

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

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

Data analysis

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](#)

All population-level resequencing data, the raw data associated with the *N. pictus* genome assembly, the transcriptome data, and the raw sequencing data for linked-read genome assemblies representing the entire genus *Neobatrachus* have been deposited in the European Nucleotide Archive under BioProjects PRJEB49532, PRJEB101309, and PRJEB101307, respectively.

The 18 linked-read genome assemblies of the genus *Neobatrachus*, as well as the reference genome sequence, gene model annotation, GO annotation, and IPR annotation files for the diploid *N. pictus*, have been deposited at <https://doi.org/10.6084/m9.figshare.30576644>. Custom scripts will be available at <https://>

github.com/novikovalab/Neobatrachus_pictus.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender NA

Reporting on race, ethnicity, or other socially relevant groupings NA

Population characteristics NA

Recruitment NA

Ethics oversight NA

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

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 This work represents a genomic study on a population level. We sequenced whole genomes of all Neobatrachu species with different technologies (10x Genomics, HiC, stLFR, paired short-read WGS) and conducted selectscion scans

Research sample Neobatrachus genus: N. pictus, N. albipes, N. wilsmorei, N. sutor, N. fulvus, N. pelobatoides, N. sudellae, N. kunapalari and N. aquilonius

Sampling strategy We obtained fresh field material from N. pictus to build a high-quality refence genome and annotation. For the population-scale sampling of all the species we used museum collections of Neobatrachus frogs (South Australian Museum and Western Australian Museum). The samples were chosed on their species assignments and geolocations to cover the species range.

Data collection The metadata is present in the Supplementary Table 4 and linked to the raw sequencing data on ENA

Timing and spatial scale The first N. pictus sample for HMW DNA extraction was collected in 2017. The sampling of fresh material was dictated by the weather conditions and availability for field work. The sampling of the museum material was conducted depending on the availability of funding.

Data exclusions One of the samples was excluded from the study (described in Supplementary Table 4), because the sequencing results did not match the original species assignment and it was not possible to assign to any of the Neobatrachus species unambiguously

Reproducibility No experimental tests where conducted in this study. All the analysis is reproducible with the raw data and the code we make available

Randomization Randomization was used for modeling the diploid ancestries of the tetraploids, randomly combining two diploid samples within and between species

Blinding Blinding is not relevant for our study

Did the study involve field work? Yes No

Field work, collection and transport

Field conditions Collections where conducted at night time during the rain, as those are the activity conditions for Neobatrachus frogs

Location Supplementary Table 4

Access & import/export It is assured that the study followed existing national regulations. All the procedures involving animals were performed in Australia in

accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. None of the *Neobatrachus* species are endangered, in total, we use up to 10 field-collected individuals of *N. pictus* with the permit from the Department of Environment and Water of the Government of South Australia to Prof. Stephen Donnellan to lead the fieldwork, capture the animals by hand, euthanase and collect tissue. The permit was renewed for every collection season. The rest of the samples were obtained from the preserved tissues at the South Australian Museum and Western Australian Museum.

Disturbance

We do not anticipate any disturbance cause by our study

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	Involvement
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involvement
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

No laboratory animals were involved in the study

Wild animals

All the procedures involving animals were performed in Australia in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. None of the *Neobatrachus* species are endangered, in total, we use up to 10 field-collected individuals of *N. pictus* with the permit from the Department of Environment and Water of the Government of South Australia to Prof. Stephen Donnellan to lead the fieldwork, capture the animals by hand, euthanase and collect tissue. The permit was renewed for every collection season. The rest of the samples were obtained from the preserved tissues at the South Australian Museum and Western Australian Museum.

Reporting on sex

When the data on the sex of the used animals is available it can be traced by the internal number of the voucher stored in the South Australian Museum or Western Australian Museum

Field-collected samples

Field-collected samples were not kept in the laboratory, they were euthanased according to the Australian Code of Practice. All the vouchers are stored for long-term preservation in the SAM.

Ethics oversight

The study was performed with the permit from the Department of Environment and Water of the Government of South Australia to Prof. Stephen Donnellan to lead the fieldwork, capture the animals by hand, euthanase and collect tissue.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.