

Abschließender Sachbericht

**Graduelle Umweltveränderung *versus*
einzelnes Katastrophenereignis –
Identifizierung von Treibern der
Säugetierevolution**

ausführende Stelle: Leibniz-Institut für Zoo- und Wildtierforschung

Aktenzeichen: SAW-2013-IZW-2

Förderzeitraum: 01.04.2013- 31.03.2016 (kostenneutral verlängert bis 31.12.2016)

Ansprechpartner: Prof. Dr. Jörns Fickel

List of contents page

Aims	1
Course of work	1
Development and establishment of methods	1
Deviations from the planned project	1
Results	2
Modules 1 & 2: Analysis of mtDNA haplotypes	2
Modules 3 & 4: Reconstruction of palaeo-climatic conditions	5
Module 5: Analysis of stable isotopes	6
Module 6: Ecological niche modelling	7
Modules 7 & 8: Dynamic modelling of future distributions	8
Economic impact/applications	9
Contribution by cooperation partners	9
Newly established cooperations	9
Theses (PhD, Master, term paper)	10
List of publications generated within the project	10
Peer-reviewed, already published	10
Submissions/resubmissions:	11
in preparation:	12
Book chapter:	12
Presentations/ talks at conferences	12
Posters:	13
Immediate impact of publications	13
Measures for safe storage and to secure access to data generated within the project	13
List of selected news clips and press coverage	14
Acknowledgements	14

Executive summary (English)

The diversity of life is not evenly distributed across the planet. However, the mechanisms generating the (often complex) patterns of distribution of genetic diversity and species are still poorly understood. A way to close this gap is to investigate how environmental changes have impacted the distribution of genetic diversity and species in the past and continue to do so at present. A few areas, so-called biodiversity hotspots, harbor the majority of all species, leading to the question: *What are the drivers shaping such high biodiversity, particularly in tropical rainforests?*

The project was set out in a biodiversity hotspot: the Sunda Shelf in Southeast Asia (SE Asia) and the bordering continental mainland. By combining climate reconstructions, molecular genetic data analysis and species distribution data from the Late Pleistocene to the present (based on contemporary habitat occupancy) we wanted to evaluate the impact of two key drivers shaping biodiversity: gradual climate change *versus* a single catastrophic event, which in SE Asia was the Toba super-volcano eruption ~73.5 thousand years ago. When possible, we studied pairs of sister species (as they derived from a common ancestor) that had either taken different ecological trajectories during speciation (generalist vs. specialist) or were now allopatrically distributed.

As a final conclusion we can state that evolutionary processes were more complex and more intertwined than envisioned in our hypotheses. There was no general pattern applicable to all species we had studied. Instead, we found phylogeographic histories and distributions to be species-specific and to have been impacted by either gradual climate change, single catastrophic events, or even additional barriers. Our hypotheses predicted that the distribution of generalist species (and differentiation among their populations) under gradual climate change should be determined by adaptability only. Thus, they should be distributed across the Sunda shelf, because gene-flow among populations should have been possible during glacial periods (when low sea levels led to the emergence of Sundaland – a large landmass connecting all Sunda Islands and Thai/Malay Peninsula). This, however, was not what we found. We regularly found clear distinctions into Sunda and continental mainland clades within species, but as dating revealed, surprisingly with different histories. Some generalist species (e.g. Leopard cat, Asian golden cat) had clades, whose age fitted well with the date of the Toba eruption, while other generalist species (e.g. red muntjac, *Rusa spec.*) had clades, whose age predated (by far) the volcano eruption and could only be explained by (at least temporal) presence of additional barriers such as the Isthmus of Kra, and the central Indian dry zone (red muntjac) or by gradual climate changes leading to a drier climate on Java compared to the other major Sunda Islands (*Rusa timorensis*).

For specialist species (e.g. Bay cat) we could confirm our hypothesis of gradual climate change having influenced the evolution of the species as they became patchily distributed following the patchy distribution of their habitat.

Analysis of climate data (by applying a newly developed nonlinear time series analysis technique) led to another quite surprising result: the identification of a seesaw relationship between the East Asian and the Australian summer monsoon at millennial to sub-centennial timescales.

Within the project we have so far generated 16 peer-reviewed manuscripts and one book chapter. Four additional publications are currently in the submission/resubmission process and five further manuscripts are being prepared (the data has already been analyzed). Different aspects of the project have been presented in 14 conference talks and four posters at 12 international conferences and colloquia. Within the project, so far three PhD theses, four master theses, and one scientific term paper have been completed. One more PhD project is still ongoing.

Executive summary (deutsch)

Arten, in ihren vielfältigen Formen, sind nicht gleichmäßig über die Erde verteilt und die Mechanismen und Prozesse, die diese ungleichmäßige Verteilung von Arten und Genen (in den Populationen) beeinflussen und bedingen, sind noch immer kaum verstanden. Eine Möglichkeit, Teile dieser Lücke zu schließen, ist das Studium klimatischer Veränderungen und deren Einfluss (in Vergangenheit und Gegenwart) auf die Verteilung und Ausbreitung von Arten, insbesondere in Gebieten, die sich durch hohe Artenvielfalt und hohe genetische Vielfalt innerhalb dieser Arten auszeichnen. Die Frage lautet daher, welche evolutiven Kräfte liegen diesem Artenreichtum in eben genau diesem Gebiet zugrunde?

Das Projekt wurde im Sundaschelf in Südostasien und dem daran angrenzenden Festland durchgeführt. Durch die Kombination von Klimadaten, genetischen Daten und Rekonstruktionen von Artverteilungen vom Pleistozän bis in die Gegenwart (basierend auf heutigen Habitatnutzungsdaten) untersuchten wir den Einfluss zweier, die Artenvielfalt prägender Faktoren: allmählicher Klimawandel *versus* Umweltkatastrophen (in Südostasien der Ausbruch des Supervulkans Toba vor ~73.5 tausend Jahren). Wir haben, wenn möglich, Paare eng verwandter Arten untersucht (diese stammen unmittelbar von einem gemeinsamen Vorfahren ab), die während der Artbildung unterschiedliche ökologische Bahnen einschlugen (Habitatgeneralist vs. -spezialist), oder die räumlich getrennt wurden (allopatrische Artbildung).

Im Ergebnis unserer Studien müssen wir konstatieren, dass evolutionäre Prozesse komplexer und enger miteinander verwoben sind, als wir dies in unseren Arbeitshypothesen angenommen haben. Im Gegensatz zu einer unserer maßgeblichen Ausgangshypothesen, die für Generalisten bei allmählichem Klimawandel eine Verbreitung in geeigneten Habitaten über das gesamte Sundaschelf vorhersagte (da in Phasen pleistozäner Gletscherbildung der niedrige Meeresspiegel den Genfluss zwischen allen Populationen einer Art im trockenengefallenen Sundaschelf erlauben sollte), fanden wir auch bei Generalisten regelmäßig eine genetische Aufspaltung in eine Sunda- und eine Festland-Klade, jedoch mit für die einzelnen Arten unterschiedlichen Entstehungsszenarien, wie die molekulare Datierung ergab. Einige Generalisten (z.B. Bengalkatze, Asiatische Goldkatze) wiesen Kladen auf, deren Alter sehr gut mit dem Zeitpunkt der Toba-Eruption übereinstimmte, während die Kladen anderer Generalisten (Rotes Muntjak, Rusa-Hirsch) lange vor der Toba-Eruption entstanden waren und entweder auf graduellen Klimawandel (trockeneres Klima auf Java: Rusa) oder die Anwesenheit weiterer Ausbreitungsbarrieren wie dem Isthmus of Kra und der zentralindischen Trockenzone zurückzuführen waren (Rotes Muntjak).

Für Habitatspezialisten (z.B. Borneo-Katze) konnten wir unsere Hypothese bestätigen, wonach bei allmählichem Klimawandel die Ausbreitung der Arten der Ausbreitung bzw. Schrumpfung der Habitate folgte und nicht geeignete Habitate Barrieren darstellten.

Ein weiteres unerwartetes Ergebnis erbrachte die Analyse der holozänen Klimadaten. Durch Entwicklung einer neuen Analyseverfahren (nicht-lineare Zeitserienanalyse) konnte ein Zusammenhang zwischen den Stärken der Sommermonsun-Regen in Ostasien und Australien aufgedeckt werden.

Im Rahmen des Projektes entstanden bisher 16 begutachtete Publikationen. Weitere vier sind im Prozess des Einreichens bzw. Wiedereinreichens. Fünf weitere Manuskripte sind in Vorbereitung (die Daten sind erhoben und bereits analysiert). Verschiedene Aspekte der Ergebnisse wurden bisher in vierzehn Vorträgen und vier Postern auf insgesamt 12 internationalen Kongressen und Kolloquien vorgestellt. Außerdem wurden drei Dissertationen, vier Masterarbeiten und eine wiss. Hausarbeit erfolgreich abgeschlossen. Ein weiteres Dissertationsvorhaben läuft noch.

Final report

Aims

We wanted to know whether species richness in the Sunda shelf biodiversity hotspot was predominantly shaped by gradual climate change or whether a single catastrophe was responsible for the observed distribution and diversity patterns. Finding convincing evidence in either direction across a wide variety of species would also falsify the null hypothesis of random species assemblages (Hubbell 2001). We focused on mammals, because a sufficient number of ancient samples required for the past vs. present comparisons were available for this group. We planned to investigate carnivores, hoofed mammals and primates covering a broad spectrum of different ecological requirements across habitat, diet, and forest strata. The selection comprised habitat specialists (e.g. leopard) as well as habitat generalists (e.g. leopard cat). We expected our results to allow general conclusions about the evolution of mammals in the Sunda Shelf.

Course of work

Most of the work followed the working plan, whereby the first two years were dedicated to completing the collection of specimens via our cooperation museums and institutions (first year) and to the establishment (even development) of methods for all work modules, in particular for sequencing ancient and archival samples and for species distribution modeling (first and second year). Parts of the second and the third year were then dedicated to data analysis and writing of manuscripts (see 'List of publications').

Development and establishment of methods

In the beginning we used two sequencing platforms, an Illumina MiSeq and an IonTorrent. As library generating protocols for non-Illumina platforms had been scarce, in particular for ancient and archival samples, we developed a modified protocol, to be used for samples with degraded DNA, such as very old samples (Martins *et al.* 2017).

We established a new *in house* bioinformatics pipeline (using already existing software modules, e.g. MITObim) to allow the analysis of complete mitogenomes, even if a mitogenome from a closely related species is not available.

We also developed (together with our cooperation partners) a modified procedure and established optimal conditions for the hybridization capture of highly degraded DNA from museum samples (Paijmans *et al.* 2016).

For the analysis of available climate data, our cooperation partner PIK developed a new tool, which allows confident identification of strong versus weak monsoon phases at millennial to sub-centennial timescales (Eroglu *et al.* 2016).

Due to the detection of unexpected additional barriers to mammalian dispersal, we developed a new approach to explain how palaeo-climate and species life history traits may have shaped current distribution ranges (Radchuk *et al.*, in revision).

Deviations from the planned project

Budget: With the acceptance/approval of the project we were also informed about severe cuts in the financial budget. During the project's kick-off meeting (March 2015), we had to reassign/reshape tasks and working modules to fit the new budget.

Access to samples and climate record material: Despite having a long-standing good relationships with the local government bodies in the Sunda shelf (e.g. MoUs on the use of biological samples), we did not manage to get access to local stalactites for a more detailed reconstruction of past (late Pleistocene) local climatic conditions. Thus, we had to rely on publicly available databases for the reconstructions and therefore diverted to habitat distribution modeling. We also faced some unexpected drawbacks due to belated or no availability of samples. The British Museum in London could not grant access to its samples because of

construction work in the collection, the museum in Paris did only grant access to a single specimen per population (i.e. rhinos), but not from the localities we were interested in. The *Naturalis* museum in Leiden (which had been very helpful) had received the mammal collection from the Amsterdam museum, and thus access to the ungulate collection could only be granted a year later than originally planned (because everything was in sealed boxes). These unforeseen developments were the reason why we could not include all species we had planned to be studied.

Sequencing: With the highly degraded DNA from archival samples, we decided to extend our sequencing approach by sequencing whole mitogenomes, which in the end, allowed us much better analyses (we also contributed our sequences to a general database of mitogenomes of SE Asian mammals; Salleh *et al.* 2017).

Personnel: we had to deal with the withdrawal of a PhD student after 2 years. A comprehensive publication by another group on her PhD topic [phylogeny and phylogeography of SE Asian primates] led, in combination with personal reasons, to that decision. Thus, we did not yet finish the analysis of the data we have retrieved from the primate samples. However, from the remaining salary budget we were able to hire a postdoc for several months to at least continue with the analysis of the isotope data. The corresponding manuscript is in preparation (Soto *et al.* 2017).

Results

Modules 1 & 2 (Analysis of mtDNA haplotypes)

These modules largely went according to plan. We analysed the phylogeny and phylogeographic history of numerous SE Asian mammals according to our research plan. As a final conclusion we can state that evolutionary processes were more complex and more intertwined than envisioned in our hypotheses. There was no general pattern applicable to all species we had studied. Instead we found phylogeographic histories and distributions to be species-specific and to have been impacted by either gradual climate change, single catastrophic events, or even additional barriers. Our hypotheses predicted that the distribution of generalist species (and differentiation among their populations) under gradual climate change should be determined by adaptability only. Thus, they should be distributed across the Sunda shelf, because gene-flow among populations should have been possible during glacial periods (when low sea levels led to the emergence of Sundaland – a large landmass connecting all Sunda Islands and Thai/Malay Peninsula). This, however, was not what we found. We regularly found clear distinctions into Sunda and mainland (continental) clades within species, but as dating revealed, surprisingly with different histories. Some generalist species (e.g. Leopard cat, Asian golden cat) had clades, whose age fitted well with the date of the Toba eruption, while other generalist species (e.g. red muntjac, *Rusa spec.*) had clades, whose age predated (by far) the volcano eruption and could only be explained by (at least temporal) presence of additional barriers such as the Isthmus of Kra, and the central Indian dry zone (red muntjac) or by gradual climate changes leading to a drier climate on Java compared to the other major Sunda Islands (*Rusa timorensis*).

For specialist species (e.g. Bay cat) we could confirm our hypothesis of gradual climate change having influenced the evolution of the species as they became patchily distributed following the patchy distribution of their habitat.

There were, however, several unexpected results with strong consequences for species conservation. For instance, we were able to show that the contemporary tiger (*Panthera tigris*) is in fact a very young species. A precondition for a successful tiger recovery and global tiger management (including global captive breeding programs and conservation management of free-ranging meta-populations) is a consensus on the number of tiger conservation units [that is, subspecies, evolutionarily significant units (ESUs), ecotypes, or management units (MUs)] because active interventions, such as translocations or releases of captive-bred tigers, will presumably become more important in the future for reversing the decline of wild tigers. We

were able to show (by analysing the most comprehensive data set ever used on tigers), that there are actually only two tiger subspecies (with three management units). This will, if accepted, greatly facilitate captive breeding programs.

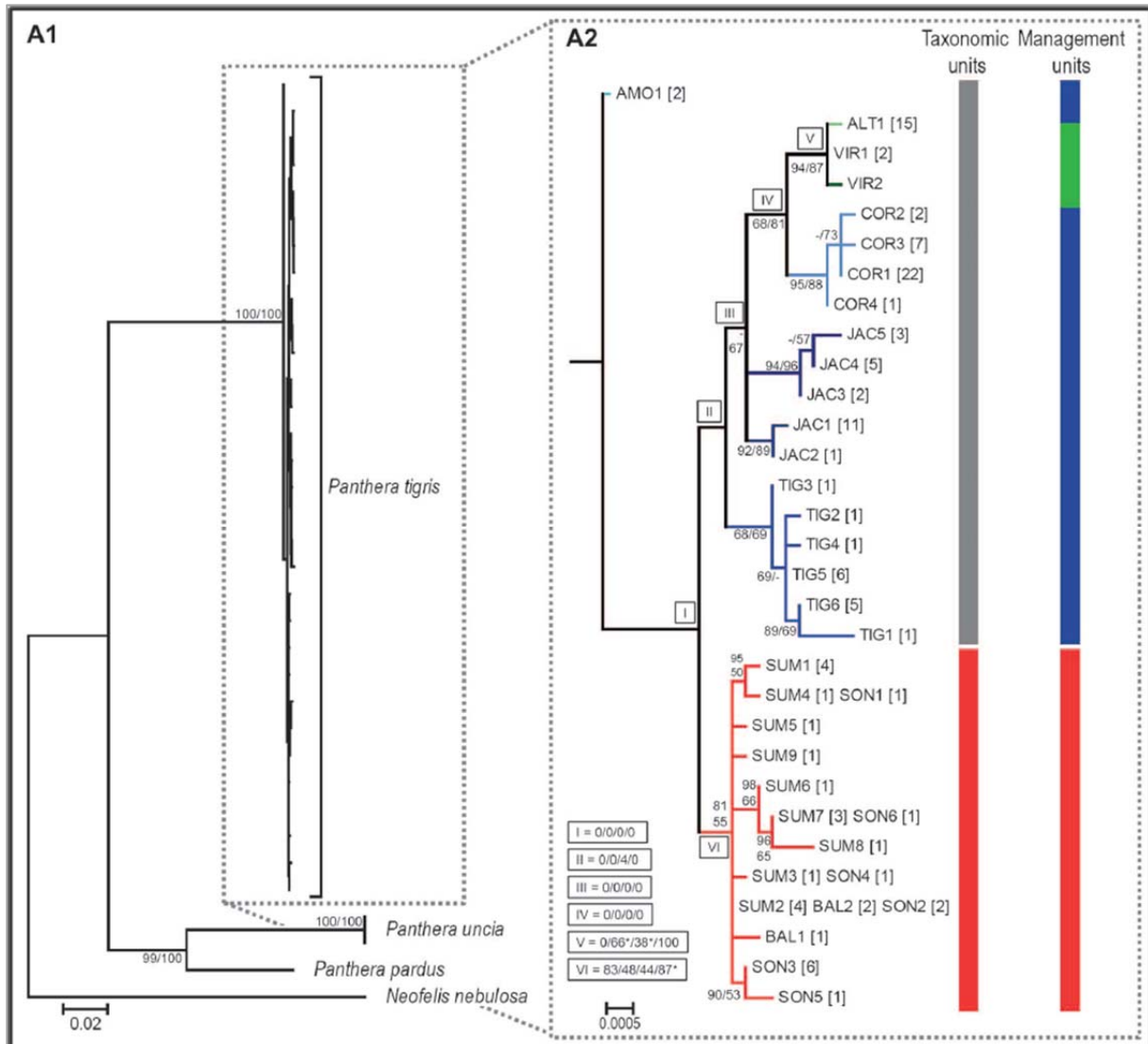


Fig. 2A from Wilting *et al.* (2015) *Science Advances* 1:e1400175 [Open Access]. Phylogenetic analyses of all nine putative subspecies using 3968 bp of mtDNA. (A1 and A2) Maximum likelihood (ML) tree of intraspecific variation among all putative tiger subspecies in relation to three pantherine cat species [snow leopard (*Panthera uncia*), leopard (*Panthera pardus*), and clouded leopard (*Neofelis nebulosa*)]. Values above or below branches show bootstrap supports for ML and Bayesian inference. (A1) ML tree including three pantherine cat species as outgroups. (A2) Enlargement of the ML tree part showing the tigers only. Roman numerals indicate bootstrap supports of nodes for skull {females} / skull {males} / skin / ecological preferences. Abbreviations for putative subspecies are given in Table 1 in the original paper. * indicates that one additional putative subspecies clusters with this group.

Another very surprising finding was the phylogeographic history of the two *Rusa* species studied in the project (*R. timorensis*, *R. unicornis*). Our results indicated that the split between the two species took place during the Pleistocene (~1.8 million years ago), possibly driven by adaptations of *R. timorensis* to the drier climate found on Java compared to the other major islands of Sundaland. Geographical structuring among populations of the widely distributed Sambar (*Rusa unicornis*) was also found, but this did only partially correspond to their geographic origin and not to the current subspecies assignments. Although mitochondrial and

nuclear markers identified two well differentiated clades, there was a largely discrepant pattern between the two markers: while the nuclear markers clearly separated the individuals into the two species (microsatellite analysis in the original paper; Martins *et al.* 2017, under review), the mitochondrial DNA revealed that all *R. timorensis* sampled to the east of the Sunda shelf (Lombok, Dompoe, Timor, Sulawesi and the Moluccas) carried haplotypes from the *R. unicolor* clade but not from the *R. timorensis* clade (Java and Bali), while one *Rusa unicolor* from South Sumatra carried a *R. timorensis* haplotype. Our results show that hybridisation occurred between these two sister species in Sundaland during the Late Pleistocene and constitute the first report of early hybrid translocation events mediated by humans (Martins *et al.*, 2017, under review).

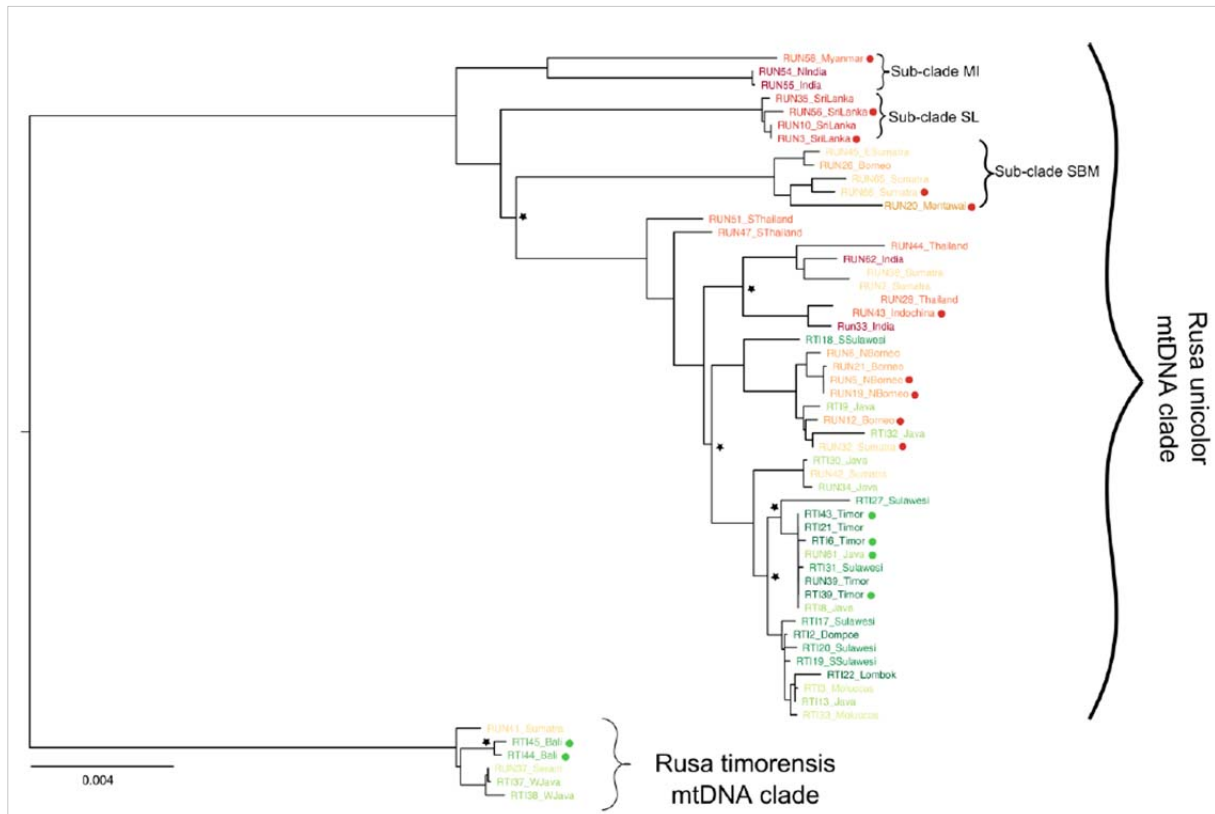


Fig. 3 from Martins *et al.* (2017), Journal of Biogeography, under review. Mitogenome ML tree of both *Rusa* species. Colors on tips represent sampling location (as in Fig. 2 of the same paper) and stars represent split events with bootstrap values/Bayesian posterior probabilities lower than 90/0.95. Red and green dots represent samples for which we obtained nDNA; red dot: assigned to the *Rusa unicolor* genotypic cluster, green dot: assigned to the *Rusa timorensis* genotypic cluster. Major mtDNA clades and sub-clades are labeled with curved brackets. The scale bar indicates the number of substitutions per position.

While studying the historical phylogeography of two SE Asian rhino species (Javan rhino *Rhinoceros sondaicus*, Sumatran rhino *Dicerorhinus sumatrensis*) we were able to match climatic events of the Pleistocene (gradual climate change) to the distribution of genetic divergence of both species, revealing that each species' ecological needs likely led to different speciation trajectories. While the Javan rhino was able to disperse through vegetation zones of the available land corridors, which were exposed during the low sea levels of glacials periods, the Sumatran rhino was likely confined to montane forest refuges. More importantly, our results also revealed a much higher genetic diversity within both species than ever reported, proving that with the decline of these species also unique genetic lineages, equalling the genetic diversity of potential subspecies had gone lost (Martins *et al.* 2017).

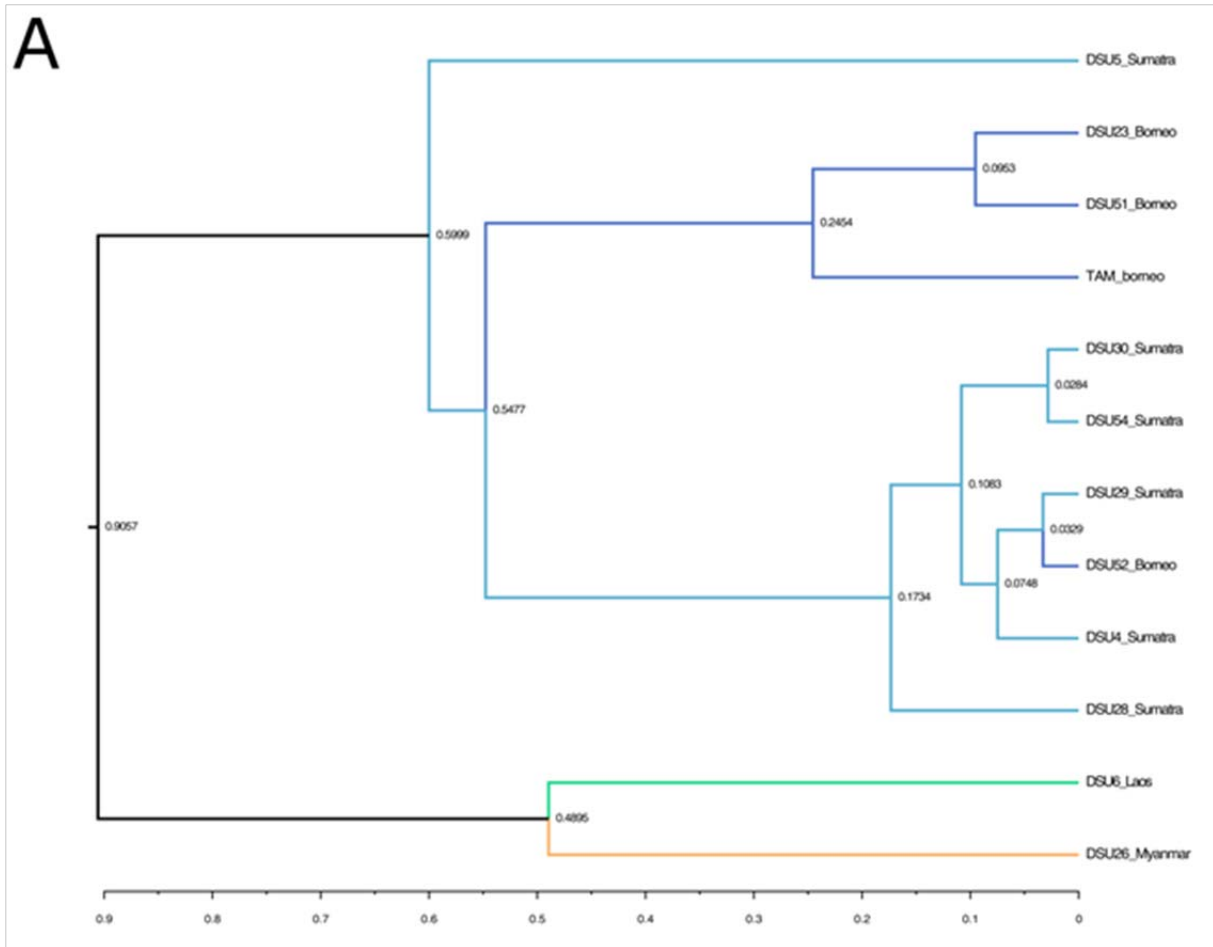
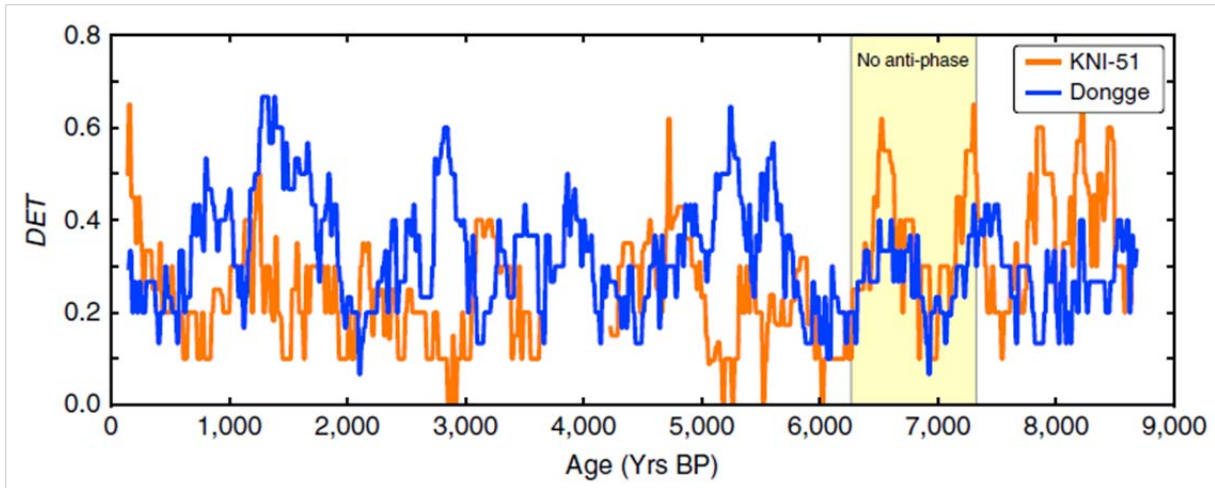


Fig. 4A from Martins *et al.* (2017), *paper awaiting approval by all co-authors*. Dated Bayesian inference gene trees for Sumatran rhinos. Scale is in millions of years before present. One can clearly see the long distinct branches of the two historical specimen from Myanmar and Laos.

Modules 3 & 4 (reconstruction of palaeo-climatic conditions)

Here, we were not able to follow our research plan as we did not succeed in getting access to the records from Borneo (Gunung cave, Partin *et al.* 2007), from the Indian subcontinent (Cave Krum Umsynrang), and from eastern China (Sanbao Cave, Wang *et al.* 2008). We only had access to the Dongge-Hulu caves from Eastern China (Wang *et al.* 2001, Yuan *et al.* 2004), whose records cover the last 9000 years (Holocene). However, using the available data, we were able to detect a climatic seesaw between the summer monsoon rainfalls in southern China and northwestern Australia (Eroglu *et al.* 2016).

Next page: Figure 3 from Eroglu *et al.* (2016) Nature Communications 7:12929 [Open access]. Determinism of KNI-51 (NW Australia) and Dongge Cave (S China) highlighting the antiphase relationship. There is a gap in the data of KNI-51 ~4,000 years before present. Contrary to the general antiphase relation of the two determinism time series of KNI-51 and Dongge Cave proxies, the region ~7,000 years before present shows an in-phased relationship (yellow).



Module 5 (Analysis of stable isotopes)

Due to the withdrawal of the PhD student assigned to this part of the project, we were not able to complete the module. However, the isotopic ratio data for all carnivores has already been measured and analysed (Soto et al. 2017, in prep.). Our results on carnivores clearly show ecological niche adaptation for several carnivore species (Fig. 1 from Soto *et al.* 2017, in prep.). We also detected area specific niche adaptation in several of the carnivores studied (Fig. 2 from Soto *et al.* 2017, in prep.).

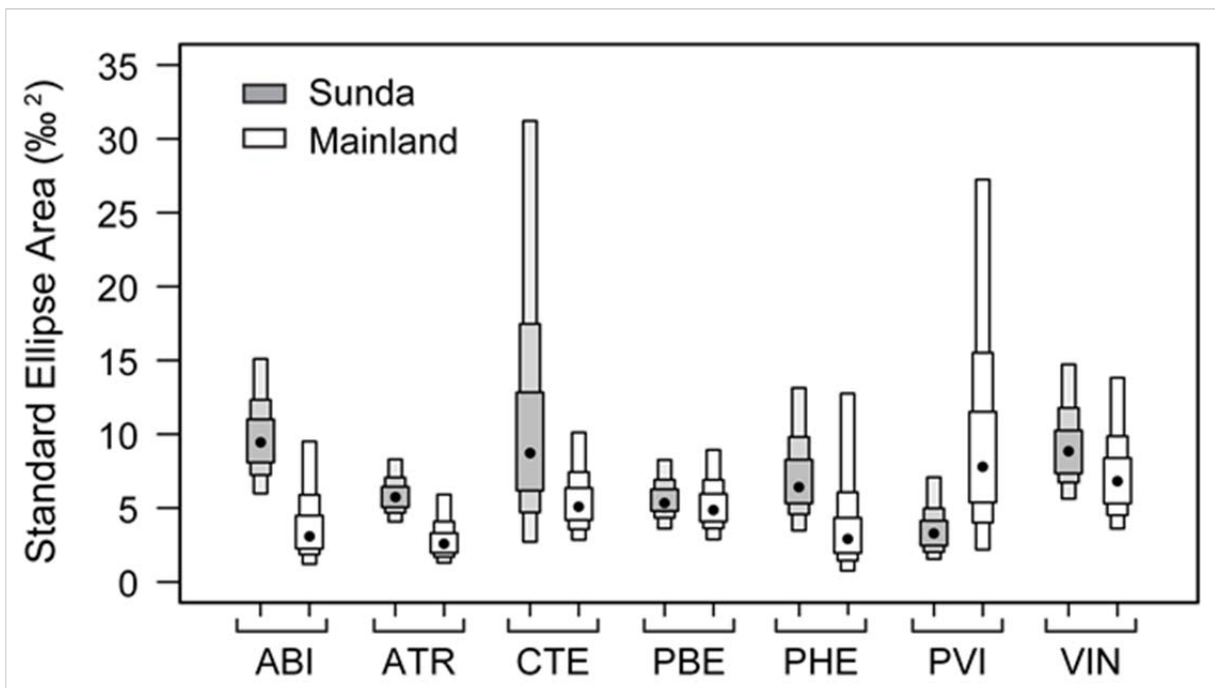


Fig. 1 from Soto *et al.* (2017, in prep.). Bayesian results for Standard Ellipse Area (SEA_B) of the carnivore mammal species in SE Asia (Sunda Shelf vs. Mainland). Boxes indicate the 50%, 75%, and 95% credibility intervals. Black dots are the modes for each group as a measure of central tendency. ABI: *Arctictis binturong* (Binturong), ATR: *Arctogalidia trivirgata* (small-toothed palm civet), CTE: *Catopuma temminckii* (Asian golden cat), PBE: *Prionailurus bengalensis* (Leopard cat), PHE: *Paradoxurus hermaphroditus* (Asian palm civet), PVI: *Prionailurus viverrinus* (Fishing cat), VIN: *Viverricula indica* (small Indian civet).

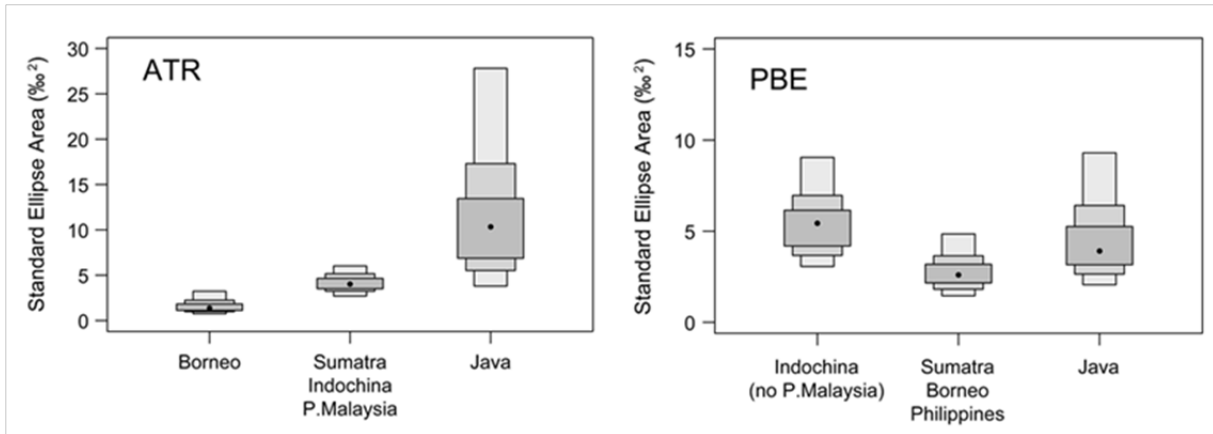
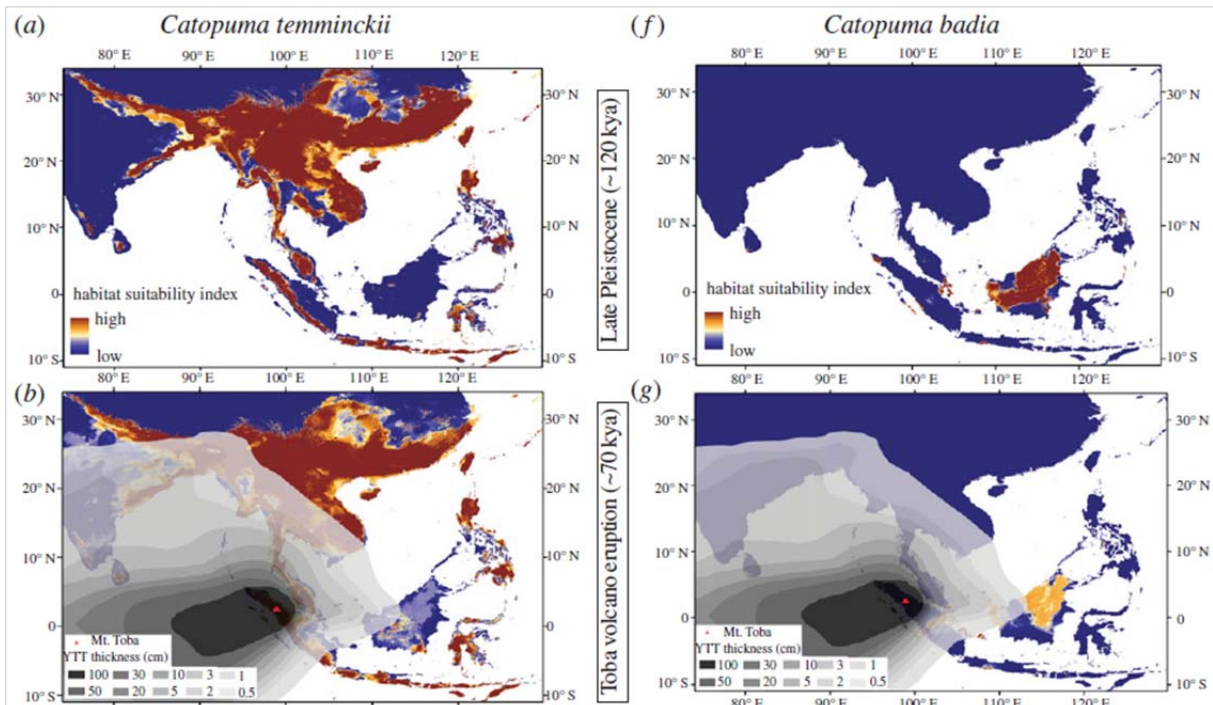


Fig. 2 from Soto *et al.* (2017, in prep.). Bayesian results for Standard Ellipse Area (SEA_B) of two SE Asian carnivore species with contrasting genetic regions. Boxes indicate the 50%, 75%, and 95% credibility intervals. Black dots are the modes for each group as a measure of central tendency. ATR: *Arctogalidia trivirgata* (small-toothed palm civet), PBE: *Prionailurus bengalensis* (Leopard cat).

We will continue with the analysis for the other two mammal groups of the project (ungulates, primates).

Module 6 (ecological niche modeling)

This module was successfully completed according to the work plan. We were able to reconstruct past habitats and thus past distribution areas (e.g. Patel *et al.* 2016, Wilting *et al.* 2016).



Upper part of Fig. 5 from Patel *et al.* (2016) R. Soc. open sci 3: 160350 [Open Access].

The figure depicts the projected distribution of the Asian golden cat (*Catopuma temminckii*, left) and the Bay cat (*Catopuma badia*, right) for two selected time periods (more time periods in the publication). Current distributions (to serve as ‘occurrences’ for model-fitting procedures) were taken from the IUCN/SSC Red List of Threatened species.

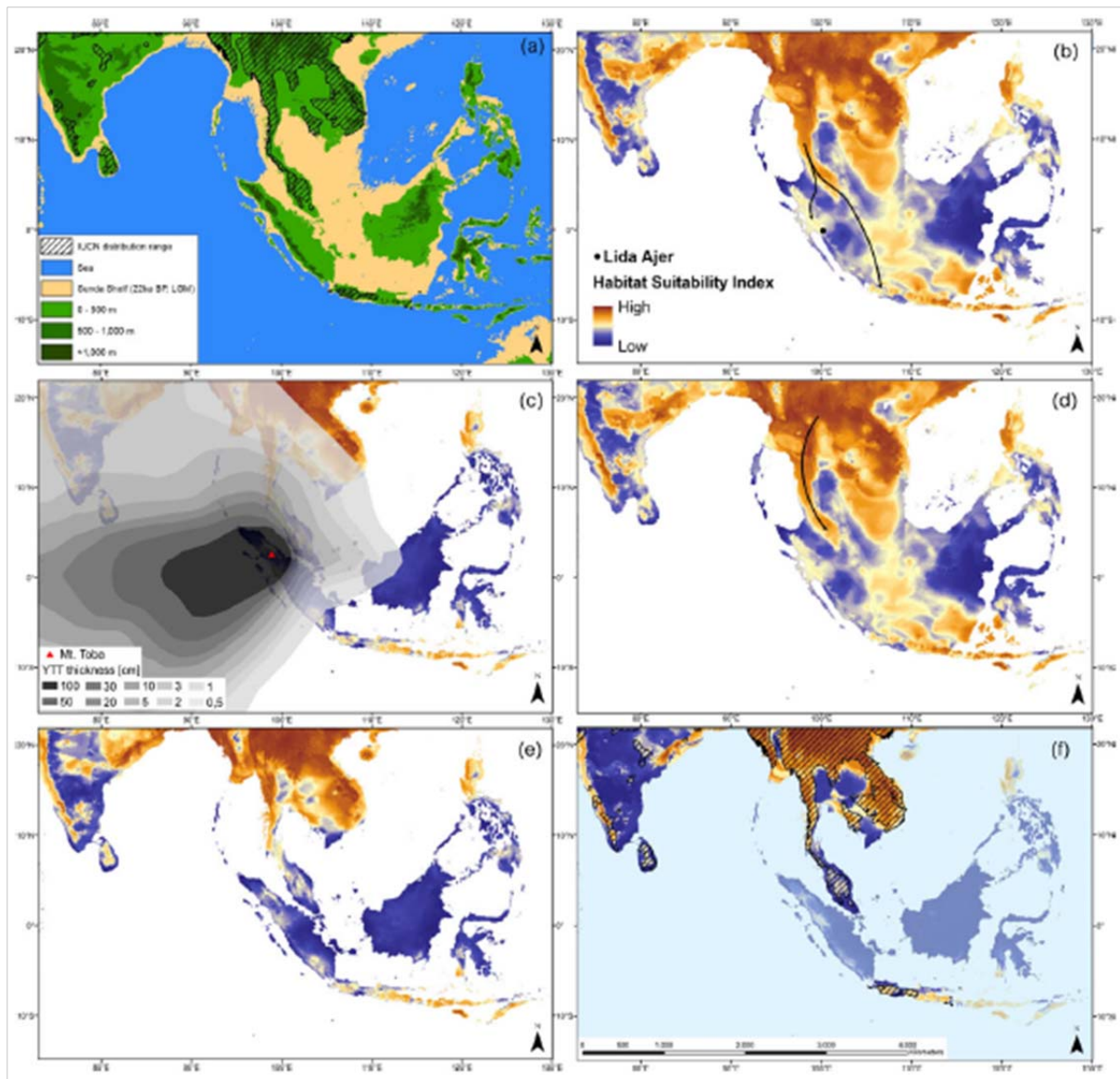


Figure 1 from Wilting *et al.* (2016) *Journal of Zoology* 299: 239-250. (a) Map of the exposed Sunda Shelf during the Last Glacial Maximum (LGM) ~22 kya. Sea levels were 116 m below present day level. The map is based on data from Voris (2000) and on the ETOPO1 digital elevation model (Amante & Eakins, 2009). (b–f) Projected South and Southeast Asian leopard distribution for (b) the Middle Pleistocene (~600 kya) using the LGM projections; (c) Late Pleistocene (~74 kya) using the Mid-Holocene projections. The thickness of the Young Toba Tuffs (YTT) were superimposed on the projection to indicate the severity of impact of the Toba super volcanic eruption. In (b) we added the pre-Toba fossil site Lida Ajer (80 kya or 128–118 kya, see Louys, 2012) in Sumatra, and in (f) we indicated the species distribution according to the IUCN Red List of Threatened Species as used for the modelling with a hatched pattern while areas outside of the natural (pre-human) distribution range were shaded in light blue. Black arrows in (b) and (d) indicate inferred dispersal directions of leopards.

Modules 7 & 8 (dynamic modelling of future distributions)

This is work still in progress. First results (as proof of principle) have already been published on Orang-utans (*Pongo*): Struebig MJ, Fischer M, Gaveau DL, Meijaard E, Wich SA, Gonner C, Sykes R, Wilting A, Kramer-Schadt S (2015) anticipated climate and land-cover changes reveal refuge areas for Borneo's orang-utans. *Global Change Biology* 21: 2891-2904 [Open

access]. However, as this research was not initiated from the SAW project, this publication is not included in the output list.

Economic impact/applications

We are not aware of any immediate economic impact and there is no planned economic application (no patents, and no cooperation with industry). However, nature conservation is costly and our contribution to the removal of numerous taxonomic uncertainties will have indirect economic consequences.

Contributions by cooperation partners

The cooperation with the PIK did not work out as planned, because we could not get access to the climatic record (e.g. stalactites) of the Sunda shelf itself. Thus, we could only work with Holocene data from caves in China and Australia. Nevertheless, this work led to the detection of a seesaw relation in the summer monsoon rainfalls of China and Australia.

The museum *Naturalis* (Leiden, The Netherlands) granted access to its very large SE Asian mammal collection, including access to fossil material. Close collaboration (e.g. with Dr. Niels Raes) led to an improvement in our habitat distribution modeling approach.

Dr. Christine Hertler from the Senckenberg Gesellschaft für Naturforschung in Frankfurt/M. not only shared her extensive knowledge on human dispersal in SE Asia (which for some of our species was very important as they did not reach certain areas of SE Asia by their own dispersal but by translocation by humans) but also organized access to the museum's collection. Through her own network she provided us with the contact address of an Australian climatologist so that we could relay that information to our partners from PIK.

Dr. Frieder Mayer and his colleagues from the 'Museum für Naturkunde' (MfN) Berlin shared the museum's extensive collection on SE Asian mammals with us, as well as taxonomic information and distribution data. For instance, most of the muntjac specimens came from the MfN. Through our own connections to RJ Timmins (the IUCN/SSC Muntjac specialist), we were able to improve the assignment of several specimens from the muntjac collection.

A very close cooperation was established with the University of Potsdam (Prof. Michael Hofreiter, Genomics Lab). Their experience in whole genome sequencing as well as their sharing of ideas, protocol improvements and bioinformatics scripts were a great help in the molecular work modules of the project.

Newly established cooperations

1. Peter J. van Coverden de Groot – Queens University, Ontario, Canada
2. Love Dalén and Johanna von Seth – Swedish Museum of Natural History, Stockholm, Sweden
3. Robert J. Timmins – Muntjac specialist at the IUCN Red List of Threatened Species
4. Douglas W. Yu (Kunming University), visit by Renata Martins to Center for Evolution and Ecology of Kunming, to set up a clean lab and transfer knowledge regarding hybridisation capture techniques.
5. Thomas Marques-Bonet (University Pompeu Fabra/CSIC): he is interested in cooperating with us on SE Asian primates. As our data have not yet been published, we are likewise interested

Theses

Ph.D./ Dr.rer.nat.

1. Deniz Eroglu (2016): Entrapping hidden changes in nature – understanding the past with the power of recurrences. Ph.D. thesis (Dr. rer. nat.), PIK and Humboldt-University Berlin, already defended
2. Riddhi Patel (2017): Generalists & Specialists: Closely Related Felidae Species exhibit Diverse Evolutionary Histories. Ph.D. thesis (Ph.D.), IZW and Dahlem Research School at Freie University Berlin
3. Renata Martins (2017): Deciphering evolutionary histories of Southeast Asian Ungulates: comparative phylogeography in a Biodiversity Hotspot, Ph.D. thesis (Dr. rer. nat.), IZW and University Potsdam
4. Kunde M (2017), still ongoing, working title: Evolutionary history and genetic diversity of sun bears (*Helarctos malayanus*). Ph.D. thesis (Ph.D.), IZW and Griffith University, Australia

Master of Science (MSc)

1. Wiebke Sickel (2014): Phylogeography of the Marbled Cat (*Pardofelis marmorata*, Martin 1837) in South East Asia. IZW and University of Central Lancashire (UCLAN), School of Forensic and Applied Sciences, Preston, Lancashire, UK
2. Julia Canitz (2014): Biogeography of *Arctogalidia trivirgata* (Gray, 1832) at a biodiversity hotspot – A model identifying drivers of evolution. IZW and Georg-August-University Göttingen
3. Laura Werner (2015): Die Phylogeographie der Asiatischen Goldkatze (*Catopuma temminckii*) in Südostasien. [Phylogeography of Asian golden cat (*Catopuma temminckii*) in South East Asia], IZW and Trier University
4. Katja Winter (2017): Development and implementation of a bioinformatics pipeline for the analysis of mitogenome data (was planned for 2016, but due to prolonged sickness not finished yet), IZW and Potsdam University

Scientific term paper [credited], (German: wiss. Hausarbeit)

1. Katja Winter (2015): Gene oder Genome? Spielt die Länge der DNA-Sequenzen eine Rolle für die Genauigkeit phylogeographischer Analysen? [Gene or genome? How important is sequence length for phylogeographic analyses?] IZW and Potsdam University

List of publications generated within this project

Peer-reviewed, already published

1. Salleh FM, Ramos-Madriral J, Peñaloza F, Liu S, Sinding MHS, Patel RP, Martins RR, Lenz D, Fickel J, Roos C, Shamsir MS, Azman MS, Lim BK, Rossiter SJ, Wilting A, Gilbert MTP (2017) An expanded mammal mitogenome dataset from Southeast Asia. [GigaScience Database](#), in press, [OPEN ACCESS](#)
2. Patel RP, Lenz D, Kitchener AC, Fickel J, Förster DW, Wilting A (2017) Threatened but understudied – supporting conservation by understanding the genetic structure of the flat-headed cat. *Conservation Genetics*, early online, doi: 10.1007/s10592-017-0990-2.
3. Patel RP, Wutke S, Lenz D, Mukherjee S, Ramakrishnan U, Veron G, Fickel J, Wilting A, Förster DW (2017) Genetic structure and phylogeography of the Leopard cat (*Prionailurus bengalensis*) inferred from mitochondrial genomes. *JOURNAL OF HEREDITY* **108**(4): 349-360. [OPEN ACCESS](#)

4. Gaubert P, [Patel RP](#), Veron G, Goodman SM, Willsch M, Vasconcelos R, Lourenço A, Sigaud M, Justy F, Joshi BD, [Fickel J](#), [Wilting A](#) (2017) Phylogeography of the small Indian civet and origin of introductions to western Indian Ocean islands. *JOURNAL OF HEREDITY* **108**(3): 270-279. [OPEN ACCESS, DRYAD](#)
5. Martins RF, Fickel J, Le M, van Nguyen T, Nguyen HM, Timmins R, Gan HM, Rovie-Ryan JJ, Lenz D, Förster DW, Wilting A (2017). Phylogeography of red muntjacs reveals three distinct mitochondrial lineages. *BMC EVOLUTIONARY BIOLOGY* **17**: 34. [OPEN ACCESS](#)
6. [Patel RP](#), Förster D, Kitchener A, Rayan M, Mahamed SW, Werner L, [Lenz D](#), Pfestorf H, [Kramer-Schadt S](#), [Radchuk V](#), [Fickel J](#), [Wilting A](#) (2016) Two species of the genus *Catopuma* with diverging histories: An island endemic forest specialist and a wide-spread habitat generalist. *ROYAL SOCIETY OPEN SCIENCE* **3**:160350. [OPEN ACCESS](#)
7. [Wilting A](#), [Patel R](#), Pfestorf H, Kern Ch, Sultan K, Ario A, Peñaloza F, [Kramer-Schadt S](#), [Radchuk V](#), Foerster DW, [Fickel J](#) (2016) Evolutionary history and conservation significance of the Javan leopard *Panthera pardus melas*. *JOURNAL OF ZOOLOGY* **299**: 239–250.
8. Pajmans JLA, [Fickel J](#), Courtiol A, Hofreiter M, Förster DW (2016) Impact of enrichment conditions on cross-species capture of fresh and degraded DNA. *MOLECULAR ECOLOGY RESOURCES* **16**(1): 42-55.
9. [Wilting A](#), Courtiol A, Christiansen P, Niedballa J, Scharf AK, Orlando L, Balkenhol N, Hofer H, [Kramer-Schadt S](#), [Fickel J](#), Kitchener AC (2015) Planning tiger recovery: Understanding intraspecific variation for effective conservation. *SCIENCE ADVANCES* **1**(5): e1400175. [OPEN ACCESS](#)
10. Veron G, Willsch M, Dacosta V, Patou M-L, Seymour A, Bonillo C, Couloux A, Siew Te Wong, Jennings AP, [Fickel J](#), [Wilting A](#) (2014) The distribution of the Malay civet *Viverra zibetha* (Carnivora: Viverridae) across Southeast Asia: natural or human-mediated dispersal? *ZOOLOGICAL JOURNAL OF THE LINNEAN SOCIETY LONDON* **170**: 917-932.
11. [Eroglu D](#), McRobie FH, Ozken I, Stemper T, Wyrwoll KH, Breitenbach SFM, [Marwan N](#), [Kurths J](#) (2016) See-saw relationship of the Holocene East Asian-Australian summer monsoon. *NATURE COMMUNICATIONS* **7**: 12929. [OPEN ACCESS](#)
12. Schultz P, Peron T, [Eroglu D](#), Stemler T, Avila GMR, Rodrigues FA, [Kurths J](#) (2016) Tweaking synchronization by connectivity modifications. *Physical Review E* **93**: 062211
13. Afsar O, [Eroglu D](#), [Marwan N](#), [Kurths J](#) (2015) Scaling behavior for recurrence-based measures at the edge of chaos. *EUROPHYSICS LETTERS* **112**(1): 10005.
14. Ozken I, [Eroglu D](#), Stemler T, [Marwan N](#), Bagci GB, [Kurths J](#) (2015) Transformation-cost time-series method for analyzing irregularly sampled data. *PHYSICAL REVIEW E* **91**: 062911
15. [Eroglu D](#), [Marwan N](#), Prasad S, [Kurths J](#) (2014) Finding recurrence networks' threshold adaptively for a specific time series. *NONLINEAR PROCESSES IN GEOPHYSICS* **21**: 1085-1092. [OPEN ACCESS](#)
16. [Eroglu D](#), Peron TKDM, [Marwan N](#), Rodrigues FA, Costa L da F, Sebek M, Kiss IZ, [Kurths J](#) (2014) Entropy of weighted recurrence plots. *PHYSICAL REVIEW E* **90**: 042919

Submissions, resubmissions:

1. [Martins RF](#), Schmidt A, [Lenz D](#), [Wilting A](#), [Fickel J](#) (2017) Human mediated introduction of introgressed deer across Wallace's line: historical biogeography of *Rusa unicorn* and *R. timorensis*. Submitted to *JOURNAL OF BIOGEOGRAPHY*. *under review*

2. Martins RF, Lenz D, Förster D, von Seth J, Dalén L, Nathan S, Goossens B, van Coeverden de Groot P, Fickel J, Wilting A (2017) The lost genetic legacy of Sumatran and Javan rhinoceros: phylogeography and the genetic diversity of historical rhinoceros populations. *Final draft waiting for approval by all authors, ready to be submitted*
3. Radchuk V, Kramer-Schadt S, Fickel J, Wilting A (2017) Current distribution ranges of mammals in South East Asia are shaped by the legacy of paleoclimate and species life history traits. (rejected from ECOGRAPHY, resubmission to another journal in preparation)

in preparation:

1. Soto DX, Maaß J, Voigt CC, Wilting A, Fickel J (2017) Intra-specific dietary and isotopic specialization in small carnivorous mammals: The benefit of dispersal.
2. Patel RP, Lenz D, Förster D, Wutke S, Wilting A, Fickel J (2017) How did fishing cat arrive on Java? Natural dispersal or hitchhiking with traders and settlers? (Data from mitogenome and nuclear markers [exons and low coverage genomes])
3. Patel RP, Lenz D, Förster D, Wilting A, Fickel J (2017) Phylogeography and evolutionary history of the marbled cat (*Pardofelis marmorata*) from mitogenome and pelage data.
4. Martins RF, Lenz D, Förster DW, Wilting A, Fickel J (2017). Phylogeny, genetic diversity and cryptic lineages among muntjacs of South and Southeast Asia.
5. Kunde M, Lenz D, Fickel J, Förster D (2017). Genetic analysis of sun bears (*Helarctos malayanus*) – relevance for conservation and management.

Book chapter

1. Martins RF, Kampmann M-L, Förster DW (2017) Library preparation from degraded samples for non-Illumina platforms. *Methods in Molecular Biology: aDNA Methods and Protocols*, 2nd Edition, Springer Protocols. *in press*

Presentations/ talks at conferences

1. Patel RP (2016) Young and colourful – Evolutionary history of the Asian golden cat. ConGenomics, May 3rd – 6th, Vairão, Portugal
2. Martins RF (2016) How many red muntjac species are there? Insights from mitogenome data of archival samples. ConGenomics, May 3rd – 6th, Vairão, Portugal [[3rd prize for “Best flash talk”](#)]
3. Radchuk V, Wilting A, Pfestorf H, Fickel J, Kramer-Schadt S (2016) Learn from paleoclimates: semi-permeability of Isthmus of Kra for mammal movements during pleistocene. European Conference of Tropical Ecology and Annual Conference of the Society for Tropical Ecology, Feb 23rd – 26th, Göttingen, Germany
4. Martins RF (2016) Evolution in a Biodiversity Hotspot – insights from archival DNA of red muntjacs. Graduate Meeting Evolutionary Biology & Ecology, May 7th – 9th, Greifswald, Germany
5. Wilting A (2016) Zoological colloquium: Past, present and future of southeast Asian mammals, University Hamburg, June 22nd, Hamburg, Germany
6. Patel RP (2015) Young and colourful – Evolutionary history of the Asian golden cat. 10th International Conference on Behaviour, Physiology and Genetics of Wildlife, Sept 28th – Oct 1st, Berlin, Germany.
7. Patel RP (2015) Young and colourful – Evolutionary history of the Asian golden cat. 2nd International Symposium at the Berlin Center for Genomics in Biodiversity Research, Sept 23rd, 2015, Berlin, Germany

8. Martins RF (2015) Biogeography of Ungulates of Southeast Asia. 2nd International Symposium at the Berlin Center for Genomics in Biodiversity Research, Sept 23rd, 2015, Berlin, Germany
9. Patel RP (2015) Hybrid capture & Next Generation Sequencing in museum samples, August 10th -21st, Workshop at the National Centre for Biological Sciences, Bangalore, India
10. Wilting A (2015) Past, presence and future of southeast Asian mammals, Sumatran Wildlife Symposium, Andalas University, Nov 3rd, Padang, Indonesia
11. Wilting A (2015) Past, presence and future of southeast Asian mammals, June 18th, University Trier, Germany
12. Wilting, A (2015) Past, presence and future of southeast Asian mammals, Dept. of Wildlife and National Parks (PERHILITAN), Oct 26th, Kuala Lumpur, Malaysia
13. Wilting A What is a tiger? TRAFFIC Southeast Asia, Oct 27th, Kuala Lumpur, Malaysia
14. Patel RP (2014) Fishing deep from a shallow pond –Next Generation sequencing approach for degraded DNA material. 1st International Symposium at the Berlin Center for Genomics in Biodiversity Research, Sept 26th, Berlin, Germany

Poster

1. Eroglu D, Ozken I, McRobie F, Stemler T, Marwan N, Wyrwoll K-H, Kurths J (2016) A solar variability driven monsoon see-saw: switching relationships of the Holocene East Asian-Australian summer monsoons. European Geosciences Union General Assembly, April 17th-22nd, Vienna, Austria
2. Pajmans J, Fickel J, Courtiol A, Hofreiter M, Förster DW (2015) Impact of enrichment conditions on cross-species capture of fresh and degraded DNA. Annual Meeting of the Society for Molecular Biology and Evolution, July 12th – 16th, Vienna, Austria.
3. Martins RF, Förster DW, Fickel J, Wilting A. (2015) Mitogenome data from archival samples help solving the species boundaries of Red Muntjacs. 10th International Conference on Behaviour, Physiology and Genetics of Wildlife, Sept 28th – Oct 1st, Berlin, Germany
4. Pajmans J, Fickel J, Courtiol A, Hofreiter M, Förster DW (2015) Impact of enrichment conditions on cross-species capture of fresh and degraded DNA. 10th International Conference on Behaviour, Physiology and Genetics of Wildlife, Sept 28th – Oct 1st, Berlin, Germany.

Immediate impact of publications

Three out of the papers mentioned above (Tigers [*Panthera tigris*], Leopard cat [*Prionailurus bengalensis*], Asian golden cat [*Catopuma temminckii*]) have even led to changes in cat taxonomy as endorsed by the Cat specialist group of IUCN. Reference: CATNEWS SPECIAL ISSUE 11 WINTER 2017

Measures for safe storage and to secure access to data generated within the project

Storage (IZW)

1. All raw data as well as all processed data generated via the 'bioinformatic pipeline' are stored on our own server. All data and results are backed up monthly to magnetic backup tapes. We apply the following system: 'Tandberg LTO-5 HH drive SAS 6GB/s

extern' for storage media 'Ultrium 5 (LTO5) - 1.5 TB/3.0 TB'[LTO: linear tape-open technology]. According to the vendor, the system is designed to store data for up to 30 years. We do not overwrite older data but acquire new tapes. Upon finishing of a project, all data belonging to that project will be moved to backup tapes to free space on the server's hard drives.

2. The storage/backup system is administered by our bioinformatician (Dorina Lenz) and allows reading access for the director, the department head and members of the department (personal login for projects required). Writing access is granted on a 'per project' basis.
3. We also use DRYAD and GenBank (NCBI) to store molecular data.

Access (IZW)

1. In general, we tried to publish in Open Access journals (eight out of the 16 already published papers were published as OA). Unfortunately, press companies charge ridiculously high 'Article Processing Charges' (APCs) that did not allow us to publish every paper as OA publication.
2. We also use self-archiving permissions by press companies. After an embargo period (12 months) papers will be uploaded to repositories such as Researchgate and others. Prior to that papers are also being sent out (with our own layout) to people contacting us via email.

List of selected list news clips and press coverage

1. <https://www.naturetoday.com/intl/en/nature-reports/message/?msg=23230>
2. <https://www.sciencedaily.com/releases/2016/12/161222094845.htm>
3. <https://www.sciencedaily.com/releases/2016/05/160504085111.htm>
4. <https://www.sciencedaily.com/releases/2015/06/150627081055.htm>
5. <http://blog.nus.edu.sg/leopardcat/author/leopardcat/>
6. <https://www.washingtonpost.com/news/speaking-of-science/wp/2015/06/26/to-save-big-cats-from-extinction-scientists-say-we-need-to-redefine-tiger/>
7. <https://phys.org/news/2015-06-tiger-subspecies.html>
8. <http://www.sonnenseite.com/de/wissenschaft/monsunregen-in-china-und-australien-riesige-wippe-entdeckt.html>
9. <http://www.asianscientist.com/2016/05/in-the-lab/javan-leopard-populations-urgent-conservation/>
10. <http://derstandard.at/2000036304090/Woher-die-kleinsten-Leoparden-der-Welt-kamen>

Acknowledgements

On behalf of all applicants and all our cooperation partners I would like to thank all the colleagues and institutions who supported us with granting us access to their SE Asian mammal collections, who shared their ideas with us and helped us to overcome the numerous impediments. Thank you so much.