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## The Australian Barrineans and Their Relationship to Southeast Asian Negritos: An Investigation using Mitochondrial Genomics

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# The Australian Barrineans and Their Relationship to Southeast Asian Negritos: An Investigation using Mitochondrial Genomics

## **Abstract**

The existence of a short-statured Aboriginal population in the Far North Queensland (FNQ) rainforest zone of Australia's northeast coast and Tasmania has long been an enigma in Australian anthropology. Based on their reduced stature and associated morphological traits such as tightly curled hair, Birdsell and Tindale proposed that these "Barrinean" peoples were closely related to "negrito" peoples of Southeast Asia and that their ancestors had been the original Pleistocene settlers of Sahul, eventually displaced by taller invaders. Subsequent craniometric and blood protein studies, however, have suggested an overall homogeneity of indigenous Australians, including Barrineans. To confirm this finding and determine the degree of relatedness between Barrinean people and Southeast Asian negritos, we compared indigenous Australian mitochondrial DNA (mtDNA) sequences in populations from the FNQ rainforest ecozone and Tasmania with sequences from other Australian Aboriginal populations and from Southeast Asian negrito populations (Philippines Batek and Mamanwa, and mainland Southeast Asian Jahai, Mendriq, and Batak). The results confirm that FNQ and Tasmanian mtDNA haplogroups cluster with those of other Australian Aboriginal populations and are only very distantly related to Southeast Asian negrito haplogroups.

## **Keywords**

Barrineans, Negritos, mtDNA, Trihybrid Thesis, Sahul, Australian Aborigines, Sahul, Phylogeography

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## **Brief Communication**

### ***The Australian Barrineans and Their Relationship to Southeast Asian Negritos: An Investigation using Mitochondrial Genomics***

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*Abstract* The existence of a short-statured Aboriginal population in the Far North Queensland (FNQ) rainforest zone of Australia's northeast coast and Tasmania has long been an enigma in Australian anthropology. Based on their reduced stature and associated morphological traits such as tightly curled hair, Birdsell and Tindale proposed that these "Barrinean" peoples were closely related to "negrito" peoples of Southeast Asia and that their ancestors had been the original Pleistocene settlers of Sahul, eventually displaced by taller invaders. Subsequent craniometric and blood protein studies, however, have suggested an overall homogeneity of indigenous Australians, including Barrineans. To confirm this finding and determine the degree of relatedness between Barrinean people and Southeast Asian negritos, we compared indigenous Australian mitochondrial DNA (mtDNA) sequences in populations from the FNQ rainforest ecozone and Tasmania with sequences from other Australian Aboriginal populations and from Southeast Asian negrito populations (Philippines Batek and Mamanwa, and mainland Southeast Asian Jahai, Mendriq, and Batak). The results confirm that FNQ and Tasmanian mtDNA haplogroups cluster with those of other Australian Aboriginal populations and are only very distantly related to Southeast Asian negrito haplogroups.

The existence of a distinct population of short-statured Aboriginal inhabitants of the Far North Queensland (FNQ) rainforest ecozone (the 4,600 km<sup>2</sup> area between Mossman, Cardwell, and the Atherton Tablelands) was first noticed by travelers on Captain Cook's exploratory voyage in the 1770s (Parkinson 1984). It was not, however, until the Harvard/Adelaide Museum anthropological expedition of

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KEY WORDS: BARRINEANS, NEGRITOS, MTDNA, TRIHYBRID THESIS, SAHUL, AUSTRALIAN ABORIGINES, SAHUL, PHYLOGEOGRAPHY.

Norman Tindale and Joseph Birdsell visited the area in 1938 that anthropological science officially recorded their existence. Tindale and Birdsell (1940), after taking anthropometric measurements and oral history from some 600–700 people at two FNQ missions, Yarrabah and Mona Mona, confirmed that the “pygmy” Aboriginal people there were indeed marked by much shorter stature than other Aboriginal people and by features such as lighter (though still black) skin and “frizzly” [*sic*] hair. Describing these traits as “negrito” features, Birdsell (1949) theorized that the “Barrinean” people (named after nearby Lake Barrine) represented the remnants of a primordial “pygmy” settlement of Sahul, whose closest affiliations lay with other negrito peoples in Southeast Asia. This colonizing wave, Birdsell wrote, had been followed by secondary and tertiary waves of taller “Murrayians” and “Carpentarians,” accounting for what he called the trihybrid nature of Australian Aboriginal people. Based on historical accounts describing allegedly similar physical features among Tasmanian Aboriginal people, Birdsell also included them in the “Barrinean” category.

Research subsequent to Birdsell and Tindale’s work, however, has cast doubt on their identification of a distinct population of Barrinean negritos, instead emphasizing the homogeneity of Australian Aboriginal peoples. A craniometric survey by Larnach and Macintosh (1970), for example, was unable to distinguish skulls from the Queensland rainforest zone from other Australian crania. Classical blood group marker studies similarly failed to support a separate, non-Aboriginal negrito origin for Barrineans (Graydon et al. 1958; Balakrishnan et al. 1975; Kirk 1983). Such evidence has led most researchers to abandon Birdsell and Tindale’s Barrinean classification in favor of a shared Aboriginal Australian origin for the FNQ and Tasmanian rainforest populations and an adaptive explanation of their morphology. No genetic analyses, however, have yet been undertaken.

Accordingly, we compared Aboriginal mitochondrial DNA (mtDNA) from FNQ samples volunteered for the National Geographic Genographic Project and published Tasmanian Aboriginal sequences with those of several Southeast Asian negrito and other Australian Aboriginal groups. mtDNA is commonly used in human ancestry studies because its haploid nature ensures that it does not lose mutations via recombination and hence accurately traces the maternal line of descent (Chinnery et al. 1999). In common with standard procedures for mtDNA ancestry studies, our analysis focused on hypervariable segments 1 and 2 (HVS1 and HVS2), where available, using these sequences to generate individual haplotypes. In addition, specific-coding-region single nucleotide polymorphisms (SNPs) were typed to allow, in combination with the HVS haplotypes, identification of particular mtDNA haplogroups. These haplotypes and haplogroups were then compared with published mtDNA data on the groups targeted for comparison: Southeast Asian negritos and non-FNQ and non-Tasmanian mainland Australian Aboriginal populations.

Research on Australian mtDNA lineages to date has established that all seem to be distinct localized variants (usually unique to Australia but sometimes shared with Papua New Guinea, Island Melanesia, and the Philippines) of macrohaplogroups M, N, and R, such as M42a, S and O, and P4 and P8 (van Holst

Pellekaan et al. 1998, 2006; Ingman and Gyllensten 2003; Hudjashov et al. 2007). One Q haplogroup, distinct from those reported from Papua New Guinea, has also been found by Hudjashov et al. (2007). Within these few haplogroups, however, Australian Aboriginal populations show a very high level of diversity and great time depth.

Studies of Southeast Asian negrito mtDNA sequences, on the other hand, show equal diversity but across many more haplogroups. Hill et al. (2006), for example, found novel M, N, and R lineages among mainland Southeast Asian Semang populations but also low levels of the Asian-specific B haplogroup, most probably from introgression. Scholes et al. (2011) also found multiple novel variants of the basal M, N, and R macrohaplogroups among Batak negritos of Palawan in the Philippines but also varieties of the Asian-specific F haplogroup, as well as haplogroup Y. Heyer et al. (this issue) have likewise reported M7 lineages among Aeta and Agta negritos and N11 among Mamanwa negritos but also lineages from the B, E, F, Y, and D haplogroups. For the purposes of this analysis, Asian-specific haplogroups such as these last few have been removed from consideration since by definition they do not show any relationship with Australia (where they have not been found to date) and do not appear among the Barrineans. Interestingly, however, Heyer et al. also report P10 haplogroups among the Aeta and Agta, and P9 among the latter, and this sharing of haplogroup P with Australia and Papua New Guinea is considered below.

One caveat to our study, raised by Heyer et al.'s (this issue) example, is the problem of potential sex bias in an exclusively mtDNA survey. For example, Heyer et al. found very different ratios of male to female effective population sizes in the Aeta and Agta negrito populations they sampled, possibly due to differing rates of introgression of Austronesian mitochondrial lineages. Conversely, the well-established "conquerors' signature," where male effective population size is dramatically reduced through the extermination of indigenous Y-chromosome lineages by a few invading ones, can also reveal demographic processes inaccessible to/through an mtDNA analysis. This, however, is precisely the problem for this study: Y-chromosome data for FNQ or Tasmanian populations is difficult to find because most such lineages have been replaced by European and other Y-chromosome lineages, as has also occurred in other Australian Aboriginal populations over the past 150 years (Taylor et al. 2012). This does mean, however, that this study relies more than would be ideal on information encoded in maternally inherited lineages.

## **Materials and Methods**

Until recently, not even mtDNA sequences of people indigenous to the FNQ rainforest ecozone have been available. With the successful completion of the first phase of the National Geographic Genographic Project, however, nine such samples from FNQ volunteers have now been collected. To ensure that these samples genuinely represent people whom Birdsell and Tindale considered Barrinean, selection from the Genographic Project database was restricted to two of the twelve "pygmy"

tribal groups the two anthropologists identified, the Yidindji and Gungandji, and one “pygmoid” group, the Yelandji (identified as Irukandji by Birdsell; Tindale and Birdsell 1940). Although verifying tribal affiliations among Aboriginal people can be difficult owing to the extreme dislocation of colonization (which has tragically resulted in many Aboriginal people being forcibly removed from their traditional country and losing knowledge of their own grandparentage), this situation does not apply in the FNQ rainforest ecozone, for three reasons. First, this area was settled remarkably late in Australian history; the first European settlers did not infiltrate the area until the late 1800s. Second, in FNQ the church missions to which the local Aboriginal people were relocated were actually established on their own traditional country. Third, the comprehensive genealogical survey undertaken by Birdsell and Tindale at the Yarrabah and Mona Mona missions in the 1930s (when a large number of residents were still born either before colonization or in the first generation after mission settlement), whose card records are currently housed in the South Australian Museum, allowed rigorous cross-checking of family lineages and oral histories reported by volunteers in this study. This guaranteeing that the samples included are all from individuals bearing unbroken maternal lineages descending from FNQ Barrinean ancestors. Thus, though the number of FNQ samples in this study is small, the quality is high.

Very few genetic data are available for Aboriginal Tasmanians, and no new Tasmanian samples are included in this study. Instead, HVS sequences from the individuals reported in Presser et al. (2002) are cited for comparison. Similarly, all non-FNQ and non-Tasmanian Aboriginal sequences are taken from those published in van Holst Pellekaan et al. (2006), Ingman and Gyllensten (2003), and Hudjashov et al. (2007). Southeast Asian negrito sequences are likewise taken from published literature. Sequences for Malaysian Jahai, Mendriq, and Batek groups (collectively called Semang) are taken from Hill et al. (2006). Philippine negrito sequences are taken from Gunnarsdottir et al. (2010) for the Mamanwa and from Scholes et al. (2011) for the Batak, Aeta, and Agta.

Samples were collected using Oragene collection kits (DNA Genotek Inc., Ontario, Canada) after obtaining informed consent and then extracted following the manufacturer’s protocol. HVS1 sequences were typed by Sanger sequencing and aligned to the Revised Sapiens Reference Sequence (Behar et al. 2012) using MEGA, version 5 (Tamura et al. 2011). Coding region SNPs were typed using TaqMan (Applied Biosystems, Victoria, Australia). The mitochondrial SNP hierarchical typing protocol was as follows: All samples were typed for basal M, N, and R haplogroup affiliation (nucleotides 14783, 10873, 8701, 9540, and 12705). Haplogroup Ms were then typed for M42a (12771) and M29/Q (13500), Ns were typed for haplogroup S (8404), and Rs typed for haplogroup P and H (15607 and 7028). In the case of the Tasmanian individuals, Presser et al. (2002) reported three distinct haplotypes from their samples: T1a and T1b, T2, and T3 (together with one known to be from Victoria—a mainland state). The mtDNA haplogroup predictor program HaploGrep (Kloss-Brandstatter et al. 2011), which interrogates the current Phylotree (van Oven and Kayser 2009), was applied to the HVS1

**Table 1. Mitochondrial Haplogroups of FNQ, Tasmanian, Other Australian Aboriginal, and Southeast Asian Negrito Groups**

| MITOCHONDRIAL<br>HAPLOGROUP | POPULATION <sup>a</sup> |                  |                   |                  |                  |                   |                  |                  |                   |                   |
|-----------------------------|-------------------------|------------------|-------------------|------------------|------------------|-------------------|------------------|------------------|-------------------|-------------------|
|                             | FNQ                     | TAS <sup>1</sup> | AA <sup>2,3</sup> | MAM <sup>4</sup> | BTK <sup>5</sup> | BTKM <sup>6</sup> | JAI <sup>6</sup> | MEN <sup>6</sup> | AGTA <sup>7</sup> | AETA <sup>7</sup> |
| P                           | 5                       | 1                | 55                | 0                | 0                | 0                 | 0                | 0                | 26                | 6                 |
| Q                           | 0                       | 0                | 1                 | 0                | 0                | 0                 | 0                | 0                | 0                 | 0                 |
| S                           | 0                       | 1                | 39                | 0                | 0                | 0                 | 0                | 0                | 0                 | 0                 |
| M42a                        | 2                       | 0                | 32                | 0                | 0                | 0                 | 0                | 0                | 0                 | 0                 |
| M*                          | 1                       | 1                | 11                | 2                | 0                | 0                 | 0                | 0                | 0                 | 0                 |
| N*                          | 1                       | 0                | 7                 | 15               | 0                | 0                 | 0                | 0                | 0                 | 0                 |
| Others <sup>b</sup>         | 0                       | 0                | 7                 | 65               | 31               | 29                | 48               | 32               | 33                | 51                |

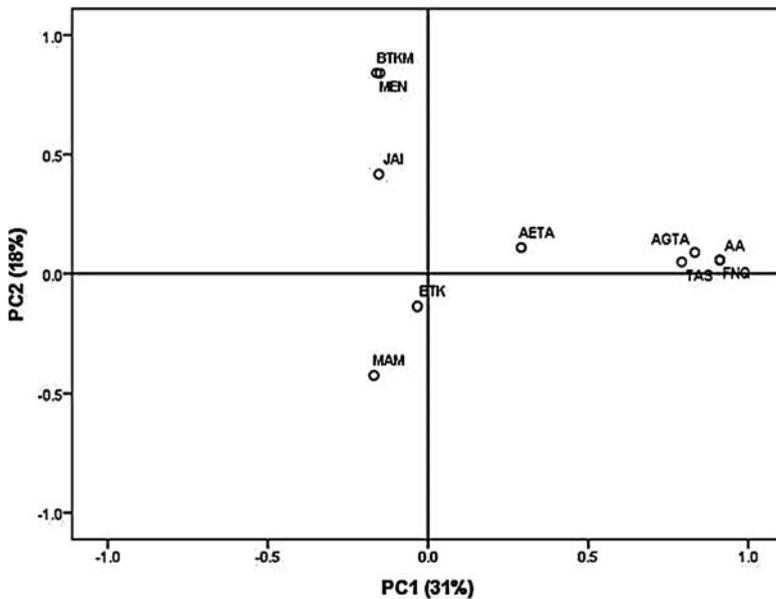
<sup>a</sup>Population abbreviations: FNQ, Barrinean; TAS, Tasmanian; AA, other Aboriginal Australian; MAM, Mamanwa; BTK, Batak (Philippines); BTKM, Batek (Malaysia); JAI, Jahai; MEN, Mendriq). Numbers indicate data sources: 1, Presser et al. (2002); 2, Hudjashov et al. (2007); 3, van Holst Pellekaan et al. (2006); 4, Gunnarsdottir et al. (2010); 5, Scholes et al. (2011); 6, Hill et al. (2006); 7, Heyer et al. this issue.

<sup>b</sup>Includes haplogroups frequent in negrito groups, such as B, E, F, D, R, Y, M19, and M80, but not shared with any Australian Aboriginal, FNQ, or Tasmanian group.

and HVS2 haplotypes of these sequences, as well as to our FNQ samples, to see if it could identify the haplogroups with higher resolution. The haplogroup data comprising all the samples were then subjected to principle components analysis (PCA) and multidimensional scaling (MDS) analyses in order to define the degree of relatedness among them. A PCA plot was generated using SPSS, version. 20 (IBM Corp. 2011), and Arlequin version 3.5 (Excoffier and Lischer 2010) was used to determine  $F_{ST}$  indices from which an MDS plot was drawn with SPSS.

## Results

The distribution of mtDNA haplogroups in the populations analyzed in this study is shown in Table 1. Application of HaploGrep provided no further resolution of the nine FNQ haplotypes except for one P, which was classified as P1d1 with high confidence. This is an interesting result given that haplogroup P1 has been found in Melanesia (Friedlaender et al. 2005) but not to date in Australia. HaploGrep also gave ambiguous results when applied to the Tasmanian haplotypes. Haplotypes T1a and T1b were allocated to very different haplogroups, M33c and U, respectively. However, a Tasmanian in Presser et al. (2002) with affiliation to the Wyerlooiber tribe (T1b) and identical in HVS haplotype to that of T1b was recently sequenced for the whole mitochondrial genome and found to belong to a novel P subtype. Therefore, we feel confident in assigning T1a and T1b to haplogroup P. T2 belongs to the uniquely Australian haplogroup S and is most likely S1a (PhyloTree, Build 15) because of the presence of mutations 16075, 16172, and 16301. T3 is assigned to R8 (a haplogroup that has its highest frequencies in Eastern India but not reported in negrito groups), but because R8 has not otherwise been reported in Australian



**Figure 1.** PCA analysis of mtDNA haplogroups in 10 populations, as defined in Table 1.

Aboriginals, T3's assignment remains speculative. It was therefore not included in further analyses.

Other than at the most basal, very ancient clades of macrohaplogroups M and N, the Southeast Asian negrito groups analyzed here share only one haplogroup (P) with either FNQ or Tasmania. [The M and N haplogroup subtypes found among the Mamanwa by Gunnarsdottir et al. (2010) were not found by this study in FNQ or Tasmanian people.] Haplogroup P also appears among the mainland Australian Aboriginal population (Hudjashov et al. 2007), however, so it seems that it, too, may be an ancestral haplogroup that evolved some time before, or on the cusp of, the original human settlement of Sahul. Haplogroup P has relatively high frequencies in Aeta (10%) and Agta (44%) (Heyer et al. this issue) and is reported in much lower frequencies in a general Philippine sample (Tabbada et al. 2010). These frequencies of P among Filipinos and negritos tend to negate the hypothesis of a recent back-migration of P people from Sahul. Further, Filipino subtypes P9 and P10 did not appear in any of the FNQ samples or in Tasmanians, are not seen in Papua New Guinea or Melanesia (van Oven and Kayser 2009), and are very distant from any P subtypes found in Australian Aboriginals to date (van Holst Pellekaan et al. 2006; Hudjashov et al. 2007). Thus, it appears that the P haplogroup, too, is shared only between Southeast Asian negritos and FNQ/Tasmanian populations at the most ancient, basal level.

By contrast, FNQ and Tasmanian Aborigines share many more haplogroups

with other mainland Australian Aboriginal populations. The phylogeny of haplogroup P is yet to be fully established in Australia (all but one of the HVS haplotypes of the FNQ P sequences in this study are novel; see Table S1), so the inferences that can be drawn from the multiple P haplogroup lineages shared among FNQ, Tasmanian, and other Australian Aboriginal groups are limited. Several other Australian-specific haplogroups, however, are shared among all three population groups. Both haplogroups M42a and S, for example, which are known to be specific to Australian Aboriginal populations, are found in FNQ and Tasmanian populations, respectively.

Principal components analysis (PCA) likewise indicates that all Australian samples, including FNQ rainforest groups and Tasmanians, have much more in common with each other than with any Southeast Asian negrito group (Figure 1). The first two components account for 49% of the variation. The second component places the Agta of the Philippines close to Australians due to their high frequency of haplogroup P (the analysis was performed at haplogroup level), but, as mentioned above, this is misleading because the two subtypes diverge strongly from all Australian P subtypes.

The MDS plot gives a similar picture with dimension 1 splitting the Southeast Asian negrito groups from all the Australian Aboriginals (Figure 2). Once again,

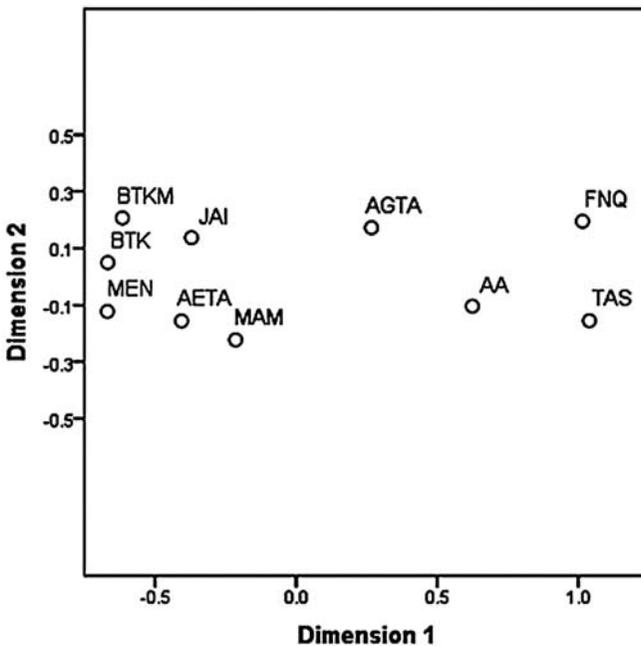


Figure 2. MDS plot of the 10 populations shown in Figure 1.

**Table S1. HVS1 Haplotypes of Barrinean Participants**

| BARRINEAN PARTICIPANT NUMBER | MTDNA HAPLOGROUP | REVISED SAPIENS REFERENCE SEQUENCE   |                                 |
|------------------------------|------------------|--|---------------------------------|
|                              |                  | HVS1 MUTATIONS   | HVS2 MUTATIONS                  |
| 1                            | P                | 16093C 16129G 16176T<br>16187C 16223C 16230A<br>16266T 16270T 16278C<br>16311T 16357C                  | 146T 195T 212C 247G             |
| 2                            | P                | 16187C 16223C 16230A<br>16258C 16263C 16278C<br>16295T 16311T 16362C                                   | 146T 153G 195T 247G             |
| 3                            | P                | 16129G 16187C 16223C<br>16230A 16231C 16271C<br>16278C 16309G 16311T                                   | 146T 153G 195C 247G             |
| 4                            | M42a             | 16129G 16184T 16187C<br>16188T 16189C 16223T<br>16230A 16278C 16287T<br>16294T 16311T 16356C           | 146T 185A 195T 247G             |
| 5                            | M*               | 16129G 16187C 16223T<br>16230A 16278C 16289G<br>16311T 16357C  | 64T 146T 195C 247G              |
| 6                            | M42a             | 16129G 16184T 16187C<br>16188T 16189C 16223T<br>16230A 16278C 16287T<br>16294T 16311T 16356C           | 146T 185A 195T 247G             |
| 7                            | P                | 16129A 16187C 16223C<br>16230A 16258C 16263C<br>16278C 16295T 16311T<br>16362C                         | 64T 143A 146T 153G 195T<br>247G |
| 8                            | P                | 16129A 16187C 16193T<br>16207G 16223C 16230A<br>16256T 16258C 16263C<br>16278C 16295T 16311T<br>16362C | 146T 153G 195C 247G             |
| 9                            | N*               | 16066G 16129G 16187C<br>16230A 16278C 16311T   | 152C 247G                       |

the Agta are closest to the Australians because of their high frequency of P. The stress is 0.011.

## Discussion

No mtDNA haplogroups are shared between Southeast Asian negrito groups and FNQ/Tasmanian populations, except at the most basal level, and those that are shared are also common to other Australian Aboriginal populations. By contrast, the mtDNA haplogroups found by this study in FNQ and Tasmanian samples are

also those found in other Australian Aboriginals. Some of these (M42a and S) are presently viewed as unique to Aboriginal Australians and must have evolved from within ancestors arriving in Sahul carrying the basal haplogroups M (M42a) and N (S), respectively. Haplogroup P is more widespread, having been reported in high frequencies in Papua New Guinea, parts of Island Melanesia (Friedlaender et al. 2005), and some negrito groups in the Philippines (Heyer et al. this issue), as well as in low frequency in a large general Filipino sample (Tabbada et al. 2010). However, as reported here, the P haplogroups found in Australia differ from those found in the Philippines. The finding of P1d1, previously reported only in Papua New Guinea, is noteworthy, and it is interesting to speculate whether it represents recent introgression or an ancient lineage present in both Papua New Guinea and Australia.

In summary, the evidence of this mtDNA study overall suggests that there is no genetic relationship between Southeast Asian negrito and FNQ Barrinean Aboriginal groups closer than the late Pleistocene connections that all Australian Aboriginal people have to some Southeast Asian negritos. Thus, Tindale and Birdsell's hypothesis of a Barrinean population distinct from other Australian Aboriginal people and closely related to Southeast Asian negritos receives no support from this study of mtDNA lineages. However, that the genetic origins of the ancestors of contemporary Australians (including those of FNQ and Tasmania) are far from clearly understood, as well as the extent and degree of isolation since their arrival, is illustrated by the recent discoveries reported by Pugach et al. (2013). Their genome-wide analysis of Northern Territory Aboriginal samples confirms the ancient separation of Filipinos from Australians but demonstrates that a substantial portion of the latter's genome (~11%) is of Indian origin and that it entered Australia only approximately 4,200 years ago. It will be of considerable interest to determine if this Holocene Indian introgression is evident in samples from other regions of Australia and is of the same magnitude.

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