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Morphometric Variation in the *pusillus* Group of the Genus *Rhinolophus* (Mammalia: Chiroptera: Rhinolophidae) in East Asia

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Based on 203 specimens belonging to the *Rhinolophus* "*pusillus* group" (Mammalia: Chiroptera: Rhinolophidae), univariate and multivariate morphometric analyses using 19 characters were performed to assess the confused species taxonomy. The results indicated that *R. pusillus* (including *calidus*, *parcus*, and *szechuanus*) in the continental region and Hainan Island of China and "*R. cornutus*" in Japan are morphologically divergent species. *Rhinolophus cornutus* should be further split into *R. cornutus* (including *orii*, *pumilus*, and *miyakonis*) in the main islands of Japan, the Amami and Okinawa Group of the central Ryukyu Archipelago, and Miyako Group of the southern Ryukyus; and *R. perditus* and *R. imaizumii* from the Yaeyama Group in the southern Ryukyus. *Rhinolophus monoceros* from Taiwan is morphologically more similar to species in Japan than to *R. pusillus*. In addition to *R. pusillus*, another form that is morphologically similar to species in Japan was recognized from Langzhong in Sichuan Province; this may represent an undescribed species, and further examination is necessary to determine its taxonomic status. Specimens from Guang'an in Sichuan Province, China, are also different from the others, and are characterized by the smallest skull size. Although further studies are required, these specimens were tentatively identified as *R. subbadius*.

Key words: Chiroptera, *Rhinolophus pusillus* group, taxonomy, East Asia, morphometrics

INTRODUCTION

Within the East Asian horseshoe bats belonging to the *Rhinolophus pusillus* group (Mammalia: Chiroptera: Rhinolophidae), several named forms are recognised (*R. pusillus*, *R. cornutus*, *R. pumilus*, *R. perditus*, *R. imaizumii*, and *R. monoceros*), however there is disagreement and confusion over the taxonomic status of each (review in Csorba et al., 2003). There have been numerous phylogenetic and taxonomic studies, but no consensus exists regarding their taxonomy (Hill and Yoshiyuki, 1980; Yoshiyuki, 1989, 1990; Bogdanowicz, 1992; Corbet and Hill, 1992; Koopman, 1994; Maeda, 1996; Csorba, 1997; Zhang, 1997; Csorba et al., 2003; Wang, 2003; Abe, 2005; Simmons, 2005; Li et al., 2006; Smith and Xie, 2008; Xu et al., 2008; Sano and Armstrong, 2009; Sun et al., 2009).

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Rhinolophus pusillus and *R. cornutus* were originally described by Temminck (1834) from Java and Japan, respectively (Csorba et al., 2003; Simmons, 2005). The populations on the main islands of Japan (Hokkaido, Honshu, Shikoku, Kyushu, and offshore islands) have been referred to *R. cornutus* sensu stricto (Csorba et al., 2003; Sano and Armstrong, 2009), while several species or subspecies were originally described from the Ryukyu Islands: *R. cornutus orii* from Tokunoshima Island in the Amami Group of the central Ryukyus (Kuroda, 1924), *R. cornutus pumilus* from Okinawajima Island in the Okinawa Group of the central Ryukyus (Andersen, 1905), *R. miyakonis* from Miyako Island in the Miyako Group of the southern Ryukyus (Kuroda, 1924), *R. perditus* from Ishigaki Island in the Yaeyama Group of the southern Ryukyus (Andersen, 1918), and *R. imaizumii* from Iriomote Island of the Yaeyama Group (Hill and Yoshiyuki, 1980). Taxonomic arrangements of these Japanese species are variable, and two to four species with different combinations have been recognized. Yoshiyuki (1989) recognized four species, *R. cornutus* (including *orii* as a subspecies), *R. pumilus* (including *miyakonis* as a subspecies), *R. perditus*, and *R. imaizumii*. Csorba et al. (2003) and Simmons (2005) recognized two species, *R. cornutus* (including *orii*, *pumilus*, *miyakonis*, and *perditus* as junior synonyms or subspecies) and *R. imaizumii*, while Sano and Armstrong (2009) recognized three species, *R. cornutus* (including *orii* as a junior synonym), *R. pumilus* (including *miyakonis* as a junior synonym), and *R. perditus* (including *imaizumii* as a junior synonym).

The species *R. pusillus* is thought to be distributed in China, and *szechuanus* from Chunking (Andersen, 1918), *calidus* from Yenping, Fujian (Allen, 1923), and *parcus* from Nodoa, Hainan Island (Allen, 1928) have been considered junior synonyms or subspecies within China (Csorba et al., 2003; Simmons, 2005). Corbet and Hill (1992) suggested that *pusillus* and *cornutus* are conspecific, and Simmons (2005) citing Corbet and Hill (1992) suggested that *R. pusillus* may include *cornutus*, *pumilus*, and *perditus*. In addition, *R. monoceros* is endemic to Taiwan (Andersen, 1905). Recently, Li et al. (2006), Xu et al. (2008), and Sun et al. (2009) used mitochondrial cytochrome *b* gene sequences to reconstruct the phylogenetic relationships of the *pusillus* group from China and Japan, and suggested that *R. monoceros*, *R. pusillus*, and *R. cornutus* are a monophyletic group forming a single species.

Given the prevailing level of confusion on the taxonomic status of the various named forms throughout China and Japan, and the lack of a comprehensive morphological assessment that has included all of these, we conducted a morphometric study of skull characters to examine the variation within the group, and make comments regarding taxonomy.

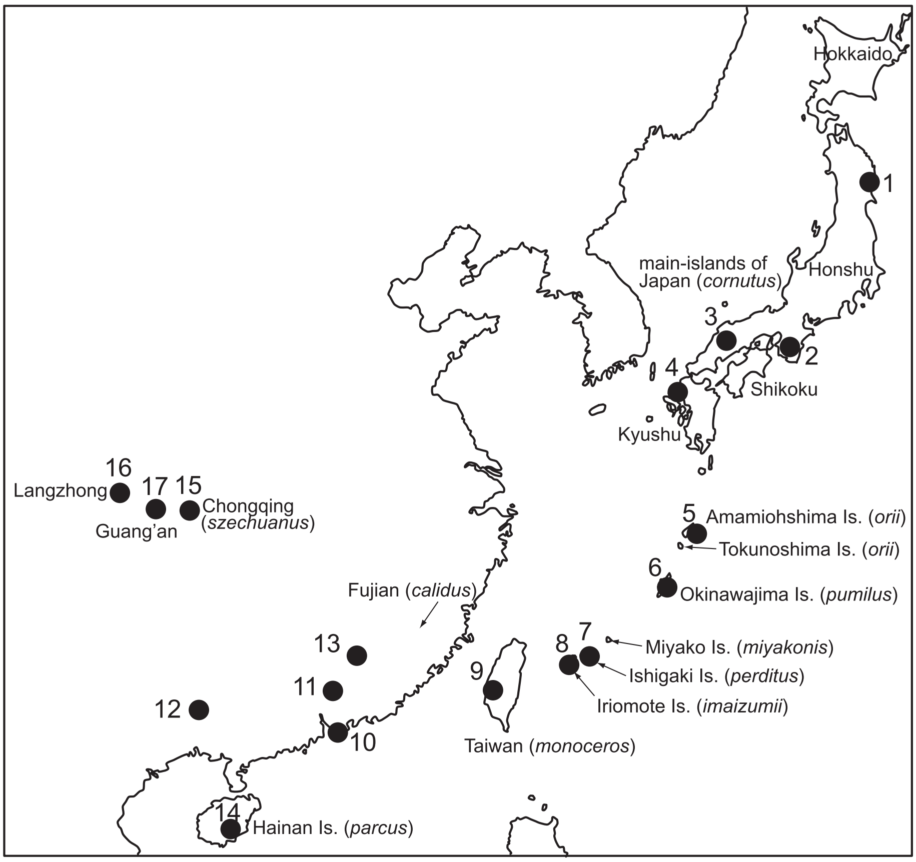

Fig. 1. Map of East Asia showing sampling localities of the *pusillus* group used in this study.

MATERIALS AND METHODS

All 203 specimens used in this study have been deposited at Guangzhou University, Guangzhou, China (GZHU); China West Normal University, Sichuan, China (CWNU); Guangdong Entomological Institute, Guangdong Academy of Science, Guangzhou, China (GEI); Kyoto University Museum, Kyoto, Japan (KUZ); Graduate School of Medicine, Osaka City University, Osaka, Japan (OCU); and National Museum of Nature and Science, Tokyo, Japan (NSMT). The specimens examined in this study were as follows (asterisks indicate specimens used in the principal component analyses) (Fig. 1): *R. cornutus* (n = 40), 1 Kesennuma, Miyagi Pref. (OCU 8586* through 8595*); 2 Ryujin, Wakayama Pref. (OCU 8568*, 8571*, 8573* through 8580*); 3 Tojyo, Hiroshima Pref. (OCU 4771* through 4776*, 5016* through 5018*, 5020*); 4 Ibarayama, Maebaru City, Fukuoka Pref. (OCU 5223* through 5228*, 5230*, 5232*, 5234*, 5238*); 5 *R. cornutus orii* (n = 11) from Naze and Tatsugo, Amamiohshima Island, Kagoshima Pref. (OCU 6112*, 6115*, 6120* through 6122*, 8969, 8977*, 8980*, 8982*, 8983*, KUZ M4946*); 6 *R. pumilus* (n = 11) from Gushito, Okinawajima Island, Okinawa Pref. (KUZ M4947*, OCU 8689*, 8691* through 8699*); 7 *R. perditus* (n = 16) from Ishigaki Island, Okinawa Pref. (NSMT 3540*, 3653*, 3654*, 18087*, 18088, 18089, 18090*, 18092, 23948*, 23952*, 24236*, 24243*, 33318* through 33320*, 33323*); 8 *R. imaizumii* (n = 12) from Iriomote Island, Okinawa Pref. (OCU 6023* through 6025*, 6027*, 6037*, NSMT 24246*, 24253*, 24261*, 24268*, 25259*, KUZ M4948*, 4949*); 9 *R. monoceros* (n = 10) from Nantou, Taiwan (OCU T12* through T18*, T20*, T22*, T23*); 10 *R. pusillus* (n = 77) from Hong Kong (GZHU 0443*, 0444*, 0453*, 0454*); 11 Yingde and Longmen, Guangdong Province (GZHU 99064*, 99065*, 99121*, 99122, 99123, 99137*, 99138*, 00167, 00202*, 01004*, 01006*, 01007*, 01056*, 03006*, 0411* through 0416*, 0419* though 0423*, 0428*, 0429*); 12 Guangxi Province (GEI 9912098*, 9912248*, GZHU 04238*, 04240* through 04242*, 4244, 04245*, 04247* through 04249*); 13 Xiaoping, Jiangxi Province (GEI 3133*, 3135* through 3139*, 3143*, 3145*, 3146*, 3150* through 3152*); 14 Lingshui, Hainan Province (GZHU 04173*, 04174*, 04177*, 04180*, 08015* through 08024*); 15 Wanxian, Chongqing City (GZHU 5020* through 5026*, 5028*, 5029*). 16 Langzhong population (see discussion, n = 16) from Langzhong (CWNU 90072* through 90078*, 90079, 90080*, 90083*, 90085*) and Mianyang (GZHU 07029* through 07031*, 003* through 005*), Sichuan Province. 17 Guang'an population (see discussion, n = 10) from Guang'an, Sichuan (CWNU 13, 14*, 15, 17*, 19* through 24*).

Specimens were carefully identified based on their external and cranial characters following Csorba et al. (2003). Specimens of *miyakonis* were restricted to two specimens used in the original description by Kuroda (1924), which were destroyed by fire in 1945 (Yoshiyuki, 1989; Maeda, 1996; Motokawa and Maeda, 2002).

Therefore, we could not examine specimens of *miyakonis* in the present study.

Nineteen cranial and dental measurements were taken by the senior author using a digital caliper to the nearest 0.01 mm as follows: GSL, greatest skull length; CCL, length from the front of canines to occiput; CH, cranium height; RH, rostral height; CB, cranial breadth; MB, mastoid breadth of the skull; ZW, width of the skull between zygomata; IOB, width of the interorbital constriction; TBB, tympanic bulla breadth; COL, cochlea length; PBL, palatal bridge length; C1M3L, crown length from the upper canine to the third molar; M1M3L, upper tooth row length between M1 and M3; CCW, width of the rostrum between the outer margins of the crown of canines; M3M3W, width of the rostrum between the outer margins of the crown of the third upper molar; c1m3L, crown length from the lower canine to the third molar; m1m3L, lower tooth row length between m1 and m3; DL, length of the mandible between the hindermost portion of the articular process and anterior-most edge of first incisor alveolus; and RAP, distance from the ramus to the angular tip. Measurements except for CCW followed the definition by Armstrong (2002).

Principal component analysis (PCA) was performed with the PRINCOMP procedure of SAS version 8 (SAS Institute Inc., 1990) based on the correlation matrix of 19 cranial measurements. The measurements were log-transformed, and specimens with missing values were excluded from the PCA. Different pairs among samples from Japan and Taiwan, and among samples of *R. pusillus* (*calidus*, *parcus*, *szechuanus*) were examined by ANOVA and Tukey's test ($P < 0.05$).

RESULTS

Cranial measurements for a total of 203 specimens are listed in Tables 1 and 2. Overall skull size, represented by GSL and CCL, was greater for *imaizumii* than the other specimens, and its ranges overlapped only with those of *perditus*. Similar trends were also observed in other measurements, such as RH, ZW, PBL, M1M3L, CCW, and M3M3W. The values of C1M3L and DL were greater for *imaizumii* than for the other specimens, with no range overlaps.

Specimens from Guang'an showed the smallest values of CCL and ZW among the samples examined. The range of CCL in the Guang'an population overlapped only with

Table 1. Cranial measurements (mm) of the *pusillus* group from Japan. Values are given as means ± SD, followed by sample sizes in parentheses in the upper column and the ranges in the lower column. See text for character abbreviations.

Character	*cornutus* 1–4	*orii* 5	*pumilus* 6	*perditus* 7	*imaizumi* 8
GSL	16.08 ± 0.22 (41)	15.75 ± 0.23 (11)	15.84 ± 0.23 (11)	16.38 ± 0.38 (16)	17.47 ± 0.23 (12)
	15.47–16.52	15.41–16.10	15.55–16.30	15.81–17.33	17.17–17.87
CCL	14.23 ± 0.22 (41)	13.75 ± 0.21 (11)	13.86 ± 0.16 (11)	14.46 ± 0.35 (16)	15.44 ± 0.15 (12)
	13.61–14.69	13.22–13.94	13.61–14.03	14.03–15.35	15.10–15.68
CH	6.30 ± 0.12 (41)	6.19 ± 0.12 (11)	6.27 ± 0.07 (11)	6.20 ± 0.21 (16)	6.68 ± 0.30 (12)
	6.04–6.72	5.93–6.34	6.14–6.35	5.93–6.66	6.18–7.11
RH	4.87 ± 0.16 (41)	4.74 ± 0.18 (11)	4.89 ± 0.14 (11)	5.14 ± 0.20 (16)	5.56 ± 0.14 (12)
	4.44–5.14	4.52–5.06	4.64–5.13	4.92–5.54	5.32–5.85
CB	6.62 ± 0.20 (41)	6.38 ± 0.17 (11)	6.51 ± 0.22 (11)	6.38 ± 0.16 (16)	6.74 ± 0.24 (12)
	6.28–7.10	6.12–6.75	6.21–6.83	5.91–6.60	6.37–7.08
MB	7.90 ± 0.13 (41)	7.59 ± 0.08 (11)	7.68 ± 0.07 (11)	7.94 ± 0.15 (16)	8.28 ± 0.13 (12)
	7.51–8.14	7.48–7.77	7.57–7.80	7.59–8.28	8.03–8.46
ZW	7.66 ± 0.14 (41)	7.38 ± 0.15 (11)	7.72 ± 0.05 (11)	8.09 ± 0.29 (16)	8.48 ± 0.14 (12)
	7.30–7.96	7.15–7.57	7.64–7.80	7.60–8.63	8.30–8.72
IOB	2.28 ± 0.10 (41)	2.05 ± 0.07 (11)	2.05 ± 0.09 (11)	2.25 ± 0.11 (16)	2.24 ± 0.09 (12)
	2.08–2.44	1.93–2.15	1.86–2.20	2.05–2.46	2.10–2.43
TBB	7.79 ± 0.15 (41)	7.55 ± 0.09 (11)	7.55 ± 0.14 (11)	7.52 ± 0.15 (15)	7.92 ± 0.33 (12)
	7.51–8.15	7.41–7.70	7.37–7.76	7.31–7.78	7.46–8.35
COL	2.90 ± 0.08 (41)	2.95 ± 0.09 (11)	2.95 ± 0.09 (11)	2.97 ± 0.10 (15)	3.06 ± 0.09 (12)
	2.71–3.06	2.84–3.08	2.84–3.15	2.85–3.21	2.94–3.23
PBL	4.98 ± 0.19 (41)	4.80 ± 0.13 (11)	4.81 ± 0.12 (11)	5.23 ± 0.33 (15)	5.60 ± 0.14 (12)
	4.59–5.34	4.48–4.95	4.60–5.02	4.66–5.95	5.38–5.87
C1M3L	5.76 ± 0.10 (41)	5.58 ± 0.10 (11)	5.64 ± 0.08 (11)	6.07 ± 0.25 (16)	6.59 ± 0.09 (12)
	5.54–5.99	5.35–5.72	5.49–5.76	5.47–6.33	6.48–6.72
M1M3L	3.44 ± 0.12 (41)	3.38 ± 0.08 (11)	3.36 ± 0.10 (11)	3.63 ± 0.15 (16)	3.94 ± 0.13 (12)
	3.20–3.63	3.28–3.52	3.19–3.51	3.36–3.89	3.76–4.20
CCW	3.71 ± 0.11 (41)	3.62 ± 0.12 (11)	3.97 ± 0.12 (11)	3.86 ± 0.37 (16)	4.33 ± 0.13 (12)
	3.50–3.95	3.49–3.82	3.78–4.12	3.04–4.35	4.15–4.60
M3M3W	5.74 ± 0.11 (41)	5.54 ± 0.10 (11)	5.62 ± 0.11 (11)	5.92 ± 0.32 (16)	6.43 ± 0.16 (12)
	5.55–5.91	5.40–5.73	5.48–5.84	5.18–6.29	6.12–6.60
c1m3L	5.97 ± 0.13 (41)	5.88 ± 0.11 (11)	6.08 ± 0.22 (11)	6.24 ± 0.37 (16)	6.93 ± 0.26 (12)
	5.58–6.20	5.77–6.17	5.78–6.66	5.66–6.96	6.61–7.48
m1m3L	3.87 ± 0.14 (41)	3.78 ± 0.12 (11)	3.84 ± 0.13 (11)	4.03 ± 0.23 (16)	4.41 ± 0.14 (12)
	3.57–4.17	3.59–4.04	3.67–4.09	3.64–4.34	4.21–4.70
DL	10.17 ± 0.24 (41)	9.85 ± 0.24 (11)	10.22 ± 0.24 (11)	10.71 ± 0.45 (16)	11.55 ± 0.12 (12)
	9.40–10.92	9.33–10.11	9.71–10.51	9.79–11.27	11.31–11.68
RAP	3.01 ± 0.18 (41)	2.91 ± 0.16 (10)	3.05 ± 0.15 (10)	3.23 ± 0.19 (16)	3.52 ± 0.27 (12)
	2.77–3.50	2.52–3.08	2.90–3.42	3.01–3.60	3.02–3.80

Table 2. Cranial measurements (mm) of the *pusillus* group from China. Values are given as means ± SD, followed by sample sizes in parentheses in the upper column and the ranges in the lower column. See text for character abbreviations.

Character	*monoceros* 9	*calidus* 10–13	*parcus* 14	*szechwanus* 15	Langzhong population 16	Guang'an population 17
GSL	15.26 ± 0.16 (10)	15.46 ± 0.36 (54)	15.29 ± 0.17 (14)	15.46 ± 0.25 (9)	15.47 ± 0.31 (16)	14.42 ± 0.41 (10)
	15.04–15.56	14.76–16.31	15.01–15.49	15.05–15.81	15.03–16.09	13.56–14.91
CCL	13.36 ± 0.17 (10)	13.66 ± 0.41 (53)	13.48 ± 0.20 (14)	13.76 ± 0.23 (9)	13.48 ± 0.20 (15)	12.48 ± 0.33 (10)
	13.12–13.67	12.52–14.33	13.21–13.84	13.48–14.19	13.2–13.76	11.95–13.00
CH	6.08 ± 0.07 (10)	6.68 ± 0.20 (54)	6.66 ± 0.13 (14)	6.65 ± 0.18 (9)	5.95 ± 0.19 (16)	5.52 ± 0.15 (10)
	6.01–6.18	6.25–7.07	6.45–6.93	6.45–6.88	5.49–6.33	5.22–5.70
RH	4.80 ± 0.22 (10)	4.31 ± 0.21 (54)	4.26 ± 0.11 (14)	4.33 ± 0.11 (9)	4.81 ± 0.20 (16)	4.39 ± 0.12 (8)
	4.56–5.37	3.95–4.78	4.13–4.59	4.14–4.48	4.48–5.25	4.19–4.57
CB	6.45 ± 0.16 (10)	6.21 ± 0.28 (54)	6.08 ± 0.09 (14)	6.30 ± 0.21 (9)	6.33 ± 0.35 (16)	5.81 ± 0.21 (10)
	6.15–6.70	5.58–6.71	5.82–6.21	6.02–6.62	5.54–6.84	5.49–6.19
MB	7.35 ± 0.09 (10)	7.62 ± 0.22 (54)	7.44 ± 0.14 (14)	7.55 ± 0.15 (9)	7.42 ± 0.18 (16)	6.94 ± 0.17 (10)
	7.22–7.55	7.17–8.10	7.19–7.66	7.42–7.73	7.14–7.71	6.66–7.13
ZW	7.40 ± 0.12 (10)	7.51 ± 0.23 (54)	7.36 ± 0.14 (14)	7.37 ± 0.11 (9)	7.21 ± 0.23 (16)	6.43 ± 0.25 (10)
	7.21–7.58	7.08–8.02	7.15–7.62	7.28–7.53	6.85–7.60	6.05–6.66
IOB	2.17 ± 0.10 (10)	2.27 ± 0.14 (54)	2.17 ± 0.12 (14)	2.16 ± 0.06 (9)	2.13 ± 0.15 (16)	1.99 ± 0.13 (10)
	2.07–2.38	1.92–2.64	1.96–2.33	2.08–2.24	1.83–2.42	1.87–2.23
TBB	7.33 ± 0.10 (10)	7.35 ± 0.30 (54)	7.25 ± 0.12 (14)	7.38 ± 0.12 (9)	7.30 ± 0.18 (16)	6.89 ± 0.19 (10)
	7.15–7.45	6.64–7.86	7.08–7.47	7.29–7.62	7.06–7.65	6.58–7.14
COL	2.83 ± 0.12 (10)	3.10 ± 0.14 (54)	3.16 ± 0.05 (14)	3.10 ± 0.13 (9)	2.90 ± 0.09 (16)	2.71 ± 0.17 (10)
	2.51–2.97	2.80–3.38	3.06–3.22	2.95–3.40	2.76–3.10	2.49–2.97
PBL	4.49 ± 0.21 (10)	4.23 ± 0.31 (54)	4.35 ± 0.18 (14)	4.25 ± 0.23 (9)	4.35 ± 0.20 (16)	4.28 ± 0.21 (10)
	4.24–4.91	3.54–5.13	4.10–4.70	3.85–4.58	3.98–4.73	4.00–4.71
C1M3L	5.56 ± 0.14 (10)	5.52 ± 0.20 (54)	5.55 ± 0.10 (14)	5.59 ± 0.14 (9)	5.57 ± 0.12 (16)	5.15 ± 0.12 (10)
	5.42–5.79	4.95–5.96	5.41–5.85	5.44–5.80	5.27–5.81	5.03–5.33
M1M3L	3.44 ± 0.09 (10)	3.38 ± 0.14 (54)	3.39 ± 0.07 (14)	3.35 ± 0.07 (9)	3.36 ± 0.18 (16)	2.91 ± 0.11 (10)
	3.27–3.55	3.15–3.71	3.29–3.49	3.24–3.45	3.01–3.75	2.76–3.13
CCW	3.59 ± 0.11 (10)	3.32 ± 0.17 (54)	3.61 ± 0.19 (14)	3.39 ± 0.14 (9)	3.48 ± 0.19 (16)	2.77 ± 0.15 (10)
	3.48–3.84	3.03–3.61	3.25–3.84	3.12–3.58	3.12–3.96	2.55–3.00
M3M3W	5.56 ± 0.15 (10)	5.60 ± 0.18 (54)	5.58 ± 0.12 (14)	5.51 ± 0.12 (9)	5.43 ± 0.19 (16)	4.66 ± 0.21 (10)
	5.27–5.76	5.23–5.94	5.37–5.85	5.31–5.70	4.94–5.71	4.34–4.92
c1m3L	5.66 ± 0.17 (10)	5.68 ± 0.15 (54)	5.85 ± 0.15 (14)	5.69 ± 0.14 (9)	5.82 ± 0.10 (16)	5.47 ± 0.17 (10)
	5.44–5.97	5.27–6.06	5.58–6.11	5.62–5.92	5.59–5.98	5.24–5.78
m1m3L	3.79 ± 0.12 (10)	3.91 ± 0.17 (54)	3.78 ± 0.11 (14)	3.88 ± 0.12 (9)	3.79 ± 0.22 (16)	3.56 ± 0.12 (10)
	3.60–3.97	3.61–4.34	3.62–3.92	3.66–4.08	3.39–4.15	3.32–3.72
DL	9.48 ± 0.28 (10)	9.99 ± 0.32 (54)	9.69 ± 0.28 (14)	9.86 ± 0.16 (9)	9.77 ± 0.27 (16)	9.14 ± 0.20 (10)
	9.06–9.95	9.44–10.78	9.22–10.19	9.63–10.10	9.06–10.32	8.88–9.43
RAP	2.76 ± 0.21 (10)	3.14 ± 0.24 (52)	3.02 ± 0.18 (14)	3.18 ± 0.09 (9)	2.76 ± 0.10 (16)	2.44 ± 0.15 (10)
	2.42–3.13	2.45–3.59	2.73–3.33	3.03–3.29	2.65–2.94	2.15–2.69

calidus, and ZW did not overlap with the other specimens.

The cranium height (CH) and cochlea length (COL) of *R. pusillus* (*calidus*, *parcus*, and *szechuanus*) tended to be larger than those of the Japanese specimens (*cornutus*, *orii*, *pumilus*, *perditus*, *imaizumii*) and *monoceros*, while the palatal bridge length (PBL) and the crown length from the lower canine to the third molar (c1m3L) were smaller in the former than in the latter.

The results of the PCA are shown in Tables 3 and 4. The first three principal component axes explained 57.7%, 13.1%, and 5.5% of the total variation, respectively. In the first axis, all the variables showed similar positive loading. CH (positive) and COL (positive) in the second axis and IOB (positive) and TBB (positive) in the third axis indicated relatively large loading. In the PC1 axis, greater values were found in *imaizumii*, with intermediate values in *perditus*, followed by *cornutus*, *pumilus*, *orii*, *pusillus* (*calidus*, *szechuanus*, *parcus*), *monoceros*, and the Langzhong population. The Guang'an population showed the smallest values and was completely separated from the other samples. In the second axis, the values were positive in the *pusillus* group (*calidus*, *parcus*, *szechuanus*) and were distinct from the others (*cornutus*, *orii*, *pumilus*, *perditus*, *imaizumii*, *monoceros*, the Langzhong population, and the Guang'an population), which had negative mean values.

Due to these differences, *pusillus* (*calidus*, *parcus*, *szechuanus*), *imaizumii*, the Guang'an population, and others (*cornutus*, *orii*, *pumilus*, *perditus*, *monoceros*, and the Langzhong population) were separated from each other in the scatterplots of the first and second axes (Fig. 2). Three samples of *pusillus* showed extensive overlap, and significant differences in PC3 scores were found only between *calidus* and *parcus* (Table 4). A range of differentiation as well as overlap in the PC3 score was observed among the last group (*cornutus*, *orii*, *pumilus*, *perditus*, *monoceros*, and the Langzhong population). The patterns of differentiation were attributable to overall size differences as represented by PC1 and proportional differences between *cornutus* versus *orii*, *pumilus*, and *perditus* as represented by PC3 (Table 4).

DISCUSSION

In the present study, 77 specimens from mainland China (Fig. 1; 10–13, 15) and Hainan Island (14) are distinguished from the other specimens in having positive and greater values in the second axis of PCA, as well as greater values in CH and COL. Based on these clear distinct morphological characters, we consider that *R. pusillus* is found in mainland China and Hainan Island, and clearly different from *R. monoceros* from Taiwan and species from Japan.

In China, Wang (2003) and Smith and Xie (2008) recognized four subspecies of *R. pusillus*: *R. pusillus szechuanus* Anderson, 1918 distributed in Xizang, Sichuan, Guizhou, and Hubei; *R. pusillus calidus* Allen, 1923 in Fujian, Guangdong, Guizhou, and Guangxi; *R. pusillus parcus* Allen, 1928 on Hainan Island; and *R. pusillus lakkhanae* Yoshiyuki, 1990 in Yunnan. In the present study, specimens of the former three subspecies were examined: *R. pusillus szechuanus* from Wanxian (= Wanhsien), Chongqing (type locality, n = 9); *R. pusillus calidus* from Hong Kong, Guangdong, Guangxi, and Jiangxi (n = 54); and *R. pusillus parcus* from Hainan Island (n = 14). As no morphometric differences were detected among these three subspecies, and *szechuanus* and *calidus* were nested within the score range of *parcus* in the first two principal component axes, we suggest that the differences among these three subspecies are negligible. This view is also in agreement with the previous report of Li et al. (2006) discussing low differentiation and overlap among *R. pusillus* subspecies in mitochondrial DNA sequences and echolocation call frequencies. Although the

Table 3. Eigenvectors of the first three principal component axes based on cranial characters. See text for character abbreviations.

Character	PC1	PC2	PC3
GSL	0.286	–0.062	–0.029
CCL	0.286	–0.007	0.001
CH	0.121	0.507	0.046
RH	0.209	–0.374	–0.101
CB	0.199	–0.127	0.357
MB	0.277	0.056	0.210
ZW	0.281	0.090	0.010
IOB	0.107	0.279	0.628
TBB	0.213	–0.084	0.382
COL	0.067	0.481	–0.313
PBL	0.222	–0.305	0.022
C1M3L	0.272	–0.067	–0.125
M1M3L	0.214	0.037	–0.050
CCW	0.254	–0.125	–0.091
M3M3W	0.273	0.106	–0.019
c1m3L	0.237	–0.149	–0.191
m1m3L	0.217	0.146	–0.266
DL	0.268	0.016	–0.198
RAP	0.194	0.293	–0.063
Eigenvalue	10.968	2.487	1.048
Proportion	0.577	0.131	0.055

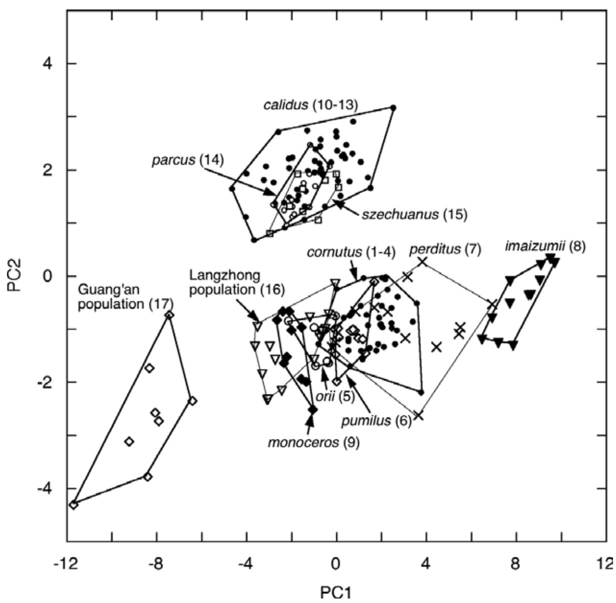

Fig. 2. Scatterplots of scores on the first two principal component axes based on cranial characters.

Table 4. Mean, minimum, and maximum values of the first three principal component scores for each sample. Different pairs among samples from Japan and Taiwan, and among samples of *R. pusillus* (*calidus*, *parcus*, *szechuanus*) were examined by ANOVA and Tukey's test (P < 0.05).

Sample	PC1			PC2			PC3		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
cornutus 1–4 [c]	1.631	–0.791	3.779	–0.904	–2.196	–0.023	1.088	0.055	1.854
*orii*5 [o]	–0.698	–2.117	–0.037	–1.140	–1.699	–0.733	–0.355	–1.343	0.387
pumilus 6 [u]	0.671	–0.040	1.673	–1.142	–1.981	–0.106	–0.614	–2.098	0.679
perditus 7 [e]	2.873	–0.286	6.925	–0.926	–2.620	0.275	–0.421	–2.761	2.219
imaizumii 8 [i]	8.198	6.463	9.708	–0.432	–1.311	0.326	–1.139	–1.736	–0.412
monoceros 9 [m]	–1.926	–2.650	–1.049	–1.373	–2.513	–0.662	0.367	–0.717	1.328
Differences	i > ecuom, e > uom, c > om, u > m				i > m			c > oeui, m > i	
calidus 10–13 [c]	–1.043	–4.634	2.538	1.945	0.671	3.161	0.050	–2.323	2.447
parcus 14 [p]	–1.713	–2.799	–0.285	1.500	0.954	2.462	–0.756	–1.861	0.167
szechuanus 15 [s]	–1.233	–2.978	0.084	1.485	0.801	1.925	–0.330	–1.306	0.366
Differences		None			None			c > p	
Langzhong 16	–1.946	–3.634	–0.065	–1.351	–2.337	–0.145	–0.222	–2.207	1.598
Guang'an 17	–8.442	–11.710	–6.418	–2.664	–4.304	–0.732	–0.372	–1.748	0.862

further comparison with *R. pusillus* specimens from Java which is the type locality (Csorba et al., 2003) is necessary, we recognize no subspecies within *R. pusillus* in China.

For specimens from Japan, our PCA results (Fig. 2) showed that *imaizumii* is quite distinct from the others in having greater values on the first axis, while the plots for *cornutus*, *orii*, and *pumilus* overlapped with each other, showing smaller values in the first axis. The first axis of PCA is interpreted to represent the overall size, because all the characters showed positive loadings for PC1 (Table 3). Yoshiyuki (1989) suggested presence of a cline in *R. cornutus* sensu strict for overall size increase from south to north. In contrast, there was no such clinal change among four localities (1–4) both in PC1 and GSL in the present study, and *orii* had smaller overall size (PC1 and GSL) than *cornutus* as pointed out by Yoshiyuki (1989). An interesting pattern was found in the plots of *perditus* specimens from Ishigaki Island, with values between those of the *imaizumii* plots and the *cornutus–orii–pumilus* plots, with wide ranges in both the first and second axes.

Rhinolophus pumilus was originally described by Andersen (1905) from Okinawajima Island in the Okinawa Group of the central Ryukyus. Two main views were held regarding its taxonomic status: i.e., *R. pumilus* was suggested to be a valid full species (including *miyakonis* as a junior synonym or subspecies) (Yoshiyuki, 1989; Abe, 2005; Sano and Armstrong, 2009) distributed on the islands of Okinawajima, Iheya, Tokashiki, and Kume in the Okinawa Group and the islands of Miyako and Irabu of the Miyako Group in the southern Ryukyus (Sano and Armstrong, 2009), or a subspecies or junior synonym of *R. cornutus* (Hill and Yoshiyuki, 1980; Corbet and Hill, 1992; Csorba et al., 2003; Simmons, 2005). Because the present study did not support the morphological differentiation between *R. pumilus* and *R. cornutus*, we consider *R. pumilus* together with *miyakonis* as junior synonyms of *R. cornutus*. We also consider *orii* distributed on the islands of Amamiohshima, Kakeroma, Tokunoshima, and Okinoerabujima in the Amami Group of the central Ryukyus to be a junior synonym of *R. cornutus*, following most authors who also consider it as a junior synonym (Corbet and Hill, 1992; Csorba et al., 2003; Abe, 2005; Sano and Armstrong, 2009) or subspecies (Hill and Yoshiyuki, 1980; Yoshiyuki, 1989; Simmons, 2005) of *R. cornutus* with no evidence of divergence.

Hill and Yoshiyuki (1980) described *R. imaizumii* as a new species from Iriomote Island in the Yayema Group of the southern Ryukyus. This species is morphologically distinct from *R. cornutus* (including *pumilus*) as well as from *R. monoceros* in its larger size and several other characters. Therefore, Yoshiyuki (1989), Csorba et al. (2003), and Simmons (2005) recognized *R. imaizumii* as a valid species, although Sano and Armstrong (2009) considered *imaizumii* to be a junior synonym of *R. perditus* originally described from Ishigaki Island in the Yaeyama Group. As *perditus* has been considered a valid species endemic to Ishigaki Island (Yoshiyuki, 1989), a valid species endemic to Ishigaki and Iriomote islands (*imaizumii* to be a junior synonym: Abe, 2005; Sano and Armstrong, 2009), or a junior synonym of *R. cornutus* together with *pumilus* (Hill and Yoshiyuki, 1980; Csorba et al., 2003; Simmons, 2005), its taxonomic status is still problematic. In the present study, the plots of *perditus* were intermediate in position between those of the *imaizumii* and *cornutus–orii–pumilus* clusters. The Ishigaki Island *perditus* is distinct from *R. pumilus* in the Okinawa Group in skull morphology (the present study), as well as echolocation call characteristics (Sano and Armstrong, 2009). Although similarities in morphology and echolocation call characteristics with the Iriomote Island population (i.e., *imaizumii*) have been reported (Sano and Armstrong, 2009), the present study found differences between specimens from Ishigaki and Iriomote islands, which are separated only by about 20 km of ocean. Therefore, we recognize both species as valid: *R. perditus* on Ishigaki Island and *R. imaizumii* on Iriomote Island. As the plot range of *R. perditus* is somewhat wider in PCA on the first two axes, the Ishigaki Island population may consist of two species due to migration of *R. imaizumii* from Iriomote Island in the west and *R. cornutus* from the east and north; but this scenario seems to be less plausible. Future detailed genetic studies should be explored for the divergence of *R. cornutus* (including *orii* and *pumilus*), *R. perditus*, and *R. imaizumii* in the central and southern Ryukyus.

Andersen (1905) described *R. monoceros* as an insular endemic species distributed in Taiwan, and it is differentiated from *R. cornutus* by the shape of the lancet in the nose leaf. However, the shape of the lancet in the nose leaf of *R. cornutus* shows variation between different individuals and populations, and therefore, several authors have suggested that *R. monoceros* may be conspecific with *R. cornutus* or *R. pusillus* (Corbet and Hill, 1992; Koopman, 1994; Csorba, 1997). According to the PCA in the present study, *R. monoceros* plots were close to those of *orii* (= *R. cornutus*), but with little overlap. As the distribution of *R. monoceros* is far from that of *orii* and extensive morphometric differentiation was found in *R. monoceros* compared to the geographically closer species *R. imaizumii* and *R. perditus*, we suggest that *R. monoceros* is a distinct insular endemic species.

Specimens from Langzhong in Sichuan Province are distinct from Chinese *R. pusillus* in having lower PC2 scores and lying close to the plots of *R. monoceros*, and *R. cornutus* (including *orii* and *pumilus*). In addition to *R. blythi* (= *R. pusillus*), Allen (1938) listed *R. cornutus pumilus* as distributed in China based on previous records (Andersen, 1905; Thomas, 1911, 1912; Mell, 1922) from Foochow (= Fuzhou, Fujian Province), Kiatingfu (Sichuan Province), Penhsien (35 km north of Chengdu, Sichuan Province), and Kwangtung (= Guangdong Province) without direct examination of these specimens. Wang et al. (1962) also reported both *R. cornutus pumilus* and *R. blythi* (= *R. pusillus*) from Guangxi. Considering the differences among specimens from Okinawa *pumilus*, Guangdong *pusillus*, and the Langzhong population in CH (6.27 ± 0.07 mm in Okinawa, 6.71 ± 0.22 in Guangdong, 5.95 ± 0.19 in Langzhong) and ZW (7.72 ± 0.05, 7.60 ± 0.23, and 7.21 ± 0.23, respectively), we do not believe that "*pumilus*" is also distributed in China. Instead, the Langzhong population could be an undescribed form. The recent first record of *R. monoceros* from mainland China based on a specimen from Guizhou Province (Zhou and Yang, 2010) may be conspecific with the Langzhong specimens. Further detailed morphological and genetic examinations are necessary for description after comparison with related taxa from Asia.

Specimens from Guang'an, Sichuan Province in China are also different from Chinese *R. pusillus* specimens in having much lower first PC scores as well as small univariate measurements. Measurements of the Guang'an population are very similar to those of *R. subbadius* reported by Bates and Harrison (1997) and Csorba et al. (2003), except that ZW in the Guang'an population (6.43 ± 0.25) is smaller than the value reported by Csorba (2003) (7.10 ± 0.29). After *R. subbadius* was named by Blyth (1844) in Nepal, it has been recorded in Nepal, north Myanmar, and India (Csorba et al., 2003). Hill (1962) reported specimens of *R. subbadius* from Yunnan, China, but there have been no subsequent reports of the occurrence of this species in China. We have tentatively identified the Guang'an population as *R. subbadius*. Further taxonomic study of Guang'an specimens by direct comparison with well identified *R. subbadius* specimens is required.

In conclusion, we recognize seven species among the specimens examined from China and Japan: *R. cornutus* (including *orii*, *pumilus*, *miyakonis* as junior synonyms), *R. perditus*, *R. imaizumii*, *R. monoceros*, *R. pusillus*, *R. subbadius* tentatively identified, and possible undescribed species in Langzhong in Sichuan Province. Genetic studies using all of these species are required to test this revised taxonomic arrangement as well as phylogenetic relationships and zoogeography of these species in East Asia.

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