

Results

Based on analyses in Cervus and GENEPOP, two loci (TF064 and TF163) showed significant differences between observed and expected heterozygosities in *T. viridipunctatus* and four (TF098, TF100, TF121 and TF167) in *T. luyeanus* (Table 1, Table 2). The deviation to Hardy-Weinberg equilibrium (HWE) is probably due to the Wahlund effect (Wahlund 1928) because samples from several geographically isolated populations were put together in HWE detection (Lin *et al.* 2006).

In testing linkage by GENEPOP, all of the *p*-value statistics were larger than 0.0006, the critical value of *p* after being proofread by Bonferroni test, indicating unlinked loci.

No pairwise comparison between loci showed significant gametic disequilibrium. Heterozygotes were discovered in both genders for all these loci, showing that no locus is sex-linked.

Determination hybrids by mitochondrial DNA data

Using specific primers to process PCR, one individual (1/299), which was collected from the southern-bank of the Liwu River, where *T. luyeanus* is usually found, carries the genetic characteristics of *T. viridipunctatus* (Figure 3, Figure 4).

Determination hybrids by microsatellite data

Using RstCalc to calculate R_{st} values between *T. viridipunctatus* and *T. luyeanus*, six loci out of 13 show significant differentiation (Table

3).

Two populations which conform to Hardy-Weinberg equilibrium were detected both in the 13-loci and 6-loci analyses. Five and eight individuals were assigned as hybrids in the 13-loci and 6-loci analyses, respectively. Among these individuals assigned hybrid status, two are shared between the analyses, giving a total of 11 individuals of potential hybrid origin of the north bank. On the other hand, 11 and 15 individuals were respectively assigned to be hybrids from the south bank. Six among them are shared by these two analyses, representing the potential hybrids of this region to be 20 (Figure 3, Figure 4).

Summary of determination hybrids

Based on microsatellite data, levels of introgressive hybridization of *T. viridipunctatus*. and *T. luyeanus* are around 3% (nine individuals of 299 samples) ~ 10% (31 individuals of 299 samples) (Table 4). This is in contrast to be low proportions of hybrids detected by mitochondrial DNA. Due to the conflicting nuclear and mitochondrial DNA data, it is presumed that a large proportion of hybrids are caused by male introgression (Table 5).

There was no statistically significant difference of the levels of introgressive hybridization between these two species (chi-square test, $p < 0.05$). The mean migration rate between the two species is 0.166, calculated with BayesAss. The migration rate of *T. luyeanus* to *T. viridipunctatus* is 0.004, and *T. viridipunctatus* to *T. luyeanus* is 0.008 (Table 6). Rates of these two directions did not show statistic difference. These values are comparatively much lower than the intraspecific

migration rates of *T. viridipunctatus* (0.996) and *T. luyeanus* (0.992). The two species distributed around river have a high genetic distance $(\delta\mu)^2$ of 3.4301 (Goldstein *et al.* 1995), and $R_{st} = 0.1346$ ($p < 0.005$) and $Nm = 1.6068$ calculated by RstCalc and $F_{st} = 0.0659$ calculated in GENEPOP (Table 7).