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# Differentiation in stag beetles, *Neolucanus swinhoei* complex (Coleoptera: Lucanidae): Four major lineages caused by periodical Pleistocene glaciations and separation by a mountain range

Q1 Cheng-Lung Tsai [a], Xia Wan [b], Wen-Bin Yeh [a,*]

[a] *Department of Entomology, National Chung Hsing University, 250, Kuo Kuang Rd., Taichung 40227, Taiwan*
[b] *Department of Ecology, School of Resources and Engineering, Anhui University, Jiulong Road 111, 230601 Hefei, PR China*

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ABSTRACT

Taxonomic debates on *Neolucanus swinhoei* complex consisting of *N. swinhoei*, *N. doro doro*, *N. doro horaguchii*, and *N. euganiae*, distributed exclusively in Taiwan, have been ongoing for several decades because of their overlapping morphological characters. To clarify their taxonomic status and phylogeographical history, we analyzed nine morphological characteristics and four molecular amplicons. Phylogenetic inferences based on COI + 16S rDNA + *wingless* showed one eastern and three western lineages, with the latter consisting of one low-hill and two montane lineages. Intermingled DNA sequences from different populations within each lineage, many low $F_{ST}$ values, and a high variance component between lineages indicate the possibility of gene flow among populations. However, positive relationships were observed between the genetic divergences of 16S rDNA and its $F_{ST}$ values with geographic distance. A divergence estimation based on COI + 16S revealed that these beetles might have originated from Asian mainland and differentiated into western and eastern lineages ca. 1 Mya, with the differentiation of the western lineages occurring approximately 0.50–0.75 Mya. Isolation by mountain ranges and limited flying capability of these beetles as well as populations retreat to and expansion from refugia in response to glaciation cycles have resulted in the current distribution of *N. swinhoei* complex. Although most morphological characters are variable and undistinguishable, multi-dimensional scaling analysis based on measurable characteristics could recognize hill *N. swinhoei* as a cluster distinct from the others. However, based on the realities of genetic admixture, shared phylogeographical history and overlapping characteristics, all of these stag beetles should be regarded as *Neolucanus swinhoei* Bates, 1866.

## 1. Introduction

The island of Taiwan, situated in both subtropical and tropical regions, was formed approximately six million years ago by the collision of the Philippine Sea plate and Eurasian plate (Huang et al., 1997). The drastic uplift of mountain ranges about 2.5–1 Mya (Teng, 1990; Huang et al., 1997) resulted in the appearance of the Central Mountain Range (CMR) forming a north–south axis of an altitude up to 3952 m, as well as the branch ranges of Xueshan, Yushan, and Alishan with more than 200 peaks higher than 3000 m in elevation. Diverse topography and climate have boasted a rich biodiversity of Taiwan. Mountain range topography was the major driving force of diversification on Taiwanese biota, as proposed in some population genetic studies on a number of plants, invertebrates, fishes, amphibians, and reptiles (Yang et al., 1994; Toda et al., 1998; Lin et al., 2002; Su et al., 2003; Yeh et al., 2004, 2009; Cheng et al., 2005a,b; Shih et al., 2006, 2007; Huang and Lin, 2010; Wu et al., 2010).

Pleistocene climatic fluctuation was another important factor that has significant impact on the distribution and differentiation of extant organisms in Taiwan. During glaciations, decreasing temperature combined with different topography and latitude would have affected population dynamics. Glacial cycles might have repeatedly promoted the isolation of populations in refugia and induced their secondary contacts due to range expansion during the warm period (Cheng et al., 2005a,b; Chiang et al., 2006; Yeh et al., 2009; Huang and Lin, 2010; Wu et al., 2010). In a glaciation scenario, montane populations might have dispersed to lower altitudes and/or refugia; and then they migrated to higher altitudes in different mountain or northern areas during interglacial periods.

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Q2 * Corresponding author. Fax: +886 4 22875024.
*E-mail address:* wbyeh@nchu.edu.tw (W.-B. Yeh).

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Phylogeographical studies focused on the relationship between genetic lineages and geographical distribution of closely related species would help us understand their evolutionary history (Avise, 2000). In Taiwan, among the hypotheses proposed to explain the population differentiation caused by glaciation events and/or the CMR interruption, the common one suggests a recent colonization or range expansion deduced by the last glacial period, leaving little or no divergence within the vagile species, e.g., cricket, butterfly, wood spider, fish, and bat (Wang et al., 1999; Chen and Shih, 2003; Lee et al., 2004; Wang et al., 2004; Yeh et al., 2004, 2005, 2006; Chen et al., 2006; Lin et al., 2008). The second hypothesis suggests that differentiation of populations with specific geographical distribution could be accounted for by glaciations events and the CMR isolation, as observed in many plants, invertebrates, fishes, and amphibian (Yang et al., 1994; Lu et al., 2002; Yeh et al., 2004; Lin et al., 2008; Chiang et al., 2010; Huang and Lin, 2010, 2011). For example, the cyprinid fishes introduced during glaciations to Central Taiwan and then colonized in southern and northern Taiwan were separated by mountain ranges. The third hypothesis suggests that closely related species or populations could be isolated in different mountain ranges, as reported by Lai and Lue (2008) and Li et al. (2011) for salamanders which are found to distribute in a limited area in elevation higher than 1500 m. Finally, differentiated populations could be confined in small areas, i.e., refugia, where they were protected throughout the glaciation events. Examples include *Oncorhynchus masou formosanus* salmon in Chichiawan Stream, *Lucanus formosana* stag beetle in the ALS range, *Cunninghamia konishii* fir and *Castanopsis carlesii* beech in the Xueshan range, crabs and cycad in southeastern Taiwan (Huang et al., 2001; Chung et al., 2004; Cheng et al., 2005a,b; Shih et al., 2006, 2007; Huang and Lin 2010).

The stag beetles of *Neolucanus*, which are distributed in Oriental and Sino-Japanese regions (Krajcik, 2001; Holt et al., 2013), are defined taxonomically by the equal length of the head and elytra, a completely protruding canthus, and serrate teeth on the inner side of the non-protruding mandibles (Miwa, 1932; Yang, 1963). In Taiwan, taxonomic debates among *N. swinhoei* Bates, *N. eugeniae* Bomans, *N. doro doro* Mizunuma, and *N. doro horaguchii* Nagai have been ongoing for a long time because of their simple original description and overlapping characteristics (Mizunuma and Nagai, 1994; Nagai, 2001). According to the original description, *N. swinhoei* is distributed in lower hills (Bates, 1866), while the remaining three mainly are distributed in cloud forests. *N. eugeniae* occurs only in southern Taiwan at elevations of approximately 1000 m (Bomans, 1991); *N. d. doro* is confined to west-central Taiwan, i.e., Nantou County, between elevations of 1600 and

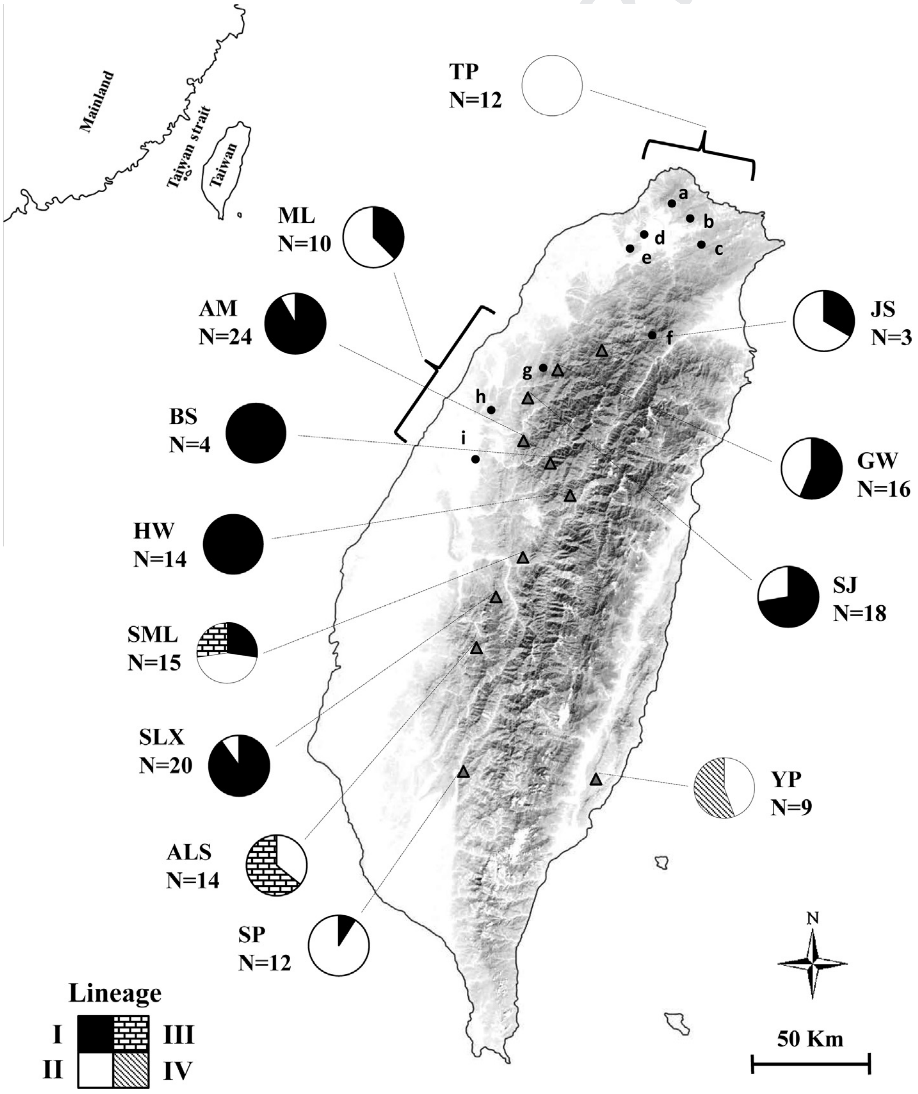

**Fig. 1.** Collection localities of *N. swinhoei* complex. Abbreviations of populations are the same in Table 1. Individuals and phylogenetic lineages composition of each population based on the phylogenetic analyses of COI + 16S rDNA + *wingless* are shown (see Fig. 3).

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