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Climate, not Quaternary biogeography, explains skull morphology of the long-tailed macaque on the Sunda Shelf

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ABSTRACT

Sundaland, comprising the low-lying Sunda Shelf, the major islands of Borneo, Sumatra, and Java, as well as many smaller surrounding islands, formed a contiguous landmass through much of the Pliocene and Pleistocene. Subsequent late-Pleistocene rising sea levels have often been invoked as major zoogeographic barriers to gene flow and associated phenotypic and taxonomic divergence of Sundaic mammals. However, previous examinations of mammalian biogeography have painted a complex picture, suggesting that mammalian diversification in the region cannot easily be explained by a single overarching biogeographical phenomenon. Instead, it may be that the significant climatic changes and their concomitant impacts on local environments were the more significant control. In this context, we study adult skull morphology of a widely distributed primate in insular and continental Southeast Asia, the long-tailed macaque (Macaca fascicularis), using a 3D geometric morphometric (GM) approach. We used principal component analysis and linear discriminant function analysis to analyze the associations between morphological divergence and island biogeography, taxonomy, and phylogeny. Reduced rank regressions (RRR) were employed to investigate associations with climate of the present and last interglacials and the last glacial maximum (LGM). There was very poor morphological divergence and discrimination among specimens from different biogeographical zones. There was also large overlap between M. fascicularis subspecies in skull size and shape, with M. f. fascicularis occupying the largest range of size and shape variation, consistent with its wide geographical distribution. Nonetheless, sundaic M. f. fascicularis and M. f. philippinensis, restricted to the Philippines, were successfully discriminated on size and shape, supporting the latter as a valid subspecies. There was moderate morphological differentiation associated with the deepest split in mitochondrial DNA lineages (M. f. aureus versus the rest) in skull size and shape. Contrary to biogeography, we detected strong associations between skull morphology and climate. Skull form (size and shape combined) was better explained by climate closer to present conditions and correlated most strongly with the current interglacial, even though past climatic conditions do show correlations with cranial morphology. We found no strong evidence that cranial and mandibular variation in *M. fascicularis* consistently tracks insularity caused by Ouaternary sea level change. Instead, the morphological overlap evident in our sample indicates connections between subspecies and is consistent with the history of Sundaland as a single landmass for large portions of the Quaternary.

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1. Introduction

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Sundaland, the geographical subregion comprising the lowlying Sunda Shelf, the major islands of Borneo, Sumatra, and Java, as well as many smaller islands surrounding, is a region characterised by a complex geological, biogeographical, and climatic

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history. Although currently composed of islands and shallow seas, during much of the Pleistocene Sundaland was a single exposed landmass that connected the mainland Malaysian peninsula to Borneo, Java, Sumatra and many of the smaller islands in the region (Sarr et al., 2019).

Pliocene and early Pleistocene connections between what is now considered mainland Southeast Asia and Sunda allowed the dispersal of a diverse suite of mammals from the Indochinese subregion into Sundaland, with most divergence dates of Sundaic species occurring during this time (e.g. Patou et al., 2010; Liedigk et al., 2015; Yao et al., 2017). Such exchanges may have been facilitated by the development of extensive grassland environments that stretched from southern China through to Java (Louys and Roberts, 2020). The subsidence of the Sunda Shelf from ~0.4 Ma (Sarr et al., 2019) onwards meant that during major interglacials (including the present Holocene), which were less frequent than glacials, higher sea levels split Sundaland into separate islands, in part resulting in extensive losses of open forests and grasslands (Louys and Roberts, 2020). This significantly late date for the full development of the Sundaic islands requires a re-evaluation of traditional assumptions of faunal evolution and diversification in the region (Husson et al., 2020). By the late Pleistocene, Southeast Asia had largely assumed its current geographic configuration and rainforest environments became dominant, although significant instances of continental shelf exposure and subsequent grassland expansion have been recorded, most notably for the Last Glacial Maximum (Heaney, 1991; Bird et al., 2005; Nguyen et al., 2022; Cheng et al., 2023).

Previous examinations of mammalian biogeography in the region have painted a complex picture of the evolutionary effects of the fragmentation of Sundaland and the environmental changes that took place in the region (Meijaard, 2004a; Louys and Meijaard, 2010). The Isthmus of Kra is considered one of the most significant biogeographical divides, and imperfectly separates mainland (Indochinese subregion) from island (Sundaic subregion) Southeast Asia (Parnell, 2013). Morphological divergence between mainland and island Southeast Asia has been observed in tigers (Mazák, 2010), leopards (Meijaard, 2004b), tree shrews (Endo et al., 2000), and macaques (Hamada et al., 2008), amongst others. Conversely, other studies have recorded no differences between mainland and island Southeast Asian populations, for example in sun bears (Meijaard, 2004c) and clouded leopards (Wilting et al., 2011).

The isolation of the larger islands of Sundaland, Java, Borneo, and Sumatra from rising sea levels is often invoked as a major zoogeographic barrier to gene flow, and thus also as an explanation of the morphological and taxonomic divergence observed in their resident mammals. Morphological differences between the major islands of Sundaland have also been observed for some species. Javan slow lorises are considered morphologically distinct enough from Bornean and Sumatran populations to be elevated to species level, with significant subspecific variation observed between Sumatran and Bornean populations (Groves and Maryanto, 2008). Bornean sun bears are smaller than other Sundaic individuals (Meijaard, 2004c). Javan and Sumatran palm civets are morphologically and genetically distinct from Bornean palm civets (Patou et al., 2010), and Sumatran tiger skull morphology has been considered intermediate between mainland and Javanese/Balinese, though morphologically distinct from both (Mazák, 2010). Conversely, while Malay and Bornean bearded pigs are more closely linked genetically to each other than to Sumatran groups, none show any significant morphological differentiation (Lucchini et al., 2005).

Morphological differences between smaller and larger islands, or within larger islands themselves have also been reported. Groves and Maryanto (2008) reported that slow lorises from Bangka were morphologically more similar to Bornean populations than Sumatran, despite the proximity and the shallow water separation between Bangka and Sumatra. They also reported no differences between northern and southern Sumatran slow lorises, contrary to differences observed in Sumatran squirrels in the genus Hylopetes (Rasmussen and Thorington, 2008). In this latter group, craniodental characters successfully distinguished northern Sumatran from southern Sumatran species, consistent with a significant zoogeographic divide between north and south Sumatra (Whitten et al., 1984). In the tree shrews from the Philippines (genus *Tupaia*), however, craniometrics failed to differentiate some species but did identify a unique morphotype from the island of Balabac (Sargis et al., 2014). In palm civets, the Malay Peninsula, Java, and Sumatra formed a lineage separate from Borneo, the Philippines, and the Mentawais (islands off the western coast of Sumatra) based on genetic data and dental morphology (Patou et al., 2010).

Analysis of the environmental preferences of mammals on the smaller islands of Southeast Asia indicate that in Sundaland, forest-dependent species are today largely confined to islands off the west coast of Sumatra (e.g. Mentawai Islands), the Lingga and Riau archipelagos, Palawan, and Bunguran Island but absent from those in the Java Sea, the Sunda Strait, and the east coast of eastern Borneo (Meijaard, 2003). These can be related to island size and latitude (Meijaard, 2003). Similarly, the number of primate species found on Southeast Asian islands may be controlled by island size and inversely related to latitude and longitude (Nijman and Meijaard, 2008). Interestingly, the degree of isolation of the islands seemed to have limited bearing on species numbers (Nijman and Meijaard, 2008).

These conflicting patterns of zoogeographic and morphological divergences and convergences between the different island groups of Sundaland suggest that any overarching biogeographical control on the evolutionary trajectory of mammals is unlikely. Rather, it may be that the significant climatic changes that have been recorded for the region, and their concomitant impacts on local environments, were the more significant control of taxonomic and/or morphological diversity. While recent sea level fluctuations have produced increased levels of insularity and isolation in mammalian populations, it remains largely untested what impacts climatic changes observed between the last glaciation and the penultimate interglacial had on mammal evolution for this region.

In this context, we examine morphological differentiation in Macaca fascicularis, the long-tailed macaque. The M. fascicularis range is encompassed largely by the Sunda Shelf, which makes it an ideal taxon to explore competing hypotheses about mammalian differentiation in Southeast Asia during the Pleistocene. It was not included in the Husson et al. (2020) meta-analysis as it is not confined to evergreen forest. Modern M. fascicularis occupies a large number of different forested and shrubland habitats, including mangroves, riverine forest and primary and secondary rainforest (Fooden, 1995). The diet of modern M. fascicularis is opportunistic, flexible and remarkably broad (Lucas and Corlett, 1991; Fooden, 1995), consistent with its occupation of a range of habitats. It forages on shorelines, and is also adept at exploiting anthropogenically disturbed and urban habitats (Fooden, 1995). Indeed, it is remarkably flexible in its habitat use (Fooden, 1995; Eudey et al., 2020). Although predominantly arboreal, with corresponding skeletal adaptations (Rodman, 1979), it is not exclusively so, coming to the ground to forage, including in fairly open areas such as cultivated fields, and also travels short distances, for example along riverbanks (Fooden, 1995; Eudey et al., 2020). In common with most primates, M. fascicularis is ecologically dependent on trees, and so in the past probably would not have spread across extensive open areas, but if its ancient conspecifics were ecologically similar, it would not have been confined to primary

rainforest and probably moved quite effectively in transitional habitats.

1.1. Evolutionary background of the long-tailed macaque

Extant *M. fascicularis* is found on continental Asia in southern Bangladesh, Myanmar, and Lao PDR, as well as central, east and south Thailand, and across Vietnam, Cambodia, and mainland Malaysia, plus Singapore and other islands off the mainland coast. It also inhabits Nicobar, Borneo, Sumatra, Java, the Lesser Sunda Islands, and the Philippines, but not Sulawesi. Seven of its ten subspecies (M. f. umbrosa, M. f. fusca, M.f. condorensis, M. f. tua, M. f. lasiae, M. f. karimondjawae, M. f. atriceps) are endemic to small islands or island groups but the other subspecies (M. f. aureus, M. f. philippinensis and M. f. fascicularis) are spread over much wider geographic areas (Fooden, 1995; Groves, 2001), with potential intergradation between M. f. fascicularis and M. f. philippinensis or *M. f. aureus* where their respective ranges meet (Fooden, 1995). Its fossil record is quite sparse, with the earliest certain fossil occurrence being from Java and dated to ~0.9 Ma (Aimi, 1981; Fooden, 2006). Although other fossils have been recovered (e.g. from the late Pleistocene Niah Cave [Harrison, 2000; Piper and Rabett, 2016]), much of what is known about its evolutionary history and dispersal has been inferred from molecular studies. As M. fascicularis is philopatric, mtDNA clades should reflect geography (Yao et al., 2017) and Y chromosome data give a picture of gene flow between populations (Tosi and Coke, 2007). However, molecular sampling has not covered the full *M. fascicularis* range comprehensively, and to date subspecies recognized morphologically cannot be mapped fully onto mtDNA or other clades derived from molecular data.

The ancestor of *M. fascicularis* originated in the late Pliocene (2.9-4 Ma; Liedigk et al., 2015; Yao et al., 2017). Where M. fascicularis originated, and its subsequent spread across Sundaland and parts of mainland southeast Asia, is not well understood. One possibility is that the ancestor of the *fascicularis* group, originally from a mainland source population that moved overland during low sea levels, originated on Java, before spreading back onto the mainland and into other areas of Sundaland (Delson, 1980). Some Y chromosome analysis lends support to an insular origin (Rovie-Ryan et al., 2021). Another scenario, implied by Fooden (1995), is that M. fascicularis evolved on mainland Southeast Asia then spread to what are now the islands of the Sunda Shelf. Y chromosome data indicate that there was secondary contact between M. fascicularis populations, with males moving from the mainland back onto the Sunda Shelf (after initial divergence), possibly in the Last Glacial Maximum (Tosi and Coke, 2007).

Diversification within M. fascicularis and any subsequent movements are also far from clear. Based on both mtDNA and Y chromosome data *M. fascicularis* is paraphyletic, with *M. f. aureus* from Myanmar and Thailand sister to a clade that includes M. f. fascicularis and closely related macaque species (e.g. M. mulatta, M. cyclopis, M. fuscata [Bunlungsup et al., 2016; Matsudaira et al., 2018]). The respective clades to which M. f. aureus and M. f. fascicularis belong appear to have diverged ~4 Ma or ~2.6 Ma based on mtDNA and Y chromosomal data, respectively (Matsudaira et al., 2018). Based on mtDNA analyses, divergence between the major clades within M. fascicularis (but excluding M. f. aureus), occurred ~1.3-1.9 Ma (Liedigk et al., 2015; Yao et al., 2017; Matsudaira et al., 2018). Y chromosome analyses indicate an initial split between the Indochinese mainland (above the Thani-Krabi depression) and Sunda Shelf forms ~2.35 Ma, with further divergence between Sunda Shelf mainland and island clades ~2 Ma (Rovie-Ryan et al., 2021). All these estimates fall well before the Middle Pleistocene separation of Sundaland into islands as indicated by Sarr et al.

(2019). Two separate mtDNA lineages (hereinafter Clades A and B [Liedigk et al., 2015]) are found in Sumatra, with northern Sumatran M. f. fascicularis individuals forming a group (Clade A) with mainland specimens from the same subspecies (Yao et al., 2017). The deepest split in that clade is in the mainland specimens, indicating a spread from the mainland to Sumatra (Yao et al., 2017). Clade B includes specimens from southern Sumatra. Simeulue (an oceanic island off the west coast of Sumatra). Java. Bali. Lesser Sunda, Borneo and the Philippines assigned to M. f. fascicularis, M. f. fusca, and M. f. philippinensis (Liedigk et al., 2015; Yao et al., 2017). A recent study using nuclear DNA has recovered the same two clades (Yao et al., 2020). In each main mtDNA clade, rapid diversification (within ~200,000 years) occurred ~1 Ma (Liedigk et al., 2015; Schillaci et al., 2017; Yao et al., 2017), again before the subsidence of the Sunda shelf, with M. f. fascicularis fanning out across Sundaland and diversifying further. *M. fascicularis* arrived in the Philippines, probably from Borneo, between 0.43 and ~0.06 Ma (Yao et al., 2017). Human agency has been implicated in the presence of M. fascicularis on islands east of the Wallace line, such as Timor (Fooden, 1995), but the evidence for this is equivocal (Liedigk et al., 2015; Yao et al., 2017), and M. fascicularis is absent from archaeological sites until the Holocene (Glover, 1986).

Sea level changes and topographic barriers are the factors most often assumed to be the primary drivers of geographic separation of Pleistocene macaques (Fooden, 1995; Schillaci et al., 2007), and Abegg and Thierry (2002) provide a comprehensive review of potential scenarios of dispersal and divergence. Further, some local tectonically-driven events (such as the super Toba eruption) have been suggested to have created biogeographic barriers (Yao et al., 2017, 2020). As the major mtDNA splitting events within M. fascicularis occurred well before 0.4 Ma, it is unlikely that substantial diversification between the main clades was caused by isolation on newly-formed islands of the Sunda Shelf. Of the subspecies endemic to islands or small island groups, three (M. f. umbrosa [on the Nicobar Islands], M. f. fusca [on Pulau Simeulue] and *M. f. tua* [on Pulau Maratua]) are found on islands defined as 'deep water' by Fooden (1995), so divergence based on insularity is more plausible. Of these three subspecies, molecular data are available only for M. f. fusca, which is nested within clade B and sister to specimens classified as M. f. fascicularis (Groves, 2001), from the adjacent island of Nias (Yao et al., 2017). MtDNA data indicate that these split from Javanese M. f. fascicularis ~0.9 Ma (Yao et al., 2017).

Over its range *M. fascicularis* shows phenotypic variation in pelage, which has been used to demarcate subspecies (Abegg and Thierry, 2002; Fooden, 1995). Some further morphological variation is attributed to insularity, but this pattern is not consistent. For example, Groves (2001) (p. 226) commented that "tail length differentiates the restricted peripheral and insular populations from each other, but its variation in the widespread M. f. fascicularis (67–150% of head plus body) covers the entire range". Trends in M. fascicularis tail length may conform to Allen's rule (Fooden and Albrecht, 1999). Indeed, the role of climate in morphological differentiation in *M. fascicularis* has been the subject of several studies, which nonetheless reveal patterns that are ambiguous. Skull length in *M. fascicularis* has been interpreted to conform to Bergmann's rule based on a relationship with latitude over part of its range (Fooden and Albrecht, 1993), with a separate study (Schillaci et al., 2009) finding latitudinal trends that could not be explained by temperature. Grunstra et al. (2018), however, found no association between craniodental size and climate, longitude, or latitude in long-tailed macaques across insular Sundaland. To provide further context, in several groups of comparable African monkeys, there is little evidence for latitudinal trends in cranial form (size and shape; Cardini et al., 2013), and a relatively weak association between temperature and form in vervets and baboons (Cardini et al., 2007; Dunn et al., 2013). Rainfall is more strongly associated with cranial form in these taxa, which may be due to differences in primary productivity (Cardini et al., 2007). Alternatively, year to year predictability in food resources may explain size differences in baboons (Jolly, 2012), close relatives of macaques. Observed differences in body masses and growth trajectories between *M. f. fascicularis* from Thailand and Singapore have been attributed to nutritional differences (Schillaci et al., 2007). This indicates that although insularity and biogeography may have shaped the phenotypic and taxonomic diversity evident in modern *M. fascicularis*, the role of other extrinsic factors such as rainfall (important in the climate of Southeast Asia) and food availability must be explored in greater detail when seeking to understand its Quaternary evolution and differentiation.

1.2. Aims

In this study, we use a large geometric morphometric dataset to quantify fine-scale cranial and mandibular variation within *M. fascicularis*, and investigate the roles of insularity and climate in patterns of morphological change. To understand whether Quaternary sea level fluctuation helped to determine modern longtailed macaque skull (cranial and mandibular) morphology, we explore morphological divergence in the context of geography, taxonomy, and phylogeny and quantify morphological divergence among M. fascicularis from different regions of Sundaland. Geographic isolation on islands through sea level change may result in genetic isolation and impact phenotypic divergence, so it is possible that M. fascicularis on different islands in Sundaland are different morphologically. Indeed, subspecies designations of *M. fascicularis* have been defined largely on the basis of soft tissue differences linked to geography. Here, we investigate whether subspecific taxonomy is reflected in skull morphology, expecting subspecies, especially those on deep water islands, to differ significantly. Based on previous observations, we nonetheless predict that the morphospace occupied by the nominate subspecies (*M. f. fascicularis*) will span most of the range of variation exhibited by the species. We also investigate phenotypic divergence in association with major biogeographic zones/islands without taking subspecific taxonomy into account.

Another possibility is that as Sundaland was a connected landmass for much of the Pleistocene, with only occasional and fairly short-lived disconnection because of rising sea levels in interglacial periods from the Middle Pleistocene onwards, skull variation in modern forms reflects the major division between mtDNA clades but otherwise shows considerable overlap among subspecies, reflecting the potential for gene flow across subspecies for much of the Quaternary history of *M. fascicularis*. The major mitochondrial divisions in long-tailed macaques occurred before the Quaternary sea level fluctuations, and understanding how skull morphology maps to major genetic divergences thus provides important background to our primary aim. If so, skull morphology might diverge according to mitochondrial lineage. Since morphological change tends to accumulate with time and more closely related lineages are typically more similar phenotypically than distant relatives (Harvey and Pagel, 1991), the more recently diverged mtDNA clades A and B should be more similar to each other morphologically than either is to the outgroup, *M. f. aureus* (e.g., Matsudaira et al., 2018).

The effects of environmental variation (elevation and climate) may be a plausible alternative explanation to sea level change for morphological differentiation within long-tailed macaques. We thus investigate the degree to which different environmental variables can explain skull form and shape during three major climatic phases: the current interglacial, the Last Glacial Maximum (LGM) and the Last Interglacial period (LI). Specifically, we compare the explanatory power and patterns of climate from these three time periods to evaluate how long-tailed macaque skull morphology has tracked environmental change through time. We anticipate that the pattern of climatic effects will be similar for interglacial (current and LI) environments compared to the LGM, as interglacial environments should be more similar to each other than either is to glacial conditions. Further, if skull morphology in *M. fascicularis* is influenced by thermal influences and the spatiotemporal distribution of food resources, then we predict skull form and shape to be better explained by the climate of the current interglacial than past climates, because if animals' phenotypes are adapted to the environment (e.g., adapted for resource utilisation), they are expected to track the animals' environment and thus climate closely in time and space. Moreover, we expect climatological variables related to rainfall and seasonality - and thus habitat productivity and resource availability - to be important in driving associations between climate and skull shape because of presumed dietary adaptations in skull shape. In contrast, variables important for thermoregulation, notably temperature and elevation, might be more important in explaining skull form, which includes size, given that mammalian body size is known to be subject to thermoregulatory constraints.

2. Materials and methods

2.1. Materials and methods

The sample included eight of the 10 recognized subspecies of *M. fascicularis* (Table 1, Fig. 1). We studied adult cranial and mandibular variation using a 3D geometric morphometric (GM) approach. We analysed a total of 75 anatomical landmarks on crania (p = 54) and mandibles (p = 21) of 187 female crania and 307 male crania, recorded on osteological specimens housed at various natural history collections (see Appendix A for a full list) by means of a 3D digitiser (MicroScribe 3DX, Immersion Corporation). Landmarks were a subset of those used by Cardini et al. (2007), and digitised by Andrea Cardini on the left side only to avoid redundancy and maximise the number of specimens measured. A list of landmark definitions can be found in Appendix B.

Island categories were designed to represent distinct biogeographical regions, resulting in (1) mainland Southeast (SE) Asia (including small, fringing islands), (2) Borneo (including fringing islands), (3) Sumatra (including fringing islands), (4) Philippines, (5) Java and Bali (including fringing islands), (6) Wallacea (a group of islands east of the Asian continental shelf including Flores and Timor), (7) Simeulue, and (8) Nicobar islands (Fig. 1).

Elevation and climate data, selected from among the 'bioclimatic' variables, were collected from the WorldClim database (www.worldClim.org; Fick and Hijmans, 2017; Hijmans et al., 2005; Otto-Bliesner et al., 2006). The *M. fascicularis* specimens in our sample had associated locality data, on which we determined their

Table 1

Maximum sample sizes by sex and subspecies; sample sizes differed slightly depending on the analysis.

Subspecies	Males (N)	Females (N)		
M. f. atriceps	1	2		
M. f. aureus	2	7		
M. f. condorensis	5	1		
M. f. fascicularis	250	143		
M. f. fusca	7	4		
M. f. philippinensis	39	29		
M. f. tua	1	1		
M. f. umbrosa	2	0		
Total	307	187		



Fig. 1. Distribution of specimens used in the present study across mainland Southeast Asia, Sundaland (the Malaysian peninsula along with Sumatra, Java, Borneo and surrounding smaller islands), the Philippines and Wallacea. Regions and islands that constitute distinct biogeographic zones are named individually.

geographical coordinates. For these point coordinates we used QGIS 3.1 (OGIS Development Team, 2020) to extract elevation and climate data for three time periods: the current interglacial (based on present-day climate, i.e., from 1970 to 2000; WorldClim 2, Fick and Hijmans, 2017), the Last Glacial Maximum (LGM, ~22 ka; WorldClim 1.4, Hijmans et al., 2005), and the Last Interglacial (LI, i.e., Marine Isotope Stage 5 climate at ~120–140 ka; WorldClim 1.4, Otto-Bliesner et al., 2006). Elevation data were extracted at 2.5 arcminutes, and since elevation was only available for the present we treated it as constant across the three time periods. The present climate data were available at 30 arc-seconds as well as at 2.5 arcminutes, the LGM data only at 2.5 arc-minutes and the LI data only at 30 arc-seconds. The 30-sec and 2.5-min data for present climate were highly correlated (0.98 < r < 1.00 among the bioclimatic variables), so we felt confident that data of different spatial resolutions could be combined. Present-day climate data at a resolution of 2.5 arc-minutes were used in the analysis. Elevation and climate parameters used in this study are presented in Table 2.

2.2. Analysis

We analysed the cranium and the mandible separately, as the cranium and mandible potentially carry different signals (Caumul and Polly, 2005). We also separated the sample by sex, because similar work on African cercopithecids has suggested that a corrected, sex-pooled sample may obscure subtle differences in male and female cranial morphology in relation to environmental variation (Cardini et al., 2010), and a sample separated by sex does not require sexual dimorphism to be accounted for. We furthermore analysed shape and form (shape and size) separately to investigate if these attributes have responded differently to the environment and to appreciate the role of size. Form (or size-and-shape) space was constructed by multiplying the Procrustes shape coordinates with centroid size to obtain Boas coordinates (Bookstein, 2021). This yields results that are indistinguishable from those based on size-shape space constructed by adding log centroid size, but Boas coordinates are easier when visualizing form changes

Table 2

Elevation and nine non-redundant climate variables analysed in this study, from WorldClim (Fick and Hijmans, 2017; Hijmans et al., 2005; Otto-Bliesner et al., 2006).

Variable	Description
Elevation	Altitude (measured above sea level), in m
Annual mean temperature	Mean annual temperature, in °C
Mean diurnal range	Mean of monthly differences between maximum and minimum temperature, in °C
Temperature seasonality	Standard deviation of monthly temperatures x 100, in °C
Maximum temperature	Maximum temperature of warmest month, in °C
Minimum temperature	Minimum temperature of coldest month, in °C
Annual precipitation	Total annual precipitation, in mm
Maximum precipitation	Precipitation of wettest month, in mm
Minimum precipitation	Precipitation of driest month, in mm
Precipitation seasonality	Coefficient of variation

(Mitteroecker et al., 2013; Mitteroecker and Schaefer, 2022).

A small percentage of specimens had missing data. Among females, at most six specimens (1.6% of the female sample) had missing data for a given cranial landmark; at most three specimens were missing data for a given mandibular landmark. 31 out of 54 cranial landmarks and 12 out of 21 mandibular landmarks had no missing data whatsoever. Among males, at most nine specimens (3.0% of the male sample) had missing data for a given cranial landmark; at most three male specimens were missing a given mandibular landmark. 33 cranial and 14 mandibular landmarks had no missing data whatsoever. We estimated missing coordinates using the function 'estimate.missing' from the package 'geomorph' (Adams et al., 2019) in RStudio (RStudio Team, 2019). This algorithm estimates missing coordinates based on the thin-plate spline by minimising the bending energy (Gunz et al., 2009). We imputed missing landmarks for females and males separately so as not to let sexual dimorphism influence the data estimation. Among the specimens that had missing data, the majority was missing coordinates for a single landmark, though some for up to five (mandible) or six landmarks (cranium). Following missing data estimation, all specimens were subjected to Generalised Procrustes Analysis (GPA) by sex and by skull part using the function 'gpagen' from package 'geomorph' in R.

Next, we conducted principal component analysis (PCA), by means of the native R function 'prcomp', to explore the patterns and dimensionality of skull shape and form variation and inspect whether morphological differentiation of long-tailed macaques is in line with phylogeny, subspecific taxonomy, or biogeography. To explore phylogenetic structure in skull variation, specimens were assigned to one of three mtDNA clades: *M. f. aureus*, clade A, or clade B, with *M. f. aureus* being sister to the other two clades. Assignment followed from subspecies taxonomy (e.g. specimens labelled as *M. f. aureus*) and geography. For the latter we followed the known geographic distribution of clades A and B from earlier work (Liedigk et al., 2015; Yao et al., 2017) and assigned specimens in our sample on the basis of their localities. Specimens that could not confidently be assigned to a clade based on their geographic occurrence were labelled as "unknown".

We used discriminant analysis to understand how individuals classified into groups based on taxon (subspecies), mtDNA clade and island group for both cranial and mandibular shape, with analyses performed separately for males and females. Groups with samples <10 were excluded from all analyses. The small sample sizes for most subspecies meant that only two of them, M. f. fascicularis and M. f. philippinensis, could be included robustly in the discriminant analysis based on taxon. In the clade analysis, two groups (clade A and clade B) were included. In the island group analysis, individuals from Andaman and Nicobar, Simeulue, and Wallacea were excluded because of small sample sizes. Principal component scores of shape were used as the independent variables. Normality was evaluated using Shapiro Wilk tests (alpha = 0.05), and variables that were not normally distributed were excluded. For each analysis, sample size determined the number of variables included in the model (following convention, the sample size of the smallest group was at least ~ five times the number of variables). Forward stepwise analyses were used to select the most informative (and highly significant) variables for each model. Box's M tests were used to inspect equivalence of covariance matrices (with, per convention, alpha set at 0.001 to account for the test's sensitivity). When covariance matrices were not equivalent, quadratic (QDA) rather than linear discriminant analysis (LDA) was used. Group membership was determined using unequal priors based on group sizes, and all models used leaveone-out cross validation. Shapiro-Wilk and Box's M tests were run in IBM SPSS (version 26), as was the cross-validated LDA.

Variable selection for QDA was undertaken in JMP Pro (version 15), with cross-validation performed in the MASS package of R (Venables and Ripley, 2002). Tables 3–5 detail the model (QDA or LDA), the PCs and sample sizes used in each analysis. Following Kovarovic et al. (2011), various sensitivity analyses were performed to assess the reliability of the models. In addition, we used Kruskal-Wallis tests performed in SPSS to evaluate differences in cranial and mandibular centroid sizes (the square root of the sum of all squared distances between the landmarks and their centroid) between subspecies, split by sex.

Finally, we used reduced rank regression (RRR) to analyze the environmental effect (i.e., of elevation and climate) on morphology. Reduced rank regression is an extended version of a multiple multivariate regression that includes a decomposition step to find linear combinations (dimensions) among a set of response variables that show maximum change as a result of a unit change among a set of predictor variables (Mitteroecker et al., 2016; Grunstra et al., 2018; Stansfield et al., 2021). First, a multiple multivariate regression is carried out, resulting in a *pxq* matrix of *p* partial regression coefficients (i.e. slopes) for q dependent variables in response to *p* predictors. Each regression coefficient represents the change in a given response variable (here, a form or shape coordinate) resulting from a unit change in a given predictor (here, an environmental variable) while holding other predictors constant. Next, a singular value decomposition (SVD) of the pxq matrix is computed, which results in pairs of linear combinations or latent variables (LVs) that successively maximise the regression slope of the dependent LV (morphology) on the independent LV (environment) (Stansfield et al., 2021). As is usual, the loadings of the original variables onto these LVs represent their contribution to the obtained associations. For our purposes, RRR has the following advantages over other dimension-reduction techniques such as partial least squares analysis (PLS): (1) it finds axes of successively maximum effect of combinations of presumed driving factors on response variables rather than the *covariance* between them; (2) the use of a regression technique enables us to determine the unique effect of correlated climate predictors, revealing causal effects while controlling for conditional effects of one predictor on another with which it is highly correlated as, for example, in the case of temperature variables (with dimension reduction nonetheless allowing us to understand how a complex, multivariate factor such as climate affects the phenotype); (3) it is not sensitive to differences in variance of the different predictors, something that can affect PLS even after variance-standardisation; and (4) it allows for a direct comparison of the importance of climate in explaining morphology (measured by the coefficient of determination, R^2) between different time periods, sexes and skull parts (Stansfield et al., 2021; Mitteroecker and Schaefer, 2022). Reduced rank regression was performed separately for shape and form, by sex, and by skull part (cranium vs. mandible). To this end, RRR was conducted on the Procrustes coordinates representing shape and on Boas coordinates representing form. A few cases with missing climate data were removed prior to analysis. All climate variables and elevation were mean-centred and variance-standardised to remove differences in scale and unit of measurement.

3. Results

3.1. Geography, taxonomy, phylogeny

There was poor morphological divergence and discrimination among macaques from different island groups, with extensive overlap and highly similar group means observed between different biogeographical zones in cranial form and shape space, among both females and males (Figs. 2–3, Figs. C.1-C.4 in Appendix

Table 3

Discriminant function analyses for biogeographical zone (island group) classification, based on a stepwise cross-validation approach. Model and variables entered given in parentheses in footnotes. QDA - quadratic discriminant analysis, LDA - linear discriminant analysis. Priors based on group sizes in all cases. Numbers in the table represent count of specimens (with percentages in parentheses).

	Indochina & Malay peninsula	Sumatra	Borneo	Java & Bali	Philippines	Ν
Female cranium ^a						
Indochina & Malay peninsula	6 (25.0%)	6 (25.0%)	3 (12.5%)	8 (33.3%)	1 (4.2%)	24
Sumatra	2 (5.7%)	13 (37.1%)	15 (42.9%)	1 (2.9%)	4 (11.4%)	35
Borneo	2 (3.2%)	12 (19.4%)	28 (45.2%)	5 (8.1%)	15 (24.2%)	62
Java & Bali	6 (31.6%)	1 (5.3%)	5 (26.3%)	7 (36.8%)	0 (0%)	19
Philippines	1 (2.5%)	0 (0%)	12 (30.0%)	3 (7.5%)	24 (60.0%)	40
Male cranium ^b						
Indochina & Malay peninsula	4 (9.1%)	26 (59.1%)	3 (6.8%)	0 (0%)	11 (25.0%)	44
Sumatra	6 (6.1%)	64 (65.3%)	18 (18.4%)	4 (4.1%)	6 (6.1%)	98
Borneo	4 (5.3%)	38 (50.0%)	28 (36.8%)	0 (0%)	6 (7.9%)	76
Java & Bali	0 (0%)	11 (45.8%)	3 (12.5%)	1 (4.2%)	9 (37.5%)	24
Philippines	4 (7.7%)	9 (17.3%)	4 (7.7%)	2 (3.8%)	33 (63.5%)	52
Female mandible ^c						
Indochina & Malay peninsula	10 (41.7%)	1 (4.2%)	9 (37.5%)	1 (4.2%)	3 (12.5%)	24
Sumatra	2 (5.7%)	14 (40.0%)	15 (42.9%)	0 (0%)	4 (11.4%)	35
Borneo	2 (3.2%)	5 (8.1%)	41 (66.1%)	2 (3.2%)	12 (19.4%)	62
Java & Bali	1 (5.3%)	2 (10.5%)	13 (68.4%)	1 (5.3%)	2 (10.5%)	19
Philippines	1 (2.5%)	2 (5.0%)	12 (30.0%)	0 (0%)	25 (62.5%)	40
Male mandible ^d						
Indochina & Malay peninsula	1 (2.3%)	27 (61.4%)	12 (27.3%)	2 (4.5%)	2 (4.5%)	44
Sumatra	2 (2.0%)	59 (60.2%)	24 (24.5%)	5 (5.1%)	8 (8.2%)	98
Borneo	6 (7.9%)	36 (47.4%)	24 (31.6%)	0 (0%)	10 (13.2%)	76
Java & Bali	0 (0%)	16 (66.7%)	1 (4.2%)	3 (12.5%)	4 (16.7%)	24
Philippines	2 (3.8%)	7 (13.5%)	5 (9.6%)	0 (0%)	38 (73.1%)	52

^a 43.3% correctly classified (cross-validated QDA, PC3 + PC4 + PC7 + PC8).

^b 44.2% correctly classified (cross-validated LDA, PC1, PC2, PC12, PC10, PC13).

^c 50.6% correctly classified (cross-validated LDA, PC1, PC16, PC2, PC33).

^d 42.5% correctly classified (cross-validated LDA, PC2, PC6, PC12, PC7, PC21).

Table 4

Discriminant function analyses for subspecies classification, based on a stepwise cross-validation approach. Model and variables entered given in parentheses in footnotes. QDA - quadratic discriminant analysis, LDA - linear discriminant analysis. Priors based on group sizes in all cases. Numbers in the table represent count of specimens (with percentages in parentheses).

	M. f. fascicularis	M. f. philippinensis	Ν
Female cranium ^a			
M. f. fascicularis	141 (98.6%)	2 (1.4%)	143
M. philippinensis	2 (6.9%)	27 (93.1%)	29
Male cranium ^b			
M. f. fascicularis	243 (97.2%)	7 (2.8%)	250
M. philippinensis	6 (12.8%)	33 (87.2%)	39
Female mandible ^c			
M. f. fascicularis	135 (94.4%)	8 (5.6%)	143
M. philippinensis	8 (27.6%)	21 (72.4%)	29
Male mandible ^d			
M. f. fascicularis	239 (95.6%)	11 (4.4%)	250
M. philippinensis	15 (38.5%)	28 (61.5%)	39

^a 97.7% correctly classified (cross-validated QDA, PC1, PC2, PC3, PC4, PC14, PC45).
^b 95.8% correctly classified (cross-validated LDA, PC3, PC1, PC7, PC11, PC12, PC6, PC10, PC2).

^c 90.7% correctly classified (cross-validated LDA, PC1, PC6, PC31, PC12, PC19, PC23).

^d 91.0% correctly classified (cross-validated QDA, PC3, PC2, PC42, PC21, PC4, POC36, PC10, PC8, PC14, PC45, PC29).

C). Results for the mandible were highly similar (not shown). A few additional PCs were inspected, accounting for a very small fraction of the total variation, and they also revealed no, or very poor, morphological separation between biogeographical groups (not shown). In discriminant analysis of skull shape (Table 3), correct classifications to island groups ranged from 42.5% (male mandible) to 50.6% (female mandible). Misclassifications occurred among and into all groups. Specimens from the Philippines were less likely to be misclassified than individuals from other islands.

There was strong overlap between *M. fascicularis* subspecies in skull (i.e. cranial and mandibular) form and shape, with *M. f. fascicularis* consistently occupying the largest range of form and shape variation, encompassing most or much of the morphological variation exhibited by all other subspecies on PC 1 versus 2 and PC 3 versus 4 (Figs. 2–3, Figs. C.1-C.4 in Appendix C). The taxon that diverged most from *M. f. fascicularis* was *M. f. philippinensis*. The cranial shape distinction between *M. f. fascicularis* and *M. f. philippinensis* was particularly evident on PC 3, for both females and males (Fig. 3 and Fig. C.4 in Appendix C). Small samples for most of the subspecies prevented their inclusion in discriminant analysis, but misclassification rates for cranial specimens were very low for *M. f. fascicularis* and *M. f. philippinensis* (Table 4), with 97.7% of

Table 5

Results of the Kruskal-Wallis tests for significant pairwise differences among *M. fascicularis* subspecies in centroid size. An insufficient number of male *M. f. aureus* specimens precluded comparisons with this subspecies in the male sample.

	Ν	df	H ^a	Significant pairwise differences (subspecies)
Female cranium	187	6	68.36*	fascicularis-philippinensis, fascicularis-aureus
Male cranium	307	7	51.64*	fascicularis-philippinensis
Female mandible	187	6	54.79*	fascicularis-philippinensis, fascicularis-aureus
Male mandible	307	7	43.73*	fascicularis-philippinensis

^a Adjusted for ties* p < 0.001.



Fig. 2. Female cranial shape variation along PCs 1 and 2 with *M. fascicularis* specimens classified by mtDNA clade (top), subspecies (middle), and island group (bottom). The shape deformations associated with the PCs are magnified by a factor of two. "Unknown" mtDNA specimens represent *M. fascicularis* individuals that could not be assigned to an mtDNA clade. Mandibular shape yielded highly similar results (not shown).

females and 95.8% of males correctly classified. Mandibular misclassification was slightly higher (Table 4).

In terms of cranial and mandibular size, represented by centroid

size, females and males of *M. f. fascicularis* are significantly smaller than *M. f. philippinensis* (Table 5, Fig. 4), though the former spans a larger range of size variation, similar to skull shape (Fig. 4). There is



Fig. 3. Female cranial shape variation along PCs 3 and 4 with *M. fascicularis* specimens classified by mtDNA clade (top), subspecies (middle), and island group (bottom). The shape deformations associated with the PCs are magnified by a factor of two. "Unknown" mtDNA specimens represent *M. fascicularis* individuals that could not a priori be assigned to an mtDNA clade. Mandibular shape yielded highly similar results (not shown).



Fig. 4. Boxplots showing the variation in centroid size of the cranium (left) and mandible (right) among *M. fascicularis* subspecies. Boxes represent the interquartile range (IQR, 25th to 75th percentiles), the solid horizontal line inside each box is the median, and the whiskers extend to \pm 1.5*IQR, with individual specimens outside this range denoted by asterisks. For each subspecies, females are on the left ("F") and males on the right ("M"). Sample sizes were below eight for all subspecies except *M. f. fascicularis* and *M. f. philippinensis* and so the results should be interpreted with caution.

also a significant difference in female cranium and mandible centroid size between the larger *M. f. aureus* and *M. f. fascicularis*.

Among females, mtDNA clades A and B were more similar in mean cranial form and shape compared to M. f. aureus (Figs. 2-3, Figs. C1-2 in Appendix C). However, there was considerable overlap in morphospace between M. f. aureus and the other clades. The exception was PC 1 in female form space, in which mtDNA clade A and *M. f. aureus* occupied separate parts of morphospace (Fig. C.1 in Appendix C). The same patterns were observed for the mandible (not shown). Since skull form was dominated by size effects (r > 0.996, p = 0.001 between PC 1 and centroid size in all cases), this reflects moderate divergence in female body size between these lineages. In discriminant analysis (Table 6), 82.1% of cranial and 82.7% of mandibular specimens in females were classified correctly, although this figure was driven by the high probability of correct classification of Clade B individuals. In contrast, Clade A females were more likely to be misclassified as Clade B than assigned correctly to their own clade. Overall classification rates in the male discriminant analysis (Table 6) were very similar to those seen in females, although clade A specimens were less likely to be misclassified.

3.2. Climate and elevation

Long-tailed macaque skull morphology was found to be significantly explained by climate and elevation, ranging from ~7 to ~27% based on R^2 calculated in the multiple multivariate regressions (Table 7). We detected differences in the amount of morphological variation that was accounted for by the environment from different time periods, as well as between form and shape (Table 7). Variation in form was consistently better explained by present climate compared to past climates (LGM and LI), whereas shape variation was approximately equally well explained by present and LGM climate compared to LI climate. Overall, the environment had

Table 6

Discriminant function analyses for mtDNA clade classification, based on a stepwise cross-validation approach. Model and variables entered given in parentheses in footnotes. LDA - linear discriminant analysis. Priors based on group sizes in all cases. Numbers in the table represent count of specimens (with percentages in parentheses).

	Clade A	Clade B	Ν
Female cranium ^a			
Clade A	18 (48.6%)	19 (51.4%)	37
Clade B	11 (8.4%)	120 (91.6%)	131
Male cranium ^b			
Clade A	58 (65.2%)	31 (34.8%)	89
Clade B	27 (14%)	166 (86%)	193
Female mandible ^c			
Clade A	14 (37.8%)	23 (62.2%)	37
Clade B	6 (4.6%)	125 (95.4%)	131
Male mandible ^d			
Clade A	53 (59.6.7%)	36 (40.4%)	89
Clade B	23 (11.9%)	170 (88.1%)	193

^a 82.1% correctly classified (cross-validated LDA, PC2, PC26, PC40, PC13, PC121, PC134, PC72).

^b 79.4% correctly classified (cross-validated LDA, PC3, PC8, PC20, PC34, PC7, PC14, PC18, PC2, PC12, PC9, PC28, PC5, PC16, PC23, PC53, PC92, PC11, PC35).

^c 82.7% correctly classified (cross-validated LDA, PC1, PC37, PC5, PC27, PC14, PC10, PC52).

^d 79.1% correctly classified (cross-validated LDA, PC3, PC2, PC42, PC21, PC4, POC36, PC10, PC8, PC14, PC45, PC29).

highly similar explanatory power in the cranium and mandible (Table 7). However, females consistently showed a stronger association between environment and cranial and mandibular morphology than males (Table 7). Individual predictors' coefficients of determination are presented in Appendix C (Tables C.1-C.3).

The RRR results yielded further insight into how elevation and the nine climate variables act together in affecting morphology and what the magnitude of their individual contribution is to each LV (which is different from the proportion of explained variance, or

Table 7

Coefficients of determination (R^2) as calculated from the multiple multivariate regressions. N = 176 for females (F) and N = 294 for males (M). See also Tables C.1-C.3 for R^2 for each predictor and associated *p*-values (Appendix C).

	Cranial form		Mandi form	Mandibular form		Cranial shape		Mandibular shape	
	F	М	F	М	F	М	F	М	
Current interglacial									
R ²	0.27	0.14	0.26	0.15	0.14	0.09	0.12	0.08	
LGM									
R ²	0.24	0.12	0.23	0.13	0.14	0.09	0.12	0.08	
Last interglacial									
R ²	0.2	0.1	0.22	0.12	0.11	0.07	0.1	0.07	

 R^2). First, after decomposing the effects of the environmental predictors into latent variables (LVs) that successively maximise the regression slope of skull morphology on the environment, the first two LVs summarised ~70–90% of the morphological variation that was accounted for by the environment, depending on time period, sex, skull part, and form vs. shape. LV 3 explained an additional ~5–15% of the overall environment-morphology association, translating to ~1–3% of total form or shape variation.

Second, the loading patterns of the climatic variables and elevation on these dimensions were similar between form and shape. There was also strong congruence between the environmental patterns associated with cranial and mandibular variation, and since environment explained similar amounts of cranial and mandibular variation (Table 7), we focus on the results for the cranium henceforth. (As we are primarily concerned with the role of climate on macaque morphological variation, we focus on the climatic latent variables rather than the associated patterns of morphological change. Nonetheless, the associated form and shape changes were similar across the interglacials and glacial.) Environmental latent variable (LV) 1, which has the maximum effect on morphological LV 1, was especially associated with temperature variables across all three time periods for both females and males and in form as well as in shape (Fig. 5).

The strongest effect on morphology, i.e. by current interglacial environments (Table 7), was characterised by warmer and somewhat drier conditions despite low maximum and minimum temperatures and high maximum and minimum precipitation levels (Fig. 5). Annual and minimum rainfall contributed more to the association with form than to shape, whereas minimum temperature had a larger effect on shape than on form. The pattern of environmental influence along LV 1 was very similar between the sexes for the current interglacial but less so for past climates (Fig. 5). The LV 1 loading patterns were similar for the mandible in form and shape, in both sexes, across all three time periods (not shown).

The next largest proportion of morphological variation was explained by LV 2, with current climate once again predicting morphology better than past climates. LV 2 of the current interglacial included a negative effect of maximum temperature and a positive effect of minimum temperature, combined with positive contributions of annual mean temperature, diurnal range, and temperature seasonality (Fig. 6). In other words, LV 2 represents tempered hot and cold annual extremes despite a greater discrepancy between monthly minima and maxima (diurnal range) and moderate temperature seasonality, in association with the effect of moderately high annual temperatures. Along LV 2, cranial variation is thus more affected by temperature variability on shorter rather than on longer timescales. Patterns were again largely similar between the sexes. Mandibular form (but not shape) was less affected by mean temperature than cranial form and shape, but otherwise the LV 2 loading patterns were similar for the

mandible in form and shape in both sexes for the current interglacial. Discrepancies in individual variable contributions to LV 2 were found between the cranium and mandible in past climates (not shown), more so in males, possibly due to the weaker relationship between morphology and past climates.

Environmental LV 3 was dominated by rainfall variables, with different variables being the strongest contributors depending on sex and form vs. shape. However, LV 3 only explained a minor fraction of total morphological variation (\sim 1–3%) in both the cranium and the mandible, especially for the current interglacial, for which only LVs 1 and 2 were relevant (as judged by scree plots).

Overall, how the environment has affected skull morphology, as evident from the loading patterns, was found to be highly consistent between the sexes and between the cranium and mandible for the current interglacial, as would be expected. Visual inspection of how specimens contributed to the environment-morphology associations along each LV (not shown) revealed that minor differences between the sexes can likely be partly explained by a few specimens from geographically peripheral locations that contributed strongly to the associations but which were unequally sampled among males and females. Such "peripheral" specimens notably include *M. f. aureus* from mainland SE Asia, *M. f. umbrosus* from the Nicobar islands, *M. f. condorensis* from Con Son Island (Vietnam), and *M. f. fascicularis* from the lesser Sunda islands (i.e., Bali, Lombok, Flores and Timor), all of which may be associated with more divergent climate values.

4. Discussion

4.1. Geography, taxonomy, phylogeny

We found no strong evidence that cranial and mandibular variation in *M. fascicularis* consistently tracks insularity caused by Quaternary sea level change. The poor morphological divergence and discrimination among macaques from almost all different island groups contradict our expectation that long-tailed macaques from different islands exhibit morphological divergence as a result of isolation during the Quaternary. Instead, the morphological overlap evident in our sample indicates connections between subspecies, with the relatively poor discrimination among specimens from different islands consistent with the history of Sundaland as a single landmass for large portions of the Quaternary. Although sample sizes are small, specimens of the three subspecies found on 'deep water' islands (M. f. umbrosa, M. f. fusca and M. f. tua, as identified by Fooden [1995]), generally fall within the M. f. fascicularis variation for cranial and mandibular form and shape. This indicates that even in subspecies endemic to islands that did not form part of the Sunda landmass during glacial periods, cranial and mandibular differences may not be related primarily to insularity. Indeed, consistent with Groves' (2001) observation on tail length, most cranial and mandibular size and shape variation among M. fascicularis subspecies is subsumed into the variation found within the most widespread subspecies, M. f. fascicularis. Similarly, our results concur with those of Insani and Takai (2020), whose M. fascicularis specimens (housed in Singapore and Indonesia) showed complete overlap between mainland and island populations along the first two principal axes describing cranial shape variation. These authors also did not find significant differences in cranial size between island-dwelling and mainland long-tailed macaques. Their work further elucidated the lack of such mainland-island distinctions in two other cercopithecid primates, Macaca nemestrina and Presbytis femoralis.

Subspecies of *M. fascicularis* are diagnosed largely on discontinuous soft tissue characters rather than hard tissue (Fooden, 1995; Groves, 2001). Within the African guenon tribe, there is



Environment LV 1

Fig. 5. Environmental latent variable (LV) 1 for cranial form and shape for the two interglacials and Last Glacial Maximum (LGM) obtained in reduced rank regressions (RRR). Grey bars represent females and checkered bars males, and the proportion of total cranial variation explained by LV 1 is displayed. This dimension, along which cranial morphology has a maximal regression slope on the environment, is characterised by a pronounced contrast between mean and minimum temperature and additionally, in form, a moderate contrast between annual and minimum rainfall variables for the current interglacial. Loading patterns for the other time periods deviate somewhat and exhibit some discrepancies between the sexes.

evidence that species diagnosed on the basis of soft tissue have distinctive cranial morphologies, indicating that reproductive isolation translates to hard tissue divergence even in ecologically similar and sympatric animals (Cardini and Elton, 2009). Studies of widespread, polytypic baboons (Papio hamadryas) and vervets (Chlorocebus aethiops) based on geometric morphometric data similar to those used in the present study indicate cranial differences among subspecies (Elton et al., 2010; Dunn et al., 2013). Like *M. fascicularis*, baboons and vervets evolved and diverged in the late Pliocene and Pleistocene (Dolotovskaya et al., 2017). Baboon and vervet subspecies may be more accurately viewed as separate species (Groves, 2001), in contrast to M. fascicularis, which has been retained as a single widespread species with multiple subspecies (Eudey et al., 2020). Comparison of the hard tissue patterns in baboons/vervets and *M. fascicularis* suggests that there has been little, if any, reproductive isolation between the M. fascicularis subspecies,

and reinforces our view that Quaternary sea level changes and resulting periods of insularity were not particularly influential in their divergence. It seems likely that gene flow occurred between many long-tailed macaque populations and subspecies during the Pleistocene, facilitated by the connected Sunda Shelf landmass.

Specimens from the Philippines, however, do appear more distinctive morphologically than others in our sample, and there is good statistical discrimination in shape and centroid size between *M. f. philippinensis* and *M. f. fascicularis*. This indicates reduced gene flow between populations in the Philippines and Sundaland. The Philippines is an archipelago, with a complex geographic history and biogeography largely unrelated to Sundaland (Heaney, 1986). Two long-tailed macaque subspecies are found in the Philippines and reflected in our sample: *M. f. fascicularis* on the westernmost portion of Mindanao, and *M. f. philippinensis* elsewhere, including Palawan as well as the main island of Luzon. Whereas a deep water



Environment LV 2

Fig. 6. Environmental latent variable (LV) 2 for cranial form and shape for the two interglacials and Last Glacial Maximum (LGM) obtained in reduced rank regressions (RRR). Grey bars represent females and checkered bars males, and the proportion of total cranial variation explained by LV 2 is displayed. This dimension, along which cranial morphology has the next maximal regression slope on the environment, is characterised by a decreased discrepancy between maximum and minimum temperature and secondarily by an increase in mean temperature and diurnal range for the current interglacial. Loading patterns for the other time periods deviate somewhat and exhibit some discrepancies between the sexes.

barrier separated most of the archipelago from Sundaland, Palawan was probably connected to it only during OIS 12 (~0.44 Ma) and OIS 16 (~0.63 Ma) (Piper et al., 2011; Robles et al., 2015), before or just at the likely arrival of *M. fascicularis* on the Philippines between 0.43 and ~0.06 Ma (Yao et al., 2017). Deep sea channels could thus have acted as a barrier to gene flow after the initial colonisation event over a landbridge or a shallow-water crossing. Fossil macaques are recovered from at least the terminal Pleistocene on Palawan (Ochoa et al., 2022), and a Middle Pleistocene arrival on the archipelago, indicating that morphological divergence of Philippine and Sundaland populations was relatively rapid, is supported by the high species-level endemicity of Palawan combined with the high proportion of genera shared in common with Borneo (Heaney, 1986; Piper et al., 2011). Such rapid divergence may not be unusual in island primate populations, as it is also evident, for example, in insular African red colobus monkeys (Cardini et al., 2007).

Darlington (1957) suggested human translocation of pigs, monkeys, and civets to explain their presence on Luzon. Our data support Fooden's (1991) suggestion that the presence of *M. f. fascicularis* on Mindanao is a much more recent event than the dispersal of *M. f. philippinensis*; the former is possibly a human translocation event. Thus, only the Philippines provides good evidence, in *M. f. philippinensis*, of insular craniometric divergence in our sample of Southeast Asian macaques. Interestingly, although Smith et al. (2014) suggested that *M. f. philippinensis* was not genetically different enough from *M. f. fascicularis* to warrant subspecific distinction, our results show that they are distinct in skull morphology. This adds weight to previous observations of distinct pelage color and molar morphology in *M. f. philippinensis* as a valid subspecies.

Consideration of the mtDNA and Y chromosome data in

conjunction with our morphological findings are complex to interpret, MtDNA data (Liedigk et al., 2015; Yao et al., 2017) indicate that the major divisions between M. f. fascicularis clades occurred substantially before the subsidence of the continental shelf and fluctuations in sea level that created islands in the Sunda region. Contrary to expectations, our cranial and mandibular morphological results do not mirror the mtDNA split: there is little cranial or mandibular distinction between Clades A and B, with Clade A being nested inside the Clade B variation, and Clade A specimens frequently misclassified as Clade B. This may suggest that common selective pressures have acted to maintain similarity of form across the two clades. In contrast, some splits within *M. fascicularis* seem likely to have occurred because of vicariance caused by the presence of physical barriers such as rivers and mountain ranges. This is certainly implicated in the divergence of M. f. aureus, the mainland subspecies found in Bangladesh, Lao PDR, Myanmar and westcentral Thailand, found to the west of high mountains that probably acted as a barrier to gene flow (Fooden, 1995). While skull form and shape may reflect deep phylogenetic divergence within female long-tailed macaques, namely between the Burmese long-tailed macaque (M. f. aureus) and the rest, increased sampling of the former is required to confirm this. Abegg and Thierry (2002) highlight morphological discontinuities in M. fascicularis taxa from deep water islands unlikely ever to have been connected to Sundaland, and suggest dispersal via rafting, which would have created reproductive isolation of peripheral populations. Unfortunately, our sample sizes for specimens from these islands are too small for rigorous analysis of hard-tissue divergence, but should suitable samples become available in the future, it would be a worthwhile investigation.

4.2. Climate

Our findings add to the complex picture of morphological differentiation in Southeast Asian mammals and the effects of the fragmentation of Sundaland, and provide further support to the need to look for explanations alternative to sea level changes for Pleistocene mammalian differentiation in the region. Morphological variation in our sample shows interesting correlations with climatic conditions today and in the past. Today, the climate of Southeast Asia is tropical, hot and humid, and affected by monsoon, the intertropical convergence zone (ITCZ) (Chuan, 2005), and ocean circulation and surface temperature around its many islands. Unsurprisingly over such a large area, topographic differences exist, with montane climates different to those of the lowlands, with the former tending to lower temperatures and less seasonal rainfall (Corlett, 2009). Temperatures are slightly cooler further north but overall are fairly homogeneous across Southeast Asia (National Oceanic and Atmospheric Administration, 2020). There is more variation in rainfall, with the Sunda Shelf being wetter than the mainland, and with some areas of Java and Borneo being extremely wet (National Oceanic and Atmospheric Administration, 2020). Indeed, equatorial Borneo and Sumatra have probably been wet since the late Miocene (Boyd and Peacock, 1986; Morley, 2012), and stable isotope data from western Sumatra may suggest that the region was even wetter during MIS 4 than it is today (Louys et al., 2022).

Per our expectations, and despite its relative homogeneity, modern temperature was associated with latent variable (LV) 1, as were levels of rainfall, having the largest effect on skull form (size and shape together). Compared to form, however, skull shape was even more strongly affected by the contrast between mean and minimum temperature along LV 1, while rainfall was less important, somewhat contrary to our expectations. Low annual rainfall was a key component of LV 1 for cranial form in the current interglacial, but elevation was not. In contrast to the pattern seen in some African monkeys, where smaller individuals are found in drier environments (Cardini et al., 2010), there is a significant correlation between smaller cranial centroid size and wetter environments in *M. fascicularis* (r = -0.44 and -0.26 between centroid size and minimum rainfall for females and males, resp.). The multiple multivariate regressions indicate that female long-tailed macaques track their environment more closely than males, possibly because they are more sensitive to patterns of resource distribution, supporting the notion of long-tailed macaque females as the ecological sex (Wrangham, 1980; Gordon et al., 2013). However, annual and minimum rainfall and rainfall seasonality were more important in cranial form of males than of females, indicating that precipitation may be important to size growth in males.

Bird et al. (2005), in their extensive review of late Pleistocene Sundaland palaeoenvironments, report that the region was probably 2–3 °C cooler by the LGM, with reduced but more seasonal rainfall. The climate data extracted for the localities used in this study confirm this for rainfall, with temperature differences between the present and the LGM being even a bit more pronounced (not shown). Rainfall reduction in the LGM may have varied across Sundaland (Bird et al., 2005). Coefficients of determination were fairly similar across the three time periods, but with the exception of female cranial shape and male mandibular shape in the LGM, we found a stronger association between present climate and skull form and shape, followed by LGM climate and skull form/shape. This could suggest that skull form and shape have responded to climate change since the LGM. However, recent climatic modeling (Hällberg et al., 2021) has indicated that the deglacial period following from the LGM was also considerably drier than today, suggesting that the current strong association between modern climate variables and skull morphology happened after this drier deglacial phase. While we expected elevation to have a strong influence on skull form, this was only observed during the LGM, and is perhaps related to altitudinal lowering of vegetation zones under glacial climatic conditions (e.g. Werner et al., 2018). However, we do not have accurate elevation data for our localities during the LGM and LI, and we used the same data for all three periods so that our regression models were directly comparable. Elevation may covary with climate, notably temperature. Such joint effects among predictors are normally accounted for in multiple regression, but due to the presumed reduced covariation between past temperatures and present elevation, it is possible that some residual effect of modern temperatures remains in the past climate models through elevation, causing the latter to act as a proxy for modern climate to some extent. The greater contribution of elevation to the LGM models may thus reflect the influence of modern climate on cranial morphology, which is in fact consistent with our finding that current interglacial mean temperature is a consistently important predictor of long-tailed macaque skull morphology.

Over much of Southeast Asia there is rainfall rather than temperature seasonality, although the northern parts of the region do experience subtropical climates with seasonality in both variables (Corlett, 2009). Seasonality strengthened in the Plio-Pleistocene compared to earlier periods, as witnessed by fluctuating periods of cooler/drier and warmer/wetter climate commensurate with glacial cycles (Morley, 2012). Seasonality is one of the explanatory variables in our models, but has a smaller effect on morphology in all time periods than several of the other environmental variables. By the Middle Pleistocene, millennia-long intervals of reduced monsoon rainfall were precipitated by a southern fixture of the ITCZ following deglaciations (Meckler et al., 2012; Cheng et al., 2016). This period sees the peak of savannah-adapted mammals, as recorded by stable carbon isotope ratios (Louys and Roberts, 2020). The subsidence of the Sunda Shelf from approximately 400 ka (Sarr et al., 2019) decelerated drying trends by markedly reducing exposed land and thus decreasing regional albedo, leading to increased atmospheric convection and regional rainfall (Di Nezio et al., 2016). Decreasing maximum peaks in oxygen isotopes from the Middle Pleistocene would have accelerated the shift towards a wetter climate further. By the LI, models indicate no temperature change or even a slight cooling in Southeast Asia ~125 ka compared to today, as a result of increased cloud cover, with the monsoon intensifying (Otto-Bliesner et al., 2013; Pedersen et al., 2017).

Contrary to our expectations, we did not find that the LI and the current interglacial are more similar to each other, in terms of impacting cranial form and shape, than the LGM, at least not in a consistent way. We can examine the effects of changing climates on macaque cranial shape and form by noting changes in loading polarity (i.e., changes in positive/negative loadings) in the first two LVs across sexes. The clearest signal is that climate loadings on female cranial shape did not significantly change between the current interglacial, the LGM, and the last interglacial. Female cranial form, on the other hand, shows more variation in environmental effects through time. This is consistent with the observations that skull form, which includes a size component, was increasingly strongly correlated with climate from the LI to the present, reflecting a certain lability of organismal size, whereas skull shape showed a more moderate and slower response to the environment. As size responds more readily to environmental change it thus tends to show different climatic signals across (inter) glacials with different climates. Beyond the globally consistent patterns across males and females, some differences between the sexes were observed. Male cranial form and shape showed several polarity changes through the different climatic regimes, with a lot less consistency in the climate loadings between the current interglacial, the LGM, and the last interglacial in males than females. Most of the polarity changes in male crania are between the LGM and the current interglacial, which accounted for minor portions of the total variation. These sex differences in loading polarity thus indicate subtle differences in how male and female skulls have responded to climatic parameters. For both female and male skulls, temperature is the predominant factor differentiating the three climatic periods we considered. For both sexes in the LGM and LI, precipitation generally makes a much smaller overall contribution to LV 1.

Our findings underline the complexity of morphological evolution in long-tailed macaques evident from consideration of previous studies, which identified no consistent association between climate and morphology. Notwithstanding its importance in determining the climate of Southeast Asia, we found that rainfall was less strongly and consistently associated with cranial and mandibular variation than temperature in long-tailed macaques. This contrasts with studies of African monkeys that highlight the generally greater influence of rainfall over temperature in skull morphology (Cardini et al., 2007; Cardini et al., 2010; Dunn et al., 2013). Our findings also deviate from those of a recent study of predominantly long-tailed macaques and other closely related species, which found a relationship between cranial shape and rainfall and temperature only outside the tropics and which did not detect a significant effect of rainfall and temperature on male cranial size (Arenson et al., 2022). Latitudinal trends in M. fascicularis skull length may not be linked to temperature gradients (Schillaci et al., 2009; Arenson et al., 2022) but our findings demonstrate that temperature is an important influence on morphology, even if that is not linked to geography. The slightly higher classification rates for subspecies based on cranial compared to mandibular morphology detected in our study may indicate that the cranium probably carries non-environmental signals, like population history, but the mandible and cranium had very similar coefficients of determination in the environmental analysis. This contrasts with studies that have documented phylogenetic signal and population history in various non-masticatory regions in the cranium, with comparatively stronger signals related to diet and climate observed in the mandibles of humans (e.g., Smith, 2009; von Cramon-Taubadel, 2011), non-human primates (Cardini and Elton, 2009), and other mammals (Caumul and Polly, 2005).

Clearly, a deeper understanding of morphological diversification in the skulls of M. fascicularis requires further investigation of contributory factors such as diet. Nutritional factors play a role in body size and growth differentiation in long-tailed macaques (Schillaci et al., 2007), and including diet in future analyses may well help to explain greater amounts of variance than environment alone. Long-tailed macaques are generalist feeders, subsisting on a variety of plant foods (including fruit) and animal matter (small vertebrates, insects and other invertebrates, such as crustaceans and molluscs) for the bulk of their diet (Gumert et al., 2017). However, geographical variation in the proportion of food types, which differ in physical properties such as hardness and toughness, as well as in the amount of feeding time has been recorded (Fooden, 1995; Gumert et al., 2017). Thus, future research on M. fascicularis morphological evolution would benefit from greater consideration of the role that the vegetation history of the region played in adaptation and differentiation. Environmental change, including shifts in rainforest distribution, has previously been cited as contributing to shape and size variation in macaque molars (Ingicco et al., 2014), suggesting the incorporation of fossil specimens in our study would provide further support for our findings. Today, Sundaland, and indeed Southeast Asia more generally, is characterised by rainforest, albeit with substantial anthropogenic disturbance (Corlett, 2009). The modern dominance of dense tropical canopies began after the mid-Brunhes event, a time when extensive areas of open forests were replaced by grassland, with savanna reaching a peak in the Middle Pleistocene (Louys and Roberts, 2020). These types of environments were largely absent during the later Pleistocene although very likely returned during the brief glacial periods of the late Pleistocene, most notably during the LGM (Heaney, 1991; Bird et al., 2005; Nguyen et al., 2022; Cheng et al., 2023) coinciding with changes in obliquity (McCarthy et al., 2022). By the Last Glacial Maximum (LGM, ~20 ka) localised vegetation shifts responding to climatic fluctuations may also have contributed to divergence of cranial size, but the proximal factors influencing skull morphology were impacted by longer-term environmental changes initiated earlier in the Pleistocene, namely, the increased dominance of rainforests. These environmental changes had impacts on the morphological divergence of macaques that bear little resemblance to modern coastlines and borders produced by rising sea levels since the LGM.

5. Conclusions

We carried out a geometric morphometric study of cranial and mandibular morphology in the widely distributed long-tailed macaque. There was no evidence of a relationship between morphological variation and island biogeography in Sundaland, contrary to previous work on some other primate and non-primate mammals. We detected an association between morphology and subspecific taxonomy among two major subspecies, Sundaic *M. f. fascicularis* and Philippines-restricted *M. f. philippinensis*. This illustrates concordance between divergence in soft tissue morphology, on the basis of which *M. fascicularis* subspecies have been described, and hard tissue morphology, at least when comparing Sundaic and non-Sundaic subspecies. Our results indicate, firstly, that geographical boundaries imposed by recent Quaternary sea-level changes are poor predictors of variation in skull morphology of long-tailed

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macaques, reflecting the history of Sundaland as a single landmass for much of the Quaternary. Secondly, systematic relationships of long-tailed macaques are not strong correlates of morphological variation, indicating that phenotypic similarity among clades may be enforced by common selective pressures.

Conversely, we found clear relationships between skull morphology and climate parameters, particularly between skull form and mean, maximum, and minimum temperature and annual and minimum rainfall. Skull shape was similarly predicted by temperature means, maxima and minima, as well as by diurnal range, but not rainfall, highlighting the role of rainfall parameters in influencing skull size. Moreover, females consistently seemed to track their environments more closely than males. Our analysis of phenotype-climate associations across the last three (inter)glacials of the last ~140 ka revealed that size-dominated skull form tracks climate more quickly than shape, being most clearly associated with the present climate, whereas major differences in skull shape seem to have arisen by the LGM in M. fascicularis. Nevertheless, cranial morphology in *M. fascicularis* is better explained by modern climatic conditions than those present during the LGM and LI. Taken together, our findings indicate that climate, and especially temperature, likely played a relatively prominent role in phenotypic divergence among long-tailed macaques and help to explain why previous studies have found mixed results with regard to the impact of biogeography on Sundaic mammalian diversification.

Author contributions

Nicole Grunstra and Sarah Elton conceived of the study, prepared the data and carried out the analyses; Nicole Grunstra, Julien Louys and Sarah Elton, interpreted the results and wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quascirev.2023.108121.

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