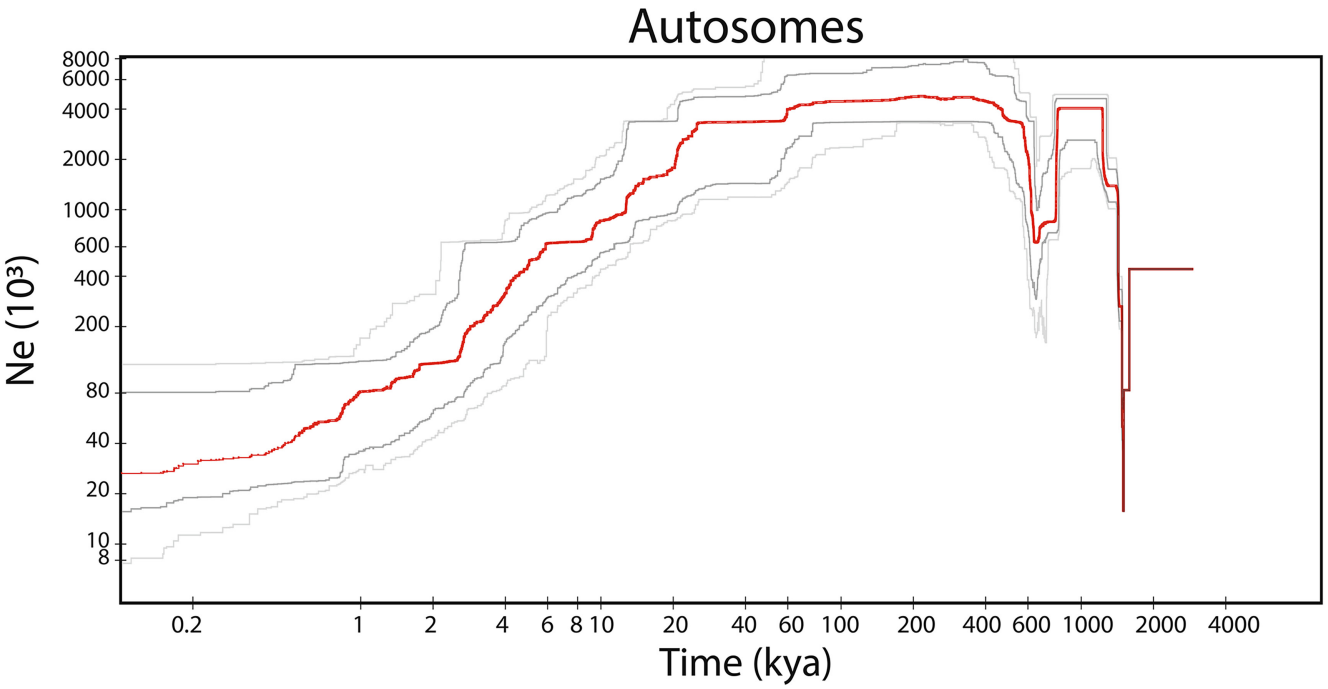

FIGURE 3 | Demographic history of the *D. silvatica* population inferred from autosomal data ($n = 24$). The red line indicates the median population size (200 bootstrap replicates). Dark grey and light grey lines delimit the 75% and the 95% bootstrap confidence intervals, respectively. Inference based on the unfolded SFS. Mutation rate per site and per generation of 6.07×10^{-9}. Generation time of 1.5 years.

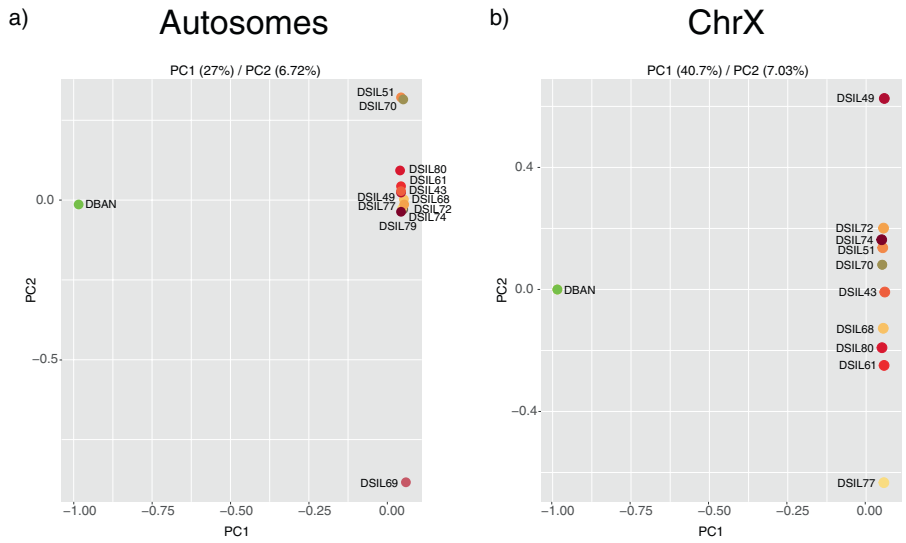

FIGURE 4 | Principal component analysis (PCA) using data from (a) autosomes and (b) X-chromosomes (data from only the 10 females).

component only explains 7% of the variance in the sample, and the individuals concerned are different in different chromosomes. The differentiation along the PC2 axis may simply be the result of different coalescence times in different unlinked regions along the genome. The lack of apparent signs of structuring in the phylogenetic tree based on mitogenomes (Figure S1) supports this scenario. All these observations, together with the fact that the variability estimated for a single individual is of the same order of magnitude as that calculated for the whole population, rule out current or ancestral population structure as being responsible for the high levels of polymorphism detected in this species. Beyond that, as expected most of the variance explained by PC1 is attributed to the divergence between species.

3.2 | Natural Selection in Recent Past of *D. Silvatica*

We identified 17 genomic regions as candidates to have been the target of positive selection in *D. silvatica*. Among them, 10 regions exhibit two or more consecutive windows with μ-*R* values above the 0.001% threshold of the empirical distribution (Tables S7 and S8). Notably, these regions are overrepresented in the X chromosome, comprising 52% of candidate regions despite accounting for only 26% of the genome assembly (chi-square test p-value $< 10^{-5}$). The windows with highest μ-*R* statistic values, as well as the largest number of consecutive significant windows, are predominantly found in chromosome 1 (Figure 5). In this chromosome, we identified a particularly strong candidate region (designated as Chr1_ID1) spanning over 146 kb with a maximum μ-*R* value of 228 (Table S8).

We identified 46 protein-coding genes annotated within the 17 candidate regions for positive selection. The GO enrichment analysis on the terms associated with these 46 genes revealed significant enrichment across 26 biological processes (BP), 14 molecular functions (MF) and 1 cellular component (CC) (p-value < 0.01; Table S9). However, after controlling for the false discovery rate (FDR), only 3 BPs and 14 MFs terms remained significant (q-value < 0.10; Table S9). The enriched biological processes are associated with genes involved in the visual system, nutrient transport, biosynthetic processes and apoptosis, among others. We identified molecular functions related to glycerol, sucrose and glutamate. However, none of these functions remained significant after correction for FDR (q-value > 0.05; Table S9B). We also found one significantly enriched cellular component, which is associated with the helicase complex, although with q-values above the significance threshold (Table S9C). Notably, only GO terms associated with the visual system and apoptosis remain significant after applying the correction for multiple testing (Table S9).

To gain further insights into the biological relevance of our results, we also performed a hierarchical clustering of the enriched GO terms across the three GO domains, a method based on a similarity index between GO terms. The names of the main clusters and the number of GO terms within each cluster varied slightly depending on whether the p-value (Figure 6) or the q-value (Figure 7) was used as the threshold to select enriched terms. However, in all cases, the main clusters of enriched GO terms are related to the regulation of photoreceptor development, biosynthesis of components of the arginine pathway, chitin biosynthesis, glycerol catabolism and sucrose transport. The GO terms associated with the visual system consistently display the lowest p- and q-values and are among those with the highest number of associated genes.

We applied the MKT to 28,900 genes (i.e., all putatively functional protein-coding genes annotated in the *D. silvatica* reference genome). Unfortunately, most of them (25,657) lacked

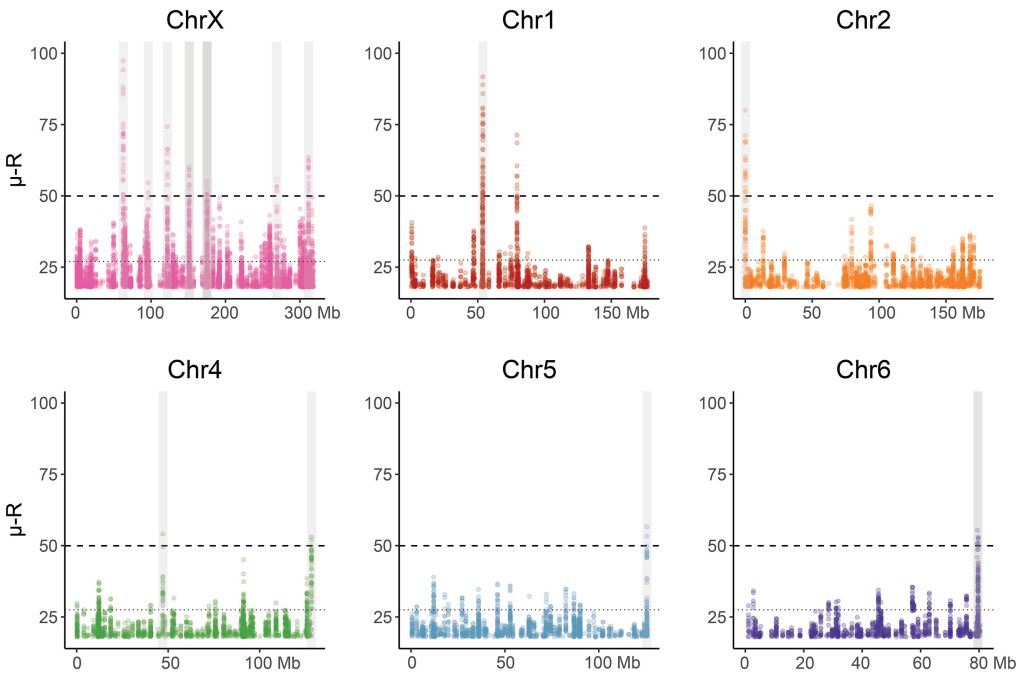

FIGURE 5 | Results of the genome scan of selection. Dots depict those values of the μ-*R* statistic. Dotted and dashed lines indicate the cut-off values at 0.0001% and 0.00001%, respectively (Table S7). Outlier regions analysed are shaded in grey.

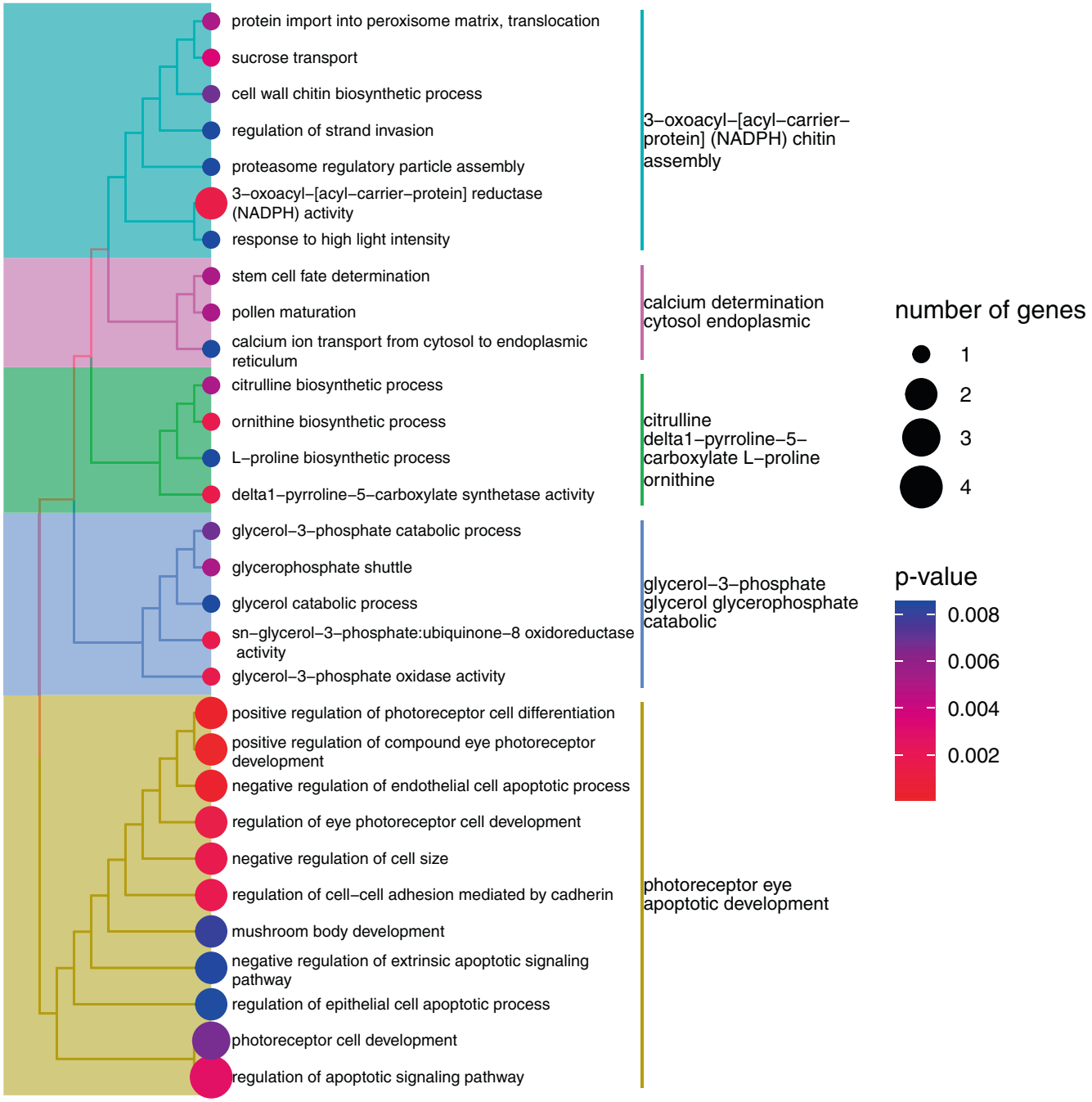

FIGURE 6 | Hierarchical clustering of significantly enriched terms (p-value <0.01). Clusters are highlighted in different colours.

sufficient divergence to obtain reliable results in this neutrality test. Among the 3243 analysed genes, 104 showed statistically significant departures from neutral expectations (p-value <0.05), with 49 and 55 of them showing signals of negative or positive selection, respectively (Table S10).

4 | Discussion

In this study, we present the first comprehensive population genomics analysis of an endemic spider from the Canary Islands, the nocturnal ground-dwelling spider *D. silvatica*. We adopted a low-coverage whole-genome resequencing strategy since it represents an optimal balance between inference power and sequencing costs, particularly suited for organisms with large genome sizes. Surprisingly, despite being an endemic species confined to three of the smallest islands of the Canarian archipelago, our analysis revealed that *D. silvatica* harbours exceptionally high nucleotide diversity levels ($\pi=0.015$; for autosomes; Table 1), which approach the median diversity observed in arthropods (Romiguier et al. 2014).

The scarcity of population genomics studies in island endemic species, other spiders, or even other chelicerates makes it difficult to contextualise the high levels of polymorphism detected in this study. Our estimates for *D. silvatica* are much higher than those calculated in the few available studies within chelicerates, namely mites ($\pi=0.0006$; Chen et al. 2020) and social

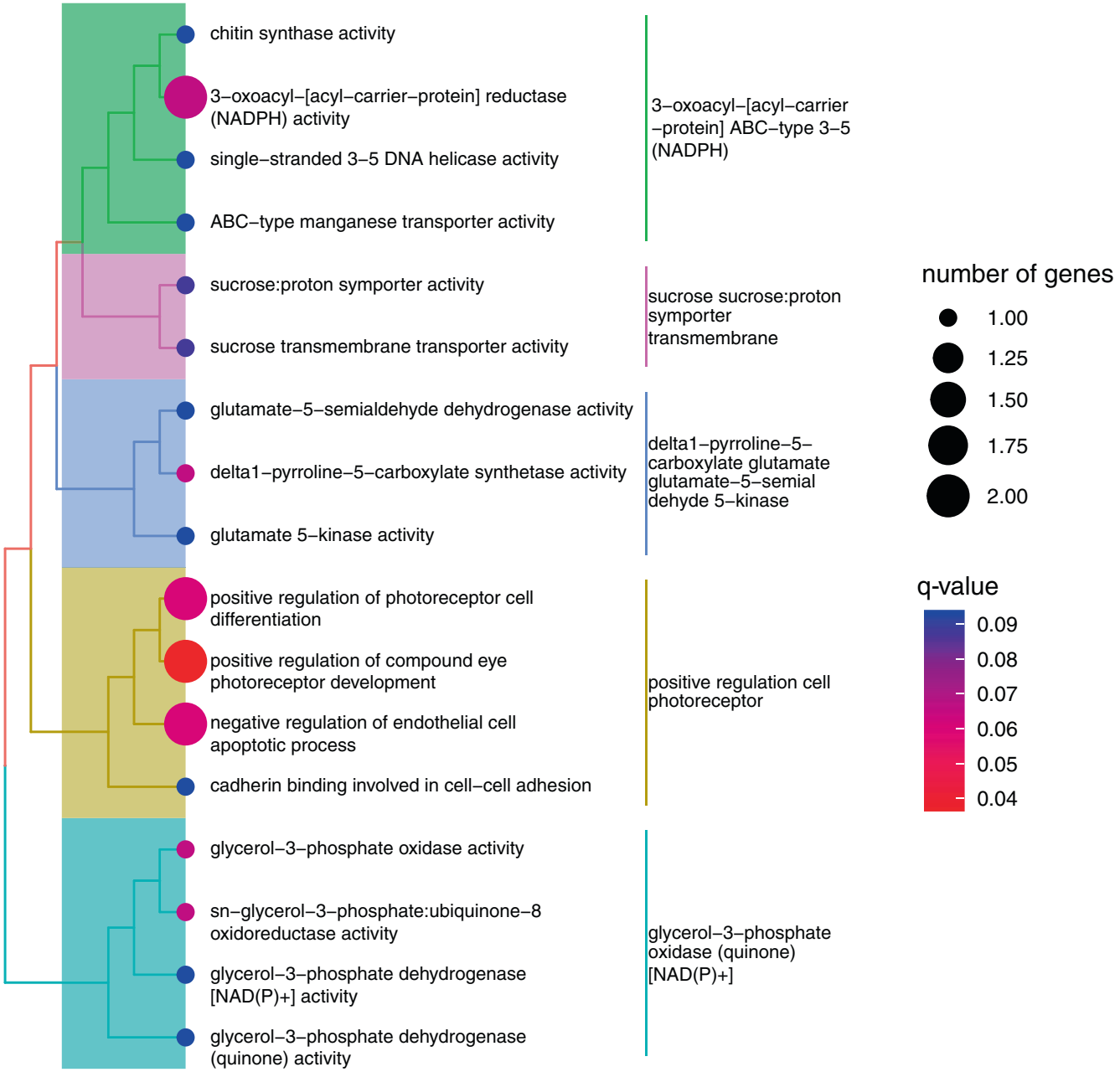

FIGURE 7 | Hierarchical clustering of significantly enriched terms after correcting by FDR. Clusters are highlighted in different colours.

spiders (e.g., *Stegodyphus sarasinorum*; $\pi = 0.0005$; Settepani et al. 2017). Indeed, these values even exceed genome-wide averages reported for the cosmopolitan fruit-fly *Drosophila melanogaster* (averages of $\pi = 0.003–0.006$) and overcome those estimated in high-recombination regions in this species ($\pi < 0.01$; Kapun et al. 2021). Under the mutation-drift equilibrium, these elevated levels of nucleotide polymorphism would indicate either large historical effective population sizes or high mutation rates in this species. Based on divergence data, we estimated the neutral mutation rate per site and per generation to be $\mu = 6.07 \times 10^{-9}$, which is a value typically observed in eukaryotes. Then, assuming a random mating, constant size population, the estimated nucleotide diversity values would imply a large effective population size ($E(N_e) = 375{,}000$ individuals). Although also high, the level of nucleotide diversity is lower than theoretically expected on the X chromosome, suggesting that mutational or selective factors acting differently on this chromosome may be operating in this population. Furthermore, our results do not support the 'faster X' hypothesis (Bechsgaard et al. 2019; Charlesworth, Campos, and Jackson 2018), as the number of protein-coding genes with evidence of positive selection inferred by the MKT is not proportionally higher on the X than on the autosomes (Table S10). However, the number of genes and substitutions used in the MKT analysis is too small to draw any firm conclusions. Data from a more distant species will be needed to extend the analysis to the remaining genes.

The enormous variability found in *D. silvatica* could be due to some population structure, which could raise nucleotide diversity in a particular locality by the introduction of variants from other highly differentiated populations, rather than to

high historical effective population sizes. Indeed, the populations of *D. silvatica* from La Gomera, La Palma and El Hierro are highly divergent lineages, and some authors have even proposed that they are different species. However, the phylogenetic results (Figure S1) and especially the PCA (Figure 4) allow us to rule out this hypothesis. Under a scenario of gene flow, introgression or admixture between islands affecting the surveyed locality, we would expect much more intrapopulation variability in the PCA analysis. Therefore, the results indicate that the high nucleotide diversity in the La Gomera population does not seem to be due to population subdivision in this species and would be more plausible under a scenario of a large stable historical population size. Indeed, demographic inference, and linkage disequilibrium and recombination rate estimates seem to anticipate even larger than the current N_e in the history of this species (see below).

Results from demographic inference based on the unfolded SFS predict large stable (at least for more than 500 ky) ancestral population sizes for *D. silvatica* (~4×10^6 individuals). Despite the recent, abrupt decline in population size, we still observe remarkable levels of nucleotide diversity in this species. Moreover, the levels of polymorphism estimated in the single individual sequenced at medium coverage (mean $\pi=0.016$ for autosomes) are congruent with those estimated for the whole sample ($n=24$), indicating that the fine structure observed in the PCA does not account for this large, unexpected levels of polymorphism. Hence, this species appears to have maintained large, constant population sizes for a long time, at least during the last 100,000 years. The small proportion of nucleotide substitutions observed in coding regions between *D. silvatica* and *D. bandamae* suggest that this demographic history may be common to other, closely related species, pointing to shared polymorphism as an important factor to consider in variability studies on this genus in the Canary Islands.

The recombination rate also appears to be noticeably high across the *D. silvatica* genome, with an LD decay at the same order as that found in species with known large population sizes (Signor, New, and Nuzhdin 2018). Inaccuracies in the estimated demographic model and/or the scaling applied internally in pyrho to convert the ρ to r, not yet validated in non-human genomic data, may influence the magnitude of the estimated r. However, direct estimates of ρ in iSMC (per 100 kb window autosomal $\rho=0.187$), a method that jointly estimates demography and recombination, are consistent with a high-recombination scenario. In any case, the estimated recombination landscape across the genome is expected to be confidently inferred. In this context, autosome recombination rates and LD are higher than those for the X chromosome (Figure 2d). Intriguingly, we have not detected visibly reduced levels of genetic recombination in the extremes of chromosomes, such as those observed in centromeric and telomeric regions in *Drosophila* (Comeron, Ratnappan, and Bailin 2012; Figure S2). In line with this finding, we also failed to observe any obvious reduction in the variability of these chromosomal regions (Figure 2a). These findings may relate to this species' unique organisation of holocentric chromosomes (Benavente and Wettstein 1980). Indeed, the presence of holocentromeres does not appear to cause a reduction in the rate of recombination. In fact, in some cases, these levels are even remarkably high (e.g., Lepidoptera, Nematoda, Carex; Escudero, Hahn, and Hipp 2018; Rockman and Kruglyak 2009; Torres et al. 2023). Investigation into the role of these chromosomal organisations in shaping genomic features deserves attention in future studies.

In this study, we have identified several genomic regions and protein-coding genes with signals of recent positive selection in *D. silvatica*. Interestingly, several GO terms enriched in these features are of biological significance for this species. It is important to clarify here that we included in the analysis all GO terms, including those with few associated genes. The main reasons for not filtering out these terms are that (i) we are dealing with a small list of candidate genes, and (ii) we are studying a non-model-organism, for which we lack precise information on the function of genes in any closely related species; ontology information is therefore primarily based on orthology relationships with distant species. Overall, these two aspects are expected to affect statistical power in the GO overrepresentation analysis negatively. We identified a correspondence between the number of genes per GO term in our analysis and those found in former studies (published between 2010 and 2024) that include the same or related terms (Figures S4 and S5). It is worth noting that those significantly enriched terms with a higher number of genes are those within the 'photoreceptor' cluster (the cluster with lowest p- and q-values). These terms are among the ones having more functional information in databases, which makes us confident that vision is the biological process with best evidence of being the target of selection in *D. silvatica*. On the other hand, it should be noted that in the absence of a database of GO terms for a closely related organism, GO terms are assigned to genes by sequence similarity. Therefore, many of the GOs associated with *D. silvatica* genes were obtained by BLAST or profile-based searches of the whole protein or some of its domains of *D. silvatica* against existing databases, which explains some associations with functions apparently not relevant to animal species.

None of the genes exhibiting footprints of natural selection in the divergence of *D. silvatica* and *D. bandamae* are within the candidate regions identified in the RAiSD analysis, suggesting that the specific targets of selection would be different at different evolutionary time scales. On the other hand, we found some GO terms, such as those related with visual systems, which are enriched among the candidates from both analyses (Table S11), suggesting that vision could have been recurrently targeted for adaptation throughout the recent history of this species, albeit mediated by different target genes.

D. silvatica is a nocturnal ground-dwelling hunting spider that shelters in silk cocoons under rocks, tree trunks and barks during daylight (Pekár, Líznarová, and Řezáč 2016). Unlike many spiders, *Dysdera* lacks the anterior median eyes (AME, principal eyes), which are anatomically and functionally different from the remaining three pairs of eyes (secondary) (Morehouse et al. 2017). Principal eyes are responsible for spatial acuity and, in some spiders, colour vision, whereas secondary eyes have less acuity and specialise in peripheral view and movement detection. In many cases, secondary eyes possess a mirror-like tapetum, a biological reflector enabling nocturnal

organisms to see in dim light. In visually guided animals, one of the evolutionary strategies to enhance the visual system is by increasing eye size (Gonzalez-bellido et al. 2022). However, in spiders, the type of eyes that undergo this modification varies depending on the ecological needs. For instance, jumping spiders of the family Salticidae have developed enlarged principal eyes to adapt to high-resolution vision in bright light conditions (Land 1971). Conversely, spiders of the genus *Deinopis* have enlarged secondary eyes, rather than principal eyes, to adapt to nocturnal foraging (Stafstrom, Michalik, and Hebets 2017; Stafstrom and Hebets 2016). Noticeably, some of the enriched terms in our positive selection analyses seem to be directly or indirectly related to eye size (*negative regulation of cell size* and *regulation of apoptotic signalling pathway*, *regulation of epithelial cell apoptotic process*, *negative regulation of endothelial cell apoptotic process*; Table S9 and Figures 6 and 7). Moreover, other significant terms are likely related to secondary eyes (*compound eye photoreceptor development* and *mushroom body development*; Table S9 and Figures 6 and 7). Since only secondary eyes direct visual information towards the mushroom bodies (Barth 2002; Strausfeld and Barth 1993), beneficial mutations causing selective sweeps in *D. silvatica* have likely been driving adaptations to enhance its visual system. This adaptation is probably mediated similarly to that observed in other species, aiding in the better detection of movement in low-light-level environments (Meece, Rathore, and Buschbeck 2021).

Several species of the genus *Dysdera* exhibit preferentially, facultatively or even nearly obligate feeding on terrestrial woodlice. Species of this genus show adaptations for nutrient extraction, particularly regarding nitrogen extraction efficiency (Toft and Macías-Hernández 2017). Some components of the nitrogen metabolism and the urea cycle (*ornithine*, *citrulline*, *pyrroline−5−carboxylate*, *glutamate* and *L-proline*) are among the significantly enriched GO terms in *D. silvatica* selective sweep candidate regions (Table S9 and Figures 6 and 7). In most animals, the urea cycle has a dual function: nitrogen excretion and arginine biosynthesis (Campbell 1973). This amino acid is considered an essential nutritional and structural requirement (Rodriguez and Hampton 1966), being involved in muscular contraction for providing rapid energy during fast movements (Laino et al. 2017) and the synthesis of proline, which is a predominant amino acid in cuticle proteins and in collagen (Cianciosi and Hird 1986; Richards and Ireland 1978). However, despite having a functional purine biosynthetic pathway, spiders and other arachnids have a non-functional or incomplete urea cycle and excrete the excess of nitrogen as guanine instead of urea (Jenkinson, Grody, and Cederbaum 1996). In these organisms, arginine becomes an essential dietary component (Campbell 1973). Our results suggest that metabolic adaptations to increase nitrogen extraction efficiency from isopods (as a source of arginine) are still ongoing in this species. In fact, most of the metabolic differences observed between *Dysdera* with distinct dietary preferences can be attributed to changes in the performance of nitrogen extraction when fed on a different prey (Toft and Macías-Hernández 2017), indicating that this trait is a major driver of species diversification in the Canary Islands. Besides, proline and arginine are also implicated in certain mechanical properties of silk. Proline can confer elasticity (Hayashi, Shipley, and Lewis 1999), while arginine may play a role in conferring resistance to excess humidity (Kim et al. 2021). Moreover, in scorpions, arginine metabolism can yield various products, some of which are involved in venom composition (Arjunwadkar and Reddy 1983). Interestingly, a comparative transcriptomic study of five species of this genus identified two putative venom toxins (Vizueta et al. 2019) as candidates for adaptive changes in these species. Lastly, arginase, the enzyme responsible for converting arginine to proline and glutamate in nitrogen metabolism, uses manganese (Mn2+) as a cofactor. Interestingly, we found the biological process of ATP-dependent pumps transporting Mn2+ among the enriched GO terms in candidate selective sweep regions in our study.

Another noteworthy GO term enriched in candidate selective sweep regions is *chitin synthase activity* (Figure 7). Chitin, a complex biopolymer of sugar products, along with proteins, serves as the primary constituent of cuticles in arthropods. This term was also identified as a candidate by Vizueta et al. (2019). The genes associated with this function could be under positive selection to enhance protection or prevent desiccation in *D. silvatica*. We also observed the function of *calcium determination cytosol endoplasmic* among the significantly enriched terms. Calcium is one of the main intracellular signalling molecules, and it is involved in crucial processes such as muscle excitation-contraction and energy metabolism. Consequently, these functions are also potential targets of molecular adaptation to enhance prey performance in this species. Finally, based on our analysis, other functions could be under positive selection in *D. silvatica*, including fatty acid metabolism, glycometabolism and the production of energy and glycerol, which could be involved in rapid or seasonal adaptive responses to desiccation (preventing water loss) and cold stress (Coulson 1990; Czajka and Lee 1990; Danks 2000; Misener, Chen, and Walker 2001; Williams, Shorthouse, and Lee 2002). However, the candidate genes associated with the enriched GO terms related to these functions show orthologies with evolutionary distant genes or are difficult to interpret from a functional point of view. Although it is necessary to be cautious with the biological interpretations derived from all these results, we strongly contemplate previously introduced candidates being considered for future functional validation.

Overall, our study has revealed unexpected aspects of the recent evolution of an endemic island spider. The specific conditions favouring the long-term predicted historical effective population sizes, and the recent population decline detected in this endemic species are particularly relevant for understanding the origin, maintenance and loss of biodiversity in island ecosystems. This knowledge is instrumental for managing and conserving biodiversity in a changing world (Mergeay and Santamaria 2012). On the other hand, selection analysis through the lens of population genomics points to traits such as vision and nitrogen extraction as targets of adaptation in this spider. The results presented here contribute to increasing our knowledge of a poorly studied arthropod lineage from the point of view of population genomics, in general and to have a clearer picture of how adaptive radiations arise in environments that are true natural laboratories of evolution.

Author Contributions

J.R., A.S.-G., and M.A.A. conceived the study. P.E., S.G.-R., and J.R. drafted the first version of the manuscript. P.E. and S.G.-R. performed the analysis. P.E., S.G.-R., A.S.-G., and J.R. interpreted the data. All authors revised and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequence reads have been deposited at the DDBJ/ENA/GenBank under the Bioproject PRJNA1075441, with the following Sequence Read Archive (SRA) accession numbers, SRR27941309-SRR27941316 and SRR27941318-SRR27941321 for the *D. silvatica* low-coverage data, and the SRR28624427 and SRR27941317, the *D. silvatica* and *D. bandamae*, medium-coverage data.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.