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Discrepancies between Mitochondrial DNA and AFLP Genetic Variation among Lineages of Sea Slaters *Ligia* in the East Asian Region

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ABSTRACT

Although sea slaters *Ligia* have a significant role in rocky shore habitats, their taxonomic entities have not been clearly understood. In this study, we investigated whether genetic variation inferred from a nuclear genetic marker, namely amplified fragment length polymorphism (AFLP), would conform to that of a mitochondrial DNA marker. Using both the mitochondrial DNA marker and the AFLP marker amplified by the six selective primer sets, we analyzed 95 *Ligia* individuals from eight locations from East Asia. The direct sequencing of mitochondrial 16S rRNA gene revealed three distinct genetic lineages, with 9.8−11.7 Kimura 2-parameter genetic distance. However, the results of AFLP genotyping analysis with 691 loci did not support those of mitochondrial DNA, and revealed an unexpectedly high proportion of shared polymorphisms among lineages. The inconsistency between the two different genetic markers may be explained by difference in DNA evolutionary history, for example inheritance patterns, effective population size, and mutation rate. The other factor is a possible genomic island of speciation, in that most of the genomic parts are shared among lineages, and only a few genomic regions have diverged.

Keywords: *Ligia*, AFLP, 16S rRNA gene, genetic variation, East Asia

INTRODUCTION

Sea slaters *Ligia* are widely distributed in rocky intertidal shores, and are significant in the study of the evolutionary transition of animals that have successfully adapted from aquatic to terrestrial environments. Several aspects of the life history of sea slaters suggest that their dispersal capability is probably extremely limited, which leads to the prediction that substantial genetic differentiation should occur throughout their range. In these matters, many phylogenetic studies have recently been conducted on various *Ligia* species worldwide (Jung et al., 2008; Hurtado et al., 2010, 2018; Markow and Pfeiler, 2010; Eberl et al., 2013; Yin et al., 2013).

Twelve species of *Ligia* has been recorded in East Asia, so far (Nunomura, 1979, 1983, 1990, 1999; Lee, 1994; Schmalfuss, 2003; Tsuge, 2008; Via and West, 2008; Nunomura et al., 2011; Via, 2012) and most of these species are allopatrically distributed. However, the two species, *Ligia exotica* Roux, 1828 and *Ligia cinerascences* Budde-Lund, 1885, are sympatrically distributed across East Asia, from the southern part of China, to Korea, Japan and Russia. Notwithstanding their wide distribution and evolutionary importance, there is still uncertainty about the identity of species composition, which cannot be resolved by traditional approaches, due to their great morphological variation, and large number of individuals (Jung et al., 2008).

Until recently, sequence analyses of mitochondrial DNA genes have been the most common method for the phylogeny and species delimitations of *Ligia*, especially using *COI* gene and 16S rRNA gene (Jung et al., 2008; Hurtado et al., 2010, 2018; Markow and Pfeiler, 2010; Eberl et al., 2013; Santamaria et al., 2013; Yin et al., 2013). Previous studies have proposed distinct genetic lineages in East Asia (Jung et al., 2008; Yin et al., 2013).

Usually, studies using only mitochondrial DNA may cause biased results because of its maternal inheritance patterns. Thus, an examination of both mitochondrial and nuclear

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Table 1. Sampled *Ligia* specimens with geographic coordinates and number of individuals of which 16S rRNA gene and AFLP are analyzed in this study

	No.	Sampling sites		Geographic coordinates	Lineages	16S rRNA[a]	AFLP[b]
South Korea							
	1	Eulwang	WG_EW	37°26′37″N, 126°22′38″E	WG	11	11
	2	Uljin	EG_UJ	36°59′15″N, 129°25′04″E	EG	12	12
	3	Jeju	EG_JJ	33°13′04″N, 126°30′49″E	EG	12	12
Japan							
	4	Niigata	EG_JNI	37°56′04″N, 139°03′08″E	EG	12	12
	5	Sendai	WG_JSN	38°19′54″N, 141°03′08″E	WG	12	12
China							
	6	Qingdao	EG_QD	36°05′25″N, 120°29′21″E	EG	12	12
	7	Zhujiazien	EX_ZH	29°52′12″N, 122°23′55″E	EX	12	12
Russia							
	8	Vladivostok	WG_VB	43°04′27″N, 131°50′32″E	WG	12	12
Totals						95	95

AFLP, amplified fragment length polymorphism; WG, western group; EG, eastern group; EX, *Ligia exotica* group.
[a]Number of individuals of which 16S rRNA is sequenced.
[b]Number of individuals of which AFLP is genotyped.

DNA is preferred to infer the overall complex phylogenetic histories of the species.

Amplified fragment length polymorphisms (AFLP) (Vos et al., 1995) have been proven to be a useful DNA marker, encompassing mostly nuclear genetic variation in diverse animal groups (Mueller and Wolfenbarger, 1999; Meudt and Clarke, 2007). Applications of these markers are effective, especially with non-model species, as there is no need for prior genomic information of the species concerned. Hence, AFLP markers are used for a variety of purposes, including population genetic diversity estimation, phylogeography, phylogenetic analyses, genome-wide mapping, and detection of signatures of selection (Mueller and Wolfenbarger, 1999; Bensch and Åkesson, 2005; Meudt and Clarke, 2007; Davey et al., 2011; Smith et al., 2011; Caballero et al., 2013), and in particular, species delimitation of closely related species (Meudt and Clarke, 2007).

To investigate whether *Ligia* displays similar genetic divergence patterns with both mitochondrial DNA and nuclear DNA, we evaluated the genetic variation of *Ligia* populations from South Korea, China, Japan and Russia, by using both mitochondrial 16S rRNA gene and AFLP markers.

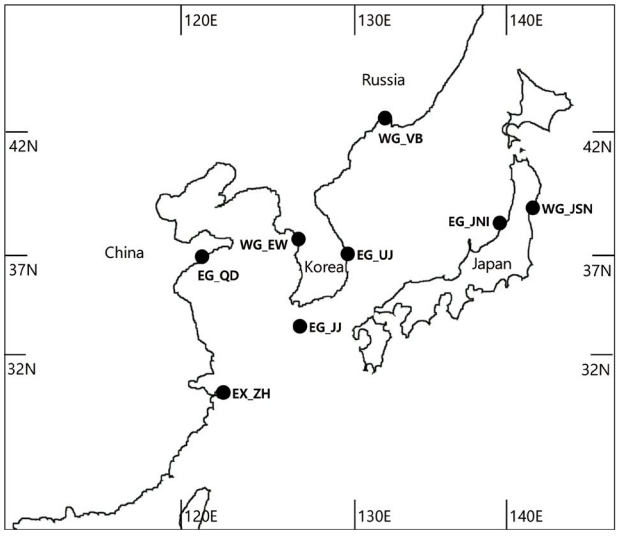

Fig. 1. Geographical locations of the *Ligia* sampling sites in a map of the East Asian region. The names of locations and related information are presented in Table 1.

MATERIALS AND METHODS

Sampling, DNA extraction, PCR and sequencing

A total of 95 individuals of *Ligia* specimens were collected by hand from eight locations in East Asia (Table 1, Fig. 1), and preserved in absolute ethanol. A total genomic DNA was extracted from the walking legs, by following Sambrook and Russell (2001), or DNeasy Blood and Tissue kit (Qiagen, USA). Part of the mitochondrial 16S ribosomal RNA gene was PCR amplified, and directly sequenced using primers 16SAR-SF and 16SBR-SF (Palumbi et al., 1991), following the protocol of Jung et al. (2008).

AFLP genotyping

The total genomic DNA of each specimen was digested by

Table 2. Genetic diversity indices in sampled *Ligia* populations

		WG_EW	WG_JSN	WG_VB	EG_UJ	EG_JJ	EG_JNI	EG_QD	EX_ZH	Mean
16S rRNA	No. of substitutions	1	2	0	1	1	0	1	6	1.5
	No. of indels	0	0	0	0	0	0	0	12	1.5
	θ (π)	0.182	0.576	0	0.167	0.167	0	0.167	3	0.532
	θ (K)	0.410	0.934	0	0.393	0.393	0	0.393	2.685	0.651
AFLP	No. of polymorphic loci	380	279	201	255	214	332	235	296	449
	Proportion of polymorphic loci (%)	54.99	40.38	29.09	36.90	30.97	48.05	34.01	42.84	65

AFLP, amplified fragment length polymorphism.

using two restriction enzymes, *Eco*RI and *Mse*I (Macrogen, Korea) for 1 h at 37°C, followed by an additional 3 h at 16°C. After inactivation of the restriction enzymes, both *Eco*RI and *Mse*I adaptors were ligated to the sticky-end of the restricted DNA fragments, by using T4 DNA ligase for 1 h at 4°C. The two primers (ECO-AXX and MSE-AXX) were used in the pre-amplification procedure. Then, three types of ECO-AXX (ECO-AGG, -AAG, -AGA) with fluorescent labels (6FAM) and two types of MSE-AXX (MSE-ACC, -AGG) were used for the selective amplification procedure. The AFLP protocols used in this study followed Jung et al. (Jung et al., 2006, 2010; Jung, 2013) (see these papers for detailed information of PCR reaction mixture compositions and experimental conditions). The PCR amplifications were performed on a GeneAmp PCR System 2700 (Applied Biosystems, USA). The selective amplification products were separated in a Genetic Analyzer 3730 (Applied Biosystems). The band sizes and genotypes were scored using the software GeneMarker 2.6.0 (Softgenetics, USA), and the existence of alleles was denoted as "1", if the band intensity ranged within 100 to 60000.

Data analyses

Multiple alignments of 16S rRNA sequences were conducted using Culstal W (Larkin et al., 2007), as implemented in the MEGA 6 packages (Tamura et al., 2013). Genetic diversity indices of each sampling sites based on 16S rRNA sequences and AFLP genotypes were calculated, using ARLEQUIN 3.5 (Excoffier and Lischer, 2010). The same software was used to infer pairwise mean Kimura 2 parameter (K2P) distance and F_{ST} among populations with permutations of 1,000, based on 16S rRNA and AFLP, respectively. MEGA 6 was used to depict phylogenetic trees of 16S rRNA gene sequences by maximum likelihood methods, with bootstrap values of 1,000 replicates. The best-fit models of nucleotide substitution for phylogenetic tree estimation were estimated using the same software packages. The principal coordinates analysis (PCoA) of AFLP genotypes was conducted in GENALEX 6.5 (Peakall and Smouse, 2012).

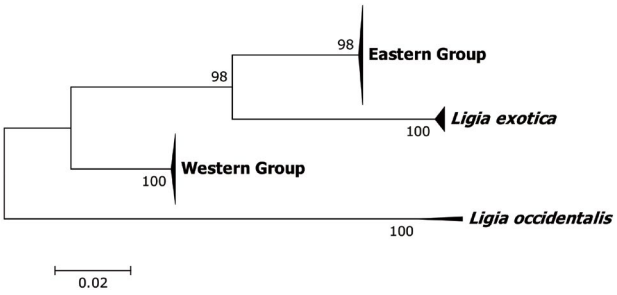

Fig. 2. A maximum likelihood tree among *Ligia* samples collected from China, Korea and Japan based on the Tamura 3-parameter obtained from 16S rRNA genes depicted with MEGA 6.06. The numbers near each node are bootstrap values, calculated using 1,000 replicates.

RESULTS

Mitochondrial 16S rRNA gene

A total of 13 haplotypes (GenBank accession number: KJ802742-KJ802854) from 95 individuals belonging to the eight populations (Table 2). Nucleotide substitutions were identified at 12 sites among 497 bp, and the number of substitutions was varied among the populations, and the indels with the value of 12 were only observed from *L. exotica* population (EX_ZH). Among the eight populations, WG_VB and EG_JNI showed the lowest diversity values, as 0 in θ (π) and θ (k); whereas EX_ZH showed the highest, as 3 and 2.685, respectively. Within the WG populations, WG_JSN showed the highest diversity values.

Three distinct genetic groups with deep divergence were identified in maximum likelihood trees based on the Tamura 3-parameter, supported by bootstrap values ranging between 95 and 100 (Fig. 2); and the Eastern group and *L. exotica* were in sister relation. The pairwise π_{ST} analysis with permutation of 1,000 (Table 3) between different lineages

Table 3. A matrix of pairwise K2P genetic distances between *Ligia* populations based on 16S rRNA (upper diagonal) and pairwise differences F_{ST} based on AFLP data (lower diagonal), respectively

	WG_EW	WG_JSN	WG_VB	EG_UJ	EG_JJ	EG_JNI	EG_QD	EX_ZH
WG_EW	–	0.1017	0.0083	**0.9965**	**0.9965**	**0.9983**	**0.9965**	**0.9893**
WG_JSN	**0.0649**	–	0.1362	**0.9926**	**0.9926**	**0.9942**	**0.9926**	**0.9863**
WG_VB	**0.1066**	**0.1583**	–	**0.9983**	**0.9983**	**1.0000**	**0.9983**	**0.9913**
EG_UJ	**0.0332**	**0.0366**	**0.1377**	–	0	0	0	**0.9866**
EG_JJ	**0.1513**	**0.2070**	**0.1309**	**0.1958**	–	0	0	**0.9866**
EG_JNI	**0.0702**	**0.1319**	**0.0648**	**0.0941**	**0.0896**	–	0	**0.9885**
EG_QD	**0.0706**	0.0059	**0.1777**	0.0294	**0.2335**	**0.1484**	–	**0.9866**
EX_ZH	**0.0113**	0.0302	**0.1063**	0.0105	**0.1762**	**0.0833**	0.0116	–

Significant values ($p<0.05$) are shaded with boldface.
K2P, Kimura 2 parameter; AFLP, amplified fragment length polymorphism.

ranged from 0.9916 to 1 with highly significant P, and the values within the same lineages were 0 to 0.1364, and statistically insignificant in all comparisons.

AFLP marker for nuclear DNA

Six hundred and ninety-one loci were detected, ranging from 52 bp to 576 bp in size (Table 3), from AFLP analysis of 95 individuals from eight populations. The number of polymorphic loci ranged from 201 (29.09%) to 380 (54.99%). Pairwise F_{ST} values of interspecific comparison ranging from 0.0000 (insignificant value = 0.0059) to 0.1777 were almost similar, with intraspecific values of WG (0.0649 to 0.1583) and EG (0.0000 to 0.2335) (Table 3). The PCoA at individual level produced a two-dimensional representation (Fig. 3), with the first two axes explaining 14.23% of the total variance, and among all three groups showed no clear cluster gathering populations.

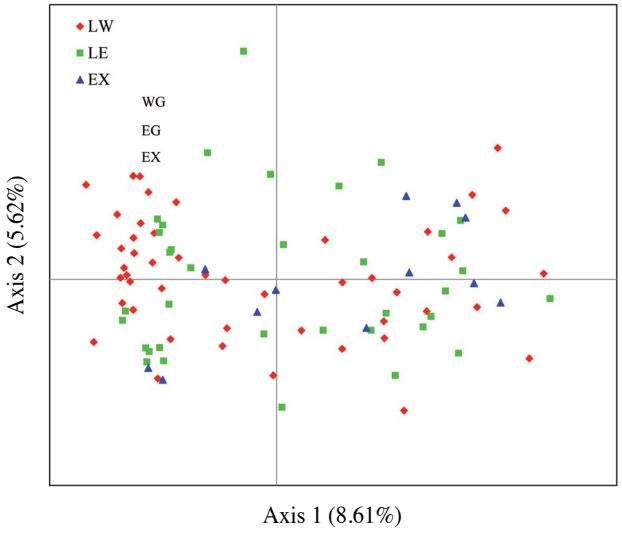

Fig. 3. A plot representing the results of principal coordinates analysis (PCoA) of amplified fragment length polymorphism genotypes from 95 *Ligia* individuals, executed with GENEALEX 6.501. Populations belonging to the Western Group, Eastern Group, and *Ligia exotica* are represented as diamond, square, and triangle, respectively.

DISCUSSION

The *Ligia* specimens found in East Asia were comprised of three genetically diverged groups, based on 16S rRNA gene differences (Table 3, Fig. 2). The mean K2P genetic distances among the three groups (9.8–11.7) were within the range of average *Ligia* between species genetic distance (5.6–25.58) (Hurtado et al., 2010). The neighbor-joining tree (Fig. 2) showed both a deep divergence between lineages, and highly significant bootstrap values (98–100%) at all nodes. These results matched well with the previous studies, using the same 16S rRNA gene (Jung et al., 2008; Yin et al., 2013) and *COI* gene (Yin et al., 2013) that identified the existence of the three distinguished genetic lineages of *Ligia* in East Asia. On the other hand, the AFLP variations between the populations within the groups (pairwise F_{ST} = 0–0.23) were similar to the variation among the groups (pairwise F_{ST} = 0–0.21), in pairwise F_{ST} analyses. Likewise, the PCoA showed overlapping individuals belonging to the three different groups, with scattered patterns, with no clear clusters observed.

This incongruence between mitochondrial and nuclear DNA markers may be explained by two different factors. First, DNAs could show different evolutionary histories, depending on the genomic regions of markers used. Because of the maternal inheritance of mitochondrial DNA, the effective population size of mitochondrial DNA is only a quarter that of nuclear DNA, and displays a faster genetic divergence, compared with nuclear DNA (Avise, 2000). Mitochondrial DNA could depict genetic divergence occurring among the three *Ligia* groups; whereas there was not enough time to fully differentiate the nuclear DNA, and

these groups still share much of the ancient polymorphisms, in that the three groups are closely related. The distinct nuclear DNA genetic variation of 49 bp size difference of nuclear 18S rRNA gene between WG and EG (Jung et al., 2008) supports this hypothesis, as nuclear rRNA regions usually experience rapid concerted evolution in metazoans (Alquezar et al., 2010). Many recent studies have also shown similar incongruence patterns in diverse animal groups, including crickets (Shaw, 2002), cichlids (Egger et al., 2007; Koblmüller et al., 2007), and lizards (Leaché, 2011).

Second is genomic islands of speciation, which could explain the observed shared polymorphisms (Turner et al., 2005). Usually, the effect of different intensity of natural selection pressure on loci during divergence may diversify the molecular evolution patterns of genes, depending on genomic regions. The wide spread of shared loci is due to only a few part of the genomic regions being highly differentiated between closely related species, these several diverged genes causing a phenomenal influence, large enough to induce a speciation process, as speciation genes causing post-zygotic inviability and hybrid sterility (Coyne and Orr, 2004; Wu and Ting, 2004; Orr, 2005; Presgraves, 2007; Rieseberg and Blackman, 2010; Nosil and Schluter, 2011).

In conclusion, the analysis of 16S rRNA revealed the existence of the three *Ligia* lineages in East Asia. Despite the discrepancies between mitochondrial DNA and AFLP results, AFLP analysis showed most of the polymorphisms were shared among lineages. Recently, in corporation with the development of new generation sequencing technology (NGS), restriction-site associated DNA sequencing has been introduced as a new and alternative method for AFLP. Thus, the results provide a basis for future study, using NGS technology to scan genome-wide patterns of differentiation, and identify specific genes that directly influence phenotypic changes, such as morphological, physiological, and behavioural changes among lineages.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

Alquezar DE, Hemmerter S, Cooper RD, Beebe NW, 2010. Incomplete concerted evolution and reproductive isolation at the rDNA locus uncovers nine cryptic species within *Anopheles longirostris* from Papua New Guinea. BMC Evolutionary Biology, 10:392. https://doi.org/10.1186/1471-2148-10-392

Avise JC, 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA, pp. 1-447.

Bensch S, Åkesson M, 2005. Ten years of AFLP in ecology and evolution: why so few animals? Molecular Ecology, 14:2899-2914. https://doi.org/10.1111/j.1365-294X.2005.02655.x

Caballero A, García-Pereira MJ, Quesada H, 2013. Genomic distribution of AFLP markers relative to gene locations for different eukaryotic species. BMC Genomics, 14:528. https://doi.org/10.1186/1471-2164-14-528

Coyne JA, Orr HA, 2004. Speciation. Sinauer Associates, Sunderland, MA, pp. 1-545.

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML, 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics, 12:499-510. https://doi.org/10.1038/nrg3012

Eberl R, Mateos M, Grosberg RK, Santamaria CA, Hurtado LA, 2013. Phylogeography of the supralittoral isopod *Ligia occidentalis* around the Point Conception marine biogeographical boundary. Journal of Biogeography, 40:2361-2372. https://doi.org/10.1111/jbi.12168

Egger B, Koblmüller S, Sturmbauer C, Sefc KM, 2007. Nuclear and mitochondrial data reveal different evolutionary processes in the Lake Tanganyika cichlid genus *Tropheus*. BMC Evolutionary Biology, 7:137. https://doi.org/10.1186/1471-2148-7-137

Excoffier L, Lischer HEL, 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10:564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Hurtado LA, Mateos M, Santamaria CA, 2010. Phylogeography of supralittoral rocky intertidal *Ligia* isopods in the Pacific region from central California to central Mexico. PLoS ONE, 5:e11633. https://doi.org/10.1371/journal.pone.0011633

Hurtado LA, Mateos M, Wang C, Santamaria CA, Jung J, Kha-

laji-Pirbalouty V, Kim W, 2018. Out of Asia: mitochondrial evolutionary history of the globally introduced supralittoral isopod *Ligia exotica*. PeerJ, 6:e4337. https://doi.org/10.7717/peerj.4337

Jung J, 2013. Population genetic structure of *Carassius auratus* (Pisces: Cypriniformes) in South Korea inferred from AFLP markers: discordance with mitochondrial genetic structure. Animal Systematics Evolution and Diversity, 29:18-22. https://doi.org/10.5635/ASED.2013.29.1.18

Jung J, Eo HS, Rho HS, Kim W, 2008. Two genetic lineages of sea slaters, *Ligia* (Crustacea: Isopoda) in South Korea: a population genetic approach. Molecules and Cells, 25:523-530.

Jung J, Han H, Ryu SH, Kim W, 2010. Amplified fragment length polymorphism analysis and genetic variation of the pinewood nematode *Bursaphelenchus xylophilus* in South Korea. Animal Cells and Systems, 14:31-36. https://doi.org/10.1080/19768351003770889

Jung J, Lee JW, Kim JP, Kim W, 2006. Genetic variations of the golden orb-web spider *Nephila clavata* (Araneae: Tetragnathidae) in Korea, using AFLP markers. Korean Journal of Genetics, 28:325-332.

Koblmüller S, Duftner N, Sefc KM, Aibara M, Stipacek M, Blanc M, Egger B, Sturmbauer C, 2007. Reticulate phylogeny of gastropod-shell-breeding cichlids from Lake Tanganyika: the result of repeated introgressive hybridization. BMC Evolutionary Biology, 7:7. https://doi.org/10.1186/1471-2148-7-7

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG, 2007. Clustal W and Clustal X version 2.0. Bioinformatics, 23:2947-2948. https://doi.org/10.1093/bioinformatics/btm404

Leaché AD, 2011. Multi-locus estimates of population structure and migration in a fence lizard hybrid zone. PLoS One, 6:e25827. https://doi.org/10.1371/journal.pone.0025827

Lee JD, 1994. A new mountain slater, *Ligia taiwanensis* (Isopoda, Ligiidae) from Taiwan. Crustaceana, 66:110-115. https://doi.org/10.1163/156854094X00198

Markow TA, Pfeiler E, 2010. Mitochondrial DNA evidence for deep genetic divergences in allopatric populations of the rocky intertidal isopod *Ligia occidentalis* from the eastern Pacific. Molecular Phylogenetics and Evolution, 56:468-473. https://doi.org/10.1016/j.ympev.2009.12.002

Meudt HM, Clarke AC, 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends in Plant Science, 12:106-117. https://doi.org/10.1016/j.tplants.2007.02.001

Mueller UG, Wolfenbarger LL, 1999. AFLP genotyping and fingerprinting. Trends in Ecology and Evolution, 14:389-394. https://doi.org/10.1016/S0169-5347(99)01659-6

Nosil P, Schluter D, 2011. The genes underlying the process of speciation. Trends in Ecology and Evolution, 26:160-167. https://doi.org/10.1016/j.tree.2011.01.001

Nunomura N, 1979. *Ligia boninensis*, a new isopod crustacean from Haha-jima, Bonin Islands, Japan. Bulletin of the Toyama Science Museum, 1:37-40.

Nunomura N, 1983. Studies on the terrestrial isopod crustaceans in Japan. I. Taxonomy of the families Ligiidae, Trichoniscidae, and Olbrinidae. Bulletin of the Toyama Science Museum, 5:23-68.

Nunomura N, 1990. Studies on the terrestrial isopod crustaceans in Japan. V. Taxonomy of the families Armadillidiidae, Armadillidae and Tylidae, with taxonomic supplements to some other families. Bulletin of the Toyama Science Museum, 13:1-58.

Nunomura N, 1999. Sea shore isopod crustaceans collected from Izu Islands, Middle Japan. Bulletin of the Toyama Science Museum, 22:7-38.

Nunomura N, Horiguchi H, Sasaki T, Hironaka M, Hariyama T, 2011. A new species of the genus *Ligia* (Crustacea: Isopoda: Ligiidae) from steep streams of Chichijima and Anijima Islands of the Ogasawara Islands. Bulletin of the Toyama Science Museum, 34:73-79.

Orr HA, 2005. The genetic basis of reproductive isolation: insights from *Drosophila*. Proceeding of the National Academy of Sciences of the United States of America, 102:6522-6526. https://doi.org/10.1073/pnas.0501893102

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G, 1991. The simple fool's guide to PCR, version 2.0. University of Hawaii Press, Honolulu, HI, pp. 1-47.

Peakall R, Smouse PE, 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28:2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Presgraves DC, 2007. Speciation genetics: epistasis, conflict and the origin of species. Current Biology, 17:R125-R127. https://doi.org/10.1016/j.cub.2006.12.030

Rieseberg LH, Blackman BK, 2010. Speciation genes in plants. Annals of Botany, 106:439-455. https://doi.org/10.1093/aob/mcq126

Sambrook J, Russell DW, 2001. Molecular cloning: a laboratory manual, Vol. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Santamaria CA, Mateos M, Taiti S, DeWitt TJ, Hurtado LA, 2013. A complex evolutionary history in a remote archipelago: phylogeography and morphometrics of the Hawaiian endemic *Ligia* isopods. PLoS One, 8:e85199. https://doi.org/10.1371/journal.pone.0085199

Schmalfuss H, 2003. World catalog of terrestrial isopods (Isopoda: Oniscidea). Stuttgarter Beitrage zur Naturkunde Serie A, 654:1-341.

Shaw KL, 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proceeding of the National Academy of Sciences of the United States of America, 99:16122-16127. https://doi.org/10.1073/pnas.242585899

Smith TA, Mendelson TC, Page LM, 2011. AFLPs support

deep relationships among darters (Percidae: Etheostomatinae) consistent with morphological hypotheses. Heredity, 107:579-588. https://doi.org/10.1038/hdy.2011.50

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30:2725-2729. https://doi.org/10.1093/molbev/mst197s

Tsuge M, 2008. A new species of the genus *Ligia* (Crustacea: Isopoda: Ligiidae) from the Lake Shinji (Shimane Prefecture), western Japan. Bulletin of the Toyama Science Museum, 31:51-57.

Turner TL, Hahn MW, Nuzhdin SV, 2005. Genomic islands of speciation in *Anopheles gambiae*. PLoS Biology, 3:e285. https://doi.org/10.1371/journal.pbio.0030285

Via S, 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. Philosophical Transactions of the Royal Society B, 367:451-460. https://doi.org/10.1098/rstb.2011.0260

Via S, West J, 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. Molecular Ecology, 17:4334-4345. https://doi.org/10.1111/j.1365-294X.2008.03921.x

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M, 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23:4407-4414. https://doi.org/10.1093/nar/23.21.4407

Wu CI, Ting CT, 2004. Genes and speciation. Nature Reviews in Genetics, 5:114-122. https://doi.org/10.1038/nrg1269

Yin J, Pan D, He C, Wang A, Yan J, Sun H, 2013. Morphological and molecular data confirm species assignment and dispersal of the genus *Ligia* (Crustacea: Isopoda: Ligiidae) along northeastern coastal China and East Asia. Zoological Journal of the Linnean Society, 169:362-376. https://doi.org/10.1111/zoj.12068

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