

Genetic relationships among Asian *Corbicula*: Thai clams are referable to topotypic Chinese *Corbicula fluminea*

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Abstract. A survey of 28 electrophoretically detected allozyme loci revealed that Thai *Corbicula* are weakly differentiated (Nei's unbiased genetic distance, $D = 0.12-0.16$) from *Corbicula fluminea* from the type locality in southern China, 1800 km away. This finding supports our earlier proposal that 20 nominal *Corbicula* species from Thailand are junior synonyms of the widespread and conchologically variable *C. fluminea*.

We have shown recently that 21 nominal species of freshwater clams, *Corbicula*, from Thailand are genetically indistinguishable and most probably referable to the widespread Asian species *C. fluminea* (Müller, 1774) (Kijviriya *et al.*, 1991). We found little variation at 24 allozyme loci in *Corbicula* collected from 40 sites up to 1500 km apart in Thailand. Thirty-five of the samples were found to be genetically identical; clustering at insignificant ($D < 0.01$) multilocus genetic distances (Nei, 1978). The remaining five samples (from northeast Thailand) were weakly differentiated from the others with $D < 0.04$. The low levels of genetic differentiation led us to suggest that 20 Thai species were junior synonyms of the earliest named local taxon, *C. fluminea*, a population of which was recognised from the Chao Phraya River, Bangkok, by Brandt (1974). We argued that our proposed revision would be supported by the demonstration that the Thai *Corbicula* are genetically similar to Chinese clams from the type locality of *C. fluminea*. We now present a genetic comparison of the Thai clams with topotypic *C. fluminea* from south China and show that the proposed synonymy was appropriate.

MATERIALS AND METHODS

To test the hypothesis that the Thai clams are genetically similar to topotypic *Corbicula fluminea* we performed a multilocus comparison of two samples of *Corbicula* from northeast Thailand and one from Hong Kong, 1800 km to the northeast. One of the Thai samples had been shown previously to be genetically identical to putative *C. fluminea* from Thailand but its relationship to topotypic clams was unknown. The three samples were:

Thai 1. Collected from Ubonrat Reservoir, Khon Kaen Province, northeast Thailand. Voucher specimens in Museum, Center for Applied Malacology and Entomology, Faculty of Science, Mahidol University (MUFS-THOO-167) and Los Angeles County Museum of Natural History (LACM 85-366.1). These clams were identified originally using Brandt's (1974) criteria as *Corbicula lydigiana* Prime, 1861, by Kijviriya (1990), and subsequently referred to as Sample 23 in Kijviriya *et al.* (1991), where they were shown to be genetically identical to putative *C. fluminea* from the Chao Phraya River, Bangkok.

Thai 2. Collected at Ban Huikom, Phibun Mangsahan District, Ubon Ratchathani Province, northeast Thailand. Conchologically very similar to Thai 1; specific identity not determined. Voucher specimens: MUFS-THOO-192 and LACM 85-367.1.

China. Collected at Ping Long Village, Lam Tseun River Valley, New Territories, Hong Kong. Shell color and morphometric variation in this population has been described in detail by Morton (1987). Voucher specimens: LACM 85-368.1.

Clams were frozen at -70°C immediately after collection and handcarried to the senior author's laboratory for genetic analysis. Starch gel electrophoretic separation of allozymes extracted from whole body homogenates was according to the methods of Kijviriya *et al.* (1991). For 20 loci the specific methods are given by Kijviriya *et al.* (1991: see Table 1 for full names and Enzyme Commission numbers): *Aat-1*, *Aat-2*, *Es-1*, *Es-4*, *Es-5*, *G6pdh*, *Gpi*, *Idh-1*, *Idh-2*, *Mdh-1*, *Mdh-2*, *Mdhp-1*, *Mdhp-2*, *Pep-A-1*, *Pep-B-2*, *Pep-B-3*, *Pgdh*, *Pgm-1*, *Pgm-2*, *Xdh*. Three additional loci [*Acp* (E.C. 3.1.3.2), *Cat* (1.11.1.6), *Ldh* (1.1.1.27)] were resolved on the TC 6.0 buffer described therein, and five others were resolved

on the TC 6.8 buffer used by Staub *et al.* (1990); *Gapdh* (1.2.1.12), *G3pdh* (1.1.1.8), *Mpi* (5.3.1.8), *Sod-1*, *Sod-2* (1.15.1.1). Data consisting of genotypes for individual clams scored at all 28 loci were analyzed and Nei's genetic identities (*I*) and unbiased genetic distances (*D*) (Nei, 1972, 1978, respectively) were calculated using the BIOSYS-1 computer programs (Swofford and Selander, 1981).

RESULTS

Allozymic variation is summarized in Table 1. Twenty-one of the 28 loci showed no detectable variation; seven loci varied geographically. Both Thai samples were isogenic. The Chinese sample showed low levels of genetic variability, with diallelic systems at four loci and genotypes in panmictic frequencies. The Chinese clams are slightly more variable than the most variable samples reported previously from Thailand (Kijviriyia *et al.*, 1991) due to the addition of another polymorphic locus, *Mpi*, to the survey.

The Thai 1 sample is genetically identical to Sample 23 of Kijviriyia *et al.* (1991), which was collected at the same reservoir. Thai 2 was found to be very similar to Sample 20 of Kijviriyia *et al.* (1991), which was collected from the same district. The small genetic distance (Table 2: $D = 0.04$) between Thai 1 and Thai 2 is similar to that reported between Samples 20 and 23 and typical of the maximum genetic differentiation detected previously among Thai *Corbicula*. The fixed difference at *Es-5* had been detected previously in Thai clams.

The Thai *Corbicula* are very similar to topotypic *C. fluminea* from Hong Kong. The mean genetic distance, $D = 0.14$, is largely due to fixed differences at *Mdh-1*, *Mpi*, and *Pgm-1*.

Table 1. Genetic variability in Asian *Corbicula**.

Sample:	Thai 1	Thai 2	China
Locus	Allele frequency		
<i>Aat-2^a</i>	1.00	1.00	0.61
<i>Es-5^a</i>	1.00	0.00	0.00
<i>Gpi^b</i>	1.00	1.00	0.75
<i>Mdh-1^a</i>	1.00	1.00	0.00
<i>Mpi^b</i>	1.00	1.00	0.5/0.5
<i>Pcp-A-1^a</i>	1.00	1.00	0.64
<i>Pgm-1^a</i>	1.00	1.00	0.00
Summary statistics			
<i>N</i>	16	17.5	17.7
<i>A</i>	1.0	1.0	1.1
<i>P</i>	0.00	0.00	0.14
<i>H</i>	0.00	0.00	0.07

*Summary statistics are: *N*, mean sample size; *A*, mean no. of alleles per locus; *P*, proportion of loci polymorphic; *H*, mean individual heterozygosity. Each variable locus has two alleles except for triallelic *Mpi*, where alleles *a* and *c* co-occur in the Chinese sample.

Table 2. Matrix of genetic distance values.

Sample	Thai 1	Thai 2
Thai 1	---	
Thai 2	0.04	---
China	0.16	0.12

DISCUSSION

The genetic identity among the Asian clams studied here is slightly higher than that reported in an earlier 12-locus comparison of samples from Hong Kong and the United States (Smith *et al.*, 1979): $I = 0.87$ vs. 0.84. The Thai *Corbicula fluminea* are thus more similar to those of China than the latter are to their trans-Pacific derivatives.

Although there is no simple relationship between genetic distance and taxonomic level the $D = 0.14$ value reported here is within the range found among conspecific populations of geographically widespread molluscs and other animals (Davis, 1983; Thorpe, 1983; Woodruff *et al.*, 1988). Cases of interspecific comparisons with $D < 0.14$ are known but, in such cases, species status rests on other criteria (especially behavioral traits that could function as reproductive isolating mechanisms or species recognition cues, post-mating sterility barriers, chromosomal reorganization, etc.) and genetic similarity merely reflects the recency of cladogenesis. We know of no such criteria which might affect our interpretation of the genetic similarities discovered among Asian *Corbicula*. Detailed studies of anatomical variation in clams from China (Morton, 1987) and Thailand (Kijviriyia, 1990) failed to reveal any taxonomically significant variation. Only in the case of conchological features (shell size, shape, sculpture and color), has significant local and geographic variation been documented. Such conspicuous conchological variation, employed by Brandt (1974) to recognize 28 *Corbicula* species in Thailand, is now seen to be taxonomically irrelevant. Morton's (1986) opinion, that most or all of Brandt's taxa are junior synonyms of *C. fluminea*, is supported. We reaffirm our recommendation (Kijviriyia *et al.*, 1991) that 20 nominal Thai taxa for which we have genetic data be synonymized with *C. fluminea* (Müller, 1774).

Finally, our finding of only moderate differentiation within geographically widespread populations of *Corbicula fluminea* on the Asian mainland contrasts with the higher levels of differentiation ($I = 0.3$) reported between Japanese and Philippine clams by Smith *et al.* (1979). A comprehensive allozymic survey in Asia is now required to establish whether the island populations merit specific recognition. Such a survey could also resolve two issues involving the North American *C. fluminea*: the identification of the source population(s) and the number of successful colonizations. Although available genetic data support an Asian mainland origin

hypothesis, they do not preclude an island origin, or multiple introductions from either source. Given the apparent genetic uniformity of the Asian mainland populations, it could be difficult, however, to reconstruct the events surrounding their introduction to North America using allozymes.

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