Genetic and Phenetic Differentiation among Three Forms of Korean Salamander *Hynobius leechii*

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Genetic and morphological variations of Korean *Hynobius leechii* were surveyed. Starch gel electrophoresis was used to study genetic variation at 23 loci. *H. leechii* is genetically divided into three groups, each of which is named as Form-A, Form-B, and Form-C. These forms are clearly separated with 4 - 5 fixed or nearly fixed allelic differences. At a sympatry near Jangseong, Jollanam-do, a significant deviation from random mating between Form-A and Form-B was observed. Therefore, they are considered as two distinct taxa of specific rank. Form-A is widely distributed in the inland of Korea, whereas Form-B is distributed in the islands or near the coastal regions of Western and Southern Korea. Form-B had, in addition, a significant morphological difference from Form-A. On the other hand, Form-C was restricted to an area near Kori atomic power plant, Kyongsangnam-do, and the genetic similarities between this form and Form-A and Form-B were 0.742 and 0.736, respectively. Moreover, Form-C had a unique color pattern, short body and small coccyx. Thus, it seems reasonable to assign this form as another new species.

The genus *Hynobius* which is distributed in several countries of east Asia differentiated into many species. One of them, *Hynobius leechii* Boulenger (1887), is common in Korea (Kang and Yoon, 1975: Yang et al., 1982).

The taxonomic status on *Hynobius leechii* have been reported by many authors (Boulenger, 1887; Mori, 1928; Okada, 1935; Sato, 1943; Shannon, 1956; Yang et al., 1982; Seto et al., 1986; Kohno et al., 1987; Uh et al., 1992; Lee and Jung, 1993; Seto and Ilzuka, 1993). Mori (1928) considered the Cheju population as a distinct taxon and proposed the subspecific name *H. l. quelpartensis*. Whereas Sato (1937, 1943) treated it as a mere local variety of *H. leechii*. Lately, Yang et al. (1982) reported that the Cheju population had shown genetic differentiation of specific level from populations in mainland Korea. Thereafter, Uh et al. (1992) also noticed that the Cheju population differs morphologically from mainland populations.

In this paper, we report the results of isozymic and morphological analyses designed to calibrate the extent of divergence among the *H. leechii* populations to elucidate their taxonomic status in Korea.

Materials and Methods

Collection

A sum of 815 adults were collected from 30 localities in Korea (Fig. 1, Table 1). During most collecting trips, color notes of each specimens were taken for comparisons of body color among populations.

Protein electrophoresis

Live samples were transported to the laboratory and were stored at -70℃ until use. In the laboratory, the tissues of liver, heart and skeletal muscle from each specimens were removed and homogenized by glass homogenizer in half volume of distilled water and were centrifugated at 18,000 rpm for 30 min at 4℃ to obtain the supernatant for electrophoresis. Voucher specimens were fixed in 10% formalin, preserved in 70% ethanol, and deposited in Yang's collection at Inha University. The supernatant was subjected to horizontal starch-gel (12%) electrophoresis and histochemical staining procedures (Selander et al., 1971; Yang et al., 1982: Buth, personal communication; Table 2). Multiple loci were numbered sequentially, and alleles were designated alphabetically with "a" being the fastest migrant. Individual genotypes were used to calculate allele frequencies for each population, these in turn were used to estimate the degree of genic variability and to calculate matrices of genetic similarity (Rogers, 1972) and genetic distance (Nei, 1972). Rogers' (1972) similarity coefficients were then clustered by the unweighted pair group method using arithmetic averages linkage (UPGMA: Sneath and Sokal, 1973) to provide a general estimate of the overall genetic relationships among populations. In addition, the analysis included a sample of 40 specimens from Jangseong, where two forms (genetically

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Table 1. Collection localities, collection dates and sample sizes for electrophoresis (E) and morphological analysis (M) of the *Hynobius leechii* complex examined in this study

Collection localities	Date	Sample size E	Sample size M
Form-A			
1. Koseong: Kanseong-eup, Koseong-gun, Kangwon-do	Apr. 15, 1995	27	27
2. Inje: Yongdae-ri, Buk-myon, Inje-gun, Kangwon-do	Apr. 14, 1995	30	30
3. Paju: Beobweon-eup, Paju-gun, Kyunggi-do	Mar. 30, 1995	30	30
4. Yangpyeong: Yangpyeong-eup, Yangpyeong-gun, Kyeonggi-do	Mar. 29, 1995	22	22
5. Myeongju: Sangye-ri, Oggye-myon, Myeongju, Kangwon-do	Mar. 27, 1994	8	8
6. Samcheok: Uji-dong, Samcheok-gun, Kangwon-do	Mar. 24, 1995	30	30
7. Jeongseon-O.: Oban-ri, Jeongseon-gun, Kangwon-do	Apr. 15, 1995	21	22
8. Jeongseon-H.: Hanso-ri, Baekjeon-myon, Jeongseon-gun, Kangwon-do	Apr. 16, 1995	38	40
9. Tanyang: Bohyon-sa, Danyang-gun, Chungchongbuk-do	Apr. 17, 1995	30	30
10. Uljin: Weolsong-ri, Pyeonghae-eup, Uljin-gun, Kyongsangbuk-do	Mar. 23, 1995	30	30
11. Chungju: Sangkumgok-ri, Chungju-shi, Chungchongbuk-do	Mar. 17, 1995	30	30
12. Yeongpung: Taejang-ri, Punggi-eup, Yeongpung-gun, Kangwon-do	Apr. 19, 1995	30	30
13. Cheongju: Sangdang-dong, Cheongju-shi, Chungchongbuk-do	Mar. 17, 1995	30	30
14. Cheongyang: Machi 1-gu, Jeongsan-myon, Cheongyang-gun, Chungchongnam-do	Mar. 17, 1995	30	30
15. Sangju: Yeongheung-sa, Sangju-gun, Kyongsangbuk-do	Mar. 18, 1995	29	27
16. Muju: Kucheon-dong, Seolcheon-myon, Muju-gun, Jollabuk-do	May 18, 1995	5	5
17. Yeongcheon: Umi 2-dong, Yeongcheon-shi, Kyongsangbuk-do	Mar. 22, 1995	35	30
18. Seongju: Jeogsong-ri, Suryun-myon, Seongju-gun, Kyongsangbuk-do	Apr. 9, 1995	20	17
19. Kyeongju: Yangdong-ri, Kangdong-myon, Kyeongju-shi, Kyongsangbuk-do	Mar. 9, 1995	20	19
20. Kurye: Hwaom-sa, Kurye-gun, Jollanam-do	Mar.10, 1995	25	23
Form-B			
21. Puan: Naeso-sa, Jinseo-myon, Puan-gun, Jollabuk-do	May 12, 1995	30	30
22. Keoje Isl.: Yongju-sa, Sinhyeon-eup, Keoje-gun, Kyongsangnam-do	Mar. 25, 1995	30	30
23. Namhae Isl.: Sannae-ri, Samjang-myon, Namhae-gun, Kyongsangnam-do	Apr. 9, 1995	30	30
24. Haenam: Jangchon-ri, Masan-myon, Haenam-gun, Jollanam-do	Mar. 18, 1995	30	30
25. Chindo Isl.: Sacheon-ri, Euisin-myon, Chindo-gun, Jollanam-do	Mar. 10, 1995	30	26
26. Cheju Isl.-C.: Ara 2-dong, Cheju-shi, Cheju-do	Mar. 31, 1995	30	30
27. Cheju Isl.-S.: Jangsu-gyo, Seoguipo-shi, Cheju-do	Apr. 1, 1995	30	30
Form-C			
28. Kori-H.: Hyoam-ri, Jangan-eup, Yangsan-gun, Kyongsangnam-do	Mar. 10, 1995	15	20
29. Kori-K.: Kilcheon-ri, Jangan-eup, Yangsan-gun, Kyongsangnam-do	Mar. 10, 1995	30	30
Sympatry of Form-A and Form-B			
30. Jangseong: Jajang-dong, Bukha-myon, Jangseong-gun, Jollanam-do	Mar. 18, 1995	40	-
Total		815	766

divergent groups) are sympatric (see Table 1, Fig. 1). To manifest the extent of assortative mating and to test for departures from Hardy-Weinberg equilibrium at there, we employed F-statistics (Vithayasai, 1973).

Morphological analysis

A total of 766 adults from 29 population were used for morphological analysis (Table 1). All measurements and counts were taken on preserved specimens. For the anaysis, external and skeletal characters were

Table 2. Buffer systems and enzymes for electrophoresis

Buffer system	E. C. No.*	Enzyme	Condition
Continuous tris citrate II (pH 8.0)	1.1.99. 5	α-Glycerolphosphate dehydrogenase *(αGpd)*	100V/3h
	1.1. 1.42	Isocitrate dehydrogenase *(Idh)*	
	1.1. 1.40	Malic enzyme *(Me-1,2)*	
	2.7. 4. 3	Adenylate kinase *(Ak)*	
	2.7. 3. 2	Creatine kinase *(Ck)*	
	1.1. 1.47	Glucose dehydrogenase *(Gdh)*	
	1.1. 1.14	Sorbitol dehydrogenase *(Sdh)*	
	3.4.11. 1	Leucine amino-peptidase *(Lap)*	
LiOH (pH 8.1)	2.6. 1. 1	Glutamate oxaloacetate isomerase *(Got-1,2)*	300V/3h
	N. S.**	General protein *(Gp-2,3)*	
	1.1. 1.27	Lactate dehydrogenase *(Ldh-1,2)*	
	3.4.11.11	Peptidase *(Pept)*	
Discontinuous tris citrate (pH 8.2)	1.1. 1.37	Malate dehydrogenase *(Mdh)*	300V/3h
	5.3. 1. 9	Phosphoglucose isomerase *(Pgi)*	
	4.2. 1. 3	Aconitate hydratase *(Aco-1,2)*	
Tris maleic EDTA (pH 7.4)	5.3. 1. 8	Mannose phosphate isomerase *(Mpi)*	100V/5h
	1.1. 1.43	6-Phosphogluconate dehydrogenase *(6Pgd)*	
	1.2. 1.37	Xanthine dehydrogenase *(Xdh-2)*	

* E. C. No.: Enzyme commission number
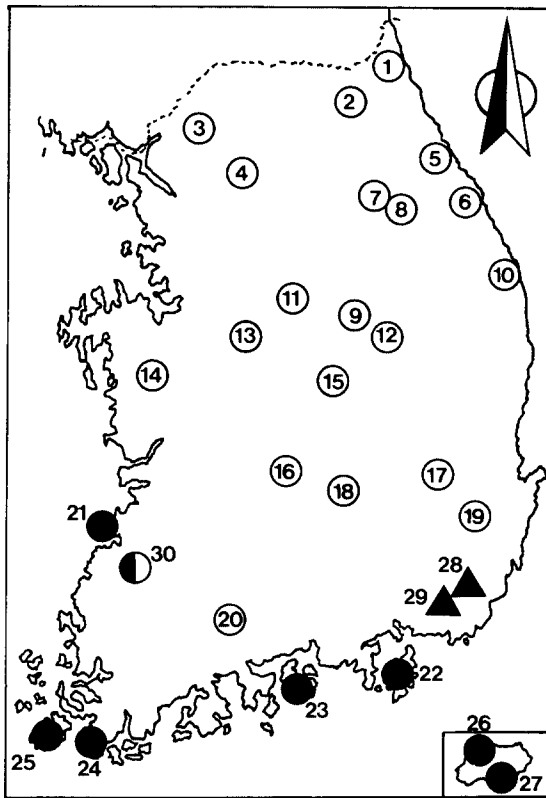** N. S.: Non specific

Fig. 1. Collection localities and distribution of the *Hynobius leechii* complex in Korea. Population numbers refer to Table 1. ○, Form-A; ●, Form-B; ▲, Form-C; ◐, sympatry of Form-A and Form-B.

chosen. Super soft X-ray apparatus (Softex C-60) was employed to radiograph skeletal characters of samples. Radiograph was conducted under the condition of 35 kVp, 1 mA and 3 min onto FUJI Medical X-ray Film (ID No. 036040, FUJI Photo Film Co., Ltd.), and then all films were developed in FUJI Rendol developing solution and FUJI Renfix fixing solution. The following characters based on the methods of Trueb (1977) were taken: snout-vent length (SVL), head length (HL), head width (HW), tail length (TL), femur length (FL), tibia length (TbL), hindlimb length (HLL), inter orbital width (IOW), inter nostril width (INW), number of vomerin teeth (NVT), number of vertebrae (NV), number of coccyx (NC), and number of costal grooves (NCG). Measurements were performed by digital calipers (Mitutoyo; 1/100 mm unit) and counts were performed under microscope (Olympus SZH-10). For comparison of measured characters, percentage ratios of each character dimension relative to SVL were calculated. T-tests were performed using Origin software on an Adtec 5500 PC computer to detect the significance of differences among popultions on all selected characters. Multivariate analysis of the morphometric data was conducted using the principal component analysis (PCA; Eisenhour, 1995) to eliminate overall size effects. Statistical Analysis System (SAS Institute, Inc., 1982) was

then used as available software.

Results

Genetic analysis

Of the 23 presumptive loci scored, 3 loci (*Ck, Gdh, Gp-3*) were monomorphic across all populations. The remaining 20 loci were polymorphic at the $P_{0.99}$ criterion level (Table 3). As shown in Table 3, 4 and Fig. 2, genetic assay revealed that the *Hynobius leechii* complex were clearly divided into three genetic groups (Form-A, Form-B and Form-C) in Korea. Genetic dissimilarities between Form-A (populations 1-20; Table 3) and Form-B (pops. 21-27) include completely different alleles at *Ldh-1* locus and diagnostic differences at the 95% confidence level (Ayala and Powell, 1972) at *Ldh-2, Gp-2, Sdh,* and *Got-2* loci. Form-A and Form-C (pops. 28 and 29) include completely different alleles at *Ldh-1* and diagnostic differences at *Ldh-2, Gp-2*
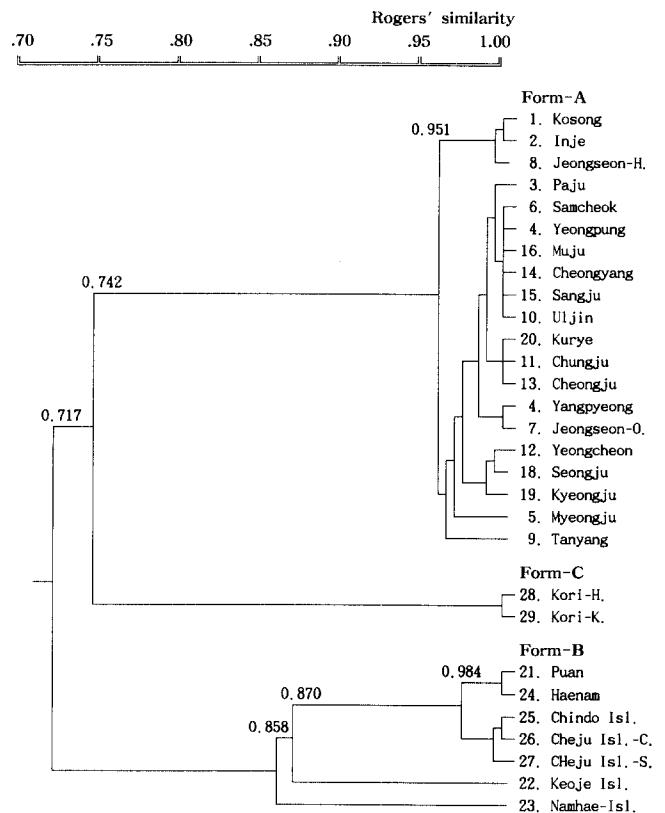

Fig. 2. A Dendrogram of 29 allopatric populations in three forms of the *Hynobius leechii* complex based on Rogers' genetic similarity coefficients (1972).

and *Xdh-2*. Completely different alleles at *Ldh-1, Ldh-2* and diagnostic differences at *Got-2* and *Xdh-2* were found in comparison between Form-B and Form-C (Table 3). Since each form has significant allelic differ-

Table 3. Allele frequencies of 29 populations in the *Hynobius leechii* complex

Locus	*Hynobius leechii* Form-A														
	1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ck	a*	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Gp-3	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Gdh	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ldh-1	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Ldh-2	b(0.61)*** d(0.39)	b(0.60) d(0.40)	b	b	b	b	b	b	b	b	b	b	b	b	b(0.97) d(0.03)
Got-2	a(0.02) b(0.98)	a(0.13) b(0.87)	a(0.02) b(0.98)	a(0.36) b(0.64)	b	b	a(0.38) b(0.62)	a(0.04) b(0.96)	a(0.30) b(0.70)	a(0.03) b(0.97)	b	b	a(0.03) b(0.97)	b	b
Gp-2	c	c	c	c	c(0.94) d(0.06)	c	c	c	e	c	c(0.87) e(0.13)	c	c	b(0.03) c(0.97)	c
Sdh	b	b	b	b	b(0.69) e(0.31)	b(0.98) e(0.02)	b(0.88) e(0.12)	b	b	a(0.03) b(0.97)	b(0.72) e(0.28)	b	b(0.98) c(0.02)	b(0.98) c(0.02)	b
Xdh-2	c	c	b(0.02) c(0.98)	c	c	a(0.17) c(0.83)	a(0.02) b(0.07) c(0.91)	a(0.10) c(0.90)	c(0.80) d(0.20)	a(0.05) c(0.95)	c	c	b(0.08) c(0.74) d(0.18)	c	b(0.14) c(0.86)
Ak	c	c	c(0.72) d(0.28)	c(0.84) d(0.16)	c	c	c	c	c	c	c	c	c	c	c
αGpd	c	c	c	c	c	c	c	c	c	c	c	c	c	c(0.95) d(0.05)	c
Idh	b	b	b	b(0.98) c(0.02)	b	b	b	b	b	b	b	b	b	b	b
Lap	c	b(0.03) c(0.97)	b	b	b	b	b	c	b	b	b	b	b	b	b
Me-1	b(0.93) c(0.07)	b(0.92) c(0.08)	b(0.97) c(0.03)	b(0.86) c(0.14)	b(0.12) c(0.88)	b(0.93) c(0.07)	b(0.71) c(0.29)	b	b(0.97) c(0.03)	b(0.92) c(0.08)	b(0.97) c(0.03)	b	b(0.98) c(0.02)	b(0.95) c(0.05)	b
Me-2	b	b	b	b	a(0.06) b(0.88) c(0.06)	b	b(0.91) c(0.09)	b	b	a(0.02) b(0.96) c(0.02)	a(0.07) b(0.93)	a(0.07) b(0.86) c(0.07)	b	a(0.06) b(0.80) c(0.14)	b
Got-1	b	b	b	b(0.89) c(0.11)	b	b	b(0.95) d(0.05)	b(0.96) c(0.01) d(0.03)	a(0.10) b(0.90)	a(0.02) b(0.98)	b	b	b(0.98) c(0.02)	b	b
Pept	b	b	b(0.73) d(0.27)	b	b	b	b	a(0.01) b(0.99)	b	b	b	b	b	b	a(0.03) b(0.97)
Aco-1	c	c	c	c	c	c	c	c	c	c	c	c	c	b(0.03) c(0.97)	c
Aco-2	b	b	b	b	b	b	b	b	b	b	b	b	b	a(0.07) b(0.93)	b
Mdh	c	c	c	c	c	c	c	a(0.07) c(0.93)	a(0.12) c(0.88)	c(0.98) e(0.02)	c	c	c(0.78) e(0.22)	c(0.98) e(0.02)	c(0.97) e(0.03)
Pgi	d	a(0.02) d(0.98)	d	d	d	d	d	b(0.04) d(0.96)	d	b(0.18) d(0.74) g(0.08)	b(0.02) d(0.98)	b(0.02) d(0.98)	d	b(0.05) d(0.95)	c(0.03) d(0.88) f(0.09)
Mpi	b	b	b	b	b	b	b	b	b	a(0.05) b(0.90) c(0.05)	b	a(0.02) b(0.98)	b	b	b(0.92) c(0.08)
6Pgd	c	c	c	c	a(0.06) c(0.94)	c	c	c	c	c(0.92) d(0.08)	b(0.08) c(0.92)	c	c	b(0.03) c(0.97)	b(0.09) c(0.91)

* Allele, ** population number, *** allele frequency.

(Continued)

Locus	Hynobius leechii Form-A					Form-B							Form-C	
	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Ck	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Gp-3	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Gdh	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ldh-1	c	c	b(0.25) c(0.75)	b(0.15) c(0.85)	c	d	d	d	d	a(0.15) d(0.85)	d	d	f	e(0.22) f(0.78)
Ldh-2	b	b	b	b	b	a	a(0.90) b(0.10)	a	a(0.93) d(0.07)	a(0.93) d(0.07)	a	a	c	b(0.07) c(0.93)
Got-2	b	a(0.03) b(0.97)	a(0.03) b(0.97)	a(0.03) b(0.97)	b	a	a(0.92) b(0.08)	a(0.93) b(0.07)	a	a	a	a(0.95) b(0.05)	b	a(0.03) b(0.97)
Gp-2	c	b(0.34) c(0.66)	c	c	c(0.98) d(0.02)	b	b	b	b	b(0.93) c(0.07)	b	b	b	a(0.20) b(0.73) c(0.07)
Sdh	b	a(0.04) b(0.96)	b	b	b(0.76) c(0.24)	e	b(0.80) c(0.05) e(0.15)	b(0.18) e(0.82)	e	e(0.95) f(0.05)	b(0.02) c(0.02) d(0.06) e(0.90)	e(0.45) f(0.55)	b(0.67) c(0.27) e(0.06)	b(0.72) c(0.22) e(0.06)
Xdh-2	c	a(0.04) b(0.20) c(0.73) d(0.03)	a(0.10) c(0.90)	b(0.05) c(0.95)	c	b(0.10) c(0.90)	a(0.02) b(0.30) c(0.68)	c	c	c(0.97) d(0.03)	c	c(1.00)	a(0.13) b(o.87)	a(0.18) b(0.82)
Ak	c	a(0.01) c(0.99)	c	c(0.45) d(0.55)	c	b(0.03) c(0.97)	d	d	c	c	c	c(1.00)	d(1.00)	d(1.00)
αGpd	c	c(0.97) d(0.03)	b(0.02) c(0.98)	b(0.07) c(0.80) d(0.13)	c	c(0.95) d(0.05)	c	a(0.03) b(0.02) c(0.95)	c	c	c	c(0.97) d(0.03)	c(1.00)	c(1.00)
Idh	b	b	b	b	b	b	b	b	b	a(0.02) b(0.98)	b	b(1.00)	b(1.00)	b(1.00)
Lap	b	b	b	b	b	a(0.07) b(0.93)	b(0.98) c(0.02)	b	b(0.97) c(0.03)	b	b	b(0.98) c(0.02)	b(0.60) c(0.40)	b(0.82) c(0.18)
Me-1	b	b	b	b(0.98) c(0.02)	b	b	a(0.05) b(0.95)	a(0.13) b(0.87)	b	b(0.97) c(0.03)	b	b(1.00)	b(0.87) c(0.13)	b(0.97) c(0.03)
Me-2	a(0.10) b(0.90)	a(0.11) b(0.69) c(0.20)	b	a(0.02) b(0.83) c(0.15)	b	a(0.98) b(0.02)	a(0.37) b(0.63)	a(0.93) b(0.07)	a(0.85) b(0.15)	b(0.97) c(0.03)	b(0.88) c(0.12)	a(0.05) b(0.95)	b(1.00)	b(0.97) c(0.03)
Got-1	b	a(0.14) b(0.86)	b(0.88) c(0.12)	b	b	a(0.07) b(0.93)	b	a(0.18) b(0.52) d(0.30)	a(0.05) b(0.93) d(0.02)	b	a(0.23) b(0.75) c(0.02)	a(0.03) b(0.97)	b(1.00)	b(0.98) d(0.02)
Pept	b	b	b	b	b(0.88) c(0.12)	b	b	b	b	b	b	b(0.93) d(0.07)	b(1.00)	b(1.00)
Aco-1	c	c	c(0.55) d(0.45)	c	c	c	a(0.12) b(0.60) d(0.28)	c	c(0.92) d(0.08)	b(0.03) c(0.97)	c	c(1.00)	c(1.00)	b(0.02) c(0.98)
Aco-2	b	a(0.09) b(0.91)	b	a(0.13) b(0.80) c(0.07)	b	b(0.97) c(0.03)	b(0.93) d(0.07)	b(0.98) d(0.02)	b	b(0.90) d(0.10)	b(0.90) d(0.10)	b(1.00)	b(1.00)	b(1.00)
Mdh	c	b(0.10) c(0.90)	c(0.95) e(0.05)	a(0.05) c(0.95)	c	c	c	b(0.02) c(0.96) d(0.02)	b(0.02) c(0.90) e(0.08)	c(0.93) e(0.07)	c	c(0.98) e(0.02)	a(0.07) b(0.03) c(0.90)	c(0.98) d(0.02)
Pgi	b(0.10) d(0.90)	d(0.91) e(0.06) g(0.03)	b(0.07) d(0.80) f(0.130	d(0.98) g(0.02)	b(0.04) d(0.96)	b(0.08) d(0.92)	d(0.82) f(0.13) g(0.05)	b(0.08) d(0.69) f(0.23)	d(0.95) f(0.05)	a(0.03) d(0.78) f(0.17) g(0.02)	b(0.90) d(0.10)	d(1.00)	d(1.00)	b(0.12) d(0.85) f(0.03)
Mpi	b	b	b	b	b	a(0.07) b(0.93)	a(0.03) b(0.97)	a(0.47) b(0.53)	a(0.07) b(0.93)	b	b	a(0.02) b(0.98)	b(0.93) c(0.07)	b(0.93) c(0.07)
6Pgd	c	c	b(0.02) c(0.98)	c	c	c(0.90) d(0.10)	c	c(0.93) d(0.07)	c(0.52) d(0.48)	c(0.92) d(0.08)	c	b(0.02) c(0.98)	b(0.07) c(0.83) d(0.10)	b(0.10) c(0.85) d(0.05)

Table 4. Rogers' (1972) genetic similarities (below diagonal) and Nei's (1972) genetic distances (above diagonal) for 29 populations of three forms in the Hynobius leechii complex

Columns 1–20 = Form-A; columns 21–27 = Form-B; columns 28–29 = Form-C.

Form and locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Hynobius leechii Form-A																													
1. Koseong	-	.001	.061	.061	.090	.054	.064	.008	.110	.057	.059	.053	.058	.056	.055	.054	.069	.070	.075	.057	.360	.351	.442	.357	.283	.297	.277	.270	.257
2. Inje	.999	-	.059	.056	.088	.052	.058	.009	.105	.055	.057	.052	.057	.054	.053	.052	.068	.069	.073	.055	.345	.337	.427	.342	.268	.283	.264	.271	.257
3. Paju	.941	.943	-	.011	.045	.008	.017	.054	.061	.010	.012	.007	.011	.009	.009	.008	.020	.022	.012	.009	.327	.277	.363	.326	.250	.263	.243	.248	.207
4. Yangpyeong	.941	.946	.989	-	.039	.009	.004	.054	.052	.010	.013	.009	.012	.010	.011	.009	.021	.022	.019	.012	.288	.257	.337	.287	.213	.225	.211	.268	.226
5. Myeongju	.914	.915	.956	.962	-	.036	.027	.090	.092	.037	.034	.040	.044	.037	.043	.040	.052	.058	.059	.041	.322	.347	.397	.320	.253	.269	.236	.305	.274
6. Samcheok	.947	.949	.992	.991	.965	-	.010	.036	.053	.004	.006	.002	.004	.003	.003	.002	.012	.014	.020	.005	.314	.299	.397	.314	.239	.252	.236	.261	.220
7. Jeongseon-O.	.938	.944	.983	.996	.973	.990	-	.010	.054	.012	.014	.011	.013	.011	.013	.012	.021	.026	.029	.005	.279	.274	.351	.279	.209	.221	.208	.277	.237
8. Jeongseon-H.	.992	.991	.948	.947	.914	.955	.944	-	.100	.058	.052	.047	.050	.049	.048	.047	.060	.061	.067	.013	.371	.359	.454	.371	.296	.309	.289	.278	.262
9. Tanyang	.896	.900	.941	.949	.912	.948	.947	.905	-	.057	.053	.054	.052	.055	.048	.054	.048	.069	.074	.056	.292	.279	.364	.291	.222	.229	.216	.273	.235
10. Uljin	.944	.946	.990	.990	.963	.996	.988	.952	.945	-	.007	.003	.010	.004	.003	.002	.015	.015	.022	.006	.371	.303	.380	.311	.240	.256	.239	.276	.231
11. Chungju	.943	.944	.988	.987	.967	.994	.986	.949	.948	.993	-	.005	.005	.001	.003	.000	.015	.020	.024	.005	.276	.295	.352	.273	.210	.224	.214	.266	.229
12. Yeongpung	.948	.949	.993	.991	.961	.998	.989	.954	.948	.997	.995	-	.005	.007	.004	.006	.015	.017	.024	.005	.303	.296	.373	.303	.239	.251	.235	.271	.219
13. Cheongju	.943	.945	.989	.988	.957	.996	.987	.952	.948	.993	.990	.993	-	.001	.005	.005	.010	.015	.018	.005	.319	.301	.395	.317	.242	.257	.239	.259	.231
14. Cheongyang	.946	.947	.991	.990	.964	.997	.989	.952	.949	.996	.990	.996	.996	-	.004	.006	.010	.015	.023	.004	.301	.294	.373	.301	.240	.251	.236	.273	.217
15. Sangju	.947	.948	.991	.989	.958	.997	.987	.953	.947	.997	.990	.998	.995	.996	-	.004	.010	.015	.017	.004	.318	.299	.388	.315	.241	.257	.239	.259	.229
16. Muju	.948	.949	.992	.991	.960	.998	.988	.954	.947	.998	.989	1.000	.987	.991	.988	-	.011	.016	.021	.002	.303	.296	.373	.304	.239	.252	.235	.272	.196
17. yeongcheon	.933	.935	.980	.979	.949	.988	.980	.942	.947	.985	.985	.974	.985	.984	.985	.989	-	.027	.019	.011	.269	.267	.339	.274	.222	.227	.216	.232	.234
18. Seongju	.932	.934	.978	.978	.944	.986	.974	.941	.954	.985	.980	.969	.983	.983	.979	.986	.974	-	.027	.027	.327	.271	.395	.320	.245	.261	.245	.280	.185
19. Kyeongju	.928	.929	.988	.981	.943	.980	.971	.935	.934	.978	.976	.982	.977	.978	.979	.982	.973	.969	-	.023	.327	.250	.331	.331	.262	.273	.257	.227	.227
20. Kurye	.945	.946	.991	.988	.960	.995	.988	.951	.928	.994	.995	.996	.992	.995	.995	.996	.984	.982	.978	-	.306	.302	.380	.305	.230	.243	.226	.268	.234
Hynobius leechii Form-B																													
21. Puan	.698	.708	.721	.750	.725	.730	.756	.690	.747	.730	.759	.739	.727	.740	.728	.739	.764	.721	.721	.737	-	.147	.120	.010	.049	.044	.057	.363	.343
22. Keoje Isl.	.704	.714	.758	.773	.707	.742	.760	.698	.757	.738	.744	.744	.740	.745	.741	.744	.766	.763	.779	.739	.863	-	.153	.153	.133	.133	.126	.226	.203
23. Namhae Isl.	.643	.653	.696	.714	.672	.679	.704	.635	.695	.684	.703	.689	.674	.689	.678	.689	.713	.673	.718	.684	.887	.859	-	.133	.166	.163	.180	.258	.248
24. Haenam	.700	.710	.722	.751	.726	.731	.756	.690	.747	.733	.761	.739	.728	.740	.729	.738	.760	.726	.718	.737	.990	.858	.875	-	.045	.045	.057	.370	.352
25. Chindo-Isl.	.753	.765	.779	.808	.776	.787	.811	.744	.801	.787	.810	.787	.785	.787	.786	.787	.801	.782	.770	.794	.952	.875	.847	.956	-	.007	.016	.305	.283
26. Cheju Isl.-C.	.743	.754	.769	.799	.764	.777	.801	.734	.796	.774	.800	.778	.774	.778	.774	.777	.797	.770	.761	.784	.957	.875	.850	.956	.993	-	.015	.312	.293
27. Cheju Isl.-S.	.758	.768	.784	.810	.771	.790	.812	.749	.806	.787	.808	.791	.787	.790	.787	.790	.806	.783	.773	.798	.944	.881	.836	.945	.984	.985	-	.294	.276
Hynobius leechii Form-C																													
28. Kori-H.	.763	.763	.780	.765	.737	.770	.758	.757	.761	.759	.766	.762	.772	.761	.772	.762	.793	.756	.797	.765	.696	.797	.772	.691	.737	.732	.745	-	.009
29. Kori-K.	.773	.774	.813	.798	.760	.803	.789	.770	.790	.794	.798	.795	.803	.794	.805	.795	.822	.791	.831	.797	.710	.817	.781	.703	.753	.746	.759	.991	-

Table 5. Genetic variation of three forms in the *Hynobius leechii* complex

Form and locality	N	Mean no. of alleles (A)	% Polymor- phism (P)	Mean heterozygosity	
				Observed (Ho)	Expected (He)
Form-A					
1. Koseong	27	1.1	8.7	.029	.029
2. Inje	30	1.2	13.0	.046	.042
3. Paju	30	1.2	8.7	.033	.041
4. Yangpyeong	22	1.2	17.4	.053	.054
5. Myeongju	8	1.3	21.7	.038	.051
6. Samcheok	30	1.1	8.7	.019	.019
7. Jeongseon-O.	21	1.3	21.7	.068	.068
8. Jeongseon-H.	38	1.3	8.7	.026	.025
9. Tanyang	30	1.2	17.4	.042	.053
10. Uljin	30	1.6	21.7	.058	.056
11. Chungju	30	1.3	17.4	.028	.045
12. Yeongpung	30	1.2	4.3	.014	.014
13. Cheongju	30	1.3	8.7	.032	.041
14. Cheongyang	30	1.5	21.7	.026	.045
15. Sangju	29	1.3	17.4	.037	.043
16. Muju	5	1.1	8.7	.017	.017
17. Yeongcheon	35	1.7	30.4	.087	.102
18. Seongju	20	1.4	26.1	.046	.082
19. Kyeongju	20	1.6	30.4	.078	.092
20. Kurye	25	1.2	8.7	.030	.031
Mean		**1.3**	**16.1**	**.040**	**.048**
Form-B					
21. Puan	30	1.4	30.4	.048	.051
22. Keoje Isl.	30	1.7	43.5	.119	.132
23. Namhae Isl.	30	1.7	39.1	.109	.118
24. Haenam	30	1.5	34.8	.074	.073
25. Chindo Isl.	30	1.7	34.8	.054	.076
26. Cheju Isl. C.	30	1.3	17.4	.041	.044
27. Cheju Isl. S.	30	1.4	17.4	.039	.047
Mean		**1.5**	**31.1**	**.069**	**.077**
Form-C					
28. Kori-H.	15	1.4	30.4	.093	.091
29. Kori-K.	30	1.8	39.1	.091	.126
Mean		**1.6**	**34.8**	**.092**	**.109**

Table 7. Inbreeding coefficients (Fis: by measuring the deviation of genotypic frequencies from Hardy-Weinberg equilibrium) on diagnostic loci between Form-A and Form-B at sympatry
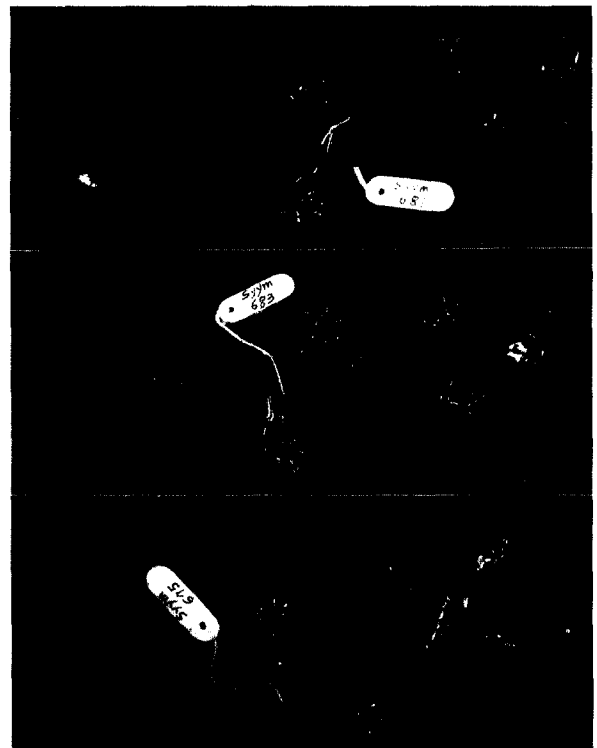
Locus	Jangseong (N=40)			x^2 value(df)
	Ho	He	Fis	
Sdh	10	19.49	0.487	4.620(2)
Got-2	10	19.35	0.483	4.518(1)
Gp-2	11	16.13	0.318	1.632(1)

clustering based on Rogers' genetic similarities shows the level of dissimilarities among the forms as well as the similarities among populations within the same nominal forms (Fig. 2).

Although Form-C occupies a relatively restricted area, this form shows higher genic variation (A=1.6, P=34.8, Ho=0.092, He=0.109) than that of Form-A (A=1.3, P= 16.1, Ho=0.040, He=0.048) or that of Form-B (A=1.5, P=31.1, Ho=0.069, He=0.077: Table 5).

The geographic distributions of each form can be summarized as follows; Form-A is widely distributed in inland of Korea, Form-B is restricted to the islands or the coastal regions of western and southern parts of Korea and Form-C is relic to an area near Kori atomic power plant, Kyongsangnam-do (Fig. 1).

A sympatry of Form-A and Form-B is discovered at Jangseong (pop. 30). This population is located at a neighboring area of two forms in distribution (see Fig.

ences at the loci mentioned above, we were convinced that these loci are significant diagnostic loci to discriminate the *Hynobius leechii* forms.

Based on allelic frequencies listed in Table 3, average genetic similarities and distances among populations of the *H. leechii* complex were estimated (Table 4). Rogers' genetic similarities between regional populations of the same nominal form were high (Form-A: ≥0.905, Form-B: ≥0.836, Form-C: ≥0.991), whereas the average genetic similarities among three forms (SA-B=0.718, SA-C=0.742, SB-C=0.736) were notably lower. The UPGMA

Table 6. The genotype frequencies of three diagnostic loci at Jangseong population, which was compared with some allopatric populations of two forms

Locus	Form-A		Sympatry of Form-A and B	Form-B	
	Koseong (N=27)	Sangju (N=29)	Jangseong (N=40)	Puan (N=30)	Cheju Island (N=30)
Sdh	bb(27)	bb(29)	bb(18) be(10) ee(12)	ee(30)	ee(09) ef(09) ff(12)
Got-2	ab(01) bb(26)	bb(29)	bb(18) ab(10) aa(12)	aa(30)	aa(30)
Gp-2	cc(27)	cc(29)	cc(23) cb(11) bb(06)	bb(30)	bb(30)

Fig. 3. Photographs showing typical specimens of each form in the *Hynobius leechii* complex. A, Form-A. B, Form-B. C, Form-C.

Table 8. The measurements (mean ± SD, in mm) of snout-vent length (SVL) and the percentage ratios (mean ± SD) of each character dimension to SVL in the three forms of the *Hynobius leechii* complex

| Form and locality	N	SVL	[1]INW/SVL	[2]HLL/SVL	[3]HL/SVL	[4]HW/SVL	[5]TL/SVL	[6]FL/SVL	[7]TbL/SVL	[8]IOW/SVL
Form-A
1	27	59.60±4.66	6.99±0.63	28.40±2.09	18.34±0.81	17.39±0.99	80.31±6.92	10.17±0.83	6.77±0.56	5.50±0.39
2	30	58.90±3.96	6.91±0.54	29.98±2.40	18.73±0.93	18.15±0.81	82.97±8.31	9.89±1.07	7.12±0.53	5.49±0.42
3	30	53.30±3.07	6.47±0.36	28.62±2.44	19.40±0.79	18.62±0.82	86.86±9.24	10.20±0.70	6.98±0.38	5.95±0.48
4	22	51.22±3.62	6.50±0.34	31.41±2.82	19.95±0.94	18.53±0.94	93.09±6.92	10.74±0.87	7.28±0.63	6.37±0.57
5	8	70.51±3.04	6.56±0.38	24.69±8.65	18.01±0.47	16.95±0.84	77.70±5.58	10.03±0.86	7.12±0.34	4.97±0.40
6	30	57.34±4.78	6.40±0.44	27.55±1.53	19.24±0.88	18.18±0.75	81.11±5.32	9.44±0.77	6.64±0.69	5.38±0.39
7	22	57.42±5.01	6.88±0.54	29.57±3.41	19.55±1.04	17.77±1.18	78.52±9.99	10.67±0.98	7.55±0.55	5.66±0.39
8	40	65.60±6.17	6.78±0.48	29.80±2.99	18.08±0.74	17.57±0.98	85.38±7.44	9.82±1.17	6.89±0.63	5.28±0.48
9	30	54.10±4.52	6.65±0.52	30.54±1.88	19.44±0.98	17.35±1.18	86.47±9.11	9.74±1.22	6.42±1.25	5.67±0.41
10	30	51.40±2.57	6.62±0.34	28.23±1.76	19.33±0.68	18.37±0.69	81.27±4.94	9.16±0.88	6.49±0.47	5.43±0.38
11	30	56.49±3.83	6.82±0.92	29.99±2.67	18.76±0.93	17.54±1.05	92.51±8.08	10.32±0.88	7.04±0.60	5.52±0.44
12	30	55.06±2.81	6.34±0.48	29.25±1.58	18.94±0.66	17.60±0.73	84.13±6.91	9.86±0.75	7.09±0.72	5.39±0.37
13	30	53.51±5.09	6.63±0.51	29.72±2.75	19.24±1.68	17.90±1.52	86.83±9.59	10.22±0.72	6.90±0.68	5.58±0.45
14	30	55.75±3.62	6.42±0.49	29.85±2.36	18.76±1.32	17.46±0.90	78.70±9.26	10.10±1.17	7.09±0.47	5.61±0.45
15	27	56.93±5.87	6.92±0.53	29.47±2.34	19.16±1.08	17.92±0.89	81.22±7.63	10.71±1.47	7.15±0.69	5.65±0.48
16	5	52.19±0.48	7.10±0.75	30.74±1.47	19.71±0.72	18.49±0.81	80.29±2.20	10.40±0.88	7.34±0.40	6.08±0.33
17	30	53.53±5.48	6.60±0.53	27.52±2.09	18.54±0.99	17.21±0.79	80.00±9.82	9.06±0.96	6.44±0.61	5.63±0.53
18	17	51.95±5.60	6.42±0.63	29.58±1.69	18.74±0.99	18.00±0.64	80.04±8.13	9.21±0.82	6.40±0.50	5.61±0.44
19	19	52.78±5.53	6.51±0.70	27.69±2.33	19.19±0.85	17.98±1.02	77.38±9.72	9.27±0.79	6.54±0.49	5.47±0.34
20	23	57.56±4.38	6.24±0.70	28.44±2.76	19.23±1.29	18.01±1.25	83.44±7.14	10.65±0.92	7.19±0.50	5.59±0.60
Form-B
21	30	53.51±3.49	7.06±0.67	30.19±2.42	19.60±0.91	18.13±1.16	88.14± 6.10	10.97±0.92	7.24±0.61	5.85±0.39
22	30	56.12±4.68	6.18±0.51	27.52±2.66	18.30±0.95	17.03±1.22	81.32± 7.68	9.54±0.82	6.77±0.43	5.60±0.40
23	30	50.33±4.09	6.16±0.55	27.62±2.35	19.22±0.84	17.62±0.92	77.83± 9.34	9.33±0.84	6.46±0.36	6.24±2.52
24	30	57.28±3.25	6.34±0.52	26.82±1.82	18.53±1.06	17.27±1.07	75.03± 9.76	9.55±0.96	6.62±0.52	5.78±0.47
25	26	54.53±3.69	5.97±0.34	24.92±2.19	19.38±0.97	16.97±1.16	69.69±12.05	9.86±1.13	6.55±0.62	5.85±0.50
26	30	55.11±4.66	7.14±0.71	27.80±1.92	19.47±1.08	17.97±1.03	87.85± 9.20	10.17±0.90	7.29±0.50	6.01±0.48
27	30	55.15±2.73	6.57±0.57	29.50±1.82	19.38±0.96	18.19±0.94	72.37± 8.10	10.03±0.97	7.14±0.41	5.93±0.39
Form-C
28	20	48.85±5.60	6.63±0.55	26.08±1.60	19.35±1.07	17.69±0.96	84.48±10.40	8.43±0.76	6.36±0.56	6.28±0.56
29	30	47.83±3.86	6.31±0.64	27.78±2.53	20.01±1.60	18.47±1.34	75.90± 9.69	9.66±1.24	6.58±0.82	6.30±0.59

[1]Inter nostril width, [2]hindlimb length, [3]head length, [4]head width, [5]tail length, [6]femur length, [7]tibia length, [8]inter orbital length.

1). In order to investigate the level of reproductive isolation between two forms in this area, three diagnostic loci (*Gp-2, Sdh,* and *Got-2*) were analyzed (Table 6) and tested for departure from Hardy-Weinberg equilibrium by F-statistics (Table 7). In Wright's inbreeding coefficients (F_{IS}) (Wright, 1965) '1' indicates perfect random mating and '0' indicates perfect inbreeding. At Jangseong, the values of F_{IS} of *Sdh, Got-2,* and *Gp-2* between two forms were 0.487, 0.483, and 0.318, res-

pectively, and it suggests that they are deviated significantly ($P < 0.05$) from the expectation under random mating (Table 7).

Morphological comparisons

The color patterns of Form-A and Form-B are similar in general, that is, brown (dark brown) dorsal part with fine black speckles and, sides (excepting around
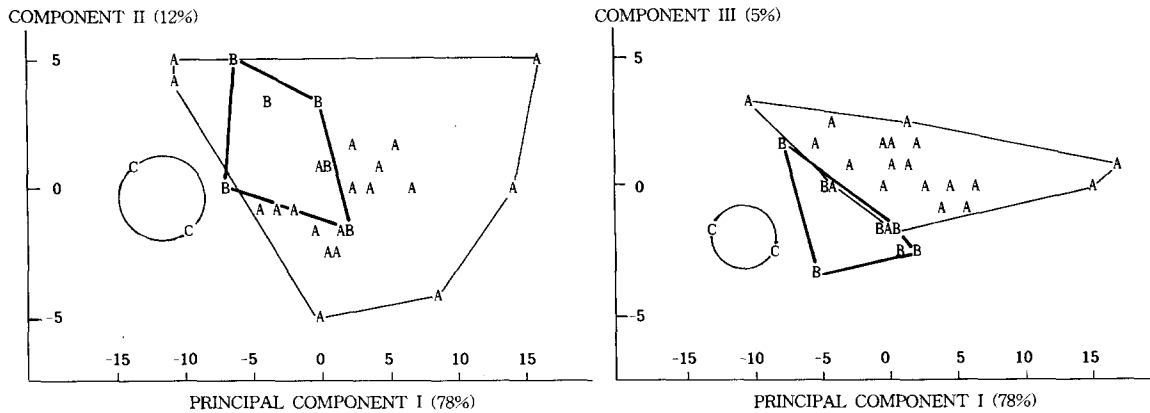
Fig. 4. Bivariate plots of the first three principal components for 13 morphological characters of 29 populations among three forms of the *H. leechii* complex. The proportion of the total variance accounted for by each component is indicated in parentheses. Operational genetic units are Form-A (A), Form-B (B), and Form-C (C).

Table 9. Comparisons of meristic characters (means ± SD) in the three forms of the *Hynobius leechii* complex

Form and locality	N	Vomerine teeth	Costal grooves	Vertebrae	Coccyges
Form-A					
1. Koseong	27	32.42±3.44	11.14±0.52	18.14±0.44	26.10±2.74
2. Inje	30	35.06±4.74	10.57±0.50	18.03±0.18	26.87±2.43
3. Paju	30	33.80±3.61	11.13±0.50	18.06±0.25	29.16±1.55
4. Yangpyeong	22	36.38±3.94	10.76±0.54	18.00±0.32	27.76±2.28
5. Myeongju	8	35.50±2.31	11.75±0.46	18.13±0.35	28.63±3.66
6. Samcheok	30	30.88±2.81	10.81±0.56	18.00±0.48	26.59±2.55
7. Jeongseon-O	22	33.50±3.78	10.30±0.47	18.25±0.55	27.90±2.07
8. Jeongseon-H	40	31.86±3.43	11.00±0.51	18.05±0.32	27.63±2.83
9. Tanyang	30	31.22±3.24	11.18±0.55	18.14±0.36	28.11±0.00
10. Uljin	30	33.36±2.95	10.43±0.63	17.97±0.32	27.47±2.32
11. Chungju	30	33.14±2.08	11.47±0.51	18.07±0.25	29.50±2.96
12. Yeongpung	30	31.60±2.39	11.10±0.48	17.97±0.18	29.10±2.73
13. Cheongju	30	33.60±3.08	11.60±0.67	18.14±0.44	30.03±4.06
14. Cheongyang	30	34.34±3.67	11.27±0.45	17.97±0.18	28.83±2.25
15. Sangju	27	33.24±3.40	11.07±0.59	18.04±0.33	28.93±3.18
16. Muju	5	33.20±1.10	11.20±0.45	18.00±0.00	29.60±2.41
17. Yeongcheon	30	33.78±3.17	11.31±0.47	18.46±0.51	28.89±1.64
18. Seongju	17	32.82±2.84	11.18±0.53	18.06±0.24	29.18±3.71
19. Kyeongju	19	34.42±2.45	11.84±0.37	18.21±0.54	28.63±3.48
20. Kurye	23	35.22±4.50	11.17±0.78	18.04±0.21	27.57±4.07
Form-B					
21. Puan	30	39.80±3.50	10.90±0.48	18.03±0.32	28.83±2.98
22. Keoje Isl.	30	38.54±3.40	10.83±0.59	18.00±0.37	26.30±2.85
23. Namhae Isl.	30	36.14±3.25	11.66±0.61	18.14±0.35	29.76±3.23
24. Haenam	30	38.80±4.16	10.97±0.49	18.13±0.35	26.03±2.72
25. Chindo Isl.	26	42.16±5.01	11.12±0.91	18.04±0.34	24.27±2.32
26. Cheju Isl.-C.	30	39.66±4.74	10.36±0.87	18.03±0.17	27.40±2.10
27. Cheju Isl.-S.	30	37.54±3.44	11.30±0.47	18.07±0.45	26.40±3.28
Form-C					
28. Kori-H.	20	35.60±3.72	10.87±0.52	17.93±0.26	25.60±1.92
29. Kori-K.	30	35.34±3.61	11.27±0.52	18.03±0.32	24.97±3.15

costal grooves) and underside are lighter than the dorsal part (Fig. 3-A, 3-B), whereas Form-C has light yellowish brown dorsal without fine speckles (Fig. 3-C).

For morpometric comparisons of the three genetic forms, a sum of 766 adult specimens from 29 populations were used, and significant differences among three forms were detected. Form-C had relatively shorter body (SVL) than Form-A and Form-B (see Table 8). By comparing percentage ratios of each character dimension relative to SVL, a significant difference (p<0.05) between Form-B and Form-C was detected on inter nostril width (INW: Table 10). In countable characters, the three forms did not differ in the number of costal grooves and the number of vertebrae, and most specimens had 11 costal groove and 18 vertebrae (Table 9). However, significant differences among the three forms were found in the number of vomerine teeth and the number of coccyges (Table 10).

Based on skeletal and external characters listed in Table 8 and Table 9, multivariate analysis for all variables

Table 10. The correlaton of statistically significant characters (t-Test) among the three forms of the *Hynobius leechii* complex

Characters	Paired forms		
	A - B	A - B	B - C
INW/SVL	1.19	1.86	2.68*
No. of vomerine teech	7.97**	1.92	2.47*
No. of coccyx	-1.87	-3.95**	-1.22

* P<0.05, ** P<0.001.

were performed. The first three principal components account for 95.1% of the total variation (Fig. 4). Factor loadings of each variables are not presented, but may be obtained upon request from the senior author. The variables most positively correlated with the first factor (correlation coefficient >0.60) are, in decreasing order of importance, Tail length (0.72) and Snout-vent length (0.63). This factor is interpreted as a body size component. The second factor is also defined mainly by Snout-vent length (0.71), and is also body size component. High loadings on the third factor are found for the number of coccyx (0.68). Two projections of 29 population of three forms onto the three principal-component axes seperate Form-C from the other forms (Fig. 4) because of its small body size (Table 8, Fig. 4a) and few coccyx number (Table 9, Fig. 4b). Form-B was also set apart from Form-A on the third axis mainly because of the relatively few coccyx (Table 9, Fig. 4b). Finally, combined morphological characteristics of the three forms are sufficiently divergent to make them distinct on one or two projections (Fig. 4).

Discussion

As a result of electrophoretic analysis, we confirmed that Korean salamander *H. leechii* Boulenger (1887) comprises of three genetically distinct forms which show considerable genetic differentiation. Five diagnostic loci between Form-A and Form-B, four diagnostic loci between Form-A and Form-C, and five diagnostic loci

between Form-B and Form-C were detected. The amount of genetic divergence among three forms (S_{A-B}=0.718, S_{A-C}=0.742, S_{B-C}=0.736) are interspecific level of differencies for the salamander species (Yang et al., 1982; Wake and Yanev, 1986; Matsui, 1987; Hayashi and Matsui, 1988). Divergent time estimation (Nei, 1975) of the three forms indicates that they diverged during 1.2-1.5 million years before present (MYBP).

Dobzhansky (1940) defined speciation as "... fixation of discontinuity among organisms. Discontinuity is maintained by isolating mechanisms that prevent the interbreeding of different adaptive complexes of genes." Since the separation of gene pools is the essence of species formation, a study of speciation must involve the examination of the level of reproductive isolation between the taxa. Electrophoretic analysis has been used extensively for such an examination at sympatry (Dowling and Moore, 1985). At sympatry, a significant deviation from random mating between Form-A and Form-B clearly indicates that isolating mechanism is operating. Two forms also differ in the number of vomerine teeth (Table 10) and their geographic distribution (Fig. 1). Therefore we conclude that they are two distinct taxa of specific rank.

Mori (1928) described the Cheju population, one of the Form-B, as a distinct subspecies of *Hynobius* (*H. leechii quelpatensis*), but Sato (1937, 1943) and Kang and Yoon (1975) did not admit its subspecific status and treated it as a mere local variety of *H. leechii*. Yang et al. (1982) and Uh et al. (1992), however, treated the Cheju population as distinct species based on the genetic difference although the studies were carried out with a limited number of specimens.

Since sympatry between Form-C and the other forms was not discovered, the level of reproductive isolation among them was not investigated. However, Mayr and Ashlock (1991) suggested a means of settling for such a taxonomic problem as "When direct proof is unavailable, it becomes nessary to decide the status of isolated populations by means of inference..... This amount of difference between good species can be used to determine the status of isolated populations in the same genus". Actually it has been well documented in numerous reports especially through the comparison of the level of genetic differentiation between taxa (Ayala, 1975; Avise, 1976; Yang et al., 1982, 1991, 1994; Matsui, 1987; Yang and Min, 1987; Hayashi and Matsui, 1988; Kim and Yang, 1995).

As the results mentioned above, since Form-C has distinct genetic differences, unique body color, morphological differences and restricted distribution from the other forms, it seems reasonable to assign this form as a new species.

Acknowledgements

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