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Article *in* Amphibia-Reptilia · November 2017
DOI: 10.1163/15685381-00003130

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Amphibia-Reptilia (2017) DOI:10.1163/15685381-00003130

Resurrection of genus *Nidirana* (Anura: Ranidae) and synonymizing *N. caldwelli* with *N. adenopleura*, with description of a new species from China

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Abstract. The taxonomy of *Babina* sensu lato was controversial in the past decades. In this study, the phylogeny of genus *Babina* sensu lato was re-constructed based on genetic analysis, morphological comparison and advertisement call analysis. We found that *Babina* sensu stricto and previous subgenus *Nidirana* should be two distinct genera in the family Ranidae. *N. caldwelli* is confirmed to be a synonym of *N. adenopleura* because of the small genetic divergence and the lack of distinct morphological differences. A new species, *Nidirana nankunensis* sp. nov. is described based on a series of specimens collected from Mt. Nankun, Guangdong Province, China, which can be distinguished from other known congeners by having a behavior of nest construction, distinctive advertisement calls, significant divergence in the mitochondrial genes, and a combination of morphological characters. Currently, the genus *Babina* contains two species and the genus *Nidirana* contains eight species.

Keywords: *Babina*, bioacoustic, mitochondrial DNA, morphology, *Nidirana nankunensis* sp. nov., phylogeny.

Introduction

The ranid genus *Babina* was established and described on the basis of *Rana holsti* Boulenger, 1892 (type species) and *Rana subaspera* Barbour, 1908 by Thompson (1912). Dubois (1992) established subgenus *Nidirana* under the genus *Rana* Linnaeus, 1758, based on morphological characters including presence of large suprabrachial gland in males, absence of a thumb-like structure on finger I and presence of well-developed dorsolateral folds. *Rana psaltes* Kuramoto, 1985 (type species), *Rana adenopleura* Boulenger, 1909, *Rana caldwelli* Schmidt, 1925, *Rana chapaensis* Bourret, 1937, *Rana daunchina* Chang, 1933 and *Rana pleuraden* Boulenger, 1904 were placed in this subgenus. Moreover, *Babina* was considered as a subgenus of *Rana*, with the characters of presence of large suprabrachial gland in males, presence of a thumb-like structure on finger I and absence of well-developed dorsolateral folds (Dubois, 1992). Subsequently, *Nidirana* was recognized as a separate genus by Chen et al. (2005), based on a molecular phylogenetic tree of Southeast Asian ranids that only included one *Nidirana* species – *R.* (*N.*) *chapaensis*. Frost et al. (2006) considered that subgenus *Babina* was a distinct genus, based on a molecular analysis which included only two species of subgenus *Nidirana* – *R.* (*N.*) *adenopleura* and *R.* (*N.*) *chapaensis*, and merged *Nidirana* with genus *Babina* because *Nidirana* has no characters that suggest it as a monophyletic group from *Babina*. Chuaynkern et al. (2010) disagreed with the arrangement of Frost et al. (2006) and followed Dubois's (1992) opinions, considering that these two groups had distinct morphotypes which indicated different adaptative niches. Fei et al. (2010) provided a phylogenetic systematics of Ranidae, in which *Babina* and *Nidirana* were regarded as two genera and established a new genus *Dianrana* Fei, Ye, and Jiang, 2010, but was not widely accepted (Frost, 2017). Kakehashi et al. (2013) provided molecular studies including three species (*B. holsti*, *B. subaspera* and *B. okinavana*), which indicated the genus *Babina* sensu lato (*Babina* sensu stricto and *Nidirana*) was monophyletic

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 DOI:10.1163/15685381-00003130

and was the sister taxon to genus *Odorrana* Fei, Ye, and Huang, 1990.

At present, the monophyletic *Babina* s. l. is recognized to contain 10 species occurring in eastern and southeastern Asia, namely *B. holsti* from Okinawa of central Ryukyu, *B. subaspera* from Amami of central Ryukyu, *B. okinavana* (Boettger, 1895) from Yaeyama of southern Ryukyu and eastern Taiwan, *B. adenopleura* from Taiwan, *B. caldwelli* from southeastern mainland China, *B. hainanensis* (Fei, Ye, and Jiang, 2007) from Hainan, *B. daunchina* from western China, *B. chapaensis* and *B. lini* (Chou, 1999) from northeastern Indochina Peninsula, and *B. pleuraden* from southwestern China (Matsui, 2007; Fei et al., 2012; Frost, 2017). Nevertheless, there is still no consensus on the phylogenetic placement between *Babina* s. s. and *Nidirana*, for lacking convictive molecular supports.

In this study, we re-constructed the phylogeny of *Babina* s. l., using mitochondrial data from all known congeners. Besides, during our herpetological field surveys in Mt. Nankun (MNK), south China, we have found a small-sized frog firstly assigned to *Babina* s. l. by possessing large suprabrachial gland in breeding males. Morphological, genetic and acoustic analysis indicated that the frog is distinctive from all known species of *Babina* s. l. Therefore, we describe it as a new species below.

Material and methods

Taxon sampling

A total of 39 samples from five known species (*Babina caldwelli*, *B. daunchina*, *B. hainanensis*, *B. lini* and *B. pleuraden*) and an unnamed species of genus *Babina* s. l. from China were used for molecular analysis. All samples were preserved in 95% ethanol and stored at −40°C. In addition, five sequences from other five known *Babina* s. l. species were obtained from GenBank and incorporated into our dataset. Eleven Ranid and two Dicroglossid species obtained from GenBank were used as out-groups. Detail information of these materials is shown in table 1 and fig. 1.

Extraction, PCR amplification, and sequencing

Genomic DNA were extracted from muscle tissue, using DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Three mitochondrion genes namely 16S ribosomal RNA gene (16S), 12S ribosomal RNA gene (12S) and cytochrome C oxidase 1 gene (CO1) were amplified. Primers used for 16S were L3975 (5′-CGCCTGTTTACCA AAAACAT-3′) and H4551 (5′-CCGGTCTGAACTCAGAT CACGT-3′), for 12S were L33 (5′-CTCAACTTACAMATG CAAG-3′), H56 (5′-CGATTATAGAACAGGCTCCT-3′), L1091 (5′-CAAACTGGGATTAGATACCCCACTAT-3′) and H1478 (5′-TGACTGCAAGGTGACGGGCGGTGTG T-3′), and for CO1 were Chmf4 (5′-TYTCWACWAAYCAY AAAGAYATCGG-3′) and Chmr4 (5′-ACYTCRGGRTGR CCRAARAATCA-3′). PCR amplifications were processed with the cycling conditions that initial denaturing step at 95°C for 4 min, 35 cycles of denaturing at 94°C for 40 s, annealing at 53°C for 40 s and extending at 72°C for 1 min, and final extending step at 72°C for 10 min. PCR products were purified with spin columns and then sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit per the guidelines, on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co., Ltd. All sequences were deposited in GenBank (table 1).

Molecular phylogenetic analysis

DNA sequences were aligned in MEGA 6 (Tamura et al., 2013) by the Clustal W algorithm with default parameters (Thompson et al., 1997). Three gene segments, which are 1032 base pairs (bp) of 16S, 754 bp of 12S, and 562 bp of CO1, were concatenated seriatim into a 2348-bp single partition. The data was tested in jmodeltest v2.1.2 with Akaike and Bayesian information criteria, resulting the best-fitting nucleotide substitution model is GTR+I+G. Sequenced data was analyzed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al., 2012), and maximum likelihood (ML) implemented in RaxmlGUI 1.3 (Silvestro and Michalak, 2012). Three independent runs were conducted in BI analysis, each of which was performed for 2 000 000 generations and sampled every 1000 generations with the first 25% samples were discarded as burn-in. In ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. Pairwise distances (p-distance) were calculated in MEGA 6 using the uncorrected p-distance model.

Bioacoustic analysis

Bioacoustic data of known *Babina* s. l. species were obtained from literature (Matsui and Utsunomiya, 1983; Chou, 1999; Chuaynkern et al., 2010; Chuang et al., 2016) for comparisons. Advertisement calls of the unnamed *Babina* s. l. species from MNK and its three congeners (*B. caldwelli*, *B. daunchina* and *B. hainanensis*) were recorded in the field by SONY PCM-D50 digital sound recorder, at the air temperature 18-20°C. The sound files in wave

Table 1. Localities, voucher information and GenBank numbers for all samples used in this study.

ID	Species	Localities ("*" indicates the type localities)	Specimen no.	16S	12S	CO1	Cites
1	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a003618	MF807828	MF807906	MF807867	this study
2	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a003619	MF807829	MF807907	MF807868	this study
3	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a004906	MF807835	MF807913	MF807874	this study
4	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a004907	MF807836	MF807914	MF807875	this study
5	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a004914	MF807837	MF807915	MF807876	this study
6	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a005717	MF807838	MF807916	MF807877	this study
7	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a005718	MF807839	MF807917	MF807878	this study
8	*Nidirana nankunensis* sp. nov.	*China: Mt. Nankun, Guangdong	SYS a005719	MF807840	MF807918	MF807879	this study
9	*Nidirana adenopleura*	China: New Taipei City, Taiwan	UMMZ 189963	DQ283117	DQ283117	/	Frost et al. (2006)
10	*Nidirana caldwelli*	China: Jingning County, Zhejiang	SYS a002725	MF807827	MF807905	MF807866	this study
11	*Nidirana caldwelli*	China: Jinggangshan Nature Reserve, Jiangxi	SYS a004025	MF807830	MF807908	MF807869	this study
12	*Nidirana caldwelli*	China: Jinggangshan Nature Reserve, Jiangxi	SYS a004026	MF807831	MF807909	MF807870	this study
13	*Nidirana caldwelli*	China: Jinggangshan Nature Reserve, Jiangxi	SYS a004027	MF807832	MF807910	MF807871	this study
14	*Nidirana caldwelli*	China: Jiangshi Nature Reserve, Fujian	SYS a004112	MF807833	MF807911	MF807872	this study
15	*Nidirana caldwelli*	China: Jiangshi Nature Reserve, Fujian	SYS a004132	MF807834	MF807912	MF807873	this study
16	*Nidirana caldwelli*	China: Mt. Yashu, Fujian	SYS a005891	MF807841	MF807919	MF807880	this study
17	*Nidirana caldwelli*	China: Mt. Yashu, Fujian	SYS a005901	MF807842	MF807920	MF807881	this study
18	*Nidirana caldwelli*	China: Mt. Yashu, Fujian	SYS a005902	MF807843	MF807921	MF807882	this study
19	*Nidirana caldwelli*	*China: Yanping District, Nanping City, Fujian	SYS a005911	MF807844	MF807922	MF807883	this study
20	*Nidirana caldwelli*	*China: Yanping District, Nanping City, Fujian	SYS a005912	MF807845	MF807923	MF807884	this study
21	*Nidirana caldwelli*	*China: Yanping District, Nanping City, Fujian	SYS a005913	MF807846	MF807924	MF807885	this study
22	*Nidirana caldwelli*	*China: Yanping District, Nanping City, Fujian	SYS a005914	MF807847	MF807925	MF807886	this study
23	*Nidirana caldwelli*	*China: Yanping District, Nanping City, Fujian	SYS a005915	MF807848	MF807926	MF807887	this study
24	*Nidirana caldwelli*	*China: Yanping District, Nanping City, Fujian	SYS a005916	MF807849	MF807927	MF807888	this study
25	*Nidirana caldwelli*	China: Mt. Wuyi, Fujian	SYS a005939	MF807850	MF807928	MF807889	this study
26	*Nidirana caldwelli*	China: Mt. Wuyi, Fujian	SYS a005940	MF807851	MF807929	MF807890	this study
27	*Nidirana caldwelli*	China: Mt. Wuyi, Fujian	SYS a005941	MF807852	MF807930	MF807891	this study
28	*Nidirana caldwelli*	China: Mt. Wuyi, Fujian	SYS a005942	MF807853	MF807931	MF807892	this study
29	*Nidirana caldwelli*	China: Mt. Wuyi, Fujian	SYS a005943	MF807854	MF807932	MF807893	this study

Table 1. (Continued.)

ID	Species	Localities ("*" indicates the type localities)	Specimen no.	16S	12S	CO1	Cites
30	*Nidirana chapaensis*	*Vietnam: Sapa, Lao Cai	T2483/2000.4850	KR827711	/	KR087625	Grosjean et al. (2015)
31	*Nidirana daunchina*	*China: Mt. Emei, Sichuan	SYS a004594	MF807822	MF807900	MF807861	this study
32	*Nidirana daunchina*	*China: Mt. Emei, Sichuan	SYS a004595	MF807823	MF807901	MF807862	this study
33	*Nidirana daunchina*	China: Hejiang County, Sichuan	SYS a004930	MF807824	MF807902	MF807863	this study
34	*Nidirana daunchina*	China: Hejiang County, Sichuan	SYS a004931	MF807825	MF807903	MF807864	this study
35	*Nidirana daunchina*	China: Hejiang County, Sichuan	SYS a004932	MF807826	MF807904	MF807865	this study
36	*Nidirana hainanensis*	*China: Mt. Diaoluo, Hainan	SYS a003741	MF807821	MF807899	MF807860	this study
37	*Nidirana lini*	*China: Jiangcheng County, Yunnan	SYS a003967	MF807818	MF807896	MF807857	this study
38	*Nidirana lini*	*China: Jiangcheng County, Yunnan	SYS a003968	MF807819	MF807897	MF807858	this study
39	*Nidirana lini*	*China: Jiangcheng County, Yunnan	SYS a003969	MF807820	MF807898	MF807859	this study
40	*Nidirana okinavana*	*Japan: Iriomote Island, Okinawa	not given	NC022872	NC022872	NC022872	Kakehashi et al. (2013)
41	*Nidirana pleuraden*	China: Mt. Gaoligong, Yunnan	SYS a003775	MF807816	MF807894	MF807855	this study
42	*Nidirana pleuraden*	China: Mt. Gaoligong, Yunnan	SYS a003776	MF807817	MF807895	MF807856	this study
43	*Babina holsti*	*Japan: Okinawa	not given	NC022870	NC022870	NC022870	Kakehashi et al. (2013)
44	*Babina subaspera*	*Japan: Amami Island, Kagoshima	not given	NC022871	NC022871	NC022871	Kakehashi et al. (2013)
45	*Amolops mantzorum*	China: Mt. Xiling, Sichuan	not given	NC024180	NC024180	NC024180	Shan et al. (2016)
46	*Amolops wuyiensis*	not given	not given	NC025591	NC025591	NC025591	Huang et al. (2016)
47	*Hylarana taipehensis*	not given	not given	KX022002	KX022002	KX022002	Chen et al. (2016)
48	*Odorrana margaretae*	China	HNNU1207003	NC024603	NC024603	NC024603	Chen et al. (2015)
49	*Odorrana nasica*	Vietnam: Mt. Pomu, Ha Tinh	AMNH A161169	DQ283345	DQ283345	/	Frost et al. (2006)
50	*Odorrana schmackeri*	not given	not given	KP732086	KP732086	KP732086	Bu et al. (*unpub.*)
51	*Pelophylax nigromaculatus*	not given	not given	KT878718	KT878718	KT878718	Jiang et al. (2017)
52	*Pelophylax plancyi*	not given	not given	NC009264	NC009264	NC009264	Nie et al. (*unpub.*)
53	*Lithobates catesbeiana*	Japan: IABHU Lab. strain	living individual	AB761267	AB761267	AB761267	Kakehashi et al. (2013)
54	*Rana japonica*	Japan: Hiroshima	IABHU 17624	AB511305	AB511305	AB511305	Kurabayashi et al. (2010)
55	*Sylvirana hekouensis*	not given	not given	KX021954	KX021954	KX021954	Chen et al. (2016)
56	*Limnonectes fujianensis*	not given	not given	NC007440	NC007440	NC007440	Nie et al. (*unpub.*)
57	*Quasipaa boulengeri*	China: Sichuan	XM3632	KX645665	KX645665	KX645665	Yuan et al. (2016)

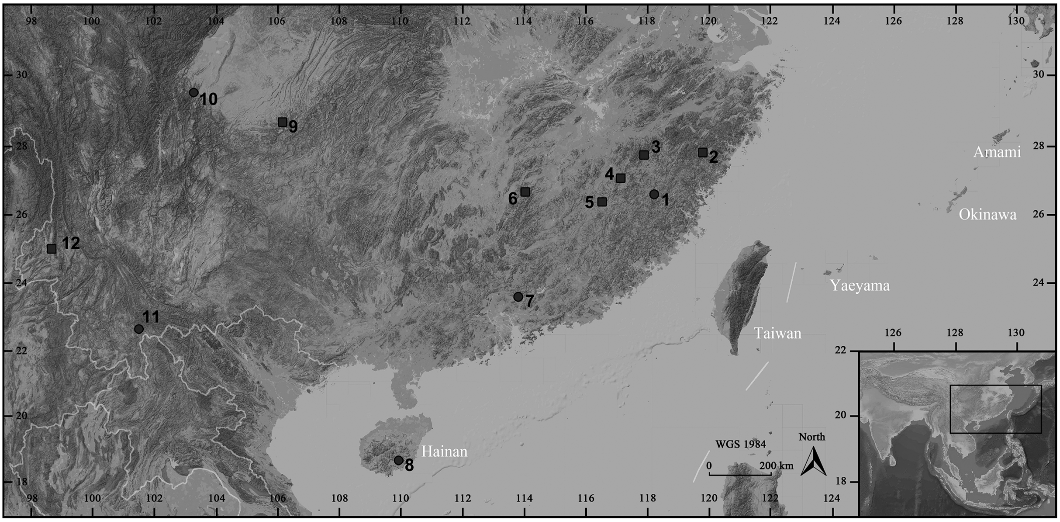

Figure 1. Collecting localities of the samples used in this study. Circles represent type localities. (1) Yanping District, Nanping City, Fujian; (2) Jingning County, Zhejiang; (3) Mt. Wuyi, Fujian; (4) Jiangshi Nature Reserve, Fujian; (5) Mt. Yashu, Fujian; (6) Jinggangshan Nature Reserve, Jiangxi; (7) Mt. Nankun, Guangdong; (8) Mt. Diaoluo, Hainan; (9) Hejiang County, Sichuan; (10) Mt. Emei, Sichuan; (11) Jiangcheng County, Yunnan; (12) Mt. Gaoligong, Yunnan.

format were sampled at 48 kHz with 24 bits in depth. Raven pro 1.5 (Cornell Lab of Ornithology, 2003-2014) was used to output the spectrograms and to measure interrelated parameters with Fast Fourier transform of 512 points and a 50% overlap (the calls of *B. hainanensis* were of poor quality to measure so that only spectrogram was outputted).

The following measurements were taken for each call: notes number, duration (the difference between begin time and end time for a selected call/note), peak frequency (PF; the frequency at which peak power occurs within the selected call/note), inter-quartile range bandwidth (IQR-BW; the difference between the first and third quartile frequencies within the selected call/note), bandwidth 90% (BW-90%; the difference between the 5% and 95% frequencies of a selected call/note). The first notes of the calls of *Babina daunchina* and the unnamed species from MNK were unique, so we measured them in the same parameters as the call; for other notes and the intervals between notes, we only measured the duration parameter. As for the call of *B. caldwelli* whose notes were without significant differences, we only measured the duration for each note and interval.

Morphometrics

Diagnostic characters of all known *Babina* s. l. species were obtained from literature for comparisons (Fei et al., 2009; Chuaynkern et al., 2010). Totally 43 specimens of five known species (*B. caldwelli*, *B. daunchina*, *B. hainanensis*, *B. lini* and *B. pleuraden*) were examined (Appendix).

External measurements were made with digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper, USA) to the nearest 0.1 mm, including snout-vent length (SVL) from tip of snout to posterior margin of vent in adult frog; head length (HDL) from tip of snout to the articulation of the jaw; head width (HDW) at the commissure of the jaws; snout length (SNT) from tip of snout to the anterior corner of the eye; internasal distance (IND); interorbital distance (IOD); eye diameter (ED) from the anterior corner of the eye to posterior corner of the eye; tympanum horizontal diameter (TD); tympanum-eye distance (TED) from anterior edge of tympanum to posterior corner of the eye; hand length (HND) from distal end of radioulna to tip of distal phalanx III; radioulna length (RAD) from the flexed elbow to the base of the outer palmar tubercle; foot length (FTL) from distal end of tibia to tip of distal phalanx IV; and tibial length (TIB) from the outer surface of the flexed knee to the heel; body length (BL) from the tip of snout to the origin of tail, and tail length (TL) from the origin to the tip of tail in tadpole.

We determined sex by secondary sexual characters, i.e. the presence of nuptial pads, vocal sacs or suprabrachial glands in males. Webbing formula was written according to Savage (1975) and tadpole stage was identified followed Gosner (1960).

All specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol. All studied specimens and all muscle tissues samples are deposited in The Museum of Biology, Sun Yat-sen University (SYS) and Chengdu Institute of Biology, the Chinese Academy of Sciences (CIB), P.R. China.

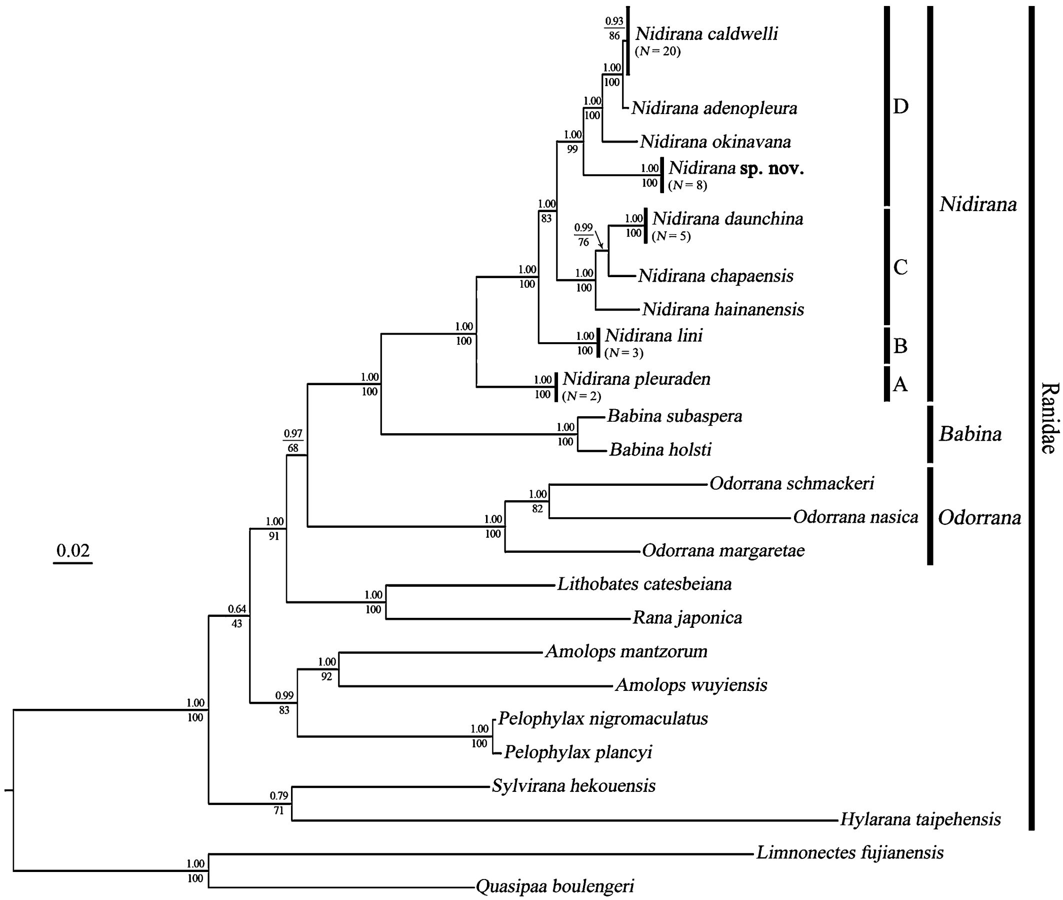

Figure 2. Bayesian inference and maximum-likelihood phylogenies. Numbers above branches indicate Bayesian posterior probabilities and numbers below branches are bootstrap support for maximum likelihood (1000 replicates) analysis.

Results

Phylogenetic analysis

The ML and BI analyses resulted in essentially identical topologies which were integrated in fig. 2. All major nodes were sufficiently supported with the Bayesian posterior probabilities (BPP) > 0.95 and the bootstrap supports for maximum likelihood analysis (BS) > 70. The p-distances among all samples are given in the online supplementary table S1.

All 10-known species of *Babina* s. l. and the population from MNK grouped in a single clade, which was the sister taxon of the genus *Odorrana* within Ranidae. The clade was further divided into two major, deeply divergent (p-distance 7.0%-12.4%), and strongly supported monophyletic groups, which completely correspond to two former subgenera *Babina* and *Nidirana* (designated here as *Babina* group and *Nidirana* group). The *Babina* group was composed of two original members, *B. holsti* and *B. subaspera* from central Ryukyu. The *Nidirana* group consisted of the remaining eight known species and an undescribed species from MNK, which were further divided into four subclades (designated here as A, B, C and D). The phylogenetic interrelationships of *Babina* s. l. inferred as following: *Babina* group + *Nidirana* group (subclade A + (subclade B + (subclade C + subclade D))).

Within subclade D, *Babina adenopleura* from Taiwan and *B. caldwelli* from east mainland

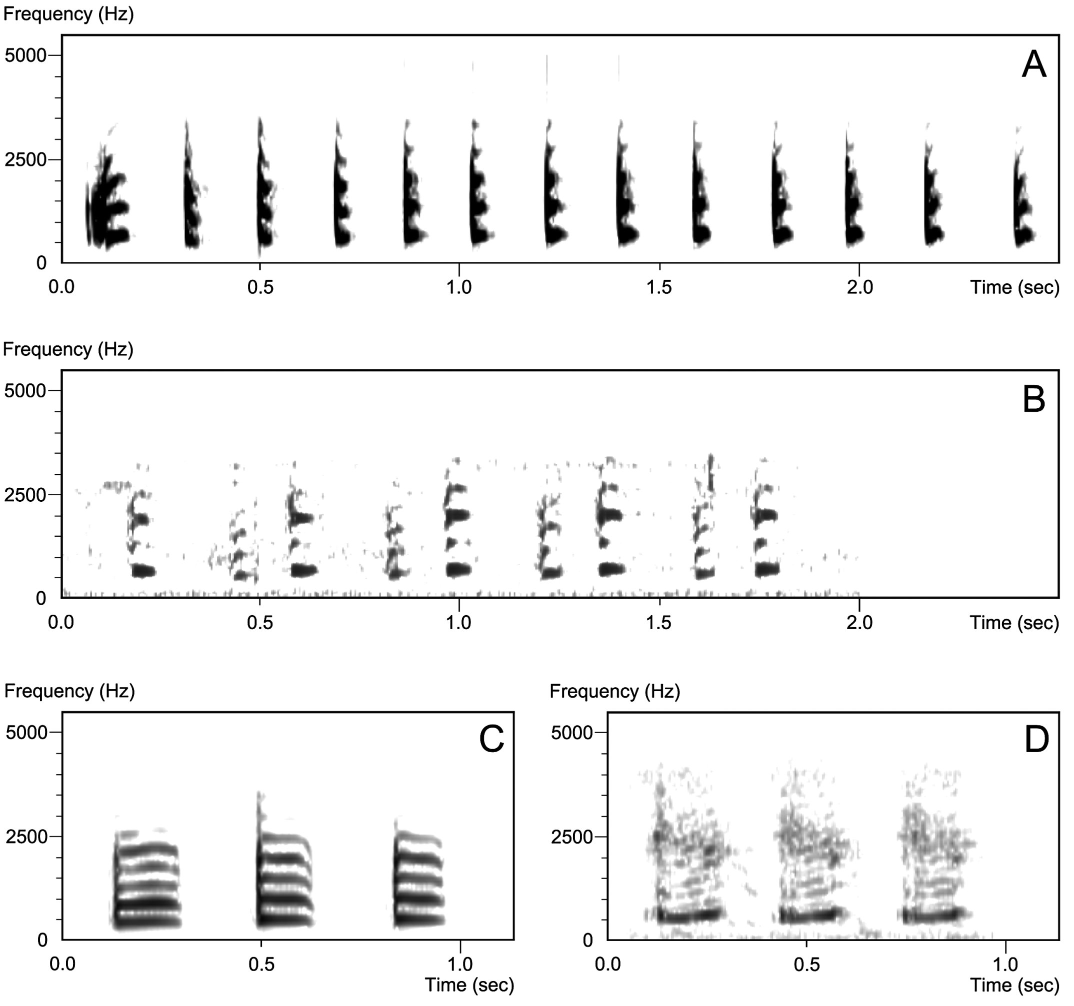

Figure 3. Advertisement call spectrograms. (A) *Nidirana nankunensis* sp. nov. from Mt. Nankun; (B) *N. hainanensis* from Mt. Diaoluo; (C) *N. daunchina* from Hejiang County; (D) *N. caldwelli* from Yanping District.

China significantly clustered into a monophyletic branch with strong supports and small divergence (*p*-distance 0.3%-1.0%). Furthermore, the individuals from MNK grouped into a monophyletic branch with strong supports and small divergence (*p*-distance 0.0%-0.3%), forming the sister taxon to ((*B. caldwelli* + *B. adenopleura*) + *B. okinavana*). The significantly deep divergences between this branch and all other species of *Nidirana* group (the smallest interspecific *p*-distance is 4.8%-5.1% between branch of MNK and *B. okinavana*) indicated that this population represented a separately evolving lineage.

Bioacoustic analysis

The call spectrograms were shown in fig. 3. The literature and bioacoustic analysis inferred the following call pattern differences among species of *Babina* group and *Nidirana* group.

Within *Babina* group, breeding males emitted short advertisement calls (0.27 ± 0.01 s for both species) composed of a single note (Matsui and Utsunomiya, 1983); whereas within *Nidirana* group, all mating calls composed of several notes (Chuaynkern et al., 2010).

Among species of *Nidirana* group, there are three advertisement call patterns differing remarkably from each other. The first pattern contains a first note which is significantly different in timbre characteristics, such as the calls from MNK (MNK call; fig. 3A) and that of *Babina daunchina* (fig. 3C): MNK call lasts about 2.30-2.74 s (2.52 ± 0.17 s, $n = 11$) and contains 13-15 (14.0 ± 0.94, $n = 11$) fast-repeated notes, of which the first note continues for 108-135 ms (122.9 ± 8.4 ms, $n = 11$) while others continue for 38-56 ms (46.1 ± 3.7 ms, $n = 50$) and the intervals between notes last 12-166 ms (138.7 ± 10.5 ms, $n = 50$); the call of *B. daunchina* lasts 0.51-1.57 s (1.01 ± 0.42 s, $n = 11$) and contains 2-5 (3.5 ± 1.21, $n = 11$) notes, of which the first note continues for 162-197 ms (182.7 ± 12.4 ms, $n = 11$) while others continue for 131-150 ms (140.6 ± 5.6 ms, $n = 23$) and the intervals between notes last 159-259 ms (193.6 ± 26.3 ms, $n = 23$). *B. hainanensis* (fig. 3B) has a unique call pattern that contains 2-4 fast-repeated double-notes. *B. caldwelli* (fig. 3D) has the third call pattern which contains several almost identical regular notes: 2-3 (2.27 ± 0.45, $n = 30$) notes with the call duration of 0.53-0.90 s (0.63 ± 0.14 s, $n = 30$), the notes continuing for 159-252 ms (209.9 ± 22.5 ms, $n = 68$) and the intervals between notes lasting 98-213 ms (122.4 ± 23.1 ms, $n = 38$). From the literatures (Chou, 1999; Chuang et al., 2016), the remaining congeners are recognized to have similar call pattern to *B. caldwelli*: 3 notes in *B. chapaensis*; 5-7 notes in *B. lini*; 17-25 fast-repeated notes in *B. okinavana*; 4-7 notes in *B. pleuraden*; 3-5 notes in *B. adenopleura* with the call duration of 0.65-1.18 s and the notes continuing for 115-182 ms.

Morphological comparison

Measurements and body proportions of examined specimens of *Babina caldwelli*, *B. daunchina*, *B. hainanensis*, *B. lini*, *B. pleuraden* and that from MNK were given in table 2 and the detail comparative data of species of *Nidirana* group were shown in table 3. The sisters *Babina* and *Nidirana* group shared synapomorphy of suprabrachial gland, and showed distinct morphological differences from each other in following characters: body size large in *Babina* group (SVL 90-140 mm in adult *B. subaspera* and 100-125 mm in adult *B. holsti*) vs. body size small or moderate in *Nidirana* group (maximum SVL 68.6 mm in adult female *B. lini*); presence of a thumb-like structure on finger I in *Babina* group vs. absent in *Nidirana* group; dorsolateral folds not well developed in *Babina* group vs. well developed in *Nidirana* group.

Discussion

Babina caldwelli *is synonymous with* B. adenopleura

The *Babina caldwelli* was originally described as *Rana caldwelli* based on one holotype from the locality probably near Yenping (=Yanping District, Nanping City), Fujian, China, differing from *R. adenopleura* by more projecting snouts, rougher skin, and posteriorly broken up dorsolateral glandular folds (Schmidt, 1925). Subsequently, Pope (1931) placed it in the synonymy of *R. adenopleura* and was followed by several authors (Liu, 1950; Kuramoto, 1985; Chou, 1999; Fei et al., 2009; Fei et al., 2012). Dubois (1992) and Chuaynkern et al. (2010) resurrected population from Fujian as a valid species *R. caldwelli* and considered *R. adenopleura* to be restricted in Taiwan, further designating that *R. caldwelli* differs by having shorter loreal region, shorter forelimb, wider shank, narrower disc of the fifth toe and spinules on the posterior half of dorsum (vs. spinules scattered from tip of dorsal snout to vent in *R. adenopleura*) (Chuaynkern et al., 2010); besides, labial tooth row formula in tadpoles of *R. caldwelli* was 1:0+0/1+1:2 (Pope, 1931) compared to 1:1+1/1+1:2 of *R. adenopleura* (Chou and Lin, 1997).

Table 2. Measurements (in mm; minimum-maximum, mean ± 1SD) and body proportions of the examined specimens of *Babina* sensu lato. F: females; M: males.

	N. nankunensis sp. nov.		*N. caldwelli*		*N. daunchian*		*N. hainanensis*	*N. lini*		*N. pleuraden*	
	M (n = 10)	F (n = 2)	M (n = 18)	F (n = 3)	M (n = 4)	F (n = 1)	M (n = 1)	M (n = 2)	F (n = 1)	M (n = 3)	F (n = 1)
SVL	33.3-37.1 (35.6 ± 1.3)	37.8-39.5 (38.6 ± 1.2)	44.5-57.6 (51.4 ± 3.8)	57.6-60.7 (58.9 ± 1.6)	42.9-47.9 (45.8 ± 2.5)	49.6	44.4	60.1-63.1 (61.6 ± 2.2)	68.6	47.5-53.0 (50.2 ± 2.8)	59.4
HDL	12.4-14.2 (13.2 ± 0.7)	13.8-14.8 (14.3 ± 0.7)	17.0-21.4 (19.1 ± 1.3)	20.3-22.5 (21.4 ± 1.1)	15.5-18.4 (16.8 ± 1.6)	18.4	17.5	21.5-22.5 (22.0 ± 0.8)	25.0	18.6-19.0 (18.9 ± 0.2)	21.1
HDW	11.0-13.0 (11.9 ± 0.7)	12.0-13.3 (12.6 ± 0.9)	15.1-19.5 (17.6 ± 1.2)	19.0-20.9 (19.9 ± 1.0)	13.2-16.8 (14.8 ± 1.8)	16.4	16.1	18.1-19.6 (18.8 ± 1.1)	21.0	16.3-16.6 (16.5 ± 0.1)	19.1
SNT	5.2-6.0 (5.6 ± 0.3)	5.8-6.4 (6.1 ± 0.4)	6.7-8.5 (7.8 ± 0.6)	7.5-8.8 (8.3 ± 0.7)	6.3-7.7 (7.0 ± 0.6)	7.8	7.3	9.2-9.3 (9.2 ± 0.0)	10.3	7.3-7.6 (7.5 ± 0.2)	8.1
IND	4.2-4.8 (4.5 ± 0.2)	4.6-4.6 (4.6 ± 0.0)	4.8-6.7 (5.6 ± 0.5)	5.9-6.4 (6.1 ± 0.2)	4.8-6.1 (5.5 ± 0.6)	6.1	5.6	6.2-6.4 (6.3 ± 0.1)	7.2	4.9-5.3 (5.1 ± 0.2)	6.6
IOD	3.3-4.1 (3.7 ± 0.3)	3.9-4.0 (3.9 ± 0.1)	4.0-5.4 (4.6 ± 0.4)	4.8-5.1 (5.0 ± 0.2)	4.1-4.2 (4.2 ± 0.1)	4.4	4.8	5.4-5.6 (5.5 ± 0.1)	5.6	3.7-4.4 (4.0 ± 0.3)	4.6
ED	3.8-4.2 (4.0 ± 0.1)	4.1-4.1 (4.1 ± 0.0)	4.7-6.0 (5.2 ± 0.3)	5.4-5.9 (5.7 ± 0.3)	4.7-5.1 (4.9 ± 0.2)	5.0	5.3	5.6-5.8 (5.7 ± 0.1)	6.4	5.2-5.3 (5.3 ± 0.0)	5.4
TD	3.2-3.4 (3.3 ± 0.1)	3.3-3.4 (3.3 ± 0.1)	3.4-5.2 (4.4 ± 0.5)	4.5-5.2 (4.9 ± 0.4)	3.8-4.4 (4.1 ± 0.3)	4.4	4.5	4.9-5.9 (5.4 ± 0.7)	4.6	4.1-4.3 (4.2 ± 0.1)	4.9
TED	1.0-1.2 (1.2 ± 0.1)	1.2-1.2 (1.2 ± 0.0)	1.3-1.5 (1.4 ± 0.1)	1.5-1.7 (1.6 ± 0.1)	1.5-1.5 (1.5 ± 0.0)	1.6	1.2	1.9-2.0 (2.0 ± 0.1)	2.4	1.8-1.9 (1.8 ± 0.0)	1.8
HND	8.9-10.2 (9.5 ± 0.4)	9.6-10.2 (9.9 ± 0.4)	11.6-15.0 (13.6 ± 1.0)	14.0-17.2 (15.2 ± 1.8)	10.6-13.2 (12.0 ± 1.1)	12.4	11.9	14.1-16.1 (15.1 ± 1.4)	17.3	12.0-13.9 (12.9 ± 1.0)	14.6
RAD	6.0-6.4 (6.2 ± 0.1)	6.4-6.6 (6.5 ± 0.1)	18.0-25.0 (21.9 ± 1.7)	20.0-26.2 (23.6 ± 3.2)	17.1-21.9 (19.7 ± 2.0)	21.3	20.1	25.3-25.6 (25.5 ± 0.2)	29.7	19.3-21.8 (20.2 ± 1.4)	22.9
FTL	25.6-28.3 (27.3 ± 0.8)	27.7-29.8 (28.8 ± 1.5)	23.0-29.9 (27.3 ± 1.8)	28.8-33.5 (30.8 ± 2.4)	23.5-27.2 (24.8 ± 1.7)	24.6	25.3	29.8-32.6 (31.2 ± 1.9)	36.8	22.5-25.0 (23.8 ± 1.3)	29.3
TIB	17.7-18.7 (18.4 ± 0.3)	18.8-20.8 (19.8 ± 1.4)	34.3-43.7 (39.6 ± 2.6)	42.5-48.5 (44.7 ± 3.3)	33.3-37.9 (35.1 ± 2.1)	36.1	35.6	43.0-50.6 (46.8 ± 5.4)	53.5	34.7-37.6 (36.0 ± 1.5)	42.2
HDL/SVL	0.35-0.39 (0.37 ± 0.01)	0.36-0.37 (0.37 ± 0.01)	0.35-0.39 (0.37 ± 0.01)	0.35-0.37 (0.36 ± 0.01)	0.35-0.38 (0.37 ± 0.02)	0.37	0.39	0.36-0.36 (0.36 ± 0.00)	0.36	0.36-0.39 (0.38 ± 0.02)	0.36

Table 2. (Continued.)

	N. nankunensis sp. nov.		*N. caldwelli*		*N. daunchian*		*N. hainanensis*	*N. lini*		*N. pleuraden*	
	M ($n = 10$)	F ($n = 2$)	M ($n = 18$)	F ($n = 3$)	M ($n = 4$)	F ($n = 1$)	M ($n = 1$)	M ($n = 2$)	F ($n = 1$)	M ($n = 3$)	F ($n = 1$)
HDW/SVL	0.32-0.35 (0.33 ± 0.01)	0.32-0.34 (0.33 ± 0.01)	0.32-0.37 (0.34 ± 0.01)	0.33-0.34 (0.34 ± 0.01)	0.30-0.35 (0.32 ± 0.02)	0.33	0.36	0.30-0.31 (0.31 ± 0.01)	0.31	0.31-0.35 (0.33 ± 0.02)	0.32
HDW/HDL	0.87-0.92 (0.90 ± 0.02)	0.87-0.90 (0.89 ± 0.02)	0.87-0.95 (0.92 ± 0.02)	0.92-0.93 (0.93 ± 0.01)	0.85-0.92 (0.88 ± 0.03)	0.89	0.92	0.84-0.87 (0.86 ± 0.02)	0.84	0.86-0.89 (0.87 ± 0.02)	0.91
SNT/HDL	0.41-0.45 (0.43 ± 0.01)	0.42-0.43 (0.43 ± 0.01)	0.36-0.43 (0.41 ± 0.02)	0.37-0.40 (0.39 ± 0.02)	0.40-0.43 (0.42 ± 0.02)	0.42	0.42	0.41-0.43 (0.42 ± 0.01)	0.41	0.38-0.41 (0.40 ± 0.02)	0.38
SNT/SVL	0.15-0.16 (0.16 ± 0.00)	0.15-0.16 (0.16 ± 0.01)	0.14-0.16 (0.15 ± 0.01)	0.13-0.15 (0.14 ± 0.01)	0.14-0.16 (0.16 ± 0.01)	0.16	0.16	0.15-0.15 (0.15 ± 0.00)	0.15	0.14-0.16 (0.15 ± 0.01)	0.14
IND/HDW	0.37-0.40 (0.38 ± 0.01)	0.35-0.38 (0.37 ± 0.02)	0.28-0.37 (0.32 ± 0.02)	0.30-0.31 (0.30 ± 0.01)	0.35-0.38 (0.37 ± 0.02)	0.37	0.35	0.33-0.34 (0.34 ± 0.01)	0.34	0.29-0.33 (0.31 ± 0.02)	0.35
IOD/HDW	0.29-0.33 (0.31 ± 0.01)	0.30-0.32 (0.31 ± 0.01)	0.24-0.30 (0.26 ± 0.02)	0.24-0.27 (0.25 ± 0.02)	0.25-0.32 (0.28 ± 0.03)	0.27	0.30	0.27-0.31 (0.29 ± 0.03)	0.27	0.22-0.27 (0.24 ± 0.03)	0.24
ED/HDL	0.27-0.32 (0.30 ± 0.02)	0.28-0.29 (0.29 ± 0.01)	0.24-0.31 (0.27 ± 0.02)	0.26-0.27 (0.27 ± 0.01)	0.28-0.32 (0.30 ± 0.02)	0.27	0.30	0.26-0.26 (0.26 ± 0.00)	0.26	0.27-0.28 (0.28 ± 0.01)	0.26
ED/SVL	0.11-0.12 (0.11 ± 0.00)	0.10-0.11 (0.11 ± 0.01)	0.09-0.11 (0.10 ± 0.01)	0.09-0.10 (0.10 ± 0.01)	0.10-0.11 (0.11 ± 0.01)	0.10	0.12	0.09-0.09 (0.09 ± 0.00)	0.09	0.10-0.11 (0.10 ± 0.01)	0.09
TD/ED	0.80-0.85 (0.83 ± 0.02)	0.81-0.83 (0.82 ± 0.01)	0.67-0.99 (0.85 ± 0.09)	0.76-0.96 (0.86 ± 0.10)	0.78-0.86 (0.84 ± 0.04)	0.88	0.85	0.86-1.02 (0.94 ± 0.11)	0.72	0.78-0.81 (0.80 ± 0.02)	0.91
TED/TD	0.32-0.38 (0.35 ± 0.02)	0.35-0.37 (0.36 ± 0.01)	0.28-0.38 (0.33 ± 0.03)	0.29-0.34 (0.32 ± 0.03)	0.34-0.40 (0.37 ± 0.03)	0.36	0.27	0.34-0.39 (0.37 ± 0.04)	0.52	0.42-0.46 (0.44 ± 0.02)	0.37
HND/SVL	0.24-0.28 (0.26 ± 0.01)	0.25-0.26 (0.26 ± 0.01)	0.24-0.29 (0.26 ± 0.01)	0.24-0.28 (0.25 ± 0.02)	0.25-0.28 (0.27 ± 0.01)	0.25	0.27	0.23-0.25 (0.24 ± 0.01)	0.25	0.25-0.26 (0.26 ± 0.01)	0.25
RAD/SVL	0.17-0.18 (0.18 ± 0.01)	0.17-0.17 (0.17 ± 0.00)	0.39-0.46 (0.43 ± 0.02)	0.35-0.43 (0.40 ± 0.04)	0.40-0.46 (0.43 ± 0.03)	0.43	0.45	0.41-0.42 (0.42 ± 0.01)	0.43	0.39-0.41 (0.40 ± 0.01)	0.39
FTL/SVL	0.72-0.80 (0.77 ± 0.02)	0.73-0.75 (0.74 ± 0.01)	0.47-0.58 (0.53 ± 0.03)	0.50-0.55 (0.52 ± 0.03)	0.52-0.57 (0.54 ± 0.02)	0.50	0.57	0.50-0.52 (0.51 ± 0.01)	0.54	0.45-0.50 (0.47 ± 0.03)	0.49
TIB/SVL	0.50-0.54 (0.52 ± 0.01)	0.50-0.53 (0.52 ± 0.02)	0.70-0.87 (0.77 ± 0.05)	0.74-0.80 (0.76 ± 0.03)	0.74-0.79 (0.77 ± 0.02)	0.73	0.80	0.72-0.80 (0.76 ± 0.06)	0.78	0.71-0.73 (0.72 ± 0.01)	0.71

Table 3. Diagnostic characters separating *Nidirana nankunensis* sp. nov. from its congeners.

Characteristics	*N. nankunensis*	*N. adenopleura*	*N. chapaensis*	*N. daunchina*	*N. hainanensis*	*N. lini*	*N. okinavana*	*N. pleuraden*
SVL of male	33.3-37.1	43.1-57.6	35.5-42.5	40.6-51.0	32.8-44.4	44.1-63.1	35.5-42.8	45.4-58.7
SVL of female	37.8-39.5	47.6-60.7	41.0-51.8	44.0-53.0	?	57.7-68.6	44.6-48.8	45.5-62.5
Body habitus	stocky	elongated	stocky	stocky	stocky	elongated	stocky	elongated
Fingers tips	dilated	dilated	dilated	dilated	dilated	dilated	dilated	not dilated
Lateroventral groove on fingers	present or absent	present or absent	present or absent	absent or rarely present	present	present or absent	present or absent	absent
Relative length of fingers	II < I < IV < III	II < I < IV < III	II < I = IV < III	II < I < IV < III	II < I < IV < III	II < I < IV < III	II < I < IV < III	II < I < IV < III
Toes tips	dilated	dilated	dilated	dilated	dilated	dilated	dilated	not dilated
Lateroventral groove on toes	present	present	present	present	present	present	present	absent
Relative length of toes	I < II < V < III < IV	I < II < V < III < IV	I < II < V < III < IV	I < II < V < III < IV	I < II < V < III < IV	I < II < V < III < IV	I < II < V < III < IV	I < II < V < III < IV
Tibio-tarsal articulation	nostril	snout tip or eye-snout	nostril	nostril	nostril	beyond snout	eye center-near nostril	eye-snout
Subgular vocal sacs	present	present	present	present	present	present	absent	present
Nuptial pad	one	one	two	one	absent	one	poorly one	one
Spinules on dorsal skin	absent or few above vent	entire or posterior dorsal skin	absent or few above vent	absent	absent	posterior dorsal skin	absent	posterior dorsal skin
Nest construction	present	absent	present	present	?	absent	present	absent
Calling	13-15 fast-repeated notes	2-4 notes	3 notes	2-5 notes	2-4 fast-repeated double-notes	5-7 notes	17-25 fast-repeated notes	4-7 notes
Tadpole labial tooth row formula	1:1+1/1+1:2	1:1+1/1+1:2 or 1:0+0/1+1:1	1:1+2/1+1:2	1:1+1/1+1:2 or 1:1+1/2+2:1	?	1:1+1/1+1:2	1:1+1/1+1:2	1:1+1/1+1:2 or 1:1+1/2+2:1
Cites	this study	this study; Pope (1931); Chuaynkern et al. (2010)	Chuaynkern et al. (2010)	this study; Liu (1950); Fei et al. (2009)	this study; Fei et al. (2009)	this study; Chou (1999); Fei et al. (2009)	Matsui and Utsunomiya (1983); Chuaynkern et al. (2010)	this study; Fei et al. (2009)

Our examined specimens of *Babina caldwelli* from Fujian, Zhejiang and Jiangxi did not show significant differences from the revision from Chuaynkern et al. (2010), in which the frogs from continent and island were found several differences in statistically morphometric data. However, we found that the dorsolateral glandular folds were not steadily broken up or continuous in Taiwanese *B. adenopleura* (Xiang et al., 2009; Lin, personal communication), which was not mentioned by the original description (Boulenger, 1909). In addition, the labial tooth row formula of tadpoles from Mt. Wuyi were 1:0+0/1+1:2 (SYS a005942) or 1:1+1/1+1:2 (SYS a005943), which were both identified as *B. caldwelli* in the molecular trees. Therefore, the characteristics separating *B. caldwelli* and *B. adenopleura* were not diagnostic, though there were statistically morphometric variations between continent and island populations. Further, the molecular analysis suggested that the samples from both Taiwan and mainland gather together with strong supports (BPP = 1.00, BS = 100) and small divergence (*p*-distance 0.3%-1.0%). Hence, we regard the populations from Taiwan and mainland China as the same species and *B. caldwelli* is synonymous with *B. adenopleura*.

Babina *and* Nidirana *are two distinct genera*

The *Babina* group, including *B. holsti* and *B. subaspera*, occurs in central Ryukyu, sharing the derived characters of large-sized body and thumb-like structure on finger I which is important during mating and regarded as the secondary sexual characteristics (Iwai, 2012). The *Nidirana* group is sister taxon to *Babina* with strongly supported and deep divergences (*p*-distance 7.0%-12.4%) in our molecular phylogenetic trees; morphologically, *Nidirana* significantly differs from *Babina* by the absence of the thumb-like structure, having relatively small-sized body and well developed dorsolateral folds; bioacoustically, as a symbol for reproductive isolation, *Nidirana* has obviously different call pattern from *Babina*; geographically, basal lineages A, B and C of *Nidirana* group occur from Sichuan, Yunnan and Hainan of China and extend to Indochina peninsula, showing that *Nidirana* and *Babina* have been isolated and undergone different geographical evolution processes.

The definition of a genus should fulfill the following three criteria to be descriptively useful: monophyletic, reasonably compact, and ecologically, morphologically or biogeographically distinct (Gill et al., 2005). Based on our data, *Nidirana* and *Babina* groups can be easily and steadily distinguished by the morphological and bioacoustic differences which are indicating that these two groups have distinct adaptative niches. Though the genetic divergence between them is relatively small when compared with other ranid genera, but the result exposed different geographical evolution processes of the two groups. Therefore, we consider the two groups should be treated as two genera within family Ranidae.

We here resurrect the genus *Nidirana* Dubois, 1992, and suggest its English common name "Music Frogs" and Chinese name "Qin Wa Shu (琴蛙属)" (Fei et al., 2012), including seven known species: *Nidirana okinavana* comb. nov. (Ryukyu Music Frog), *Nidirana adenopleura* (East China Music Frog), *Nidirana hainanensis* (Hainan Music Frog), *Nidirana chapaensis* (Chapa Music Frog), *Nidirana daunchina* (Emei Music Frog), *Nidirana lini* (Lin's Music Frog) and *Nidirana pleuraden* (Yunnan Music Frog).

The population from MNK is a new member of genus Nidirana

The comprehensive evidences of molecular analysis, morphological comparison and bioacoustics support the population from MNK to be a distinct species of genus *Nidirana* and we describe it as a new species *Nidirana nankunensis* sp. nov. as below.

Taxonomic account

Nidirana nankunensis sp. nov.

Holotype. SYS a005719 (fig. 4), adult male, collected by Zhi-Tong Lyu (ZTL) and Jian Wang (JW) on 9 April 2017 from Mt. Nankun (23°38′12″N, 113°51′15″E; 506 m a.s.l.), Longmen County, Guangdong Province, China.

Paratypes. Eleven adult specimens from the same locality as the holotype (500-600 m a.s.l.). SYS a003615, 3617-3620, five adult males, collected on 14-15 April 2015 by Run-Lin Li, ZTL and JW; SYS a004019/CIB 106879, adult male, collected on 10 June 2015 by Ying-Yong Wang (YYW), ZTL and JW; SYS a004905-4907, three adult males, collected on 5 June 2016 by ZTL and YYW; SYS a005717-5718, two adult females, collected on 9 April 2017 by ZTL and JW.

Other examined materials. SYS a004914, two tadpoles, collected from the same locality as the holotype by ZTL and YYW on 5 June 2016.

Etymology. The specific name "*nankunensis*" refers to the type locality of the new species, the Mt. Nankun. We suggest its English common name "Mt. Nankun Music Frog" and Chinese name "Nan Kun Shan Qin Wa (南昆山琴蛙)".

Diagnosis. *Nidirana nankunensis* sp. nov. is distinguished from its congeners by following combination of the morphological characteristics: (1) body small and stocky, with SVL 33.3-37.1 mm in adult males and 37.8-39.5 mm in adult females; (2) disks of digits dilated, pointed; (3) lateroventral grooves present on every digit except finger I; (4) heels meeting; (5) tibio-tarsal articulation reaching forward the nostril; (6) mid-dorsal stripe present; (7) spinules on dorsal skin absent or few above vent; (8) a pair of subgular vocal sacs present; (9) one single distinct prominent nuptial pad present on the first finger, nuptial spinules invisible; (10) suprabrachial gland present and large; (11) nest construction behavior present; (12) tadpole labial tooth row formula: 1:1+1/1+1:2; (13) calling: 13-15 fast-repeated notes.

Comparisons. *Nidirana nankunensis* sp. nov. differs from *Nidirana* congeners by following characteristics: (1) small-sized body, SVL 37.8-39.5 mm in adult females, 33.3-37.1 mm in adult males vs. 57.7-68.6 mm in adult females and 44.1-63.1 mm in adult males in *N. lini* (this study; Chuaynkern et al., 2010); 47.6-60.7 mm in adult females and 43.1-57.6 mm in adult males in *N. adenopleura* (this study; Chuaynkern et al., 2010); 45.5-62.5 mm in adult females and 45.4-58.7 mm in adult males in *N. pleuraden* (this study; Chuaynkern et al., 2010); 44.0-53.0 mm in adult females and 40.6-51.0 mm in adult males in *N. daunchina* (this study; Chuaynkern et al., 2010); 44.6-48.8 mm in adult females in *N. okinavana* (Chuaynkern et al., 2010); 41.0-51.8 mm in adult females in *N. chapaensis* (Chuaynkern et al., 2010); (2) tibio-tarsal articulation reaching forward the nostril vs. beyond the snout tip in *N. lini*; at the snout tip or eye-snout in *N. adenopleura*; eye-snout in *N. pleuraden*; eye center-near nostril in *N. okinavana*; (3) the presence of a single nuptial pad well developed, raised vs. poorly developed in *N. okinavana*; divided into two parts in *N. chapaensis*; absent in *N. hainanensis*; (4) having a behavior of nest construction vs. absent in *N. adenopleura*, *N. lini* and *N. pleuraden*; (5) the tadpole labial tooth row formula 1:1+1/1+1:2 vs. 1:1+2/1+1:2 in *N. chapaensis*; 1:1+1/1+1:2 or 1:0+0/1+1:1 in *N. adenopleura*; 1:1+1/1+1:2 or 1:1+1/2+2:1 in *N. pleuraden*. Further, *N. nankunensis* sp. nov. is different from *N. pleuraden* by its dilated digits (vs. not dilated) and lateroventral grooves present on digits expect finger I (vs. all absent); from *N. chapaensis* by its finger IV longer than finger I (vs. equal); from *N. okinavana* by having a pair of subgular vocal sacs (vs. absent); from *N. daunchina* by presence of lateroventral grooves on fingers expect finger I (vs. absent

Figure 4. Morphological features of the adult male holotype SYS a005719 of *Nidirana nankunensis* sp. nov. in life. (A) dorsolateral view; (B) ventral view; (C) right hand; (D) nuptial pad; (E) right foot; (F) posterior part of dorsal surface.

or rarely present); from *N. hainanensis* by absence of lateroventral grooves on finger I (vs. present).

Description of holotype. Adult male. Body stocky, SVL 36.3 mm; head longer than wide (HDL/HDW 1.10), flat above; snout rounded in dorsal and lateral views, slightly protruding beyond lower jaw, longer than horizontal diameter of eye (SNT/ED 1.33); canthus rostralis distinct, loreal region concave; nostril round, directed laterally; a longitudinal mandibular ridge extending from tip of snout through lower edges of nostril, eye and tympanum to axial region, forming a maxillary gland in posterior corner of mouth; supratympanic fold absent; interorbital space flat, narrower than internarial distance (IND/IOD 1.17); pupil elliptical, horizontal; tympanum distinct, round, TD/ED 0.81, and close to eye, TED/TD 0.32; pineal ocellus present, in middle point between anterior borders of eyelids; vomerine ridge present, bearing numerous small teeth; tongue large, cordiform, longer than wide, notched behind.

Forelimbs moderately robust, lower arm 17% of SVL and hand 28% of SVL; fingers thin, relative finger lengths II $<$ I $<$ IV $<$ III; tip of fingers weakly dilated, forming elongated and pointed disks; lateroventral grooves on finger II, III and IV, but absent on finger I; lateroventral grooves not meeting at the tip of disks; fingers free of webbing, with weak lateral fringes; subarticular tubercles present, prominent and rounded; palmar tubercles three, large, distinct and elliptic.

Hindlimbs relatively robust, tibia 51% of SVL and foot 78% of SVL; heels meeting when hindlimbs flexed at right angles to axis of body; tibio-tarsal articulation reaching forward the nostril when hindlimb is stretched along the side of the body; toes relatively long and thin, relative lengths I $<$ II $<$ V $<$ III $<$ IV; tip of toes weakly dilated, forming significantly elongated and pointed disks; distinct lateroventral grooves on toes; webbing moderate, webbing formula: I 2-2$\frac{1}{2}$ II 1$\frac{2}{3}$-3 III 2$\frac{1}{3}$-3$\frac{1}{2}$ IV 3$\frac{1}{2}$-2 V; toes with lateral fringes, distinct dermal flap running along lateral edge of 5th toe; subarticular tubercles oval or rounded, prominent; inner metatarsal tubercle elliptic, three times as long as wide; outer metatarsal tubercle indistinct, small and rounded; tarsal folds absent, tarsal tubercle present.

Dorsal skin of head and body smooth with tiny granules on dorsal head and body, excluding the snout; posterior part of back with several tubercles, not bearing horny spinules; developed intermittent dorsolateral fold from posterior margin of upper eyelid to above groin; flanks smooth, a large and smooth suprabrachial gland behind base of forelimb; two longitudinal ridges on dorsal side of upper arm and slightly extending on to lower arm; several longitudinal dermal ridges with horny spinules on the dorsal surfaces of thigh, tibia and tarsus. Ventral surface of head, body and limbs smooth, large flattened tubercles densely arranged on the rear of thigh and around vent.

Measurement of holotype (in mm). SVL 36.3; HDL 13.2, HDW 12.0; SNT 5.6; IND 4.7; IOD 4.0; ED 4.2; TD 3.4; TED 1.1; HND 10.2; RAD 6.0; FTL 28.3; TIB 18.6.

Color in life of holotype. Dorsal surface of head and body light brown; light brown middorsal stripe edged with broad dark brown stripes on two sides from pineal ocellus to vent; several black spots on the top of tip of snout, upper eyelids and posterior dorsum of body; dorsolateral fold bicolor, red brown upper and black lower part; upper flank light brown with large black spots; lower flank yellowish white; suprabrachial gland pale brown, feebly tinged with pink. Dorsal forelimbs reddish brown, with a black crossbar on the lower arm; one black stripe in front of the base of forelimb; irregular black marks on lower arm and dorsal hand; dorsal hindlimbs non-uniform reddish brown, three black crossbars edged with light-colored on the thigh, two on the tibia and three on the tarsus; irregular black marks on dorsal toes. Dark brown stripe from tip of snout through nostril to anterior border of eye; tympanum and temporal region dark brown; pupil black, upper $\frac{1}{3}$ iris bright brownish white and lower $\frac{2}{3}$ iris dark red; lips yellowish white with dense tiny black specks; maxillary gland white. Ventral surface creamy white; faint dark stripes on the throat; rear thigh pale yellow, tinged with pink; ventral hand pale white with dense tiny black specks; ventral foot mottled with grey-brown.

Color in preservative of holotype. Dorsal surface faded lighter, but dark brown stripes on two sides of the mid-dorsal stripe more distinct; black spots on dorsum more distinct; limbs

Figure 5. Paratypes and tadpoles of *Nidirana nankunensis* sp. nov. (A) adult female paratype SYS a005717; (B) adult male paratype SYS a004905; (C) dorsolateral view of the 36th stage tadpole; (D) dorsolateral view of the 29th stage tadpole; (E) labial tooth row formula of the 29th stage tadpole.

faded light brown and the crossbars becoming clearer; ventral surface faded pale.

Variation. Measurements of type series are given in table 2. All specimens were similar in morphology and color pattern. Body size of two females (SYS a005717, 5718) are larger than that of males (minimum 37.8 mm vs. maximum 37.1 mm in males) and the skin around vent are more rough in the females. Mid-dorsal stripe extends to the tip of snout in SYS a003615, 3617, 3618, 5717 (fig. 5A), 5718. White horny spinules present above the vent in SYS a003618, 5717, 5718. Longitudinal dermal ridges on limbs not well developed in SYS a004905 (fig. 5B), 4906, 5717. Contorted mid-dorsal stripe and dark brown upper flank in SYS a004905.

Male secondary sexual characteristics. A pair of subgular vocal sacs, a pair of slit-like openings at posterior of jaw; a creamy white single nuptial pad prominent on the dorsal surface of first finger, nuptial spinules invisible; suprabrachial gland present.

Tadpole. Body oval, flattened above; snout rounded in dorsal aspect and profile; eyes lateral; labial tooth row formula: 1:1+1/1+1:2 (fig. 5E); spiracle on left side of body, directed dorsoposteriorly; vent tube long, dextral, attached to ventral fin; tail depth slightly larger than body depth; dorsal fin arising just before

Figure 6. (A) Habitat of *Nidirana nankunensis* sp. nov. in the type locality from Mt. Nankun; (B) The nest opening at the bottom of the pond; (C) The nest opening on the bank; (D) The holotype SYS a005719 in the wild.

origin of tail, maximum depth near mid-length, tapering gradually to narrow pointed tip; BL 16.3 mm and TL 36.1 mm in the 36th stage tadpole (fig. 5C); BL 7.1 mm and TL 14.5 mm in the 29th stage tadpole (fig. 5D).

Vocalization. Advertisement calls of *Nidirana nankunensis* sp. nov. were recorded on 9 April 2017 by ZTL from MNK at the air temperature 18°C. The call of *N. nankunensis* sp. nov. has a duration of 2.30-2.74 s (2.52 ± 0.17 s, $n = 11$) and consisted of 13-15 (14.0 ± 0.94, $n = 11$) fast-repeated notes. The PF of calls is 1406.2 Hz generally (91%). The IQR-BW of calls is 281.2 Hz and the BW-90% is 1312.5 Hz. The first note of a call is obviously different from others. The first note lasts for 108-135 ms (122.9 ± 8.4 ms, $n = 11$), with IQR-BW 375 Hz generally (82%) and BW-90% 1218.8 Hz generally (91%). The PF of the first note is equal to that of the call generally (73%). Other notes of the call each have a duration of 38-56 ms (46.1 ± 3.7 ms, $n = 50$) and the interval between them lasts for 112-166 ms (138.7 ± 10.5 ms, $n = 50$).

Distribution and ecology. Currently, *Nidirana nankunensis* sp. nov. is known only from the type locality, the Mt. Nankun in southern China. This frog appeared to be very rare which is found in only four unconnected ponds and under the threats of tourism development and road construction. The extent of occurrence is estimated to be less than 5000 km^2, and the area of occupancy is estimated to be less than 500 km^2.

It was only found in small ponds with sludge bottom and covered by plants (fig. 6A). From April to June, males call from the dusk to midnight, more active during rainfall. Females bear pigmented oocytes with white animal pole and brown vegetative pole brown in the oviduct on April. The frog was observed to have the behavior of nest construction for oviposition. The nest is a soil burrow, 60 mm of diameter and located at the bottom of the pond bank. Half of the burrow is filled with water. The burrow is connected by two narrow tunnels in opposite directions to two openings: the small one is 25 mm of diameter and open on the bank and exposed (fig. 6C); another one is 40 mm of diameter and open at the bottom of the pond for water diversion (fig. 6B).

Acknowledgements. We thank Si-Min Lin from National Taiwan Normal University for his help in providing images and videos of *Nidirana adenopleura* from Taiwan. We thank Yik-Hei Sung from Hong Kong Baptist University for his help in polishing the article. This work was partially supported by Project of Comprehensive Scientific Survey of Luoxiao Mountains Region (No. 2013FY111500) of Ministry of Science and Technology of P.R. China to Ying-Yong Wang.

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Submitted: July 2, 2017. Final revision received: September 6, 2017. Accepted: October 6, 2017.
Associate Editor: Matthias Stöck.

Appendix. Specimens examined

Nidirana caldwelli (*N. adenopleura*) (29): China: Fujian Province: Nanping City: Yanping District (type locality): SYS a005911-5916; Wuyishan City: Mt. Wuyi: SYS a005939-5943; Shaowu City: Jiangshi Nature Reserve: SYS a004112, 4132; Ninghua County: Mt. Yashu: SYS a005890-5891, 5901-5902; Jiangxi Province: Guangfeng County: Tongboshan Nature Reserve: SYS a001663-1665, 1667, 1698; Guixi City: Yangjifeng Nature

Reserve: SYS a0000317, 0334; Jinggangshan City: Jinggangshan Nature Reserve: SYS a004025-4027; Zhejiang Province: Jingning County: Dongkeng Town: SYS a002725-2726.

Nidirana daunchina (5): China: Sichuan Province: Emeishan City: Mt. Emei (type locality): SYS a004594-4595; Hejiang County: Zihuai Town: SYS a004930-4932.

Nidirana hainanensis (1): China: Hainan Province: Lingshui County: Mt. Diaoluo (type locality): SYS a003741.

Nidirana lini (4): China: Yunnan Province: Jiangcheng County: Hongjiang Town (type locality): SYS a003967-3970.

Nidirana pleuraden (4): China: Yunnan Province: Tengchong City: Mt. Gaoligong: SYS a003775-3778.