

# Appendix

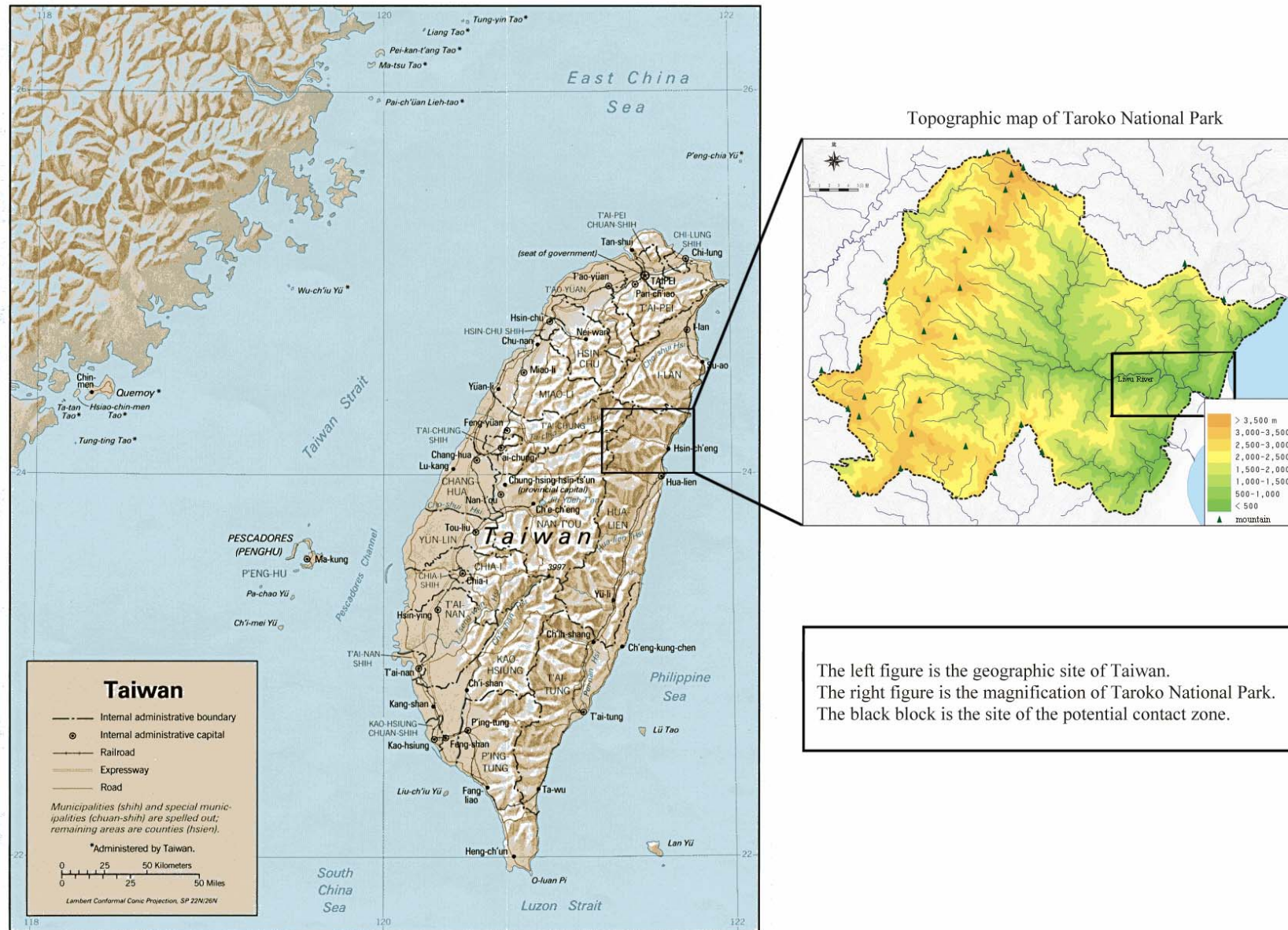


Figure 1. The geographic site of the potential contact zone of *Takydromus viridipunctatus* and *Takydromus luyeanus*.

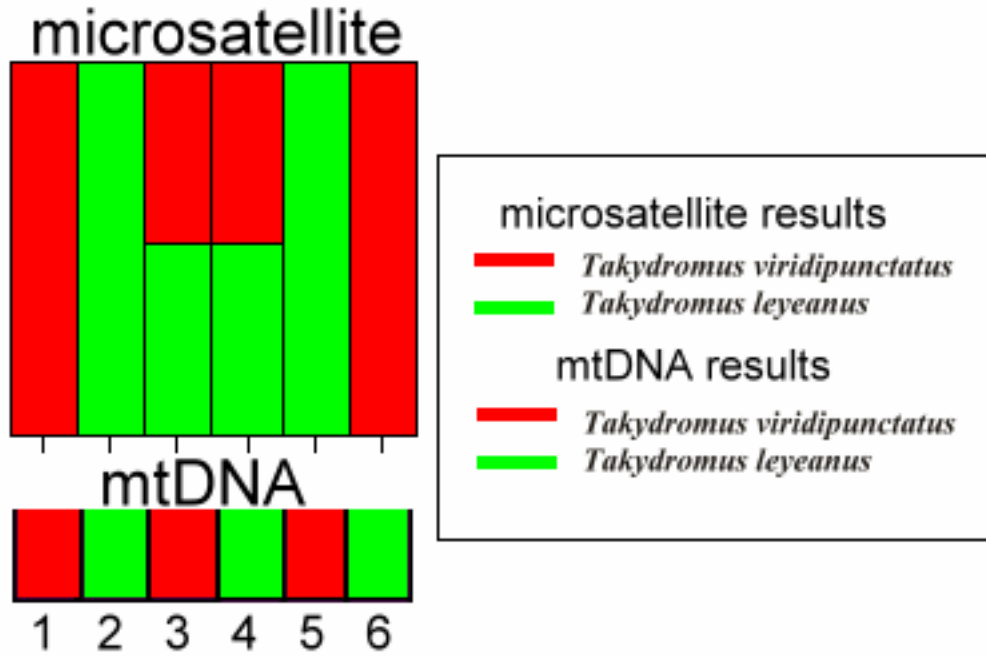


Figure 2. Hypothetical molecular results and their interpretation: Individual 1 and 2 carry the genetic character of the same species with both mitochondrial DNA and microsatellite loci, which were assigned to the pure species individuals of *Takydromus viridipunctatus* and *Takydromus lueyanus*. The other individuals carry partial of genetic character of another species, which were determined to be hybrids.

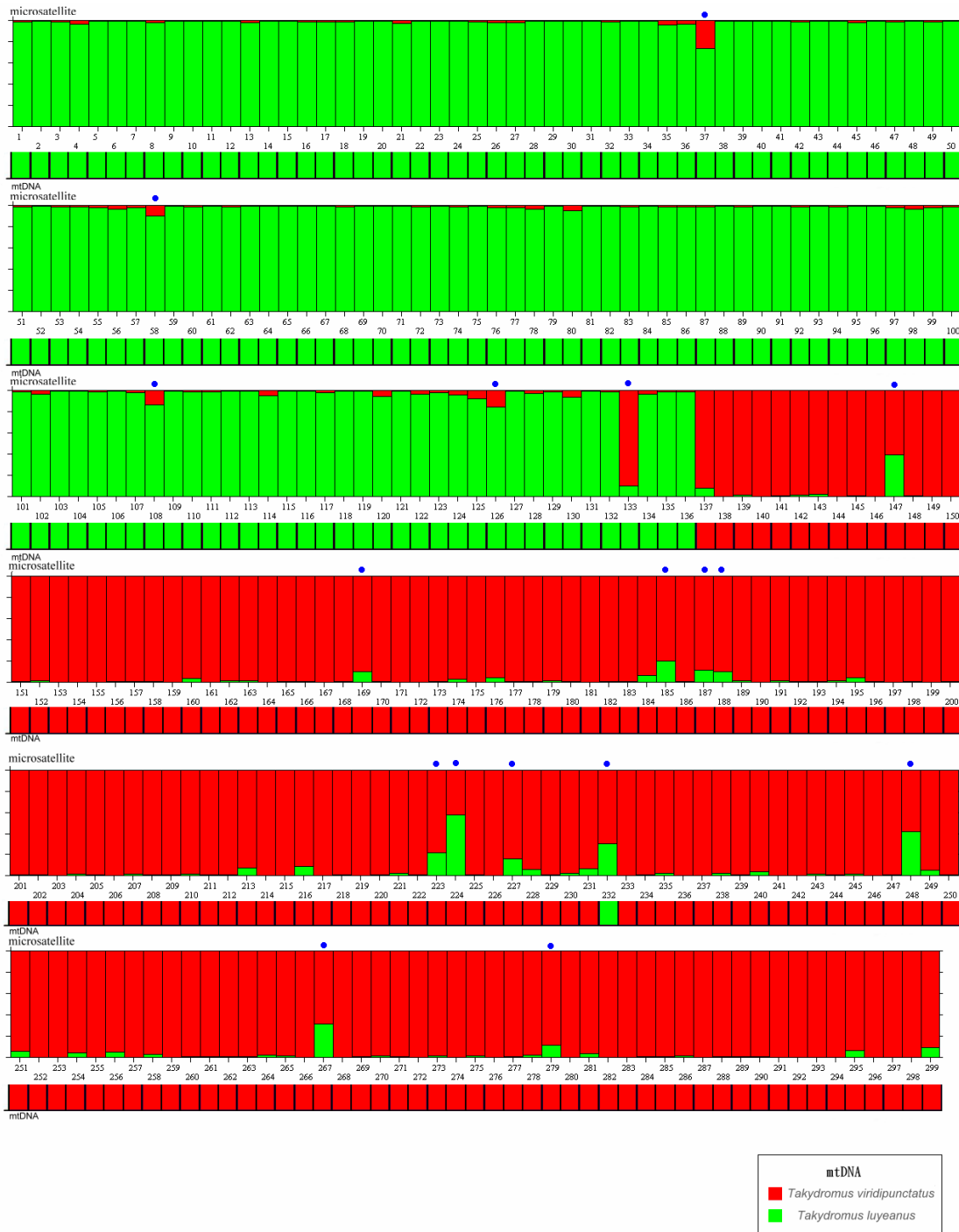


Figure 3. The combination of mitochondrial DNA (below) and microsatellite data with 13-loci analysis (above). The blue points indicate hybrids of *Takydromus viridipunctatus* and *Takydromus luyeanus* in the contact zone.

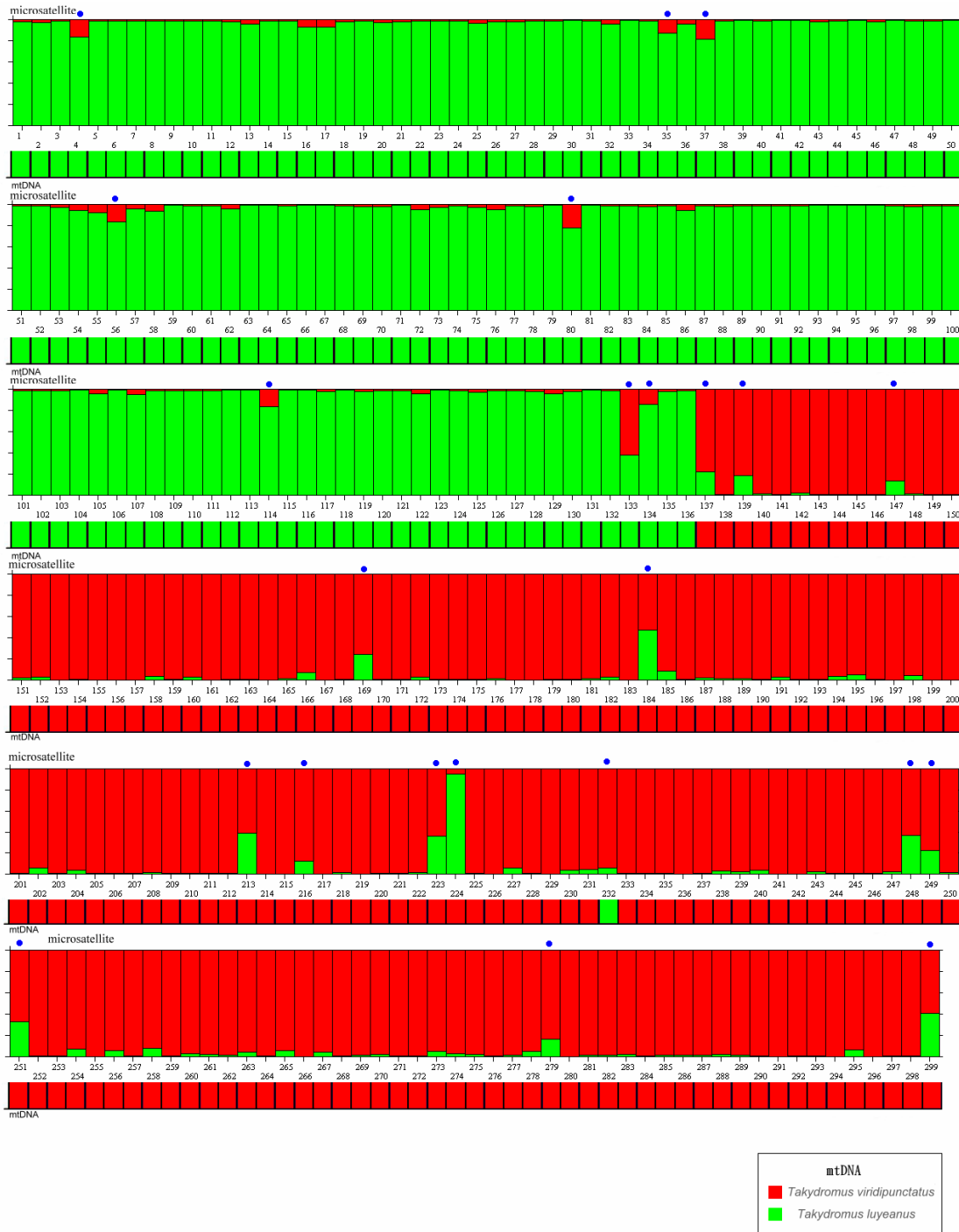


Figure 4. The combination of mitochondrial DNA (below) and microsatellite data with 6-loci analysis (above). The blue points indicate hybrids of *Takydromus viridipunctatus* and *Takydromus luyeanus* in the contact zone.

Table 1. microsatellite primers and conditions used for pure *Takydromus viridipunctatus* populations.

Locus	Repeat motif	Primer sequences(5'-3')	Range of allele sizes (bp)	Ta(°C)	No. of allele	H <sub>O</sub>	H <sub>E</sub>	P values (HWE)
TF013	(GATA) <sub>21</sub>	F: ATTCCGTGGCACTTGGCAGG R: TGGCTCATAGTATTGGCTTGC	179-223	58°C	21	0.818	0.890	0.0192
TF046	(GATA) <sub>22</sub>	F:ACAGAAGA AACTTGGGTCTCCAG R: GCTCTCCAGCCAGGTGTCAC	211-331	58°C	27	0.923	0.944	0.0084*
TF064	(GAAA) <sub>14</sub> GAAA(GAAA) <sub>5</sub>	F: CGACTCATCTCCCGTGCCAG R: ATTTCTCAAGGTGACATTGG	226-290	58°C	18	0.846	0.898	0.0920
TF092	(GAAA) <sub>11</sub> GATA(GAAA) <sub>2</sub>	F: AAACAAACATTCCGCTATCG R: TATTATGTCAAGTGCCATGC	183-283	58°C	22	0.867	0.925	0.0360
TF098	(GAAA) <sub>18</sub>	F: ATCTAAATGGTCCACTGATC R: ACTGAAGGGATGGCAATGAG	209-265	58°C	14	0.833	0.888	0.2287
TF100	(GAAA) <sub>23</sub>	F: GTTCCCTGGGGCTACCACAG R: AATGGATAACTGGCTTCCTG	202-298	58°C	19	0.870	0.890	0.8197
TF121	(GAAA) <sub>21</sub>	F: TCCACAAATCAGGGCACTGC R: ACACTCAA AATCCATCACAG	174-238	58°C	16	0.849	0.905	0.0730
TF130	(GAAA) <sub>24</sub>	F: AAGAACTGTckGGCTCTGTC R: TGTCTGAAATCAGTGGCAAC	170-266	58°C	22	0.907	0.921	0.0690
TF159	(GAAA) <sub>27</sub>	F: CCTGTTAGATTCTGCCATTC R: TGCCATACAAATTCCCACACC	216-324	58°C	31	0.909	0.950	0.1050
TF163	(GAAA) <sub>14</sub>	F: TGGAAACACTGGCAAAGGAG R: TTCTCTCATTCGGGGGTATC	222-298	58°C	18	0.610	0.923	0.0000*
TF167	(GAAA) <sub>15</sub>	F: GTGACCTCGTCCATGTGTCG R: CAAAGTCTATCGCAGCACTG	261-363	58°C	18	0.817	0.900	0.3456
TF084	(GAAA) <sub>17</sub>	F:GTGTTCTCAAGATTTCTAAG R: GACTGAACCTCCGACCACGG	97-181	58°C	18	0.743	0.880	0.0138
TF102	(GAAA) <sub>16</sub>	F:CAGCCTGGTATCACTACTGCAC R:TCCAGAGCAATTGAAATGCCTG	204-253	58°C	15	0.808	0.885	0.0283

H<sub>O</sub>: observed heterozygosity, H<sub>E</sub>: expected heterozygosity

The significant difference between H<sub>O</sub> and H<sub>E</sub> by critical p-value (p<0.05) are marked with \*

Table 2. microsatellite primers and conditions used for pure *Takydromus luyeanus* populations

Locus	Repeat motif	Primer sequences(5'-3')	Range of allele sizes (bp)	Ta(°C)	No. of allele	H <sub>O</sub>	H <sub>E</sub>	P values (HWE)
TF013	(GATA) <sub>21</sub>	F: ATTCCGTGGCACTTGGCAGG R: TGGCTCATAGTATTGGCTTGC	166-204	58°C	13	0.763	0.799	0.1725
TF046	(GATA) <sub>22</sub>	F:ACAGAAGAACTTGGGTCTCCAG R: GCTCTCCAGCCAGGTGTCAC	239-411	58°C	41	0.957	0.961	0.0278
TF064	(GAAA) <sub>14</sub> GAAA(GAAA) <sub>5</sub>	F: CGACTCATCTCCCGTGCCAG R: ATTTCTCAAGGTGACATTGG	230-294	58°C	15	0.758	0.885	0.0181
TF092	(GAAA) <sub>11</sub> GATA(GAAA) <sub>2</sub>	F: AAACAAACATTCCGCTATCG R: TATTATGTCAAGTGCCATGC	179-247	58°C	16	0.817	0.901	0.1615
TF098	(GAAA) <sub>18</sub>	F: ATCTAAATGGTCCACTGATC R: ACTGAAGGGATGGCAATGAG	185-323	58°C	17	0.809	0.909	0.0099*
TF100	(GAAA) <sub>23</sub>	F: GTTCCCTGGGGCTACCACAG R: AATGGATAACTGGCTTCCTG	198-378	58°C	28	0.842	0.927	0.0031*
TF121	(GAAA) <sub>21</sub>	F: TCCACAAATCAGGGCACTGC R: ACACTCAAATCCATCACAG	162-246	58°C	22	0.571	0.700	0.0001*
TF130	(GAAA) <sub>24</sub>	F: AAGAACTGTcGGCTCTGTC R: TGTCTGAAATCAGTGGCAAC	158-222	58°C	17	0.884	0.909	0.0113
TF159	(GAAA) <sub>27</sub>	F: CCTGTTAGATTCTGCCATTC R: TGCCATACAAATTCCCACACC	202-266	58°C	28	0.904	0.907	0.2965
TF163	(GAAA) <sub>14</sub>	F: TGGAAACACTGGCAAAGGAG R: TTCTCTCATTCCGGGGTATC	194-262	58°C	17	0.895	0.894	0.6079
TF167	(GAAA) <sub>15</sub>	F: GTGACCTCGTCCATGTGTCTG R: CAAAGTCTATCGCAGCACTG	255-283	58°C	7	0.215	0.331	0.0013*
TF084	(GAAA) <sub>17</sub>	F:GTGTTCTCAAGATTTCTAAG R: GACTGAACCTCCGACCACGG	93-165	58°C	17	0.900	0.903	0.4929
TF102	(GAAA) <sub>16</sub>	F:CAGCCTGGTATCACTACTGCAC R:TCCAGAGCAATTGAAATGCCTG	208-262	58°C	22	0.894	0.933	0.5455

$H_O$ : observed heterozygosity,  $H_E$ : expected heterozygosity

The significant difference between  $H_O$  and  $H_E$  by critical  $p$ -value ( $p < 0.05$ ) are marked with \*

Table 3. The  $R_{st}$  value between *Takydromus viridipunctatus* and *Takydromus luyeanus*. The significant  $p$ -values ( $p < 0.05$ ) are marked with \*

<b>locus</b>	<b><math>R_{st}</math></b>
TF013	0.14612*
TF046	0.12111*
TF064	0.26554*
TF092	0.07618
TF098	0.01785
TF100	0.11614*
TF121	0.00205
TF130	0.02439
TF159	0.43724*
TF163	0.00166
TF167	0.55329*
TF084	0.08206
TF102	0.05513

Table 4. The proportion of pure individuals, migrants, and hybrids of *Takydromus viridipunctatus* and *Takydromus luyeanus* around Liwu River. The range of proportion is the estimation of 13-loci and 6-loci analyses.

	<b>pure speices individuals</b>	<b>migrants</b>	<b>hybrids</b>
<b>North bank</b>	125~134		2~11
<b>N = 136</b>	(91.9%~98.5%)	0	(1.5%~8.1%)
<b>South bank</b>	143~156		7~20
<b>N = 163</b>	(87.7%~95.7%)	0	(4.3%~12.3%)
<b>Total</b>	268~290		9~31
<b>N = 299</b>	(89.6%~97.0%)	0	(3.0%~10.4%)



Table 5. The potential hybrids number of *Takydromus viridipunctatus* and *Takydromus luyeanus* based on mitochondrial DNA and microsatellites data.

	<b>introgression in mtDNA</b>	<b>introgression in microsatellites</b>	<b>hybrids</b>
<b>North bank</b>			
<b>N = 136</b>	0	2~11	2~11
<b>South bank</b>			
<b>N = 163</b>	1	6~19	7~20
<b>Total</b>	1	8~30	9~31

Table 6. Migration rate of interspecific and intraspecific *Takydromus viridipunctatus* and *Takydromus luyeanus* by

BayesAss		
	<i>Takydromus viridipunctatu</i>	<i>Takydromus luyeanus</i>
<i>Takydromus viridipunctatu</i>	0.996	0.004
<i>Takydromus luyeanus</i>	0.008	0.992

Table 7. The genetic distance  $(\delta\mu)^2$ ,  $R_{st}$  and  $Nm$  of *Takydromus viridipunctatus* and *Takydromus luyeanus*, which occupy either the north or south side of the river bank.

<b>Genetic distance (by BayesAss)</b>	<b><math>R_{st}</math> (by BayesAss)</b>	<b><math>F_{st}</math> (by GENEPOP)</b>	<b><math>Nm</math> (by BayesAss)</b>
3.4301	0.1346 (p<0.05)	0.1333	1.6068

Table 8. The  $R_{st}$  of pure *Takydromus viridipunctatus* populations. The significant  $p$ -values ( $p < 0.05$ ) is marked with \*

	<b>YM</b>	<b>BD</b>	<b>WL</b>	<b>FS</b>	<b>CL</b>	<b>SA</b>	<b>TU</b>	<b>NAN</b>	<b>NAS</b>	<b>HJ</b>
<b>BD</b>	0.123*									
<b>WL</b>	0.101*	0.070*								
<b>FS</b>	0.221*	0.109*	0.075*							
<b>CL</b>	0.270*	0.123*	0.093*	0.044 ( $p=0.05$ )						
<b>SA</b>	0.248*	0.140*	0.141*	0.062*	0.114*					
<b>TU</b>	0.371*	0.174*	0.230*	0.104*	0.108*	0.061*				
<b>NAN</b>	0.156*	0.136*	0.121*	0.124*	0.180*	0.204*	0.259*			
<b>NAS</b>	0.051 ( $p=0.06$ )	0.205*	0.142*	0.245*	0.295*	0.233*	0.367*	0.155*		
<b>HP</b>	0.167*	0.198*	0.159*	0.197*	0.270*	0.244*	0.32*	0.009 ( $p=0.25$ )	0.093*	
<b>HJ</b>	0.151*	0.106*	0.174*	0.150*	0.179*	0.191*	0.231*	0.111*	0.165*	0.118*

(YM=YangMingShan, BD=Badouzi, WL=WuLia, FS=FuSan, CL=ChiLan, SA=SuAo, TU=TnooUo, NAN=North-NanAo, NAS=South-NanAo, HP=HoPing, HJ=HoJen)

Table 9. The  $R_{st}$  of pure *Takydromus luyeanus* populations. The significant  $p$ -values ( $p < 0.05$ ) is marked with \*

	<b>SF</b>	<b>WR</b>	<b>GF</b>	<b>YL</b>	<b>CK</b>	<b>KS</b>	<b>LY</b>
<b>WR</b>	0.037 ( $p=0.16$ )						
<b>GF</b>	0.207*	0.335*					
<b>YL</b>	0.105*	0.204*	0.050 ( $p=0.10$ )				
<b>CK</b>	0.074 ( $p=0.06$ )	0.2*	0.242*	0.125 ( $p=0.06$ )			
<b>KS</b>	0.203*	0.275*	0.135*	0.030 ( $p=0.26$ )	0.163*		
<b>LY</b>	0.237*	0.325*	0.003 ( $p=0.36$ )	0.040 ( $p=0.20$ )	0.281*	0.084 ( $p=0.09$ )	
<b>TT</b>	0.020 ( $p=0.19$ )	0.100*	0.209*	0.095*	0.065 ( $p=0.10$ )	0.202*	0.233*

(SF=ShouFeng, WR=WanRung, GF=GunangFu, YL=YuLi, CK=ChengKung, KS=KwanShan, LY=LuYea, TT=TaiTung)