

Phylogenetic position of *Odorrana macrotympana* (Yang, 2008) (Anura, Ranidae) and extension of its geographical distribution

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http://zoobank.org/14210910-7D4B-4B80-85D0-76B70E939916

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Abstract

Based on a 16S rRNA gene fragment, a molecular phylogeny for the genus *Odorrana* Fei, Ye & Huang, 1990 was reconstructed, the validity of the poorly-known ranid species *O. macrotympana* (Yang, 2008) was confirmed and its phylogenetic position was evaluated. In addition, we report the first country record of *O. macrotympana* from Myanmar, based on our new records from Htamanthi Wildlife Sanctuary, Sagaing Division and present a supplementary description of this species. This report also constitutes the first record of *O. macrotympana* from outside of China.

Key Words

16S rRNA, China, Htamanthi Wildlife Sanctuary, morphology, Myanmar, new record, western Yunnan

Introduction

Odorrana macrotympana is a poorly-known ranid species described, based on only two specimens from Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China, close to the border with Myanmar (Yang and Rao 2008). This species was originally placed in the genus *Rana* Linnaeus, 1758 and was transferred into the genus *Odorrana* by Frost (2009).

Odorrana macrotympana most closely resembles *O. tiannanensis* (Yang & Li, 1980), but it is characterised by the following characters: lips uniform white without any spots or streaks, dorsal skin smooth and intermittent dorsolateral folds present, tympanum large and external vocal sac present in males (Yang and Rao 2008). *Odorrana macrotympana* was previously known only from the type locality and there have been few records of this species since its original description (Fei et al. 2009, 2012; AmphibiaChina 2021; Frost 2021).

As no molecular data were available for *Odorrana macrotympana* so far, the phylogenetic position of *O. macrotympana* has never been inferred and, thus, its

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systematic affinities remain unresolved. During our field surveys in Yunnan Province, China and in northern Myanmar from 2009 to 2021, we collected one topotype of *O. macrotympana* from its type locality, Yingjiang County, Dehong Prefecture, Yunnan Province, China; five specimens of *Odorrana* cf. *macrotympana* from Htamanthi Wildlife Sanctuary, northern Myanmar; and four topotypes of *O. tiannanensis* from its type locality, Hekou County, Honghe Prefecture, Yunnan Province, China, respectively. We collected DNA sequence data of these specimens in order to reconstruct a molecular phylogeny and evaluate the phylogenetic position of *O. macrotympana*.

In addition, our phylogenetic analysis of molecular data demonstrated that the specimens from northern Myanmar should be assigned to *Odorrana macrotympana*. Thus, this report constitutes the first record for the species outside China and from the country of Myanmar.

Materials and methods

Taxon sampling

Field survey in Yingjiang County, Yunnan Province, China, was conducted under permits from Tongbiguan Provincial Natural Reserve Management and Protection Bureau; field survey in Hekou County, Yunnan Province, China, was conducted under permits from Honghe Prefecture Forestry and Grassland Bureau of Yunnan Province; and the field survey in northern Myanmar was undertaken at the invitation of the Republic of the Union of Myanmar, Ministry of Natural Resources and Environmental Conservation, Forest Department, Forest Research Institute. After live photographs were taken, specimens were collected, euthanised with ethyl acetate and fixed in 75% ethanol for storage. Liver tissue samples were preserved in 99% ethanol for molecular analysis. The specimens from China were deposited in Kunming Institute of Zoology, Chinese Academy of Sciences (abbreviation: KIZ; address: Kunming, Yunnan, China) and specimens from Myanmar were deposited in Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (abbreviation: SEABRI; address: Yezin, Nay Pyi Taw, Myanmar).

Morphology

Measurements were taken with a digital caliper to the nearest 0.1 mm by Shuo Liu. We measured: snout-vent length (SVL, from tip of snout to vent); head length (HL, from tip of snout to rear of jaw); head width (HW, width of head at widest point); snout length (SL, from tip of snout to anterior border of eye); eye diameter (ED, diameter of exposed portion of eyeball); tympanum diameter (TD, maximum diameter of tympanum); forearm and hand length (FHL, from elbow to tip of third finger); hand length (HAL, from the base of the outer palmar tubercle to the tip of third finger); total leg length (LEG, from vent to tip of fourth toe); tibia length (TL, distance from knee to heel); and tarsus and foot length (TFL, from heel to the tip of fourth toe). Morphological data of the type specimens of *Odorrana macrotympana* were taken from Yang and Rao (2008).

Collection and analysis of molecular data

Total genomic DNA was extracted from liver tissues using the DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA). A fragment encoding mitochondrial 16S rRNA gene was amplified and sequenced. All new sequences have been deposited in GenBank, other sequences being downloaded from GenBank (Table 1). Sequences of Rana chensinensis David, 1875 and Pelophylax nigromaculatus (Hallowell, 1861) were used as outgroups (Chen et al. 2013; Feng et al. 2017; Yuan et al. 2019; Liu et al. 2021; Zhang et al. 2021). The primers L2188 (Matsui et al. 2006): 5'-AAAGTGGGCCTAAAAGCAGCCA-3' and 16H1 (Hedges 1994): 5'-CTCCGGTCTGAACTCAGATCAC-GTAGG-3' were used in amplification and cycle sequencing. Amplified DNA was produced in 25 µl reactions after 30 cycles of denaturation for 60 sec at 94 °C, annealing for 60 sec at 43–46 °C and extension for 1 min at 72 °C. PCR products were isolated through 1% agarose gel electrophoresis and further purified using Millipore Microcon Kits. Purified PCR products were sequenced by Davis Sequencing and sequences were edited and manually managed using SeqMan in Lasergene 7.1 (DNASTAR Inc., Madison, WI, USA) and MEGA X (Kumar et al. 2018).

Sequences were aligned using MAFFT 7 (Katoh and Standley 2013) with default parameters. The best substitution models were selected using the Bayesian Information Criterion (BIC) in ModelFinder (Kalyaanamoorthy et al. 2017) as implemented in IQ-TREE 1.6.12 (Nguyen et al. 2015). Bayesian Inference (BI) was performed in MrBayes 3.2.7 (Ronquist et al. 2012), based on the GTR+F+I+G4 substitution model and the Markov chains were run for 10,000,000 generations and sampled every 1,000 generations. Maximum Likelihood (ML) analysis was performed in IQ-TREE 1.6.12 (Nguyen et al. 2015), based on the TIM2+F+R4 substitution model and 1,000 bootstrap pseudoreplicates via the ultrafast bootstrap approximation algorithm were used to construct a final consensus tree. The genetic divergences (uncorrected p-distance) were calculated in MEGA X (Kumar et al. 2018).

Results

Phylogenetic analyses

The sequence alignment is 1095 bp in length. Topologies resulting from BI and ML analyses were consistent. The sequences of the specimens from Myanmar clustered with the sequence of our single topotype of *Odorrana macrotympana*; together, they represent a distinct lineage in the genus *Odorrana*, which is sister to *O. tiannanensis* with strong support (Fig. 1).

| Odorrana dabitaKe Sap, Xe Kong, Laos*FIMIL258109EU861542Odorrana animajensisTokmoshima, Ryaky, JapanKUIIF24635AB200947Odorrana animagensisAnlong, Guizbau, China*HINU10081109KF185049Odorrana bacboensisKhe Moi, Nghe An, Vietnam*FMNID25611DQ650569Odorrana bacboensisTaran Lap, Gia Lai, VietnamAMNI A161439DQ28372Odorrana chapaensisLai Chua, VietnamAMNI A161439DQ28372Odorrana chapaensisDarjeeing, Viest Bengal, India*BMNI 1947.2.28.6DQ650594Odorrana duiongensisDalongijang, Yunnan, China*RIX00502KF183056Odorrana duiongensisBulo B, Ha Giang, VietnamROM70052KF183056Odorrana grahaniGao B, Ba Giang, VietnamINNU2957 kKF183051Odorrana grahaniKurming, Yunnan, China*HINU10060123KF185051Odorrana grahaniKurming, Yunnan, China*HINU10060123KF185051Odorrana grahaniKurming, Yunnan, China*HINU10060123KF185052Odorrana hainanensisWuzhishan, Hainan, China*HINU10060123KF185052Odorrana hainanensisHuzishan, Hainan, China*HINU10060123KF185054Odorrana hainanensisJungan, Sichuan, China*HINU10060101KF185054Odorrana hainanensisJungan, Sichuan, China*HINU10060101KF185054Odorrana hainanensisJungan, Sichuan, China*HINU100607001KF185054Odorrana hainanensisJungan, Sichuan, China*HINU100607001KF185054 <tr< th=""><th>Species</th><th>Locality</th><th>Voucher No.</th><th>GenBank No.</th></tr<> | Species | Locality | Voucher No. | GenBank No. |
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| Odorrana animensisTokunoshima, Pyukyu, JapanKUHE24635AB200947Odorrana animegenisisAnlone, Guizbou, China*HNNU10081109KF185049Odorrana aureadaPhu Luang, Loci, Thailand*ZMKU AM 01137KT002162Odorrana bachoornanKhe Mo, Nghe An, Yietmam*ROM17472AF206487Odorrana choonanaDarjeeling, Weitmam*ROM17472AF206487Odorrana choonanaDarjeeling, Weit Bergal, India*BMNII 164139DQ281372Odorrana choonanaDarjeeling, Weit Bergal, India*BMNII 164139DQ281372Odorrana choonanaSa Pa, Lao Cai, VietnamROM19053AF206482Odorrana dolongensisDalioongjiang, Yumana, China*HNNU0607032KF185056Odorrana geminaaCao Bo, Ha Giang, VietnamANNI 165782EU861546Odorrana hejiangensisWuzhishan, Hainan, China*HNNU060105KF185052Odorrana hejiangensisWuzhishan, Hainan, China*HNNU1007102KF185059Odorrana hejiangensisKula Lampur, MalaysiaLBBHU 21004AB511284Odorrana hejiangensisWurjshan, Fujian, China*HNNU06006105KF185059Odorrana hejiangensisJingdong, Yuana, China*HNNU0600010KF185059Odorrana hangegangensisJungdong, Yuana, China*HNNU000000105KF185054 </td <td>Odorrana absita</td> <td>Xe Sap, Xe Kong, Laos*</td> <td>FMNH258109</td> <td>EU861542</td> | Odorrana absita | Xe Sap, Xe Kong, Laos* | FMNH258109 | EU861542 |
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| Odorrana ishkawaeAmarni Island, Ryukyu, JapanNo voucherAB511282Odorrana jingdongensisJingdong, Yunan, China*20070711017KF185050Odorrana kuangwuensisJunlian, Sichuan, China*HNNU002KF185034Odorrana kuangwuensisNanjiang, Sichuan, China*HNNU009811185KF185034Odorrana kuengwuensisShilam, Meitan, Guizbou, ChinaCIBGYU20130917004MH195348Odorrana lipuensisLipu, Guilin, Guangxi, China*HNNU10081099KF185036Odorrana lipuensisLipu, Guilin, Guangxi, China*HNNU10081099KF185036Odorrana lingshengensisLongsheng, Guangxi, China*HNNU70028KF185054Odorrana nacrotympanaYingjiang, Yunnan, China*HNNU70028KF185054Odorrana macrotympanaHamanthi, Sagaing, MyanmarSEABRI 2019120040OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831013Odorrana margoretaeEmei, Sichuan, China*HNNU20050032KF185035Odorrana nargiangensisNanjiang, Sichuan, China*HNNU1071291KF185047Odorrana narijangensisNanjiang, Sichuan, China*HNNU071291KF185047Odorrana narinaOkinawa Island, Ryukyu, JapanNo voucherAB511287Odorrana narinaOkinawa Island, Ryukyu, JapanNo voucherAB511287Odorrana narina< | Odorrana huanggangensis | Wuyishan, Fujian, China* | HNNU0607001 | KF185059 |
| Odorrana jingdongensisJingdong, Yunan, China*20070711017KF185050Odorrana junlianensisJunlian, Sichuan, China*HNNU002KF185058Odorrana kuangvuensisNanjiang, Sichuan, China*HNNU090811185KF185034Odorrana kweichowensisShilan, Meitan, Guizbou, ChinaCIBGYU20130917004MH193548Odorrana leporipesShaoguan, Guangdong, China*NHNU10081099KF185036Odorrana lipuensisLipu, Guilin, Guangxi, China*NHMG1306002KM388699Odorrana lingshengensisLongsheng, Guangxi, China*NHNU70028KF185054Odorrana nacrotympanaYingjiang, Yunan, China*KIZ 2009051020OL831010Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120041OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana marotfatiTram Lap, Gia Lai, VietnamROM7446AF206484Odorrana narigaretaeEmei, Sichuan, China*HNNU10071291KF185053Odorrana nasicaHa Tinh, VietnamAMNH A161169D02283345Odorrana nasicaWazhishan, Hainan, China*HNNU090811349KF185047Odorrana sugrahensisSangzhi, Hunan, China*HNNU09081349KF185047Odorrana sugraharina <td< td=""><td>Odorrana ishikawae</td><td>Amami Island, Ryukyu, Japan</td><td>No voucher</td><td>AB511282</td></td<> | Odorrana ishikawae | Amami Island, Ryukyu, Japan | No voucher | AB511282 |
| Odorrana junlianensisJunlian, Sichuan, China*HNNU002KF185038Odorrana kuangwuensisNanjiang, Sichuan, China*HNNU0908II185KF185034Odorrana kweichowensisShilian, Meitan, Guizhou, ChinaCIBGYU20130917004MH193548Odorrana lipuensisLipu, Guilin, Guangxi, China*HNNU10081099KF185036Odorrana lipuensisLipu, Guilin, Guangxi, China*HNNU10081099KF185054Odorrana linguensisLongsheng, Guangxi, China*BMNFI 1889.2.25.48DQ650615Odorrana nacrotympanaYingjiang, Yunnan, China*HNNU70028KF185054Odorrana macrotympanaYingjiang, Yunnan, China*KIZ 2009051020OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana nargaretaeEmei, Sichuan, China*HNNU10071291KF185035Odorrana narjiangensisNanjiang, Sichuan, China*HNNU10071291KF185042Odorrana nasitaWuzhishan, Hainan, China*HNNU00811349KF185047Odorrana nasitaMuzhishan, Huinan, China*HNNU00811349KF185034Odorrana sungzeinensisSangzhi, Huana, China*HNNU090811349KF185047Od | Odorrana jingdongensis | Jingdong, Yunan, China* | 20070711017 | KF185050 |
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| Odorrana kveichowensisShilian, Meitan, Guizhou, ChinaCIBGYU20130917004MH193548Odorrana leporipesShaoguan, Guangdong, China*HNNU10081099KF185036Odorrana linjuensisLipu, Guilin, Guangxi, China*NHMG1306002KM388699Odorrana lingshengensisLongsheng, Guangxi, China*NHNU70028KF185054Odorrana macrotympanaYingjiang, Yunnan, China*HNNU70028KF185054Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831010Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120041OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana marcotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana nargaretaeEmei, Sichuan, China*HNNU10071291KF185042Odorrana narijangensisNanjiang, Sichuan, China*HNNU10071291KF185042Odorrana nasicaHa Tinh, VietnamAMNH A161169DQ283345Odorrana nasutaWuzhishan, Hainan, China*HNNU10081119KF185047Odorrana sustaWuzhishan, Hainan, China*HNNU1090811349KF185047Odorrana sustaNantou, Taiwan, China*HNNUTW9KF185046Odorrana sustaNantou, Taiwan, China*HNNUTW9KF185046Odorrana supranarinaHekou, Yunnan | Odorrana kuangwuensis | Nanjiang, Sichuan, China* | HNNU0908II185 | KF185034 |
| Odorrana leporipesShaoguan, Guangdong, China*HNNU10081099KF185036Odorrana lipuensisLipu, Guilin, Guangxi, China*NHMG1306002KM388699Odorrana lingshengensisLongsheng, Guangxi, China*BMNH 1889.2.25.48DQ650615Odorrana lungshengensisLongsheng, Guangxi, China*HNNU70028KF185054Odorrana macrotympanaYingjiang, Yunnan, China*KIZ 2009051020OL831010Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120041OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120041OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120041OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana margaretaeEmei, Sichuan, ChinaHNNU20050032KF185035Odorrana narigangensisNanjiang, Sichuan, China*HNNU10071291KF185042Odorrana nariaOkinawa Island, Ryukyu, JapanNo voucherAB511287Odorrana nasitaWukihshan, Hinanan, China*HNNU007119KF185053Odorrana sangzhiensisSangzhi, Hunan, China*HNNU00811349KF185057Odorrana sustaWukihshan, Hinana, China*HNNU09811349KF185047Odorrana sustaVichang, Hubei, China*HNNU09811349KF185047Odorrana sustaIriomotejima, Ryukyu, JapanNHMG1303018KT315390Odorrana supranarinaIri | Odorrana kweichowensis | Shilian, Meitan, Guizhou, China | CIBGYU20130917004 | MH193548 |
| Odorrana lipuensisLipu, Guilin, Guangxi, China*NHMG1306002KM388699Odorrana lividaThagata Juwa, Myanmar*BMNH 1889.2.25.48DQ650615Odorrana lungshengensisLongsheng, Guangxi, China*HNNU70028KF185054Odorrana macrotympanaYingijang, Yunnan, China*KIZ 2009051020OL831010Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana margaretaeEmei, Sichuan, China*HNNU10007291KF185035Odorrana nanjiang, Sichuan, China*HNNU1001291KF185042Odorrana nariaOkinawa Island, Ryukyu, JapanNo voucherAB511287Odorrana nasicaHa Tinh, VietnamAMNH A161169DQ283345Odorrana sangzhiensisSangzhi, Hunan, China*HNNU051119KF185053Odorrana sangzhiensisSangzhi, Hunan, China*HNNU05119KF185047Odorrana sangzhiensisSangzhi, Hunan, China*HNNU051119KF185047Odorrana supranarinaIriomotejima, Ryukyu, JapanKUHE2898AB200950Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274OL831007Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274 | Odorrana leporipes | Shaoguan, Guangdong, China* | HNNU1008I099 | KF185036 |
| Odorrana lividaThagata Juwa, Myanmar*BMNH 1889.2.25.48DQ650615Odorrana lungshengensisLongsheng, Guangxi, China*HNNU70028KF185054Odorrana macrotympanaYingjiang, Yunnan, China*KIZ 2009051020OL831010Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana margaretaeEmei, Sichuan, ChinaHNNU20050032KF185035Odorrana nanjiangensisNanjiang, Sichuan, China*HNNU10071291KF185042Odorrana nasicaHa Tinh, VietnamAMNH A161169DQ283345Odorrana nasicaHa Tinh, VietnamAMNH A161169DQ283345Odorrana sughiensisSangzhi, Hunan, China*HNNU090811349KF185037Odorrana sughiensisSangzhi, Hunan, China*HNNU090811349KF185047Odorrana sughiensisSangzhi, Hunan, China*HNNU1909811349KF185047Odorrana sughiensisHekou, Yunnan, China*KIZ20193272OL831009Odorrana tiannanensisHekou, Yunnan, China*KIZ20193273OL831008Odorrana tiannanensisHekou, Yunnan, China*KIZ20193273OL831007Odorrana tiannanensisHekou, Yunnan, China*KIZ | Odorrana lipuensis | Lipu, Guilin, Guangxi, China* | NHMG1306002 | KM388699 |
| Odorrana lungshengensisLongsheng, Guangxi, China*HNNU70028KF185054Odorrana macrotympanaYingijang, Yunnan, China*KIZ 2009051020OL831010Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120041OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana margaretaeEmei, Sichuan, China HNNU20050032KF185035Odorrana nanjiangensisNanjiang, Sichuan, China*HNNU10071291KF185042Odorrana narinaOkinawa Island, Ryukyu, JapanNo voucherAB511287Odorrana nasicaHa Tinh, VietnamAMNH A161169DQ283345Odorrana sangzhiensisSangzhi, Hunan, China*HNNU051119KF185053Odorrana suitaWuzhishan, Hainan, China*HNNU090811349KF185046Odorrana svinhoanaNantou, Taiwan, China*HNNUTW9KF185046Odorrana svinhoanaNantou, Taiwan, China*NHMG1303018KT315390Odorrana tiannanensisHekou, Yunnan, China*KIZ20193272OL831009Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274OL831006Odorrana tiannanensisHekou, Yunnan, China* | Odorrana livida | Thagata Juwa, Myanmar* | BMNH 1889.2.25.48 | DQ650615 |
| Odorrana macrotympanaYingjiang, Yunnan, China*KIZ 2009051020OL831010Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120041OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaTram Lap, Gia Lai, VietnamROM7446AF206484Odorrana nanjiangensisNanjiang, Sichuan, China*HNNU10071291KF185042Odorrana narinaOkinawa Island, Ryukyu, JapanNo voucherAB511287Odorrana nasutaOkinawa Island, Ryukyu, JapanNo voucherAB511287Odorrana asutaWuzhishan, Hainan, China*HNNU051119KF185053Odorrana sugzhiensisSangzhi, Hunan, China*HNNU0908II349KF185047Odorrana sugranarinaIriomotejima, Ryukyu, JapanKUHE2898AB200950Odorrana swinhoanaNantou, Taiwan, China*HNNU190908II349KF185046Odorrana tiannanensisHekou, Yunnan, China*KII220193272OL831009Odorrana tiannanensisHekou, Yunnan, China*KII220193274OL831007Odorrana tiannanensisHekou, Yunnan, China*KII220193274OL831007Odorrana tiannanensisHekou, Yunnan, China*No voucherD831006Odorrana tiannanensisHekou, Yunnan | Odorrana lungshengensis | Longsheng, Guangxi, China* | HNNU70028 | KF185054 |
| Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120040OL831011Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831012Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120072OL831013Odorrana macrotympanaHtamanthi, Sagaing, MyanmarSEABRI 2019120073OL831014Odorrana macrotympanaNanjiang, Sichuan, China*HNNU10003032KF185035Odorrana nasicaNanjiang, Sichuan, China*HNNU051119KF185053Odorrana sangchiensisSangzhi, Hunan, China*CSUFT 4305220051MW4648655Odorrana sugnanarinaIriomotejima, Ryukyu, JapanKUHE2898AB200950Odorrana sugnanarinaIriomotejima, Ryukyu, JapanKUHE2898AB200950Odorrana tiannanensisHekou, Yunnan, China*NHMG1303018KT315390Odorrana tiannanensisHekou, Yunnan, China*KIZ20193272OL831008Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274OL831007 </td <td>Odorrana macrotympana</td> <td>Yingjiang, Yunnan, China*</td> <td>KIZ 2009051020</td> <td>OL831010</td> | Odorrana macrotympana | Yingjiang, Yunnan, China* | KIZ 2009051020 | OL831010 |
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| Odorrana tiannuliItanmushan, Zhejiang, China*NHMG1303018R1315390Odorrana tiannanensisHekou, Yunnan, China*KIZ20193272OL831009Odorrana tiannanensisHekou, Yunnan, China*KIZ20193273OL831008Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274OL831007Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274OL831006Odorrana tiannanensisHekou, Yunnan, China*KIZ20215191OL831006Odorrana tiannanensisHekou, Yunnan, China*SCUM50510CHXEF453751Odorrana tormotaHuangshan, Anhui, China*No voucherDQ835616Odorrana trankieniTa Xua, Son La, Vietnam*VNMN04035KX893900Odorrana utsunomiyaorumIriomotejima, Ryukyu, JapanKUHE12896AB200952 | Odorrana swinnoana | Nantou, Taiwan, China | HNNU1W9 | KF185046 |
| Odorrana tiannanensisHekou, Yunnan, China*KIZ20193272OL831009Odorrana tiannanensisHekou, Yunnan, China*KIZ20193273OL831008Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274OL831007Odorrana tiannanensisHekou, Yunnan, China*KIZ2015191OL831006Odorrana tiannanensisHekou, Yunnan, China*SCUM50510CHXEF453751Odorrana tiannanensisHekou, Yunnan, China*No voucherDQ835616Odorrana trankieniTa Xua, Son La, Vietnam*VNMN04035KX893900Odorrana utsunomiyaorumIriomotejima, Ryukyu, JapanKUHE12896AB200952 | Odorrana tianmuii | Tianmushan, Zhejiang, China* | NHMG1303018 | K1315390 |
| Odorrana tiannanensisHekou, Yunnan, China*KIZ20193273OL831008Odorrana tiannanensisHekou, Yunnan, China*KIZ20193274OL831007Odorrana tiannanensisHekou, Yunnan, China*KIZ20215191OL831006Odorrana tiannanensisHekou, Yunnan, China*SCUM50510CHXEF453751Odorrana tormotaHuangshan, Anhui, China*No voucherDQ835616Odorrana trankieniTa Xua, Son La, Vietnam*VNMN04035KX893900Odorrana utsunomiyaorumIriomotejima, Ryukyu, JapanKUHE12896AB200952 | Odorrana tiannanensis | Hekou, Yunnan, China* | KIZ20193272 | OL831009 |
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| Odorrana tormotaHuangshan, Anhui, China*No voucherDQ835616Odorrana trankieniTa Xua, Son La, Vietnam*VNMN04035KX893900Odorrana utsunomiyaorumIriomotejima, Ryukyu, JapanKUHE12896AB200952Olama kitikaKatika Katika Kat | Odorrana tiannanensis | Hekou, Yunnan, China* | SCUM50510CHX | EF453751 |
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| | Odorrana utsunomiyaorum | Iriomotejima, Ryukyu, Japan | KUHE12896 | AB200952 |
| <i>Odorrana versabilis</i> Leishan, Guizhou, China* HNNU003 LS KF185055 | Odorrana versabilis | Leishan, Guizhou, China* | HNNU003 LS | KF185055 |
| Odorrana wuchuanensis Wuchuan, Guizhou, China* HNNU019 L KF185043 | Odorrana wuchuanensis | Wuchuan, Guizhou, China* | HNNU019 L | KF185043 |
| Odorrana yentuensis Tay Yen Tu, Bac Giang, Vietnam* IEBRA.2015.38 KX893891 | Odorrana yentuensis | Tay Yen Tu, Bac Giang, Vietnam* | IEBRA.2015.38 | KX893891 |
| Odorrana vizhangensis Yizhang, Hunan, China* HNNU10081075 KF185048 | Odorrana yizhangensis | Yizhang, Hunan, China* | HNNU1008I075 | KF185048 |
| Odorrana yunnanensis Longchuan, Yunnan, China* HNNU001YN KF185057 | Odorrana yunnanensis | Longchuan, Yunnan, China* | HNNU001YN | KF185057 |
| Pelophvlax nigromaculatus Aichi, Japan No voucher LC389208 | Pelophylax nigromaculatus | Aichi. Japan | No voucher | LC389208 |
| Rana chensinensis Ningshan, Shanxi, China HNNU20060359 KF185061 | Rana chensinensis | Ningshan, Shanxi, China | HNNU20060359 | KF185061 |

Genetic distances

Genetic divergence (uncorrected p-distance) between the specimens from Myanmar and the topotype of *Odorrana macrotympana* was only 0.43% (Suppl. material 1: Table S1). Therefore, we considered the Myanmar population to be conspecific with *O. macrotympana*. Genetic divergence (uncorrected p-distance) between *O. macrotympana* and other species of *Odorrana* ranged from 9.51% to 16.87% (Suppl. material 1: Table S1), suggesting that *O. macrotympana* is a valid species.



Figure 1. Phylogenetic tree of *Odorrana*, inferred from BI analysis of a 1095 bp fragment of the 16S rRNA. Numbers before slashes indicate Bayesian posterior probabilities (≥ 0.90) and numbers after slashes indicate bootstrap support from a separate ML analysis (≥ 90).

Taxonomic accounts

Odorrana macrotympana (Yang, 2008)

Suggested common English name "Large Eared Odorous Frog" Figs 2–6 and 8B

Holotype. KIZ 94001, adult female.

Paratype. KIZ 94002, adult male.

Type locality. Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China.

Other materials. KIZ 2009051020, One adult female, collected by Dingqi Rao from Xueli Village, Taiping Town, Yingjiang County, Dehong Prefecture, Yunnan Province, China (24°26'29"N, 97°33'42"E, 330 m elevation), on 10 May 2009. SEABRI 2019120040, one adult male and SEABRI 2019120041, one adult female, collected by Shuo

Liu from Nam Pa Gon section, Htamanthi Wildlife Sanctuary, Sagaing Division, Myanmar (25°19'3"N, 95°31'43"E, 140 m elevation), on 2 December 2019. SEABRI 2019120072 and SEABRI 2019120074, two adult males and SEABRI 2019120073, one adult female, collected by Shuo Liu from Nam E Zu section, Htamanthi Wildlife Sanctuary, Sagaing Division, Myanmar (25°28'8"N, 95°38'57"E, 130 m elevation), on 9 December 2019.

Description of the specimens from Myanmar. Measurements and the comparison with the type specimens are represented in Table 2. Habitus moderately slender, SVL 56.7–59.1 mm in males and 91.6–111.2 mm in females; head width slightly shorter than length (HW/HL 0.89–0.97); snout obtuse, pointed in dorsal view and rounded in profile, obviously projecting beyond lower jaw; position of nostril dorsolateral, closer to snout than



Figure 2. Type specimens of *Odorrana macrotympana* in preservative: **A.** Dorsal and **B.** Ventral, view of the holotype (KIZ 94001, female); **C.** Dorsal and **D.** Ventral, view of the paratype (KIZ 94002, male); **E.** Lateral view of the head of the holotype (KIZ 94001, female); **F.** Lateral view of the head of the paratype (KIZ 94002, male). Photo by Shuo Liu.

eye; canthus rostralis distinct; loreal region concave and vertical; internarial distance greater than interorbital distance; snout length greater than eye diameter; pineal body distinct; supratympanic fold horizontal and straight; tympanum very large in males (TD/ED 0.71–0.78) and relatively small in females (TD/ED 0.56–0.62), round and transparent; vomerine teeth distinct; choanae close to the vomerine teeth. Tongue cordiform, posterior notch

Table 2. Measurements (mm) of *Odorrana macrotympana*. See text for character definitions. Data of the type specimens were obtained from the original description (Yang and Rao 2008). Notes: HAL of holotype, originally reported as "26 mm" is parenthetically included, along with the true value (16 mm).

| | KIZ 94001 | KIZ 94002 | SEABRI | SEABRI | SEABRI | SEABRI | SEABRI |
|-----|-----------|-----------|------------|------------|------------|------------|------------|
| | Holotype | Paratype | 2019120040 | 2019120041 | 2019120072 | 2019120073 | 2019120074 |
| | Female | Male | Male | Female | Male | Female | Male |
| SVL | 95 | 50 | 56.8 | 111.2 | 59.1 | 91.6 | 56.7 |
| HL | 35.5 | 20 | 22.3 | 40.6 | 24.8 | 33.4 | 23.8 |
| HW | 32 | 17 | 21.2 | 37.2 | 22.0 | 32.5 | 22.2 |
| SL | 15 | 8 | 10.1 | 18.2 | 10.3 | 15.6 | 10.8 |
| ED | 13 | 7.5 | 7.9 | 11.7 | 8.2 | 9.2 | 8.0 |
| TD | 6 | 5.5 | 5.6 | 6.6 | 5.8 | 5.7 | 6.2 |
| FHL | 41.5 | 24 | 28.5 | 50.6 | 29.7 | 45.2 | 27.2 |
| HAL | 16 (26) | 15.5 | 16.4 | 27.7 | 18.6 | 26.5 | 17.9 |
| LEG | 185 | 100 | 117.0 | 219.0 | 121.0 | 195.5 | 117.0 |
| TL | 63.5 | 32.5 | 36.2 | 68.7 | 38.5 | 60.2 | 35.9 |
| TFL | 75.5 | 42.5 | 46.2 | 84.5 | 49.7 | 76.5 | 46.3 |



Figure 3. Adult female topotype of *Odorrana macrotympana* from its type locality: A. Dorsal view; B. Ventral view. Photo by Xiaofeng Ma and Hong Hui.



Figure 4. Specimens of Odorrana macrotympana from Myanmar in preservative: A. Dorsal view; B. Ventral view. Photo by Shuo Liu.



Figure 5. Live colour variation in *Odorrana macrotympana* from Htamanthi Wildlife Sanctuary, Sagaing Division, Myanmar: A, B, C. Adult males; D. Subadult female; E, F. Adult females. Photo by Shuo Liu.

enlarged and formed as U-shaped; vocal sac openings visible on floor of mouth, in each corner; paired external vocal sacs present in males.

Forelimbs robust in males and relatively weak in females; relative lengths of fingers III > IV > I > II; all finger tips slightly expanded; lateral fringes and webbing on fingers absent; subarticular tubercle round and prominent; inner metacarpal tubercle and outer metacarpal tubercle distinct; greyish-yellow glandular nuptial pad on finger I in males. Hind limbs long, tibia slightly longer than thigh; toes long and thin, relative lengths IV > V > III > II > I; all toe tips slightly expanded; entire webbing; subarticular tubercles prominent and longitudinally ovoid; inner metatarsal tubercle prominent and oval; outer metatarsal absent.

Dorsal skin smooth, lateral skin granular, ventral skin smooth. Dorsolateral folds distinct.

Colouration in life. Dorsum greyish-brown, brownish-green or solid green with a few or many tiny black



Figure 6. Live ventral colour variation in *Odorrana macrotympana* from Myanmar: A, B. Adult males; C. Subadult female; D. Adult female. Photo by Shuo Liu.

spots and several distinct or indistinct large black or brown spots. Upper and lower lips greyish-white, yellow, light green or brown with a few or many brown or black spots. Lateral side of head and body greyish-brown with many large or tiny black spots. Dorsal surfaces of limbs greyish-brown with some brown or black bands. Ventral surface of throat, chest and belly uniform white or some stains on ventral surface of throat, chest and anterior part of belly. Ventral surface of limbs white or pink. Tympanum brown, iris greyish-brown, pupil black. **Colouration in preservative.** In preservative, dorsum fades to greyish-black. Ventral surface of throat, chest, belly and limbs pale white. Iris black, pupil white.

Sexual dimorphism. Body size much smaller in adult males than in adult females, adult males have relatively stouter forelimbs than females and greyish-yellow glandular nuptial pad on finger I, external vocal sacs present in adult males.

Natural history notes. In Myanmar, all specimens of *Odorrana macrotympana* were found on the banks of



Figure 7. Type locality in western Yunnan, China (red star) and new collection sites in northern Myanmar (red dots) summarising all known localities for *Odorrana macrotympana*.

large rivers. During December, we heard the courtship calls from males on the river banks at night, the sound of the calls being similar to those of other *Odorrana* species. We also observed males and females in amplexus on the banks of the rivers. No eggs or tadpoles were found.

Discussion

According to Yang and Rao (2008), Odorrana macrotympana is a distinct species and most closely resembles O. tiannanensis. Our molecular analyses revealed that O. macrotympana was a sister lineage of O. tiannanensis (Fig. 1) and the genetic divergence between O. macrotympana and O. tiannanensis was 9.51% (Suppl. material 1: Table S1). This study agrees with Yang and Rao (2008) that O. macrotympana is a valid species and closely related to O. tiannanensis, although they are distributed far apart (the distance between the type localities of O. macrotympana and O. tiannanensis is approximately 660 km).

Previously, Odorrana macrotympana was known only from its type locality (Yang and Rao 2008; Fei et al. 2009, 2012; AmphibiaChina 2021; Frost 2021), located by the Dayingjiang River, a tributary of the Ayeyarwady River. Here, we report collections of this species from Htamanthi Wildlife Sanctuary, northern Myanmar, located by the Chindwin River, also a tributary of the Ayeyarwady River. Our finding significantly extended the known distribution of this species, indicating that this species is widely distributed in the Ayeyarwady River system. The new Myanmar record is approximately 230 km from the type locality in China. Additionally, within the Htamanthi Wildlife Sanctuary, Myanmar, O. macrotympana was quite abundant (we observed a large population at ~ 140 m elevation). However, at the type locality in China



Figure 8. Habitat of *Odorrana macrotympana* in A. The Htamanthi Wildlife Sanctuary, Myanmar; B. Male and female in amplexus. Photo by Shuo Liu.

(\sim 300 m elevation), the species is not abundant and the original population is comparatively small. We speculate that western Yunnan forms the edge of its distribution and northern Myanmar may be closer to the centre of this species' distribution.

Morphologically, the characters of the specimens from northern Myanmar mostly agree with the original description of *Odorrana macrotympana* apart from a few differences. According to the original description of *O. macrotympana*, the dorsum is greyish-brown and upper and lower lips are uniform white without any spots or streaks (Yang and Rao 2008). However, dorsal surfaces of specimens from northern Myanmar vary from greyishbrown to brownish-green or solid green and lips vary from greyish-white to yellow, light green or brown with variable dark spots. Thus, dorsal and labial colour characteristics, originally proposed as diagnostic characters for this species, may be more variable (and non-diagnostic) than originally reported (Yang and Rao 2008).

The type specimens of *Odorrana macrotympana* were collected in December 1994 and females have been observed to deposit eggs in February of the following year (Yang and Rao 2008). We heard male courtship calls and observed pairs in amplexus during December in northern Myanmar, which is basically consistent with original reports from China (Yang and Rao 2008). It is unknown

whether this species also reproduces during months outside of this (December–February) range.

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Supplementary material 1

Table S1

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Data type: Table (MS EXCEL file)

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