

## Research



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### Authors for correspondence:

Laurent A. F. Frantz

e-mail: [laurent.frantz@qmul.ac.uk](mailto:laurent.frantz@qmul.ac.uk)

Greger Larson

e-mail: [greger.larson@arch.ox.ac.uk](mailto:greger.larson@arch.ox.ac.uk)

<sup>†</sup>Contributed equally to this study.

<sup>¶</sup>Present address: Pertamina University, Jl.

Teuku Nyak Arief, Kawasan Simprug,  
Kebayoran Lama, Jakarta Selatan 12220,  
Indonesia.

<sup>||</sup>Deceased.

<sup>‡</sup>Co-supervised the study.

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# Synchronous diversification of Sulawesi's iconic artiodactyls driven by recent geological events

Laurent A. F. Frantz<sup>1,2,†</sup>, Anna Rudzinski<sup>3,†</sup>,  
Abang Mansyursyah Surya Nugraha<sup>4,¶,†</sup>, Allowen Evin<sup>5,6,†</sup>, James Burton<sup>7,8,†</sup>,  
Ardern Hulme-Beaman<sup>2,6</sup>, Anna Linderholm<sup>2,9</sup>, Ross Barnett<sup>2,10</sup>,  
Rodrigo Vega<sup>11</sup>, Evan K. Irving-Pease<sup>2</sup>, James Haile<sup>2,10</sup>, Richard Allen<sup>2</sup>,  
Kristin Leus<sup>12,13</sup>, Jill Shephard<sup>14,15</sup>, Mia Hillyer<sup>14,16</sup>, Sarah Gillemot<sup>14</sup>,  
Jeroen van den Hurk<sup>14</sup>, Sharron Ogle<sup>17</sup>, Cristina Atofanei<sup>11</sup>, Mark G. Thomas<sup>3</sup>,  
Friederike Johansson<sup>18</sup>, Abdul Haris Mustari<sup>19</sup>, John Williams<sup>20</sup>,  
Kusdiantoro Mohamad<sup>21</sup>, Chandramaya Siska Damayanti<sup>21</sup>,  
Ita Djuwita Wiryadi<sup>||</sup>, Dagmar Obbles<sup>22</sup>, Stephano Mona<sup>23,24</sup>, Hally Day<sup>25</sup>,  
Muhammad Yasin<sup>25</sup>, Stefan Meker<sup>26</sup>, Jimmy A. McGuire<sup>27</sup>, Ben J. Evans<sup>28</sup>,  
Thomas von Rintelen<sup>29</sup>, Simon Y. W. Ho<sup>30</sup>, Jeremy B. Searle<sup>31</sup>,  
Andrew C. Kitchener<sup>32,33</sup>, Alastair A. Macdonald<sup>7,‡</sup>, Darren J. Shaw<sup>7,‡</sup>,  
Robert Hall<sup>4,‡</sup>, Peter Galbusera<sup>14,‡</sup> and Greger Larson<sup>2,‡</sup>

<sup>1</sup>School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK

<sup>2</sup>The Palaeogenomics & Bio-Archaeology Research Network, Research Laboratory for Archaeology and History of Art, University of Oxford, Oxford OX1 3QY, UK

<sup>3</sup>Research Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK

<sup>4</sup>SE Asia Research Group, Department of Earth Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK

<sup>5</sup>Institut des Sciences de l'Evolution, Université de Montpellier, CNRS, IRD, EPHE, Place Eugène Bataillon, 34095 Montpellier, Cedex 05, France

<sup>6</sup>Department of Archaeology, Classics and Egyptology, University of Liverpool, 12-14 Abercromby Square, Liverpool L69 7WZ, UK

<sup>7</sup>Royal (Dick) School of Veterinary Studies & The Roslin Institute, University of Edinburgh, Easter Bush Campus, Roslin, Edinburgh EH25 9RG, UK

<sup>8</sup>IUCN SSC Asian Wild Cattle Specialist Group and Chester Zoo, Cedar House, Caughall Road, Upton by Chester, Chester CH2 1LH, UK

<sup>9</sup>Department of Anthropology, Texas A&M University, College Station, TX 77843-4352, USA

<sup>10</sup>Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, 1350 Copenhagen K, Denmark

<sup>11</sup>Ecology Research Group, Section of Life Sciences, School of Human and Life Sciences, Canterbury Christ Church University, North Holmes Road, Canterbury CT1 1QU, Kent, UK

<sup>12</sup>Copenhagen Zoo, IUCN SSC Conservation Breeding Specialist Group—Europe, Roskildevej 38, Postboks 7, 2000 Frederiksberg, Denmark

<sup>13</sup>European Association of Zoos and Aquaria, PO Box 20164, 1000 HD Amsterdam, The Netherlands

<sup>14</sup>Centre for Research and Conservation (CRC), Royal Zoological Society of Antwerp, Koningin Astridplein 20-26, 2018 Antwerp, Belgium

<sup>15</sup>Environment and Conservation Sciences, School of Veterinary and Life Sciences, Murdoch University, Perth, Western Australia 6150, Australia

<sup>16</sup>Molecular Systematics Unit/Terrestrial Zoology, Western Australian Museum, Welshpool, Western Australia, Australia

<sup>17</sup>Edinburgh Medical School: BMTO, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK

<sup>18</sup>Gothenburg Natural History Museum, Box 7283, 402 35 Gothenburg, Sweden

<sup>19</sup>Department of Forest Resources Conservation and Ecotourism, Faculty of Forestry, Bogor Agricultural University, PO Box 168, Bogor 16001, Indonesia

<sup>20</sup>Davies Research Centre, School of Animal and Veterinary Sciences, Faculty of Sciences, University of Adelaide, Roseworthy, Southern Australia 5371, Australia

<sup>21</sup>Faculty of Veterinary Medicine, Bogor Agricultural University, Jalan Agatis, IPB Campus, Darmaga, Bogor 16680, Indonesia

<sup>22</sup>Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Ch. Deberiotstraat 32, 3000 Leuven, Belgium

<sup>23</sup>Institut de Systématique, Évolution, Biodiversité, ISYEB - UMR 7205 - CNRS, MNHN, UPMC, EPHE, Ecole Pratique des Hautes Etudes, 16 rue Buffon, CP39, 75005 Paris, France

<sup>24</sup>EPHE, PSL Research University, Paris, France

<sup>25</sup>No affiliation

<sup>26</sup>Department of Zoology, State Museum of Natural History Stuttgart, Rosenstein 1, 70191 Stuttgart, Germany

<sup>27</sup>Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720, USA

<sup>28</sup>Department of Biology, McMaster University, Hamilton, Ontario, Canada

<sup>29</sup>Museum für Naturkunde - Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

<sup>30</sup>School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales 2006, Australia

<sup>31</sup>Department of Ecology and Evolutionary Biology, Cornell University, Corson Hall, Ithaca, NY 14853, USA

<sup>32</sup>Department of Natural Sciences, Chambers Street, National Museums Scotland, Edinburgh EH1 1JF, UK

<sup>33</sup>Institute of Geography, School of Geosciences, University of Edinburgh, Drummond Street, Edinburgh EH8 9XP, UK

 LAFF, 0000-0001-8030-3885; AE, 0000-0003-4515-1649; AH-B, 0000-0001-8130-9648; DJS, 0000-0003-2016-1541; GL, 0000-0002-4092-0392

The high degree of endemism on Sulawesi has previously been suggested to have vicariant origins, dating back to 40 Ma. Recent studies, however, suggest that much of Sulawesi's fauna assembled over the last 15 Myr. Here, we test the hypothesis that more recent uplift of previously submerged portions of land on Sulawesi promoted diversification and that much of its faunal assemblage is much younger than the island itself. To do so, we combined palaeogeographical reconstructions with genetic and morphometric datasets derived from Sulawesi's three largest mammals: the babirusa, anoa and Sulawesi warty pig. Our results indicate that although these species most likely colonized the area that is now Sulawesi at different times (14 Ma to 2–3 Ma), they experienced an almost synchronous expansion from the central part of the island. Geological reconstructions indicate that this area was above sea level for most of the last 4 Myr, unlike most parts of the island. We conclude that emergence of land on Sulawesi (approx. 1–2 Myr) may have allowed species to expand synchronously. Altogether, our results indicate that the establishment of the highly endemic faunal assemblage on Sulawesi was driven by geological events over the last few million years.

## 1. Introduction

Alfred Russel Wallace was the first to document the 'anomalous' biogeographic region in Island Southeast Asia (ISEA) now known as Wallacea [1,2]. This biodiversity hotspot [3] is bounded by Wallace's Line in the west and Lydekker's Line in the east [4]. It consists of numerous islands in the Indonesian Archipelago, all of which boast a high degree of endemism. For example, on Sulawesi, the largest island in the region, at least 61 of the 63 non-volant mammalian species are endemic [5] and this figure is likely to be an underestimate.

The geological origins of Wallacea are as complex as its biogeography. Until recently, Sulawesi had been regarded as the product of multiple collisions of continental fragments from the Late Cretaceous [6–9]. This assumption has been challenged and a recent reinterpretation suggests instead that the island began to form as the result of continental collisions during the Cretaceous, which were then followed by Eocene rifting of the Makassar Strait. This process led to the isolation of small land areas in western Sulawesi from Sundaland. In the Early Miocene (approx. 23 Ma), a collision between the Sula Spur (a promontory of the Australian continent) and north Sulawesi led to uplift and emergence of land [10–12]. Later tectonic movements led to the present-day configuration of islands between Borneo and Australia [13,14].

A previous interpretation, involving the assembly of multiple terranes by collision, was used to suggest that Sulawesi's peculiar species richness resulted from vicariance and amalgamation over long geological time periods [10,15,16]. However, recent molecular-clock analyses suggest that a dispersal, starting in the Middle Miocene (approx. 15 Ma) from both Sunda and Sahul, is a more plausible explanation [17–19]. These conclusions suggest a limited potential for animal dispersal to Sulawesi prior to approximately 15 Ma. Rapid tectonic changes, coupled with the dramatic sea-level fluctuations over the past 5 Myr [20] might also have affected land availability and influenced patterns of species dispersal to Sulawesi, intra-island species expansion and speciation.

The hypothesis of a recent increase in land area [19] can be tested by comparing the population histories of multiple species on the island. Analyses of genetic and morphometric variability can be used to infer the timing and trajectories of dispersal, and the geographical and temporal origins of expansion. For example, if land area had increased from a single smaller island, extant species now living on Sulawesi would all have expanded from the same area. In addition, under this assumption, within the same geographical region their respective diversifications would be expected to have been roughly simultaneous.

Here, we focus on three large mammals endemic to Sulawesi: the babirusa (*Babyrousa* spp.), the Sulawesi warty pig (SWP, *Sus celebensis*) and the anoa, a dwarf buffalo (*Bubalus* spp.). The babirusa is a suid characterized by wrinkled skin and two extraordinary curved upper canine tusks displayed by males [21–23]. It represents a 'ghost lineage', because there are no closely related extant species outside Sulawesi (e.g. African suids are more closely related to all other Asian suids than to the babirusa) and the babirusa is unknown in the fossil record outside Sulawesi [24]. Three extant species of babirusa (distributed primarily in the interior of Sulawesi and on surrounding islands [21–23]) have been described: *Babyrousa babyrussa* (Buru and Sulu Islands), *Babyrousa celebensis* (mainland Sulawesi) and *Babyrousa togeanensis* (Togian Island) [25].

The anoa is an endemic 'miniature buffalo' related to indigenous bovines in the Philippines and East Asia [26,27]. It stands approximately 1 m tall, weighs 150–200 kg and mostly inhabits pristine rainforest [28]. Although the subgenus *Anoa* has been divided into two species, the lowland anoa (*Bubalus depressicornis*) and the highland anoa (*Bubalus quarlesi*) [29], this classification is still contentious [27]. In contrast with anoa and babirusa, the SWP occupies a wide range of habitats, from swamps to rainforests. This species is closely related to the Eurasian wild pig (*Sus scrofa*), from which it

diverged during the Early Pleistocene (approx. 2 Ma) [24,30]. The SWP has been found on numerous islands throughout ISEA, probably as the result of human-mediated dispersal [31]. As its name implies, male SWPs develop facial warts. These cultural icons (e.g. SWP/babirusa and anoa are represented in the oldest prehistoric cave paintings [32,33]) have undergone recent and significant population reduction and range contraction due to overhunting and conversion of natural habitat for agricultural use.

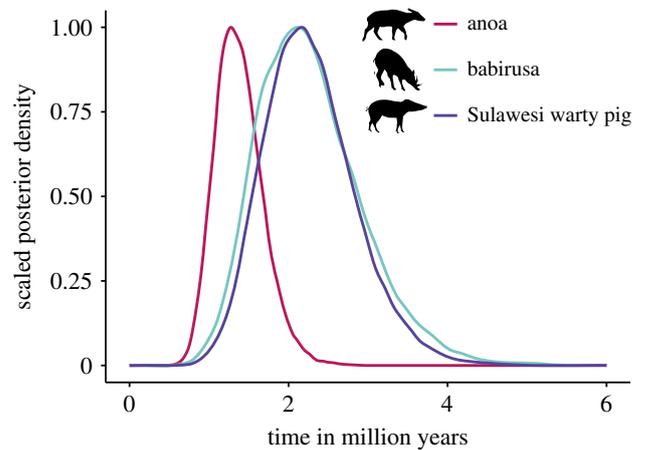
Here, we establish when Sulawesi gained its modern shape and size, including connectivity between its constituent peninsulae, and assessed the impact of island formation on the evolution of Sulawesi's biodiversity. To do so, we used new reconstructions of the island's palaeogeography that allowed us to interpret the distribution of land and sea over the last 8 Myr at 1 Myr intervals. To determine the timings of diversification of the three largest endemic mammals on the island, we generated and analysed genetic and/or morphometric data from a total of 1289 samples of the SWP, anoa and babirusa obtained from museums, zoos and wild populations (456, 520 and 313 samples, respectively; electronic supplementary material, table S1). More specifically, we measured a total of 356 teeth from 227 specimens (357 babirusa and 191 SWP) using a geometric morphometric approach. In addition, we sequenced mitochondrial loci (cytb and/or control region) from 142 anoas, 213 babirusa and 230 SWP. Lastly, we typed 13 microsatellite loci from 163 anoa, 14 loci from 238 SWP and 13 from 182 babirusa (see the electronic supplementary material for more information). Although these taxa have been divided into multiple species (see taxonomic notes in the electronic supplementary material), for the purpose of this study, we treated SWP, anoa and babirusa as single taxonomic units.

## 2. Results and discussion

### (a) Contemporaneous divergence

We generated mitochondrial DNA (mtDNA) sequences and/or microsatellite data from 230 SWPs, 155 anoas and 213 babirusa sampled across Sulawesi and the neighbouring islands (electronic supplementary material, figure S1 and table S1). Using a molecular-clock analysis, we inferred the time to the most recent common ancestor (TMRCA) of each species. The estimates from this method represent coalescence times, which provide a reflection of the crown age of each taxon. The closer relationship between babirusa and SWP (approx. 13 Ma) [34], compared with the divergence of either species from the anoa (approx. 58 Ma) [35] allowed us to align sequences from babirusa and SWP alongside one another, and jointly infer their relative TMRCA. Separate analyses were performed for the anoa. The inferred TMRCA of SWP was 2.19 Myr (95% credibility interval (CI) 1.19–3.41 Myr; electronic supplementary material, figure S2) and of babirusa was 2.49 Myr (95% CI 1.33–3.61 Myr) (figure 1; electronic supplementary material, figure S2). The inferred TMRCA of anoa was younger (1.06 Myr; figure 1; electronic supplementary material, figure S3), though its 95% CI (0.81–1.96 Myr) overlapped substantially with the TMRCA of the other two species.

The relatively recent divergence between babirusa and SWP also allowed us to compare their TMRCA using identical microsatellite loci. To do so, we computed the average square distance (ASD) [36,37] between every pair of individuals



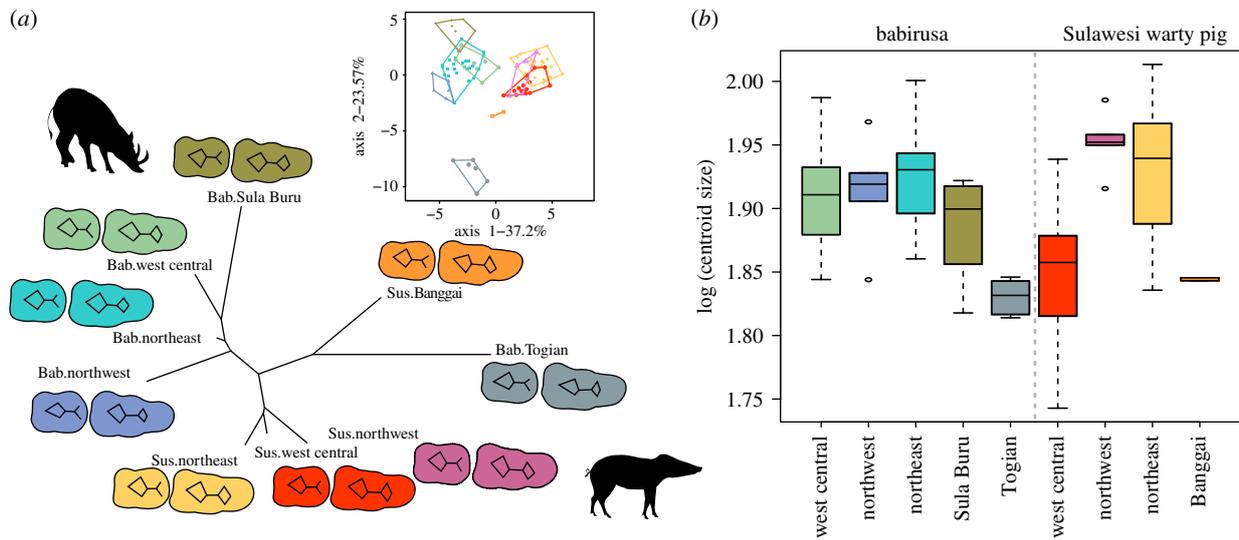
**Figure 1.** Time to the most recent common ancestor (TMRCA) for three mammal species on Sulawesi. Posterior densities of the TMRCA estimates for anoa, babirusa and Sulawesi warty pig.

within each species at the same 13 microsatellite loci. Although such an analysis might be affected by population structure (see below), we found that the distributions of ASD values were not significantly different between these two species (Wilcoxon signed-rank test,  $p = 0.492$ ). This is consistent with the mitochondrial evidence for the nearly identical TMRCA in the two species.

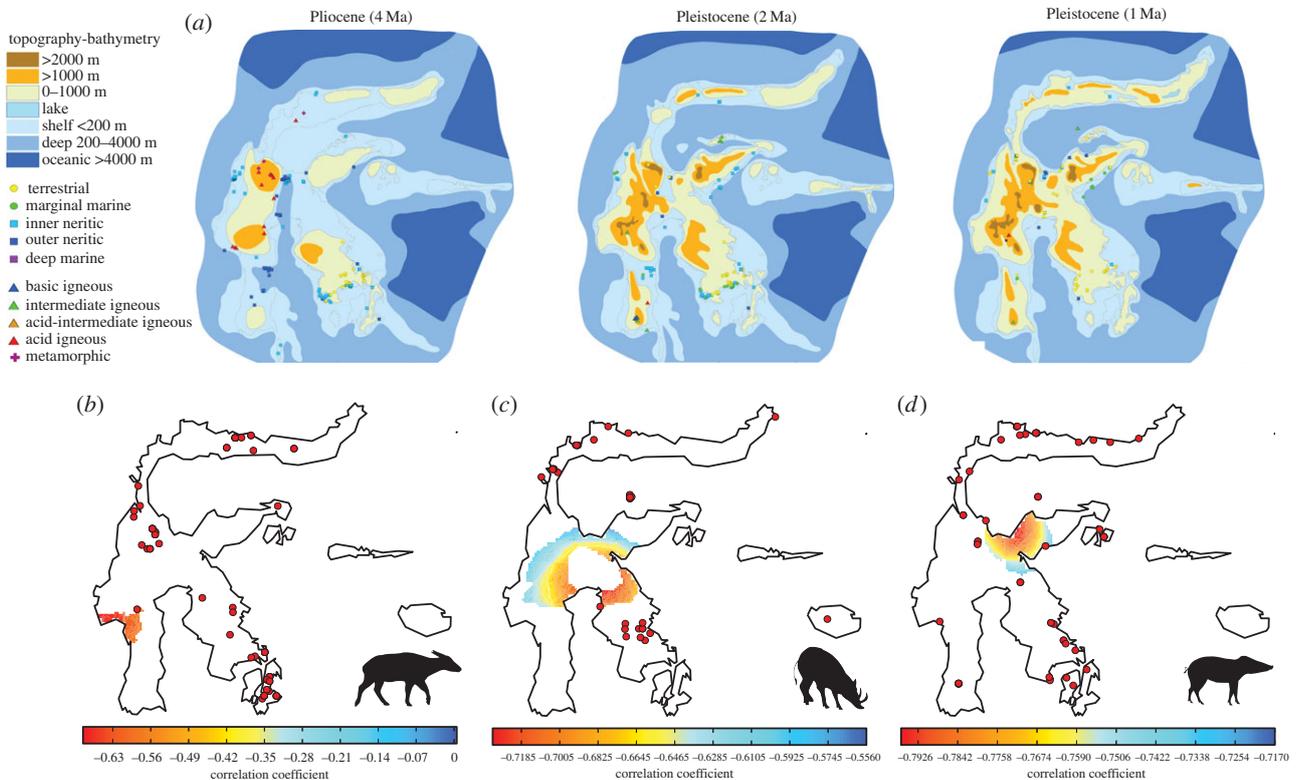
Recent molecular analyses have indicated that babirusa may have colonized Wallacea as early as 13 Ma, whereas SWP and anoas appear to have only colonized Sulawesi within the last 2–4 Myr [17,30,32,34]. An early dispersal of babirusa to Sulawesi (Late Palaeogene) has also been suggested on the basis of palaeontological evidence [19]. In addition, our data corroborate previous studies in indicating that both SWP and babirusa are monophyletic with respect to their most closely related taxa on neighbouring islands (e.g. Borneo), which is consistent with only one colonization of Sulawesi (electronic supplementary material, figure S4–S6) [30].

We then examined whether patterns of morphological diversity in these taxa are consistent with the molecular date estimates. To do so, we obtained measurements of 356 second and third lower molars (M2 and M3) from 95 babirusa and 132 SWPs. SWP and babirusa do not overlap morphologically (figure 2a), and we were thus able to assign each specimen to its correct species with success rates of 94.3% (CI: 92.7%–95.5%, distribution of leave-one-out cross validation of a discriminant analysis based on a balanced sample design) [38] and 94.7% (CI: 93.8%–96.7%) based on their M2 and M3, respectively. Our results also indicate that babirusa did not accumulate more tooth shape variation within Sulawesi (Fligner–Killeen test  $\chi^2 = 1.04$ ,  $p = 0.3$  for M2,  $\chi^2 = 3.45$ ,  $p = 0.06$  for M3). The data instead suggest that SWP has greater variance in the size of its M3 ( $\chi^2 = 4.52$ ,  $p = 0.03$ , but not in the size of the M2,  $\chi^2 = 3.44$ ,  $p = 0.06$ ), and that the population from west central Sulawesi has an overall smaller tooth size than the two populations from northwest and northeast Sulawesi (figure 2b; electronic supplementary material, table S2). While these results may result from different selective constraints, they indicate that babirusa did not accumulate greater morphological variation in tooth shape than did the SWP, despite arriving on Sulawesi up to 10 Myr earlier.

Altogether our analyses suggest that although the three species are believed to have colonized the island at different



**Figure 2.** Population morphological variation inferred from geometric morphometric data. (a) Neighbour-joining network based on Mahalanobis distances measured from second and third lower molar shapes and visualization of population mean shape. Bab, babirusa; Sus, Sulawesi warty pig. (b) Variation of third molar size per population (log centroid size).



**Figure 3.** Geological maps of Sulawesi and the geographical origin of expansion. (a) Reconstruction of Sulawesi over the last 5 Myr (adapted from [39]) and potential origin of expansion of (b) anoa, (c) babirusa and (d) SWP. Red dots represent the location of the samples used for this analysis. Low correlation values (between distance and extrapolated genetic diversity; see electronic supplementary material) represent most likely origin of expansion.

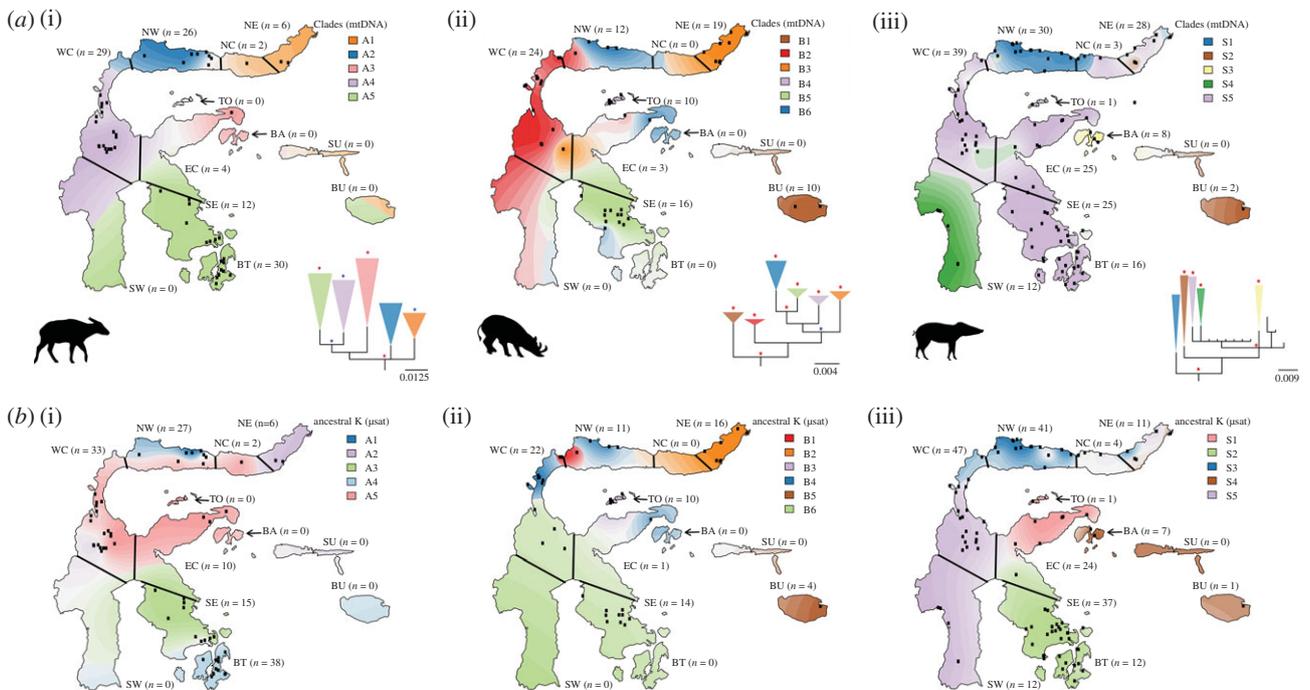
times, their similar degrees of morphological diversity and their nearly synchronous TMRCAs raise the possibility that they (and possibly other species) responded to a common mechanism that triggered their contemporaneous diversification.

### (b) Past land availability correlates with the expansion origins

Increasing land area may have promoted a simultaneous diversification and range expansion in babirusa, SWPs and anoas. To test this hypothesis, we used a new reconstruction that depicts

land area in the Sulawesi region through time using information from the geological record. The reconstructions in 1 Myr increments (figure 3a; electronic supplementary material, figure S7) [39] support a scenario in which most of Sulawesi was submerged until the Late Pliocene to Early Pleistocene (2–3 Ma). Large-scale uplifts over the last 2–3 Myr would have rapidly and significantly increased land area, making it possible for non-volant species to expand their ranges.

To further assess whether these Plio-Pleistocene uplifts were responsible for a synchronous expansion, we inferred the most likely geographical origin of expansion using microsatellite



**Figure 4.** Population structure and geographical patterning of three mammal species on Sulawesi inferred from mitochondrial and microsatellite DNA. (a) A tessellated projection of sample haplogroups in each region of endemism and phylogeny of (i) anoa, (ii) babirusa and (iii) Sulawesi warty pig. Each region is labelled with the number of samples used for the projection. The projection extends over regions with no samples (e.g. the southwest peninsula for babirusa and anoa) and the population membership affinities for these regions are, therefore, unreliable. Red and blue stars on the phylogenetic trees correspond to posterior probabilities greater than 0.9 and 0.7, respectively. (b) Tessellated projection of the STRUcTURE analysis, using the microsatellite data, for (i) anoa, (ii) babirusa and (iii) Sulawesi warty pig. The best  $K$ -value for each species was used ( $K = 5$  for anoa;  $K = 6$  for babirusa;  $K = 5$  for Sulawesi warty pig; electronic supplementary material, figure S8). NE, northeast; NC, north central; NW, northwest; TO, Togian; BA, Banggai Archipelago; EC, east central; WC, west central; SU, Sula; BU, Buru; SE, southeast; SW, southwest; BT, Buton.

data under a model of spatial loss of diversity with distance from expansion origin (electronic supplementary material). These estimates were obtained independently of, and uninformed by, either the geological reconstructions or modern phylogeographical boundaries inferred from other species. We deduced that the most likely origin for both SWP and babirusa was in the east central region of Sulawesi (figure 3*c,d*), and the most likely origin of anoa was in the west central region (figure 3*b*).

The origins of the population expansions of both SWP and babirusa occurred in an area of Sulawesi that only emerged during the Late Pliocene to Early Pleistocene (figure 3*a*; electronic supplementary material, figure S7). On the other hand, the anoa's most likely origin of diversification lies in a region that was submerged until the Pleistocene, consistent with palaeontological evidence [32] and with the slightly more recent TMRCA inferred for this species (figure 1). Thus, for all three species, the inferred geographical origins of their range expansions match the land availability derived from our geological reconstruction of Sulawesi.

### (c) Geological history of past land isolation correlates with zones of endemism

Previous studies have identified endemic zones that are common to macaques, toads [18,40], tarsiers [41–44] and lizards [45]. We tested whether the same areas of endemism are linked to the population structure in our three species by generating a phylogenetic tree for each species using mtDNA and defined five to six haplogroups per species based on well-supported clades (figure 4*a–c*; electronic supplementary

material, figure S4–6). We found that haplogroup proportions were significantly different between previously defined areas of endemism in all three species (Pearson's  $\chi^2$ -test;  $p < 0.001$ ), suggesting population substructure.

We also used STRUcTURE [46] to infer population structure from microsatellite data. The optimum numbers of populations ( $K$ ) were 5, 6 and 5 for anoa, babirusa and SWP, respectively (electronic supplementary material, figure S8; figure 4*b*). Plotting the proportion of membership of each sample onto a map revealed a strong correspondence with the previously described zones of endemism (figure 4*b*). Using an analysis of molecular variance (AMOVA), we found that these areas of endemism explained approximately 17%, 27% and 5% of the variance in allele frequencies in anoa, babirusa and SWP, respectively (electronic supplementary material, table S5). Populations of babirusa and SWP in these zones of endemism were also strongly morphologically differentiated (figure 2).

Altogether, these data and analyses indicate that, despite some differences, the zones of endemism identified in tarsiers, macaques, toads and lizards [18,40–45,47] are largely consistent with the population structure and morphological differentiation in the three species studied here. This is particularly striking for the north arm of Sulawesi (NW, NC and NE in figure 4), where we identify two highly differentiated populations (reflected in both mtDNA and nuclear datasets) in all three taxa. This pattern could result from either adaptation to local environments or from isolation due to the particular geological history associated with the northern arm. Geological reconstructions (figure 3*a*) indicate that although land was present in this region during the past

4 Myr, it was often isolated from the rest of Sulawesi until the mid-Pleistocene. Thus, the combined geological and biological evidence presented here indicate that the high degree of divergence observed in the northern-arm populations in a multitude of species (e.g. three ungulates, macaques and tarsiers) might have been shaped by isolation from the rest of the island until the last 1 million years (figure 3a).

#### (d) Recent and contemporary land isolation also affected morphological evolution including dwarfism

Similar isolation is likely to have influenced the populations inhabiting the smaller islands adjacent to Sulawesi, including the Banggai Archipelago, Buru, Togian and Sula Islands. Interestingly, our geometric morphometric analyses demonstrated that these island populations of SWP and babirusa are the most morphologically divergent (figure 2a). For example, the insular populations from the Togian Islands (babirusa) and the Banggai Archipelago (SWP) were found to have much smaller tooth sizes than their counterparts on the mainland (figure 2b).

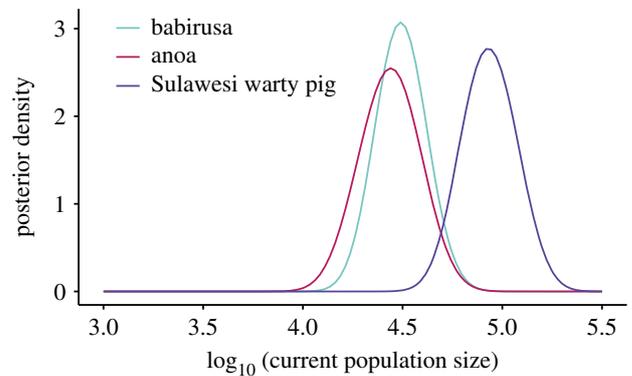
The significant morphometric divergences between populations on various islands are consistent with the genetic differentiation between babirusa/SWP on Togian, Sula and Buru (figure 4; electronic supplementary material, figure S9 and figure S10) and between island populations of SWP on Banggai Archipelago, Buton and Buru (figure 4; electronic supplementary material, figure S9 and figure S10).

Together, these results show that while suture zones between tectonic fragments are consistent with genetic and morphometric differentiation within Sulawesi, isolation on remote islands is likely to have had a much greater effect on morphological distinctiveness. Rapid evolution on islands has been described in many species (e.g. [48]), including in pigs [49] where island populations are known to have smaller tooth sizes than their mainland counterparts [50,51].

#### (e) Demographic history

Isolation of subpopulations across Sulawesi might also be linked to recent anthropogenic disturbances, especially for anoa and babirusa that occupy pristine forest or swamps [21,28]. In order to assess the impact of recent anthropogenic changes on the three species, we inferred their demographic history using approximate Bayesian computation (ABC). We fitted various demographic models to the genetic data (combining both mtDNA and microsatellite data; electronic supplementary material; figure S11). The best-supported demographic model involved a long-term expansion followed by a recent bottleneck in all three species (electronic supplementary material, table S3), corroborating the results of recent analyses of the SWP genome [30].

While our ABC analysis had insufficient power to retrieve the time of expansion (electronic supplementary material, table S4), it provided relatively narrow estimates of the current effective population sizes (figure 5; electronic supplementary material, table S4). We inferred a larger effective population size in SWP (83 021; 95% CI 46 287–161 457) than in babirusa (30 895; 95% CI 17 522–54 954) or anoa (27 504; 95% CI 13 680–54 056). Sulawesi warty pig occupies a wide range of habitats, including agricultural areas [52]. Thus, this species is likely to be less affected by continuing deforestation than babirusa or



**Figure 5.** Posterior distribution of the current population size ( $N_e$ ) of each species as inferred via ABC.

anoa, which is typically restricted to less disturbed forest and swamps [21,26]. Phylogenetic analyses of microsatellite data indicate more geographical structuring in babirusa and anoa than in SWP (electronic supplementary material, figure S12 and table S5). Altogether, these results are consistent with species-specific responses to habitat loss.

### 3. Conclusion

Our results indicate that, while the different geological components of Sulawesi were assembled at about 23 Ma, the island only acquired its distinctive modern form in the last few million years. By 3 Ma there was a large single island at its modern centre, but the complete connection between the arms was established more recently. The increasing land area associated with Plio-Pleistocene tectonic activity is likely to have provided the opportunity for a synchronous expansion in the three endemic mammal species in this study, as well as numerous other species. Interestingly, both our Pleistocene geological reconstruction and our proposed origins of expansion in the centre of the island closely resemble maps inferred from a study of tarsier species distribution on Sulawesi [42].

Furthermore, the recent emergence of connections between Sulawesi's arms coincides with a faunal turnover on the island and the extinction of multiple species. The geological reconstruction, and in particular, the recent elimination of the marine barrier at the Tempe depression separating the southwest and central regions, fits well with suggested replacement in tarsier species that occurred in the last approximately 1 million years [41]. The dispersal of our three species from the central region of Sulawesi may therefore have played a role in other local extinctions, such as the extinct suid known from southwest Sulawesi, *Celebochoerus*.

Sulawesi's development by emergence and coalescence of islands had a significant impact on the population structure and intraspecific morphological differentiation of Sulawesi's three largest mammals and many other endemic taxa. Thus, while most of Sulawesi's extant fauna arrived relatively recently, the more ancient geological history of the island (collision of multiple fragments) might have also affected patterns of endemism. Many aspects of Sulawesi's interconnected natural and geological histories remain unresolved. Integrative approaches that combine biological and geological datasets are therefore essential for reconstructing a comprehensive evolutionary history of Wallace's most anomalous island.

**Data accessibility.** All datasets, including microsatellites, mitochondrial, morphometric and meta data, are available on Dryad (<http://>

dx.doi.org/10.5061/dryad.dv322) [53]. The mitochondrial data are also available on GeneBank (accession MH021990–MH022712).

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## References

- Wallace AR. 1863 On the physical geography of the malay archipelago. *J. R. Geogr. Soc. Lond.* **33**, 217–234. (doi:10.2307/1798448)
- Wallace AR. 2012 *Island life*. Cambridge, UK: Cambridge University Press.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. 2000 Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858. (doi:10.1038/35002501)
- Lohman DJ, de Bruyn M, Page T, von Rintelen K, Hall R, Ng PKL, Shih H-T, Carvalho GR, von Rintelen T. 2011 Biogeography of the Indo-Australian Archipelago. *Annu. Rev. Ecol. Evol. Syst.* **42**, 205–226. (doi:10.1146/annurev-ecolsys-102710-145001)
- Musser GG. 1987 The mammals of Sulawesi. In *Biogeographical evolution of the Malay Archipelago* (ed. TC Whitmore), pp. 73–93. Oxford, UK: Oxford University Press.
- Smith RB, Silver EA. 1991 Geology of a Miocene collision complex, Buton, eastern Indonesia. *Geol. Soc. Am. Bull.* **103**, 660–678. (doi:10.1130/0016-7606(1991)103<0660:GOAMCC>2.3.CO;2)
- Hall R. 1996 Reconstructing Cenozoic SE Asia. In *Tectonic evolution of SE Asia* (eds R Hall, DJ Blundell), pp. 153–184. London, UK: Geological Society of London.
- Hamilton W. 1979 *Tectonics of the Indonesian region*. Geological Survey Professional Paper 1078. Washington, DC: US Government Printing Office.
- Davidson JW. 1991 The geology and prospectivity of Buton Island, Southeast Sulawesi, Indonesia. In *Proceedings, 20th annual convention, Jakarta*, vol. 1, pp. 209–234. Jakarta, Indonesia: Indonesian Petroleum Association.
- Moss SJ, Wilson MEJ. 1998 Biogeographic implications from the Tertiary palaeogeographic evolution of Sulawesi and Borneo. In *Biogeography and geological evolution of SE Asia* (eds R Hall, JD Holloway), pp. 133–163. Leiden, The Netherlands: Backhuys Publishers.
- Hall R. 1998 The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In *Biogeography and geological evolution of SE Asia* (eds R Hall, JD Holloway), pp. 99–131. Leiden, The Netherlands: Backhuys Publishers.
- Hall R. 2013 The palaeogeography of Sundaland and Wallacea since the Late Jurassic. *J. Limnol.* **72**, 1–17. (doi:10.4081/jlimnol.2013.s2.e1)
- Spakman W, Hall R. 2010 Surface deformation and slab–mantle interaction during Banda arc subduction rollback. *Nat. Geosci.* **3**, 562–566. (doi:10.1038/ngeo917)
- Hall R. 2011 Australia-SE Asia collision: plate tectonics and crustal flow. In *The SE Asian gateway: history and tectonics of Australia-Asia collision* (eds R Hall, MA Cottam, MEJ Wilson), pp. 75–109. London, UK: Geological Society of London.
- Michaux B. 1996 The origin of southwest Sulawesi and other Indonesian terranes: a biological view. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **122**, 167–183. (doi:10.1016/0031-0182(95)00100-X)
- de Boer AJ, Duffels JP. 1996 Historical biogeography of the cicadas of Wallacea, New Guinea and the West Pacific: a geotectonic explanation. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **124**, 153–177. (doi:10.1016/0031-0182(96)00007-7)
- Stelbrink B, Albrecht C, Hall R, von Rintelen T. 2012 the biogeography of Sulawesi revisited: is there evidence for a vicariant origin of taxa on wallace's 'Anomalous Island'? *Evolution* **66**, 2252–2271. (doi:10.1111/j.1558-5646.2012.01588.x)
- Evans BJ, Supriatna J, Andayani N, Setiadi MI, Cannatella DC, Melnick DJ. 2003 Monkeys and toads define areas of endemism on Sulawesi. *Evolution* **57**, 1436–1443. (doi:10.1111/j.0014-3820.2003.tb00350.x)
- van den Bergh GD, de Vos J, Sondaar PY. 2001 The Late Quaternary palaeogeography of mammal evolution in the Indonesian Archipelago. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **171**, 385–408. (doi:10.1016/S0031-0182(01)00255-3)
- Miller KG *et al.* 2005 The Phanerozoic record of global sea-level change. *Science* **310**, 1293–1298. (doi:10.1126/science.1116412)
- Macdonald AA, Burton JA, Leus K. 2008 *Babyrousa babyrussa*. The IUCN Red List of Threatened Species. (doi:10.2305/IUCN.UK.2008.RLTS.T2461A9441445.en)
- Macdonald A, Leus K, Masaaki I, Burton J. 2016 *Babyrousa togeanensis*. The IUCN Red List of Threatened Species. (doi:10.2305/iucn.uk.2016-1.rlts.t136472a44143172.en)
- Leus, K., Macdonald, A., Burton, J. & Rejeki, I. 2016 *Babyrousa celebensis*. The IUCN Red List of Threatened Species. (doi:10.2305/iucn.uk.2016-1.rlts.t136446a44142964.en)
- Frantz L, Meijaard E, Gongora J, Haile J, Groenen MAM, Larson G. 2016 The evolution of Suidae. *Ann. Rev. Anim. Biosci.* **4**, 61–85. (doi:10.1146/annurev-animal-021815-111155)
- Meijaard E, Groves C. 2002 Upgrading three subspecies of babirusa (*Babyrousa* sp.) to full species level. *Asian Wild Pig News* **2**, 33–39.
- Semiadi G, Mannullang B, Burton JA, Schreiber A, Mustari AH. 2008 *Bubalus depressicornis*. The IUCN Red List of Threatened Species. (doi:10.2305/IUCN.UK.2008.RLTS.T3126A9611738.en)
- Burton JA, Hedges S, Mustari AH. 2005 The taxonomic status, distribution and conservation of the lowland anoa *Bubalus depressicornis* and mountain anoa *Bubalus quarlesi*. *Mamm. Rev.* **35**, 25–50. (doi:10.1111/j.1365-2907.2005.00048.x)
- Burton JA, Wheeler P, Mustari A. 2016 *Bubalus depressicornis*. The IUCN Red List of Threatened Species. (doi:10.2305/IUCN.UK.2016-2.RLTS.T3126A46364222.en)
- Groves CP. 1969 Systematics of the anoa (Mammalia, Bovidae). *Beaufortia* **17**, 1–12.
- Frantz LA *et al.* 2013 Genome sequencing reveals fine scale diversification and reticulation history during speciation in *Sus*. *Genome Biol.* **14**, R107. (doi:10.1186/gb-2013-14-9-r107)

31. Groves CP. 1984 Of mice and men and pigs in the Indo-Australian Archipelago. *Canberra Anthropol.* **7**, 1–19. (doi:10.1080/03149098409508559)
32. Rozzi R. 2017 A new extinct dwarfed buffalo from Sulawesi and the evolution of the subgenus *Anoa*: an interdisciplinary perspective. *Quat. Sci. Rev.* **157**, 188–205. (doi:10.1016/j.quascirev.2016.12.011)
33. Aubert M *et al.* 2014 Pleistocene cave art from Sulawesi, Indonesia. *Nature* **514**, 223–227. (doi:10.1038/nature13422)
34. Gongora J *et al.* 2011 Rethinking the evolution of extant sub-Saharan African suids (Suidae, Artiodactyla). *Zool. Scr.* **40**, 327–335. (doi:10.1111/j.1463-6409.2011.00480.x)
35. dos Reis M, Inoue J, Hasegawa M, Asher RJ, Donoghue PCJ, Yang Z. 2012 Phylogenomic datasets provide both precision and accuracy in estimating the timescale of placental mammal phylogeny. *Proc. R. Soc. B.* **279**, 3491–3500. (doi:10.1098/rspb.2012.0683)
36. Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL, Feldman MW. 1995 An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**, 463–471.
37. Sun JX, Mullikin JC, Patterson N, Reich DE. 2009 Microsatellites are molecular clocks that support accurate inferences about history. *Mol. Biol. Evol.* **26**, 1017–1027. (doi:10.1093/molbev/msp025)
38. Evin A, Cucchi T, Cardini A, Strand Vidarsdottir U, Larson G, Dobney K. 2013 The long and winding road: identifying pig domestication through molar size and shape. *J. Archaeol. Sci.* **40**, 735–743. (doi:10.1016/j.jas.2012.08.005)
39. Nugraha AMS, Hall R. 2016 Late Cenozoic palaeogeography of Sulawesi, Indonesia. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **490**, 191–209. (doi:10.1016/j.palaeo.2017.10.033)
40. Evans BJ, Supriatna J, Andayani N, Melnick DJ. 2003 Diversification of Sulawesi macaque monkeys: decoupled evolution of mitochondrial and autosomal DNA. *Evolution* **57**, 1931–1946. (doi:10.1111/j.0014-3820.2003.tb00599.x)
41. Driller C, Merker S, Perwitasari-Farajallah D, Sinaga W, Anggraeni N, Zischler H. 2015 Stop and go—waves of tarsier dispersal mirror the genesis of Sulawesi Island. *PLoS ONE* **10**, e0141212. (doi:10.1371/journal.pone.0141212)
42. Merker S, Driller C, Perwitasari-Farajallah D, Pamungkas J, Zischler H. 2009 Elucidating geological and biological processes underlying the diversification of Sulawesi tarsiers. *Proc. Natl Acad. Sci. USA* **106**, 8459–8464. (doi:10.1073/pnas.0900319106)
43. Burton JA, Nietsch A. 2010 Geographical variation in duet songs of Sulawesi Tarsiers: evidence for new cryptic species in South and Southeast Sulawesi. *Int. J. Primatol.* **31**, 1123–1146. (doi:10.1007/s10764-010-9449-8)
44. Shekelle M, Meier R, Wahyu I, Ting N. 2010 Molecular phylogenetics and chronometrics of Tarsiidae based on 12S mtDNA haplotypes: evidence for Miocene origins of crown tarsiers and numerous species within the Sulawesi clade. *Int. J. Primatol.* **31**, 1083–1106. (doi:10.1007/s10764-010-9457-8)
45. McGuire JA, Linkem CW, Koo MS, Hutchison DW, Lappin AK, Orange DI, Lemos-Espinal J, Riddle BR, Jaeger JR. 2007 Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotophytid lizards. *Evolution* **61**, 2879–2897. (doi:10.1111/j.1558-5646.2007.00239.x)
46. Pritchard JK, Stephens M, Donnelly P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.
47. Evans BJ, McGuire JA, Brown RM, Andayani N, Supriatna J. 2008 A coalescent framework for comparing alternative models of population structure with genetic data: evolution of Celebes toads. *Biol. Lett.* **4**, 430–433. (doi:10.1098/rsbl.2008.0166)
48. van der Geer A. 2010 *Evolution of island mammals: adaptation and extinction of placental mammals on islands*. New York, NY: Wiley-Blackwell.
49. Evin A, Dobney K, Schafberg R, Owen J, Vidarsdottir US, Larson G, Cucchi T. 2015 Phenotype and animal domestication: A study of dental variation between domestic, wild, captive, hybrid and insular *Sus scrofa*. *BMC Evol. Biol.* **15**, 6. (doi:10.1186/s12862-014-0269-x)
50. Kruska D, Röhrs M. 1974 Comparative–quantitative investigations on brains of feral pigs from the Galapagos Islands and of European domestic pigs. *Z. Anat. Entwicklungsgesch.* **144**, 61–73. (doi:10.1007/BF00518633)
51. McIntosh GH, Pointon A. 1981 The Kangaroo Island strain of pig in biomedical research. *Aust. Vet. J.* **57**, 182–185. (doi:10.1111/j.1751-0813.1981.tb00505.x)
52. Burton JA, Macdonald AA. 2008 *Sus celebensis*. The IUCN Red List of Threatened Species. (doi:10.2305/IUCN.UK.2008.RLTS.T41773A10559537.en)
53. Frantz LAF *et al.* 2018 Data from: Synchronous diversification of Sulawesi's iconic artiodactyls driven by recent geological events. Dryad Digital Repository. (<https://doi.org/10.5061/dryad.dv322>)