

Molecular Phylogeny and Phylogeography of Suidae

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Abstract: Although the Suidae animals were one of the most prosperous mammals, their taxa and phylogeny were poorly studied. To bridge this research gap, the complete mitochondrial DNA *cyt b* sequences (1 140 bp) of one red river pig (*Potamochoerus porcus*), one bearded pig (*Sus barbatus*), and several Eurasian wild boars (*Sus scrofa*) were determined with direct PCR sequencing method. Incorporated with the sequences of other Suidae species obtained from GenBank, the phylogenetic trees of Suidae species were constructed by Neighbor-Joining and Most Parsimony method. Our results showed that the babirousa was sister group of all other species. The African species clustered into a single clade, while the Eurasian species formed the other clade. The phylogenetic positions of the *Sus* species were consistent with their geographical distribution. Based on our phylogeographical results, the *Sus* species could be classified into three groups: (1) the relatively primitive animals from Philippine Islands (such as *S. cebifrons* and *S. philippensis*), Sulawesi and its adjacent islands (*S. celebensis*), (2) the Eurasian wild boars (*S. scrofa* and the mysterious *S. salvanius*), and (3) other pigs (such as *S. barbatus* and *S. verrucosus*), which might further diverged as the islands type (such as *S. barbatus* from Sumatra and Borneo) and the continental type (such as Malay Peninsula wild boar).

Key words: Suidae; Phylogeny; Phylogeography; Mitochondrial DNA; Cytochrome *b*; Pigs

猪科的系统发育与系统地理分化

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摘要: 猪科动物是最为昌盛的哺乳动物之一, 但它们的分类及系统发育关系却尚待解决。为此, 用 PCR 直接测序法测定了一个红河猪 (*Potamochoerus porcus*)、一个马来西亚野猪 (*Sus barbatus*) 以及数头欧亚野猪 (*Sus scrofa*) 线粒体细胞色素 *b* 全序列 1 140 bp。结合从 GenBank 中获得的其他猪科物种细胞色素 *b* 序列, 用邻接法和最大简约法构建了猪科物种的系统发育关系, 其结果显示苏拉威西野猪与其他猪科物种为姐妹群的关系。非洲物种疣猪和河猪聚为一枝, 而欧亚猪属物种聚为另一支。各物种在系统发育关系中的位置与它们的地理分布对应。猪属物种可以被划分为 3 个种组: 分布于菲律宾群岛、苏拉威西岛及其邻近岛屿的猪属物种形成一个种组, 包括苏拉威西岛疣猪、菲律宾疣猪和印度尼西亚疣猪; 欧亚野猪种组, 包括欧亚野猪和姬猪; 须猪和爪哇疣猪形成一个种组。

关键词: 猪科; 系统发育; 系统地理学; 线粒体 DNA; 细胞色素 *b*; 猪

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The family Suidae animals were widely distributed in old world continents and Southeast Asian Islands.

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Simpson (1945) recognized three subfamilies from the extant species: Suinae (including genus *Sus*, *Potamochoerus*, and *Hylochoerus*), Phacochoerinae (*Phacochoerus*) and Babyrousinae (*Babyrousa*). The babirusa (*Babyrousa babyrussa*) was distributed only on Sulawesi and some neighboring islands in Indonesia, while Phacochoerinae (warthog) was distributed in tropical area of Africa. Among the Suinae, both *Potamochoerus* and *Hylochoerus* were only distributed in Africa. The *Sus* were one of the most widely distributed and prosperous mammals, but the number of species in genus *Sus* is unclear. According to Groves (1981), at least 5 species and 24 subspecies are recognized, while Hardjasmita (1987) recognized several additional species. Later, Groves (1997, 2001) and Oliver (2001) classified the genus *Sus* into three species groups: (1) the *S. verrucosus* group, including 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 *S. celebensis*,

and the Visayan pig *S. cebifrons*; (2) the *S. philippensis* group, and (3) the *S. scrofa* group, including Eurasia wild boar *S. scrofa* and the pygmy hog *S. salvanius*. By analyzing morphologic and molecular data, Lucchini et al (2005) disapproved with this species group classification. In summary, the phylogenetic relationships among the species, species groups, genus and subfamilies of Suidae are not clear (Groves, 2001; Lucchini et al, 2005). To bridge this research gap, we analyzed the complete mtDNA *cyt b* sequences of the species in Suidae.

1 Materials and Methods

1.1 Sampling

One red river pig *Potamochoerus porcus*, one Malaysia wild boar *Sus barbatus*, and several Eurasian wild boars and domestic pigs *Sus scrofa*, were collected for sequencing. The sequences of other species (Kijas & Andersson, 2001; Randi et al, 1996, 2002) were obtained from GenBank (Tab. 1).

Tab. 1 Location and GenBank accession number of species used in the phylogenetic analysis

Common name	Species	Location	GenBank accession No.
Peccaries	<i>Tayassu pecari</i>	America	U66290
Babirusa	<i>Babyrousa babyrussa</i>	Indonesia	Z50106
Babirusa	<i>B. babyrussa</i>	Indonesia	AJ314559
Babirusa	<i>B. babyrussa</i>	Indonesia	AJ314560
Babirusa	<i>B. babyrussa</i>	Indonesia	AJ314545
Babirusa	<i>B. babyrussa</i>	Indonesia	AJ314546
Warthog	<i>Phacochoerus africanus</i>	Africa: Tanzania	AJ314547
Warthog	<i>P. africanus</i>	Africa: Kenya	AJ314548
Warthog	<i>P. aethiopicus</i>	Africa: Kenya	AJ314549
Warthog	<i>P. aethiopicus</i>	Africa: Kenya	AJ314550
Warthog	<i>P. aethiopicus</i>	Africa: Kenya	AJ314551
Red river pig	<i>Potamochoerus porcus</i>	Africa	DQ315602
Javan warty pig	<i>Sus verrucosus</i>		AJ314552
Javan warty pig	<i>S. verrucosus</i>		AJ314553
Bearded pig	<i>S. barbatus</i>		AJ314554
Bearded pig	<i>S. barbatus</i>		AJ314555
Bearded pig	<i>S. barbatus</i>		AY534297
Bearded pig	<i>S. barbatus</i>		Z50107
Philippine warty Pig	<i>S. philippensis</i>		AY920905
	<i>S. philippensis</i> × <i>S. barbatus</i>		AY920907
Visayan warty pig	<i>S. cebifrons</i>		AY920906
Bearded pig	<i>S. barbatus</i>	Malay Peninsula	DQ315601
Asian wild boar	<i>S. scrofa</i>	China	DQ315597
Asian wild boar	<i>S. scrofa</i>	China	DQ315598
Asian wild boar	<i>S. scrofa</i>	China	DQ315599
Asian domestic pigs	<i>S. scrofa</i>	China	DQ315600
Italian wild boar	<i>S. scrofa</i>	Italy	AF304201
Swedish wild boar	<i>S. scrofa</i>	Swedish	AF304203
Landrace	<i>S. scrofa domestic</i>	European	NC-000845

1.2 DNA amplification and sequencing

Genomic DNA was extracted by standard phenol/chloroform method. The complete mtDNA *cyt b* sequences were amplified and sequenced by the following primers L15280: (5'-GGA ATC TAA CCA CGA CCA A-3'), H15829: (5'-AAA AGC CCC CTC AGA TTC-3'); L15562: (5'-ATT ACG GAT GAG TTA TTC GCT ACC-3'), H16123 (5'-GGG TTT GCT GGG GTG TAG TTG TCT-3'); L15971 (5'-AAT CTC ATC AGA CAT AGA C-3'), H16543 (5'-AGT CTC CTT CCT TGA GTC TTG G-3'); L16254 (5'-GCC CAT ACT ACA CAC ATC CA-3'), H180 (5'-GTT AAT GCA CGA CGT ACA TAG-3'). L and H refer to light and heavy strands, respectively. PCR were performed in a 50 μ L volume [500 mmol/L Tris-HCl (pH 8.3), 0.1% Triton X-100, 2.5 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.5 mmol/L of each dNTP, 10 μ mmol/L of each primer, and 1 unit of *Taq* polymerase (*S*_{ABC})] following 35 cycles of 1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C. PCR products were purified on spin columns (Watson BioTechnologies Inc., Shanghai) and were directly sequenced for both strands by using BigDye™ Terminator Cycle Sequence Kit (ABI Applied Biosystems) according to the manufacturer's manual.

1.3 Data analysis

Sequences were edited and aligned by DNASTAR package (DNASTAR Inc.). The species, *Tayassu pecari*, that the most closely related to Suidae, was used as the outgroup in the phylogenetic analysis. The neighbor-joining (NJ) tree (Saitou & Nei, 1987) was constructed using Kimura 2-parameter included in MEGA version 3.0 (Kumar et al, 2004). The most parsimony tree was constructed with PAUP 4.0 β (Swofford, 1998) using the branch-and-bound search with TBR branch-swapping option. Robustness of the nodes was assessed by the bootstrap method (Felsenstein, 1985) after 1 000 replications (bootstrap option with heuristic search in PAUP) by adding sequences random.

2 Result and Analysis

2.1 The phylogenetic relationships among the subfamilies and genus

To investigate the phylogenetic relationships among the subfamilies and genus, the phylogenetic trees of Suidae species were constructed by both Neighbor-Joining and Most Parsimony method. In NJ tree (Fig. 1a), Phacochoerus and Suidae clustered into a large branch, which became the sister group of the clade solely composed of Babyrousa. However, the sister relationship between *Potamochoerus* and *Phacochoerus* received low

statistic support (BP = 28). MP analysis of the dataset (1 140 nucleotide characters with 363 variable sites and 261 parsimony-informative sites) generated 10 MPTs (CI = 0.655, RI = 0.827, RC = 0.542, *L* = 667). The strict consensus tree of these 10 MPTs was produced as shown in Fig. 2b. Both methods produced essentially identical tree topologies. In MP analysis, the sister relationship between *Potamochoerus* and *Phacochoerus* received moderate statistic support (BP = 65). The phylogenetic status of Babyrousa was consistent with its special morphological characters, such as the more complex stomach (Langer, 1988), the remarkable canines of the male (Groves, 1981), compared with other Suidae animals. Interestingly, a recently study (Bernhard & Lowden, 2004) on suid herpesviruses also showed that the virus Babyrousa babyrousa rhabdovirus 1 (Bbab RHV-1), identified from Babyrousa, were more closely related to a ruminant gammaherpesvirus, bovine herpesvirus 4 (BoHV-4), than to the virus identified from other suid animals, which were closely related to known beta- (porcine cytomegalovirus) and gammaherpesviruses [porcine lymphotropic herpesvirus (PLHV) 1 and 3] of domestic pigs. So the molecular evidence, together with the morphological evidences support the suggestion that Babyrousa becomes an independent family. In the large clade composed of Phacochoerus and Suinae, all Eurasian species were clustered into a single clade, while the African species formed another clade with moderate support. This makes the *Sus* paraphyletic, but seems in favor of the suggestions that the African species is monophyletic (Groves & Grubb, 1993).

2.2 The phylogenetic relationships among the *Sus* species

The visayan wild pigs *S. cebifrons* branched off firstly from the Eurasian clade (Fig. 1), this was consistent with Lucchini's (2005) results. Although the *S. celebonse* was not analyzed here, but it might have the similar phylogenetic status since it retains the same plesiomorphic character with *S. cebifrons* (Groves, 1997). The next species diverged from the branch was the Philippine wild boar *S. philippensis*. The phylogenetic position of visayan and Philippine wild pigs showed that the species in Philippine Islands and sulawesi were more primitive than species from other places. The *S. barbatus* and *S. verrucosus* were clustered into a single clade, and became a sister group of the Eurasian wild boar *S. scrofa* which was clustered into another single clade. It's noteworthy that this sister group relationships didn't support the previous results that the *S. barbatus* and *S. verrucosus* were the ancestral population of

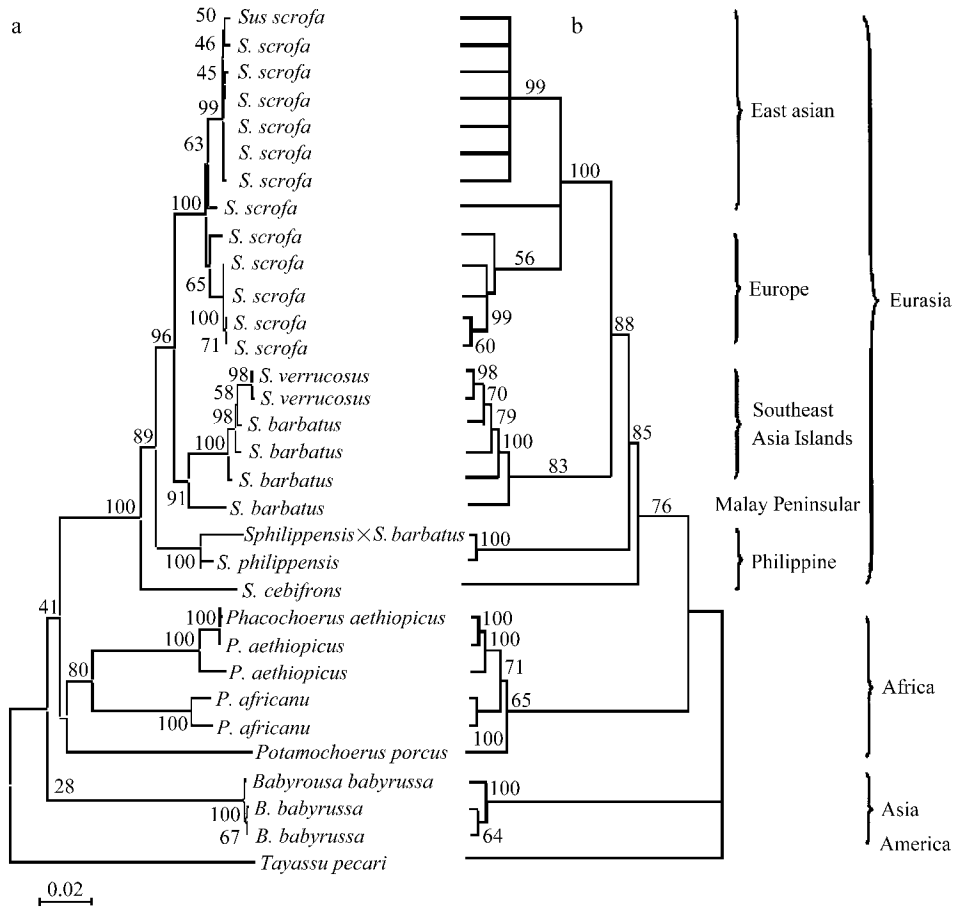


Fig. 1 Phylogenetic trees constructed by neighbor-joining method and most parsimony method. The numbers on nodes are the bootstrap value (1 000 replicates). Both trees produce the identical topology. (a) NJ tree; (b) The strict consensus tree derived from the MP analysis.

Eurasian wild boars (Larson et al., 2005). In the *Sus barbatus* clade, the pigs from Malay Peninsular occupied a distinct clade, while the animals from Islands clustered into another clade.

2.3 The species groups classification and their phylogenetic relationships

Groves (1997, 2001) classified the *Sus* species into three groups based on their morphological characters. By analyzing both morphological and molecular data, Lucchini's (2005) reclassified the species groups as follows: (1) the pigs from the Philippines (*S. cebifrons*) and Sulawesi (*S. celebensis*), (2a) the western *S. barbatus* subspecies, (2b) a group consisting of *S. scrofa*, *S. verrucosus*, and *S. cf. barbatus ahoenobarbus*. Our results showed that the phylogenetic relationships were consistent with their geographical distribution, so the *Sus* species could be classified accordingly as the following three groups: (1) the relatively primitive animals from Philippine Islands (such as *S. ceb-*

ifrons and *S. philippensis*), Sulawesi and its adjacent islands (*S. celebensis*), (2) the Eurasian wild boars (*S. scrofa* and the mysterious *S. salvanius*), and (3) other pigs (such as *S. barbatus* and *S. verrucosus*), which might further diverged as the islands type (such as *S. barbatus* from Sumatra and Borneo) and the continental type (such as Malay Peninsula wild boar). Each species group was similar but different from previous classification (Groves, 1997, 2001; Lucchini's, 2005).

In general, our phylogenetic analysis showed that the Africa species, the Sulawesi Babyrousa, and the Eurasia species, each formed an independent group that was consistent with their geographical distribution. Furthermore, the phylogenetic relationships among the species in Suidae were also consistent with their geographical distribution. This finding would be beneficial for further research on their early dispersal history, taxonomy and conservation.

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赵尔宓,男。1951年毕业于华西大学生物系。中国科学院成都生物研究所研究员,四川大学教授。2001年当选为中国科学院院士。曾为美国康乃尔大学高级访问教授、美国伯克利加州大学客座教授。

命名40个新种和亚种,发现15个我国新纪录,建立两个新属,报道我国1个科的新纪录。发表论文140余篇,指导研究生论文30余篇,出版著作40种,其中在美国出版的英文版《中国两栖爬行动物学》一书,是第一部全面系统介绍我国661种两栖和爬行动物的专著。我国首批入藏考察的两栖爬行动物学者之一,为西藏增加8个新种和10个中国或西藏新纪录种,并首次报道在墨脱希壤采到眼镜王蛇,将其分布范围向北推移了4个纬度,认为这是亚热带动物沿雅鲁藏布江大峡谷水汽通道向北扩散的证据。主要依据爬行动物的分布首先提出在动物地理区划的西南区增加一个新的“喜马拉雅南坡亚区”。1976年提出对新疆西部草原毒蛇危害的生态防治措施;1979—1980年命名新种“蛇岛蝮”,并提出它在蛇岛上的起源和演化见解;1989年对西太平洋岛链两栖爬行动物区系形成和温带东亚两栖动物的分布格局提出自己的研究见解。1992年首次提出“我国毒蛇咬伤的医学地理学”概念,指导毒蛇咬伤防治实践;筹组全国两栖爬行动物学会;创办《四川动物》、《两栖爬行动物学报》、*Asiatic Herpetological Research* 三种期刊和《蛇蛙研究丛书》(已出12辑)。