

New phylogenetic perspectives among species of South-east Asian wild pig (*Sus* sp.) based on mtDNA sequences and morphometric data

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Abstract

There are more taxa of wild pig in South-east Asia (SEA) than in any other comparable area in the world, but the number of species and subspecies is still uncertain. The taxonomy of some wild pig populations distributed in Malaysia, Indonesia and the Philippines, was investigated using molecular and morphometric techniques. Our results suggest the existence of two main evolutionary clades that are likely to have diverged during the Pliocene in SEA: one including wild pig populations distributed in the Philippines (*Sus cebifrons*) and Sulawesi (*S. celebensis*); the other including the Indonesian and Malaysian bearded pigs (*S. barbatus*), and the widespread Eurasian wild boar (*S. scrofa*). A possible scenario for pig speciation in SEA is developed, and the need for a taxonomic revision of bearded pigs suggested, particularly concerning the taxonomic status of *S. b. ahoenobarbus* from Palawan Island, and the existence a new species distributed in the Tawi Tawi Islands (Philippines). Unexpectedly, bearded pigs in the Malay Peninsula are closely related to the Bornean population, but distinct from Sumatran *S. barbatus*, and they should be considered as belonging to a different subspecies.

Key words: phylogeny, mtDNA, wild pig, Suidae, *Sus*, South-east Asia, Sundaland

INTRODUCTION

South-east Asia (SEA) has the highest wild pig diversity in the world. This was probably promoted by vicariance in archipelagos, where islands were repeatedly connected or isolated during the sea level fluctuations that have occurred since the Early Pliocene (*c.* 5.2 million years ago, Mya). Several studies have attempted to clarify the systematics of SEA wild pigs (Groves & Grubb, 1993; Grubb, 1993; Ranes, 1995; Groves, 1997; Oliver, 2001) but the number of species is still uncertain. Groves (1997) suggested that the genus *Sus* can be differentiated into three informal groups, named the *S. verrucosus*, *S. philippensis* and *S. scrofa* groups. The *S. verrucosus* group includes the Javan warty pig *S. verrucosus*, the bearded pig *S. barbatus* (from Borneo, Sumatra, Bangka, the Riau Archipelago, Palawan and the Malay Peninsula), the Sulawesi pig *Sus celebensis*, and the Visayan pig *S. cebifrons*. Males in this group have straight lower canines, in which the inferior surface is usually 120–150% of the posterior, and have warts when adult. Pigs in the

S. philippensis group, in which the canine ratio is *c.* 140–180%, have two pairs of warts, with a long thin tuft emerging from the one on the angle of the jaw. The *S. scrofa* group includes this species and the pygmy hog *S. salvanius* (endemic to India); the canine ratio is generally < 100, and there are no warts. Phylogenetic relationships among these groups are unclear (Groves, 2001).

Taxonomic uncertainty exists also at the subspecies level. Groves (1981) and Groves & Grubb (1993) recognized three subspecies of *S. barbatus*: the nominate subspecies from Borneo; *S. b. oi* from Sumatra, the Malay Peninsula, and islands of the Riau and Lingga archipelagos; *S. b. ahoenobarbus* from the Palawan, Balabac and Culion Islands, Philippines. Groves (2001) later suggested that *S. b. ahoenobarbus* should be elevated to full species. The bearded pigs from Bangka Island were named *S. barbatus edmondi* by Sody (1937), a subspecies that Groves (1981) rejected. New wild pig populations have been discovered recently, including a new species from Tawi Tawi Island and offshore islets (Oliver, 2001).

The aim of this study was to investigate phylogenetic and systematic relationships among SEA wild pig populations using a combination of morphometric and genetic analyses. An accurate taxonomy is a fundamental

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Table 1. Origin and number of samples used in this study

Species	Subspecies	Origin	Samples	Collectors ^a
Bearded pig				
<i>Sus barbatus</i>	<i>barbatus</i>	Borneo	16	E. Meijaard, C. H. Diong
	<i>barbatus</i>	Philippines: Tawi Tawi	3	K. Rose
	<i>barbatus</i>	Philippines: Sibutu	3	K. Rose
	<i>oi</i>	Malay Peninsula	24	C. H. Diong
	<i>oi</i>	Indonesia: Sumatra	6	E. Meijaard
	<i>ahoenobarbus</i>	Philippines: Palawan	3	FMNH
Eurasian wild pig				
<i>Sus scrofa</i>		Bulgaria	2	P. Genov
		Italy	2	INFS
Domestic pigs		China	4	INFS
Visayan warty pig				
<i>Sus cebifrons</i>	<i>negrinus</i>	Philippines: Negros	6	FMNH
		Philippines: Masbate	4	FMNH
		Philippines: Panay	3	FMNH
Warthog				
<i>Phacochoerus africanus</i>		Africa: Tanzania	1	G. Tosi

^a See Appendix for museum abbreviations.

prerequisite for every conservation plan in SEA, an area with high, but still poorly known, biodiversity that is rapidly declining as a result of deforestation and loss of natural habitats (Brooks, Pimms & Collar, 1997; Achard *et al.*, 1998; BirdLife International, 2001; Jepson *et al.*, 2001).

MATERIALS AND METHODS

Tissue sampling, DNA and phylogenetic analyses

The following tissue and hair samples were collected: 22 of *Sus barbatus barbatus* from Borneo, Sibutu and Tawi Tawi; 30 of *S. b. oi* from the Malay Peninsula and Sumatra; 3 of *S. b. ahoenobarbus* from Palawan; 23 of *S. scrofa*; 13 of Visayan warty pig *S. cebifrons* (Table 1; Fig. 1). The Sumatran samples were collected by EM from a bush meat market without detailed information on either specific identity or collection locality. The samples of bearded pigs from Palawan and the samples of Visayan warty pigs are from museum specimens. All samples were collected with permission from the local authorities and with the relative permits. One African warthog *Phacochoerus africanus*, the most closely related sister lineage of *Sus* (Randi, Lucchini & Diong, 1996), was used as an outgroup. DNA was extracted using a salting-out procedure (Miller, Dykes & Poleski, 1988). DNA from museum specimens was extracted following Gerloff *et al.* (1995), working in a dedicated room. Negative controls were always used to check for contamination.

We amplified two mitochondrial DNA cytochrome *b* fragments (mtDNA *cyt b*), respectively 199 and 282 nucleotides long, using primer pairs L15044 (5'-GG-GCGAGGTCTATACTACGGATCCGG-3')/*SusCybSH2* (5'-ATTCATTCTACGAGGTCTGTTCCG-3'), and L15404 (5'-CCATTTACCCATACTACTACT-3')/*DDA-cybH936* (5'-TTGTTTGGATGTGTGGAGGAAGGG-3').

Two fragments of the mtDNA control-region (CR), of 402 and 282 nucleotides, were respectively amplified using primer pairs SSCDL356 (5'-AGACTAACTCCG-CCATCA GCACCC-3')/H764 (5'-AAGCGGGTTGC-TGGTTTTCACGC-3') and L746 (5'-GCGTGAAAC CAGCAACCCGCTT-3')/SSCcrH1022 (Okumura *et al.*, 1996; Ursing & Arnason, 1998). PCRs (with 2 mM MgCl₂) were carried out with initial denaturation at 94 °C × 2 min; 30 cycles for fresh tissues or 40 cycles for museum specimens at 94 °C × 15 s; annealing at 50 °C for *cyt b*, and 55 °C for CR; extension at 72 °C × 30 s; one final extension step at 72 °C × 10 min. Cycle sequencing was performed using the ABI Dye Terminator protocol. Sequences were analysed on an ABI 3100 automated sequencer. Details of laboratory protocols are available upon request.

The sequences were aligned using CLUSTAL X (Thompson *et al.*, 1997) and manually edited with SE-AL 2.0 (A. Rambaut; <http://evolve.zoo.ox.ac.uk>). Phylogenetic congruence of the combined *cyt b* and CR alignment was assessed by the partition-homogeneity test (Farris *et al.*, 1995), with heuristic search and 1000 random partitions, as implemented in PAUP* 4.10b (Swofford, 1998). Aiming to compare the results of different approaches, phylogenetic trees were obtained using maximum parsimony (MP; Swofford, 1998), genetic distances and neighbour-joining (NJ; Saitou & Nei, 1987), and maximum-likelihood (ML; Felsenstein, 1981) procedures. The MP tree was obtained by heuristic searches with characters unweighted, and using or not deletions as a fifth character. Alternative character weighting schemes (weighting transversions 5, 10 or 20 more than transitions) did not change the shape of the MP trees. The program MODELTEST 3.06 (Posada & Crandall, 1998) was used to identify the model of sequence evolution that best fits the data. The ML analysis was performed using a fast heuristic approach based on the quartet puzzling algorithm implemented in the program

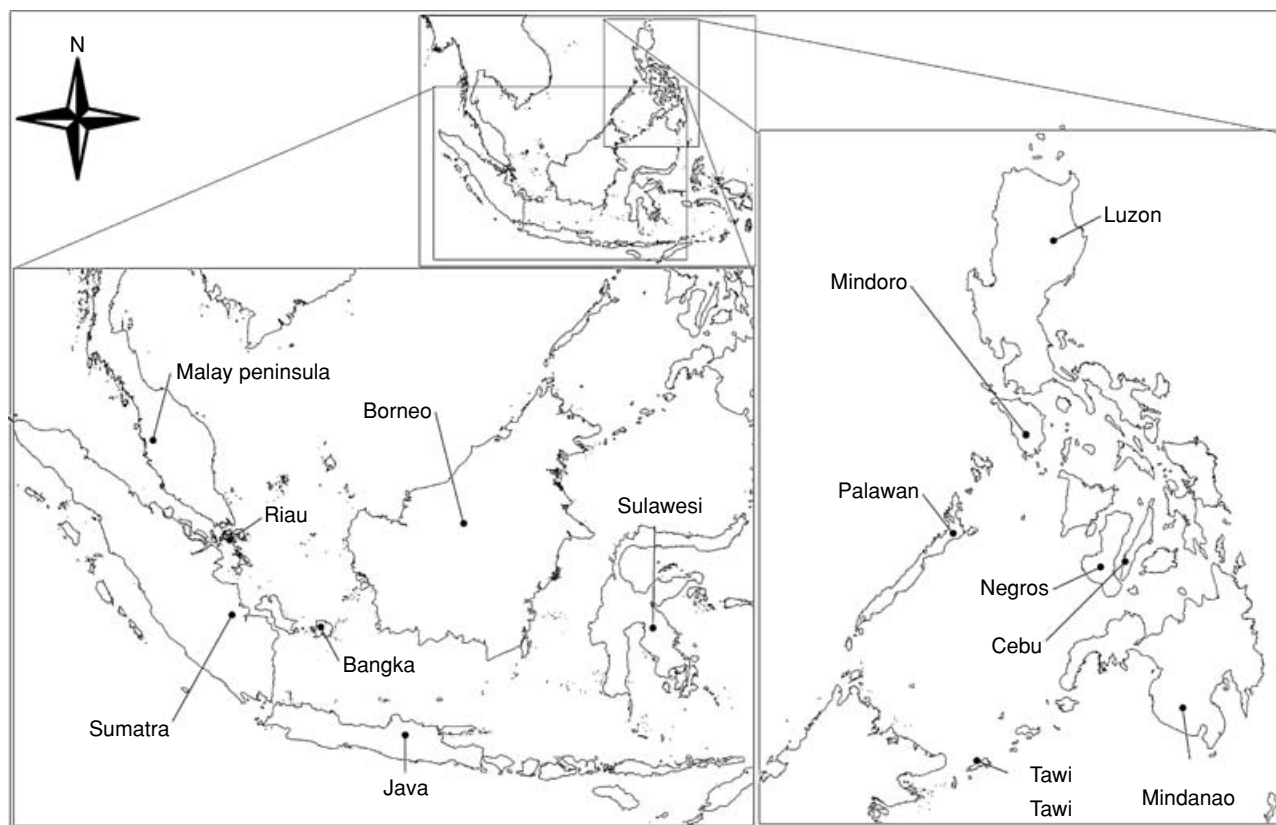


Fig. 1. Map of the islands on which South-east Asian pig species occur.

PUZZLE 4.0.1 (Strimmer & von Haeseler, 1996). In NJ tree the support of the different branches was obtained with bootstrap percentage (BP) based on 10 000 bootstrap resamplings (Felsenstein, 1985). Genetic distances between and within bearded pig, wild boar and Visayan warty pig samples were computed with MEGA 2.1 (Kumar *et al.*, 2001).

Craniometric analyses

Sixteen traits were measured by CPG in 214 pig skulls in several museum collections (Appendix). CPG selected traits after visually inspecting morphological differences between species and, based on this, choosing the measurements which best represented the observed differences. Except for 16 specimens, all were males, and therefore all statistical tests were conducted on male specimens only. Those samples in which the suture was fully fused were judged to be fully grown. Thus, only adult specimens were selected for statistical analysis. The specimens included *S. barbatus*, *S. verrucosus*, *S. philippensis*, *S. cebifrons*, *S. celebensis*, *S. scrofa*, the provisionally recognized *S. oliveri*, and one additional specimen of unknown specific identity from Tawi Tawi Island. The *S. scrofa* specimens used in this analysis originated from East and South-east Asia, including the Malay Peninsula and Singapore. The measurements

included: greatest skull length (GL; A in Fig. 2a), condylobasal length (CBL; B in Fig. 2a), basal length (BL; C–C' in Fig. 2b), bizygomatic width (ZW; D in Fig. 2b), occipital breadth (OB; E in Fig. 2b), palate length (PL; F–C' in Fig. 2b), length of upper tooth row (UTR; G in Fig. 2b), length of upper molar row (MR; H in Fig. 2b), post-orbital breadth (PO; I in Fig. 2c), width across post-orbital processes (POP; J in Fig. 2c), length of frontal + parietal (FP; K in Fig. 2c), nasal length (NL; L in Fig. 2c), occipital height from basion (OHB; not shown; measured from the lower ridge of the foramen magnum to the upper side of the occiput), skull height (SH; not shown; if placed on its mandible on a flat surface, measured from the highest point on the skull to the surface), length of lower tooth row (LTR; not shown), and length of upper incisor row (IR; not shown). All measurements were made with an accuracy of 0.1 mm with a pair of Vernier callipers (precision 0.05 mm).

Skull measurements were analysed with SPSS 11.0 software (SPSS Inc., Chicago). Classifications were made between and within the subspecies using the multivariate techniques of principal components analysis (PCA) and discriminant analysis (DA). The phenetic relationships between different species were analysed using MEGA 2 (Kumar *et al.*, 2001), which has an algorithm for handling morphometric data. Unstandardized canonical discriminant functions evaluated at group centroids were obtained, which provided the input in a dissimilarity

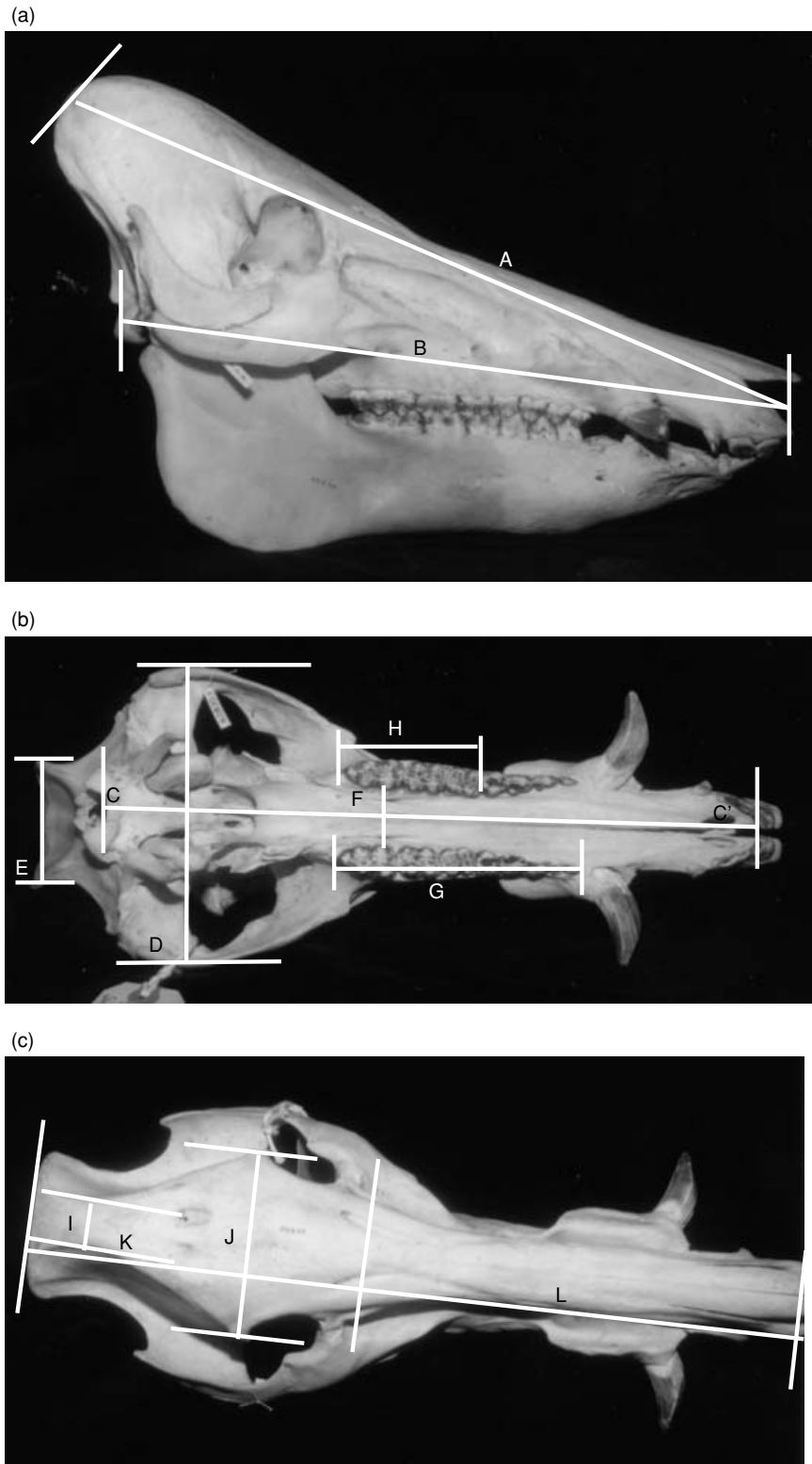


Fig. 2. *Sus ahoenobarbus* skull, with skull diagrams of measurements as explained in the text: (a) lateral view; (b) ventral view; (c) dorsal view.

matrix based on the square root of the sum of the squared Euclidean distance between species. These data were used to generate species phenograms based on craniometric similarities. The phenetic relationships between the pig

taxa were analysed by neighbour-joining (NJ) and unweighted pair group method with arithmetic mean (UPGMA) analyses using the group centroids for 5 discriminant functions.

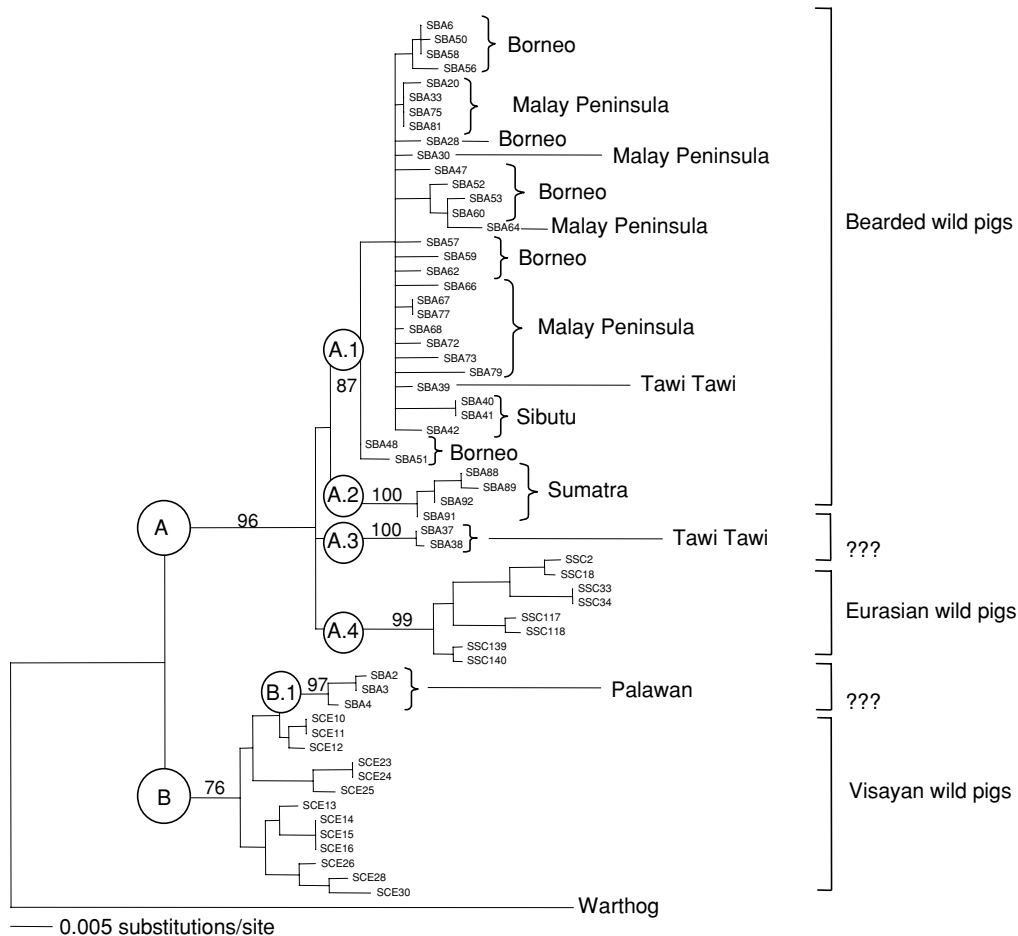


Fig. 3. Neighbour-joining phylogenetic tree obtained from the analysis of 968 mtDNA nucleotides. All the main clades were identified by different phylogenetic methods (MP, ML and NJ; see Materials and methods), and are indicated by open circles. NJ bootstrap values after 10 000 replicates are indicated on their branches.

RESULTS

DNA analyses: phylogenetic relationships and genetic divergence in SEA wild pigs

The *cyt b* sequences were carefully inspected. No internal stop-codon or frameshift mutations were observed, suggesting that only mitochondrial functional genes were amplified. The partition homogeneity test indicated that *cyt b* and CR display a congruent phylogenetic signal (P value = 1.00). Thus, a concatenated sequence alignment of 968 nucleotides (385 *cyt b* + 583 CR nucleotides) was used, which showed 62 distinct haplotypes defined by 121 variable sites (12.5%), 104 (10.7%) of which were parsimony informative. The transition/transversion ratio, estimated by maximum-likelihood, was $Ts/Tv = 17.14$. Among the CR sequences of bearded pigs there were two single base insertions in all Sumatran bearded pigs and in two bearded pigs from Tawi Tawi (SBA37 and SBA38). Five additional indels differentiated the bearded pig from all the other sequences.

The MP, NJ and ML trees were very similar. Figure 3 shows a rooted NJ tree computed with Tamura–Nei (Tamura & Nei, 1993) pairwise distances (d) with a

gamma distribution of mutation rate (α parameter = 0.7). The mtDNA haplotypes were split into two main clades: clade A includes all the sequences of Eurasian wild pigs and the bearded pigs from Borneo, Sibutu, Tawi Tawi, the Malay Peninsula and Sumatra; clade B includes the bearded pigs from Palawan (*ssp. ahoenobarbus*) and all the Visayan warty pigs. Clade A is further subdivided into four strongly supported clades: clade A.1 comprises the haplotypes sampled from populations of bearded pigs from Borneo, the Malay Peninsula, Sibutu and one sample from Tawi Tawi; clade A.2 includes only the bearded pig haplotypes sampled from Sumatra; clade A.3 includes two pigs sampled on Tawi Tawi; and clade A.4 includes all the Eurasian wild pig samples. The distinct populations of bearded pigs included in clade A.1 do not show reciprocal monophyly with any phylogenetic method. The Sumatran bearded pig (clade A.2) is the sister clade of Bornean and Malaysian bearded pigs (clade A.1) in the NJ tree, but not in MP and ML trees. These findings confirm that the Sumatran samples are bearded pigs that are assigned to a phylogenetically unique clade (A.2). The bearded pigs from Palawan were grouped into clade B.1, which was nested within the Visayan warty pigs (clade B) in all the phylogenetic analysis.

Table 2. Matrix of inter- and intra-population Tamura & Nei (1993) genetic distances among SEA wild pigs. Lower triangular matrix, mean genetic distances; upper triangular matrix, standard errors; diagonal, average genetic diversity within each population (SE in parentheses)

Population	1	2	3	4	5	6	7	8
1 <i>S. barbatus</i> (Borneo)	0 (0)	0.001	0.004	0.005	0.002	0.008	0.007	0.006
2 <i>S. barbatus</i> (Malay Peninsula)	0.006	0.005 (0.001)	0.004	0.005	0.002	0.008	0.007	0.006
3 <i>S. barbatus</i> (Sumatra)	0.019	0.019	0.004 (0.002)	0.005	0.005	0.008	0.007	0.005
4 <i>S. barbatus</i> (Tawi Tawi; A.3)	0.021	0.021	0.026	0 (0)	0.005	0.008	0.007	0.005
5 <i>S. barbatus</i> (Sibutu/Tawi Tawi; A.1)	0.008	0.007	0.022	0.0021	0.007 (0.002)	0.008	0.007	0.006
6 <i>S. barbatus</i> (Palawan)	0.045	0.046	0.051	0.047	0.043	0.003 (0.001)	0.004	0.008
7 <i>S. cebifrons</i>	0.045	0.045	0.051	0.044	0.045	0.022	0.017 (0.003)	0.007
8 <i>S. scrofa</i>	0.032	0.032	0.032	0.031	0.031	0.053	0.0052	0.016 (0.003)

Table 2 shows the genetic distances within and between wild pig populations. The Malay Peninsula bearded pigs are genetically less divergent from Bornean ($d = 0.006 \pm 0.001$) and Sibutu/Tawi Tawi ($d = 0.007 \pm 0.002$) bearded pigs than from Sumatran ($d = 0.019 \pm 0.004$) ones. Two wild pigs sampled in Tawi Tawi were genetically distinct from all the other bearded pigs analysed in this study ($d = 0.021 \pm 0.047$). Moreover the bearded pigs from Palawan were less distinct from the Visayan warty pigs ($d = 0.022 \pm 0.004$) than from the other of bearded pig samples ($d = 0.044 \pm 0.055$).

Craniometrics

A PCA of a restricted set of skull measurements (OHB, SH, and LTR) were initially left out because of many missing values) resulted in the separation of four main groups: (1) *S. barbatus*; (2) *S. ahoenobarbus* mixed with *S. scrofa*; (3) *S. verrucosus* overlapping with *S. barbatus*; (4) *S. philippensis*, *S. cebifrons*, *S. celebensis*, and *S. oliveri* (Fig. 4a). The positive correlation of the variables with the first component, which predicted 80% of the total variance, indicated that the difference between these groups was primarily based on overall size. *Sus verrucosus* is separated from *S. barbatus* by the significantly (ANOVA, $P < 0.001$) lower values for CBL, BL, SH, FP, and NL, and significantly (ANOVA, $P < 0.001$) higher values for OB.

The differences between the pig taxa were further investigated in a DA. Differences between the means of the species groups were significant ($P < 0.001$) as expressed by the low value of Wilks' lambda ($= 0.01$) for the first function, which predicted 81% of the total variance, and for the second function ($= 0.14$) which predicted 12% of the total variance; the third function predicted 3% of the total variance. The resulting graphs (Fig. 4b, c) again show that the western *barbatus* group from Borneo, Sumatra, and the Malay Peninsula is distinct from the Palawan taxon (*ahoenobarbus*), and a one-way analysis of variance (ANOVA) showed that *ahoenobarbus* was significantly ($P < 0.01$) smaller for all measurements. The western *barbatus* group is differentiated from all other species by a longer snout (PL, NL, and GL), and larger teeth (MR, IR, UTR, and LTR). A group with intermediate values for these characters consists of *S. scrofa*, *S. verrucosus*, and

S. barbatus ahoenobarbus. *Sus scrofa* is separated from the other two taxa by low values of the third function (Fig. 4c), which correlates with relatively low values for UTR, NL, OHB, and ZW. *Sus barbatus ahoenobarbus* is separated from the other two by low values of the second function, indicating a relatively short tooththrow (MR and UTR), and a relatively long palate (PL). The smallest skulls belong to the Philippine (except Palawan) and Sulawesi pigs. *Sus philippensis* together with *S. cebifrons* are separated from *S. celebensis* by the third function (Fig. 4c), with significantly higher values ($P < 0.01$) for the former two for NL and UTR and significantly lower ($P < 0.01$) for PO. When our only specimen from Tawi Tawi Island was entered, a PCA grouped it with the other Philippine pigs species (except *ahoenobarbus*), but it was not possible to determine to which species the Tawi Tawi specimen was morphologically most similar (see arrow, Fig. 4d). The three *S. oliveri* specimens grouped mostly with the *S. philippensis* specimens, but an ANOVA could not significantly differentiate means of measurements for *S. oliveri* from those of *S. cebifrons*.

Variation within the western *S. barbatus* group was also investigated, but neither a PCA nor a DA of skull measurements resulted in significant separation of the specimens from Borneo, Sumatra, and the Malay Peninsula. When the smaller Philippine pigs were left out and the Malay Peninsula and Bangka specimens entered as unknowns, more structure became apparent (Fig. 4e). The Bangka specimens were located in the border area between Sumatran and Bornean specimens. Two specimens from the Malay Peninsula grouped with the Sumatran group and one Malay Peninsula specimen (Singapore Museum, labelled 'Selangor') was close to the *S. scrofa* group. One Bornean *barbatus* specimen (Cibinong Museum, labelled 'Barabai', which is in South Kalimantan) grouped with *S. scrofa*. The grouping of the latter two specimens suggests that these may either have been misclassified or mislabelled; inspection by CPG of the original data confirms that the Barabai skull is indeed a *S. scrofa*, presumably a feral pig since no true wild pigs except for *S. barbatus* are known from Borneo, although there seemed no particular problem with the Selangor skull. The Sumatran specimens show some distinction from the Bornean ones in Fig. 4(e), primarily because of the relatively low values for function two. This especially correlates with larger means for SH

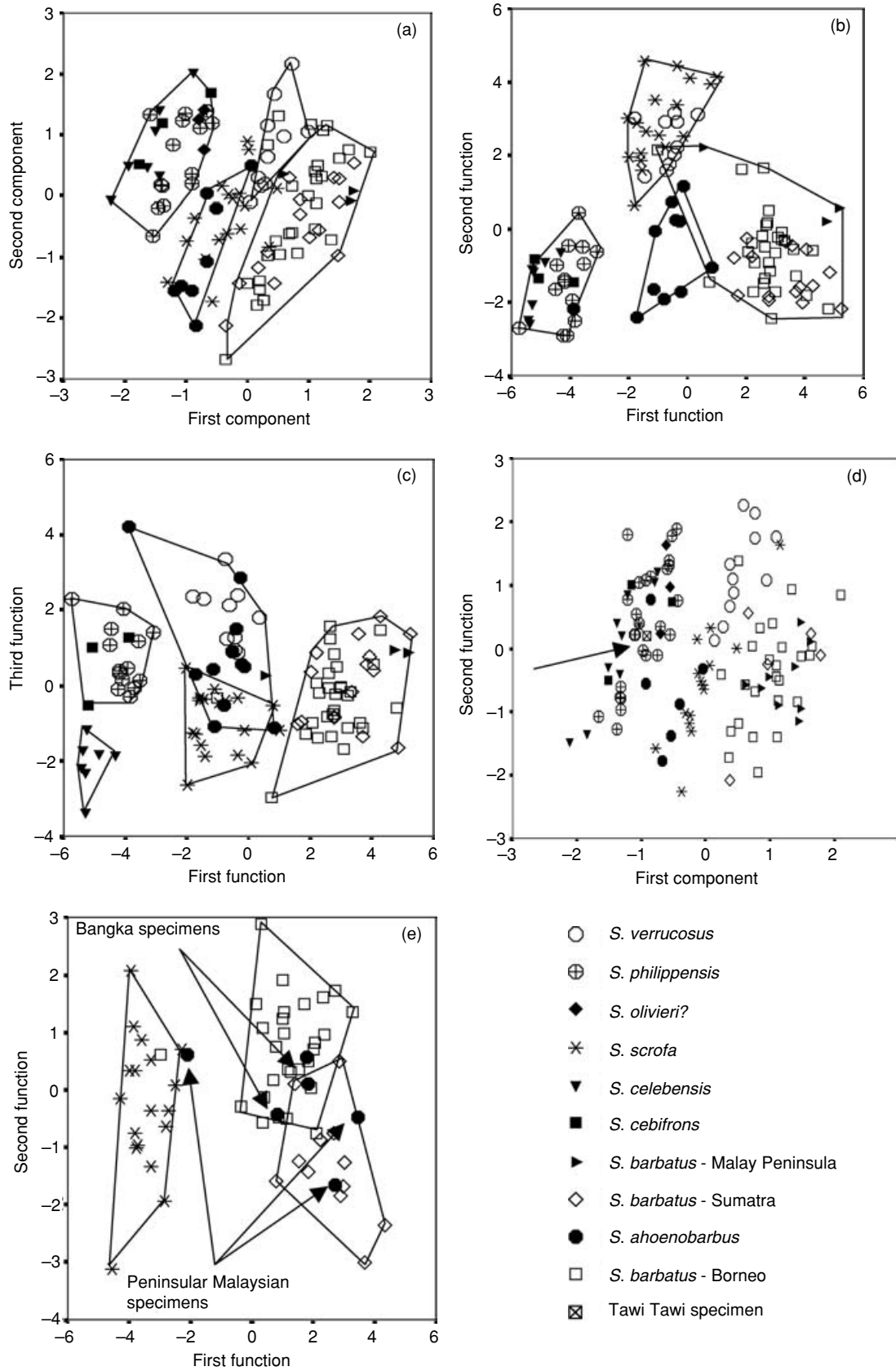


Fig. 4. Principal component analysis (PCA) and discriminant analysis (DA). (a) PCA of pig skull measurements; (b) DA of pig skull measurements, first and second functions; (c) DA of pig skull measurements, first and third functions; (d) PCA of several pig taxa, including the unknown taxon from the Tawi Tawi Islands (arrow); (e) DA of *barbatus* and *scrofa* specimens, with specimens from Bangka and the Malay Peninsula entered as unknowns (see text for description).

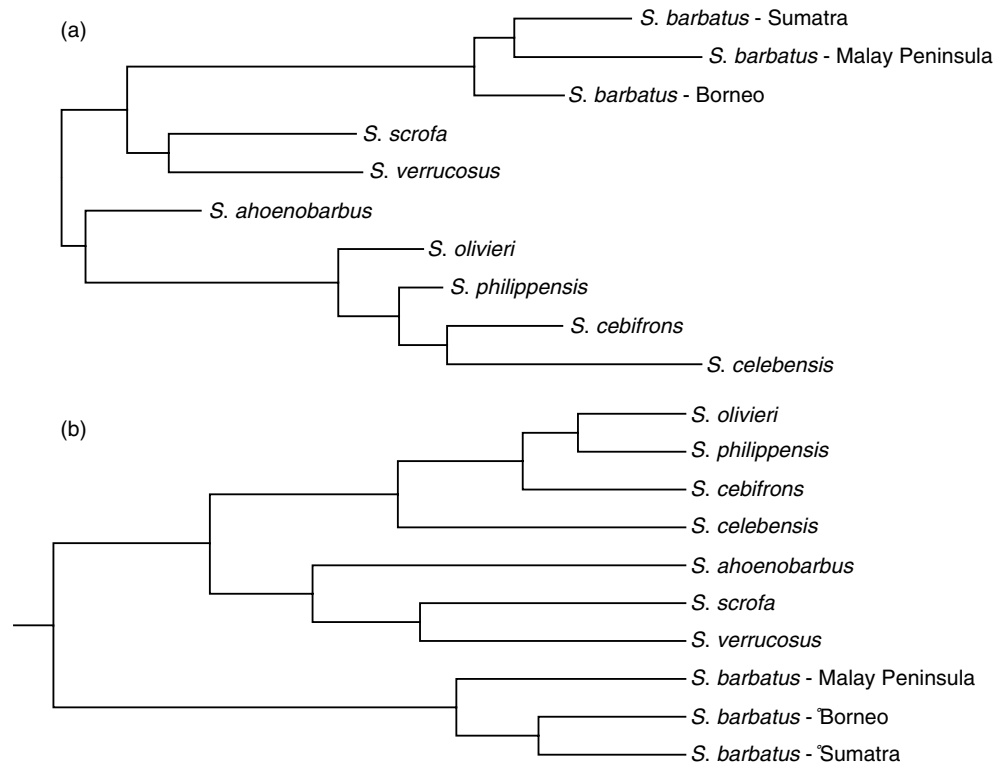


Fig. 5. An NJ (a) and UPGMA (b) tree based on cranial measurements.

(Sumatra average = 248.67, $n = 9$; Borneo average = 237.94, $n = 35$); CBL (Sumatra average = 422.88, $n = 8$; Borneo average = 406.87, $n = 31$); and IR (Sumatra average = 58.56, $n = 9$; Borneo average = 55.03, $n = 33$); in an ANOVA none of these differences were significant at a $P = 0.05$ level.

The PCA and DA diagrams (Fig. 4a–c) have directionality; the diagrams can be seen as having phylogenetic polarity. This is indicated by the consistent closeness of species not otherwise related (*celebensis* and *cebifrons/philippensis*; and generally *scrofa* and *verrucosus*) towards the left side of the diagrams. In contrast, closeness could only indicate that small species group with other small species, and large species group with large species, considering that most of the variation in first functions/components was determined by size. Different skull measurements, however, contributed differentially to the PC1 and DF1 scores, suggesting that shape components also affected the described morphometric relations. The NJ dendrogram (Fig. 5a), which was rooted at the split between the Philippine/Sulawesi pigs and those from the west of the region, resulted in a species tree that grouped together all Philippine species and *S. celebensis*. Geographic populations of the western *barbatus* group were closely related, and *S. scrofa* and *S. verrucosus*, surprisingly, grouped closely together. The UPGMA (Fig. 5b) of all the species differed slightly from the NJ tree, with *celebensis* at a more basal position in the Philippine/Sulawesi group, and *ahoenobarbus* grouping with the *scrofa/verrucosus* group.

DISCUSSION

Evolutionary history

Molecular and morphometric techniques used in this study revealed unexpected intraspecific relationships between SEA wild pigs. The three groups (*verrucosus*, *philippensis*, *scrofa*) described by Groves (1981) are not supported by our data, which instead suggest the following groups:

- (1) pigs from the Philippines (*S. cebifrons*) and Sulawesi (*S. celebensis*) that retain plesiomorphic characters (as discussed by Groves, 1997), and from which *celebensis* diverges most (although *S. celebensis* was not analysed genetically);
- (2a) the western *S. barbatus* subspecies, among which little morphological differentiation was found, but which separate into a Sumatran group and a Malaysian/Bornean group based on DNA sequences;
- (2b) a group consisting of *S. scrofa*, *S. verrucosus*, and *S. cf. barbatus ahoenobarbus*, in which the first two especially seem to be morphometrically similar (although *S. verrucosus* was not analysed genetically). The molecular analysis groups *ahoenobarbus* with group 1 (*S. cebifrons*).

The first group is similar to the one proposed by Sanborn (1952), who referred all Philippine pigs to *S. celebensis*. Later Groves (1997) separated *S. philippensis* as a distinct group related to *S. scrofa*, and placed *S. celebensis*

(from Sulawesi) and *S. cebifrons* in his *S. verrucosus* group, although suggesting that the two species were distantly related to *S. scrofa*. Our data suggest that Philippine and Sulawesi species (Group 1) are primitive and have a relict distribution. Species in Group 1 probably separated quite early from an ancestral species that occurred in Sundaland and that later separated into the *barbatus* (Group 2a) and *verrucosus* lineages (Group 2b above). *Sus scrofa* is a relatively recent species that probably entered Sundaland c. 70 000 years ago (van den Bergh, de Vos & Sondaar, 2001), whereas the Javan *S. verrucosus* lineage (including the Javan fossil species *S. macrognathus* and *S. brachygnathus*; see Hardjasamita, 1987) has been present on the island for at least 2 Myr (e.g. Aimi, 1989). Randi, Lucchini & Diong (1996) found that *S. scrofa* diverged from *S. barbatus* between 1.3 and 6.6 Mya, which might suggest that *S. scrofa* diverged from the *verrucosus/barbatus* lineage either on the Asian mainland before dispersing into Sundaland, or else as a vicariant event between Sundaland and Continental Asia.

The close morphometric similarity between *scrofa* and *verrucosus* found in the present research is surprising, because these two were considered to be quite distinct (Groves, 1981). Fistani (1996), however, reported on the close similarity between *S. strozii*, the Early Pleistocene precursor of *S. scrofa* in Europe, and the *verrucosus* lineage. After its divergence from *scrofa*, the *verrucosus* lineage may have further evolved on the Asian mainland, if Indochinese *S. bucculentus* is indeed related to it. Groves *et al.* (1997) and Groves & Schaller (2000) affirmed that in its morphology *S. bucculentus* resembles *S. verrucosus* (in agreement with Heude, 1892), and there are considerable differences between 12S ribosomal gene sequences of *S. bucculentus* and *S. scrofa*, indicating that *S. bucculentus* is not closely related to *S. scrofa* (note, however, that *S. verrucosus* was not included in this molecular research).

A possible evolutionary scenario for the SEA pigs would be as follows. Some time during the Pliocene (5.3–1.8 Mya) an ancestral pig species crossed from Sundaland to the Philippines. This could either have happened via Sulawesi or via Palawan, much more probably the latter; the source population in either case would have been in what is now Borneo. The morphometric distinctness of Palawan's *ahoenobarbus*, its basal position in a phenetic species tree (Fig. 5a, b) and its phylogenetic relationships to *S. cebifrons*, indicate that this dispersal went via Palawan to the Philippines, while *celebensis* crossed from Sundaland into Sulawesi. After dispersal to Sulawesi and the Philippines, the *verrucosus* and *barbatus* lineages diverged. Considering that the former is restricted to Java and the latter to Borneo/Sumatra/Malay Peninsula, the divergence probably happened as the result of Java's separation from the rest of Sundaland.

Finally, as suggested by the molecular work, there seems to be a clear split within *S. barbatus*, with the Bornean and the Malay Peninsula populations in one group and the Sumatran in another. Considering the lack of morphological differentiation between the populations,

we think that the divergence between them happened relatively recently, probably during the Late Pleistocene. During the last glacial maximum (LGM), evergreen forest areas in Sundaland were probably restricted to several refuges (Meijaard, 2003) and *barbatus* could have been separated into different populations. If indeed the Malay Peninsula population is more closely related to the Bornean than to the Sumatran, the population of the Malay Peninsula may have become extinct during the LGM and reinvaded the Peninsula from Borneo when climatic conditions became wetter once more, but before rising sea levels separated Borneo from the Malay Peninsula. In relation to this it should also be mentioned that *barbatus* is absent from northern Sumatra, which suggests that the Sumatran population was restricted to the southern part of Sumatra, and did not reinvade the northern parts after the LGM.

Taxonomic implications

Our data confirm that *ahoenobarbus* is morphologically and genetically distinct from *barbatus*. Using a phylogenetic species concept (Cracraft, 1983; see also Groves, 2001), assigning full species status to the Palawan pig, as *Sus ahoenobarbus* is recommended, irrespective of whether *Sus barbatus* might be paraphyletic if *ahoenobarbus* were included.

Our material was insufficient to assess the taxonomic status of *barbatus* from Bangka Island, and discriminant analyses based on skull measurements (Fig. 4e) indicated a position intermediate between Borneo and Sumatra for the three Bangka specimens. There is a slightly closer similarity between the Bangka and Borneo specimens, suggesting that this population reached Bangka from Borneo across the Karimata land bridge. A similar scenario, in which Bangka populations are more closely related to those of distant Borneo than to those of nearby Sumatra, can be observed for some other Sundaland species, such as *Nycticebus coucang* according to Groves (1971).

The current taxonomy of the *barbatus* populations from Malaysia, Borneo and Sumatra seems to be incorrect in the light of our molecular data. The populations from Malaysia and Sumatra, considered to be the same subspecies *oi*, are genetically very divergent. Sumatran samples constitute a single monophyletic clade separate from Malaysian samples, which are grouped together with the Bornean samples that belong to the subspecies *barbatus*. Therefore a possible taxonomic revision of the *barbatus* species would be to restrict subspecies *oi* to the Sumatran population and to assign the Malaysian population to the subspecies *barbatus* together with the Bornean population. As the pelage features considered to differentiate Bornean and Sumatran subspecies of *Sus barbatus* by Groves (1981) may not be quite so consistent as once thought (C. P. Groves & E. Meijaard, pers. obs.), there would be no phenotypic objections to such a reassignment.

Morphological and genetic data confirm the existence of a new species of wild pig on Tawi Tawi. Its phylogenetic

position could not, however, be resolved in this research because of the contrasting molecular and morphological results and the small sample size. Therefore, further field work and more samples are needed to clarify its status.

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APPENDIX. List of museums in which pig skulls were measured

American Museum of Natural History, New York
Natural History Museum, London (BM)
Natural History Museum, Cambridge (NHMC)
Field Museum of Natural History, Chicago (FMNH)
Zoological Museum Cibinong (MZB)
National Museum of Natural History, Leiden (NRML)

Natur-Museum und Forschung-Institut Senckenberg,
Frankfurt-an-Main (SMF)
Sarawak Museum, Kuching (SMK)
National Museum of Natural History, Smithsonian
Institution, Washington (USNM)
Zoological Museum Amsterdam (ZMA)
Museum für Naturkunde, Universität Humboldt,
Berlin (ZMB)
Raffles Museum of Biodiversity Research,
Singapore (RMBR)