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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All the skin morphological characteristics were quantified by Pro-Plus® (IPP) version 6.0. Compound Discoverer 3.1 was used to perform peak alignment, peak picking, and quantitation for each metabolite. SOAPnuke was used to filter adapter and low-quality data for long-read sequencing.

Data analysis	The peaks of metabolite were matched against the mzCloud, mzVault and Mass List database to obtain the accurate qualitative and relative quantitative results. Statistical analyses were performed using the statistical software R (version R-3.4.3), Python (v 2.7.6) and CentOS (CentOS release 6.6). The metabolites were annotated using the KEGG database, HMDB database and LIPIDMaps database. Principal components analysis (PCA) and Partial least squares discriminant analysis (PLS-DA) were performed at metaX. PacBio long-read data were de novo assembled with Falcon. Both PacBio long-read data and BGISEQ short-read data were polished with Pilon. BUSCO was used to evaluate the finally obtained contig-level assembly. Lachesis was used to cluster, order, and orient the contigs based on valid reads of Hi-C. Annotation of protein-coding genes was performed based on de novo prediction (using Augustus), homology-based (with BLAST+ and GeneWise) and RNA sequences-based (with GMAP) methods, and genes predicted from these methods were integrated by EVidenceModeler. SwissProt, TrEMBL, KEGG, Gene Ontology and NR were selected for gene functional annotation. RepeatMasker and RepeatProteinMask were used to annotate the repetitive sequences in the N. parkeri genome. Clean reads of RNA-seq were de novo assembled using Trinity and redundant contigs were eliminated using CD-HIT. The TGICL pipeline was used to cluster and assemble sequences with CAP3 and TransRate was used to evaluate the quality of the reference transcript. "crb-blast" (version 0.6.9) method was used to identify orthologous genes and then were aligned with FasParser. The coding region of assembled contig was determined using TransDecoder (version 3.0.1). Prank and Gblocks were used to align each gene into a consensus 1:1 orthologous gene set. All of the clean reads obtained from mRNA-seq were mapped to the reference genome using HISAT2 or mapped to reference transcript using Bowtie2. StringTie was used to calculate transcript expression levels and a Python script (prepDE.py) was used to extract read count information. The expression of transcript was calculated using RSEM. STEM (version 1.3.12) was used to classify significant temporal gene expression groups and co-expressed genes were identified using WGCNA. Filtered miRNA-seq reads were mapped to the genome or reference transcripts using Bowtie and analyzed with miRDeep2. The mRNA targets of miRNAs were predicted with TargetScan (version 7.1). An improved branch-site model in PAML was used to identify genes showing positive selection along the N. parkeri branch.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data are available in the Genome Sequence Archive (GSA) in National Genomics Data Center (NGDC), China National Center for Bioinformation / Beijing Institute of Genomics (CNCB/BIG), Chinese Academy of Sciences (CAS) at https://ngdc.cncb.ac.cn/gsa/ (GSA accessions: CRA004362, CRA004830 and CRA005235), the Genome Warehouse (GWH) of NGDC at https://bigd.big.ac.cn/gwh (accession: GWHBCKU00000000) and Figshare (https://figshare.com/projects/Genomic_data_of_Nanorana_parkeri/116061).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	n = 21–28 individuals of the highest-elevation N. parkeri and its lower-elevation relatives (N. phrynoides and Q. spinosa) were treated with a time course of UV exposure experiment (0, 0.5 h, 1 h, 2 h, 4 h, 6 h, and 8 h). Dorsal skin sample from each individual was used for morphological observation (skin structures quantification and ABTS+-scavenging test), molecular identification (metabolomics analysis for other 20 individuals of each species) and genetic analysis (genome sequencing, gene expression tracing from RNA-seq data and no-coding RNAs identification from miRNA-seq data). Substitutions of genes that are under positive selection in the highest-elevation frog were identified by evolutionary analysis and the affinity of substitutions-bearing genes were tested in vitro.
Research sample	The research samples are from the Nanorana parkeri, which is the highest-elevation frog in the world, and its lower-elevation relatives (N. phrynoides and Q. spinosa). Frogs of the genus Nanorana (Dicroglossidae) have colonized the Qinghai-Tibetan Plateau (at elevations up to 5,000 m) over the past 8–9 million years, and N. parkeri experiences much stronger UV radiation during daytime insolation than its lower-elevation relatives. Such long-term selective pressure acting on populations of N. parkeri resulted in a serious of phenotypic adaptations in this species to potential UV defense, including increased skin pigmentation compared to its lower-elevation relatives. Thus, N. parkeri is an ideal model organism for studying the long-term adaptive strategies and the underlying genetic and molecular mechanisms related to UV-radiation protection. Male adults of each species were collected for this study.
Sampling strategy	For generated morphological and molecular data, no statistical methods were used to predetermine sample size. In order to eliminate noise of individual difference, at least 54 images (9 images x 6 time points) were captured from each species to statistically quantify the skin morphology. 3–4 skin samples at each time point were used for RNA-seq and miRNA-seq separately.
Data collection	Ting-Ting Fu and Chun-Hua Yang performed UV exposure experiments on studied frogs in lab and isolated dorsal skin samples for

	morphological observation and genetic analyses. The skin characteristics were quantified based on images of HE stained sections. Xin-Wang Yang performed the ABTS+-scavenging test for skin secretions. Cheng-Bo Long conducted enzyme kinetic analyses. Metabolite identification was based on the LC-MS/MS system. The N. parkeri genome was sequenced from a combination of long-range PacBio RS II, short-read Illumina platform and Hi-C technique. The transcriptome data was sequenced on an Illumina platform to generate 150 bp paired-end reads and miRNA data was sequenced using an Illumina platform to generate 50 bp single-end reads.
Timing and spatial scale	The studied individuals were collected from Tibet (~ 4,500m), Yunnan Province (~ 1,700m) and Anhui Province from 2017 to 2018. All the individuals were collected during their reproductive periods at the same time of each year. The experiments and data analyses were performed from 2018 to 2019.
Data exclusions	No data were excluded from the analyses.
Reproducibility	We repeated experiments on different individuals and all the results come to the same conclusion. For data analyses pipeline, we repeated the analyses procedures independently and all attempts to repeat the analyses were successful.
Randomization	The individuals used in experiments were randomly allocated into groups with or without UV exposure.
Blinding	Investigators were blinded to group allocation during data collection and analysis.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Climate on Qinghai-Tibetan Plateau is dry and with a large temperature difference between morning and evening. The daily UV radiation on it in May is around 3.5 mW/cm2.
Location	N. parkeri individuals were collected at ~4,500 m on Qinghai-Tibetan Plateau with daily UV radiation of ~ 3.5 mW/cm2. N. phrynoides individuals were collected at ~1,700m in Yunnan Province with daily UV radiation of ~3.5 mW/cm2. Q. spinosa individuals were collected at ~118m in Anhui Province with daily UV radiation of ~0.4 mW/cm2.
Access & import/export	We planned routes before sampling and search every possible habitats along with routes that maybe accessible for studied frogs. The collecting permit and all study protocols were approved by the Animal Care and Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences, and were conducted in strict accordance with the guidelines for Animal Care and Use at the Kunming Institute of Zoology (SMKX-20160301-03).
Disturbance	After sampling collection, we recovered the habitats as possible.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	The study did not involve laboratory animals
Wild animals	To exclude potential influences of sex differences and developmental stages, male adults during their reproductive periods were collected for all the studied frogs. Captured frogs were housed in artificial ponds with sufficient food at appropriate temperature. Mean snout-vent lengths of each species were measured using a digital Vernier caliper to ensure they were of similar age. After the frogs were sacrificed, their dorsal skin was isolated rapidly for subsequent analyses.
Field-collected samples	Captured frogs were housed in artificial ponds with sufficient food at appropriate temperature. Water in the ponds was changed everyday to keep fresh.
Ethics oversight	The collecting permit and all study protocols were approved by the Animal Care and Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences, and were conducted in strict accordance with the guidelines for Animal Care and Use at the

Kunming Institute of Zoology (SMKX-20160301-03).

Note that full information on the approval of the study protocol must also be provided in the manuscript.