TEXT S1

Genomic DNA Extraction, Polymerase Chain Reaction (PCR) and Sequencing

The genomic DNA was extracted from 1.0-2.0 mm of the preserved fin clips of each specimen using a modified CTAB extraction method. The extracted genomic DNA were stored in -20°C until amplification. The PCR amplification targeting cytochrome *c* oxidase I, [*COI*], cytochrome *b* [*cytb*] and recombination activating 1 [*rag1*] genes (Table S1) were performed on BioRad Thermocycler (BioRad Laboratories Inc., USA) in a total volume of 25 µL reactions. The PCR reactions consist of 5.5 µL of 5× MyTaq Red Reaction buffer (Bioline Reagents Ltd, UK), 0.5 µL of 10 uM forward and reverse primers, 0.25 µL of i-Taq™ DNA polymerase (iNtRON Biotechnology, Inc., Gyeonggi-do, Korea), 17.25 µL of distilled water and 2.0 µL template DNA. The PCR reactions were same for each gene. The thermal conditions for *COI* gene were: 4 min of pre-denaturation at 94°C, followed by 40 cycles of 30 s of denaturation at 94°C, 50 s annealing at 47.9°C and 3 min of initial extension at 72°C, and 7 min of final extension at 72°C. Thermal conditions for *cytb* gene were: 2 min of pre-denaturation at 95°C, followed by 40 cycles of 45 s of denaturation at 94°C, 45 s annealing at 55°C and 1 min of initial extension at 72°C, and 7 min of final extension at 72°C. Thermal conditions for *rag1*gene were: 2 min of pre-denaturation at 95°C, followed by 40 cycles of 45 s of denaturation at 94°C, 45 s annealing at 48.9°C and 1 min of initial extension at 72°C, and 7 min of final extension at 72°C. The samples were stored at 4°C before gel electrophoresis. All PCR products were visualized on 1.7% (w/v) agarose gel stained with 1.0-1.5 µL of RedSafe™ Nucleic Acid Staining Solution (iNtRON Bio-technology, Inc., Gyeonggi-do, Korea) in 0.5× Tris-borate-EDTA (TBE). The PCR products with clear bands were considered as successfully amplified and were then sequenced by First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia) using an ABI3730XL capillary sequencer (Applied Biosystems, USA).

TABLE S1

Table S1. Primer pairs used to target mitochondrial DNA genes (*COI* and *cytb*) and nuclear DNA gene (*rag1*)

DNA target	Targeted gene (size, reference)	Primer sequences
Mitochondrial DNA	***COI*** (650 bp) (Ward *et al.*, 2005)	FishF1: 5'- TCA ACC AAC CAC AAA GAC ATT GGC AC -3' FishR1: 5'- TAG ACT TCT GGG TGG CCA AAG AAT CA -3' FishF2: 5'- TCG ACT AAT CAT AAA GAT ATC GGC AC -3' FishR2: 5'- ACT TCA GGG TGA CCG AAG AAT CAG AA -3'
	cytb (1200 bp) (Newly designed)	Sclero_CytbF: 5'- ACG GCC TGA AAA ACC GTT GTT GCA TTC -3' Sclero_CytbR: 5'- TTA GCT TTG GGA GTT AAG GGC GGG AGT T -3'
Nuclear DNA	***rag1*** (800-1500 bp) (Li and Ortí 2007, Lopez et al. 2004)	RAG1-2510F: 5'- TGG CCA TCC GGG TMA ACA C -3' RAG1-3222F: 5'- TCY TTC CGC TTY CAC TTC CG -3' RAG1-4078R: 5'- TGA GCC TCC ATG AAC TTC TGA AGR TAY TT -3'

Reference for primers:

Li C, Ortí G (2007) Molecular phylogeny of Clupeiformes (Actinopterygii) inferred from nuclear and mitochondrial DNA sequences. Mol Phylogenet Evol 44:386–398 doi:10.1016/j.ympev.2006.10.030.

López JA, Chen WJ, Ortí G (2004) Esociform phylogeny Copeia 2004:449–464

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD (2005) DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci 360:1847–1857 doi:10.1098/rstb.2005.1716.

FIGURE S1

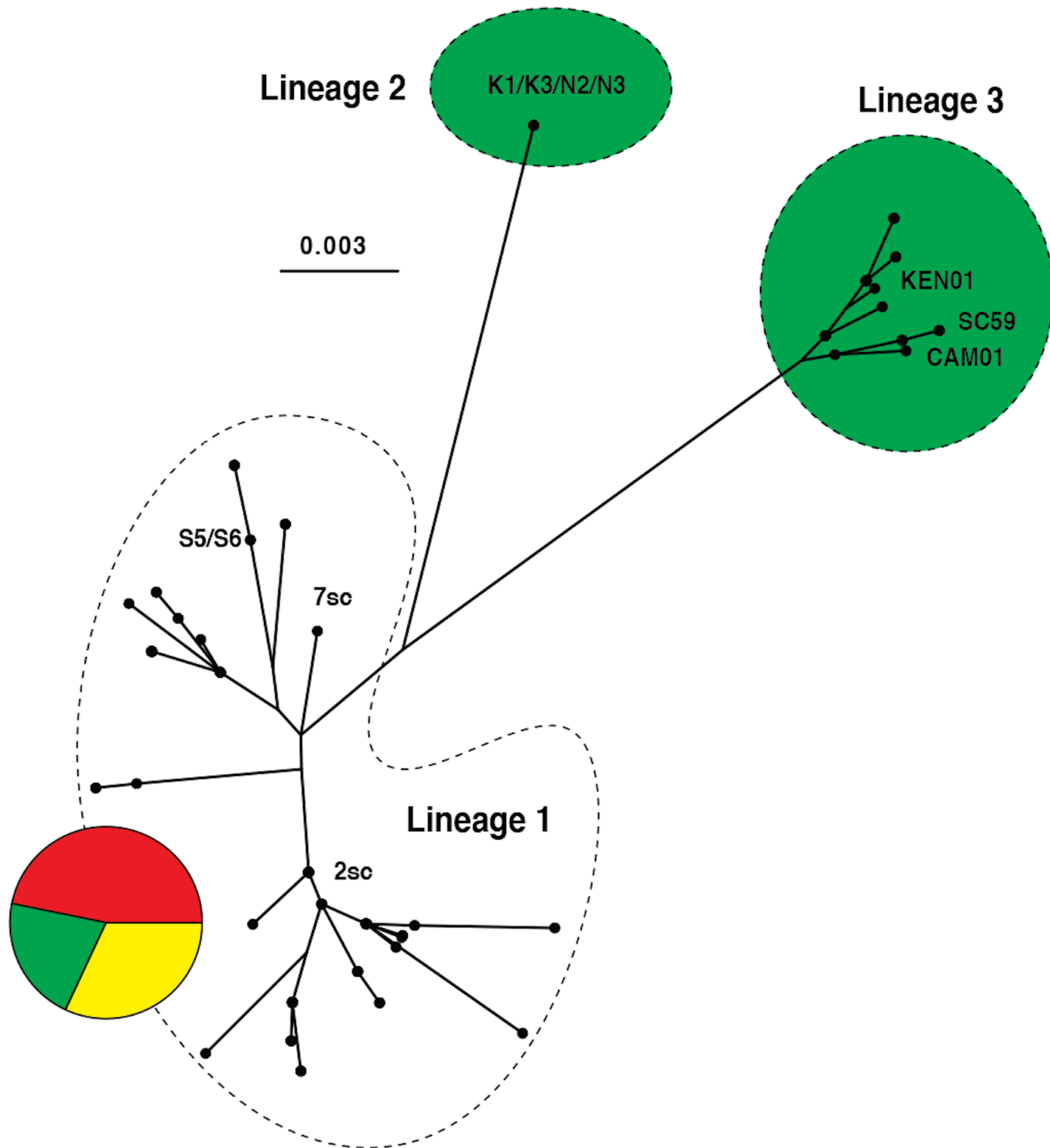

Figure S1. Unrooted maximum likelihood trees for GenBank archived sequences of *cytochrome b, mitochondrial* gene (total: 180 haplotypes) for which the color-variety of the specimens of *Scleropages formosus* are indicated. Most of these sequences are from cultivated specimens without natural geographical origin (therefore not used in the main part of this study which focuses on phylogeography). These trees were built using the software RaxML. Only the names of specimens newly sequenced in this study are shown. This tree demonstrates that our limited-size dataset not only captures the known genetic variability within *Scleropages formosus* but also extends the variability in revealing a new lineage (lineage 2).

FIGURE S2

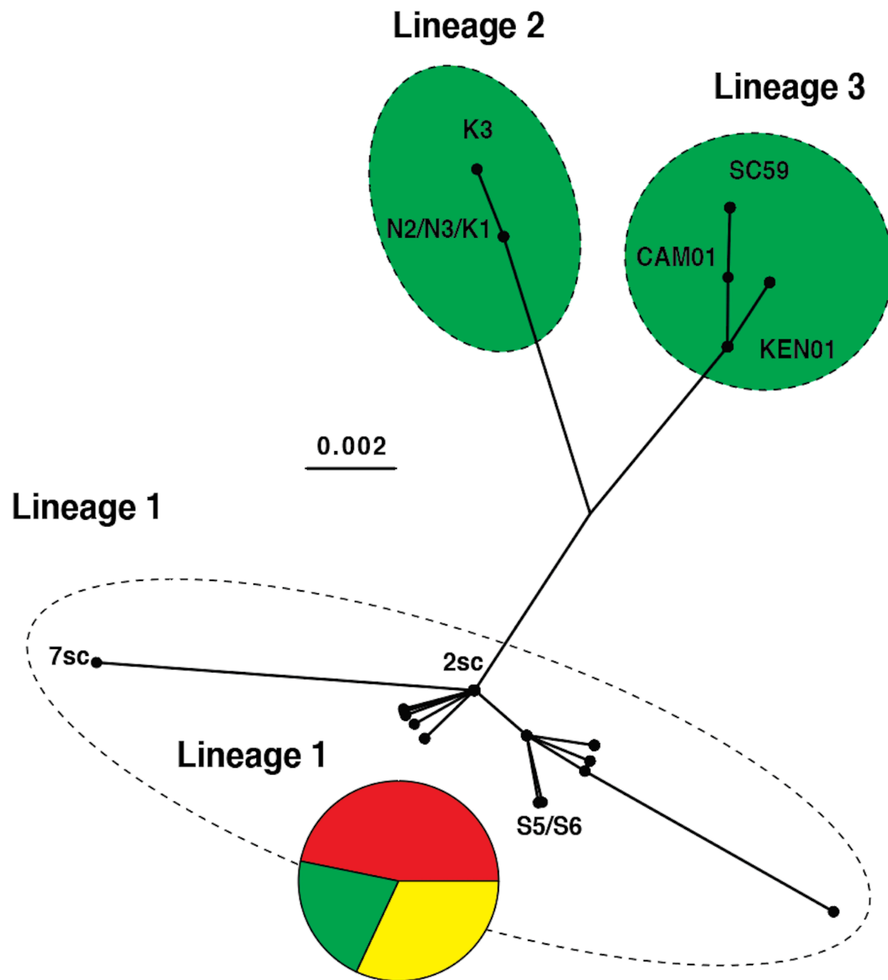

Figure S2. Unrooted maximum likelihood trees for GenBank archived sequences of *cytochrome c oxidase I, mitochondrial* gene (total: 171 haplotypes) for which the color-variety of the specimens of *Scleropages formosus* are indicated. Most of these sequences are from cultivated specimens without natural geographical origin (therefore not used in the main part of this study which focuses on phylogeography). These trees were built using the software RaxML. Only the names of specimens newly sequenced in this study are shown. This tree demonstrates that our limited-size dataset not only captures the known genetic variability within *Scleropages formosus* but also extends the variability in revealing a new lineage (lineage 2).