

Orogeny of Ethiopian sky islands and Pliocene climate shifts promoted budding speciation and complex admixture in flightless *Calosoma* ground beetles

Cody Raul Cardenas^{1,*}, J  r  my Gauthier^{1,2}, Julia Bilat¹, Marjorie Lab  dan³, Yeshitla Merene⁴, Joachim Schmidt⁵, Emmanuel F.A. Toussaint¹

¹Department of Entomology, Natural History Museum of Geneva, Geneva, 1208, Switzerland

²Natur  um—State Museum of Natural Sciences, Lausanne, 1005, Switzerland

³Department of Ecology and Evolution, University of Lausanne, Lausanne, 1015, Switzerland

⁴Crop Research Directorate, Amhara Agricultural Research Institute (ARARI), Bahir Dar, Amhara, 6000, Ethiopia

⁵Institute of Biosciences, General and Systematic Zoology, University of Rostock, Rostock, 18055, Germany

*Corresponding author. Natural History Museum of Geneva, Geneva, Switzerland. E-mail: nepenthesbaphomet@outlook.com, cody.cardenas@unige.ch

Abstract

Flight loss, through its effect on the resulting dispersal capacity and gene flow, has been proposed as a driver of diversification. Ground beetles of the genus *Calosoma* are unique among the predominantly flightless Carabinae, as nearly half of the species retain flight-capability. Most flightless *Calosoma* occur in extreme, often isolated habitats such as montane ecosystems. Species in this genus provide an ideal system to investigate the impact of flight loss on diversification across geographical scales. Here, we focus on the flightless species *Calosoma* (*Carabophanus*) *gestroi*, which occurs at high elevations in the southern mountains surrounding the Great Rift Valley of Ethiopia. We investigate genetic differentiation among populations and the biogeographical history of this species across its geographical range using HyRAD-X data. Our results indicate that populations from different mountains form distinct clades, exhibiting both deep genetic differentiation and signatures of recent admixture. The combination of warmer Pliocene climatic conditions and the ongoing orogeny of the Ethiopian highlands promoted an ideal setup for complex cycles of population isolation and admixture. Warm periods drove genetic fixation on either side of the Great Rift Valley, whereas glacial oscillations across emerging sky islands in the Eastern Mountains facilitated genetic homogenization through cycles of allopatric isolation and secondary contact.

Keywords African mountains, beetle phylogeography, Carabidae evolution, Great Rift Valley biogeography, population genomics, tropical mountain phylogeography

Introduction

Flight is a key innovation that has enabled pterygote insects to diversify over hundreds of millions of years (Kingsolver and Koehl 1989, Misof *et al.* 2014, Schachat *et al.* 2023, Treidel *et al.* 2024). It provides species with the capacity to disperse and colonize new habitats, find mates, and access resources (Dudley 2000). However, secondary flight loss through modifications of wing morphology is widespread across Pterygota, and it is estimated that ~5% of insect species have lost

functional wings (Wagner and Liebherr 1992, Roff 1994). This striking evolutionary pattern and its underlying drivers have long attracted attention and remain actively debated. The loss of flight is frequently hypothesized to promote diversification, because populations are no longer capable of readily dispersing and exchanging genes (Roff 1990, Ikeda *et al.* 2012, Leihy and Chown 2020, Greig *et al.* 2025). Accordingly, genetic structure in flightless lineages is expected to be stronger than in their flight-capable counterparts (Salces-Castellano *et al.* 2021,

Received: 1 February 2026. Revised: 23 April 2026. Accepted: 28 May 2026

   The Author(s) 2026. Published by Oxford University Press on behalf of The Linnean Society of London.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Suárez *et al.* 2022). This pattern is partly explained by the habitat stability hypothesis, which predicts that lineages inhabiting stable ecosystems are more likely to lose flight capability compared to lineages occurring in less stable ecosystems (Darlington 1943, Slater 1977, Roff 1990). These predictions and the subsequent impact of flight on micro- to macro-evolutionary patterns have been investigated in some detail, although significant gaps remain in understanding the link between wing evolution and fine-scale evolutionary patterns across insects. Of particular interest are clades that have lost the capacity to fly and have also been exposed to climatic oscillations through time, rendering their habitats less stable than predicted by theory. In such frameworks, investigating the response of flightless lineages to climatic fluctuations can provide interesting new insights into our understanding of the intricate interplay between ecology and flight loss.

Changes in habitat suitability can be effectively examined in mountain systems, where climatic oscillations and geological reconfigurations are frequent. For instance, the importance of Pleistocene temperature changes in shaping biodiversity across montane landscapes is well documented (Hewitt 1996, Bennett and Provan 2008, Stewart *et al.* 2010, Knowles and Massatti 2017, Flantua *et al.* 2019, Fonseca *et al.* 2021, Ortego and Knowles 2022). During cooler periods, lineages adapted to high-altitude ecosystems are forced to shift their altitudinal ranges downslope, which can promote range expansion and create opportunities for secondary contact and gene flow (Weng *et al.* 2016, Chala *et al.* 2017, Stone and Wolfe 2021, Gao *et al.* 2022). Conversely, during climatic warming events, lineages tend to track their ecological preferences towards higher altitudes, which is accompanied by a contraction in habitable range, promoting genetic isolation and strong selective pressure (e.g. Weng *et al.* 2024). Such expansions and contractions can drive population fragmentation in mountainous landscapes, promote allopatric speciation, and cause local extinctions (Avice 2000, Hewitt 2000, 2003, 2004, Coyne and Orr 2004, Schluter 2009). Reconstructing the fine-scale evolutionary history of montane lineages in response to climatic shifts is an active area of research, particularly in light of current global warming and theoretical predictions regarding highland biota (Laurance *et al.* 2011, Chan *et al.* 2024). Yet, several mountainous regions of the world remain surprisingly under-studied, despite potentially representing ideal laboratories for testing theoretical hypotheses across parallel geographical frameworks.

One of the most prominent rift systems on Earth occurs in East Africa. Extending over 3000 km long and traversing the Ethiopian and Kenyan plateaus, the East African Rift was formed through intense geological processes that not only created extreme geological features, but also harbours and exceptionally high species richness concentrated in the Eastern Afromontane Biodiversity Hotspot (Mittermeier *et al.* 2011). Since the Miocene, Ethiopia has been progressively shaped by this rift, which currently divides the northwestern and southeastern highlands, and is characterized by moderately elevated plateau landscapes from which high volcanic mountains protrude, some of which reach over 4000 m (Billi 2015). These periods of intense geological activity, coupled

with palaeoclimatic changes, have played a central role in shaping the high biodiversity observed in Ethiopia today (deMenocal 2004, Trauth *et al.* 2009, Couvreur *et al.* 2021). In the late Miocene, the climate of Ethiopia was warmer and wetter than today (Andrews and VanCouvering 1975). Subsequently, the Pleistocene climate was characterized by oscillating glacial cycles, leading to expansions and contractions of environments (deMenocal 2004). During warm interglacial periods, grasslands and savannahs contracted, while moist forests expanded into higher-altitude montane environments. In contrast, during glacial periods, when the climate was cool and dry, forests contracted to lower altitudes, driven partly by the appearance of glaciers on mountain peaks (Coetzee 1964, deMenocal 2004, Groos *et al.* 2021). The impact of palaeogeography and palaeoecology on phylogeographical patterns and processes in species groups inhabiting the highland ecosystems surrounding the Great Rift Valley remains poorly investigated. Previous studies have focused primarily on plants and vertebrates (Gottelli *et al.* 2004, Kebede *et al.* 2007, Freilich *et al.* 2016, Mairal *et al.* 2017, Mizerovská *et al.* 2023, Dianat *et al.* 2024). Brühl (1997) highlighted the importance of East African Rift volcanoes as Pleistocene refugia for wingless insect lineages, based on a comparison of regional faunas. However, few studies exist on high-altitude Rift arthropods and invertebrates.

Ethiopia harbours a rich diversity of ground beetles (Coleoptera: Carabidae; Merene *et al.* 2023). However, aside from studies on the Trechinae fauna, regional ground beetles have received limited recent attention (Pawłowski 2003, Ortuño and Novoa 2011, Quéinnec *et al.* 2021, Faille *et al.* 2023, Schmidt *et al.* 2024, Schmidt and Merene 2024a, 2024b). Among the ground beetles of the Ethiopian highlands is the cosmopolitan genus *Calosoma* Weber, 1801 represented by the subgenus *Carabophanus* Kolbe, 1895. Kolbe (1895) originally described this lineage as a potential morphological intermediate between the genera *Calosoma* and *Carabus*. Kolbe further noted that the peculiarities of these beetles, specifically their restriction to highlands above ~3000 m and the loss of functional wings, were traits shared with other flightless highland *Calosoma* in Africa and the Americas. Indeed, the four species belonging to *Carabophanus* are brachypterous like other African subgenera *Carabomorphus* Kolbe, 1895, *Orinodromus* Kolbe, 1895, as well as *Carabomimus* Kolbe, 1895 from Mexico (Jeannel 1940, Bruschi 2013, Toussaint and Gillett 2018).

The subgenus *Carabophanus* consists of four morphologically distinct species: *C. antinorii* Gestro, 1871, *C. arrowi* Breuning, 1928, *C. gestroi* Breuning, 1928, and *C. raffrayi* Fairmaire, 1883, all restricted to the Ethiopian highlands (see Fig. 1; Bruschi 2013, Merene *et al.* 2023). All four *Carabophanus* species are exclusively found in the afromontane and afroalpine zones and have micropterous wings that are completely reduced to stubs, resulting in flightlessness and reduced dispersal capacity (Bruschi 2013). They prefer habitats with high insolation, such as grasslands and shrublands, and frequently occur in cultivated or fallow fields (our personal observations; Bruschi 2013). While *C. arrowi*, *C. raffrayi*, and *C. antinorii* occur in the northern and

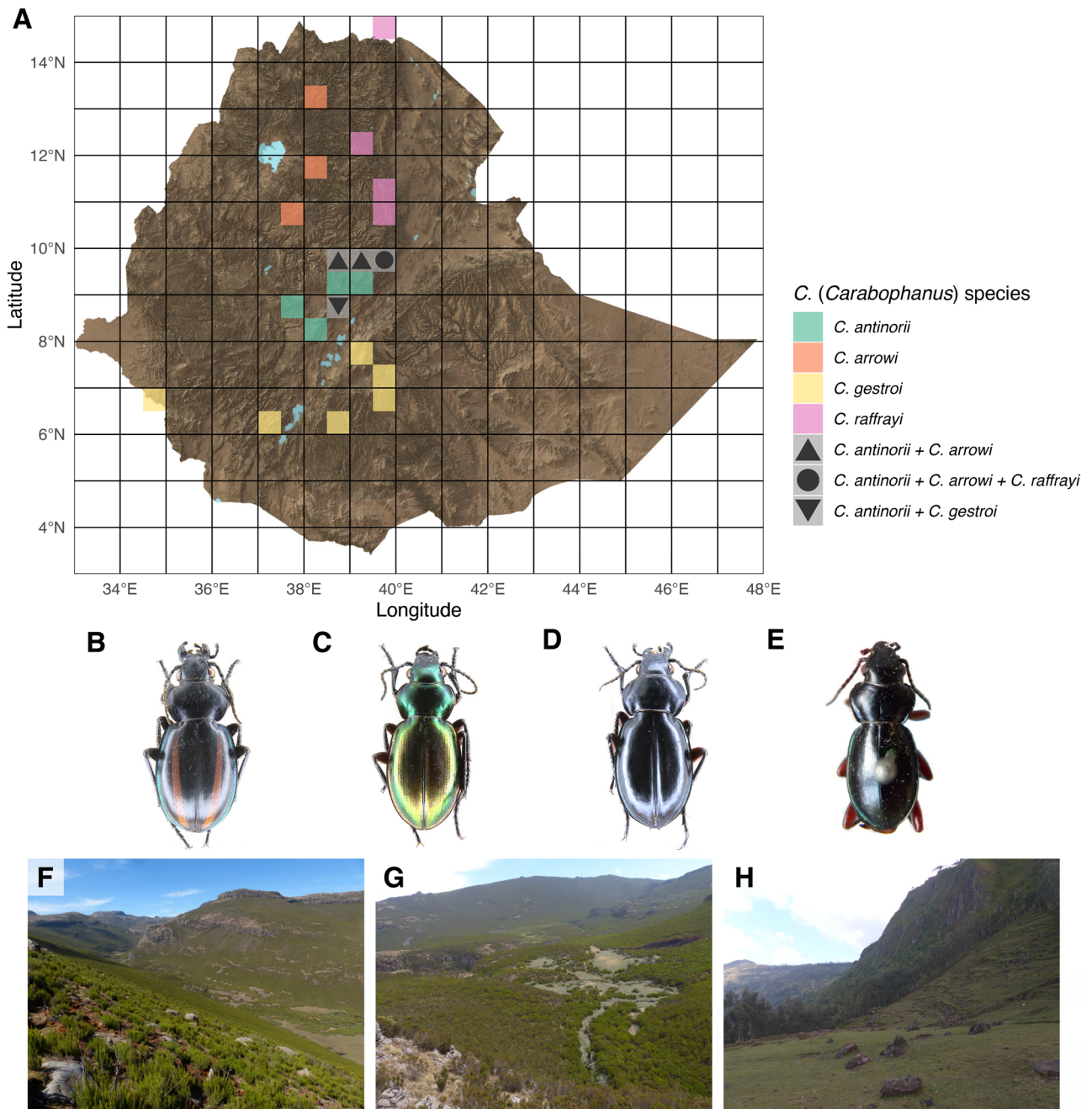


Figure 1 *Calosoma* (*Carabophanus*) distribution. A, elevation map of Ethiopia using data from [WorldPop \(2018\)](#), illustrating the distribution of *Carabophanus* species; modified from [Merene et al. \(2023; fig. 14\)](#). Each 0.5-degree cell indicates presence or absence of a *Carabophanus* species. When more than one species is present, those localities are indicated in the key. Images of each *Carabophanus* species are presented, from ([Bruschi 2013](#)): B, *C. antinorii*; C, *C. arrowi*; D, *C. gestroi*; and E, *C. raffrayi*. Typical habitat of *C. gestroi*: F, upper Tegona valley, northern macro-slope of Bale Mts, ~3600 m; G, Crater valley, Mt Chilalo, ~3900 m; and H, western slope of Mt Gughe, ~2900 m. Photo credit Joachim Schmidt. This figure was stylized using Inkscape (v.1.4.2; [Inkscape Project 2025](#)).

central mountains northwest of the Rift Valley, the distribution of *C. gestroi* is restricted to the southern mountains of Ethiopia ([Merene et al. 2023](#)). Notably, *C. gestroi* is the only *Carabophanus* species that occurs on both sides of the Rift Valley and has a significant part of its distribution in the southeastern mountains of Ethiopia. In the north, *C. gestroi* occurs across the Arsi Mountains, notably on the isolated stratovolcanic Mt Chilalo (4036 m) and Mt Kaka (4184 m). The

species is also found across the Bale Mountains just south of the Arsi massif, with a widespread distribution in the highlands. Additionally, *C. gestroi* is found in highlands west of the Rift Valley and the Abaya and Chamo Lakes, particularly in the Gughe Highlands, with elevations reaching ~3500 m. Both *C. arrowi* and *C. gestroi* are documented as preferring humid environments, with *C. gestroi* occurring in seasonally swampy moorlands (our personal observations; [Bruschi 2013](#)).

Calosoma gestroi occurs across the widest elevational range within the subgenus *Carabophanus* (2800–4000 m), whereas other species have a much narrower documented altitudinal range (Bruschi 2013).

Given the natural history and geographical distribution of *C. gestroi*, this species is an ideal study system to explore how the complex palaeogeographical and palaeoclimatic history of the Ethiopian mountain ranges may have driven its current distribution and shaped its evolutionary history. This work specifically investigates the genetic diversity, divergence times, and the biogeographical history of the flightless *C. gestroi* across the Ethiopian highlands. We therefore aim to assess how genetically diverse this species is and identify the biogeographical processes that may have led to its current distribution.

Methods

Taxon sampling

Between 2016 and 2020, samples of *Calosoma gestroi* were collected at 2780–4100 m elevation in the mountains surrounding the Southern Great Rift Valley of Ethiopia (Fig. 1). Two *Calosoma* (*Carabophanus*) *arrowi*, 23 *C. (Carabophanus) gestroi* (Fig. 2; Supplementary Fig. S1), one *C. (Carabophanus) raffrayi*, and three *C. (Carabops) abyssinicum* Gestro, 1880 were collected in 96% EtOH. For *C. gestroi*, sampling sites were Mt Chilalo (MtChi $N = 4$), Mt Kaka (MtKak $N = 4$), Bale Mountains (North, West, and South; BalMN $N = 4$, BalMW $N = 3$, and BalMS $N = 6$; respectively), and the Gughe Highlands ($N = 2$). For

phylogenomic purposes, publicly available data were included for the following outgroups: *Carabus* (*Platycarabus*) *depressus* (Bonelli, 1810) (GCA_048127345.1), *Calosoma* (*Castrida*) *granatense* Géhin, 1885 (GCA_022063505.1), *Calosoma* (*Calosoma*) *frigidum* Kirby, 1873 (SRR2083640), *Calosoma* (*Callistenia*) *wilkesii* LeConte, 1852 (GCA_044734065.1), and *Calosoma* (*Chrysostigma*) *tepidum* LeConte, 1852 (GCA_044734075.1) (McKenna *et al.* 2019, Vangestel *et al.* 2024, Gauthier *et al.* 2025, Gauthier *et al.* 2025).

Genome sequencing workflow

Since no well-preserved specimens of *C. gestroi* were available to generate a reference genome, a closely related *Calosoma* (*Carabophanus*) species was used to improve locus recovery and variant calling. A specimen of *Calosoma* (*Carabophanus*) *arrowi* (NCBI Biosample SAMN60565131; CBX0265) was captured on 30 April 2022 from Mt Choke in Ethiopia and kept frozen at -80°C until DNA extraction. High-molecular-weight DNA was extracted from dissected tissue following the protocol described in Gauthier *et al.* (2025). The remaining post-extraction tissues (mostly elytra) were mounted on pinned cardboard for dry archival and deposited at the Natural History Museum of Geneva with the label ‘Toussaint lab DNA Voucher CBX0265’ for future reference. A SMARTbell library was prepared with the SMRTbell prep kit 3.0 (Pacific Biosciences, Menlo Park, CA, USA), according to the whole-genome protocol from PacBio (PN: 102–166–600). The polymerase SMRT library complex was prepared using the Sequel® II Binding Kit 3.2,

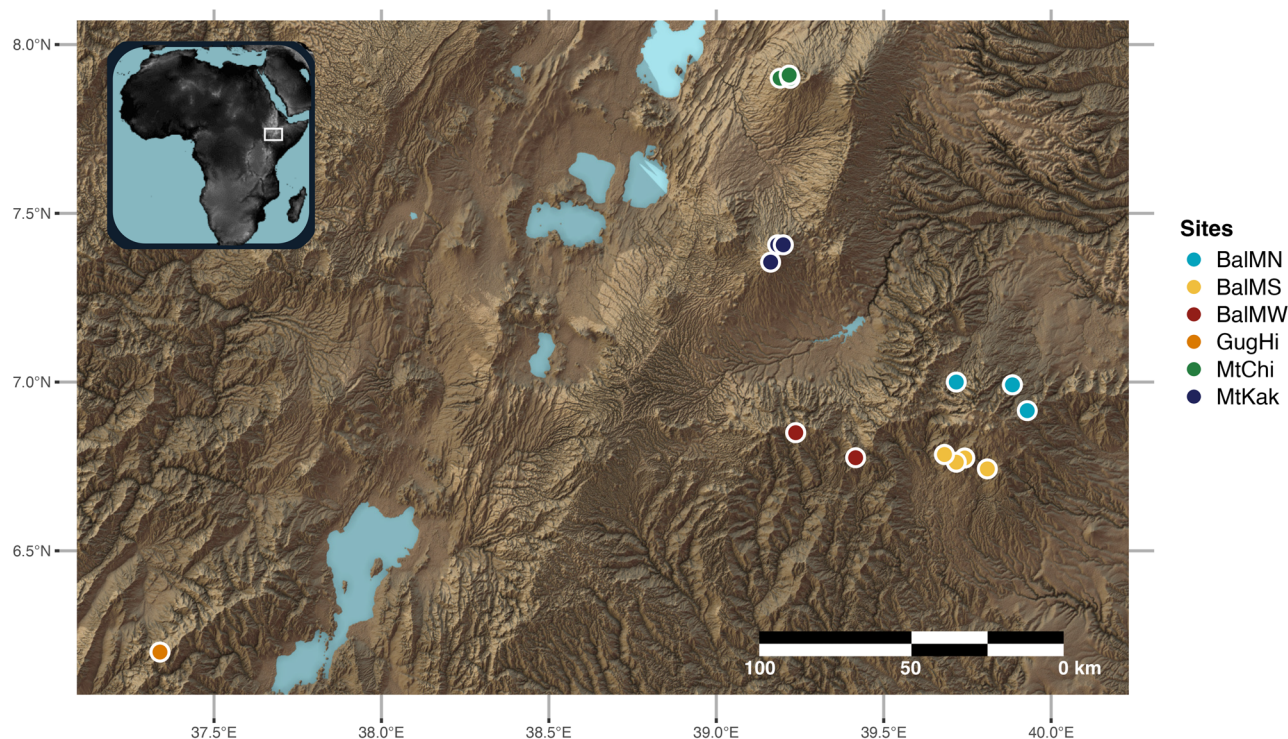


Figure 2 Sampling sites. Sampling localities for *Calosoma gestroi* across the southern Ethiopian mountain ranges surrounding the Great Rift Valley. Sampling sites include Mt Chilalo (MtChi), Mt Kaka (MtKak), Bale Mountains (North, West, and South; BalMN, BalMW, and BalMS, respectively), and the Gughe Highlands (GugHi). The figure was generated using the package rayshader (v.0.37.3; Morgan-Wall 2018) using elevation data from WorldPop (2018), and stylized using Inkscape.

and sequencing was performed on the Sequel IIe System (Pacific Biosciences) at Genesupport LifeScience (Fasteris, Plan-les-Ouates, Switzerland). Assembly was performed using Hifiasm (v.0.19.8; Cheng *et al.* 2021), and duplications were removed using purge_dups (Guan *et al.* 2020). Completeness of the genome was evaluated using BUSCO (Seppey *et al.* 2019), and the insecta_odb10 dataset, which contains 1367 genes. Genomic completeness is summarized in a snail plot generated using BlobToolKit2 (Challis *et al.* 2020).

DNA extraction and HyRAD-X methods

Genomic DNA was obtained via destructive extraction of a single leg for each specimen ($N = 26$) using a QIAmp DNA Micro Kit (Qiagen, Hilden, Germany) and eluted in 20 μ L of molecular-grade water. DNA quantification was assessed using a Fragment Analyzer 5200 (Agilent). Shotgun library preparation and HyRAD-X capture followed the protocol described in previous studies (Toussaint *et al.* 2021, Pauli *et al.* 2024). In April 2022, paired-end library sequencing (150 cycles) was performed on an Illumina NovaSeq 6000 SP (Lausanne Genomic Technologies Facility, Switzerland).

In silico probes

We modified the PHyRAD (Gauthier *et al.* 2020) pipeline to integrate a closely related reference genome for improved variant identification and locus identification. The original probeset generated for use in Toussaint *et al.* (2021), which was used to capture the genomic data, was mapped against the assembled *C. arrowi* genome; using BWA mem (v.0.7.17; Li and Durbin 2009; see ‘Genome workflow’). The mappings were cleaned to remove duplicates and realign indels using the Picard suite (v.2.20.2; Broad Institute 2019) and Samtools v.1.4 (Li 2011). A final *in silico* probe file was generated using a combination of Bedtools functions (<https://bedtools.readthedocs.io>). All scripts are available at https://github.com/crcardenas/Ethiopian_Calosoma.

HyRAD-X bioinformatic workflow

We processed and mapped sample reads; first, raw reads were demultiplexed and technical sequences were trimmed using CutAdapt2 v.2.0 (Martin 2011). Read quality was assessed using FastQC v.0.11.8 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Trimmed reads were collapsed using AdapterRemoval—combine (v.2.3.4; Schubert *et al.* 2016). The long reads from the *C. arrowi* reference genome, together with the long reads from *Carabus* and *Calosoma* outgroups, were processed into 100-bp ‘pseudo-short reads’ so they could be integrated into the PHyRAD bioinformatic pipeline using a custom Python script (‘simulate_reads.py’). These pseudo-reads were summarized using SeqKit (v.2.8.0; Shen *et al.* 2024) and integrated into downstream mapping steps.

All paired-end reads were mapped against the *C. arrowi* *in silico* probes using BWA mem. For variant calling, GATK HaplotypeCaller (v.4.5; Poplin *et al.* 2017) was used to produce a VCF file which was subsequently filtered using VCFtools (v.0.1.16; Danecek *et al.* 2011) with the following

flags: `—max-alleles 2—min-alleles 2—max-missing 0.5—minDP 6—thin 1000—remove-indels` to recover unlinked biallelic single nucleotide polymorphisms (SNPs) with a minimum coverage of six SNPs found in at least half of the samples. Samples with high proportions of missing data were removed from the VCFtools analysis. For phylogenetic analysis, we used the previously mapped reads and a modified PHyRAD pipeline (Gauthier *et al.* 2020) with a minimum read coverage of 3, and a minimum consensus length of 100 bp (‘modified_ortho_consensus.sh’ script).

Population genetic analysis

We broadly summarized the genetic data recovered from the *C. gestroi* samples by performing a principal components analysis (PCA) on SNP variation. We generated two VCF files: the first containing all recovered SNPs (dataset PCA all) and the second with thinned SNPs (dataset PCA filtered) to remove potential linkage (e.g. only one SNP per locus; see the previous section ‘HyRAD-X bioinformatic workflow’). PCA was performed using PLINK (v.1.90b6.2; Chang *et al.* 2015) and all recovered SNPs with the following flags: `—double-id—allow-extra-chr—set-missing-var-ids @:# —make-bed—pca`, to allow for multiple loci and individual samples to be used in a PCA. The output was visualized in R (v.4.5.1; R Core Team 2025).

Genetic clusters were estimated using STRUCTURE (v.2.3.4; Pritchard *et al.* 2000) with the thinned *C. gestroi* SNP dataset. The VCF file was converted into a ‘genind’ object (adegetnet v.2.1.11; Jombart 2008, Jombart and Ahmed 2011) for downstream analyses and then converted into a STRUCTURE-compatible file (Clark 2017). Ten independent replicates were performed with admixture, 100 000 steps, a 50 000-step burn-in, and clusters (K) ranging from one to six. Analyses were assessed using pophelper (v.2.3.1; Francis 2017) in R to determine the best-supported value of K (Evanno *et al.* 2005), address label switching, and visualize the structure plots.

Genetic diversity, including observed heterozygosity (H_o), expected heterozygosity (H_s), inbreeding coefficient (F_{is}), pairwise fixation indices by mountain range (F_{st}), and size-corrected fixation indices (F'_{st} and G'_{st} ; as recommended by Meirmans and Hedrick 2011) were estimated using the R package hierfstat (v.0.5–11; Goudet 2005) and the thinned dataset. Data were examined for evidence of isolation by distance by estimating pairwise geographical distances for each locality using Mercator coordinates in geosphere (v.1.5–20; Hijmans 2019). Although classic population genetic analyses typically require a minimum of three samples per locality, limited sampling in some localities could theoretically be a potential limitation. However, using thousands of loci and SNPs and as few as two samples per locality, population statistics can still provide accurate estimates (see Nazareno *et al.* 2017). Therefore, we are confident that our results provide an adequate estimate of the current genomic landscape of *C. gestroi* populations.

We further assessed the effect of geography on population structure using two analyses of molecular variance (AMOVAs;

Excoffier *et al.* 1992) with the *poppr.amova* function in the R package *poppr* (v.2.9.7; Kamvar *et al.* 2014, 2015). First, AMOVA was used to estimate genetic variation among sites relative to the samples (Sites; formula: ~site). Second, a hierarchical analysis was conducted by comparing genetic variation among mountains, among sites within mountains, and within all sites (site by mountain; formula: ~mountain/site). For each test, 10 000 permutations were performed, accounting for within-sample variation (flag *within* = *TRUE*). Significance tests were conducted with 10 000 replicates using the *rand-test* function in the R package *pegas* (v.1.3; Paradis 2010). All R scripts are available at https://github.com/crcardenas/Ethiopian_Calosoma.

Phylogenetic analyses

We performed phylogenetic analyses to understand the relationships of available *Carabophanus* taxa and the sampled *C. gestroi* localities. To generate phylogenetic matrices, recovered consensus loci were aligned using *MAFFT* (v.7.505; Katoh and Standley 2013) and summarized using *AMAS summary* (Borowiec 2016) and a custom Python script (*summary.py*; available at https://github.com/crcardenas/Ethiopian_Calosoma). Loci with less than 50% occupancy (i.e. percentage of samples present in a locus) were removed from the dataset. Taxa with high proportions of missing or ambiguous base pairs were excluded from phylogenetic analyses. Alignments were trimmed using *trimAl -automated1* (v.1.4, Capella-Gutiérrez *et al.* 2009). The trimmed alignments were concatenated using *AMAS concat* to produce a final matrix and partition scheme for phylogenetic inference.

An optimal partition scheme was identified using ModelFinder as implemented in *IQ-TREE* (v.2.3.6; Minh *et al.* 2020), and the best partition was selected based on the Bayesian information criterion (BIC) score (flags: *-m MFP+MERGE*; Chernomor *et al.* 2016, Kalyaanamoorthy *et al.* 2017). Maximum likelihood (ML) inference was performed in *IQ-TREE* with 1000 ultrafast bootstrap replicates (UFBoot; Hoang *et al.* 2018), 1000 SH-like approximate likelihood ratio tests (SH-aLRT; Guindon *et al.* 2010), optimization of UFBoot trees using nearest neighbour interchange, and 50 independent tree searches (flags: *-bb 100 -bnni -alrt 1000 -runs 50*). Branches with SH-aLRT ≥ 80 and UFBoot ≥ 95 were considered robust. All ML phylogenomic analyses were conducted on Pyrgus, the computational server at the Natural History Museum of Geneva. A SplitNetwork analysis was conducted using the filtered dataset containing only the *C. gestroi* taxa with the SplitsTree App (v.6.0; Huson and Bryant 2024) and estimated using the P-Distance method (Hamming 1950) with default options, and applying the NeighborNet method to obtain splits (Bryant and Moulton 2004, Bryant and Huson 2023).

We estimated the concordance of loci and sites for the ML phylogenetic analysis, where site and locus/gene concordance factors (sCF and gCF, respectively) describe the proportion of data concordant with the ML tree. An sCF score $\leq 33\%$ indicates that ML methods identify different topologies, while gCF scores represent the frequency with which locus trees

definitively support a branch in the ML tree (Lanfear and Hahn 2024). Locus trees were inferred IQ-TREE2 using the following flags: *-S -bb 1000 -bnni -alrt 1000*.

Finally, using the same taxa as in the ML phylogenetic inference (see phylogenetic analyses results), an SNP phylogeny was reconstructed. We retained genotypes with a maximum of 50% missing SNPs and thinned the dataset to a single SNP per locus. We used the Python scripts *Vcf2phylip.py* (v.2.0; 10.5281/zenodo.2540861) and *raxml_ascbias.py* (https://github.com/btmartin721/raxml_ascbias) to remove invariant sites and correct for heterozygous genotypes arising from IUPAC ambiguities during conversion from haplotype calls (VCF file) to PHYLIP format. We performed 50 independent runs in IQ-TREE, each with 1000 UFboot and SH-aLRT replicated, using the general time-reversible model with ascertainment correction (flags: *-m GTR + ASC*).

Coalescent analyses

To account for potential incomplete lineage sorting, we inferred phylogenies using two coalescent methods robust to missing data (e.g. taxa absent from some locus trees) and locus tree uncertainty. *wASTRAL* (v.1.22.2.4; Zhang and Mirarab 2022) was used with the locus trees inferred for concordance analysis, and with an increased number of search rounds and replacement (flags: *-R*). In *wASTRAL* analyses, a local posterior probability (LPP) ≥ 0.90 was considered robust. Second, we used *Asteroid* (v.1.0; Morel *et al.* 2023), estimating 100 random starting trees and 1000 bootstrap replicates (flags: *-r 100 -b 1000*). In *Asteroid* analyses, bootstrap values of ≥ 900 (i.e. 90%) were considered robust.

Divergence time estimation

To understand divergence times of *Carabophanus* and *C. gestroi*, we performed divergence estimates. We generated two additional data subsets, using a reduced genomic matrix containing a single representative per species and per *C. gestroi* STRUCTURE cluster. Sample selection for the cluster subset was made by choosing the sample with the highest number of loci recovered per cluster (species $N = 9$; cluster $N = 11$; *C. gestroi* cluster samples: CBX0222 MtKak, CBX0227 GugHi, and CBX0234 BalMN). The new matrices were generated using the same locus identification, alignment, and trimming pipeline as described above. Loci were identified for downstream dating analyses using a minimum length of 100 bp and at least eight taxa per locus. Two subset ML trees were inferred using this subset of taxa: first, an ML tree search as described above with 50 independent tree searches, and second, for each locus using 1000 UFBoot and SH-aLRT replicates.

For both the species and cluster divergence subsets, 300 loci were selected for divergence time estimation using a 'gene shopping' approach with *SortaDate* (Smith *et al.* 2018) and *phyx* (Brown *et al.* 2017). This method selects for clock-like loci and locus trees with similar lengths exhibiting the least topological conflict with the concatenated ML tree. Only loci containing the *Carabus* outgroup were used in *SortaDate*, identified using a custom Python script (*sortadate_helper.py*; available at https://github.com/crcardenas/Sortadate_IQTree2_helper).

PartitionFinder2 (Lanfear *et al.* 2014, 2017, Stamatakis 2014) was used to determine the optimal partitioning scheme for these 300 loci. To limit overparameterization, the following flags were applied: `—raxml—min-subset-size 2000—rcluster-max 1000—rcluster-percent 20`. ModelFinder, as implemented in IQ-TREE and restricted to substitution models available in BEAST (v.1.10; Suchard *et al.* 2018), was used to select the most likely nucleotide substitution models based on the initial PartitionFinder2 partitions; flags: `-m MF -mset K80, TN93, SYM, HKY, GTR, TNe, GTR -mrate E, I, G, I + G -merit BIC`. The subset ML tree served as a fixed topology for input in BEAST. The tree was converted into an ultrametric topology using the function *chronotree* in the R package *phytools* (v.2.2.4; Revell 2024), with nodes calibrated to median age estimates from Cardenas *et al.* (2025): root 44.09 Mya (*Calosoma* + *Carabus*) and 22.58 Mya for the crown age of *Calosoma*.

In BEAUti (v.1.10; Suchard *et al.* 2018), each partition was configured with an independent lognormal relaxed clock and ModelFinder substitution models. Two analyses were performed using Coalescent constant (Kingman 1982) and Yule process (Yule 1925, Gernhard 2008) tree priors on the fixed topology. Mean substitution priors for all partitions were assigned a uniform distribution, with a starting value of 0.01, and a range from 1.0 to 1.0×10^{-8} . Two secondary calibrations were applied, the split between *Carabus* + *Calosoma* (median 44.09 [40.53–47.78] Mya) and the crown age of *Calosoma* (median 22.58 [20.81–24.95] Mya) as reported by Cardenas *et al.* (2025). The lognormal prior distribution was configured to encompass the 95% highest posterior density (HPD) confidence interval reported by Cardenas *et al.* (2025): root age (*Calosoma* + *Carabus*) mean = 42.441, SD = 1.85, offset = 1.6; crown of *Calosoma* mean = 21.908, SD = 1.056, offset = 0.9. BEAST analyses were conducted for 100 million steps, with five independent runs sampling parameters every 1000 steps. Operator files from all analyses were initially examined to ensure optimal acceptance ratio, and log files were inspected in *Tracer* (v.1.7.2; Rambaut *et al.* 2018) to assess convergence (ESS ≥ 100). The resulting treefiles were combined in *LogCombiner* (v.1.10; Suchard *et al.* 2018) with a 20% burn-in, and annotated in *TreeAnnotator* (v.1.10; Suchard *et al.* 2018).

Results

Genome workflow

A total of 14.3 ng/ μ L, corresponding to 1307.9 ng of genomic DNA was extracted from the *C. arrowi* specimen for high-fidelity sequencing. PacBio HiFi sequencing generated 312677 reads (2.0 Gb) with an average length of 6.5 kb and an N50 of 7.5 kb, from which 9.9 Mb of duplications were removed. The final assembly comprised 5052 contigs, with an N50 of 50 kb, a total length of 196 Mb, and 82.5% completeness based on the universal single-copy orthologue gene set for Insecta (BUSCO) (Fig. 3). Based on the size of the final assembly, the sequencing depth was estimated at $\sim 9.15 \times$.

HyRAD-X data recovery

In total 314304337 (median = 8407954, SD = 9785093.42) reads were sequenced. CBX0240 was excluded due to high levels of missing data (675836 reads; see [Supplementary Material](#)). For the long-read genomic samples (GCA_044734065.1, GCA_044734075.1, and the new *C. arrowi* genome), the processed pseudo-reads retained a median of 51151002.5 (SD = 32836178.15). Overall, 6910 (72.90%) of the *in silico* probes mapped onto the *C. arrowi* reference genome. After filtering, 6211 *in silico* probes were retained, with a total length of 1038397 bp and median length of 167.2 bp. Mapping the samples against these probes recovered a median mapping of 433831.36 reads (SD = 957731.20). Variant calling of the mapped reads across all *C. gestroi* samples identified 104463 SNPs from 120865 sites. After removing insertions and deletions, allowing up to 50% missing data, and thinning SNPs, 846 SNPs remained for population genetic analysis.

We identified 6211 orthologous loci with a per-sample average of 2483 loci (SD = 1500 loci). After alignment, trimming, and applying a 50% locus occupancy threshold, 1772 loci were retained for phylogenetic analyses. Per sample a median of 1239 loci (SD = 388) were recovered, with a median of 26.185% missing data (SD = 21.902%). Sample CBX0213 was excluded from phylogenetic analyses due to low quality; after trimming, 75.77% of sites were ambiguous. Per-sample statistics are provided in the [Supplementary Material](#).

Population genetic analyses

For the unfiltered and filtered PCA, we retained three axes. PCA of the unfiltered 120865 SNPs and the filtered 846 SNPs explained 28.36% and 32.29% of the variation, respectively ([Supplementary Fig. S2](#)). Both analyses show similar patterns, with GugHi samples distinct from the rest of the *C. gestroi* samples, and high levels of overlap between the MtChi and MtKak samples ([Supplementary Fig. S3](#)). The third PCA axis of the unfiltered SNPs exhibits clearer clustering of BalMN and BalMS samples compared to the corresponding axis of the filtered SNPs ([Supplementary Fig. S3](#)).

Genetic clustering analysis using 846 SNPs in *STRUCTURE* suggested an optimal $K = 3$ ($\Delta K = 20.3148$; [Fig. 4A](#); see also [Supplementary Table S1](#)). The admixture plots do not show discrete genetic clustering by sites or mountains ([Fig. 4B](#)). For $K = 3$, BalMW, MtKak, MtChi (black), and GugHi (blue) do not cluster according to sites or mountains. For samples from the BalMN and BalMS sites (tan), there is a varying degree of admixture between the blue and black clusters.

Population genetic statistics indicate a global H_o lower than H_s , and per-site heterozygosity statistics show the same pattern of H_o being lower than H_s ([Table 1](#)). For statistics of individual sites, Gughe Highlands was excluded due to too few taxa ([Supplementary Tables S1 and S2](#)). F_{is} is high in both the global and per-site statistics (see [Supplementary Table S1](#)). Gene diversity across all taxa is relatively low, and global fixation indices indicate moderate levels of population differentiation ([Table 1](#)). Pairwise fixation indices by site indicate low to high levels of differentiation between sites ([Supplementary Table S3](#)). Low levels of differentiation are

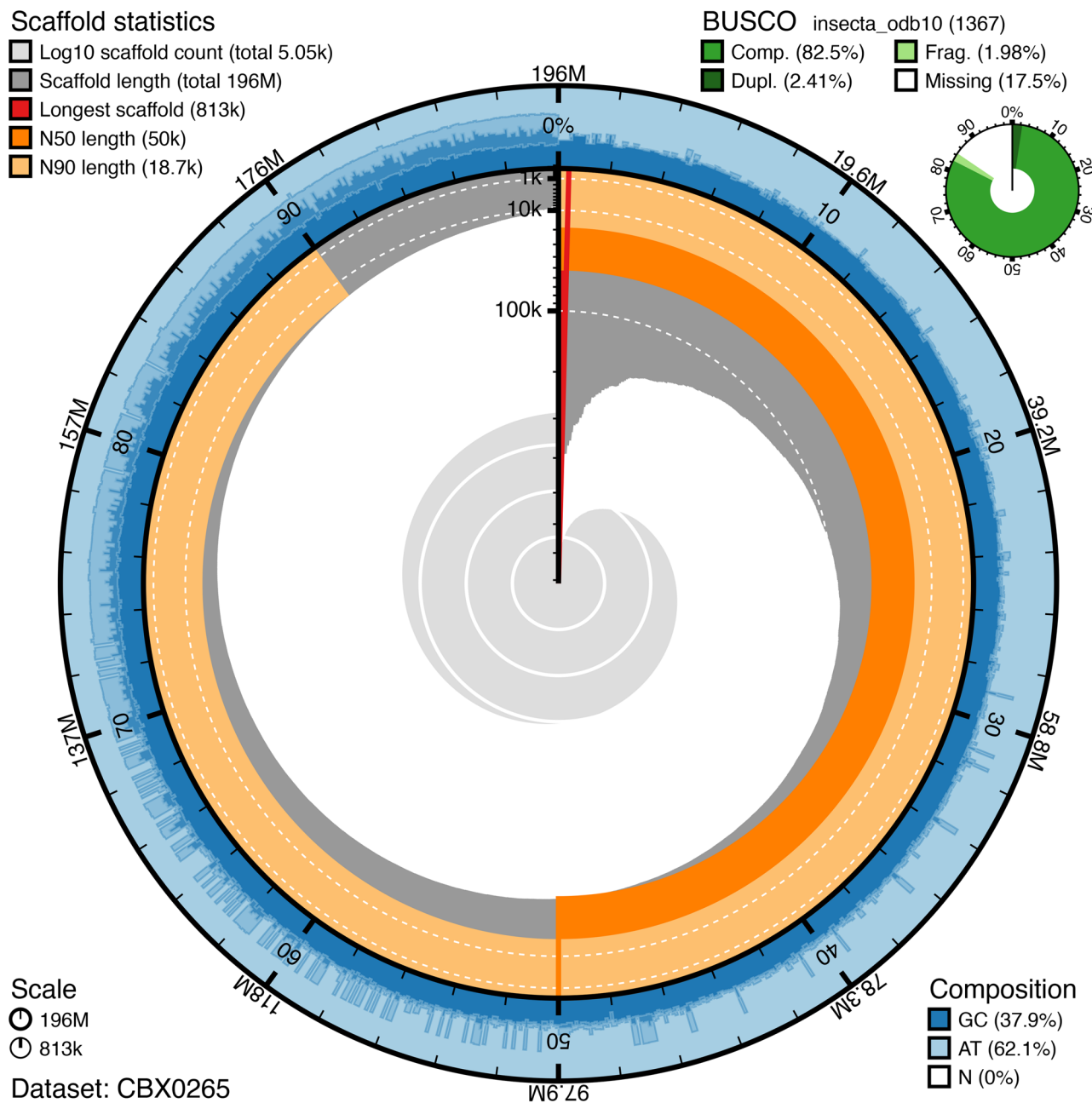


Figure 3 Snail plot summary of the *Calosoma arrowi* genome. Snail plot of the *Calosoma arrowi* genome showing the contig size distribution. Descriptive statistics presented here include total assembly length, N50 and N90 values, the longest scaffold, and the GC and AT content. BUSCO results based on the insecta_odb10 dataset are also provided.

observed between MtKak, MtChi, and BalMW (Supplementary Table S4). Pairwise comparisons of fixation indices by site, mapped against pairwise geographical distances, show evidence of isolation by distance (Supplementary Fig. S4).

The AMOVA for sites and the hierarchical stratification of geography show that the percentage of variation is moderate and is explained by geography (Table 2A, B). Similar to F_{st} , ϕ is a measure of population differentiation (Excoffier *et al.* 1992, Michalakis and Excoffier 1996). The overall percentages of variation are significant between sites (Table 2A), and within sites across mountains (Table 2B). While most of the within-population variation is found among individuals

within a given site, there is evidence of population structure between sites (Table 2A, B). For the hierarchical analysis, the comparison of variation between mountains, sites within mountains, and within sites is significantly different (Table 2B).

Phylogenetic analyses

The preliminary matrix, comprising 1772 loci, spans 291835 bp (Table 3). Among these loci, 43 contained no parsimony-informative sites, and ModelFinder identified 40 partitions. Among the 50 independent tree searches, run 26 yielded the highest log-likelihood of $\text{LnL} = -803831.4828$

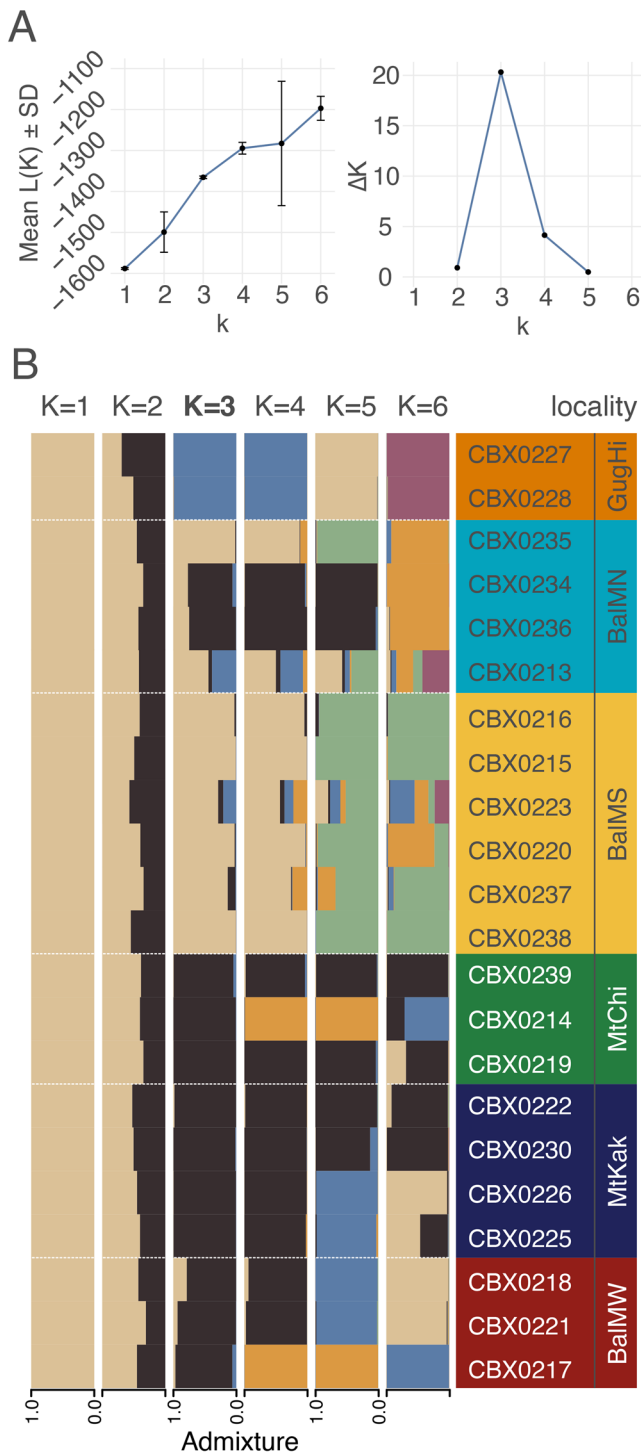


Figure 4 STRUCTURE analysis of *Calosoma gestroi*. Results of the population genetic clustering analysis obtained using *STRUCTURE*. A, mean log-likelihood of K , along with standard deviation and ΔK values, estimated using the Evano Method. B, population structure for K values ranging from 1 to 6. The optimal K value estimated using ΔK is highlighted in bold in the clustering models, and the admixture axis scale is shown for every other K estimate (e.g. $K = 1, 3, 5$), while the same scale is applied across all K values. The resulting STRUCTURE plots were stylized using Inkscape.

(SE = $1.499E + 95$; see [Supplementary Fig. S5](#)). Excluding CBX0213 only reduced the missing data and removed a few parsimony-informative sites ([Table 3](#)), while the ML

inference of the filtered data set improved phylogenetic inference ([Fig. 5](#)). The repeated analysis in ModelFinder without CBX0213 recovered 36 partitions. Among the new 50 independent tree searches, run 47 yielded the highest log-likelihood of $LnL = -801299.4099$ (SE = 0.00). This represents a marginal increase in likelihood and a substantial improvement in standard error. In the filtered dataset, deep phylogenetic relationships were generally well supported, except for the placement of *C. frigidum*. *Calosoma* (*Carabophanus*) was recovered as monophyletic, with strong support for the monophyly of species. Within *C. gestroi* we identified four well-supported clades, comprising samples from: (i) Gughe Highlands, (ii) Bale Mountain North, (iii) Bale Mountain South, and (iv) Bale Mountain West + Mount Kaka + Mount Chilalo. The splits-network analysis revealed distinct clades for Bale Mountain North, Bale Mountain South, and Gughe Highlands, whereas Mount Kaka and Mount Chilalo formed a loose clade, suggesting admixture ([Fig. 6](#)). Finally, Bale Mountain West did not form a distinct clade, but its samples showed evidence of past admixture with Gughe Highlands as well as Mount Kaka and Mount Chilalo.

The analysis of phylogenetic concordance indicated that 14–22.7% of the loci are decisive for backbone phylogenetic relationships (see [Supplementary Fig. S7](#)). The branch corresponding to the subgenus *Carabophanus* was the most decisive, with 22.7% gCF. However, less than 1% of the loci were definitive for resolving inter-locality relationships of *C. gestroi* in the ML tree. The sCF estimates indicated that most of the backbone relationships were closely concordant, indicating that most sites in the matrices agree with the separation of *Carabophanus* species (sCF = 44; see [Supplementary Fig. S8](#)). With the exception of the Gughe Highlands (sCF = 27.5), the sCF values indicated that the dataset provided reasonable resolution of the *C. gestroi* localities sampled.

A total of 1061 SNPs were identified using the same taxa as the ML tree, and filtering for invariant sites retained 995 SNPs for downstream inferences ([Supplementary Fig. S9](#)). The placement of the outgroups and of the *Carabophanus* taxa was consistent and stable across replicates, mirroring the topology recovered in the ML tree. Inter-locality relationships within *C. gestroi* were difficult to resolve using SNPs alone (UFBoot median = 51.5, SD = 25.8), and no locality was monophyletic except for the GugHi population, with most relationships mirroring the clustering observed in the STRUCTURE analysis.

Coalescent analyses

Both coalescent analyses recovered well-supported backbone relationships, except for the placement of *Calosoma* (*Calosoma*) *frigidum*, and for the monophyly of the sampled *Carabophanus* species (see [Supplementary Figs S10, S11](#)). The wASTRAL analysis recovered poorly supported nodes (median LPP = 0.711, SD = 0.138; [Supplementary Fig. S10](#)) and did not reflect the ML relationships. In contrast, the ASTEROID analysis recovers a topology similar to that of the ML tree, albeit with low branch support (median bootstrap = 268.500, SD = 189.666; [Supplementary Fig. S11](#)). Additionally, the ASTEROID analysis recovers some clades that reflect the clustering observed in STRUCTURE.

Table 1 Estimated population genetic statistics.

Population	H_o	H_s	H_t	F_{is}	H'_t	D'_{st}	F_{st}	F'_{st}
Global	0.0115	0.0352	0.0431	0.6743	0.0449	0.0097	0.1823	0.2154

Global estimates of population statistics, including observed heterozygosity (H_o), expected heterozygosity (H_s), total gene diversity (H_t), inbreeding coefficient (F_{is}), corrected gene diversity (H'_t), corrected gene diversity among samples (D'_{st}), fixation index (F_{st}), and population corrected fixation index (F'_{st}) as calculated by *hierfstat*.

Table 2 Analysis of molecular variance.

A	Sites					
Comparison of var.	d.f.	σ	% var	ϕ	Alter	P
Between sites	5	7.0196	19.0973	0.1910	Greater	.0002
Within sites	16	29.7373	80.9027	–	–	–
Total	21	36.7569	100.0000	–	–	–
B	Site by mountain					
Hierarchical comparison of var.	d.f.	σ	% var	ϕ	Alter	P
Between mountains	3	5.0340	13.1999	0.2202	Greater	.0497
Between sites within mountains	2	3.3654	8.8247	0.1017	Greater	.0318
Within sites	16	29.7373	77.9754	0.1320	Less	.0001
Total	21	38.1368	100.0000	–	–	–

Analysis of molecular variance for *C. gestroi* collected in the Highland Plateaus surrounding the Ethiopian Great Rift valley. The two AMOVAs are comparison of (A) variance by site and (B) hierarchical comparison of variance for sites within and between mountains. The analysis of variance by site has only one comparison, and no significance test could be performed for the within-site analysis. Significant *P*-values are in bold.

Table 3 Concatenated phylogenetic matrix statistics.

Dataset (N)	Loci	Missing data (%)	Alignment length (bp)	Distinct patterns	PIS	IS
Preliminary (33)	1772	43.504	291 835	109 400	19 696	248 929
Filtered (32)	1772	42.603	291 835	107 161	19 638	248 993
Species loci (9)	1202	14.658	227 093	10 541	11 088	196 006
Species SortaDate (9)	300	9.296	64 231	4008	3565	54 797
Cluster loci (11)	1841	27.59	341 069	24 169	16 569	295 634
Cluster SortaDate2 (11)	300	25.78	59 032	5536	2406	52 139

Summary of the datasets used in the phylogenetic inferences indicating the number of taxa included in each dataset. The number of loci, proportion of missing data, alignment length, and counts of parsimony-informative (PIS) and invariant sites (IS) are reported for each dataset.

Divergence time estimation

The Dating subset used for divergence time estimations comprised 1841 loci (see Table 3). Analyses of this matrix recovered a highly supported ML phylogeny, which exhibited an identical topology to that of the filtered ML tree estimate (Fig. 5; Supplementary Figs S12, S13). Among the 1841 loci, 1675 included the outgroup *Carabus* (*Platycarabus*) *depressus*. These 1675 loci were employed to select the optimal 300 loci using *SortaDate*. The ML dating subset tree length was 0.138, and the median *SortaDate* locus length was 0.148 (0.07 SD), median root-to-tip variance 2.11E-4 (3.70E-4 SD), and median bipartition 0.5 (0.11 SD, min 0.5, max 1.0). Analysis of these 300 loci with PartitionFinder2 recovered eight partitions,

and the substitution models estimated in ModelFinder were applied. Phylogenetic inference with these 300 loci recovered a topology identical to that obtained with all 1841 loci (Supplementary Figs S14, S15).

Independent BEAST divergence time analyses, employing different taxonomic sampling (STRUCTURE clusters versus species) and tree model prior (Yule speciation model versus Coalescent model), converged successfully and recovered largely overlapping credibility intervals, yielding consistent divergence time estimates (see Fig. 7; Supplementary Fig. S8). It is important to note that BEAST expects a bifurcating tree; while the ML analyses of all data reveal gene tree uncertainty, the results of the species and cluster analyses are consistent. The coalescent constant results for the cluster dataset

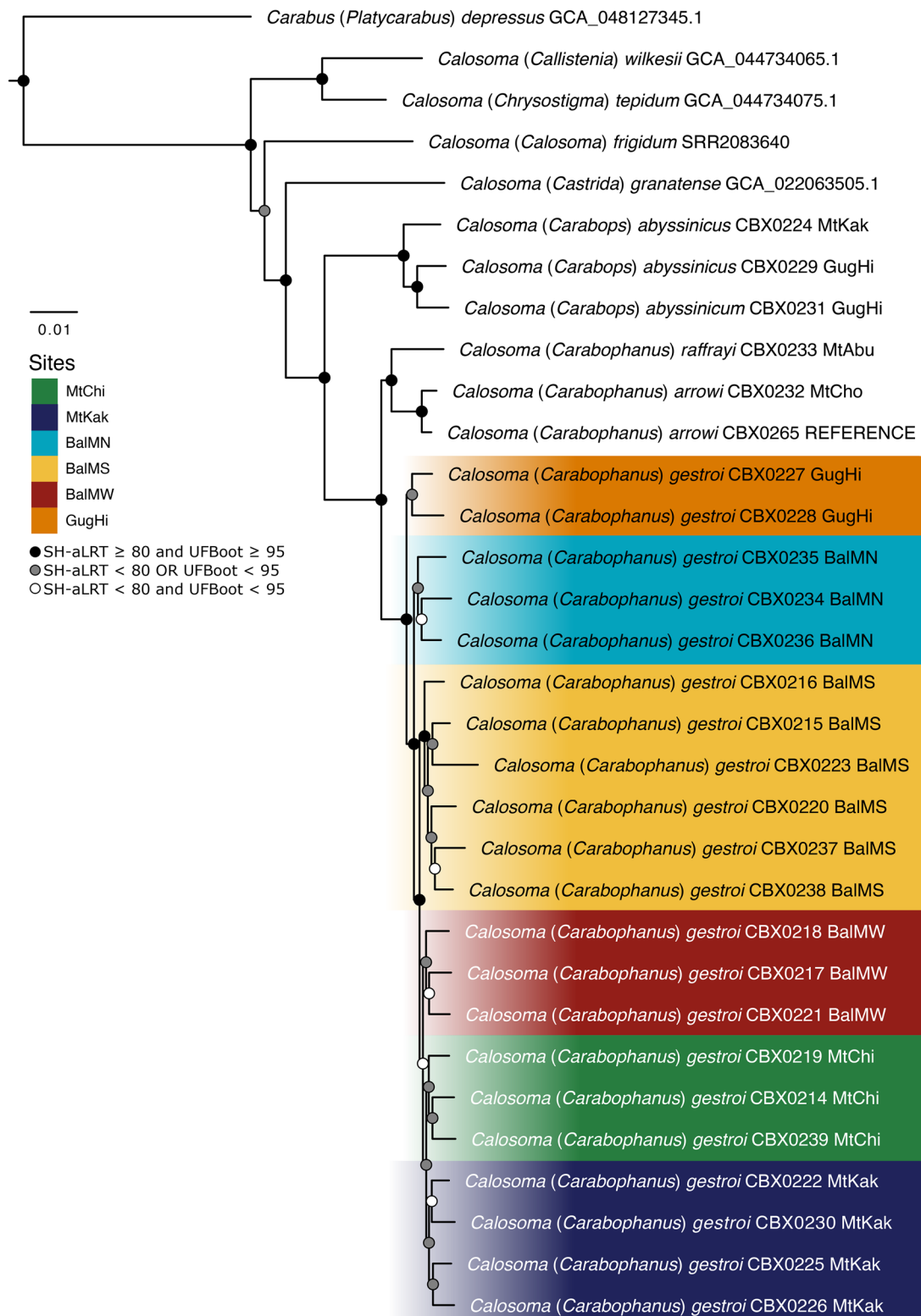


Figure 5 Maximum-likelihood inference of sampled taxa. Independent tree search 47 of 50 maximum-likelihood (ML) phylogenomic tree inferences using the filtered data in IQ-TREE. Branch support values are colour-coded as white for weak support, grey for moderate support, and black for strong support as indicated by SH-aLRT and UFBoot estimates. The ML tree was generated using FigTree (v1.4.4, 2018) and annotated using Inkscape. Branch support metrics across the tree are provided in [Supplementary Fig. S6](#).

were used as an estimate of the divergence times for the *C. gestroi* populations (Fig. 7, see also [Supplementary Fig. S16](#), and [supporting information](#) for all likelihood estimates). The

estimated divergence between *Carabophanus* and *Calosoma* (*Carabops*) *abyssinicum* occurred ~14.4–15.9 Mya during the mid-Miocene. The crown age of modern *Carabophanus* was

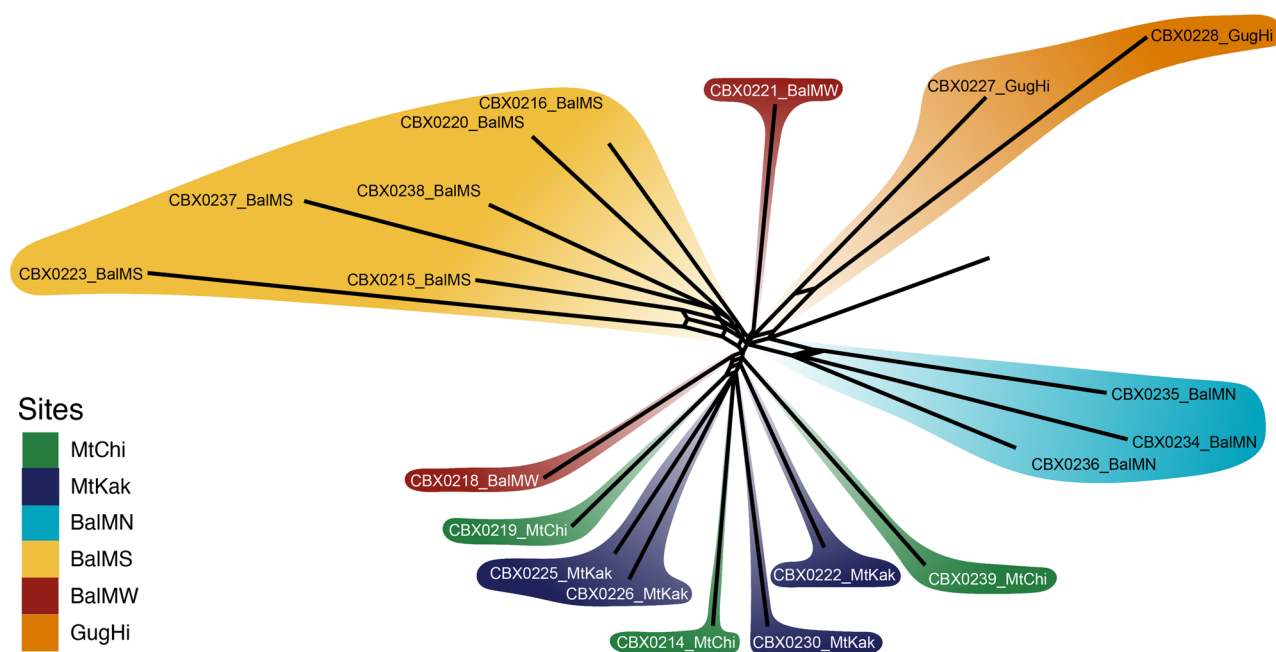


Figure 6 SplitsTree neighbour-network analysis. The neighbour-network based on the filtered dataset's uncorrected p-distances. The resulting network shows the relationships between *C. gestroi* taxa. The results were visualized in the SplitsTree app and stylized in InkScape.

estimated at ~6.4–8.3 Mya, corresponding to the divergence between *C. arrowi* + *C. raffrayi* and *C. gestroi*. Within *C. gestroi*, the BEAST analyses recovered an initial split between populations west of the Great Rift Valley (i.e. Gughe Highlands, represented by sample CBX0227) and populations east of the Great Rift Valley at ~4.1–4.6 Mya. A subsequent divergence occurred between the Arsi mountains (Mount Chilalo and Mount Kaka) and the Bale mountains at ~3.8–3.9 Mya.

Discussion

Phylogenomic inference and population genomics

Calosoma gestroi populations across the Ethiopian Highlands exhibit a gradient of structured genomic variation. Both PCA and STRUCTURE analyses distinguish localities on either side of the Great Rift Valley, with the Gughe Highland population in the west clearly separated from eastern populations. STRUCTURE analyses indicate that Gughe Highland samples share limited but detectable ancestry with eastern populations, although they consistently form a distinct cluster. Populations from mountains east of the Great Rift Valley show substantially higher admixture, suggesting recent gene flow. Population genetic statistics further support this pattern, showing high global inbreeding ($F_{is} = 0.6743$), low gene diversity (e.g. $H_t = 0.0449$ and $D_{st} = 0.0097$), and fixation among geographically distant sites ($F_{st} = 0.1823$; see Table 1). AMOVA indicates significant levels of genetic variation occur between mountains in the eastern range, while sites within mountains have lower levels of genetic differentiation.

Phylogenomic analyses support a sister relationship between *Carabops* and *Carabophanus* with strong branch support (Fig. 5). This placement is probably influenced by

insufficient taxon sampling at the subgeneric level. Although the placement of *Carabophanus* within *Calosoma* remains unclear, it has been suggested that this subgenus could be sister to the winged and widespread subgenus *Campalita* (Sota *et al.* 2022). Contrary to Jeannel's (1940) hypothesis based on morphological convergence, it is unlikely that all montane flightless *Calosoma* from the Afrotropical highlands form a clade (e.g. the subgenera *Carabops*, *Carabomorphus*, *Carabophanus*, and *Orinodromus*). Rather, these lineages probably represent derived montane-adapted taxa within otherwise winged and flight-capable *Calosoma* clades (Su *et al.* 2005, Toussaint and Gillett 2018, Toussaint *et al.* 2021, Sota *et al.* 2022). It is therefore currently unclear, in the case of *Carabophanus* and other Afrotropical highland endemic subgenera, whether winglessness evolved in the group's ancestor or at a later stage of cladogenesis. Given the strong geographical segregation observed in *Calosoma gestroi* the former is more likely. However, the placement of *Carabophanus* within *Calosoma* should be considered inconclusive and requires denser taxonomic sampling.

In his seminal monograph, Jeannel (1940) proposed that *C. antinorii* and *C. gestroi* are closely related based on the overall external morphology and their geographical proximity. Within *Carabophanus*, *C. gestroi* is inferred as sister to a clade including *C. arrowi* and *C. raffrayi*. *Calosoma antinorii* could not be included in this study due to a lack of high-quality DNA extracts. However, a robust placement of all four *Carabophanus* species remains desirable to confirm this relationship. Within *C. gestroi*, analyses recover strong branch support for the Gughe Highland population forming a distinct 'early-diverging' clade, and for Bale Mountains North as sister to the remaining eastern localities. However, resolving discrete phylogenetic relationships among populations remains difficult, with low branch support in the most derived parts

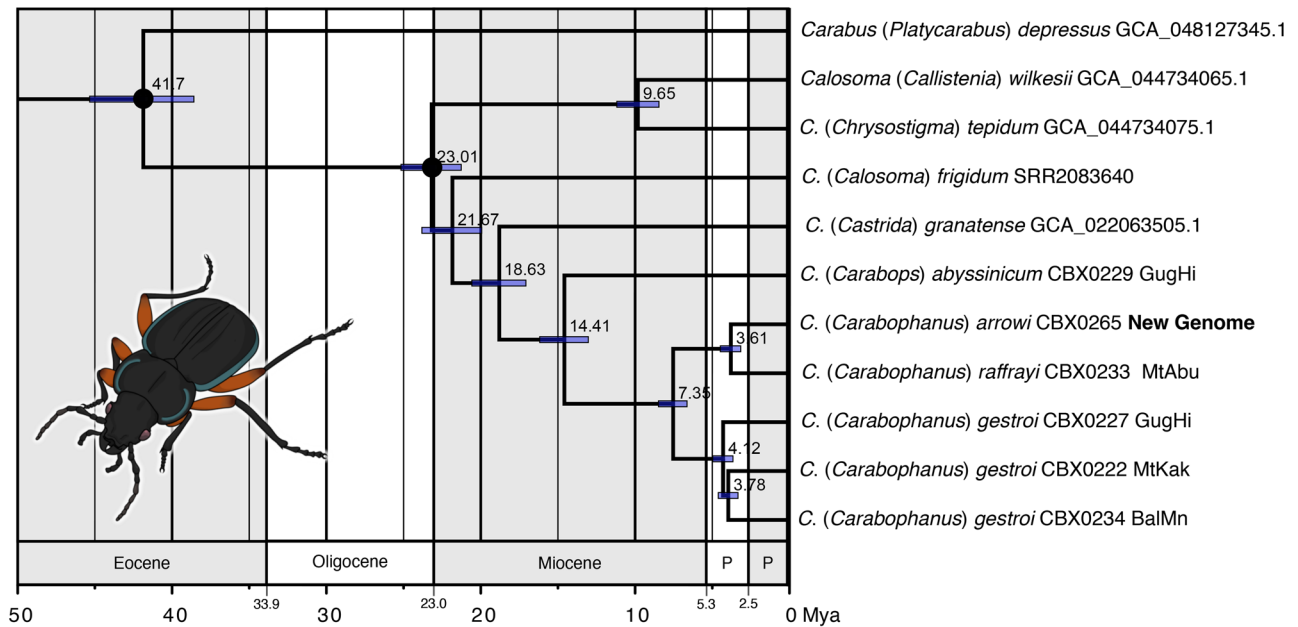


Figure 7 Time-calibrated divergence estimates of *Carabophanus*. Time-calibrated tree of *Carabophanus* using two secondary calibrations, a coalescent constant tree prior, a fixed topology, and 300 loci selected using SortaDate. The black circles represent secondary calibrations from Cardenas *et al.* (2025). Node bars represent the 95% HPD confidence intervals while node values indicate median age estimates. The geological epochs are listed along the bottom, with the final two being the Pliocene and Pleistocene; the Holocene is illustrated but not labelled. Inset in the bottom left of the tree is an illustration of *C. (Carabophanus) gestroi*; credit Cody Raul Cardenas. The tree was constructed using FigTree (v1.4.4, 2018) and annotated in Inkscape (v1.4.2; Inkscape Project 2025).

of the tree for both concatenated and coalescent phylogenetic methods. While the coalescent analyses are a more appropriate analysis given the evidence of gene flow observed in all of the analyses, the derived interspecies relationships were difficult to resolve (Supplementary Figs S10, S11). Regardless, these patterns suggest ongoing or recent gene flow, as illustrated by inconsistencies between concatenation and multispecies coalescent inferences.

Overall, population genomic patterns and phylogenomic inferences consistently recover a well-delineated split between Gughe Highland populations west of the Great Rift Valley and eastern mountain populations on the opposite side of this divide. Similarly, all approaches reveal a more complex pattern within eastern mountain populations, with substantial noise in phylogenomic inferences and a strong signal of admixture in population genomic analyses, possibly reflecting recent gene flow between the Arsi and Bale mountain ranges. The coexistence of lineage divergence across the Great Rift Valley and signature of gene flow within and between eastern mountains mirrors patterns observed in other tropical alpine ground beetles (Weng *et al.* 2016, Murienne *et al.* 2022).

Evolutionary history of *Calosoma gestroi* in Ethiopian highlands

Based on these results, the inferred patterns meet some theoretical expectations of flightlessness leading to increased genetic structure. Because *C. gestroi* is a flightless species, higher levels of differentiation among localities would be expected as a result of allopatric segregation associated with recent Quaternary glaciations (see Salces-Castellano *et al.* 2021,

Suárez *et al.* 2022). However, divergence time estimation indicates a split between *Calosoma gestroi* and the rest of the subgenus at ~6.4–8.3 Mya during the Messinian (Late Miocene), suggesting a more complex evolutionary scenario. This timing broadly corresponds to continued uplift of the East African Plateau and the emergence of highland specialists (e.g. Antonelli 2009, Peter Linder *et al.* 2013). The global climate cooled following the mid-Miocene climatic optimum, and the end of this epoch was marked by the Late Miocene Cooling between 7 and 5.3 Mya (Zachos *et al.* 2001, Herbert *et al.* 2016). Accordingly, suitable conditions for the establishment and diversification of temperate, cold-adapted species groups already existed in the Ethiopian highlands at that time. These climatic conditions may have provided the prerequisite for the establishment and spread of ancestral *Carabophanus*, as well as other extratropical ground beetle groups, such as the Holarctic genera *Amara*, *Calathus*, *Harpalus*, and *Trechus*, which today occur in the highlands with numerous endemic species but are absent from the lowlands (Merene *et al.* 2023). Although those beetles display notable ecological convergence today, the underlying diversification processes are not necessarily identical. For instance, cold-adapted *Trechus* beetles began diversifying in the Ethiopian mountains much earlier than *Carabophanus*, during the Early Miocene (Faillie *et al.* 2023). With the combined effect of plateau uplift and global cooling, ancestors of *Carabophanus* probably adapted to new ecological niches in high-mountain landscapes, potentially triggering diversification within the subgenus during the Late Miocene ~5–7 Mya (Fig. 7). A scenario involving progressive altitudinal shifts driven by passive uplift of widespread populations would explain the observed patterns (Rahbek *et al.* 2019,

Hedges *et al.* 2025, Marder *et al.* 2025). As a result, by the end of the Late Miocene Cooling event ~5 Mya, *Carabophanus* were probably increasingly cold-adapted, and an initial cladogenetic split had occurred between the ancestors of *C. arrowi* and *C. raffrayi* in the north, and those of *C. gestroi* (and potentially *C. antinorii*) in the southern Ethiopian highlands. The causes and consequences of evolutionary adaptation to cold climates and mountain ecosystems are well documented in ground beetles (Darlington 1936, 1943, den Boer *et al.* 1980, Kavanaugh 1985). By specializing in highland ecosystems, *Carabophanus* lineages probably lost functional wings through natural selection, resulting in the four cold-adapted and flightless taxa currently known from the Ethiopian highlands.

Phylogenomic and divergence time analyses suggest that the ancestors of modern *C. gestroi* populations originated ~4–4.5 Mya, probably via the separation of the Gughe lineage from the eastern mountain lineage (Fig. 7). While there is some limitation in our divergence estimates given the observed admixture in *C. gestroi*, our divergence times between *C. gestroi* and the rest of the subgenus are consistent across all analyses. More importantly, the inferred estimates within the sampled *Calosoma* species are consistent with previously work (e.g. *C. granatense* and *C. gestroi* ~28–15 Mya in Sota *et al.* 2022; see Fig. 7). However, little is known about the geomorphological structure of the Ethiopian landscape during the late Miocene. Therefore, the divergences and location of ancestral *Carabophanus gestroi* populations can only be inferred. The period from ~2 to 6 Mya was characterized by intense volcanic activity in southern Ethiopia, leading to the formation of the Arsi and Bale Mountains (Abbate *et al.* 2015). This also suggests that the Rift Valley in southern Ethiopia was less deep and extensive at the Miocene–Pliocene boundary than at the end of this orogenic phase, allowing ancestors of *C. gestroi* to disperse across the Rift. Under this scenario, cold-adapted highland ancestors of *C. gestroi* were probably widespread across the southern Ethiopian highlands at the beginning of the Pliocene ~4–5 Mya. This would have probably persisted until the Pliocene Thermal Maximum ~3.3 Mya, a relatively short interval of warmer climate (Salzmann *et al.* 2011, Fedorov *et al.* 2013). During this event, *C. gestroi* populations were probably restricted to higher elevations within each mountain range. As a result, gene flow between populations across the Great Rift Valley was probably reduced or halted, with populations evolving in isolation on so-called ‘sky islands’ (Taylor 1940, Marshall 1957, Heald 1967, McCormack *et al.* 2009, Love *et al.* 2023). Although the sky island concept has been developed primarily for temperate regions, similar processes have also been proposed in tropical montane systems (Robin *et al.* 2010, Toussaint *et al.* 2013). This period of allopatric isolation in high-altitude refugia probably fostered genetic segregation on each side of the Great Rift Valley, resulting in the present-day genetic divergence observed between the Gughe Highlands and the eastern mountains, dated to ~4.1–4.6 Mya. It is also possible that shifts in distribution across these sky islands contributed to this genetic isolation (Knowles and Massatti 2017). This pattern is consistent with both population genomic and phylogenomic results, showing low levels of admixture and

pronounced genomic sorting between the Gughe Highlands and the eastern mountains.

Population genomic and phylogenomic analyses recover structure across the eastern mountains, albeit with strong signals of recent admixture gene flow. This pattern probably reflects ancestral genomic sorting in glacial refugia, so-called sky islands, during warm periods, followed by repeated secondary contact at lower elevations during glacial periods. The divergence between the earliest eastern mountain lineages is dated to ~3.8–3.9 Mya, corresponding closely to the warmest Pliocene interval. Consequently, populations were probably restricted to even higher elevations on the highland plateaus while orogeny was occurring. The well-supported basal position of a Bale population (Bale North) within the eastern clade is consistent with both the greater age of the Bale Mountains relative to the Pliocene–Pleistocene Arsi volcanoes (Williams 2016) and the geographical proximity of the Gughe and Bale Mountains. The phylogenetic and phylogeographical structure of eastern *C. gestroi* populations further reflects the uplift processes and geomorphological conditions of the region. The Bale Mountains are characterized by an extensive high plateau (the ‘Sanetti Plateau’), which acts as an effective distributional barrier for Afrotropical species and creates significant climatic and ecological differences between the northern and southern slopes, driven by regional circulation systems (Miehe and Miehe 1994, Fazzini *et al.* 2015). The Arsi volcanoes, although similarly elevated but less extensive, were only active at the end of the Pliocene and thus became available as habitat later (Williams 2016). Divergence time estimates indicate a split between the Arsi and Bale Mountains at ~2.7–2.8 Mya, consistent with a more recent genetic divergence. Ancestors of eastern mountain populations were probably restricted to the older Bale massif during the warm Pliocene and subsequently colonized the Arsi Mountains during the Quaternary, once conditions became suitable and interglacial climates pushed these beetles downslope.

Interglacial periods probably had a stronger impact across the Rift than among eastern mountain populations, due to significant differences in altitudinal profiles. This may explain the comparatively high genetic admixture observed in the eastern mountains, probably fostered by repeated secondary contact among populations, resulting in incomplete lineage sorting and reticulation, as reflected by weak support for a fully bifurcating topology in concatenated phylogenomic inferences. Occasional ancestral secondary contact between Gughe Highlands and eastern mountain populations during the Plio–Pleistocene may explain the low but detectable admixture signatures observed in STRUCTURE plots. In summary, the Ethiopian highlands probably acted as a ‘species pump’ during the Pliocene warm epoch (Carstens and Knowles 2007, Schoville *et al.* 2012, Tonzo and Ortego 2021, Ortego and Knowles 2022), triggering incipient genetic differentiation in the Gughe Highlands population. However, those highlands also acted as a ‘melting pot’ in eastern mountains, where Quaternary altitudinal range shifts resulted in the erosion of genomic divergence accumulated during interglacial periods through gene flow following glacial range expansions

(e.g. Flantua and Hooghiemstra 2018, Flantua *et al.* 2019, Maier *et al.* 2019, Murienne *et al.* 2022). These results illustrate the complexity of evolutionary patterns in *C. gestroi*, and reaffirm the importance of the Ethiopian highlands as a natural laboratory for testing classical evolutionary models (e.g. Wallis *et al.* 2016).

Given current global warming trends, these climatic oscillations are likely to have a strong impact on genetic connectivity in *C. gestroi*, as well as in a wide range of species inhabiting Ethiopian highland ecosystems (e.g. Razgour *et al.* 2021, Berhanu *et al.* 2022, Yebeyen *et al.* 2022, Abrha *et al.* 2025, Abuhay *et al.* 2025, Mosisa and Tassie 2025). However, the pace of these climatic shifts is unprecedented, and the extent to which lineages will be able to cope with such drastic ecosystem reconfigurations remains unknown (Laurance *et al.* 2011, Chan *et al.* 2024). Additionally, excessive anthropogenic land use, even in high mountain areas, is significantly restricting the habitats of Afromontane and Afroalpine species and often extends into protected areas. As a result, the potential range of some high-altitude ground beetles is already extremely limited, meaning that populations are unlikely to respond to climate change through vertical migration and are therefore considered at risk of extinction (Schmidt and Merene 2024a, 2024b). Future research should focus on generating comprehensive genomic datasets for all *Carabophanus* species to better resolve the evolutionary history of these beetles using demographic modelling (e.g. Weng *et al.* 2024), while also integrating fine-scale occurrence data to estimate past, present and future habitat suitability in the face of global climate change.

Acknowledgements

We thank the Ethiopian Wildlife Conservation Authority, the Ethiopian Biodiversity Institute, the College of Natural and Computational Sciences, and the Department of Plant Biology and Biodiversity Management at Addis Ababa University, the Bale Mountains National Park, the Arsi Mountains National Park, and the Abune Yosef and Choke Mountain Community Conservation offices for their cooperation and permission to conduct fieldwork. We thank Ines Carrasquer-Puyal for productive discussions in developing the bioinformatic pipeline.

Author contributions

Cody Raul Cardenas (Conceptualization [Equal], Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Methodology [Equal], Software [Equal], Validation [Equal], Visualization [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), Jérémy Gauthier (Data curation [Equal], Formal analysis [Equal], Investigation [Equal], Methodology [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), Julia Bilat (Data curation [Equal], Formal analysis [Equal], Methodology [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), Marjorie Labédan (Data curation [Equal], Investigation [Equal], Methodology [Equal]), Yeshitla Merene Abebe (Data curation [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), Joachim Schmidt (Conceptualization [Equal], Data curation [Equal], Funding acquisition [Equal],

Project administration [Equal], Resources [Equal], Visualization [Equal], Writing—original draft [Equal], Writing—review & editing [Equal]), and Emmanuel F.A. Toussaint (Conceptualization [Equal], Funding acquisition [Equal], Project administration [Equal], Resources [Equal], Supervision [Equal], Visualization [Equal], Writing—original draft [Equal], Writing—review & editing [Equal])

Supplementary material

Supplementary material is available at *Evolutionary Journal of the Linnean Society* online.

Conflicts of interest

None declared.

Funding

This study was funded by a Swiss National Science Foundation grant (310030_200491) to Emmanuel F.A. Toussaint and a German Research Foundation (DFG) grant (SCHM 3005/2-1) to Joachim Schmidt.

Data availability

The data underlying this article are available in the article and in its online [supplementary material](#).

References

- Abbate E, Bruni P, Sagri M. Geology of Ethiopia: a review and geomorphological perspectives. In: Billi P (ed.), *Landscapes and Landforms of Ethiopia*. Dordrecht: Springer Netherlands, 2015, 33–64.
- Abrha AM, Rödder D, Gedeon K *et al.* Evaluating the impact of climate change on mountaintop endemic bird species in the Ethiopian highlands: insights from moorland francolin (*Scleroptila psilolaema*). *Ornithology Research* 2025;33:47. <https://doi.org/10.1007/s43388-025-00252-1>
- Abuhay ZT, Ali AM, Atickem A *et al.* Modelling of past, current and future distribution of suitable habitat for Menelik's bushbuck (*Tragelaphus sylvaticus menelik* Neumann, 1902) in the Ethiopian highlands. *BMC Ecology and Evolution* 2025;25:47. <https://doi.org/10.1186/s12862-025-02367-x>
- Andrews P, VanCouvering J. Paleoenvironments in the East African Miocene. *Contributions to Primatology* 1975;5:62–103.
- Antonelli A. Have giant lobelias evolved several times independently? Life form shifts and historical biogeography of the cosmopolitan and highly diverse subfamily Lobelioideae (Campanulaceae). *BMC Biology* 2009;7:82. <https://doi.org/10.1186/1741-7007-7-82>
- Avise JC. *Phylogeography: The History and Formation of Species*. Cambridge, MA: Harvard University Press, 2000.
- Bennett K, Provan J. What do we mean by 'refugia'? *Quaternary Science Reviews* 2008;27:2449–55. <https://doi.org/10.1016/j.quascirev.2008.08.019>
- Berhanu Y, Tassie N, Sintayehu DW. Predicting the current and future suitable habitats for endemic and endangered

- Ethiopian wolf using MaxEnt model. *Heliyon* 2022;8:e10223. <https://doi.org/10.1016/j.heliyon.2022.e10223>
- Billi P (ed.), *Landscapes and Landforms of Ethiopia*. Dordrecht: Springer Netherlands, 2015.
- den Boer PJ, Huizen T, Boer-Daanje W *et al.* Wing polymorphism and dimorphism in ground beetles as stages in an evolutionary process (Coleoptera: Carabidae). *Entomologia Generalis* 1980;6:107–34. <https://doi.org/10.1127/entom.gen/6/1980/107>
- Borowiec ML. AMAS: a fast tool for alignment manipulation and computing of summary statistics. *PeerJ* 2016;4:e1660. <https://doi.org/10.7717/peerj.1660>
- Broad Institute. Picard Toolkit, Broad Institute, GitHub repository 2019. <https://broadinstitute.github.io/picard/>
- Brown JW, Walker JF, Smith SA. Phyx: phylogenetic tools for unix. *Bioinformatics* 2017;33:1886–8. <https://doi.org/10.1093/bioinformatics/btx063>
- Brühl C. Flightless insects: a test case for historical relationships of African mountains. *Journal of Biogeography* 1997;24:233–50. <https://doi.org/10.1046/j.1365-2699.1997.00073.x>
- Bruschi S. *Calosoma of the World: (Coleoptera, Carabidae)*. Bologna (Italy): Natura edizioni scientifiche, 2013.
- Bryant D, Huson DH. NeighborNet: improved algorithms and implementation. *Frontiers in Bioinformatics* 2023;3:1178600. <https://doi.org/10.3389/fbinf.2023.1178600>
- Bryant D, Moulton V. Neighbor-net: an agglomerative method for the construction of phylogenetic networks. *Molecular Biology and Evolution* 2004;21:255–65. <https://doi.org/10.1093/molbev/msh018>
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 2009;25:1972–3. <https://doi.org/10.1093/bioinformatics/btp348>
- Cardenas CR, Gustafson G, Toussaint E. Global integration of phylogenomic data and fine-scale partitioning strategies refine the evolutionary tree of adephaga beetles. *Bulletin of the Society of Systematic Biologists* 2025;4:1–35. <https://doi.org/10.18061/bssb.v4i1.10345>
- Carstens BC, Knowles LL. Shifting distributions and speciation: species divergence during rapid climate change. *Molecular Ecology* 2007;16:619–27. <https://doi.org/10.1111/j.1365-294X.2006.03167.x>
- Chala D, Zimmermann NE, Brochmann C *et al.* Migration corridors for alpine plants among the ‘sky islands’ of eastern Africa: do they, or did they exist? *Alpine Botany* 2017;127:133–44. <https://doi.org/10.1007/s00035-017-0184-z>
- Challis R, Richards E, Rajan J *et al.* BlobToolKit – interactive quality assessment of genome assemblies. 2020;10:1361–74. <https://doi.org/10.1534/g3.119.400908>
- Chan W-P, Lenoir J, Mai G-S *et al.* Climate velocities and species tracking in global mountain regions. *Nature* 2024;629:114–20. <https://doi.org/10.1038/s41586-024-07264-9>
- Chang CC, Chow CC, Tellier LC *et al.* Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* 2015;4:s13742-015-0047–8. <https://doi.org/10.1186/s13742-015-0047-8>
- Cheng H, Concepcion GT, Feng X *et al.* Haplotype-resolved de novo assembly using phased assembly graphs with hifiasm. *Nature Methods* 2021;18:170–5. <https://doi.org/10.1038/s41592-020-01056-5>
- Chernomor O, Von Haeseler A, Minh BQ. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 2016;65:997–1008. <https://doi.org/10.1093/sysbio/syw037>
- Clark L. Ivclark/R_genetics_conv: R_genetics_conv 1.1, 2017. <https://doi.org/10.5281/ZENODO.846816>
- Coetzee JA. Evidence for a considerable depression of the vegetation belts during the Upper Pleistocene on the East African Mountains. *Nature* 1964;204:564–6. <https://doi.org/10.1038/204564a0>
- Couvreux TLP, Dauby G, Blach-Overgaard A *et al.* Tectonics, climate and the diversification of the tropical African terrestrial flora and fauna. *Biological Reviews* 2021;96:16–51. <https://doi.org/10.1111/brv.12644>
- Coyne JA, Orr HA. *Speciation*. Oxford: Oxford University Press, 2004.
- Danecek P, Auton A, Abecasis G *et al.*; 1000 Genomes Project Analysis Group. The variant call format and VCFtools. *Bioinformatics* 2011;27:2156–8. <https://doi.org/10.1093/bioinformatics/btr330>
- Darlington PJ. Variation and atrophy of flying wings of some carabid beetles (Coleoptera). *Annals of the Entomological Society of America* 1936;29:136–79. <https://doi.org/10.1093/aesa/29.1.136>
- Darlington PJ. Carabidae of mountains and islands: data on the evolution of isolated faunas, and on atrophy of wings. *Ecological Monographs* 1943;13:37–61. <https://doi.org/10.2307/1943589>
- deMenocal PB. African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letters* 2004;220:3–24. [https://doi.org/10.1016/S0012-821X\(04\)00003-2](https://doi.org/10.1016/S0012-821X(04)00003-2)
- Dianat M, Konečný A, Lavrenchenko LA *et al.* How to cross the desert if you are small and need mountains? Out-of-Ethiopia dispersal in Afromontane shrews. *Journal of Biogeography* 2024;51:230–45. <https://doi.org/10.1111/jbi.14748>
- Dudley R. *The Biomechanics of Insect Flight: Form, Function, Evolution*. Princeton, NJ: Princeton University Press, 2000.
- Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* 2005;14:2611–20. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 1992;131:479–91. <https://doi.org/10.1093/genetics/131.2.479>
- Faillie A, Hofmann S, Merene Y *et al.* Explosive radiation versus old relicts: The complex history of Ethiopian Trechina, with description of a new genus and a new subgenus (Coleoptera, Carabidae, Trechini). *Deutsche Entomologische Zeitschrift* 2023;70:311–35. <https://doi.org/10.3897/dez.70.107425>

- Fazzini M, Bisci C, Billi P. The climate of Ethiopia. In: Billi P (ed.), *Landscapes and Landforms of Ethiopia*. Dordrecht: Springer Netherlands, 2015, 65–87.
- Fedorov AV, Brierley CM, Lawrence KT *et al*. Patterns and mechanisms of early Pliocene warmth. *Nature* 2013;**496**:43–9. <https://doi.org/10.1038/nature12003>
- FigTree. Rambaut, GitHub repository 2018. <https://github.com/rambaut/figtree>
- Flantua SGA, Hooghiemstra H. Historical connectivity and mountain biodiversity. In Hoorn C, Perrigo A, Antonelli A (eds.), *Mountains, Climate and Biodiversity*. Oxford: Wiley-Blackwell, 2018, 171–85.
- Flantua SGA, O'Dea A, Onstein RE *et al*. The flickering connectivity system of the north Andean páramos. *Journal of Biogeography* 2019;**46**:1808–25. <https://doi.org/10.1111/jbi.13607>
- Fonseca EM, Colli GR, Werneck FP *et al*. Phylogeographic model selection using convolutional neural networks. *Molecular Ecology Resources* 2021;**21**:2661–75. <https://doi.org/10.1111/1755-0998.13427>
- Francis RM. pophelper: an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources* 2017;**17**:27–32. <https://doi.org/10.1111/1755-0998.12509>
- Freilich X, Anadón JD, Bukala J *et al*; Evolutionary Genetics - Class of 2013. Comparative phylogeography of Ethiopian anurans: impact of the Great Rift Valley and Pleistocene climate change. *BMC Evolutionary Biology* 2016;**16**:206. <https://doi.org/10.1186/s12862-016-0774-1>
- Gao K, Hua Y, Xing L-X *et al*. Speciation of the cold-adapted scorpionfly *Cerapanorpa brevicornis* (Mecoptera: Panorpidae) via interglacial refugia. *Insect Conservation and Diversity* 2022;**15**:114–27. <https://doi.org/10.1111/icad.12519>
- Gauthier J, Blanc M, Toussaint EFA; [dataset: GCA_044734065.1, GCA_044734075.1] Chromosome-scale genomes of the flightless Caterpillar Hunter Beetles *Calosoma tepidum* and *Calosoma wilkesii* from British Columbia (Coleoptera: Carabidae). *Genome Biology and Evolution* 2025;**17**:evae247. <https://doi.org/10.1093/gbe/evae247>
- Gauthier J, Cardenas CR, Nari M; [dataset: GCA_048127345.1] *et al*. Draft genome of the endemic alpine ground beetle *Carabus (Platycarabus) depressus* (Coleoptera: Carabidae) from long-read sequencing of a frozen archived specimen. *G3: Genes, Genomes, Genetics* 2025;**15**:jkaf027. <https://doi.org/10.1093/g3journal/jkaf027>
- Gauthier J, Pajkovic M, Neuenschwander S *et al*. Museomics identifies genetic erosion in two butterfly species across the 20th century in Finland. *Molecular Ecology Resources* 2020;**20**:1191–205. <https://doi.org/10.1111/1755-0998.13167>
- Gernhard T. The conditioned reconstructed process. *Journal of Theoretical Biology* 2008;**253**:769–78. <https://doi.org/10.1016/j.jtbi.2008.04.005>
- Gottelli D, Marino J, Sillero-Zubiri C *et al*. The effect of the last glacial age on speciation and population genetic structure of the endangered Ethiopian wolf (*Canis simensis*). *Molecular Ecology* 2004;**13**:2275–86. <https://doi.org/10.1111/j.1365-294X.2004.02226.x>
- Goudet J. hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes* 2005;**5**:184–6. <https://doi.org/10.1111/j.1471-8286.2004.00828.x>
- Greig KJ, Buckley TR, Holwell GI *et al*. Flight loss decreases diversification in New Zealand beetles (Coleoptera: Zopheridae). *Biological Journal of the Linnean Society* 2025;**145**:blaf021. <https://doi.org/10.1093/biolinnean/blaf021>
- Groos AR, Akçar N, Yesilyurt S *et al*. Nonuniform Late Pleistocene glacier fluctuations in tropical Eastern Africa. *Science Advances* 2021;**7**:eabb6826. <https://doi.org/10.1126/sciadv.abb6826>
- Guan D, McCarthy SA, Wood J *et al*. Identifying and removing haplotypic duplication in primary genome assemblies. *Bioinformatics* 2020;**36**:2896–8. <https://doi.org/10.1093/bioinformatics/btaa025>
- Guindon S, Dufayard J-F, Lefort V *et al*. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 2010;**59**:307–21. <https://doi.org/10.1093/sysbio/syq010>
- Hamming RW. Error detecting and error correcting codes. *Bell System Technical Journal* 1950;**29**:147–60.
- Heads M, Marinov M, Nicol DA *et al*. Passive uplift of montane biotas: recent advances. *Cladistics* 2025;**42**:63–78. <https://doi.org/10.1111/cla.70014>
- Heald WF. *Sky Island*. Princeton, NJ: Van Nostrand, 1967.
- Herbert TD, Lawrence KT, Tzanova A *et al*. Late Miocene global cooling and the rise of modern ecosystems. *Nature Geoscience* 2016;**9**:843–7. <https://doi.org/10.1038/ngeo2813>
- Hewitt G. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* 1996;**58**:247–76. <https://doi.org/10.1006/bijl.1996.0035>
- Hewitt G. The genetic legacy of the Quaternary ice ages. *Nature* 2000;**405**:907–13. <https://doi.org/10.1038/35016000>
- Hewitt GM. Ice Ages: their impact on species distributions and evolution. In: Rothschild LJ, Lister AM (eds.), *Evolution of Planet Earth*. New York: Academic Press, 2003.
- Hewitt GM. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* 2004;**359**:183–95. <https://doi.org/10.1098/rstb.2003.1388>
- Hijmans RJ. Geosphere: spherical trigonometry. R package, 2019. <https://github.com/rspsatial/geosphere>
- Hoang DT, Chernomor O, von Haeseler A *et al*. UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 2018;**35**:518–22. <https://doi.org/10.1093/molbev/msx281>
- Huson DH, Bryant D. The SplitsTree App: interactive analysis and visualization using phylogenetic trees and networks. *Nature Methods* 2024;**21**:1773–4. <https://doi.org/10.1038/s41592-024-02406-3>
- Ikeda H, Nishikawa M, Sota T. Loss of flight promotes beetle diversification. *Nature Communications* 2012;**3**:648. <https://doi.org/10.1038/ncomms1659>
- Inkscape Project. Inkscape, 2025. <https://inkscape.org/>
- Jeannel R. Les Calosomes. *Mémoires du Muséum national d'histoire naturelle* 1940;**13**:1–240.

- Jombart T. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 2008;**24**:1403–5. <https://doi.org/10.1093/bioinformatics/btn129>
- Jombart T, Ahmed I. Adegnet 1.3.1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* 2011;**27**:3070–1. <https://doi.org/10.1093/bioinformatics/btr521>
- Kalyaanamoorthy S, Minh BQ, Wong TKF *et al.* ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 2017;**14**:587–9. <https://doi.org/10.1038/nmeth.4285>
- Kamvar ZN, Brooks JC, Grünwald NJ. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics* 2015;**6**:208. <https://doi.org/10.3389/fgene.2015.00208>
- Kamvar ZN, Tabima JF, Grünwald NJ. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2014;**2**:e281. <https://doi.org/10.7717/peerj.281>
- Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 2013;**30**:772–80. <https://doi.org/10.1093/molbev/mst010>
- Kavanaugh D. On wing atrophy in carabid beetles (Insecta: Coleoptera: Carabidae), with special reference to Nearctic *Nebria*. *Taxonomy, Phylogeny, and Zoogeography of Beetles and Ants: A Volume Dedicated to the Memory of Philip J. Darlington, Jr.* 1904–1983. Dordrecht: Dr. W. Junk Publishers, 1985, 408–31.
- Kebede M, Ehrlich D, Taberlet P *et al.* Phylogeography and conservation genetics of a giant lobelia (*Lobelia giberroa*) in Ethiopian and Tropical East African mountains. *Molecular Ecology* 2007;**16**:1233–43. <https://doi.org/10.1111/j.1365-294X.2007.03232.x>
- Kingman JFC. The coalescent. *Stochastic Processes and Their Applications* 1982;**13**:235–4. [https://doi.org/10.1016/0304-4149\(82\)90011-4](https://doi.org/10.1016/0304-4149(82)90011-4)
- Kingsolver JG, Koehl MAR. Selective factors in the evolution of insect wings: response to Kukalová-Peck. *Canadian Journal of Zoology* 1989;**67**:785–7. <https://doi.org/10.1139/z89-114>
- Knowles LL, Massatti R. Distributional shifts—not geographic isolation—as a probable driver of montane species divergence. *Ecography* 2017;**40**:1475–85. <https://doi.org/10.1111/ecog.02893>
- Kolbe HJ. Über die in Africa gefundenen montanen und subalpinen Gattungen der mit Calosoma verwandten Coleopteren. In: Jahrgang (ed.), *Sitzungsberichte Der Gesellschaft Naturforschender Freunde Zu Berlin*, Vol 15. Berlin: Gesellschaft Naturforschender Freunde, 1895, 50–68.
- Lanfear R, Calcott B, Kainer D *et al.* Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology* 2014;**14**:82. <https://doi.org/10.1186/1471-2148-14-82>
- Lanfear R, Frandsen PB, Wright AM *et al.* PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 2017;**34**:msw260. <https://doi.org/10.1093/molbev/msw260>
- Lanfear R, Hahn MW. The meaning and measure of concordance factors in phylogenomics. *Molecular Biology and Evolution* 2024;**41**:msae214. <https://doi.org/10.1093/molbev/msae214>
- Laurance WF, Carolina Useche D, Shoo LP *et al.* Global warming, elevational ranges and the vulnerability of tropical biota. *Biological Conservation* 2011;**144**:548–57. <https://doi.org/10.1016/j.biocon.2010.10.010>
- Leihy RI, Chown SL. Wind plays a major but not exclusive role in the prevalence of insect flight loss on remote islands. *Proceedings of the Royal Society. B: Biological Sciences* 2020;**287**:20202121. <https://doi.org/10.1098/rspb.2020.2121>
- Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetic parameter estimation from sequencing data. *Bioinformatics* 2011;**27**:2987–93. <https://doi.org/10.1093/bioinformatics/btr509>
- Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 2009;**25**:1754–60. <https://doi.org/10.1093/bioinformatics/btp324>
- Love SJ, Schweitzer JA, Woolbright SA *et al.* Sky islands are a global tool for predicting the ecological and evolutionary consequences of climate change. *Annual Review of Ecology, Evolution, and Systematics* 2023;**54**:219–36. <https://doi.org/10.1146/annurev-ecolsys-102221-050029>
- Maier PA, Vandergast AG, Ostojia SM *et al.* Pleistocene glacial cycles drove lineage diversification and fusion in the Yosemite toad (*Anaxyrus canorus*). *Evolution* 2019;**73**:2476–96. <https://doi.org/10.1111/evo.13868>
- Mairal M, Sanmartín I, Herrero A *et al.* Geographic barriers and Pleistocene climate change shaped patterns of genetic variation in the Eastern Afrotropical biodiversity hotspot. *Scientific Reports* 2017;**7**:45749. <https://doi.org/10.1038/srep45749>
- Marder E, Smiley TM, Yanites BJ *et al.* Direct effects of mountain uplift and topography on biodiversity. *Science* 2025;**387**:1287–91. <https://doi.org/10.1126/science.adp7290>
- Marshall JT. *Birds of Pine-Oak Woodland in Southern Arizona and Adjacent Mexico*. Berkeley, CA: Cooper Ornithological Society, 1957
- Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal* 2011;**17**:10. <https://doi.org/10.14806/ej.17.1.200>
- McCormack J, Huang H, Knowles L. Sky islands. In: Gillespie RG, Clague DA (eds.), *Encyclopedia of Islands*. Berkeley: University of California Press, 2009, 841–43.
- McKenna DD, Shin S, Ahrens D; [dataset: SRR2083640] *et al.* The evolution and genomic basis of beetle diversity. *Proceedings of the National Academy of Sciences* 2019;**116**:24729–37. <https://doi.org/10.1073/pnas.1909655116>
- Meirmans PG, Hedrick PW. Assessing population structure: FST and related measures. *Molecular Ecology Resources* 2011;**11**:5–18. <https://doi.org/10.1111/j.1755-0998.2010.02927.x>
- Merene Y, Lorenz W, Opgenoorth L *et al.* Ground and tiger beetles (Coleoptera: Carabidae, Cicindelidae) of the Federal Democratic Republic of Ethiopia: a provisional faunistic checklist based on literature data. *Zootaxa* 2023;**5247**:1–345. <https://doi.org/10.11646/zootaxa.5247.1.1>

- Michalakis Y, Excoffier L. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* 1996;142:1061–4. <https://doi.org/10.1093/genetics/142.3.1061>
- Miehe S, Miehe G. *Ericaceous Forests and Heathlands in the Bale Mountains of South Ethiopia: Ecology and Man's Impact*. Reinbek: Warnke, 1994.
- Minh BQ, Schmidt HA, Chernomor O *et al*. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 2020;37:1530–4. <https://doi.org/10.1093/molbev/msaa015>
- Misof B, Liu S, Meusemann K *et al*. Phylogenomics resolves the timing and pattern of insect evolution. *Science* 2014;346:763–7. <https://doi.org/10.1126/science.1257570>
- Mittermeier RA, Turner WR, Larsen FW *et al*. Global biodiversity conservation: the critical role of hotspots. In: Zachos FE, Habel JC (eds.), *Biodiversity Hotspots*. Berlin: Springer, 2011, 3–22.
- Mizerovská D, Martynov AA, Mikula O *et al*. Genomic diversity, evolutionary history, and species limits of the endemic Ethiopian laminate-toothed rats (genus *Otomys*, Rodentia: Muridae). *Zoological Journal of the Linnean Society* 2023;199:1059–77. <https://doi.org/10.1093/zoolinnean/zlad063>
- Morel B, Williams TA, Stamatakis A. Asteroid: a new algorithm to infer species trees from gene trees under high proportions of missing data. *Bioinformatics* 2023;39:btac832. <https://doi.org/10.1093/bioinformatics/btac832>
- Morgan-Wall T. rayshader: Create Maps and Visualize Data in 2D and 3D, R Package, 2018. <https://doi.org/10.32614/cran.package.rayshader>
- Mosisa GB, Tassie N. Climate change and the future of the endemic Ethiopian Siskin: a looming extinction crisis. *Journal of Ornithology* 2025;166:511–24. <https://doi.org/10.1007/s10336-024-02239-z>
- Muriene J, Barragán A, Manzi S *et al*. How tectonic, volcanic and climatic processes in Andean 'sky islands' shaped the diversification of endemic ground beetles. *Journal of Biogeography* 2022;49:2077–90. <https://doi.org/10.1111/jbi.14495>
- Nazareno AG, Bemmels JB, Dick CW *et al*. Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species. *Molecular Ecology Resources* 2017;17:1136–47. <https://doi.org/10.1111/1755-0998.12654>
- Ortego J, Knowles LL. Geographical isolation versus dispersal: Relictual alpine grasshoppers support a model of interglacial diversification with limited hybridization. *Molecular Ecology* 2022;31:296–312. <https://doi.org/10.1111/mec.16225>
- Ortuño VM, Novoa F. A new species of *Trechus* from the Ethiopian Highlands (Coleoptera: Carabidae: Trechinae) and key to the *Trechus* species of Ethiopia. *Annals of the Entomological Society of America* 2011;104:132–40. <https://doi.org/10.1603/an10122>
- Paradis E. pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics* 2010;26:419–20. <https://doi.org/10.1093/bioinformatics/btp696>
- Pauli MT, Gauthier J, Labédan M *et al*. Museomics of *Carabus* giant ground beetles shows an Oligocene origin and in situ alpine diversification. *Peer Community Journal* 2024;4. <https://doi.org/10.24072/pcjournal.445>
- Pawłowski J. Preliminary report on Ethiopian species of the genus *Trechus* Clairville, 1806 (Coleoptera: Carabidae). *Baltic Journal of Coleopterology* 2003;3:157–60.
- Peter Linder H, Antonelli A, Humphreys AM *et al*. What determines biogeographical ranges? Historical wanderings and ecological constraints in the danthonioid grasses. *Journal of Biogeography* 2013;40:821–34. <https://doi.org/10.1111/jbi.12070>
- Poplin R, Ruano-Rubio V, DePristo MA *et al*. Scaling accurate genetic variant discovery to tens of thousands of samples, 2017. <https://doi.org/10.1101/201178>.
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;155:945–59. <https://doi.org/10.1093/genetics/155.2.945>
- Quéinnec E, Ollivier E, Reeb C. Insights on Ethiopian montane ground beetles biodiversity: taxonomic study of afro-alpine and sub-alpine Trechini (Coleoptera: Carabidae: Trechinae). *Journal of Insect Biodiversity* 2021;27. <https://doi.org/10.12976/jib/2021.27.1.1>
- R Core Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing, 2025.
- Rahbek C, Borregaard MK, Antonelli A *et al*. Building mountain biodiversity: Geological and evolutionary processes. *Science* 2019;365:1114–9. <https://doi.org/10.1126/science.aax0151>
- Rambaut A, Drummond AJ, Xie D *et al*. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 2018;67:901–4. <https://doi.org/10.1093/sysbio/syy032>
- Razgour O, Kasso M, Santos H *et al*. Up in the air: threats to Afromontane biodiversity from climate change and habitat loss revealed by genetic monitoring of the Ethiopian Highlands bat. *Evolutionary Applications* 2021;14:794–806. <https://doi.org/10.1111/eva.13161>
- Revell LJ. phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and other things). *PeerJ* 2024;12:e16505. <https://doi.org/10.7717/peerj.16505>
- Robin VV, Sinha A, Ramakrishnan U. Ancient geographical gaps and paleo-climate shape the phylogeography of an endemic bird in the sky islands of Southern India. *PLoS One* 2010;5:e13321. <https://doi.org/10.1371/journal.pone.0013321>
- Roff DA. The evolution of flightlessness in insects. *Ecological Monographs* 1990;60:389–421. <https://doi.org/10.2307/1943013>
- Roff DA. The evolution of flightlessness: is history important? *Evolutionary Ecology* 1994;8:639–57. <https://doi.org/10.1007/BF01237847>
- Salces-Castellano A, Andújar C, López H *et al*. Flightlessness in insects enhances diversification and determines assemblage structure across whole communities. *Proceedings of*

- the Royal Society B: Biological Sciences 2021;288:20202646. <https://doi.org/10.1098/rspb.2020.2646>
- Salzmann U, Williams M, Haywood AM *et al.* Climate and environment of a Pliocene warm world. *Palaeogeography, Palaeoclimatology, Palaeoecology* 2011;309:1–8. <https://doi.org/10.1016/j.palaeo.2011.05.044>
- Schachat SR, Goldstein PZ, Desalle R *et al.* Illusion of flight? Absence, evidence and the age of winged insects. *Biological Journal of the Linnean Society* 2023;138:143–68. <https://doi.org/10.1093/biolinnean/blac137>
- Schluter D. Evidence for ecological speciation and its alternative. *Science* 2009;323:737–41. <https://doi.org/10.1126/science.1160006>
- Schmidt J, Merene Y. *Trechus* species from Mt. Choke of northern Ethiopia related to *T. niloticus* (Quéinnec & Ollivier) with notable male genital morphology (Carabidae: Trechini). *Zootaxa* 2024a;5492:343–55. <https://doi.org/10.11646/zootaxa.5492.3.3>
- Schmidt J, Merene Y. Taxonomy, distribution and habitat of the giant trechus beetles endemic to Mt. Choke, Ethiopia (Coleoptera: Carabidae). *Taxonomy* 2024b;4:27–50. <https://doi.org/10.3390/taxonomy4010003>
- Schmidt J, Merene Y, Faille A. *Trechus hauthi* sp. n., a microphthalmic ground beetle from Mt. Choke, Ethiopia (Coleoptera: Carabidae: Trechini). *Integrative Systematics: Stuttgart Contributions to Natural History* 2024;7:1–7. <https://doi.org/10.18476/2024.867729>
- Schoville SD, Roderick GK, Kavanaugh DH. Testing the ‘Pleistocene species pump’ in alpine habitats: lineage diversification of flightless ground beetles (Coleoptera: Carabidae: *Nebria*) in relation to altitudinal zonation. *Biological Journal of the Linnean Society* 2012;107:95–111. <https://doi.org/10.1111/j.1095-8312.2012.01911.x>
- Schubert M, Lindgreen S, Orlando L. AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Research Notes* 2016;9:88. <https://doi.org/10.1186/s13104-016-1900-2>
- Seppey M, Manni M, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness. In: Kollmar M (ed.), *Gene Prediction: Methods and Protocols*. New York, NY: Springer, 2019, 227–45.
- Shen W, Sipos B, Zhao L. SeqKit2: a Swiss army knife for sequence and alignment processing. *iMeta* 2024;3:e191. <https://doi.org/10.1002/imt2.191>
- Slater JA. The incidence and evolutionary significance of wing polymorphism in lygaeid bugs with particular reference to those of South Africa. *Biotropica* 1977;9:217. <https://doi.org/10.2307/2388139>
- Smith SA, Brown JW, Walker JF. So many genes, so little time: A practical approach to divergence-time estimation in the genomic era. *PLoS One* 2018;13:e0197433. <https://doi.org/10.1371/journal.pone.0197433>
- Sota T, Takami Y, Ikeda H *et al.* Global dispersal and diversification in ground beetles of the subfamily Carabinae. *Molecular Phylogenetics and Evolution* 2022;167:107355. <https://doi.org/10.1016/j.ympev.2021.107355>
- Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–3. <https://doi.org/10.1093/bioinformatics/btu033>
- Stewart JR, Lister AM, Barnes I *et al.* Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* 2010;277:661–71. <https://doi.org/10.1098/rspb.2009.1272>
- Stone BW, Wolfe AD. Phylogeographic analysis of shrubby beardtongues reveals range expansions during the Last Glacial Maximum and implicates the Klamath Mountains as a hotspot for hybridization. *Molecular Ecology* 2021;30:3826–39. <https://doi.org/10.1111/mec.15992>
- Su Z-H, Imura Y, Osawa S. Evolutionary history of Calosomina ground beetles (Coleoptera, Carabidae, Carabinae) of the world as deduced from sequence comparisons of the mitochondrial ND5 gene. *Gene* 2005;360:140–50. <https://doi.org/10.1016/j.gene.2005.06.028>
- Suárez D, Arribas P, Jiménez-García E *et al.* Dispersal ability and its consequences for population genetic differentiation and diversification. *Proceedings of the Royal Society B: Biological Sciences* 2022;289:20220489. <https://doi.org/10.1098/rspb.2022.0489>
- Suchard MA, Lemey P, Baele G *et al.* Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 2018;4:vey016. <https://doi.org/10.1093/ve/vey016>
- Taylor EH. ‘Island’ faunas on the Mexican Plateau. *Nature* 1940;146:467. <https://doi.org/10.1038/146467a0>
- Tonzo V, Ortego J. Glacial connectivity and current population fragmentation in sky islands explain the contemporary distribution of genomic variation in two narrow-endemic montane grasshoppers from a biodiversity hotspot. *Diversity and Distributions* 2021;27:1619–33. <https://doi.org/10.1111/ddi.13306>
- Toussaint EFA, Gauthier J, Bilat J *et al.* HyRAD-X exome capture museomics unravels giant ground beetle evolution. *Genome Biology and Evolution* 2021;13:evab112. <https://doi.org/10.1093/gbe/evab112>
- Toussaint EFA, Gillett CPDT. Rekindling Jeannel’s Gondwanan vision? Phylogenetics and evolution of Carabinae with a focus on *Calosoma* caterpillar hunter beetles. *Biological Journal of the Linnean Society* 2018;123:191–207. <https://doi.org/10.1093/biolinnean/blx128>
- Toussaint EFA, Sagata K, Surbakti S *et al.* Australasian sky islands act as a diversity pump facilitating peripheral speciation and complex reversal from narrow endemic to widespread ecological supertramp. *Ecology and Evolution* 2013;3:1031–49. <https://doi.org/10.1002/ece3.517>
- Trauth MH, Larrasoana JC, Mudelsee M. Trends, rhythms and events in Plio-Pleistocene African climate. *Quaternary Science Reviews* 2009;28:399–411. <https://doi.org/10.1016/j.quascirev.2008.11.003>
- Treidel LA, Deem KD, Salcedo MK *et al.* Insect flight: state of the field and future directions. *Integrative and Comparative Biology* 2024;64:533–55. <https://doi.org/10.1093/icb/icae106>
- Vangestel C, Swaegers J, De Corte Z; [dataset: GCA_022063505.1] *et al.* Chromosomal inversions from an initial ecotypic divergence drive a gradual repeated radiation

- of Galápagos beetles. *Science Advances* 2024;**10**:eadk7906. <https://doi.org/10.1126/sciadv.adk7906>
- Wagner DL, Liebherr JK. Flightlessness in insects. *Trends in Ecology & Evolution* 1992;**7**:216–20. [https://doi.org/10.1016/0169-5347\(92\)90047-F](https://doi.org/10.1016/0169-5347(92)90047-F)
- Wallis GP, Waters JM, Upton P *et al.* Transverse alpine speciation driven by glaciation. *Trends in Ecology & Evolution* 2016;**31**:916–26. <https://doi.org/10.1016/j.tree.2016.08.009>
- Weng Y-M, Kavanaugh DH, Schoville SD. Evidence for admixture and rapid evolution during glacial climate change in an alpine specialist. *Molecular Biology and Evolution* 2024;**41**:msae130. <https://doi.org/10.1093/molbev/msae130>
- Weng Y-M, Yang M-M, Yeh W-B. A comparative phylogeographic study reveals discordant evolutionary histories of alpine ground beetles (Coleoptera, Carabidae). *Ecology and Evolution* 2016;**6**:2061–73. <https://doi.org/10.1002/ece3.2006>
- Williams FM. *Understanding Ethiopia*. Cham: Springer International Publishing, 2016.
- WorldPop. Global 100m Covariates. 2018. <https://doi.org/10.5258/SOTON/WP00644>
- Yebeyen D, Nemomissa S, Hailu BT *et al.* Modeling and mapping habitat suitability of highland bamboo under climate change in Ethiopia. *Forests* 2022;**13**:859. <https://doi.org/10.3390/f13060859>
- Yule GU. A mathematical theory of evolution, based on the conclusions of Dr. J. C. Willis, F.R.S. *Philosophical Transactions of the Royal Society of London Series B, Containing Papers of a Biological Character* 1925;**213**:21–87.
- Zachos J, Pagani M, Sloan L *et al.* Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 2001;**292**:686–93. <https://doi.org/10.1126/science.1059412>
- Zhang C, Mirarab S. Weighting by gene tree uncertainty improves accuracy of quartet-based species trees. *Molecular Biology and Evolution* 2022;**39**:msac215. <https://doi.org/10.1093/molbev/msac215>