

1 **Multi-layered population structure in Island Southeast Asians**

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3 **Running title: Population structure in Island Southeast Asia**

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1 **ABSTRACT**

2 The history of human settlement in Southeast Asia has been complex and involved several
3 distinct dispersal events. Here we report the analyses of 1825 individuals from Southeast Asia
4 including new genome-wide genotype data for 146 individuals from three Mainland Southeast
5 Asian (Burmese, Malay and Vietnamese) and four Island Southeast Asian (Dusun, Filipino,
6 Kankanaey and Murut) populations. While confirming the presence of previously recognized
7 major ancestry components in the Southeast Asian population structure, we highlight the
8 Kankanaey Igorots from the highlands of the Philippine Mountain Province as likely the
9 closest living representatives of the source population that may have given rise to the
10 Austronesian expansion. This conclusion rests on independent evidence from various analyses
11 of autosomal data and uniparental markers.

12 Given the extensive presence of trade goods, cultural and linguistic evidence of Indian
13 influence in Southeast Asia starting from 2.5kya we also detect traces of a South Asian
14 signature in different populations in the region dating to the last couple of thousand years.

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16 **Keywords:** population genetics, Southeast Asia, India, Austronesian expansion, Kankanaey

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1 INTRODUCTION

2 Mainland (MSEA) and Island Southeast Asia (ISEA) are home to hundreds of different ethno-
3 linguistic groups each displaying a complex demographic history.¹ Previous studies have
4 revealed strong genetic correlations between populations which are geographically and
5 linguistically close and suggested a common origin of all Southeast Asian and East Asian
6 populations from a single migration wave.² It is well known, however, that in the more recent
7 past the populations living in this region have undergone major demographic changes,
8 particularly during the last five thousand years in association with the spread of the Neolithic
9 cultural complex and Austronesian languages.³ Wollstein and colleagues⁴ reported significant
10 genetic contributions from people currently inhabiting the Borneo (used as a proxy for Asian
11 influence) and Papua New Guinea islands into Malayo-Polynesians (Austronesians who
12 migrated beyond Taiwan) from Near and Remote Oceania. These admixture events were
13 dated to approximately 3 kya, consistently with similar population movements involving
14 people of Asian ancestry moving through ISEA dated around 4-3 kya.⁵ More recent studies^{6,7}
15 have distinguished at least three major ancestral components in MSEA and ISEA in
16 association with Papuan, Austro-Asiatic and Austronesian speaking populations. However the
17 analyses aiming to identify the likely source regions of these dispersals are confounded by
18 recent admixture in most modern ISEA populations with groups originating from other
19 regions including MSEA^{2,8} (see Text S1 for more details on the candidate populations
20 included in this study).

21 In addition to the migratory events involving South East Asian sources, more recent South
22 Asian influences in forms of cultural and trading networks, starting more than 2kya, in ISEA
23 and MSEA have been well established from historical and archaeological data.⁹⁻¹²

24 Exemplary for these developments are sites the sites of Khao Sam Kaeo and Phu Khao Thong
25 from Peninsular Thailand yielding archaeological evidence dating to 2.3-1.2kya.. They
26 confirm the earliest trade networks with India, which include rouletted ware, semi-precious

1 stone beads and artefacts, and Indian crops.¹³ In ISEA, one finds evidence of Indian trade
2 either directly or via peninsular Thailand. Coastal sites located in Northern Bali dating to 2.1
3 kya yielded pottery of East Indian or Sri Lankan production, gold and carnelian objects from
4 North India and mung bean.¹⁴ Furthermore epigraphy indicates a strong Indian impact on the
5 nascent political structures of the region¹⁵ and provides records of Brahmanic rituals and
6 animal sacrifices¹⁶.

7 Linguistic evidence also supports early interethnic contact between Indian and Southeast
8 Asian populations. Apart from the ubiquitous influence of Sanskrit¹⁷ where it is difficult to
9 distinguish ancient from more recent borrowings, analyses of the earliest Maritime Southeast
10 Asian literature demonstrate that it already exhibits signs of Tamil influence from South
11 India, much of which most likely spread across the region through pre-existing local
12 networks.¹⁸ Traces of paternal (Y chromosomes) and maternal (mtDNA) Indian ancestry have
13 been detected across several Indonesian islands at low frequency (<5%).¹⁹⁻²² The influx of
14 Indian ancestry is detectable in some genome-wide analyses of low density autosomal SNP
15 data² while being restricted to just a few populations from western Indonesia (Sumatra).
16 Contrarily to that, a more recent study²³ using medium density SNP data could not find a
17 South Asian genetic signature in South East Asia. The same authors however inferred gene
18 flow from the Indian sub-continent to Aboriginal Australian populations and dated it at
19 around 4kya. In the absence of a similar South Asian component in SEA this finding was
20 interpreted to require a direct sea route bypassing Southeast Asia to explain such a signature
21 in Australasia.

22 In order to refine the current understanding on the source of the Austronesian expansion and
23 to further explore potential South Asian genetic contributions in MSEA and ISEA, we
24 generated high density (730K) SNP Chip data for a panel of 196 individuals from 10
25 populations including 50 of which (from the Bajo and Lebbo populations) are published
26 already⁷ and 146 new (Burmese and Vietnamese from MSEA, Ilocano, Tagalog and

1 Kankanaey from the Philippines, Murut, Malay and Dusun from ISEA plus 4 Australian
2 Aborigines). We merged the newly generated dataset with those available from the literature
3 (cf. Material and Methods) and i) investigated the existence of signs of South Asian admixture
4 in our new SEA populations, ii) refined current knowledge on the putative source of the
5 Austronesian expansion; iii) determined the extent to which signs of local adaptation are
6 shared across local populations, as function of their common demographic history.

7

8 **MATERIAL AND METHODS**

9 **Samples, genotyping and phasing**

10 The newly generated dataset for this study consists of 150 individuals from 9 Southeast Asian
11 and one Australian population (Figure 1 and Table S10). DNA was extracted from saliva
12 samples collected from healthy adult donors who signed an informed consent form. The study
13 was approved by local Research Ethics Committees (SingHealth Centralised Institutional
14 Review Board and the Medical and Health Research Ethics Committee of the National Cancer
15 Centre, Brunei Darussalam), the Cambridge Ethics Committee (HBREC.2011.01) and the
16 ERC Ethics Panel. Southeast Asian samples were genotyped using Illumina OmniExpress
17 Bead Chips for 730 525 SNPs. They are accessible together with 50 Bajo and Lebbo samples
18 under the GEO accession number GSE77508. For the three Australian samples the Illumina
19 Human 660K Quad Bead Chip yielded 655 215 SNPs, while for one Australian the 610K
20 version of the latter chip gave 616 795 variants. These four samples are accessible under the
21 accession number EGAS00001001738 in the European Genome-phenome Archive.

22 Before the analyses as such data filtering and quality checks using PLINK 1.07²⁴ were
23 performed. Firstly, only autosomal SNPs with a genotyping success rate greater than 98%
24 were included. PLINK was also utilized to remove all individuals more closely related than
25 first degree cousins. This was done by estimating pairwise identity by descent (IBD)

1 iteratively; individuals with an IBD > 0.125 were excluded. Following these quality controls
2 haplotypes were inferred from genotype data with SHAPEIT.²⁵
3 Furthermore, 8 full mitochondrial Kankanaey genomes were sequenced by Complete
4 Genomics (Mountain View, California, USA) using CG software version 2.4. Access to the
5 sequences is provided under the GenBank accession numbers KU752558 to KU752565. All
6 novel data from this paper will also be available under www.ebc.ee/free_data in the PLINK
7 (genotype data) and fasta (mitochondrial genomes) formats respectively.

8

9 **Demographic analyses**

10 To get a first overview for the novel Southeast Asian data we merged them with four
11 reference populations from the HapMap 3 panel²⁶ and the HGDP Papuans²⁷ to obtain a set of
12 307 625 common SNPs. Runs of Homozygosity (rOH), average observed heterozygosity and
13 IBD were obtained using PLINK default parameters. Furthermore pairwise F_{ST} was calculated
14 using an *ad hoc* Perl script implementing an estimator for Wright's formula.²⁸

15 To address more specific questions regarding the ancestries of our novel populations we
16 performed two distinct ADMIXTURE^{29,30} analyses. For comparative purposes publicly
17 available genotype data from the HapMap²⁶, HDGP²⁷ and the Pan-Asian Consortium² projects
18 was added to 185 individuals from 9 SEA populations (the divergence from the original
19 number of 196 is due to the removal of close relatives). Additionally we used SNP data from
20 studies focused on Indian populations.^{31,32} This resulted in a dataset consisting of 1099
21 individuals.

22 For further verification of our ADMIXTURE analysis, we assembled a second panel of 1010
23 samples including 187 samples from our 9 SEA populations, and 4 Australian Aborigines,
24 which are newly reported here. The samples, populations and references for both analyses are
25 listed in Table S10. A detailed description of the merging and data curation for ADMIXTURE
26 can be found in the Text S2.

1 Effective population size for our 9 SEA populations was estimated by analyzing LD patterns
2 with the NeON R package.³³ To further investigate genetic structure and gene flow between
3 populations we used the TreeMix v1.1 software package.³⁴ To measure how well the trees
4 with different numbers of migration events (N) reflect the relationship between population
5 groups we calculated the fraction f of explained variance as described by the original authors
6 of the method. We used MEGA v6.0.6³⁵ to create a graphic representation of the TreeMix
7 output. For specific admixture events of interest suggested by the ADMIXTURE plots the
8 respective sets of recipient and source populations were tested with the three populations test
9 (f_3).^{34,36} The population trios yielding a Z-score smaller than -2 were considered significantly
10 admixed. These were then analyzed with ALDER³⁷ to date the putative admixture event.
11 Furthermore we used the f_4 -ratio test³⁸ to obtain a quantitative estimate of admixture
12 percentages of interest.

13 For the analysis of the mtDNA data the haplogroup affiliation of each sample was assigned
14 using HaploGrep 2.0³⁹ and PhyloTree build 16 (as of 19/02/2014)
15 (<http://www.phylotree.org>).⁴⁰ The variants are described relative to the rCRS (GenBank
16 Accession Number NC_012920.1).⁴¹

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19 **Selection tests**

20 To capture haplotype homozygosity based signals the Integrated Haplotype Score (iHS)⁴² and
21 Cross Population Extended Haplotype Homozygosity (XP-EHH)⁴³ tests were used. Both the
22 iHS and XP-EHH statistics were calculated as in Pickrell et al. (2009)⁴⁴, yielding about 10
23 000-11 000 genomic windows for iHS and about 13,700 windows for XP-EHH for each SEA
24 population analyzed. From the top 1% of all iHS signals, putatively the strongest candidates
25 for selection, windows present in the top 5% iHS windows of the CHB population from the
26 Hap Map panel were excluded, to pick up only signals particular to SEA. However, for the

1 analysis of regional sharing patterns based on the iHS this condition did not apply. For the
2 XP-EHH the use of a reference population is inherent in the method, again CHB was chosen,
3 for similar reasons.
4 Furthermore, we computed the allele frequency based Population Branch Statistic (PBS). This
5 test statistic represents the amount of allele frequency change at a given locus in the history of
6 the test population since it diverged from other populations.⁴⁵ The outgroups for each tested
7 SEA population were the YRI and CHB populations. Pairwise F_{ST} values for the populations
8 of interest and the references were calculated following Weir and Cockerham.⁴⁶ PBS scores
9 were estimated from the pairwise F_{ST} values.⁴⁵ Based on the approach of Pickrell et al.
10 (2009)⁴⁴ the genome was divided into windows of a modified size of 100kb and the
11 maximum PBS score in each window was used as the test statistic. This resulted in between
12 26 000 and 27 000 windows for each analyzed group.

13

14 **RESULTS**

15 To investigate general patterns of population structure in our data we performed two distinct
16 ADMIXTURE analyses: the first was mainly focused on populations from Southeast Asia and
17 South Asia while the second provided the context of a broader, worldwide genetic landscape
18 and additional validation for inferences from the first analysis.

19 According to the cross-validation scores for both analyses $K=9$ admixture fractions provide
20 the best fit (for the local plot additional K s are provided in Figure S2, for the global plot K s
21 from 3 to 15 are shown in Figure S3B). The ADMIXTURE analyses of the newly generated
22 data (Figure 1A, Figure S1) recapitulate the main ancestral components associated with
23 Austronesian (k_6), Austro-Asiatic (k_5) and Papuan (k_3) populations (Figure 1, Figure S2)
24 already described in the area by previous studies.^{5,6} At lower K values the component
25 associated with the Papuans is highly prevalent in Eastern Indonesia and the Mamanwa (a

1 Negrito group from the Philippines) while at higher values it continues to persist only in the
2 Alorese and Bajo from Indonesia (Figure 1B, Figure S2).

3 Burmese and Vietnamese exhibit significant proportions of the k2 component indicating
4 shared ancestry with East Asian populations. The k4 component associated with South Asian
5 ancestry is also consistently visible in Burmese and Malays (this study) and some Indonesian
6 populations, mainly the Batak of Sumatra.² However at lower Ks this component is also
7 present in the Javanese and the Mamanwa Negritos, suggesting affinities which however
8 decline with higher Ks (Figure 1B, Figure S2). Notably, in the extended worldwide analysis
9 (Figure S3B) the Papuan-related component (red) in the Bajo and the South Asian signal
10 (green) in the Burmese and Malays were also clearly detectable. The SEA groups described
11 here exhibit a remarkable diversity from very heterogeneous groups such as the Malays to the
12 Kankanaey who appear homogenous in their ancestry composition by the ADMIXTURE
13 analyses (Figure 1B, Figure S2).

14 The Kankanaey are an indigenous population of northern Luzon, belonging to the broader
15 “Igorot” group. At K=9, the majority of Kankanaey ancestry is in the k6 component, which
16 they share with the Ami (AX-AM) and Atayal (AX-AT) from Taiwan and, hence, is
17 putatively associated with the Austronesian expansion (Figure 1A, Figure S2). When it
18 emerges as distinct from the other Asian components, the k6 brown ancestry is spread
19 throughout ISEA and remains stable in all these groups from K8-10 (Figure 1B, Figure S2).

20 Remarkably in the regional admixture plots the Kankanaey remain unadmixed throughout all
21 Ks from 2-10 (Figure S2), even though at lower Ks they do not yet have their own distinct
22 component. These findings are consistent with the Kankanaey’s geographic location, the
23 Mountain Province in the Northern Philippines (Figure 1A, Figure S1), close to Taiwan, the
24 likely center of the Austronesian expansion.^{3,6}

25 Kankanaey genome wide heterozygosity levels and extent of runs of homozygosity (rOH)
26 (Table S1) rule out potential confounders such as extreme inbreeding or genetic drift being

1 causative for their unusually homogeneous ancestry. To further explore the potential effect of
2 demographic history on population structure we estimated the effective population size of the
3 nine SEA populations presented here based on the development of linkage disequilibrium
4 (LD) patterns over time (Figure S4).³³ The mainland Burmese and Vietnamese groups exhibit
5 comparatively high effective population sizes and signs of recent expansion. This is in line
6 with their recent history of admixture with neighboring populations, whereas there is more
7 variation in the ISEA populations. Notably the Kankanaey have one of the lowest values
8 varying between 2000-3000 (6000-27000 kya). However they are not an extreme outlier and
9 are comparable to the Lebbo from Borneo (no significant difference, $p = 0.7938$), who instead
10 do not show such a homogeneous ADMIXTURE profile. Under the assumption that the
11 brown k6 component reflects ancestry connected to the Austronesian expansion, the
12 Kankanaey displayed a higher percentage of it than even Austronesian Taiwanese populations
13 (AX-AT, AX-AM, Figure 1A, Figure S2). The affinity of the Kankanaey to these groups was
14 supported by the TreeMix³⁴ analyses of 25 populations (Figures S5-S6) where the Kankanaey
15 did not cluster with other Filipinos but rather with the Taiwanese aboriginals.

16 The emerging picture seems to be compatible with a scenario of local Austroasiatic and
17 Papuan components influenced by the incoming Austronesian (brown k6, Figure 1A, Figure
18 S2) wave 4-3kya which originated from a population living in Taiwan and, perhaps, in the
19 North Philippines.⁶ The attempt to date the above admixture events using ALDER³⁷
20 highlighted a clear admixture pattern between “Kankanaey like” people and earlier substrates,
21 dated to at least 2.2 kya in the Bajo (Table 1).

22 These affinities of the Kankanaey and their potential role as a good proxy for the
23 Austronesian expansion are further highlighted when looking at uniparental markers. The
24 eight available Kankanaey mtDNA sequences (Table S2) exhibit lineages (B4a1a;M7b1a2a1)
25 which are typical markers of Malayo-Polynesian speaking populations.^{47,48}

1 Lastly, the Kankanaey cannot be modeled as any kind of mixture from 46 populations using
2 the f_3 statistic (Table S3).³⁶ Taken together, the evidence from these independent approaches
3 suggests that the Kankanaey could potentially represent an unadmixed remnant population
4 close to the source that may have given rise to the Austronesian expansion.

5 We also utilized the f_3 test together with ALDER to further contextualize the potential South
6 Asian connections of some SEA groups. Both of these statistics (Table 1) suggest the
7 presence of variable degrees of South Asian-related ancestry in the MSEA and ISEA
8 populations (Bajo, Burmese, Filipino and Malay). Assuming a generation time of 30 years⁴⁹
9 the earliest possible midpoint of the South Asian admixture is estimated at 2.4 kya. The
10 overall proportion of South Asian ancestry was further estimated by applying the f_4 statistic³⁸
11 (Table S4) according to the tree presented in Figure S7. The estimated values were 24.9% for
12 the Burmese, 8.3% for the Malays and 5.3% for the Bajo. One limitation of this approach is
13 its dependence on shared genetic drift. As the Papuans and South Indians have a similar
14 position in the phylogenetic tree relative to the other groups, Papuan ancestry could be
15 mistaken as South Indian. This has probably no effect in the Burmese and Malay, who do not
16 show Papuan admixture (Figure 1A, Figure S2) but could contribute to the South Indian
17 ancestry detected in the Bajo. True Indian ancestry in this population still seems conceivable
18 given the presence of South Asian lineages in uniparental marker analyses²²

19 These analyses indicate a South-Asian related component in the genetic make-up of at least
20 some SEA groups which entered their gene pool ca. 2.4 kya ago, being supported by
21 ADMIXTURE, f_3 and f_4 analyses for the Burmese and the Malay and by f -statistics for the
22 Bajo (f_3 , f_4) and the lowland Filipinos (f_3).

23 As an additional tool to explore relationships among populations, we examined patterns of
24 haplotype homozygosity and allelic differentiation using test statistics iHS ⁴², $XP-EHH$ ⁴³, and
25 PBS test⁴⁵ (Tables S7, S8, S9). For the iHS the amount of signal sharing between two groups
26 correlates only very weakly ($r^2=0.041$ for a linear regression) to overall genetic similarity as

1 expressed by the F_{ST} (Figure S8). However, the MSEA groups and the Han Chinese (included
2 as a reference) who share a considerable proportion of East-Asian ancestry (Figure 1A, Figure
3 S2) also show a great affinity to each other regarding haplotype homozygosity patterns (Table
4 S5). In ISEA those groups with at least three significant ancestry components at $K=9$ (Bajo,
5 Filipino, Malay, Figure 1A) exhibit more signal sharing. In contrast, Kakanaey, Lebbo and
6 Murut show reduced sharing with all other populations, which perhaps highlights phenomena
7 of deep population splits and separate demographic histories in recent times when the
8 haplotype homozygosities have accumulated.

9 However, these inferences are highly dependent upon the approach utilized. A different
10 picture presents itself for the XP-EHH, which considers both haplotype homozygosity and
11 allelic differentiation, with the Han Chinese used as outgroup. The average fraction of signal
12 sharing declines from 0.31 to 0.22, while the correlation with the F_{ST} increases considerably
13 ($r^2= 0.256$). This is probably because signals connected to shared ancestry with East Asians
14 are excluded. It causes the Burmese, who exhibit a large fraction of the $k2$ East Asian-related
15 component (Figure 1A, Figure S2) to become an outlier especially with respect to their high
16 fraction of unique top 1% XP-EHH signals, only 15% of which are shared with other groups
17 on average.

18

19 **DISCUSSION**

20 In this study we set out to explore population structure in MSEA and ISEA and more
21 specifically, to clarify the exact nature of South Asian gene flow into SEA and the presence of
22 potential un-admixed Austronesian population(s) close to the ancestral Austronesian source.

23 We detected a minor South Asian component in our ADMIXTURE analyses in MSEA and
24 ISEA populations (green $k4$, Figure 1A, Figure S2; green, Figure S3B) which was further
25 confirmed by $f3$, $f4$ and ALDER results and dated to have entered SEA from 2.4kya (Table
26 1). While this component is more widespread at lower Ks (Figure 1B, Figure S2) at the best

1 K=9 (Figure 1A) the evidence is strongest for the Burmese and the Malay and somewhat
2 weaker for Bajo and Filipinos, where it is limited to the f statistics (Table 1, Table S4). It is
3 important to explore how these results relate to the linguistic and archaeological evidences,
4 attesting a continuous presence of South Asian cultures in Southeast Asia since 2.5
5 kya.^{12,17,50,51} This should be done keeping in mind that in the majority of SEA populations the
6 Indian component is absent or below the scale of a potential error and detectability. Firstly, it
7 is most likely that the “carriers” of South Asian culture were traders, artisans⁵⁰ and at a later
8 date, religious scholars (Brahmins) who were influential as advisers to Southeast Asian rulers.
9 Some of these might have been locals educated in India who brought home Sanskrit texts and
10 Brahmanic rituals.⁵² So this rather small group would not have left a major genetic signature.
11 Secondly, the epigraphic record and evidence from monumental archaeology during the late
12 first millennium CE attests that the Indian presence is biased towards courts and generally
13 higher social strata, which can lead us to overestimate the impact on the majority of the
14 population.⁵² More generally speaking there are a wide range of scenarios relating to the
15 spread of cultural elements and gene flow and the patterns of this relationship are highly
16 complex to model (cf. the example of the Neolithization in Europe⁵³). So with the exception
17 of the Burmese, who are also geographically very close to the Indian subcontinent, the
18 evidence points to rather minor Indian gene flow, in contrast to the documented cultural
19 influence which, however, overlaps with the admixture range dated with ALDER (Table 1).
20 This low South Asian gene flow was however also detected in some other populations across
21 ISEA.^{2,19-22} Taken together these findings suggest Southeast Asia as a potential waypoint for
22 the reported South Asian migration into Australasia which was disputed by the authors who
23 proposed this migration event.²³ However the date obtained using ALDER (2.4 kya) is at least
24 1500 years posterior to the reported South Indian migration into Australasia.²³ A preliminary
25 conclusion would envisage the SEA and Australasia migrations as two separate events.
26 Besides the fact that the dating methods were different in our case and Pugach et al. (they

1 used a method based on wavelet transform analysis) at least two caveats can be brought up to
2 reconcile this fragmented scenario. Given the evidence presented here, it seems reasonable to
3 assume a constant gene flow from South Asia into SEA via land, with Australasia being only
4 a sporadic end-point. In this case the 4kya estimate provided by Pugach and colleagues would
5 be a point estimate of the sparse arrival into Australasia, while our ALDER estimate should
6 be interpreted as the midpoint³⁷ of such a flow between 4kya and more recent times.
7 Secondly, given the surprising concordance of linguistic and archaeological evidences for a
8 South Asian presence in SEA around 2.5 kya, one could imagine a particularly intense
9 corresponding gene flow during that time further biasing the ALDER estimate toward this
10 period.

11 In this study we have identified the Kankanaey from the northern Philippines as the
12 population harboring the highest reported amount of the Austronesian genomic component,
13 even higher than the ones detectable in modern aboriginal Taiwanese (Figure 1B, Figure S2).
14 This conclusion rests on evidence from several independent analyses including
15 ADMIXTURE, f3, rOH, TreeMix, N_e and uniparental markers.

16 The Kankanaey belong to the broader group of populations collectively known as Igorot
17 (Text S1). Various studies exist on the Kankanaey language⁵⁴ and customs⁵⁵, although works
18 on their prehistory are lacking. Genetic data from 30 Kankanaey-speakers was included in a
19 recent study of the mtDNA-haplotype-diversity in the Philippines.⁵⁶ There they were shown to
20 share many lineages with two other Igorot groups (Ibaloi and Ifugao) from Northern Luzon.
21 These results are broadly consistent with the uniparental data we present here (Table S2),
22 where the Kankanaey show haplotypes also found in Taiwanese aboriginals⁵⁷ and generally
23 associated with the Austronesian expansion^{47,48}. We conclude that the Kankanaey are either
24 the best preserved source of the Austronesian expansion, or a case of total replacement that
25 followed it. The dominant model suggests a southward diffusion of Austronesians from
26 Taiwan around 4000 BP, which impacted the Philippines, the north of Borneo and Sulawesi

1 between 3800–3600 BP, and later spread into the Pacific.³ Even if the modality of this
2 expansion is complex and still debated⁵⁸, the location of the Kankanaey in the northern
3 Philippines, close to Taiwan, suggests that they may be considered as one of the least admixed
4 living groups tracing their ancestry from the source populations of the Austronesian
5 expansion. Furthermore we confirm the finding of an Austro-Asiatic-related component in
6 ISEA populations (here the Dusun, Murut, Lebbo and Bajo) first reported by Lipson et al. in
7 2014⁶ and there described as unexpected due to the historically nearly exclusive presence of
8 Austro-Asiatic speakers on the mainland. Given its wide spread in MSEA and ISEA in
9 linguistically diverse groups, the explicit association of k5 with this language family should
10 be taken with caution. However, it is worthwhile noting that in India we find this component
11 specifically in Munda speaking populations. The k5 component could represent an ancestral
12 substrate, which was once distributed widely throughout SEA and was encountered by the
13 Austronesians when they spread from Taiwan. Another possibility is that there was an early
14 split into several subgroups during the Austronesian expansion and that this component
15 belongs to the ancestral make-up of a subgroup of Malayo-Polynesians who expanded into
16 western Indonesia.

17 Our comparison of haplotype-based scans of positive selection revealed that compared to
18 earlier studies on a continental level³² in a regional context in ISEA there is no good
19 correlation between haplotype sharing patterns and genetic distance as indicated by the F_{ST}
20 (Figure S8). However, as described above, the haplotype homozygosity patterns still reflect
21 demography to a considerable extent. Populations showing more diversity in the admixture
22 plots also exhibit higher levels of shared signals with other groups. Furthermore, the sharing
23 patterns proved to be very dependent on the kind of test utilized. Notably when the XP-EHH,
24 which uses the Han Chinese as outgroup, is applied, all signals shared with East Asians are
25 excluded. Intriguingly this causes the Burmese whose ancestry contains a significant South

1 Asian-related component (Figure 1A, Figure S2) to become an outlier (Table S6) potentially
2 reflecting haplotype homozygosity signals unique to their share of Indian ancestry.
3 In conclusion, we report a minor South Asian contribution to the genomes of some modern
4 MSEA and ISEA populations, mainly the Burmese and the Malay. This is in line with a
5 general cultural diffusion process to SEA, driven by smaller groups of influential individuals
6 from South Asia. Secondly, our work strongly suggests that based on the currently available
7 data the Kankanaey tribal group from Northern Luzon, Philippines are the best genetic
8 representative of the Austronesian expansion. We envisage high coverage whole genome
9 sequencing of this population as a sound approach to further explore this major peopling
10 event that shaped the genetic landscape of the broader South East Asia region.

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13 **Acknowledgements**

14 This work was supported by the European Research Council Starting Investigator grant FP7-
15 261213 to T.K. This work was supported by the French ANR grant number ANR-14-CE31-
16 0013-01 (grant OceoAdapto to F-X.R.), the French ANR-12-PDOC-0037-01 (grant
17 GENOMIX to D.P.), the Region Aquitaine of France (grant MAGE to T.L.), the French
18 Ministry of Foreign and European Affairs (French Archaeological Mission in Borneo
19 (MAFBO) to F-X.R). T.A. was supported by a Wellcome Trust Post-Doctoral fellowship
20 (WT1000MA).

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22 **CONFLICT OF INTEREST**

23

24 The authors declare no conflict of interest.

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26 Supplementary Information is available at the European Journal of Human Genetics' website.

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21 **Figure Legends**

22
23 **Figure 1:**
24 **(A) A map of Southeast Asia, displaying a subset of populations assessed in this study**
25 **and the distribution of ancestry components based on the local ADMIXTURE run with**
26 **the optimal number of ancestry components (K=9, cf. Figure S2). The figure legend on**
27 **the lower left section shows the list of genetic ancestry components whose color codes**
28 **correspond to those on the pie charts. Components k8 and k9 are mainly present in the**
29 **Yoruba and Ati Negritos respectively and do not significantly contribute to the genetic**
30 **diversity of the groups displayed in Figure 1.**

1 **The population abbreviations are as follows: Alo-Alorese, Baj-Bajo, Bat-Batak,**
2 **Bru-Brunei (Dusun, Murut), Bur- Burmese, CHB-Chinese from Beijing, Jav-Javanese,**
3 **Kan-Kankanaey Igorots, Leb-Lebbo, Mal-Malay, Mam-Mamanwa Negritos, Men-**
4 **Mentawai, Mun-Mundari, NIn-North Indians, Pap-Papuans, PhU-Philippine Urban,**
5 **SIn-South Indians, Taw-Ami and Atayal from Taiwan, Viet-Vietnamese.**
6 **Note that the symbols next to the population names reflect the linguistic affiliations.**
7 **Austro-Asiatic languages: circle, Austronesian languages: asterisk, Indo-European**
8 **languages: square, Dravidian languages: hash, Papuan languages: cross, Tibeto-Burman**
9 **languages: caret.**
10 **(B) Three graphs showing the proportions of ancestry components k3, k4 and k6 from**
11 **their emergence as independent components in the Papuans (k3, red), Indian**
12 **populations (k4, green) and the Kankanaey Igorot (k6, brown) across multiple higher K**
13 **values. All populations displayed show a percentage of at least 5% of the respective**
14 **ancestry when it emerges.**