# The importance of small island populations for the long term survival of endangered large-bodied insular mammals

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**Summary**

Small island populations, particularly those of large vertebrates, are vulnerable to extinction due to environmental stochasticity and genetic drift. Here, we combined analyses of 113 newly generated ~10x genomes of anoa (*Bubalus* spp.) and babirusa (*Babyrousa* spp.) with habitat suitability models to assess the evolutionary trajectories of small and large island populations within the Wallacea archipelago in Indonesia. Our analyses show that, compared to closely related species, populations on the largest island of Sulawesi exhibit low levels of heterozygosity, high levels of inbreeding, and a high proportion of deleterious alleles. In contrast, although populations on

smaller islands such as Buton and Togean exhibit lower heterozygosity and higher inbreeding than in larger islands, they also possess fewer deleterious mutations. Analyses of site frequency spectra analyses indicate this likely the result of stronger, long-term purging acting in population from smaller islands. Combined with species distribution models, our results show that populations on small islands have persisted over a long time frame in high quality habitat while populations in the larger island have recently suffered from low habitat quality as a result of heavy exploitation of natural resources. In the case of anoa and babirusa, preservation of small island populations should focus more on improving habitat quality in small islands and avoid translocating individuals from the larger island. In the face of ongoing land-use change on the Sulawesi mainland, we suggest that offshore islands are increasingly crucial for the long-term survival of Wallacea's iconic and indigenous mammals.

## Results and Discussion

Throughout the Quaternary, populations of large vertebrates on islands have experienced higher rates of extinction compared to their mainland counterparts[1], likely reflecting the effects of genetic drift that operate in small populations of slow-reproducing species, and the associated negative consequences for fitness and adaptability[2,3]. In more recent years, these long-term extinction risks have been further exacerbated by human activities, including deforestation, mining and hunting[4].

Anthropogenic pressures can vary widely among islands. On the Wallacea archipelago (Figure 1), a global centre of endemism, the exploitation of timber[5] and mineral resources[6] has occurred more intensively on the large island of Sulawesi than on the smaller offshore islands[7]. As a result, while small island populations are more likely to go extinct over geological time scale, the relatively low human impact on small island ecosystems makes them attractive locations for species conservation programs.

Small island populations, however, can suffer from inbreeding depression due to low genetic diversity[8]. One solution to improve their viability is to translocate individuals from other populations (e.g. from larger islands where anthropogenic pressures are more acute) to increase genetic diversity[9]. Aside from increasing numbers of individuals, translocation can lead to genetic rescue, a process through which the average fitness of individuals is improved by increasing heterozygosity and decreasing inbreeding[10].

Despite the proposed benefit of genetic rescue, both recent empirical work[11] and simulations[12] suggest that it could lead to long term adverse effects through the introduction of recessive deleterious mutations if the source population is larger than the population to be rescued. In fact, recent work has shown that in the case of historically small populations that have had time to purge highly deleterious mutations, translocation can lead to counterproductive outcomes[12]. It is therefore crucial to conduct thorough genetic evaluations prior to establishing onerous translocation interventions[13].

The two endangered forest-dependent mammalian genera anoa (*Bubalus depressicornis* and *Bubalus quarlesi*) and babirusa (*Babyrousa* spp.) expanded across the Wallacea archipelago approximately 2 Mya[14] . These taxa now occur on islands of different sizes (from Togean, ~230km$^2$, to Sulawesi ~180,000 km$^2$) where they are subject to contrasting levels of anthropogenic activity[7]. As a result,these taxa are potential targets for translocation and genetic rescue. Their evolutionary history also means they are excellent models to understand how long term (i.e. geological timescales) and recent disturbances (i.e. anthropogenic) affect microevolutionary processes in large-bodied insular species.

To assess the effect that long term isolation and recent human activities have had on the genetic variation of anoa and babirusa, we combined analyses of newly generated genomic data (113 genomes; ~10x coverage; Table S1) sampled across the larger island of Sulawesi and smaller islands of Buton and Togean with habitat suitability models. Our results provide guidance for conservation efforts, demonstrating that small island populations have persisted over a long time frame in high quality habitats, while their counterparts on larger islands have experienced diminished habitat quality as a result of heavy exploitation of natural resources.

We aligned anoa and babirusa short-read sequences to the water buffalo (*Bubalus bubalis*) and babirusa reference genome respectively for all downstream analysis, except for genomic load analyses for which we used the better annotated reference genome of cattle (*Bos taurus*) and pig (*Sus scrofa*) (Supplementary Information). Principal component analysis (Figure 1C & D), and phylogenetic trees (Figure S2) based on nuclear single nucleotide polymorphisms (SNPs), indicated the existence of four, well supported, lineages of anoa (North Sulawesi, East Central, Southeast Sulawesi and Buton) and three lineages of babirusa (North Sulawesi, Southeast Sulawesi and Togean) that map clearly onto the geography of Sulawesi and nearby islands. These results are largely in agreement with previous findings based on mitochondrial and microsatellite loci[14]. The phylogeographic structure defined here formed the basis of the population definition used in all subsequent analyses (Figure 1).

**Populations on smaller island are less genetically diverse than on mainland Sulawesi**

We computed genome-wide Watterson's $\theta$ using ROHan[16]. We found that $\theta$ on the small islands of Togean (babirusa: mean=0.29 x $10^{-3}$, SE=0.02 x $10^{-3}$) and Buton (anoa: mean=0.58 x $10^{-3}$, SE=0.02 x $10^{-3}$) was ~2 to ~6 fold lower than in populations from mainland Sulawesi (babirusa: mean=1.85 x $10^{-3}$, SE=0.1 x $10^{-3}$; anoa: mean=1.33 x $10^{-3}$, SE=0.1 x $10^{-3}$), indicating that small island populations possess less genetic diversity than their mainland counterparts (Figure 2A). On the large island of Sulawesi, however, we found that babirusa from the Southeast Sulawesi possessed lower $\theta$ (mean=1.17 x $10^{-3}$, SE=0.04 x $10^{-3}$) compared to those found on the Northern peninsula (mean=2.17 x $10^{-3}$, SE=0.06 x $10^{-3}$). Low $\theta$ in Southeast Sulawesi babirusa could be the result of a recent bottleneck.

We assessed whether any of these populations showed signs of recent bottlenecks by analysing Runs of Homozygosity (ROH) using ROHan (Supplementary Information). Compared to anoa from mainland Sulawesi, the genomes of individuals from the small island of Buton contained more ROH segments (1-267, Figure 2B), consistent with higher inbreeding levels (Figure 2C). Overall, this indicates that anoa populations from small islands are more inbred than those found on mainland Sulawesi.

For babirusa, levels of inbreeding (measured by the fraction of the genome in ROH) are highest in individuals in Southeast (mainland) Sulawesi followed by Togean (Figure 2B & C). This analysis also indicates that babirusa from both regions are more inbred than most other species of suids including endangered taxa such as the pygmy hog (*Porcula salvania*), with the exception of the critically endangered Javanese warty pig (*Sus verrucosus*) (Figure 2B). High levels of ROHs in Southeast babirusa (Figure 2C) indicate that this population likely underwent a recent population bottleneck.

To address whether this bottleneck in Southeast Sulawesi is consistent with a recent decrease of suitable habitat in the area, we constructed an ensemble species distribution model[17] based on

bioclimatic variables and forest cover[5,15] (Supplementary Information). Our models, for both anoa and babirusa, indicate that there is proportionally more highly suitable habitat (i.e. top 20% of all suitable habitat; see Supplementary Text) on smaller islands (i.e. Togean and Buton) compared to mainland Sulawesi (~1.5 fold more highly suitable area for anoa and ~1.6 for babirusa relative to North Sulawesi; Figure 1, Table S7). We also found that, for both species, habitat suitability was highly correlated with their protection status, where habitats with higher protection were also found to be more suitable (Supplementary Information, Figure S4).

On the mainland, the availability of suitable habitat was higher in the North compared to the Southeast for both taxa. This trend is consistent with recent deforestation rates (between 2000-2017), which were higher in Southeast Sulawesi compared to the North[18], suggesting that deforestation has led to the reduced habitat availability detected by our models. These data, combined with previously reported high rates of poaching[19], imply that population bottlenecks in babirusa in Southeast Sulawesi have been driven by relatively recent anthropogenic disturbances, rather than longer term evolutionary processes.

For both babirusa and anoa, we found that differences in levels of genome-wide $\theta$ (including ROH) and $\theta$ outside of ROH in small island populations were much less pronounced than in Southeast Sulawesi babirusa (Figure 2B). In Southeast Sulawesi babirusa, $\theta$ computed genome-wide (i.e. including ROHs) was significantly lower than when computed excluding ROHs ($p<0.001$; Figure 2B). This pattern implies that babirusa living in Southeast Sulawesi were part of a historically large population which underwent a bottleneck. Babirusa and anoa living on small islands (Togean and Buton), however, likely form part of a historically small, yet stable, population that inhabits a highly suitable habitat, as demonstrated by our species distribution model (Figure 1, Figure S4). Altogether, this suggests that although they are not as genetically diverse as their mainland counterparts, small island populations are likely to have been stable over geological time scales, and potentially since their expansion ~2Mya[14].

**More deleterious alleles segregate on Sulawesi than on small islands**

Recent studies indicated that other historically small populations (*e.g.* Channel Island foxes[20], Iberian lynx[21], and Indian tigers[22]) have experienced long histories of purging strongly deleterious recessive variants. To evaluate how small island populations purge deleterious mutations compared to mainland populations, we computed genomic load in individual genomes, using three conservation scores: SIFT, PhyloP and phastCons (Supplementary Information). Genomic load was computed at one-to-one pig-cow ortholog loci (Supplementary Information), to allow cross species comparison. We first computed a load score by summing conservation scores, weighted by genotype probability for derived alleles found at homozygous states across the genome (see Supplementary Information). This homozygous load represents the minimum impact of deleterious alleles on fitness (i.e. assumes that all deleterious mutations are recessive). We also computed the load by summing the impact of alleles found at both homozygous and heterozygous states (total load) across the genome. The total load represents the maximum impact of deleterious alleles on fitness (i.e. assumes that all deleterious mutations are dominant).

Both homozygous and total load were significantly lower in anoa than in babirusa ($p < 0.001$; See Supplementary Information) (Figure 3A & B). Mean total load, computed using all the individuals from the same population, was also significantly higher in large populations found on mainland Sulawesi than in smaller island populations such as Togian (babirusa) and Buton (anoa; Figure 3C, Table S1). This result indicates that deleterious mutations are more abundant in larger, mainland,

populations than in smaller, island populations, despite the fact that larger populations are expected to purge deleterious mutations more efficiently[12].

Mean homozygous load, however, was significantly higher in small island populations than in larger populations (Figure 3D). Altogether these results indicate that large populations possess more deleterious recessive alleles overall, most of which are found in heterozygous state and therefore hidden from selection. Thus, although large populations possess more deleterious alleles, the overall fitness impact of deleterious mutations is likely to be higher in smaller island populations which possess more deleterious alleles in homozygous state.

**Small island populations efficiently purge deleterious alleles**

Deleterious alleles are potentially more likely to be found in homozygous state in individuals from small island populations due to their lower levels of heterozygosity (Figure 2B). This could lead to the difference between the total and homozygous load observed in small and large populations (Figure 3C & D). To address this, we built unfolded site frequency spectra (SFS) using ANGSD[23], based on alleles that have been assigned different impact ratings by the Variant Effect Predictor (VEP)[24], *i.e.* low, modifier, moderate, and high (Figure 4, Supplementary Information). Comparing SFS provides the opportunity to assess the impact of purifying selection on the full frequency range of deleterious alleles in a population, not just those in heterozygous and homozygous states.

These SFS show an excess of low frequency deleterious alleles in mainland populations that have not experienced population bottleneck(s) such as North Sulawesi (both babirusa and anoa) and Southeast Sulawesi (anoa) (Figure 4A & B). This pattern is consistent with the effect of purging reducing the frequency of deleterious alleles in the population. The persistence of deleterious alleles at low frequency in these large, highly heterozygous, populations is likely due to their recessive nature which means they are less likely to be exposed to selection.

In contrast, we found no noticeable excess of deleterious mutations at low frequency in Southeast Sulawesi babirusa, likely due to a weaker effect of purging and lower heterozygosity than in the North, in this population that experienced a recent bottleneck. Small island populations, particularly Togean babirusa, however, possessed many fixed deleterious mutations, but much fewer deleterious mutations at low frequency than in large populations. The high degree of fixed deleterious alleles is likely the result of strong genetic drift leading to higher rate of fixation. Reduced heterozygosity in these island populations likely explains the near absence of low-frequency deleterious mutations, a result of long-term purging where recessive alleles are exposed to selection. This is in contrast with large populations, in which deleterious alleles persist at low frequency despite purging.

We then quantitatively compared the shape of these SFS across different impact categories using a composite likelihood approach adapted from Nielsen *et al*[25]. Large differences of composite likelihood values between SFS built using alleles with different levels of impact indicate substantial differences between the shape of SFS. As the low impact alleles are less likely to be affected by selection, we used the SNPs in this category as the expected SFS. This analysis shows that the shape of the SFS of high and low impact mutations are substantially different in small island populations, while very similar in mainland populations (Figure 4C & D).

The shape of the SFS across different impact categories in the small island populations suggests that although they are smaller than their mainland counterparts, purging is having a stronger distorting effect on the SFS of more impactful alleles. This is likely because recessive deleterious

alleles are more likely to be exposed to selection in a less heterozygous population. Over time, this will lead to a reduction in frequency for the most deleterious alleles in small populations, consistent with our total load scores (Figure 3A) and previous simulation studies[12].

## Conclusions

Our analyses show that anoa and babirusa populations on small islands have remained sufficiently stable to efficiently purge deleterious alleles. These populations also occupy high-quality habitats, often within protected areas (e.g. 45% of the Togean islands are protected as a national park). Small island ecosystems may thus offer a long-term solution to preserve these species, away from areas such as Southeast Sulawesi where natural resources are more heavily exploited.

In contrast, we show that populations on the larger Island of Sulawesi, such as in Southeast Sulawesi, occupy lower quality habitat due to a higher degree of anthropogenic disturbance, and show signs of strong bottlenecks and an accumulation of deleterious alleles. These findings imply that recent anthropogenic disturbances on larger islands may be reshaping the extinction dynamics previously observed during the Quaternary, a period during which populations on smaller islands have been generally presumed to be more vulnerable. Our results suggest a reversal of this trend, indicating that human activities may be rendering larger island populations more susceptible to extinction than their smaller counterparts.

The introduction of individuals from the mainland could be a solution to increase population size and improve genetic diversity on small islands with high habitat quality and low anthropogenic pressures. Our genomic data, however, indicate that individuals from mainland populations possess more deleterious mutations, which, if translocated to smaller, less heterozygous, island populations could result in fitness decline and increased risk of extinction[12]. We therefore suggest that, in the case of babirusa and anoa, conservation efforts should focus on maintaining forest habitat for these species on smaller islands, without the need for a possibly counterproductive, and onerous, translocation programme. Altogether, our study demonstrates the benefit of combining genomic information with species distribution modelling to help prediction of future anthropogenic threats inform species conservation planning in island systems.

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## Author contributions

S.G.A., R.D., S.R., S.B., L.F.: Conceptualization; S.G.A., R.D., N.J.D., M.V., D.F., D.S., R.B., A.M., G.L., P.H.A.G., J.B., M.S.: Data Curation; S.G.A., R.D., A.C., L.F.: Formal Analysis; N.L.W., N.A., J.S., M.S., S.R., S.B., L.F.: Funding Acquisition; S.G.A., R.D., K.M., W.E.P., A.H.M, J.L.W., J.B.: Investigation; S.G.A., R.D., A.C., N.J.D., M.V., M.F., D.S., D.G., M.S.: Methodology; S.G.A., R.D., D.S.P., N.L.W.. J.B., N.A., J.S., M.S., S.B., S.R., L.F.: Project Administration; M.S., D.S., K.M., W.E.P., J.L.W., R.B., A.M., G.L., P.H.A.G., J.B., M.S., L.F: Resources; S.G.A., R.D., A.C., N.J.D., M.F., D.G., L.F.: Software; S.G.A., R.D., S.B., S.R., L.F.: Supervision; S.G.A., R.D., A.C., L.F.: Validation; S.G.A., R.D., L.F.: Visualization; S.G.A., R.D., S.R., S.B., L.F.: Writing - original draft; all authors contribute to review and edit part of writing.

**Declaration of interests**

The authors declare no competing interests.

**Supplemental information**

**Document S1.** Figures S1–S4 and Table S1–S3

**Supplementary Data.** Excel file containing additional data (**Data S1-S3**)

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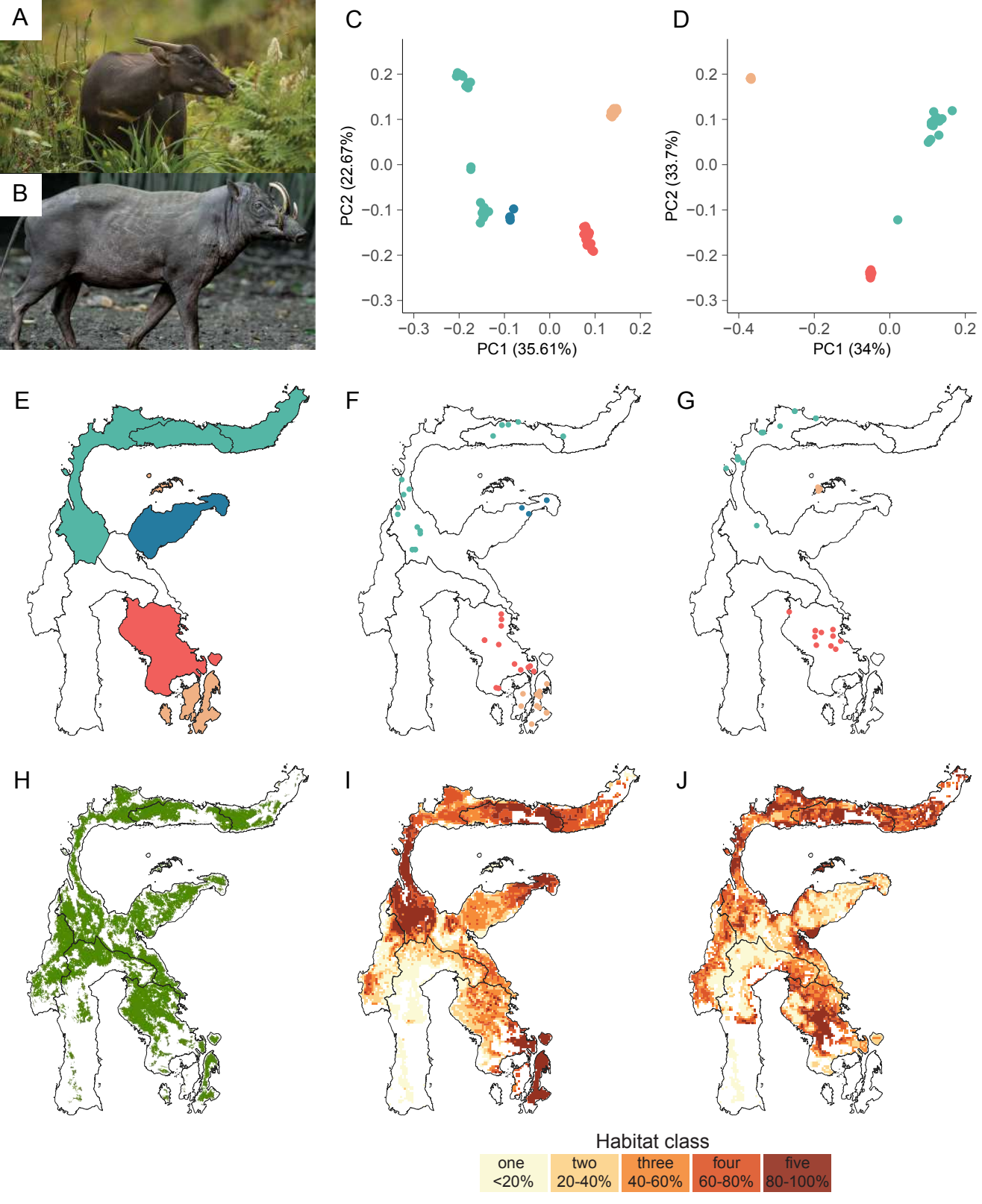
**Figure 1. Population structure and habitat suitability**. **(A)** anoa (copyright: chester zoo) and **(B)** babirusa (copyright: S.L. Mitchell). **(C)** Principal component analysis (PCA) based on SNPS from genomes (>5x) anoa (53 individuals; 1,053,534 SNPs) and **(D)** babirusa (37 individuals; 1,011,533 SNPs). **(E)** Zone of endemisms defined from Frantz *et al*[14] **(F)** Location of the samples of anoa **(G)** babirusa, coloured based on regions of endemism. **(H)** Map of Sulawesi (including Buton and Togean islands) with 2018 forest cover (Global Forest Change repository, v1.6, Hansen *et al*[15]*,* processed in Voight *et al*[5]) in green. **(I)** Ensemble distribution models constrained by forest cover, categorised by the habitat suitability score quantile (class one = least suitable habitat, class five = most suitable habitat) for anoa and **(J)** babirusa.

**Figure 2. Population-level genetic diversity and runs of homozygosity (ROH). (A)** Genome-wide Watterson's $\theta$ (i.e. including ROH; square) and excluding ROH segments (triangle), **(B)** Sum of size of ROH segments across the genome (NROH) and proportion of ROH across the genome (FROH) **(C)** distribution of ROH segment length **(D)** Map of Sulawesi with different regions considered in the analysis**.**

**Figure 3. Genomic load in babirusa, anoa and closely related species computed in individual genomes using SIFT scores. (A)** Comparison of total load of anoa and babirusa to their close relatives for alleles found at both heterozygous and homozygous states and **(B)** only homozygous. **(C)** Total load within anoa and babirusa populations at both heterozygous and homozygous states and **(D)** only homozygous.

**Figure 4. Site frequency spectra (SFS) of deleterious alleles across babirusa and anoa populations. (A)** Site frequency spectra of alleles with different impact categories rating (low, modifier, moderate, high) across populations of anoa and **(B)** babirusa. **(C)** Mean composite likelihood (CL) values in anoa and **(B)** babirusa. Composite likelihood (CL) values represent differences between the shape of the SFS computed using low impact mutation and the SFS built using moderate, modifier and high impact alleles. Mean CL was computed using 1000 bootstrap replicates and normalised using CL obtained from the SFS of low impact mutation (see Supplementary Information).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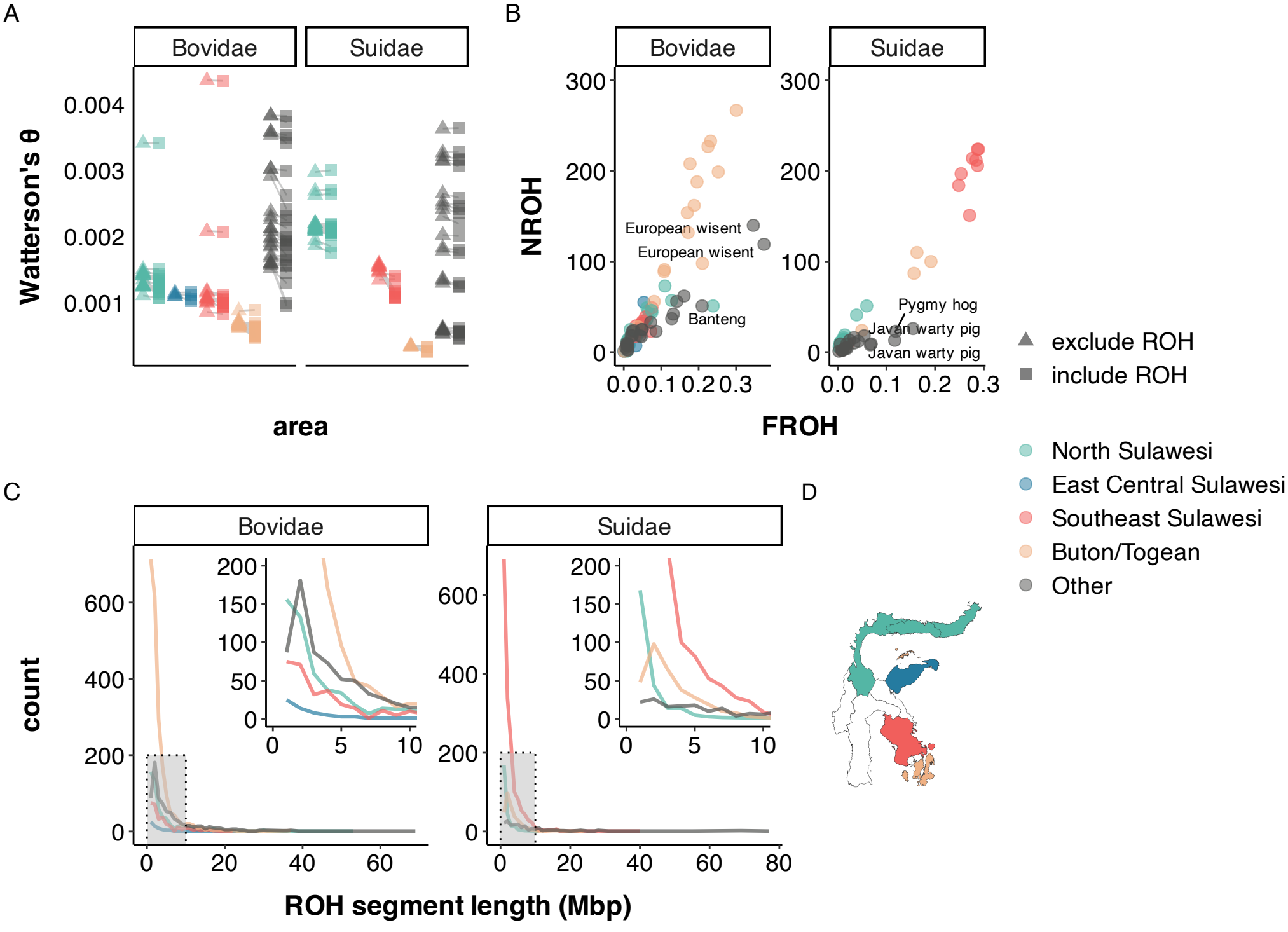
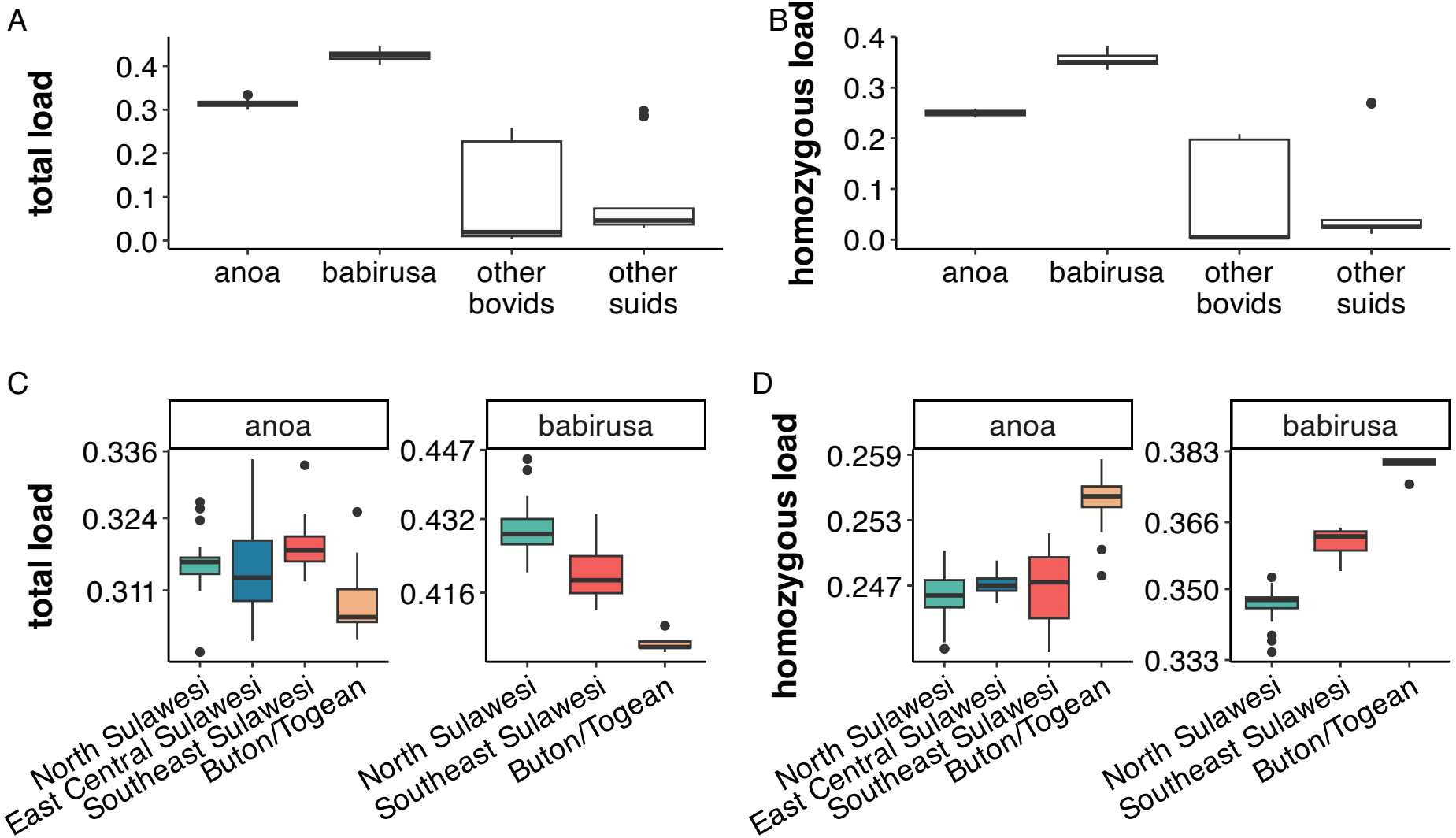
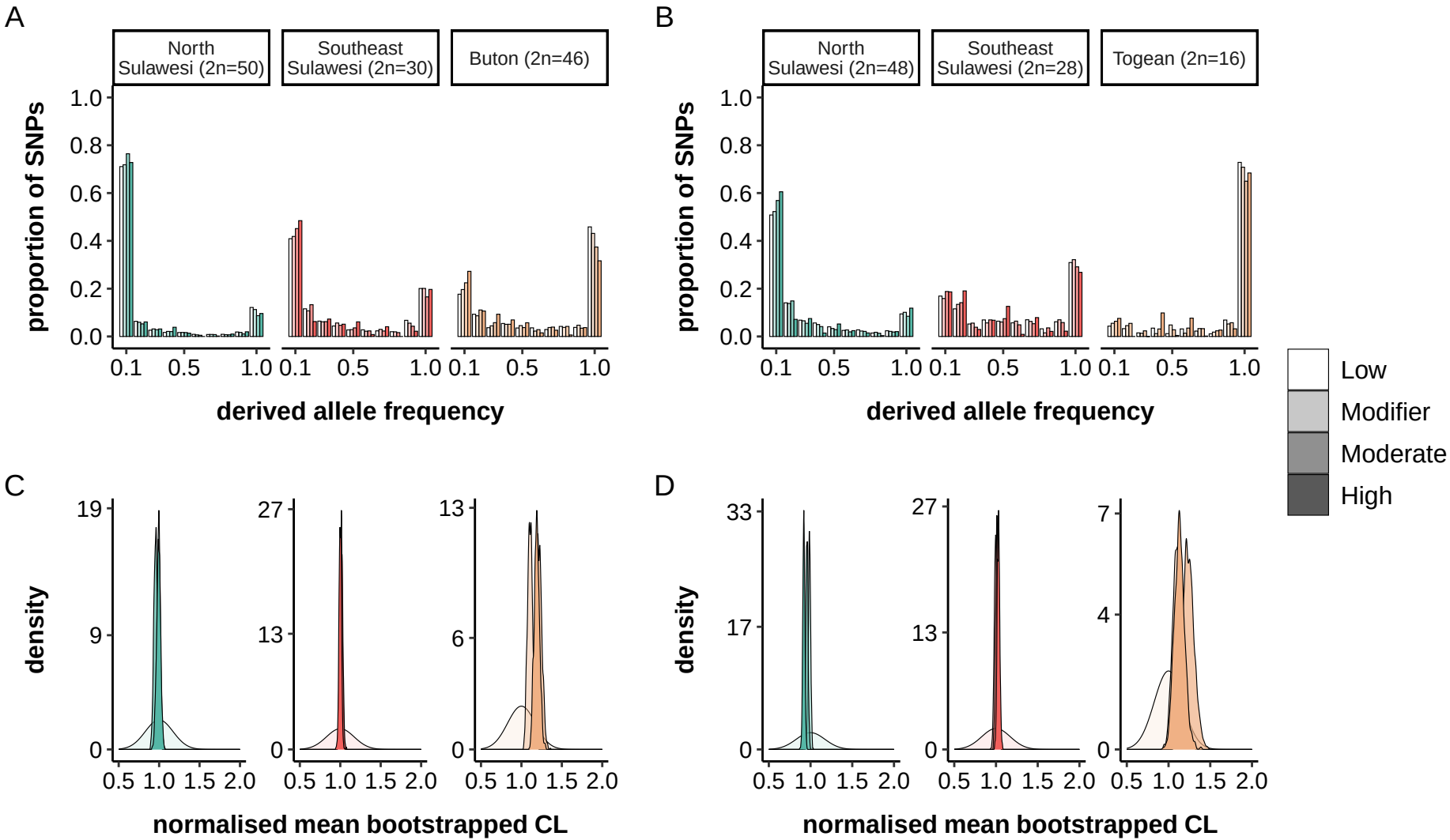
C
D
PC2 (22.67%)
PC1 (35.61%)
PC2 (33.7%)
PC1 (34%)
E
F
G
H
I
J
Habitat class
one <20%
two 20-40%
three 40-60%
four 60-80%
five 80-100%


A
Bovidae
Suidae
Watterson's θ
0.004
0.003
0.002
0.001
area
B
Bovidae
Suidae
NROH
300
200
100
0
European wisent
European wisent
Banteng
Pygmy hog
Javan warty pig
Javan warty pig
0.0 0.1 0.2 0.3
FROH
exclude ROH
include ROH
North Sulawesi
East Central Sulawesi
Southeast Sulawesi
Buton/Togean
Other
C
Bovidae
Suidae
count
600
400
200
0
150
100
50
0 5 10
0 20 40 60
0 20 40 60 80
ROH segment length (Mbp)
D


A
total load
0.4
0.3
0.2
0.1
0.0
anoa
babirusa
other bovids
other suids
B
homozygous load
0.4
0.3
0.2
0.1
0.0
anoa
babirusa
other bovids
other suids
C
total load
anoa
0.336
0.324
0.311
babirusa
0.447
0.432
0.416
North Sulawesi
East Central Sulawesi
Southeast Sulawesi
Buton/Togean
North Sulawesi
Southeast Sulawesi
Buton/Togean
D
homozygous load
anoa
0.259
0.253
0.247
babirusa
0.383
0.366
0.350
0.333
North Sulawesi
East Central Sulawesi
Southeast Sulawesi
Buton/Togean
North Sulawesi
Southeast Sulawesi
Buton/Togean


A
North Sulawesi (2n=50)
Southeast Sulawesi (2n=30)
Buton (2n=46)
proportion of SNPs
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.5
1.0
derived allele frequency
B
North Sulawesi (2n=48)
Southeast Sulawesi (2n=28)
Togean (2n=16)
proportion of SNPs
derived allele frequency
Low
Modifier
Moderate
High
C
density
19
9
0
27
13
0
13
6
0
0.5
1.0
1.5
2.0
normalised mean bootstrapped CL
D
density
33
17
0
27
13
0
7
4
0
normalised mean bootstrapped CL