



An Updated Distribution Map and a New Elevational Record of the Coastal Bullfrog (*Hoplobatrachus litoralis*)

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The Coastal Bullfrog (*Hoplobatrachus litoralis*) was first described by Hasan et al. (2012) from the coastal belt of Bangladesh (Cox’s Bazaar District) and has since been recorded from India (West Bengal, Assam, Tripura, and Mizoram), Bhutan, and Myanmar (Mondal et al. 2018; Purkayastha and Besak 2018; Bohra et al. 2019; Kundu et al. 2020; Wangyal et al. 2020). The recorded elevational range extends from 5 m asl in Bangladesh to 346 m asl in Bhutan (Al-Razi et al. 2017; Wangyal et al. 2020).



Fig. 2. An adult male Coastal Bullfrog (*Hoplobatrachus litoralis*) from Tanhril, Aizawl, Mizoram. Photograph by H. T. Lalremsanga.

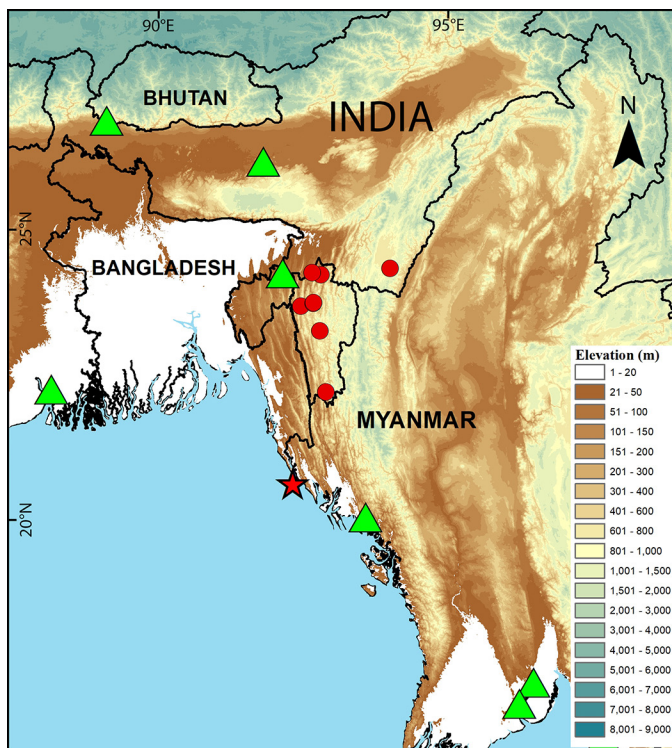


Fig. 1. Map showing distribution of the Coastal Bullfrog (*Hoplobatrachus litoralis*). The red star marks the type locality, red dots indicate the new records, and green triangles designate previously published records.

We conducted herpetofaunal Visual Encounter Surveys (VES) from January 2019 to December 2021 throughout the state of Mizoram and Chandel District in Manipur (Fig. 1). We collected eight *H. litoralis* (three males and five females) (Fig. 2) from the Dampa Tiger Reserve, Mamit District (23°41'16.59"N, 92°27'17.91"E; elev. 275 m asl); Thenzawl, Serchhip District (23°15'42.55"N, 92°47'11.94"E; 754 m asl); Pualreng Wildlife Sanctuary, Kolasib District (24°13'47.60"N, 92°48'18.08"E; elev. 670 m asl); Kolasib Zero Point, Kolasib District (24°15'42.37"N, 92°39'10.99"E; elev. 64 m asl); the Mizoram University campus, Aizawl District (23°44'4.87"N, 92°40'2.36"E; elev. 851 m asl); Tanhril, Aizawl District (23°44'35.06"N, 92°40'30.44"E; elev. 925 m asl); and the Palak National Wetland, Siaha District (22°12'17.55"N, 92°53'11.66"E; elev. 273 m asl) in Mizoram and Panchai Village, Chandel

District, in Manipur (24°20'14.76"N, 93°59'49.29"E; elev. 917 m asl). Specimens were deposited in the Departmental Museum of Zoology, Mizoram University (MZMU). Based on morphological characters identified in the original description (Hasan et al. 2012), all were tentatively identified as *H. litoralis* and this was later confirmed by Jayaditya Purkayastha.

Morphometric measurements follow Hasan et al. (2012). We extracted whole genomic DNA from liver tissue of MZMU-1777 according to the manufacturer's protocol (QIAamp DNA Mini Kit, Cat No.ID: 51306). Partial fragments of the 16S rRNA mitochondrial gene were amplified using 16S rRNA forward (L02510 - CGC CTG TTT ATC AAA AAC AT) (Palumbi 1996) and reverse (H03063 - CTC CGG TTT GAA CTC AGA TC) (Rassmann 1997) primers and sequenced at Barcode Bioscience, Bangalore, India. We aligned sequences using MUSCLE (MEGA7) and calculated K2P distances and reconstructed maximum likelihood (ML) analyses in MEGA-7 with 1,000 bootstrap replications (Kumar et al. 2016). We also estimated a Bayesian Inference (BI) tree in MrBayes 3.2.7 using the GTR+G model (Ronquist et al. 2012). We visualized the phylogenetic tree (Fig. 3) using the web-based iTOL software (Letunic and Bork 2007).

The appearance and measurements of the frogs we collected (Table 1) matched the diagnostic features for *H. litoralis* (Hasan et al. 2012). In addition, we compared the

newly generated partial 16S rRNA gene sequence of *H. litoralis* (MZMU-1777) (deposited in GenBank with accession number OM501581) with 15 congeneric sequences and that of *Leptobrachella parva* (KJ831304) as an out-group retrieved from the NCBI database. After all positions containing gaps and missing data were eliminated, a total of 515 positions remained in the final dataset. Both BI and ML analyses yielded significant relationships within the study species and its congener with well-supported values (Table 2). From the estimated K2P genetic distances we determined that the genetic distance between MZMU-1777 and *H. litoralis* (AB671181) from Cox's Bazaar, Bangladesh, was 0%. *Hoplobatrachus litoralis* formed a clade with high (BI/BS = 0.97/94) support values that is most closely related to *H. tigerinus* (AB272594) with a genetic distance of 0.026%.

A male (MZMU-2576), collected from Panchai Village, Chandel District, Manipur (917 m asl) is the first report for the state and extends the range of the species 447 km NE of the type locality. The site (Tanhril, Mizoram; 925 m asl) where we collected another male (MZMU-1848) exceeds the previous elevational record of 346 m asl from Bhutan (Wangyal et al. 2020). That collection site lies in the fringe area of a local playground, and bullfrogs were encountered in the ditches covered with grasses during July and August of the monsoon season.

Sympatric anuran species found during the surveys include *Duttaphrynus melanostictus*, *Fejervarya multistriata*, *Hoplobatrachus tigerinus*, *Minervarya asmata*, *Polypedates teraiensis*, *Raorchestes rezakhani*, and *Zhangixalus smaragdinus*. *Hoplobatrachus litoralis* and its congener, *H. tigerinus*, can easily be distinguished by the presence of a prominent band from anterior eye to snout through nostril in *H. litoralis* versus a less prominent and discontinuous band in *H. tigerinus* (Hasan et al. 2012). Vegetation of the surveyed areas is tropical semi-evergreen forest associated with moist deciduous forest corresponding to the semi-evergreen 2B/C2 forest type (Champion and Seth 1968). The survey also revealed that *H. litoralis* and sympatric frogs are confronted with the threat of illegitimate hunting and trapping for consumption as food and use as medicine by local residents.

Acknowledgements

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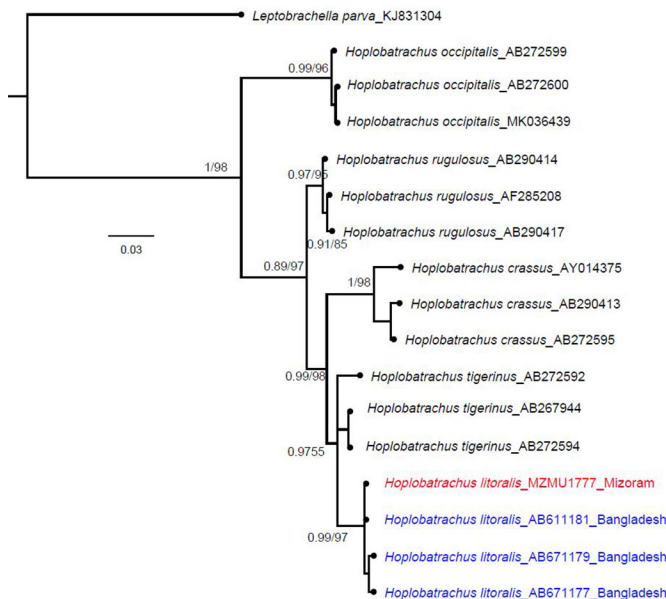


Fig. 3. Bayesian phylogram of *Hoplobatrachus* inferred from 16S rRNA sequences (GenBank accession numbers follow the name). Numbers at tree nodes indicate BI/BS support values, respectively; values >90 are considered well supported whereas values <90 indicate moderate-support. The Coastal Bullfrog (*H. litoralis*) (MZMU-1777; GenBank accession no. OM501581) indicated by the red font is from the Pualreng Wildlife Sanctuary, Kolasib District, Mizoram, and those marked by the blue font represent material from the type locality in Bangladesh (Hasan et al. 2012).

Table 1. Measurements (in mm) of Coastal Bullfrogs (*Hoplobatrachus litoralis*) from Mizoram, India. Abbreviations: SVL = snout-vent length; HL = head length; HW = head width; ED = eye diameter; EN = eye-nostril distance; NS = nostril-snout distance; SL = snout to eye distance; UEW = upper eyelid width, IOD = inter-orbital distance; IN = inter-nostril distance; TYD = tympanum diameter; TYE = tympanum-eye distance; AG = axilla-groin distance (posterior base of forelimb to anterior base of hindlimb); FLL = forelimb length; HAL = hand length; F1–F4 = length of fingers; FL = femur length; TL = tibia length; TFOL = tibia-foot length; FOL = foot length; T1–T5 = length of toes.

| Locality MZMU No. | Dampa Tiger | | Pualreng Wildlife | | | | | |
|----------------------|-----------------|-----------------|-------------------|-------------------|-----------------|---------------|-----------------|------------------|
| | Kolasib 2701 | Reserve 1744 | MZU 1849 | Sanctuary 1777 | Tanhril 1848 | Palak 1748 | Chandel 2576 | Thenzawl 2702 |
| Sex | Female | Female | Female | Male | Male | Female | Male | Female |
| SVL | 71.53 | 57.01 | 69.11 | 88.35 | 53.73 | 61.94 | 66.89 | 70.21 |
| HL | 30.88 | 23.26 | 28.79 | 34.56 | 23.86 | 28.41 | 28.72 | 29.80 |
| HW | 25.64 | 21.74 | 23.65 | 31.27 | 20.08 | 23.32 | 23.42 | 24.32 |
| ED | 6.94 | 5.81 | 6.37 | 8.09 | 4.73 | 6.31 | 6.41 | 7.09 |
| EN | 6.61 | 5.25 | 6.15 | 7.62 | 4.32 | 6.07 | 6.17 | 6.76 |
| NS | 6.46 | 3.72 | 5.22 | 6.94 | 3.67 | 5.09 | 5.15 | 6.23 |
| SL | 14.03 | 9.25 | 11.66 | 15.72 | 9.12 | 10.92 | 10.99 | 12.07 |
| UEW | 5.45 | 4.33 | 4.95 | 6.11 | 4.32 | 4.68 | 4.83 | 5.62 |
| IOD | 3.74 | 2.64 | 3.61 | 5.14 | 2.46 | 3.58 | 3.71 | 4.25 |
| IN | 5.32 | 3.12 | 4.63 | 5.65 | 3.09 | 4.16 | 4.28 | 4.65 |
| TYD | 6.27 | 4.26 | 4.54 | 6.41 | 3.58 | 4.38 | 4.41 | 5.12 |
| TYE | 3.18 | 2.72 | 3.07 | 3.64 | 2.15 | 2.97 | 2.98 | 3.13 |
| AG | 19.99 | 16.48 | 19.62 | 24.28 | 17.42 | 18.45 | 18.28 | 20.54 |
| FLL | 15.30 | 10.70 | 12.45 | 17.26 | 10.53 | 12.43 | 12.42 | 14.46 |
| HAL | 15.75 | 12.02 | 16.11 | 18.32 | 10.79 | 15.15 | 15.76 | 16.09 |
| F1 | 14.72 | 9.46 | 11.96 | 16.31 | 9.61 | 11.51 | 11.77 | 12.33 |
| F2 | 12.98 | 8.96 | 10.48 | 14.12 | 7.79 | 10.23 | 10.82 | 11.87 |
| F3 | 15.75 | 12.02 | 14.11 | 18.32 | 10.36 | 13.82 | 13.83 | 14.54 |
| F4 | 13.82 | 9.37 | 11.61 | 16.22 | 8.78 | 11.47 | 11.49 | 11.97 |
| FL | 40.39 | 26.09 | 32.44 | 43.93 | 25.53 | 31.89 | 32.12 | 35.68 |
| TL | 46.15 | 29.78 | 35.21 | 46.40 | 27.46 | 33.12 | 34.71 | 36.17 |
| TFOL | 60.24 | 43.36 | 50.16 | 69.91 | 38.91 | 48.71 | 49.81 | 50.23 |
| FOL | 40.52 | 29.27 | 33.32 | 44.77 | 27.46 | 32.75 | 32.93 | 35.18 |
| T1 | 8.89 | 8.18 | 8.43 | 10.22 | 7.26 | 8.40 | 8.42 | 9.15 |
| T2 | 14.73 | 11.66 | 13.52 | 17.57 | 10.78 | 12.56 | 13.07 | 14.86 |
| T3 | 25.23 | 18.49 | 21.64 | 30.34 | 17.57 | 20.17 | 21.08 | 23.67 |
| T4 | 33.12 | 25.12 | 29.91 | 41.32 | 22.71 | 27.53 | 28.76 | 30.11 |
| T5 | 24.41 | 17.61 | 19.54 | 29.69 | 15.13 | 18.63 | 19.21 | 21.64 |

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Table 2. The uncorrected K2p distance using a 16S rRNA partial gene sequence of a Coastal Bullfrog (*Hoplobatrachus litoralis*) (MZMU-1777; GenBank accession no. OM501581) and 15 congeneric sequences and that of *Leptobrachella parva* as an outgroup (retrieved from NCBI database).

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1 <i>Hoplobatrachus litoralis</i> OM501581 | | | | | | | | | | | | | | | | | |
| 2 <i>Hoplobatrachus litoralis</i> AB6711181 | 0.000 | | | | | | | | | | | | | | | | |
| 3 <i>Hoplobatrachus litoralis</i> AB671179 | 0.002 | 0.00 | | | | | | | | | | | | | | | |
| 4 <i>Hoplobatrachus litoralis</i> AB671177 | 0.002 | 0.00 | 0.00 | | | | | | | | | | | | | | |
| 5 <i>Hoplobatrachus tigrinus</i> AB272594 | 0.026 | 0.03 | 0.03 | 0.03 | | | | | | | | | | | | | |
| 6 <i>Hoplobatrachus tigrinus</i> AB167944 | 0.026 | 0.03 | 0.03 | 0.03 | 0.00 | | | | | | | | | | | | |
| 7 <i>Hoplobatrachus tigrinus</i> AB272592 | 0.028 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | | | | | | | | | | | |
| 8 <i>Hoplobatrachus crassus</i> AB290413 | 0.054 | 0.05 | 0.06 | 0.06 | 0.04 | 0.04 | 0.05 | | | | | | | | | | |
| 9 <i>Hoplobatrachus crassus</i> AB272595 | 0.054 | 0.05 | 0.05 | 0.05 | 0.04 | 0.04 | 0.05 | 0.00 | | | | | | | | | |
| 10 <i>Hoplobatrachus crassus</i> AY014375 | 0.062 | 0.06 | 0.06 | 0.06 | 0.05 | 0.05 | 0.06 | 0.03 | 0.03 | | | | | | | | |
| 11 <i>Hoplobatrachus occipitalis</i> AB272600 | 0.112 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.12 | | | | | | | |
| 12 <i>Hoplobatrachus occipitalis</i> AB272599 | 0.111 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.10 | 0.11 | 0.11 | 0.11 | 0.00 | | | | | | |
| 13 <i>Hoplobatrachus occipitalis</i> MK036439 | 0.111 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.12 | 0.00 | 0.00 | | | | | |
| 14 <i>Hoplobatrachus rugulosus</i> AF285208 | 0.033 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.04 | 0.05 | 0.05 | 0.06 | 0.10 | 0.09 | 0.10 | | | | |
| 15 <i>Hoplobatrachus rugulosus</i> AB290417 | 0.034 | 0.03 | 0.04 | 0.04 | 0.03 | 0.03 | 0.04 | 0.05 | 0.05 | 0.06 | 0.10 | 0.10 | 0.10 | 0.00 | | | |
| 16 <i>Hoplobatrachus rugulosus</i> AB290414 | 0.035 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.04 | 0.05 | 0.05 | 0.06 | 0.10 | 0.09 | 0.10 | 0.00 | 0.00 | | |
| 17 <i>Leptobrachella parva</i> KJ831304 | 0.205 | 0.20 | 0.21 | 0.21 | 0.21 | 0.21 | 0.21 | 0.22 | 0.22 | 0.23 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |

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