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PERMANENT GENETIC RESOURCES NOTE

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Abstract

This article documents the addition of 123 microsatellite marker loci to the Molecular Ecology Resources Database. Loci were developed for the following species: *Brenthis ino*, *Cichla orinocensis*, *Cichla temensis*, *Epinephelus striatus*, *Gobio gobio*, *Liocarcinus depurator*, *Macrolophus pygmaeus*, *Monilinia vaccinii-corymbosi*, *Pelochelys cantorii*, *Philotrypesis josephi*, *Romanogobio vladykovi*, *Takydromus luyeanus* and *Takydromus viridipunctatus*. These loci were cross-tested on the following species: *Cichla intermedia*, *Cichla ocellaris*, *Cichla pinima*, *Epinephelus acanthistius*, *Gobio carpathicus*, *Gobio obtusirostris*, *Gobio sp. 1*, *Gobio volgensis*, *Macrolophus costalis*, *Macrolophus melanotoma*, *Macrolophus pygmaeus*, *Romanogobio albipinnatus*, *Romanogobio banaticus*, *Romanogobio belingi*, *Romanogobio kesslerii*, *Romanogobio parvus*, *Romanogobio pentatrichus*, *Romanogobio uranoscopus*, *Takydromus formosanus*, *Takydromus hsuehshanesis and Takydromus stejnegeri*.

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This article documents the addition of 123 microsatellite marker loci to the Molecular Ecology Resources Database. Table 1 contains information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and

Table 1 Information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Brenthis ino	11	n/a	48990–48992, 48995–49001, 49003	JQ688092–JQ688096, JQ688098–JQ688103	Weyer, Jessica; Hochkirch, Axel; Altmann, Carolin; Hankeln, Thomas; Schmitt, Thomas; Veith, Michael
Cichla orinocensis and *C. temensis*	12	*C. ocellaris*, *C. pinima*, *C. intermedia*	49004–49015	JQ936509–JQ936520	Macrander, Jason; Willis, Stuart; Gibson, Shane; Orti, Guillermo; Hrbek, Tomas
Epinephelus striatus	10	*E. acanthistius*	49128–49137	FJ178389, FJ178391, FJ178393, FJ711588, FJ711589, JX041258–JX041260, JX041262, JX041266	Jackson, Alexis M.; Semmens, Brice X.; Bernardi, Giacomo
Liocarcinus depurator	11	n/a	49493–49503	JX104535–JX104545	Palero, F.; Garcia-Merchan, Vh.; Abelló, P.; Macpherson, E.; Pascual, M.
Macrolophus pygmaeus	9	*M. pygmaeus*, *M. costalis*, *M. melanotoma*	49096–49104	GF111951–GF111956, GF111958–GF111960	Hamdi, Faten; Clouet, Cécile; Streito, Jean-Claude; Bonato, Olivier; Gauthier, Nathalie
Monilinia vaccinii-corymbosi	21	n/a	49461–49481	JQ358947– JQ358967	Burchhardt, Kathleen M.; Cubeta, Marc A.
Pelochelys cantorii	13	n/a	49524–49536	JN016746, JN016747 See ms for details	Chen, Xiao; Ai, Weiming; Zhou, Zhiming; Chen, Zhijian; Lin, Chongwen
Philotrypesis josephi	9	n/a	49484–49492	JQ247680–JQ247688	Tian, En-Wei; Yu, Hui
Romanogobio vladykovi and *Gobio gobio*	6 novel primers, 5 cross-amplified	*G. obtusirostris*, *G.* sp. 1, *G. carpathicus*, *G. volgensis*, *R. belingi*, *R. albipinnatus*, *R. parvus*, *R. uranoscopus*, *R. banaticus*, *R. kesslerii*, *R. pentatrichus*	26381, 26383–26384, 26386–23687, 48984–48989	DQ207799, DQ207801, DQ207802, DQ207804, DQ207805, JQ993103–JQ993107	Mendel, Jan; Papoušek, Ivo; Marešová, Eva; Vetešník, Lukáš; Halačka, Karel; Nowak, Michał; Čížková, Dagmar

Table 1 (Continued)

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Takydromus viridipunctatus and *T. luyeanus*	16	*T. hsuehshanesis*, *T. formosanus*, *T. stejnegeri*	48834, 48839, 48844, 48849, 48854, 48859, 48864, 48869, 48874, 48876, 48880, 48885, 48889, 48894, 48899, 48901	See ms for details	Tseng, Shu-Ping; Wang, Hurng-Yi; Lin, Si-Min

GenBank. The authors responsible for each set of loci are listed in the final column. The MER database and GenBank accession numbers and the authors responsible are also listed. A full description of the development protocol for the loci presented here can be found on the Molecular Ecology Resources Database (http://tomato.biol.trinity.edu/).

Isolation and characterization of sixteen nuclear intron markers for a larcertid genus *Takydromus*

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Keywords: EPIC loci, gene flow, Lacertidae, speciation, *Takydromus*

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Running title: Nuclear intron markers for *Takydromus* lizards

ABSTRACT

In order to test alternative speciation hypotheses, 16 pairs of exon-primed, intron-crossing (EPIC) primers were developed for amplifying nuclear introns of *Takydromus* lizards (Squamata: Lacertidae). These primers were designed from orthologous sequences of *T. viridipunctatus* and *T. luyeanus* obtained from 454 pyrosequencing and could successfully amplify at least 5 *Takydromus* species. We will use these primers to solve the timing and the mode of speciation among several sibling species with either allopatric or parapatric distributions in East Asian islands.

Takydromus is a group of grass-living lacertid distributed in Fareast Asia, with more than half of the species occurring only on East Asian islands (Lue & Lin 2008). The species diversity occurring in this region was formed because of a series of geological events, including repetitive glaciations which have caused the connection and separation among adjacent island groups, and severe orogenesis which has led to distributional disjunctions (Lin *et al.* 2002; Ota *et al.* 2002). With six endemic species, Taiwan Island represents the hotspot of this genus and provides a valuable chance to test alternative speciation models among allopatrically or parapatrically distributed species.

In recent years, renewed analytic tools have been developed to detect probable historical gene flow between closely related taxa, such as a coalescent-based isolation with migration (IM) model (Hey & Nielsen 2004), or the likelihood methods of speciation with gene flow (Yang 2010). These new methods have led to a paradigm shift from the widely accepted hypothesis of allopatric speciation mode (a speciation with no gene flow: rate of gene exchange $m = 0$; Gavrilets 2003) to the alternative parapatric speciation mode ($m > 0$; Gavrilets 2003). In addition, an efficient and flexible method – approximate Bayesian computation (ABC) framework – has been developed to provide a chance to test alternative hypotheses for a complex speciation history under a likelihood search algorithm (Beaumont *et al.* 2002; Beaumont 2010; Bertorelle *et al.* 2010). In order to test alternative speciation hypotheses among several monophyletic sibling *Takydromus* species endemic to Taiwan,

multiple nuclear loci were needed for IM and ABC analyses. In this paper, we aim to describe and evaluate the applicability and genetic diversity of 16 nuclear loci we have newly developed for *Takydromus* lizards.

First, EST libraries of *T. viridipunctatus* and *T. luyeanus* were constructed to develop exon primed intron-crossing (EPIC) primers. Two individuals each of *T. viridipunctatus* and *T. luyeanus* with both sexes were used to construct cDNA libraries using the Poly(A) Purist^{TM} MAG kit (Ambion) and Creator SMART cDNA Library Construction Kit (Clontech). cDNA (5 μg) was sheared using nebulization and DNA sequencing was performed on the Roche454 GS-FLX sequencer (Roche Diagnostic, Germany). The previous procedures were carried out by Mission Biotech Co., Ltd (Taipei, Taiwan).

In total, 79,526 and 83,353 reads (22,497,729 and 20,899,831 base pairs) were generated for *T. viridipunctatus* and *T. luyeanus*, respectively. The reads were first trimmed of low quality sequences, and de novo assembled by using GS *de novo* Assembler software (Roche Diagnostic, Germany). After assembling, 962 contigs from *T. viridipunctatus* and 333 contigs from *T. luyeanus* with length longer than 500 base pairs were used for homologous sequence searching and primer design. Gene-specific primers were designed based on the EST database of *T. viridipunctatus*, *T. luyeanus* and the closest available genome sequence of *Anolis carolinenisis* (AnoCar 1.0 draft genome; http://www.ensembl.org/index.html). tBLASTx (Maximum E-value=E^{-10}) was used to search homologous sequences among the three species

and sequences were aligned by using clustalW (Thompson *et al.* 1994). Thereafter, the putative splice sites of *Takydromus* were obtained and EPIC markers were developed. Primers were designed for those target genes, while the program FastPCR (Kalendar *et al.* 2009) was applied to prevent primer dimmers and to obtain an optimized annealing temperature for each primer pair (Table 1).

A total of 97 grass lizards, including *T. viridipunctatus*, *T. luyeanus*, *T. hsuehshanensis*, *T. formosanus* and *T. stejnegeri* were collected in Taiwan during 2009-2010. All specimens were fixed in 95% ethanol at room temperature. Genomic DNA of *Tarkydromus* spp. was extracted from muscle tissues of lizards using EasyPure genomic DNA spin kit (Bioman) according to the manufacturer's instructions. The extracted DNA samples were stored at -20 ^{o}C for further use. Polymerase chain reactions (PCR) were carried out in a total volume of 20 μl, consisting of 1X Green GoTaq Flexi Buffer (Promega), 2.5 mM of $MgCl_2$, 0.4 μM of each primer, 0.2 mM of each dNTP, 0.5 U of GoTaq Flexi DNA Polymerase (Promega), and 0.1-0.5 ng of template DNA. The PCR conditions were set to denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, suggested annealing temperature (Tm, Table 1) for 40 s, and 72°C for 90 s, with a final extension at 72°C for 10 min using an iCycler Thermal Cycler (Bio-Rad). PCR products were checked by using electrophoresis on a 1.2 % agarose gel. Automated DNA sequencing from both directions were carried out on ABI3730 autosequencer by Genomics BioSci & Tech Co., Ltd (Taipei, Taiwan).

Sequence data from both directions was proofread and assembled by using Sequencher 4.9 (GeneCodes). Heterozygous sites were found by using the 'call the secondary peaks' feature of the 50% threshold, meaning that the secondary peak at least has half height of the primary peak. Haplotype reconstruction was performed by using PHASE 2.1 (Stephens & Scheet 2005) implemented in DnaSP v5 (Librado & Rozas 2009). Every run was set up with MCMC iterations to 100,000 and thinned every 1000 intervals. Summary statistics, including number of segregating sites, haplotype diversity, and Waterson's estimator of Theta, of these nuclear markers were carried out by using DnaSP v5 (Librado & Rozas 2009). Genetic distances were calculated using MEGA 5 (Tamura *et al.* 2011) with 100 bootstrap pseudo-replicated. The DNA sequences used in this study were submitted to GenBank (accession numbers JQ746705-JQ747474 and JQ769109-JQ769112). Blast searches were performed by using blastn for each sequence to confirm that all these sequences had never been submitted.

The information of 16 nuclear markers developed in this study are listed in Table 1. Four additional markers that had been previously published were also tested in this study (Table 1; Leaché 2009; Townsend *et al.* 2008). All 20 nuclear markers showed variations within species except *T. hsuehshanesis* at locus Taky6, *T. formosanus* at locus Taky20 and *T. stejnegeri* at locus BACH1 (Table 2). The average theta were 0.002593 for *T. viridipunctatus*, 0.00308 for *T. luyeanus*, 0.002056 for *T. hsuehshanesis* and 0.002752 for *T. formosanus*,

110 0.005753 for *T. stejnegeri*, respectively. The sixteen nuclear markers showed a board range of
111 evolution rates, and the genetic diversities of EPIC markers are usually higher than that of the
112 exon markers (Fig. 1). According the intra- and inter-species variation reported here, these
113 EPIC markers will provide wide applications in the investigation of phylogeny,
114 phylogeography and speciation hypotheses of *Takydromus* lizards.
115

116 **References**

117 Beaumont MA (2010) Approximate Bayesian computation in evolution and ecology. *Annual*
118 *Review of Ecology Evolution and Systematics*, **41**, 379-406.
119 Beaumont MA, Zhang W, Balding DJ (2002) Approximate Bayesian computation in
120 population genetics. *Genetics*, **162**, 2025-2035.
121 Bertorelle G, Benazzo A, Mona S (2010) ABC as a flexible framework to estimate
122 demography over space and time: some cons, many pros. *Molecular Ecology*, **19**,
123 2609-2625.
124 Gavrilets S (2003) Perspective: models of speciation: what have we learned in 40 years?
125 *Evolution*, **57**, 2197-2215.
126 Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates
127 and divergence time, with applications to the divergence of *Drosophila pseudoobscura*
128 and *D. persimilis*. *Genetics*, **167**, 747-760.

129 Kalendar R, Lee D, Schulman AH (2009) FastPCR software for PCR primer and probe design
130 and repeat search. *Genes, Genomes and Genomics*, **3**, 1-14.
131 Leaché AD (2009) Species tree discordance traces to phylogeographic clade boundaries in
132 north american fence lizards (*Sceloporus*). *Systematic Biology*, **58**, 547-559.
133 Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA
134 polymorphism data. *Bioinformatics*, **25**, 1451-1452.
135 Lin SM, Lue KY, Chen CA (2002) Molecular Phylogeny and Biogeography of the Grass
136 Lizards genus *Takydromus* (Reptilia: Lacertidae) of East Asia. *Molecular*
137 *Phylogenetics and Evolution*, **22**, 276-288.
138 Lue KY, Lin SM (2008). Two new cryptic species of *Takydromus* (Squamata: Lacertidae)
139 from Taiwan. *Herpetologica*, **64**, 379-395.
140 Ota H, Honda M, Chen SL, Hikida T, Panha S, Oh HS, Matsui M. (2002) Phylogenetic
141 relationships, taxonomy, character evolution and biogeography of the lacertid lizards
142 of the genus *Takydromus* (Reptilia: Squamata): a molecular perspective. *Biological*
143 *Journal of the Linnean Society*, **76**, 493-509.
144 Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotype
145 inference and missing-data imputation. *American Journal of Human Genetics*, **76**,
146 449-462.
147 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular

mvolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28,** 2731-2739.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673-4680.

Townsend TM, Alegre RE, Kelley ST, Wiens JJ, Reeder TW (2008) Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: An example from squamate reptiles. *Molecular Phylogenetics and Evolution*, **47**, 129-142.

Yang Z (2010) A likelihood ratio test of speciation with gene flow using genomic sequence data. *Genome Biology and Evolution*, **2**, 200-211.

Data Accessibility

DNA sequences: Genbank accessions JQ746705-JQ747474 and JQ769109-JQ769112

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168

169 **Figure legend**

170 **Figure 1.** Variability of 20 nuclear markers in *Takydromus* lizards in order of increasing
171 variability, plus mitochondrial 12S rRNA gene (Lin *et al.* 2002) for comparison. Divergence
172 of these markers was evaluated by (a) p-distance between the sibling species *T.*
173 *viridipunctatus* and *T. luyeanus* and (b) p-distance between *T. formosanus* species complex
174 and *T. stejnegeri*. The black bars indicate the novel nuclear marker development in this study,
175 and gray bars indicate markers from others publications.

Table 1 Primer sequences and reference information of 20 nuclear markers of *Takydromus* spp.

Locus	Primer	Reference [a]	Identity	location [b]	Primer sequence (5'—3')	Tm(°C)
DDX1	DDX1-F	*Anolis carolinensis*	exon24	1857	GATGGGTCTGGCTATTTCTCTTG	58
	DDX1-R	ENSACAT00000011315	exon25	1995	CTGCATCTCATTGTACCAAATGG	
ETFB	ETFB-F	*Anolis carolinensis*	exon5	480	GAAGGTCGAGAGGGAAGTTGAC	56
	ETFB-R	ENSACAT00000010459	exon6	657	GGTAAGTTCTACACCAAGGTCG	
FGB	FGB-F	*Anolis carolinensis*	exon7	957	TGACAAAATCAGCCAGCTTACC	55
	FGB-R	ENSACAT00000015729	exon8	1299	GGCAGAATGACAGCGATTGTA	
RPL13	RPL13-F2	*Anolis carolinensis*	exon2	208	GTCCGTGCTGGTAGAGGATTCA	56
	RPL13-R3	ENSACAT00000017140	exon5	613	GCTCTGCTGCTTCCTTGGCT	
RPS3	RPS3-F6	*Anolis carolinensis*	intron4	NA	AAACTCAGTTGGTCTTTAAGGT	62
	RPS3-R4	ENSACAT00000002165	exon5	394	TGGCTCCACTCTCCATGATGAA	
Taky2	Taky2-F	*Anolis carolinensis*	exon 1	5	CAAAGGATCTCTTGCATCCCTC	58
	Taky2-R	ENSACAT00000011606	exon 2	154	CCACTGTTTGAGCGTGACTGA	
Taky6	Taky6-F	*Anolis carolinensis*	exon4	336	GTCCTTAATCAAGCAGATTCCTCG	55
	Taky6-R2	ENSACAT00000009426	intron4	NA	TGGCTCCCGAATGCCACAAA	
Taky8	Taky8-F	*Anolis carolinensis*	exon2	139	AGTCGGAGTGAAAAGAAAGCACGG	55
	Taky8-R	ENSACAT00000009201	exon3	265	AGACATCTGGCTTGGTGATGAC	
Taky18	Taky18-F	*Anolis carolinensis*	exon3	272	TCTGCAAGATCGACAGAGAAGG	64
	Taky18-R	ENSACAT00000010504	exon4	378	CTCTTCGATGACATCTTTGGCC	
Taky22	Taky22-F	*Anolis carolinensis*	exon4	203	GTGGTCCTTACCACTTCCGT	60
	Taky22-R2	ENSACAT00000017351	exon6	366	TGCAGGGACCACCATCCTCTTG	
Taky23	Taky23-F4	*Anolis carolinensis*	exon4	591	CATGCCTGATCTCTACTTCTACC	62
	Taky23-R4	ENSACAT00000004198	exon5	674	TCCTTTGTAACCGCCTTCTCAG	
DEAH	DEAH-F	this study	NA	NA	AAGAACTACATCCGCACTTGT	56
	DEAH-R				GGCAATGATGCGGTCCAACTGT	
J7	J7-F	this study	NA	NA	CCGTTCTAACATCTCATCTAAG	62

	J7-R				TCAAAATCCACGCTCTTCCTCT	
RPL19	RPL19-F6	this study	NA	NA	CTCGTAAGTGAACATTTGCCATG	58
	RPL19-R6				GACCTAGAAGACAGTGGAAAACA	
Taky1	Taky1-F	this study	NA	NA	GGCCAAGACAGAGGCAAAATC	56
	Taky1-R				GCAACATCTAGCGCATCGTAGT	
Taky20	Taky20-F	this study	NA	NA	ACAAGTACGGAGCCACACCGA	60
	Taky20-R				TGCATCTGCCTTGCGAAATCTC	
R35 [c]	R35-F	Leaché (2009)	exon	NA	GACTGTGGAYGAYCTGATCAGTGTGGTGCC	66.5
	R35-R				GCCAAAATGAGSGAGAARCGCTTCTGAGC	
PNN [d]	PNN-F	Townsend *et al.* (2008)	exon	NA	ACAGGTAATCAGCACAATGAYGTAGA	57
	PNN-R				TCTYYTGCCTGAYCGACTACTYTCTGA	
BDNF [e]	BDNF-F	Townsend *et al.* (2008)	exon	NA	GACCATCCTTTTCCTKACTATGGTTATTTCATACTT	61
	BDNF-R				CTATCTTCCCCTTTTAATGGTCAGTGTACAAAC	
BACH1 [f]	BACH1-F	Townsend *et al.* (2008)	exon	NA	GATTTGAHCCYTTRCTTCAGTTTGC	56
	BACH1-R				ACCTCACATTCYTGTTCYCTRGC	

[a] Ensembl transcript ID of *Anolis carolinensis*; [b] 5' nucleotide position of each primer refer to the transcript of *Anolis carolinensis*; [c] R35, RNA fingerprint protein 35; [d] PNN, pinin,desmosome associated protein; [e] BACH1, basic leucine zipper transcription factor 1; [f] BDNF, brain-derived neurotrophic factor. NA: not available

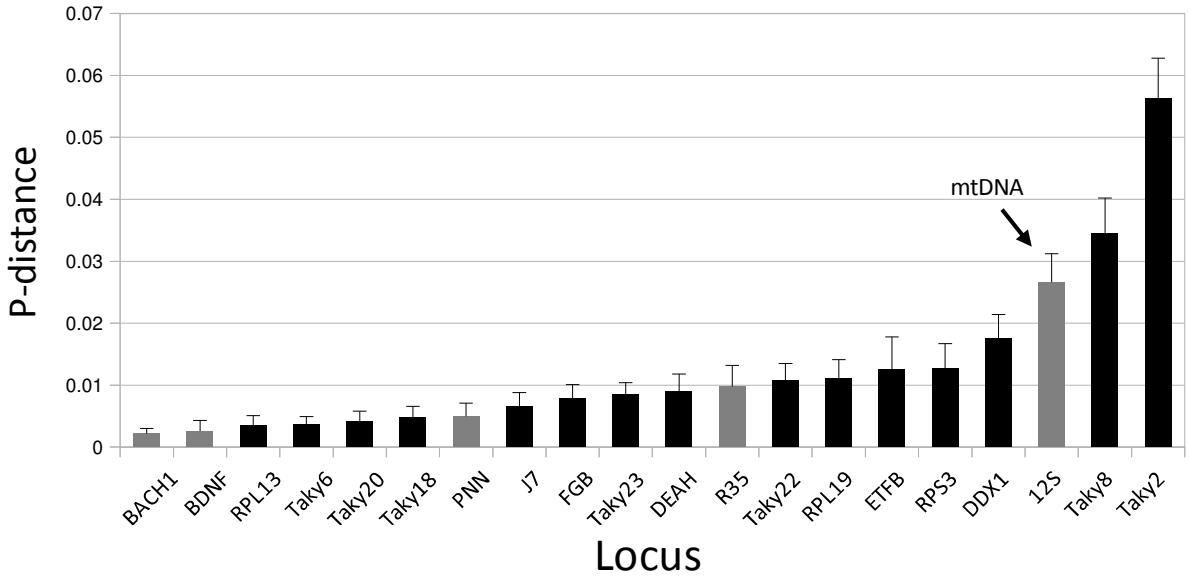
Table 2 Summary information for 20 nuclear markers in five *Takydromus* species. N: number of sequence; Site: sequence length (in bp); NetSites: sequence length with no missing data(in bp); S: number of segregating sites; Hap: number of haplotype; Hd: haplotype diversity; Theta: Waterson's estimator of Theta per site; Genbank ID: Genbank accession numbers.

Locus	Species	N	Sites	NetSites	S	Hap	Hd	Theta	Genbank ID
DDX1	*T. viridipunctatus*	16	1017	1017	8	6	0.683	0.002371	JQ746962- JQ746969
	T. luyeanus	24	901	854	13	10	0.870	0.004076	JQ746937- JQ746948
	T. hsuehshanesis	22	1001	1000	6	5	0.701	0.001646	JQ746949- JQ746959
	T. formosanus	8	1001	1000	25	6	0.929	0.009642	JQ746933- JQ746936
	T. stejnegeri	4	990	964	74	4	1.000	0.041871	JQ746960- JQ746961
ETFB	*T. viridipunctatus*	20	372	372	3	3	0.279	0.002273	JQ747036- JQ747045
	T. luyeanus	24	372	372	8	7	0.743	0.005759	JQ747009- JQ747020
	T. hsuehshanesis	22	372	372	1	2	0.485	0.000737	JQ747021- JQ747031
	T. formosanus	6	372	370	3	3	0.733	0.003551	JQ747006- JQ747008
	T. stejnegeri	8	467	466	2	2	0.536	0.001655	JQ747032- JQ747035
FGB	*T. viridipunctatus*	24	1161	1161	9	7	0.558	0.002307	JQ747072- JQ747083
	T. luyeanus	20	1161	1160	7	7	0.779	0.001701	JQ747050- JQ747059
	T. hsuehshanesis	18	1164	1164	9	6	0.817	0.002248	JQ747060- JQ747068
	T. formosanus	8	1162	1160	13	6	0.929	0.004322	JQ747046- JQ747049
	T. stejnegeri	6	1149	1149	3	3	0.600	0.001143	JQ747069- JQ747071
RPL13	*T. viridipunctatus*	16	723	723	4	4	0.442	0.001667	JQ747134- JQ747141
	T. luyeanus	14	723	723	9	6	0.604	0.003914	JQ747122- JQ747128
	T. hsuehshanesis	10	724	722	3	3	0.600	0.001469	JQ747129- JQ747133
	T. formosanus	12	1057	1057	4	5	0.803	0.001253	JQ747116- JQ747121
	T. stejnegeri	8	729	729	8	6	0.929	0.004761	JQ769109-JQ769112
RPS3	*T. viridipunctatus*	28	675	675	9	5	0.328	0.003426	JQ747217- JQ747230
	T. luyeanus	30	675	675	12	10	0.786	0.004487	JQ747189- JQ747203
	T. hsuehshanesis	18	677	677	2	3	0.627	0.000859	JQ747204- JQ747212
	T. formosanus	22	677	676	22	16	0.970	0.008928	JQ747178- JQ747188
	T. stejnegeri	8	687	687	12	3	0.464	0.006737	JQ747213- JQ747216
Taky2	*T. viridipunctatus*	12	1155	1155	19	8	0.848	0.005447	JQ747272- JQ747277
	T. luyeanus	14	1098	1070	15	8	0.890	0.004408	JQ747250- JQ747263
	T. hsuehshanesis	14	1158	1155	11	5	0.659	0.002995	JQ747264- JQ747270
	T. formosanus	8	1134	1112	12	4	0.821	0.004162	JQ747246- JQ747249
	T. stejnegeri	2	1158	1158	1	2	1.000	0.000864	JQ747271
Taky6	*T. viridipunctatus*	22	842	842	8	8	0.801	0.002606	JQ747302- JQ747312

	T. luyeanus	14	1100	1100	5	6	0.824	0.001429	JQ747283- JQ747289
	T. hsuehshanesis	16	846	835	0	1	0.000	0.000000	JQ747290- JQ747297
	T. formosanus	10	845	845	3	3	0.622	0.001255	JQ747278- JQ747282
	T. stejnegeri	8	841	841	8	4	0.643	0.003669	JQ747298- JQ747301
Taky8	*T. viridipunctatus*	20	826	826	4	5	0.732	0.001365	JQ747340- JQ747349
	T. luyeanus	16	873	873	8	7	0.825	0.002762	JQ747323- JQ747330
	T. hsuehshanesis	16	873	871	4	4	0.692	0.001384	JQ747331- JQ747338
	T. formosanus	20	824	821	4	2	0.442	0.001373	JQ747313- JQ747322
	T. stejnegeri	2	975	975	1	2	1.000	0.001026	JQ747339
Taky18	*T. viridipunctatus*	18	778	778	2	3	0.601	0.000747	JQ747359- JQ747367
	T. luyeanus	18	784	778	9	6	0.699	0.003363	JQ747350- JQ747358
Taky22	*T. viridipunctatus*	22	1168	1167	13	9	0.892	0.003056	JQ747417- JQ747427
	T. luyeanus	22	1169	1169	16	11	0.883	0.003755	JQ747397- JQ747407
	T. hsuehshanesis	18	1168	1168	11	4	0.542	0.002738	JQ747408- JQ747416
	T. formosanus	4	1201	1201	1	2	0.667	0.000454	JQ747395- JQ747396
Taky23	*T. viridipunctatus*	18	812	812	27	8	0.830	0.009667	JQ747466- JQ747474
	T. luyeanus	26	812	812	16	10	0.837	0.005164	JQ747440- JQ747452
	T. hsuehshanesis	20	811	811	4	4	0.553	0.001390	JQ747453- JQ747462
	T. formosanus	24	801	801	14	11	0.841	0.004680	JQ747428- JQ747439
	T. stejnegeri	6	809	791	10	3	0.800	0.005537	JQ747463- JQ747465
DEAH	*T. viridipunctatus*	24	863	863	7	7	0.848	0.002172	JQ746994- JQ747005
	T. luyeanus	26	857	857	9	9	0.735	0.002752	JQ746975- JQ746987
	T. hsuehshanesis	12	864	862	28	6	0.818	0.010756	JQ746988- JQ746993
	T. formosanus	10	871	858	8	5	0.889	0.003296	JQ746970- JQ746974
J7	*T. viridipunctatus*	16	697	685	7	4	0.742	0.003080	JQ747108- JQ747115
	T. luyeanus	20	685	682	13	12	0.921	0.005373	JQ747087- JQ747096
	T. hsuehshanesis	18	685	685	10	4	0.778	0.004244	JQ747097- JQ747105
	T. formosanus	6	687	687	1	2	0.533	0.000637	JQ747084- JQ747086
	T. stejnegeri	4	694	680	11	4	1.000	0.008824	JQ747106- JQ747107
RPL19	*T. viridipunctatus*	16	866	866	4	5	0.758	0.001392	JQ747170- JQ747177
	T. luyeanus	16	872	860	10	7	0.742	0.003504	JQ747147- JQ747154
	T. hsuehshanesis	24	872	872	9	6	0.442	0.002764	JQ747155- JQ747166
	T. formosanus	10	1057	1057	2	3	0.622	0.000669	JQ747142- JQ747146
	T. stejnegeri	6	878	863	22	3	0.800	0.011165	JQ747167- JQ747169
Taky1	*T. viridipunctatus*	16	404	403	1	2	0.233	0.000748	JQ747238- JQ747245
	T. hsuehshanesis	14	409	392	3	2	0.440	0.002407	JQ747231- JQ747237

Taky20	*T. viridipunctatus*	10	1012	1012	6	4	0.711	0.002096	JQ747390- JQ747394
	T. luyeanus	10	1012	1012	1	2	0.356	0.000349	JQ747374- JQ747378
	T. hsuehshanesis	18	1018	1018	3	4	0.706	0.000857	JQ747379- JQ747387
	T. formosanus	12	685	685	0	1	0.000	0.000000	JQ747368- JQ747373
	T. stejnegeri	4	682	682	0	1	0.000	0.000000	JQ747388- JQ747389
R35	*T. viridipunctatus*	34	690	690	9	9	0.829	0.003190	JQ746865- JQ746881
	T. luyeanus	32	690	690	6	5	0.468	0.002159	JQ746832- JQ746847
	T. hsuehshanesis	26	690	690	3	3	0.514	0.001139	JQ746848- JQ746860
	T. formosanus	22	689	689	5	5	0.745	0.001991	JQ746821- JQ746831
	T. stejnegeri	8	689	689	3	4	0.750	0.001679	JQ746861- JQ746864
PNN	*T. viridipunctatus*	24	944	944	5	6	0.681	0.001418	JQ746921- JQ746932
	T. luyeanus	32	944	944	4	4	0.333	0.001052	JQ746889- JQ746904
	T. hsuehshanesis	26	944	944	3	3	0.643	0.000833	JQ746905- JQ746917
	T. formosanus	14	934	934	5	5	0.736	0.001683	JQ746882- JQ746888
	T. stejnegeri	6	934	934	2	2	0.333	0.000938	JQ746918- JQ746920
BDNF	*T. viridipunctatus*	34	707	707	3	4	0.620	0.001038	JQ746804- JQ746820
	T. luyeanus	34	707	707	2	3	0.266	0.000692	JQ746770-JQ746786
	T. hsuehshanesis	26	707	707	1	2	0.492	0.000371	JQ746787-JQ746799
	T. formosanus	30	707	707	2	3	0.246	0.000714	JQ746755-JQ746769
	T. stejnegeri	8	707	707	4	3	0.607	0.002182	JQ746800- JQ746803
BACH1	*T. viridipunctatus*	22	1228	1222	8	7	0.814	0.001796	JQ746744-JQ746754
	T. luyeanus	32	1228	1228	9	8	0.808	0.001820	JQ746715-JQ746730
	T. hsuehshanesis	20	1228	1228	1	2	0.100	0.000230	JQ746731-JQ746740
	T. formosanus	20	1228	1228	4	5	0.763	0.000918	JQ746705-JQ746714
	T. stejnegeri	6	1228	1228	0	1	0.000	0.000000	JQ746741-JQ746743

(a)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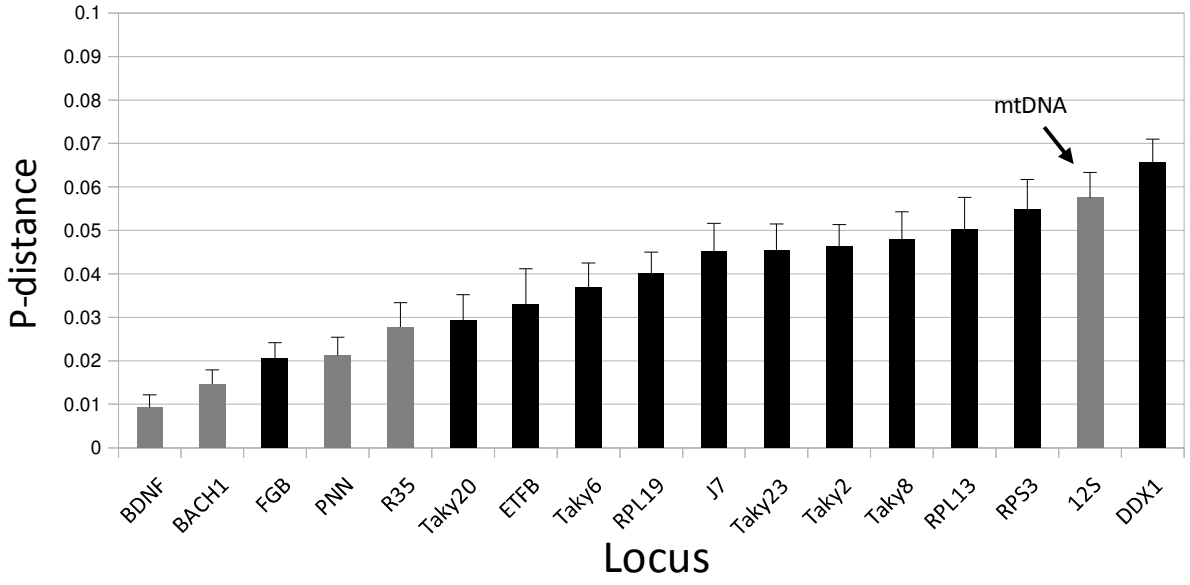
(b)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RPL13
RPS3
12S
DDX1
Locus

174 **Authors contributions box**

175 S.-M. Lin originally formulated the idea and designed the experiments. S.-P. Tseng performed

176 the experiments including primer design and molecular analyses. S.-M. Lin and S.-P. Tseng both

177 contribute to the writing. H.-Y. Wang carefully checked the data and provided all technical and

178 analytical supports needed in this study.

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